Odour signals contain multi-modal information in the banded mongoose (*Mungos mungo*).

Jessica Mitchell

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy.

October 2016

Acknowledgments

The completion of this research would not have been possible without the support and experience of the team behind Exeter University's Banded Mongoose Project. Along with access to the field site and their fully habituated animals, Mike Cant and his team allowed me to live in their research accommodation and provided logistical support throughout my field seasons. Jenni Sanderson and Harry Marshall were a great support in being around for the first couple of weeks as I settled into the site in 2014. Whilst in Mweya the support of Francis, Solomon, Kenneth and Robert was invaluable to my data collection and survival in the field. Their friendly and caring demeanour also meant I had two fantastic field seasons and really made the most of living in such a beautiful part of the world. In particular, I would like thank Francis for acting as a tour guide when my fiancé Lewis came out to visit during 2015. I would also like to thank the UWA rangers and staff for keeping me safe, and all the staff at Tembo canteen for the delicious food and drinks. Queen Elizabeth twinning project also deserve a mention here as they provided a welcome social distraction during both of my field seasons. I really enjoyed tagging along with them during their school visits and hopefully the kids enjoyed my contributions to their lessons!

Back in the UK I would again like to thank Exeter's team for their continued analytical support. Emma Vitikainen in particular has been a constant source of advice on both an academic and personal level. Although there were several disasters regarding sample storage and transport the centre for proteome research at the University of Liverpool provided a stellar service in analysing these scent samples, and helping me get the best out of this valuable data. At LJMU I was also helped by Nicola Koyama and Alan Gunn with regards to parasitology methods, identification and analyses. Dr Jerry Bird, Gill Beasly and Dave Jones were invaluable in dealing with purchases and all financial issues. My office mates throughout the years have also done a great job of keeping my spirits up when things got tough so thank you very much Sandra Edmunds, David Wells, Ed Parker, Luke Reynolds and Jim Carter.

It goes without saying that my supervisor Hazel Nichols has been a major influence on the success of this project. She has provided consistent support whilst allowing me to manage the project for myself. Her statistical and R analytics training was a major help, although quite overwhelming, in my first year and I now feel we have developed together to take ownership of this project. Finally, I would like to thank my family and friends, in particular Lewis, for putting up with me for the last three years. Although there have been some amazing highs (Free trips to Uganda) it has not been without its lows and I'm sure my turbulent emotions during the final few months were difficult to deal with so thank you all!

Contents

Abstract

Communication can be crucial to the profitability of reproduction by allowing individuals to attract and select an appropriate mate. Across mammals, successful reproduction can depend on the ability of individuals to gain information such as relatedness, health parameters and breeding status from potential mates. Although visual and auditory signals are utilised, scent is a crucial and ancient form of communication yet, with the exception of certain model systems, we understand little of how it functions in wild mammals. This thesis will focus on the mechanistic role of odour signals: what information they contain and how they may facilitate reproductive decision-making in the banded mongoose (*Mungos mungo*). I use a wild but habituated population to conduct experimental odour presentations showing these mammals are capable of discriminating scents based on sex, familiarity, relatedness and female reproductive state. The ability of odours to encode such multi-modal information suggests they may facilitate key behavioural processes such as kin recognition, mate-choice and competitive interactions. However, the discrimination of pregnancy specifically implies scent cues function within reproductive decision-making, attracting males to receptive mates. The gastro-intestinal parasite community of this banded mongoose population was also screened, allowing the ability of odour cues to advertise parasitic infection to be tested. Observations show highly parasitised individuals scent-mark less frequently, suggesting marking behaviour indicates quality in terms of parasite burdens. Furthermore, experimental odour presentations show that banded mongooses exhibit behavioural aversions toward odours of heavily infected individuals. Scent cues, in the banded mongoose system, thus appear to encode a multitude of information relevant to reproduction.

Thesis structure

This thesis presents a scheme of experimental and observational studies investigating banded mongoose scent communication. In chapter one, I begin with a brief review of functional odour communication and the lack of research on wild mammals. I then highlight the suitability of the banded mongoose as a study species for this research, and my main research aims. Chapter two outlines methodology and details of the study population and field site. Chapter three shows that odours of males and female differ in terms of their chemical composition. Odour presentations support this by demonstrating the discrimination of odours based on sex, familiarity and relatedness. However, interactions between these factors suggest banded mongooses use this information for sex-specific functions. Chapter four expands upon this point by showing that female reproductive state is discernible via scent but provokes differing reactions from the sexes. Alongside chemical analyses I also consider the protein content of odour signals and present tentative evidence for a protein-based difference between pregnant and non-pregnant scents. Chapter five is an overview of the gastro-intestinal parasite community of the banded mongoose and considers the factors responsible for variation in parasite burdens. Social, ecological and life-history factors explain only a limited amount of variation suggesting individual parameters have a greater bearing on parasite burdens. Indeed, in chapter six I discuss a submitted article where I show that genetic heterozygosity correlates with faecal egg counts in this system. Results suggest more genetically diverse individuals harbour lower parasite burdens. Chapter seven presents observational and experimental work showing that certain parasitic infections can be detected via scent. Ova burden also appears to influence scent-marking behaviour, which may have implications for mate-choice. Finally, chapter eight concludes with an overview of the information encoded by banded mongoose scent cues and how this may assist reproductive decision-making. Throughout this thesis I discuss results within the wider context of mammalian scent communication, specifying how they have advanced this field and identifying points for future research. This body of work is my own and builds upon previous scent communication research which is fully referenced throughout the thesis. For chemical and protein analyses, I collaborated with the University of Liverpool which is again acknowledged and referenced in the appropriate sections of the thesis.

Chapter 1 General introduction and background

Communication plays a fundamental role in animal societies and is particularly important in facilitating reproductive decision-making. Although a wide range of senses are used to communicate, scent represents the oldest and most widespread mechanism (Wyatt, 2014). Across social insects individuals can recognise colony members due to the specific concentration and combinations of cuticular hydrocarbons secreted on their exoskeletons (van Zweden and d'Ettorre, 2010). Such signals may also indicate an individual's role in eusocial insect societies (Martin and Drijfhout, 2009). Certain insects destroy non-Queen larvae using scent to identify them whilst other specific chemicals, known as pheromones, are secreted by the Queen and can influence the development of her workers (Holman et al., 2010; Kocher and Grozinger, 2011). Most mammals also produce olfactory cues including glandular secretions, faeces and urine. These are often deposited deliberately for communicatory purposes by scent-marking which allows odours to persist in the environment (Johnson, 1973; Ralls, 1971). Unfortunately, research into mammalian scent chemistry and functionality lags far behind that of invertebrates. Here I review the current literature regarding mammal scent communication and its caveats. I propose the banded mongoose as an ideal study species to investigate functional scent communication in mammals. Finally, detailed aims and research questions are outlined.

Introduction

Throughout this thesis I shall use the terms "odours" and "scents" interchangeably to describe the intra-specific chemical signals used by animals to communicate with conspecifics. However, there are chemical differences in the types of signal which facilitate animal communication. Scents comprise a mixture of volatile and non-volatile chemical compounds. Known as semiochemicals, their functionality can be split into two types of signal (Wyatt, 2014). Firstly, pheromones transfer information about a sender to members of the same species and comprise either a single, or suite, of specific chemicals. For example, sexually mature female silk moths produces bombykol which attracts males (Hecker and Butenandt, 1984). Being a pheromone, bombykol is present in all sexually mature females, it is the amount produced that differs between individuals. Alternatively, the second form of chemical signal "signature mixtures" are a combination of chemicals used to facilitate individual recognition or communicate membership of a particular social group. Bees, wasps and other social insects rely on a mixture of cuticular hydrocarbons, secreted from the exoskeleton, to distinguish colony mates from intruders (Wyatt, 2014). The ant, *Formica exsecta*, shows colony-specific combinations of (Z)-9-alkanes which appear to be under genetic control (Martin and Drijfhout, 2009; Martin et al., 2008). The variable combination of molecules allows individual colonies to be identifiable but it does not constitute a pheromone, which would be expected to show a uniform combination of alkanes across all colonies. Signature mixtures also allow individual recognition in mammals, for example, mouse urine contains information regarding species, sex, identity and genetic diversity (Cheetham et al., 2007; Thom and Hurst, 2004; Thom et al., 2008; Yamazaki et al., 1979). However, such odours can also encode variable parameters such as infection and reproductive status (Barnard et al., 1998; Hurst, 2009; Kavaliers et al., 2005b; Sachs, 1997).

In all previously mentioned examples, pheromones and signature mixtures were identified by gas chromatography-mass spectrometry. This method allows a "scent-profile" to be created detailing the chemical compounds present within a scent (Drea et al., 2013). However, the ability for specific chemicals to encode certain parameters such as sex, etc. must be tested via behavioural assays (Drea et al., 2013; Wyatt, 2014, 2015). For example, a pheromone has been identified in rabbit mammary glands which stimulates suckling behaviour in pups (Schaal et al.,

2003). Here a target chemical was isolated, following GC-MS analysis of mammary gland secretions, the tentative pheromone was then synthesised and behavioural assays performed on lab-housed rabbit pups to ensure this chemical elicited the suckling response (Coureaud et al., 2003; Schaal et al., 2003). Such experimental work is key to identifying pheromones as they provide evidence that the target chemical does provoke the behavioural reaction (Wyatt, 2014, 2015). However fully comprehensive studies are not always possible, particularly the synthesis of target chemicals for use in behavioural assays. Instead, odour presentations are often conducted to test an animal's response to odours of differing parameters such as sex, familiarity, and relatedness or infection status. By scoring how intensively an animal responds to an odour or for how long it remains interested or in contact with the presentation, observers can compare responses and speculate whether or not this animal is capable of determining said parameter via scent (Hurst et al., 1998). This technique has been successfully used in the house mouse and ring-tailed lemur, *Lemur catta,* where experimental presentations show that conspecifics can discriminate differences in scent upon parameters including sex, dominance and reproductive status (Beynon and Hurst, 2003; Charpentier, 2008a; Charpentier et al., 2010; Crawford et al., 2011; Hurst, 2009; Roberts et al., 2014). Chemical targets for such discriminatory behaviour can still be searched for retrospectively, using GC-MS. However, odour presentations give a strong indication of whether animals are capable of discriminating scents based upon the parameters (i.e.: sex, familiarity) in question (Beynon and Hurst, 2003; Kavaliers et al., 2005b).

In non-model and wild systems we are only just beginning to scratch the surface with regards to functional odour communication. This is due to the practical constraints of sampling multiple individuals or having access to a habituated population to conduct well-designed odour presentations. Beyond regulated laboratory environments multiple factors must be controlled for in order to understand odour communication. In asocial systems it is relatively simple to determine the function of scent signals because they tend to be decoupled from their sender, being left for interpretation in the absence of their donor (Ralls, 1971). For example, solitary female bank voles going through post-partum oestrus deposit more scent marks than pregnant or sterile females. Additionally, males show heightened marking behaviour in response to the odours of post-partum compared with odours of pregnant or sterile females (Ferkin et al., 2004). Such results suggest the reproductive state of female odours has a strong influence on recipient marking behaviour and authors attribute these differences to competitive pressures associated with reproduction. However, in social species it becomes more difficult to ascertain the function of odour signals due to various complexities. Studies similar to the bank vole example (Ferkin et al., 2004) are confounded by factors such as prior familiarity with the donor, relatedness and dominance hierarchies (Charpentier et al., 2010; Scordato et al., 2007). Such complexities mean assigning function to scent signals is difficult within social species. Social mammals may also enrich scent marking with visual and auditory displays, such as the wrist clicking of ring-tailed lemurs, which provides receivers with information on quality and dominance (Charpentier, 2008a; Kappeler, 1998; Kavaliers et al., 2003a). The importance of this information may not be realised if odour presentations focus solely on an animal's response to a scent. For example, previous research suggests health-related parameters, including parasite burdens, should be detectable via scent in the house mouse because the diversity of chemicals within the urine co-varies with diversity at immune-related genes (Cheetham et al., 2007; Hurst, 2009; Penn and Potts, 1999; Yamazaki et al., 1979). Indeed, in choice-tests, female mice preferentially mate with uninfected over infected males, suggesting odour cues facilitate a direct assessment of quality (Ehman and Scott, 2003; Kavaliers et al., 2014; Roberts et al., 2014). However prior familiarity with the odour-donor was found to be crucial for females to make this informed choice (Cheetham et al., 2008; Roberts et al., 2014). Thus functional scent discrimination may require more information than just the odour. This raises questions about the methods used to test how odour cues function and whether they account for natural scent-marking behaviour.

The over-use of choice-tests represents a limitation to current methodologies. Here, recipient animals are provided with a choice of two (or more) odours within an arena (such as a Y maze). This allows researchers to monitor the amount of time an individual spends within the vicinity of each scent and thus infer their ability to discriminate odours. Whilst ideal for testing specific hypotheses in controlled conditions, choice tests may not accurately represent the dynamics between advertisement and choice that would occur in the wild. They effectively force subjects to choose between odours when, in natural situations, ignorance of both odours may occur. Choice-tests also sanitise the scent marking environment by removing other important cues, such as visual identification of the recipient (Cheetham et al., 2008; Roberts et al., 2014) or display behaviours (Scordato et al., 2007). This may detract meaning from the signal as such additional information is often key to decision making (Charpentier, 2008a; Kappeler, 1998; Kavaliers et al., 2003a) . In recent years, experimental set-ups have become more "natural". Tests often include a neutral choice free from presented odours (Hurst et al., 1994) or refuge areas where recipients may remain and avoid both scents. Researcher's at Duke University now conduct odour presentations within their lemur's large enclosures (Charpentier, 2008a). Leaving individuals within their social group during experiments accurately replicates natural conditions but also minimises stress to focal animals. Additionally, natural investigatory behaviour is stimulated by positioning scents on experimental poles at the same height scent marks would occur on trees in the wild. (Charpentier, 2008a; Charpentier et al., 2010). Such studies on captive animals with complete life-history records are also able to test the ability of scent cues to encode multimodal information. For example, ring-tailed lemur scents are now recognised to contain a plethora of information ranging from genetic heterozygosity to sex and reproductive status (Charpentier et al., 2010; Crawford et al., 2011; Scordato et al., 2007). Additionally sex differences in odour composition may only be present during receptive periods as has been shown for other lemur species (Boulet et al., 2010). Thus, with stringent experimental design and detailed knowledge of the study species' behaviour it is possible to decipher the multimodal information encoded by scent.

Several studies convincingly show that mammalian odours can signal sex (Rasmussen et al., 1997; Scordato et al., 2007; Swaisgood et al., 1999), familiarity and relatedness (Jordan et al., 2014; Leclaire et al., 2013; Mateo, 2006), sexual receptivity (Converse et al., 1995; Ferkin et al., 2004; Hudson, 1990; Ziegler et al., 1993) and parasitic infection (Kavaliers et al., 2005a, b; Penn and Potts, 1998a). Yet how this information is utilised in behavioural decision-making is still relatively unknown beyond model organisms. In terms of the function of scent cues, most research focuses on their role within competitive interactions. In many species odours are regularly over-marked (marked on top of) by conspecifics. This is assumed to indicate one's ability to monopolise an area, as only successful competitors can keep marks recently refreshed (Gosling and Roberts, 2001). Thus, scent marking may be considered a reliable and continuous record of competitive interactions between individuals. However, if an individual can outcompete conspecifics in terms of marking frequency, and over-marking, they not only display superiority to competitors but also better advertise themselves to potential mates. Thus, within the intra-sexual competition hypothesis there is the capacity for scent cues to influence mate choice and other forms of reproductive decision making (Rich and Hurst, 1999) . Indeed, Hamilton and Zuk (1982) suggested that animals should benefit from inspecting the odours of a potential mate as a way of gauging condition. This would allow scent to function within mate-choice, directing individuals to fitter mates, yet has received little empirical attention to date. Indeed, beyond well-studied model organisms our knowledge of mammalian scent marking lags behind that of insect chemical communication. As discussed, mammalian models tend to be lab or captive populations, with wild and free-ranging systems rarely considered. However, the increasing number of well-habituated social populations, particularly for primates and

mongooses, should reduce this issue and facilitate detailed study into mammalian scent communication. In particular, wild systems should benefit our understanding of parasite detection. Laboratory trails consistently support the ability of mice to discriminate parasitic (Ehman and Scott, 2003; Kavaliers et al., 2005b; Kavaliers et al., 2014), viral (Penn and Potts, 1998a) and bacterial (Zala, 2004; Zala et al., 2015) infections via scent. However, in all cases animals have been experimentally infected and variation between "healthy" and "infected" individuals is often large (Kavaliers et al., 2005a). It would thus be useful to consider whether infection discrimination still occurs within wild systems with naturally occurring infection levels.

The banded mongoose represents an ideal target species for such research as a habituated population has been monitored in Queen Elizabeth National Park, Uganda for 20 years. The availability of long-term data, including microsatellite genotypes and life-history measures, allows a decoupling of multimodal signals as experiments can be designed to test specific functions of odour communication. Additionally, banded mongooses have a dynamic breeding system where multiple males and females can breed per reproductive bout (Cant et al., 2013). Mate-choice occurs in both sexes; males routinely invest time and energy mate-guarding females during oestrus periods, yet females may choose to "slip" their guard to breed elsewhere (Cant, 2000). As the majority of breeding happens within natal groups, inbreeding routinely occurs, although banded mongooses do attempt to avoid inbreeding within their own group (Nichols et al., 2014; Sanderson et al., 2015). Extra-group copulations provide a rare opportunity to breed with unrelated mates (Nichols et al., 2015) yet this chance often only occurs during aggressive and costly inter-group-interactions (IGIs) (Cant et al., 2002; Nichols et al., 2015). Finally, the banded mongooses highly social and aggressive nature means parasitic transmission between conspecifics is likely. As such, there appears a genuine need for a mechanism to detect the relatedness, familiarity, receptivity and infection status of conspecifics. Odour cues have such mechanistic potential as the banded mongoose is a prolific scent marker (Jordan, 2009), depositing faecal, urine and glandular secretions in regular social marking events. Previous research consistently supports a role for scent marking within intra-sexual competition as individuals show heightened interest in the scent of same-sex conspecifics (Jordan et al., 2011b; Müller and Manser, 2008). However, we currently know little of how odour cues are utilised in relation to reproductive behaviour.

This thesis investigates the information encoded in banded mongoose odour signals and how this may influence behaviour. As the most frequently deposited scent marks, anal gland secretions (AGS) are the primary focus of the following studies. The ability of AGS to encode sex, familiarity, relatedness and receptivity is assessed via chemical analyses, field-based observations and experimental odour presentations. Parasitic data is also collected for the population allowing analyses of how scent cues encode fitness-related information. Together results provide a holistic account of the information contained within scent, and the banded mongooses' ability to discriminate odours based upon such information. I also discuss the functional use of scent cues within the banded mongoose system, in particular how they may influence reproductive decision-making.

Aims of the thesis (statement of objectives)

1. To determine the information encoded in banded mongoose odours.
    a. Does scent chemistry differ based upon sex or reproductive state?
    b. Do banded mongooses discriminate scents based upon sex, familiarity, relatedness and/or reproductive state?

2. To provide an overview of the gastro-intestinal parasite community of the banded mongoose.
    a. What parasites are present within this population?
    b. How do parasite burdens vary in regards to social, life history and ecological factors?
    c. Is there a relationship between genetic diversity and/or inbreeding coefficient and parasite load in this banded mongoose population?

3. To combine parasitic and scent data to determine whether infection status is encoded in scent cues.
    a. Can banded mongooses discriminate scents based upon the infection status of their donor?
    b. Do scent-marking behaviours differ based upon parasitic infection burdens?

Chapter 2 Study species, field site and general methods.

Ethical statement

All observational and experimental procedures for this study population have been conducted under licences from the Ugandan Wildlife Authority, Ugandan National Council for Science and Technology, and have been reviewed by the University of Exeter's ethical committee (compliant with The Association for the Study of Animal Behaviour, ASAB, standards for animal ethics). Trapping procedures and anaesthesia are well-established (Jordan et al., 2010), with no adverse effect reported over the project's ~20 year history.

2.1 Study species

2.1.1 Life-history and behavioural overview

The banded mongoose (*Mungos mungo*) is a small diurnal carnivore common throughout sub-Saharan Africa. Belonging to the Herpestidea, which contains 37 known species distributed throughout Asia and Africa, banded mongooses are distinctive for being one of the few highly social species (Veron et al., 2004). They are believed to have the largest stable group size of any carnivore, with up to 75 individuals being observed within the same pack (Jordan, 2009). Median pack size is 24 individuals with a core of 2-5 breeding females and 4-12 breeding males (Cant, 2000). Packs also contain a periphery of younger individuals of both sexes (up to 15 females and 25 males) who often attempt to breed, plus pups and juveniles from recent reproductive bouts (Cant et al., 2013). Packs exhibit male-biased adult sex-ratios (Table 1) and are highly stable with most individuals of both sexes remaining in their natal groups all their lives (Cant, 2000). Younger, smaller individuals may be evicted by their social dominants however; this is often short-term with the evictees being allowed back to the pack after several days (Bell et al., 2012; Cant et al., 2010; Gilchrist, 2006; Thompson et al., 2016). Immigration of single individuals into established groups is rare with only two successful incidents recorded over the 20-year duration of this study (Bell unpublished). Hence packs are large, stable and contain closely related individuals (Nichols et al., 2012b).

Banded mongooses spend their nights in underground dens that range from dis-used aardvark burrows to crevices in derelict houses. However, they do not excavate sleeping sites themselves (Cant, 2000; Jordan, 2009). Den sites change every one to three nights but are reused regularly and groups with young pups can stay in the same den for up to a week (Cant, 2000). For the duration of this study, packs emerged from their overnight den at around 7am and spent up to an hour close to the den grooming, marking and socialising. Groups foraged for the next approximately three hours before "crashing" in the mid-day heat. "Crashing" lasts until mid-afternoon when it is cool enough to forage again. Foraging bouts focus on the acquisition of small invertebrate prey buried in the leaf litter, topsoil and herbivore dung. Bird eggs, small reptiles and human refuse will also be consumed if located (Jordan, 2009). Feeding is not a cooperative activity, except for those involved in pup care (see below), and food items are aggressively defended. After evening foraging sessions, mongooses move to a den and go below at dusk, around 7pm.

2.1.2 Reproduction

Banded mongooses are obligate cooperative breeders and display a polygynandrous mating system (Cant, 2000). The bulk of reproduction occurs within natal packs (Cant et al., 2014; Hodge et al., 2009; Nichols et al., 2015; Nichols et al., 2012b), which due to the lack of immigration and dispersal, means inbreeding is a common occurrence (Nichols et al., 2014). However, the recent genetic pedigree for this population shows evidence of non-random mating with respect to reproduction, in particular this suggests individuals attempt to avoid breeding with close kin (Sanderson et al., 2015). Indeed, 18% of pups are sired by extra-group-fathers (Nichols et al., 2015; Sanderson et al., 2015) and thus mating outsides ones natal group does seem possible. Extra-group-mating result in benefits such as faster pup growth rates and greater survival to independence (Nichols et al., 2015), however it comes at a high cost as the majority of extra-group copulations occur during aggressive inter-group-interactions (IGIs) where mongooses risk significant harm (Nichols et al., 2015).

Reproduction is monopolised by older and more dominant individuals (Nichols et al., 2010), but subordinates of both sexes will attempt to breed (Cant et al., 2010). Within-groups females enter oestrus synchronously and males will guard reproductively-active females by following and harassing them (Cant, 2000). However, younger satellite males may act as "pesterers" and attempt to intercept guarded females. As female oestrus is highly synchronised, a single male cannot monopolize all reproductive opportunities and must choose which female to guard. Larger, older females tend to be preferred and will often be mated with before younger, smaller subordinates (Nichols et al., 2010). Mate-guarding males also tend to be older individuals whom are recognised to secure more matings than "pesterers" or non-guards (Cant, 2000; Nichols et al., 2010). However, females may slip their guard to mate with other individuals suggesting this sex also engages in mate-choice (Cant et al., 2002; Nichols et al., 2010; Nichols et al., 2015).

After a gestational period of 60 days (+/- 3) (Cant, 2000) females give birth in synchrony. In 64% of breeding attempts females within the same pack give birth on the same night within the same den (Hodge et al., 2009). Such synchrony results in large communal litters of up to 23 pups (median litter size = 5) (Bell, 2007) and is recognised to reduce the risk of infanticide (Cant et al., 2014; Hodge et al., 2009). However, there is a limit to this benefit as once over 8 females are breeding, per-capita litter success begins to decline (Cant et al., 2010; Cant et al., 2014) This is likely due to increased competition between pups. Despite relatively low reproductive skew, for a cooperative breeder, banded mongoose females face intense intra-sexual competition over access to breeding resources, in particular competition between pups over carers.

Pups remain in the communal den and are 'babysat' by adults who stay behind while the rest of the pack forages (Gilchrist and Russell, 2007; Hodge, 2005; Hodge et al., 2009). At around 3 weeks of age, pups are able to move from the den and accompany the pack on foraging trips. The majority of pups develop an exclusive relationship with an adult (usually male), known as an 'escort'. Escorts feed, carry and protect their pup from predators (Gilchrist and Russell, 2007; Hodge, 2005) until the pup reaches nutritional independence at around 90 days. This is highly beneficial as escorted pups are known to have increased growth and survival rates compared to those who are not escorted (Hodge, 2005).

2.2 Field site and study population

2.2.1 Field site

This study was conducted as part of the Banded Mongoose Research Project based on the Mweya peninsular of Queen Elizabeth National Park, Uganda (0°8'2"S, 29°51'42"E). Here a population of wild banded mongoose have been habituated to human observation since 1994 allowing a wealth of life history, behavioural, genetic and physiological data to be collected. Presently the project works with nine packs spread over an approximately 15km^2 area although the number of packs monitored has varied over the duration of study (Nichols et al., 2012b).

2.2.2 Habitat

The field site comprises the Mweya peninsula that is densely vegetated primarily by *Euphorbia candelabrum and acacia (*Jordan, 200*9)*. The lower peninsula is more densely vegetated with undulating terrain, a network of game trails and deep gullies formed through erosion. Vegetation is more open on the upper peninsula (Field and Laws, 1970) and bisected by dirt roads. This area is known as Mweya village and is inhabited by ~800 people including tourists, researchers and National Park staff. The Upper peninsula is home to the Mweya safari lodge, hostel and visitor centre. There is also an airstrip, army camp, and village accommodation for those working in the tourist trade. Full details of habitat, flora and fauna are described elsewhere (Cant, 2000; Jordan, 2009).

2.2.3 Weather

Weather is recorded by a small station in the research buildings (Cant et al., 2013). Rainfall is around 800-900mm annually with two dry seasons from December to February and June to August where rainfall can be limited. Temperature and day length does not vary greatly throughout the year due to the sites' close proximity to the equator.

2.2.4 Study population

The banded mongoose project has been working on Mweya since 1994 and previous studies focused on these banded mongooses in the 1970s (Neal, 1970; Rood, 1975). During my field seasons, the project was working with nine packs. Four of these were fully habituated to human presence and the other five were at varying levels of habituation. Packs ranged in size from six to 34 individuals with mean size of 20 in 2014 and 19 in 2015 (Table 1). Across the study period, most packs displayed a male-biased adult sex ratio that is common for this species (Cant et al., 2013). Packs showed traditional compositions with a core of older breeding individuals, plus younger occasional breeders, and pups from the latest breeding attempts. Packs defended stable territories ranging between 0.3 and 2 Km^2 in size (Cant, 2000; Cant et al., 2013) which often neighboured other groups (Figure 1), thus inter group interactions (IGIs) were common.

Table 1: Pack sizes and structure for cohorts of May-August 2014 and June-August 2015.

Pack	Habituation status	Pack size		No Adult females		No Adult males		Adult sex ratio	
		2014	2015	2014	2015	2014	2015	2014	2015
1B	Full	26	34	7	14	8	20	1.14	1.42
2	Full	26	20	10	10	14	10	1.40	1.00
11	Full	23	19	4	3	19	16	4.75	5.33
1H	Full	24	25	8	12	10	13	1.25	1.08
4B	Low	6	6	1	3	5	3	5.00	1.00
7A	Low	27	26	5	8	16	18	3.20	2.25
21	Low	14	9	3	6	4	3	1.33	0.50
19	Fair	22	23	4	9	6	14	1.50	1.56
17	Fair	12	10	4	4	8	6	2.00	1.50

Pack size refers to the maximum group size noted across the above study periods. Adult numbers refer to males >6months old and females >9months old, with sex ratio representing number of adult males/number of females.

Figure 1: The territories of current banded mongoose packs.

2.2.5 Habituation

The focal population is habituated to human presence by four full-time field assistants. When approaching a pack, observers give a feeding call and throw a small amount of bait, moistened dog biscuits (Montaego Karoo pet nutrition, South Africa). The four fully habituated packs are comfortable in human presence from this initial contact. However, when walking alongside the packs a two-note habituation hum is used as reassurance when moving. The remaining packs are at varying levels of habituation; most appear in response to the bait but do not allow humans to walk with them.

2.2.6 Identification

At birth all mongooses are given a unique identification code relating to their birth pack, sex and individual identity. For visual identification, mongooses receive a shave into the fur on their backs (Figure 2) and a transponder chip that can be scanned if their shaves fade. Both are administered under anaesthesia during routine trapping events (section 2.5).

Figure 2: Banded mongooses showing fresh shaves (arrows) to enable identification.

2.2.7 Tracking

At least one mongoose per pack is fitted with a radio collar (Sirtrack Ltd. New Zealand). Collars weigh between 21 and 38g which, at around 1-2% of a mongooses' body weight, are deemed safe and unobtrusive (Cant, 2003). Collars emit a unique radio frequency and field assistants locate packs using digital telonic radios (Biotrack, UK) tuned to the specific frequency and aerial receivers to amplify the signal. Collars are fitted by trained field staff during routine capture events (see section 2.5) and are regularly monitored to ensure a comfortable fit.

2.3. Data collection

2.3.1 Life-history data

Packs are visited daily in the morning to collect life-history information including group compositions, pregnancy and oestrus states, mate-guarding information, babysitter and escort IDs. Field assistants record data using handheld tablets (Samsung, UK) which synchronize directly to the banded mongoose project network allowing data to be backed up daily.

2.3.2 Weights

Fully habituated individuals are trained to be weighed on a small electronic balance up to twice a day. Banded mongooses are baited to the scales with powdered-milk formula made up in a

rodent drinking bottle. Some mongooses can be picked up by the scruff of the neck and placed on the scales while others are lead onto the balance by following the bottle. In the four fully habituated packs over 90% of individuals can be weighed both morning and evening. Weights are recorded on handheld Samsung tablets.

2.3.3 Marking focals

All social marking bouts in the two most habituated packs (1B and 1H) were filmed during two mornings per week using a handheld video camera (Panasonic 5 Access Hybrid O.I.S, Full HD). A marking bout was defined as any type of naturally occurring marking behaviour such as latrines, novel object marking, group scent marking and territorial marking. Note that by focusing on the two most habituated packs, observers were able to gain detailed footage of marking events without causing undue stress to nervous animals. Although the majority of individuals in several other packs are well-habituated, certain older males remained shy of close observation. This would have made it difficult to quantify such individuals' presence and behavioural activity in marking bouts.

Marking bouts were filmed in their entirety and any bout starting before the observer could configure the video camera was discarded from the analysis. Videos were watched back after the field session where observers recorded; the duration of the marking bout, identities of all animals present (within 1m of the bout) and those actively involved (sniffing or depositing scent marks). For analyses in chapter 7, these data were used to calculate the frequency of group marking events where each focal mongoose was present, actively marking (sniffing or depositing at least one scent mark) or intensively marking (depositing >5 marks per bout). Intensive scent marking was defined as a when the same individual deposited more than five scent marks in a single marking bout. Preliminary analyses revealed the repeated deposition of >5 scent marks per bout was confined to 39% of the animals monitored and thus represents a relevant cut-off point to discriminate between normal marking behaviour and those individuals marking at high intensity.

2.3.4 Faecal sampling

Faecal samples for parasite-analysis are collected on a regular basis across the study population facilitating general identification of banded mongoose parasites. However, between May and July 2014, all individuals within two fully-habituated groups (1B and 1H) were sampled on a weekly basis alongside odour presentations and marking focals. Again emphasis was placed on these packs as every individual could be followed closely enough to collect the associated marking focals required for this study (Chapter 6).

Faeces for parasitic sampling were collected in morning field sessions only as previous research suggests ova burdens vary between faeces shed across the day (Rafalinirina et al., 2015; Villanua et al., 2006). Confining sampling to the three hours after mongooses left the den thus minimized the likelihood of extreme variation in ova counts. Samples were collected in small plastic bags which were turned inside-out to scoop up the sample. Faeces were homogenized inside the bag and half returned to the field so as not to disturb natural scent marking. The remaining half was labelled and stored in a thermos flask of ice until back at the field laboratory where it was transferred into a 50ml flacon tube containing approximately 20ml of 5% formalin. The sample

was broken down by mixing with a wooden skewer and tubes were labelled and stored at room temperature before being transported to the UK for analysis.

2.4 Parasitology methods

2.4.1 Parasite counts and identification

Faecal samples were analysed by a modified MacMaster salt floatation technique (Cringoli et al., 2004; Dunn and Keymer, 1986). Samples in formalin were spun down (6 minutes at 4000rpm for all spin stages) and the formalin removed by tipping off into a waste container. The sample was then washed with distilled water, spun down and drained twice more. On the final wash, 14ml of distilled water was added; the sample was then shaken and strained through a fine sieve into a clean beaker. The faecal mass was removed from the strainer, weighed and discarded while the fluid was carefully rinsed into a 15ml falcon tube and spun as previous. The resulting pellet of faecal matter was agitated with a glass rod before 15ml of fully saturated salt solution was added. The tube was then inverted 5 times before a single aliquot of the sample was pipetted from the centre of the tube to fill a 0.3ml MacMaster counting slide. After letting the slide stand for two minutes all parasite ova within each of the two chambers were counted and tentatively identified using the veterinary parasitology literature (Bowman, 2014; Leclaire and Faulkner, 2014; Urquhart et al., 1996) and communication with experts in the field. In most cases ova were identified to the genus level.

Faecal egg counts often face criticism as a measure of parasite load due to high variability within individuals sampled (Gasso et al., 2015; Villanua et al., 2006). Egg shedding loads can vary with the life stage of the parasite, co-infection, environmental conditions and the physiological condition of the host (Dorchies et al., 1997; Jolles et al., 2008; Raharivololona and Ganzhorn, 2010; Villanua et al., 2006). However for this study it would not have been feasible to sacrifice individuals to gain comprehensive adult parasite counts from the gastrointestinal tract (Poulin and Morand, 2000). As such, individual banded mongooses were sampled multiple times allowing average parasite counts to be calculated. Average egg counts provide comparable estimates of parasite load across individuals over short durations by minimising individual variation. Additionally, across targeted study periods utilising parasitic data (summer 2014) the climate remained consistently warm and dry with negligible rainfall, all banded mongoose groups patrolled consistent territories and did not experience large-scale predation or other stressful events. Thus average ova counts are unlikely to be skewed by weather fluctuations, abnormal foraging patterns, territory shifts or other known stressors.

2.4.2 Quantifying parasite load

Raw counts of all identifiable parasite ova were converted into an egg per gram figure (epg) using the following, standard McMaster equation:

$$\frac{Y*(15/0.3)}{X}$$

Here Y represents the sum of all ova counted across the two chambers of the Macmaster slide and X represents the total weight of faecal matter from which the ova were obtained (Dunn and Keymer, 1986). Epg counts for each type of identifiable ova were also calculated for each

mongoose using the above method. Parasite taxa richness (PTR) was calculated for each individual sample by considering the number of different parasite ova present.

2.5 Trapping, anaesthesia and sampling

2.5.1 General trapping protocol

Individuals in fully-habituated packs could be picked up by the scruff of the neck and transferred into a black cloth bag inside a Tomahauk box trap (67 x 23 x 23cm Tomahauk Live Trapping Company). For less-well habituated animals, traps were baited with scraps of fish and meat and left in close proximity to the morning den for around 45 minutes before observers returned to collect the trapped individuals. In all cases banded mongooses were transported back to the field laboratory within their traps which were stacked on the back of a Toyota Hilux and covered with a dark sheet.

At the laboratory mongooses were processed individually by coaxing from the trap into a dark cloth bag. The mongoose was restrained by a field assistant and anaesthetised with isoflurane, administered by positioning a gas mask over the nose and mouth area over the top of the bag. Isoflurane was initially delivered at a dosage of 5% alongside oxygen at a rate of 4L/min using a calibrated vaporiser. Isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) is a halogenated ether which vaporises quickly, although liquid at room temperature; it is routinely used for inhalation-anaesthesia as the dosage can be altered quickly and there are few known side-affects, the most common being slight respiratory aggravation (Dolan and Stevens, 1974). Once unconscious, banded mongooses were transferred to the examination bench and isoflurane flow reduced to around 3%. Field assistants collected routine samples detailed elsewhere (Jordan et al., 2010) as well as anal gland samples required for this study (below). As sampling came to an end, isoflurane was switched off and the mongooses revived by a 30-60 second exposure to pure oxygen. Mongooses were returned to traps for recovery, with a small dish of water and covered in a dark cloth. Once all trapped mongooses had been processed and regained consciousness they were driven back to the trapping location and released. Bait was also taken to the release site to ensure mongooses retained human trust, and to allow field assistants time to observe individuals as they returned to the bush.

To detect pregnancy, all mature females (>9 months old) were trapped following the end of oestrus behaviour in their social group (usually 7-14 days after oestrus began). Females would receive an ultra-sound scan and if confirmed pregnant foetuses would be counted and measured. Pregnant female's time under anaesthesia was kept to a minimum and no pregnant female was captured in late-term pregnancy (>5 weeks into gestation).

2.5.2 Odour sample collection

Banded mongooses have two anal glands, either side of the anal opening within the anal pouch (Figure 3). During routine trapping events (under anaesthesia) these glands were expressed to collect secretions for both chemical analysis and odour presentations. Secretions were collected in 2ml snap-cap glass vials (Fisher scientific) which were cleaned by soaking for several hours in methanol, air drying then soaking in detergent and warm water (1:1000 dilution), rinsing and allowing to air dry again. The anal region was cleaned with cotton wool and a glass vial placed over the gland opening. The examiner then applied gentle pressure around the gland to express

~150 µl of the secretion from each gland into the same vial. Secretions were vortexed to mix, labelled and transferred to liquid nitrogen immediately. Where possible, two vials were collected per individual, however if the glands could not be expressed with ease a single sample was split between two vials using 200µl Gilson pipettes and autoclaved pipette tips. To minimise contamination, sterile nitrile gloves were worn and changed between individual banded mongooses. Examiner's fingers never came into contact with the secretion nor the top of the glass vials.

Figure 3: Male anal region. Arrows indicate anal gland openings either side of the rectal opening within the anal pouch.

2.6 Odour presentations

2.6.1 Odour presentation protocol

Odour presentations were conducted in the field and designed to test the ability of odours to encode different information (outlined in detail across the following chapters). Odour presentations exposed the recipient banded mongoose to a single odour during natural foraging behaviour rather than simulated choice-tests or habituation-dis-habituation trials. This experimental design was considered the most reliable way to generate comparable data and to avoid habituation to the protocol. Presenting odours during natural foraging behaviour also minimised stress to the animals and ensured behavioural reactions were representative of natural scent investigation.

Odour presentations were conducted in both the morning and evening foraging sessions and each banded mongoose received a maximum of two presentations per half day to prevent habituation to the methods. Each pack was given a one-day break after every two days of presentations, again as a precaution to habituation. Odours were only presented during "normal behaviour" which was defined as when over half the pack, including the focal individual, were foraging. Presentations were not attempted during grooming or social-bonding sessions, predator alarms or for at least 20 minutes following a marking session or latrine. Odours were presented when the focal mongoose was at least 1m from conspecifics; however, there were occasions where other individuals interrupted presentations. Where these individuals did not physically interfere with the focal mongoose the presentation was recorded and scored as normal (see below). However, when other mongooses became physically involved in the presentation, and deposited

scent marks, the trial was abandoned. Finally, if an inter-group interaction (IGI) occurred, presentations were abandoned for at least 24 hours, to ensure responses were not affected by previous stimulation from the intruder group.

Individual mongooses were targeted and presented with a sample of freshly defrosted anal gland secretion applied to a clean bathroom tile (washed twice in 1:1000 diluted detergent and left to air dry) (Figure 4a). Before presentations, the selected AGS samples were removed from liquid nitrogen and stored in an iced thermos flask for transportation. Samples were defrosted by rolling between the observers' fingers for around 60 seconds and then applied to the tile using an autoclaved cotton swab. Samples were discarded after each presentation and if for any reason a presentation could not be conducted within 5 minutes of the sample defrosting it was aborted and attempted again using a fresh aliquot of anal-gland secretion. Samples were presented to the focal mongoose by placing the tile directly in their line of sight. Their following reaction was filmed on a handheld video camera (Figure 4b) and was scored later (see below). Filming continued until the focal mongoose had returned to normal foraging behaviour (as above). To ensure responses were genuinely regarding the odour and not a novel object response, mongooses were randomly presented with a clean," blank" tile with no odour. Of 31 blanks presented during preliminary work, only one provoked a scent marking response. Mean contact with blank tiles was 6.06 seconds compared to 18.07 seconds for experimental presentations and duration before returning to normal behaviour averaged 10.93 seconds for blanks but 28.03 for experimental presentations. As such, I was satisfied that banded mongooses were responding to presented odours and not the novelty of the tiles.

Figure 4a: Odour presentation kit composing of a clean tile, autoclaved swab and defrosting odour sample. Figure 4b: The presentation of an odour to a well-habituated focal individual. Once the sample was presented the observer backed away to allow the individual space to investigate the scent. For shyer mongooses the sample would be placed in their line of sight at a distance of several metres and the observer would wait for the mongoose to approach the presentation.

2.6.2 Scoring of odour presentations

Video footage was analysed by recording the following details; duration before returning to normal foraging behaviour (as defined in Table 2), duration of contact with presentation (where the mongoose was physically touching or sniffing within 5cm of the tile), and the intensity of the

response based upon how many scent marks were deposited. The total number of scent marks deposited within 30cm of the presentation were counted, however marks were further split into either direct over-marks or adjacent marks depending on whether or not they were deposited directly on top of the odour sample. This distinction between marking behaviour is critically important in terms of how results are interpreted in regard to the function of scent communication (Table 2). Direct over-marks are considered competitive, as they obliterate the odour of the previous marker (Rich and Hurst, 1999; Wolff et al., 2002). Alternatively, marks adjacent to a scent are often considered to function in self-advertisement and/or mate choice as they maximise the individual identity of both markers (Wolff et al., 2002).

Table 2: Measures of behavioural responses to presented odours

Measure	Explanation	Justification	Usage
DURATION	The duration in seconds it takes a recipient mongoose to return to normal foraging behaviour following an odour presentation.	Duration measures are often used as proxies for interest in an odour presentation. Following preliminary observations of banded mongoose scent marking individuals were observed to cease contact with the presentation but remain close to the tile in a vigilant manner. I thus felt it was important to distinguish between the time spent in contact with the odour and the time before the recipient returned to normal foraging.	Chapters 3, 4 and 7
CONTACT	The duration a recipient mongoose remains in contact with (including sniffing) tile upon which odours are presented.		Chapters 3, 4 and 7
TOTAL MARKS	The total number of olfactory marks deposited by a recipient mongoose during an odour presentation.	The number of scent marks deposited can be used to quantitatively assess the difference in response to presented odours. Generally heightened marking behaviour is considered to signal a heightened interest in the odour.	Chapters 3, 4 and 7
OVER-MARKS	The total number of olfactory marks deposited directly on top of the tile upon which the odour was presented.	Over-marks are generally considered a competitive response to a presented odour as they obliterate the original mark.	Chapters 3 and 4 (when concerning female to female presentations)
VICINITY MARKS	The total number of marks deposited within a 30cm radius of the tile upon which the odour was presented	Marks around an odour preserve the scent of both the original and recipient marker. As such this method of scent marking is often considered to function within mate-attraction, self-advertisement and is thus often linked to mate-choice.	Chapters 3 and 4 (when concerning female to male presentations). Chapter 7 when considering response to opposite-sex presentations.
NORMAL FORAGING BEHAVIOUR	Focal individual is engaged inactive foraging, digging in topsoil/dung or vegetation or eating a small food item. Individual is ~1m away from other group members but not actively engaged in social interactions such as grooming, mating, fighting or playing	Normal behaviour must be conclusively defined in order to define start and end points of odour presentations	Used throughout thesis to define when duration of interest in odour presentations ended

An explanation of terms used to measure marking responses to presented odours throughout this thesis.

2.7 Chemical analysis

2.7.1 GC-MS of anal gland secretions

All chemical analyses were performed in collaboration with Professor Rob Beynon and his team at the Centre for Proteome Research, University of Liverpool UK. In order to visualise the chemical profile of each mongooses' scent, anal gland secretions (AGS) were analysed via gas-chromatography mass-spectrometry using a hexane extraction protocol. AGS samples were weighed and diluted by adding 100uL of hexane (Merck) per 1mg of AGS. A 200ng/uL of octadecane (Sigma-Aldrich, UK) was also added as an internal standard by which to compare peaks in chemical abundances. The mixture was whirimixed for 5 minutes then allowed to stand for one hour at ambient temperature. 1uL aliquots of the extracts were analysed by gas chromatography-mass spectrometry GC-MS using a Waters GCT mass spectrometer (Waters, UK) and a 30m BPX5 (S.G.E) chromatography column. The injector temperature was 250^{o}C and the temperature programme was 50^{o}C to 285^{o}C @ 8^{o}C/min. The carrier gas was helium at a flow rate of 0.8mL/min. The mass range scanned was 40 to 500Da in a scan time of 0.9seconds. Peak areas were measured using MassLynx software using manual peak selection. Spectral identities were produced by using MassLynx to match mass spectra with entries in the NIST library.

2.7.2 Patterns of chemical similarity/differences

Following analysis via GCMS, chromatograms and peak tables detailing retention times, were compared to the National Institute of Standards and Technology (NIST) library database. This allowed tentative identification of compounds including cholesterol, cholesterol derivatives and vitamin E that were common across samples. However compound identification was not attempted extensively as the relative abundances of compounds could be compared between samples using non-parametric tests within the Primer E, version 6 Programme (Clarke and Warwick, 2001). Initially chemical data were log(x+1) transformed to create a matrix of pairwise Bray-Curtis similarity values. Such values were used to visualise individual odour signatures by creating a nonmetric multi-dimensional scaling plot. This allows chemical similarities to be visualised within a 2D scatterplot where ranked distances between individuals are equivalent to the gap between each individual point on the plot. For example, closely aggregated points represent chemically similar individuals (see Chapters 3). Differences between a-priori defined groups (sex, reproductive state etc.) were then considered using non-parametric ANOSIM (analysis of similarities) which is a permutation test that evaluates significant differences between groups of sampling units without the need for assumptions on data distribution. Finally, SIMPERs (similarity percentage analyses) were conducted to determine chemical compounds contributing to the greatest percentage similarity within and between groups. Analyses in chapter 4 were based upon older samples that had unfortunately lost some volatile compounds during storage and transport. As such analyses only considered the two most common compounds vitamin E and cholesterol and utilised non-parametric Wilcoxon tests to look for differences based upon pregnancy status.

2.7.3 Protein analysis

Following optimization, proteins were extracted from anal gland secretion samples (AGS) using a modified TCA precipitation method (Guillot et al., 2016). Briefly, AGS were suspended in 1ml of H2O and vortexed for 1 minute. Samples were then centrifuged at 16000 x *g* for 40 minutes at 4˚C (to remove lipids) and proteins were precipitated overnight on ice after adding 500µl of 30% TCA (15% TCA final concentration). Protein pellet was recovered by centrifugation (16000 x *g* for 20 minutes at 4˚C). The precipitate was re-suspended in 10µl of Laemmli SDS-PAGE buffer (Tris-HCl, 63 mM pH 6.8, SDS 2%, glycerol 10%, 0.0005% of bromephenol blue, 100mM dithiothreitol) and pH was adjusted to 6.8 by adding 1M NaOH prior to loading the gel. Two gels

(one for pregnant and one for non-pregnant samples) were run where proteins were allowed to enter the gel but not fully separate. For protein detection commasie colloidal stain was used. One centimetre gel lanes corresponding to the complete scent mark proteome per sample (as indicated by the black lined boxes, figure 5) were cut from this gel and each cut into small pieces using a scalpel blade.

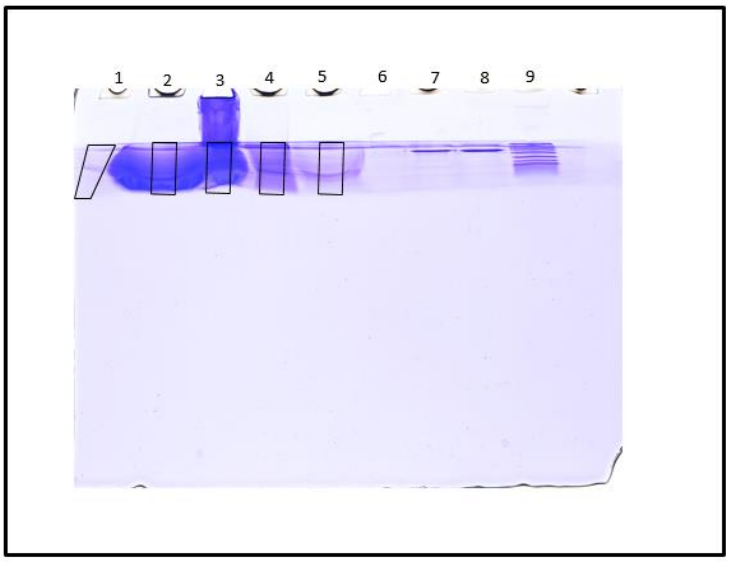


Figure 5: Gel image showing sections of protein-rich gel cut in preparation for in-gel digest with Tripsin.

Protein bands then underwent a standard in-gel triptic digest (Hayter et al., 2003). To remove the stain 50µl of acetonitrile (MeCN):100nM ammonium bicarbonate (50:50) was added to each band and incubated for 15 minutes at 37˚C. This step was repeated until bands were full de-stained. 50µl of a 10mM solution of dithiothreitol (DTT) was added to each band and incubated for 30 minutes at 37˚C, the DTT was subsequently removed. A 55mM solution of iodoacetamide (IAN) was prepared within 100mM ammonium bicarbonate and 50µl was added to each band, incubated for 60 mins at room temperature and IAN solution then removed. 20µl of ACN was added to each band and incubated for 15 minutes at 37˚C, the solvent was removed and tubes left open to allow evaporation and the gel to dehydrate. A 10µl aliquot of 10ng/µl of trypsin in 25mM ammonium bicarbonate (AMBIC) was then added to each gel and incubated for 1 hour at 37˚C. Another 10µl was then added and the solution left to incubate overnight at 37˚C. Next morning the supernatant was removed, and 20 µl of the extraction solution was added (50% MeCN: 50mM AMBIC: 0.1% FA). All samples were incubated for 30 minutes at 37˚C then the supernatant was removed and combined with o/n digest. All samples were centrifuged at 16000 x g for 30 minutes at 7˚C and the digest was then transferred to total recovery vials and analysed via injection in a Thermo QEactive HF mass spectrometer. Due to the large and variable amounts of proteins within banded mongoose AGS, samples were carefully standardised before injection to the mass spectrometer. Default peak picking parameters were applied and features with charges from 2^+ to 6^+. For peptide identification and protein inference the acquired tandem MS data were search against a mammal protein database subset from SwissProt using PEAKS search engine and. A fixed carbamidomethyl modification for cysteine

and variable oxidation modification for methionine were specified. A precursor mass tolerance of 10 ppm and a fragment ion mass tolerance of 0.01 Da were applied. The results were then filtered to obtain a peptide false discovery rate of 1%. To have a general overview of gene ontology distribution across the identified proteins in the scent mark samples, a enrichment analysis was performed using the DAVID bioinformatics resources (Huang da et al., 2009).

2.8 Statistical analysis

2.8.1 Modelling methods

Unless specified all analyses were conducted in R version 3.0.2 (R development core team, 2013) where multi-variate methods were required to deal with the complexity of the data. Due to repeated sampling across individuals, groups, litters etc. mixed models were used to control for pseudo-replication. Such models also allow random factors to be considered whilst controlling for potentially confounding effects in repeated sampling (Crawley, 2012b). Normally distributed data were analysed using linear mixed effects models (LMMs) and non-normal data with binomial distributions were analysed by generalized linear mixed models (GLMMs) using a logit link function both of which were constructed using the lme4 (Bates et al., 2008) or MASS (Venables and Ripley, 2002) packages. Other post-hoc comparison tests were used when appropriate and are described in relevant methods sections. All figures were created with the aid of ggplot2 (Wickham, 2009) unless referenced otherwise.

Initial models contained all potential explanatory factors and random effects detailed for each analysis. Second- and third-order interactions were also included when considered biologically relevant. Table 3 expands upon common terms used within models in the following chapter. The backward step-wise method of model simplification was then used to sequentially remove the least significant terms (highest p value). If removing said term caused a significant decrease in explanatory power of the model ($p<0.05$, tested via ANOVA) then it was reinstated, if not it was permanently removed from the model. Non-significant random terms were removed first, then interactions and finally non-significant fixed effects. Each dropped term was returned to the minimal model to ensure it had not been falsely excluded. Unless stated otherwise, tables in the proceeding chapters display minimal models, only including interactions where they have a significant effect. Model residuals were checked for normality following the methods of (Crawley, 2012a) to ensure data fit model assumptions, allowing confidence in the outcome. Any outlying data points were temporarily removed from models to check they did not exert a large influence on the overall model result. If any outlier was found to be highly influential, for example altering the significance of an interaction, then a decision was made as to whether or not to remove the data point from the analysis.

2.8.2 Relatedness data

The focal banded mongoose population has been tissue-sampled for DNA analysis for the past twelve years. A panel of 40 highly polymorphic microsatellite markers now exists and a 9-generation deep genetic pedigree was constructed in 2014 (Nichols et al., 2014; Sanderson et al., 2015). However, pedigree information is currently missing for individuals born after pedigree construction began in 2012. In order to maximise the dataset available for analyses in this thesis, pairwise relatedness was instead calculated based upon microsatellite markers. The

Lynch and Ritland method was utilised due to its computational simplicity yet low sampling variance (Lynch and Ritland, 1999) and the fact that it has previously been utilised for genetic studies on this banded mongoose population (Nichols et al., 2010). All genetic calculations were carried out in the InbreedR package (Stoffel et al., 2015) for R version 3.0.2.

Chapter 3 Discrimination of familiarity, relatedness and sex via scent

Abstract

Previous research on the banded mongoose has suggested odour cues primarily function within intra-sexual competition. However, newly available genetic data has broadened our understanding of this social mammal's complex breeding ecology and it would now be useful to re-asses the information encoded via scent and how this may influence behavioural decisions. Living in closely related groups with limited dispersal and immigration, banded mongooses face a real risk of inbreeding. Extra-group mating is possible, and creates fitter offspring, but it is opportunistic and costly. Thus, most individuals mate within their natal group where there is intense intra-sexual competition. Additionally, recent work reveals individuals avoid mating with very close kin suggesting a mechanism to detect the relatedness of potential mates. Using field-based odour presentations I show that banded mongooses display heightened behavioural responses to unfamiliar odours, suggesting they can detect extra-group individuals by scent. Sex differences are also apparent within both odour chemistry and behavioural discrimination. Unfamiliar female odours provoke more intense responses regardless of recipient sex. Thus although sex appears detectable via scent, males and females may utilise odours for different functions. The effect of relatedness also prompts sex-specific reactions. Males spend longer in contact with the odours of less-related group-mates whilst females increased contact toward more-related group-mates. Again, both sexes appear able to assess relatedness but may use this information in different ways. Interestingly, this result only held for familiar odours suggesting assessment of relatedness may be more important within one's social group, perhaps to avoid inbreeding or discriminate kin. Responses to unfamiliar odours however do not change relative to relatedness suggesting their novelty may be enough to signal low relatedness. Targeted research is now required to fully understand the use of odour signals in the banded mongoose. Current findings suggest they encode multi-modal information but may have sex-specific functions regarding both intra-sexual competition and mate-choice.

Introduction

Within mammalian systems scent marking is a common form of communication; individuals regularly deposit scent marks which are then investigated by conspecifics. The placement and frequency of these marks can provide a wealth of information including the identity, competitive ability and relatedness of the marker (Johnson, 1973; Wolff et al., 2002). In wild systems most research focuses on the competitive function of scent cues. Specifically, the frequency of over-marking (marking directly on top of an odour) is proposed to give an honest indication of the individuals' ability to monopolise an area. This is known as the intra-sexual competition hypothesis and pertains that individuals will over-mark same sex conspecifics most frequently, and that over-marking will be directly on-top-of, rather than next to, existing scent marks (Gosling and Roberts, 2001; Johnson, 1973). The hypothesis appears satisfied in many systems from laboratory-housed mice, *Mus musculus* (Rich and Hurst, 1999) and bank voles, *Myodes glareolus* (Ferkin et al., 2004), to captive ring-tailed lemurs, *Lemur catta* (Scordato et al., 2007) as well as wild old world primates (Heymann, 2006) and banded mongooses (Jordan et al., 2011b; Müller and Manser, 2008). However, within these wild systems there has been limited focus on the ability of scent cues to function within reproductive decision-making (Jordan et al., 2011c; Stockley et al., 2013).

For odours to function in mate-attraction and self-advertisement, theory posits that individuals should more readily investigate the scents of opposite-sex conspecifics. Here, marks should not be placed directly over a scent, but instead close by. This "vicinity" marking is proposed to maximise the identity of both the original and over-markers, allowing individuals to better assess potential mates (Wolff et al., 2002). However, repeated marking (as seen in competitive interactions) may also function as an advert to potential mates, regarding quality, receptivity or dominance (Boulet et al., 2009; Charpentier, 2008a; Cheetham et al., 2008; Hurst, 2009; Rich and Hurst, 1999; Thom et al., 2008). Indeed, Hurst and Rich (1999) expanded the intra-sexual competition theory to propose that both potential competitors and mates could use scent marks as a way of assessing the competitive ability of the donor. If an individual can outcompete conspecifics in terms of marking frequency, and over-marking, they not only display superiority to competitors but better advertise themselves to potential mates. Thus, within the intra-sexual competition hypothesis there is the capacity for scent cues to influence mate choice and other forms of reproductive decision-making. However, this has received limited empirical attention in wild systems. The banded mongoose represents an ideal target species for considering the role of scent signals within reproductive decision-making as this focal population has been habituated to humans for ~20 years and has a full life-history dataset. These animals are prolific scent markers, (Jordan, 2009) and scent chemistry is known to differ between the sexes; females have more chemically complex anal gland secretions than males (Jordan et al., 2011a). Additionally, odour-presentation experiments show that individuals over-mark same-sex odours most frequently, suggesting scent marking functions within intra-sexual competition (Jordan et al., 2011b; Müller and Manser, 2008).

At the time of previous research into banded mongoose scent communication there was no genetic information available (Jordan et al., 2011a; Jordan et al., 2011c). This lack of parentage data made it impossible to assess how relatedness influenced scent-marking behaviour, or how reproductive decisions such as mate-choice were orchestrated. A nine-generations-deep pedigree now exists providing detailed information on the breeding dynamics of this population (Nichols et al., 2015; Sanderson et al., 2015). Re-visiting the function of scent signals and testing their ability to encode relatedness would thus appear sensible. Firstly, pedigree data has demonstrated inbreeding, with 8% of the population showing inbreeding coefficients >0.25 (indicating mating between first order relatives) (Nichols et al., 2014). Although extra-group paternity (EGP) provides benefits such as increased pup heterozygosity, growth rates and survival (Nichols et al., 2015), it can only occur during inter-group interactions (IGIs) which are costly and violent (Cant et al., 2002). Thus most individuals mate within their natal group resulting in variable levels of inbreeding across the population (Nichols et al., 2015; Sanderson et al., 2015). However, evidence of non-random mating with respect to relatedness has been observed in this population, suggesting a mechanism to determine relatedness of group members may occur (Sanderson et al., 2015). Scent signals are known to encode genetic information in other mammals (Charpentier, 2008a; Cheetham et al., 2007) and thus appear a target mechanism to facilitate mate-choice and inbreeding avoidance.

Secondly, breeding competition was always recognised to be intense within the banded mongoose system. Males compete for access to suitable females but due to synchrony of females' oestrus males are limited on the number of females they can guard. However, with genetic data to confirm maternity and litter size, the intensity of female competition is now better understood (Cant et al., 2014; Cant et al., 2013; Thompson et al., 2016). Females compete for access to breeding resources and cooperative care for their pups (Hodge et al., 2009). Although communal breeding reduces the risk of infanticide (Hodge et al., 2009), when large numbers of females are breeding pup survival is reduced (Cant et al., 2014; Cant et al., 2013). Intense female-reproductive competition suggests this sex may benefit most from using

odours to assess the competitive landscape of their social group. For males, however, mate-choice via scent signals would appear highly beneficial.

In this chapter I test the ability of anal gland secretions (AGS) to contain information regarding sex, familiarity and relatedness. Secretions from the anal gland are known to be sexually dimorphic and more likely to elicit over-marking from conspecifics (Jordan et al., 2011a). Additionally, during the study period, they were the most commonly deposited odour cues, suggesting they likely encode important information regarding behavioural decision-making. In field-based odour presentations individual banded mongooses were exposed to AGS odours in separate trials and their responses filmed as detailed in section 2.3.4. Considering theories of functional scent communication, I make several predictions regarding the response to odour presentations:

1. If familiarity is detectable via scent the length and intensity of marking responses should differ between familiar and unfamiliar presentations.
2. If sex is detectable via scent banded mongooses should react differently to the odours of male and female conspecifics.
3. If banded mongooses use odour signals for competitive reasons, individuals should show heightened behavioural responses to odours from same-sex individuals.
4. If banded mongooses use odour signals within mate-choice, individuals should show heightened behavioural responses to odours from opposite-sex individuals.
5. If relatedness is detectable via scent, the length and intensity of marking responses should differ based upon the recipients' relatedness to the odour donor.
6. If banded mongooses use odour signals within mate-choice, odours from less-related individuals will arouse longer and more heightened responses than those from more closely-related donors.

Methods

Odour analysis

AGS samples were collected following the methods outlined in section 2.5.2 between May 29th and July 31st 2014. In total 49 males and 39 females were sampled from 8 social groups. Odour presentations were conducted from 1st June to 2nd August 2014 following methods described in section 2.6. Five measures of response were recorded; the duration before returning to normal behaviour (See section 2.6.1 for definition of normal), the duration of contact with the presentation (mongoose in physical contact with the tile), the total number of marks deposited, marks deposited directly on top of the odour (over-marks) and the total number of marks deposited close by but not directly over the odour (vicinity marks). Marking was categorised this way so as to maximise the power of the experiment to detect differences in the functional use of odour signals (Table 2, Chapter 2).

Odour presentations

To test the ability of banded mongooses to distinguish odours on the basis of familiarity recipients were presented (in separate trials) with odours from the following two categories: Familiar odours were acquired from individuals within the same social group as the recipient. Unfamiliar odours came from individuals within non-neighbouring groups, thus the recipient should have never encountered these scents before. At the time of presentations, full genetic analyses were not yet complete so observers were blind to the relatedness between odour

donor and recipient. This successfully removed observer and expectation bias in recording responses to odours. However, to ensure genetic information would be available, I only sampled and presented odours to individuals who had already been sampled for genotyping. As recipient and donor sex were randomised across trails, I simultaneously collected the data to test the effect of sex upon response measures. In total, 463 presentations were conducted utilising 61 male and 44 female recipients from the four most habituated study groups. All donors and recipients were over 12 months of age thus regarded as adult. No females in this study sample were pregnant or had given birth or aborted a litter within 48 hours of a presentation or odour sample collection.

Statistical analysis

Data were analysed within linear mixed models (LMMs) and general linear mixed effects models (GLMMs) all using the lme4 package (Bates et al., 2008) within R version 3.0.2 (R development core team, 2013). An initial LMM showed significant covariance between relatedness and familiarity in this system (LMM, t = -9.161, p= 1.6e-18), hence these terms could not be included in the same analysis. As such, separate GLMMs were first run to test the effect of odour familiarity on the five aforementioned response measures. Alongside odour familiarity, odour sex and recipient sex were included as explanatory factors with the identity and social group of both donor and recipient fit as random factors. The age of individuals may also impact marking behaviour due to the age-linked dominance hierarchy of banded mongooses, however when tested neither the age of odour-donor nor recipient had an effect on marking behaviours in this dataset and so were not included in the models (Appendix A, Table 2). To consider the effect of relatedness, the dataset was split into familiar and unfamiliar presentations to control for the effect of odours coming from within- and extra-group conspecifics. GLMMs then considered the effect of relatedness, odour and recipient sex upon response measures. Relatedness was calculated using the Lynch and Ritland method in the inbreedR package (Stoffel et al., 2015) as described in section 2.5.2. As before, identity and social group of both donor and recipient were included as random factors, all models were run with a Gaussian error distribution and fit by restricted maximum likelihood. Second order interactions were included in all initial models alongside a 3-way interaction between donor sex, recipient sex and either familiarity or relatedness. Non-significant terms, beginning with interactions, were sequentially removed following the backward step-wise simplification method.

Chemical analysis

41 anal gland sections (AGS) collected between May 29th and July 31st 2014 were analysed via gas chromatography – mass spectrometry (GC-MS) following the methods outline in section 2.7. I did not attempt to identify all the chemicals present in these samples but focused on assessing differences in the presence and abundance of chemicals between the sexes using ANOSIM an SIMPER analyses performed within PRIMER E (version 6). All tests followed the detailed methods outlined in section 2.7.2.

Results

Co-variance between relatedness and familiarity

Relatedness significantly co-varies with familiarity (LMM: t = -9.161, p= 1.6e-18, Table 1) for this banded mongoose population. Individuals within the same pack (familiar) are more related to one another than those of different social groups (unfamiliar).

Table 1: Co-variance between familiarity and relatedness

Fixed effect	Estimate	Standard error	t value	P value
Intercept	0.168	0.015		
Unfamiliar odour	-0.193	0.021	-9.161	1.6e-18

Output of LMM testing the relationship between familiarity and relatedness for the individuals used within this analysis. Unfamiliar odour donors were also significantly less likely to be related to recipients within this subset of the population.

Effect of familiarity and sex

Unfamiliar odours consistently provoked significantly longer reactions and more marking behaviour than familiar odours (Table 2, grey bars in Figure 1). For certain behaviours this result appears driven by the sex of the recipient; males deposited significantly more total marks (GLMM: t= -2.433, p=0.015) and over-marks (GLMM: t = -2.033, p= 0.043) toward unfamiliar odours (Figure 1c and 1d), and spent longer in contact with these scents (GLMM: t = -2.607, p = 0.009, Figure 1b). However, female recipients showed no significant difference in these response variables based on odour familiarity. Unfamiliar odours received more vicinity marks (GLMM: t= 3.178, p=0.0006) and longer durations of interest (GLMM: t = 5.490, p=6.093e-08) regardless of recipient sex. Male recipients were more likely to deposit vicinity marks than were females (GLMM: t= 3.455, p=0.042, Table 2).

Table 2: Output of GLMMs testing the effect of familiarity and sex upon response measures to presented odours

Model testing	Fixed effects	Effect size	Standard error	t value	P value
DURATION BEFORE	Intercept	21.776	1.729		
RETURN TO NORAMAL	**Unfamiliar odour**	**12.829**	**2.337**	**5.490**	**6.093e-08**
BEHAVIOUR	**Odour sex (female)**	**4.685**	**2.268**	**2.065**	**0.039**
CONTACT DURATION	Intercept	7.056	1.277		
	Unfamiliar odour	**12.471**	**1.701**	**7.331**	**8.00e-13**
	Recipient sex (female)	2.577	1.716	1.502	0.134
	Odour sex (female)	**2.693**	**1.319**	**2.041**	**0.044**
	Unfamiliar *recipient sex	**-7.244**	**2.779**	**-2.607**	**0.009**
TOTAL MARKING	Intercept	3.894	0.641		
	Unfamiliar odour	**2.500**	**0.787**	**3.178**	**0.0006**
	Recipient sex (female)	0.780	0.503	1.551	0.121
	Odour sex (female)	0.335	0.510	0.657	0.511
	Unfamiliar *recipient sex	**-2.010**	**0.826**	**-2.433**	**0.015**
	Unfamiliar *odour sex	**1.846**	**0.842**	**2.193**	**0.029**
OVER-MARKING	Intercept	4.186	0.690		
	Unfamiliar odour	**1.907**	**0.872**	**2.186**	**0.029**
	Recipient sex (female)	0.488	0.532	0.917	0.360
	Odour sex (female)	0.611	0.413	1.482	0.139
	Unfamiliar *Recipient sex	**-1.790**	**0.881**	**-2.033**	**0.043**
VICINITY MARKING	Intercept	0.672	0.221		
	Unfamiliar odour	**0.826**	**0.239**	**3.455**	**0.0006**
	Recipient sex (female)	**-0.288**	**0.141**	**-2.043**	**0.042**
	Odour sex (female)	-0.095	0.171	-0.557	0.578
	Unfamiliar *odour sex	**0.578**	**0.293**	**1.971**	**0.049**

Output of GLMMs testing the effect of odour familiarity, recipient sex, odour sex and second order interactions upon response measures to presented odours. Only interactions with significant effects are presented within the table. Non-significant fixed effects are presented alongside p-values for which they were dropped from models. Bold type denotes significant effects.

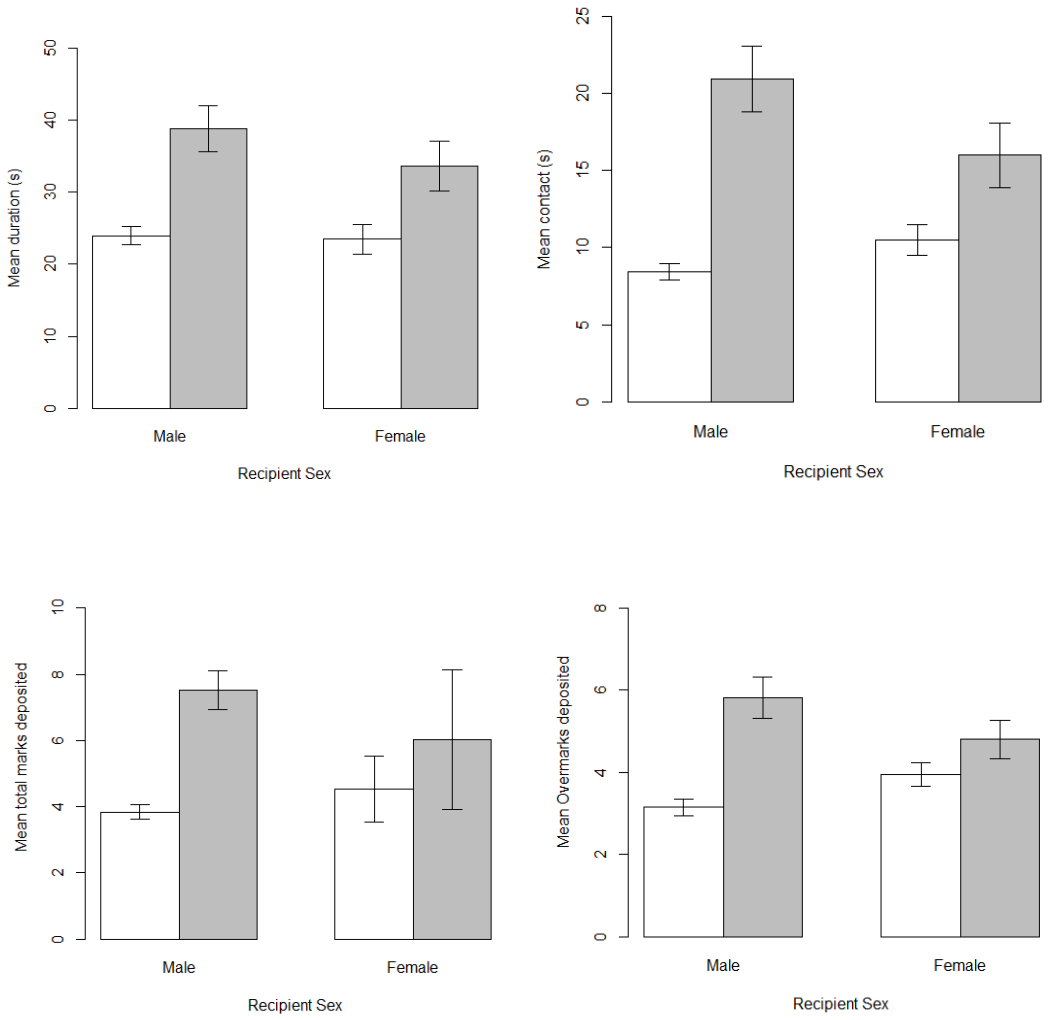


Figure 1: The interaction between odour familiarity and recipient sex upon response measures. Grey bars represent unfamiliar odours, clear bars familiar odours, error bars = standard error. Both sexes of banded mongoose take significantly longer to return to normal foraging behaviour following the presentation of unfamiliar odours (Figure 1a, top left). Male recipients also spend significantly longer in contact with unfamiliar than familiar odours (Figure 1b, top right) and deposit significantly more marks (Figure 1c, bottom left) and over-marks (figure 1d, bottom right) on unfamiliar odours; however female recipients show no such discrimination based upon odour familiarity.

Odour sex had a significant effect upon response measures to presented odours. Unfamiliar odours provoked more total marking if female (GLMM: t = 2.193, p = 0.029), however, there was no such effect of sex for familiar odours (Figure 2). Both male and female odours received more vicinity marks if unfamiliar, however this trend appeared stronger when considering female odours (GLMM: t = 1.971, p = 0.049, Figure 3). Regardless of familiarity, female odours also provoked longer durations of interest (GLMM: t = 2.065, p=0.039, Table 2) and contact (t = 2.041, p=0.044, Table 2) than did male odours. There was no significant effect of 3-way interactions or the interaction between odour and recipient sex on any response measure.

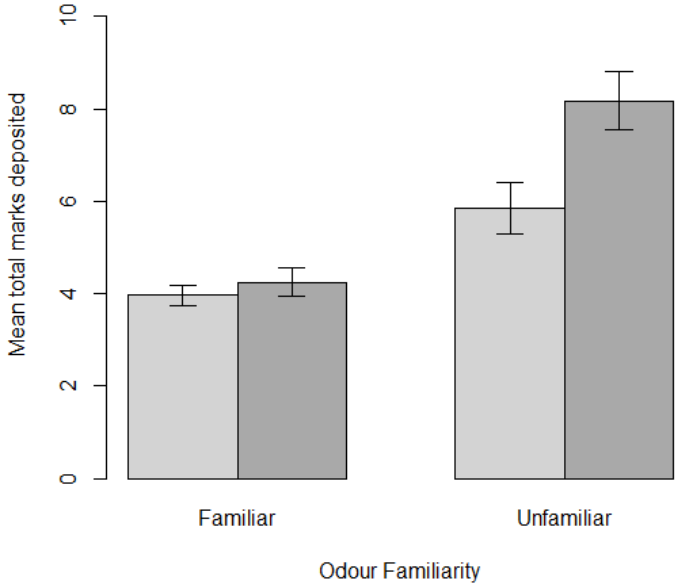


Figure 2: The interaction between odour sex and familiarity upon total marking in response to presented odours. Bar colour corresponds to odour sex, light grey for males and dark grey for female odours. Error bars = standard error. Unfamiliar odours provoke significantly more total marking if female; however familiar odours show no such sex difference.

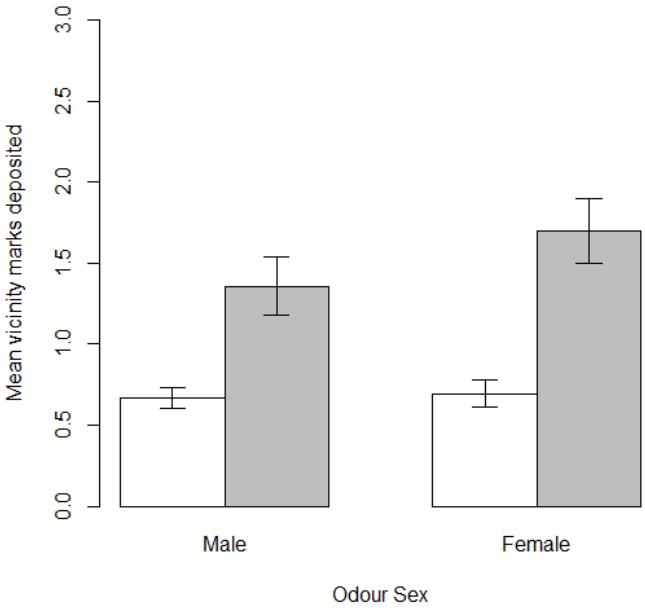


Figure 3: The interaction between odour sex and familiarity upon vicinity marking in response to presented odours. Grey bars represent unfamiliar odours and open bars familiar. Error bars = standard error. Vicinity marking is significantly higher in response to unfamiliar odours but this trend is more pronounced when odours come from female donors.

Effect of relatedness

Relatedness did not have a significant impact upon response measures to unfamilar odour presentations (Table 3). Unfamiliar female odours however did provoke significantly more

marking (GLMM:t = 2.479, p=0.014) and over-marking (GLMM: t = 2.276, p = 0.024) than did odours of unfamiliar males. With regard to familiar odours relatedness does appear detectable via scent as contact durations differed dependant upon the recipients relatedness to the odour-donor (GLMM t = 2.590, p = 0.010 Table 4). Male recipents spent less time in contact with odours as their relatedness to the odour donor increased. This trend was reversed for females, who spent longer in contact with more related familiar odours (Table 4 and Figure 5).

Table 3: The effect of relatedness and sex upon response measures to unfamiliar odours.

Model testing	Fixed effects	Effect size	SD	T-value	p-value
DURATION BEFORE RETURN	Intercept	39.461	4.164		
TO NORAMAL BEHAVIOUR	Recipient sex (Female)	-5.978	5.300	-1.128	0.261
	Relatedness			0.406	0.685
	Donor sex (female)			0.981	0.328
DURATION OF CONTACT	Intercept	16.179	2.247		
	Donor sex (female	5.360	3.056	1.754	0.081
	Recipient sex (female)			1.617	0.107
	Relatedness			-0.467	0.641
TOTAL MARKING	Intercept	5.853	0.667		
	Donor sex (female)	**2.135**	**0.861**	**2.479**	**0.014**
	Recipient sex (female)			-1.179	0.240
	Relatedness			0.278	0.781
OVER-MARKING	Intercept	4.852	0.723		
	Donor sex (female)	**1.754**	**0.770**	**2.276**	**0.024**
	Recipient sex (female)			-1.236	0.218
	Relatedness			-0.213	0.832
VICINITY MARKING	Intercept	1.616	0.253		
	Recipient sex (female)	-0.443	0.291	-1.522	0.130
	Donor sex (female)			0.975	0.331
	Relatedness			0.658	0.511

Output of GLMMs testing the effect of odour relatedness, recipient sex and odour sex upon response measures to unfamiliar odours. Only significant interactions are presented in the table, non-significant fixed effects are presented alongside p-values upon which they were removed from the model. Bold text highlights significant terms.

Table 4: The effect of relatedness and sex upon response measures to familiar odours.

Model testing	Fixed effects	Effect size	SD	T-value	p-value
DURATION BEFORE RETURN TO NORAMAL BEHAVIOUR	Intercept	22.234	1.477		
	Donor sex (female)	3.630	2.243	1.619	0.106
	Recipient sex (Female)			0.082	0.935
	Relatedness			-0.307	0.759
DURATION OF CONTACT	Intercept	9.469	0.934		
	Recipient Sex (Female)	-1.912	1.400	-1.366	0.173
	Relatedness	-5.7803	3.065	-1.886	0.060
	Relatedness* Recipient Sex	**12.965**	**5.005**	**2.590**	**0.010**
	Donor sex (female)			0.576	0.565
TOTAL MARKING	Intercept	6.196	2.134		
	Recipient sex (female)	0.683	0.524	1.305	0.193
	Relatedness			0.618	0.537
	Donor Sex (Female)			0.549	0.583
OVER-MARKING	Intercept	5.183	1.729		
	Recipient Sex (Female)	**0.844**	**0.485**	**1.739**	**0.008**
	Relatedness			0.126	0.900
	Donor sex (female)			0.001	0.999
VICINITY MARKING	Intercept	0.784	0.093		
	Recipient Sex (Female)	-0.236	0.154	-1.534	0.125
	Relatedness			-1.027	0.305
	Donor sex (female)			-0.496	0.620

Output of GLMMs testing the effect of odour relatedness, recipient sex and odour sex upon response measures to familiar odours. Only significant interactions are presented in the table, non-significant fixed effects are presented alongside p-values upon which they were removed from the model and bold text highlights significant terms.

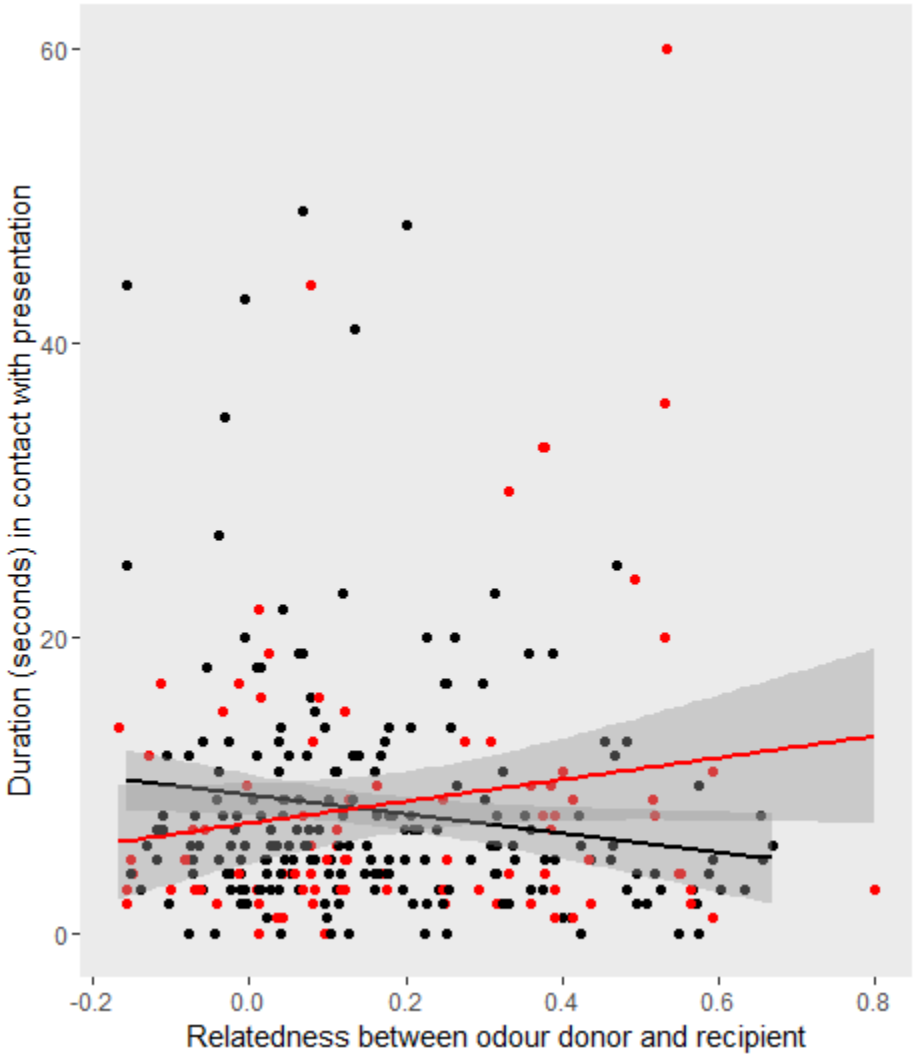


Figure 5: The effect of relatedness between odour and donor upon duration of contact with presented odours. Male recipients spend less time in contact with familiar odours as their relatedness to the odour donor increases (black points). However, females increase contact durations with familiar odours as their relatedness to the odour donor increases (red points). Lines are based up on GLMM predicting the effect of relatedness upon contact duration with familiar odours. Confidence intervals are set to 95%.

Chemical data

Banded mongoose samples contained 27 discrete compounds eluting between 8 and 42 minutes of the GC-MS run. This is fewer than research conducted by Jordan et al. in (2010) however based upon retention times, many of the compounds tentatively identified in the previous study are apparent in the current analysis. Table 3 in Appendix A details the retention times of all common compounds to allow comparison between the two analyses. I did not attempt full compound identification as this was not required to search for general differences between the composition of male and female scents using non-parametric tests (below). However, two of the most abundant compounds were realised as Vitamin E and Cholesterol which had not previously been identified. Additionally these are heavy molecules assumed to function in prolonging scent signals in other species (Scordato et al., 2007).

Chemical differences between the sexes

The chemical profiles of male and female odours differ significantly more than would be predicted by chance (ANOSIM global R = 0.356, P = 0.001). A global R value of 0.356 suggests clear separation of samples based upon sex and the p-value of 0.001 shows this chemical distinction is significant. The most appropriate way to visualise this difference between the sexes is to use non-metric multidimensional scaling based on a Bray-Curtis similarity matrix of log (x+1) transformed chemical data for each individual. The 2D scatterplot (figure 6) ranks between-individual differences in odour chemistry with points close together represent individuals with high chemical similarity. A priory defined groups (in this case sex) can be incorporated into the plot to visualise clustering, for this analysis samples clearly cluster based upon sex (Figure 6). However, two outlying female samples belong to pregnant females suggesting pregnancy causes deviations in odour chemistry.

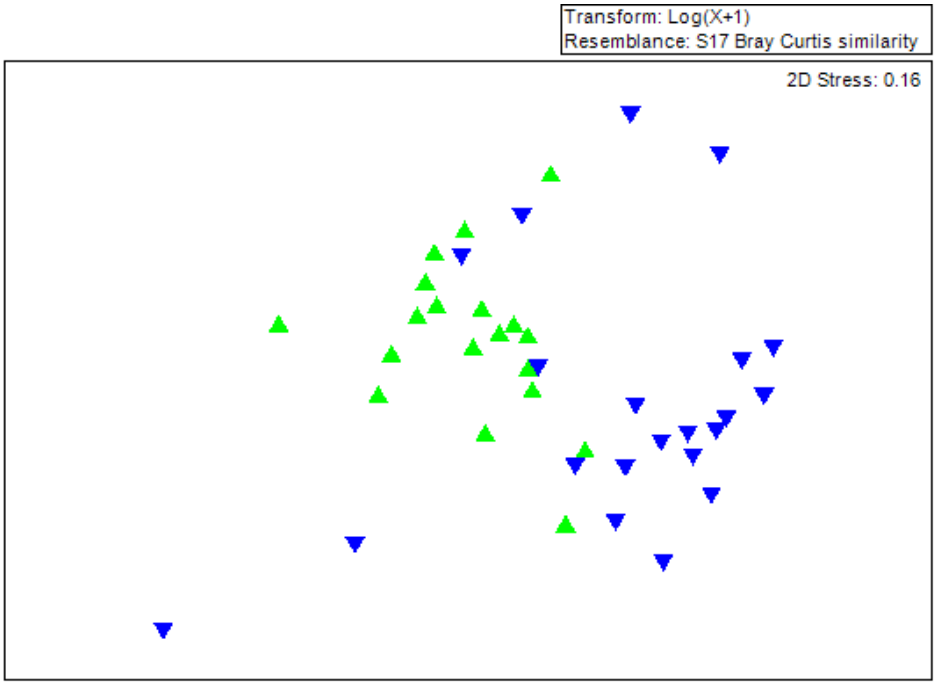


Figure 6: Plot depicting the differences between male and female odour chemistry for 41 anal gland secretions**.** Males are represented by green arrows and females by blue. The two outlying female points belong to pregnant dams indicating possible chemical differences occur during pregnancy. Plot created in Primer E, based upon a Bray-Curtis resemblance matrix of log-transformed concentrations of the chemical compounds composing banded mongooses' anal gland secretions. Note that axis are arbitrary and points cluster based upon similarities in the abundance of identified compounds within individual anal gland secretions.

Compounds contributing to male and female scents

Seven different compounds account for >90% of the chemical similarity between odours of males (Table 5). For females, just four different compounds explain >90% of the variance in their odour chemistry (Table 6). The abundance of the compounds eluting at 8.63 and 14.9 seconds account for a high percentage of both male and female odour signals, however other compounds are distinct within each sex supporting the ANOSIM result (above) and providing a mechanism by which sex may be discernible via scent.

Table 5: Chemical compounds accounting for the similarity between male odour cues

Compound retention time (s)	Average abundance	Average similarity between male odour cues	Contribution (%) to male odour cues	Cumulative contribution (%) to male odour cues
14.9	1.37	11.81	28.08	28.08
20.55	1.12	8.12	19.32	47.40
38.04	0.86	5.81	13.82	61.22
30.39	0.84	5.24	12.46	73.68
8.63	0.74	4.02	9.57	83.25
10.48	0.46	2.65	6.30	89.55
8.75	0.33	0.60	1.44	90.99

Average chemical similarity between male odours was 42.06%. Results based upon a SIMPAR analysis of similarity percentages conducted upon log transformed chemical abundance data within Primer E. Compounds identified by retention time (time in seconds to pass through the gas chromatography instrument).

Table 6: Chemical compounds accounting for the similarity between female odour cues.

Compound retention time (s)	Average abundance	Average % similarity between female odour cues	Contribution (%) to female odour cues	Cumulative contribution (%) to female odour cues
8.63	1.10	18.37	62.37	62.37
37.3	0.38	4.55	15.44	77.80
14.9	0.54	2.89	9.80	87.60
39.74	0.25	1.03	3.51	91.11

Average chemical similarity between female odours was 29.46%. Results based upon SIMPAR analysis of similarity percentages conducted upon log transformed chemical abundance data within Primer E. Compounds identified by retention time (time in seconds to pass through the gas chromatography instrument).

Discussion

In the banded mongoose, odours appear to be behaviouraly discriminated on the basis of sex, familiarity and relatedness. ANOSIM clearled showed chemical differences between the sexes, and female odours provoked more marking behaviour and longer periods of interest than did those of males. However, in contrast to previous research, I found that individuals were not more likely to over-mark same-sex odours. Instead the familiarity of odours, and sex of both donor and recipient, interacted to influence responses. Familiar odours were discriminated on the basis of relatedness yet whilst males spent longer in contact with less-related odours, females increased contact durations toward more-related odours. Unfamilar odours were not discriminated by relatedness. Such interactions suggest banded mongoose odour signals contain multi-modal information and may serve sex-specific functions.

In line with initial predications, banded mongooses discriminated odours based upon familiarity. Individuals remained in contact with unfamiliar odours for significantly longer and deposited a higher number of total marks than when investigating familiar odours. Results were stronger when considering the response of male recipients, but lay in the same direction for females. The heightened responses toward unfamiliar odours may occur for several reasons. Firstly, the novelty of unfamiliar odours may stimulate heightened reactions from recipients. Indeed, banded mongooses are frequent markers, depositing urine, faeces and gland secretions throughout their territories (Jordan, 2009; Müller and Manser, 2007). However marking behaviour is more pronounced within territory boundaries, rather than on the fringes,

suggesting scent communication is predominantly used in intra-group communication (Jordan et al., 2011b; Müller and Manser, 2008). As such, banded mongoose likely recognised the scent of their own pack by becoming familiar with them. Alternatively phenotype-matching may occur where animals learn their own phenotype, or that of their kin during, infancy and use this as a template to identify unfamiliar individuals later in life (Lacy and Sherman, 1983). Secondly, unfamiliar conspecifics represent competitive threats to banded mongooses. Intergroup interactions (IGIs) are violent and costly, resulting in 20% of pup deaths and 12% of adult deaths where the cause of mortality is known (Nichols et al., 2015) . The significant increase in over-marking behaviour toward unfamiliar odours could be an aggressive response to the cues of potential intruders. Indeed, the intra-sexual competition function of scent communication suggests that placing one's scent directly on top of another can obliterate the original mark, securing olfactory dominance for the over-marker (Gosling and Roberts, 2001; Johnson, 1973; Ralls, 1971). Thirdly, male banded mongooses spend significantly longer in contact with unfamiliar odours. Duration of contact is often used as a proxy measure for interest in an odour and suggests recipients are extracting valuable information (Hurst and Benyon, 2010). As discussed, unfamiliar conspecifics represent competitive threats however, when IGIs do occur banded mongooses also gain a rare opportunity for extra-group copulations (Nichols et al., 2015). Although conspecifics frequently breed within their natal groups, often with relatives, extra-group copulations provide known benefits. These include an increased likelihood of mating with an unrelated mate and the production of heavier offspring which have a higher chance of survival to independence (Nichols et al., 2015). Thus, intense reactions to unfamiliar odours fulfil mate-acquisition and self-advertisement theories of scent communication, suggesting recipients could be extracting information regarding mate-choice.

Explanations are not mutually exclusive; that unfamiliar female odours provoke significantly elevated marking responses from both sexes of recipient suggests the sexes utilise scent cues for different functions. For males, the assessment of unfamiliar female odours may assist in mate-choice, particularly considering the benefits of extra-group mating in this system (Nichols et al., 2015). Although both male and female banded mongoose can mate multiply, prior to copulation males invest heavily in "mate-guarding" which involves following target females to prevent access by other males. This behaviour is costly and time consuming (Cant et al., 2013; Nichols et al., 2010), thus olfactory investigation may allow males to select an appropriate target without wasting resources on an unsuitable female. Male recipients also showed heighted total marking and vicinity marking toward unfamiliar odours. Because vicinity marking is presumed to maximise identity of both the original and over-marker it is suggested to primarily function within mate-acquisition and self-advertisement (Wolff et al., 2002). Although there is no significant interaction between recipient and donor sex to satisfy predictions of mate-attraction theories, the overall excess of male vicinity marking would imply they are attempting to preserve the original scent as well as their over-mark. Indeed, males are more likely to deposit vicinity marks than are female recipients, yet female odours are more likely to receive vicinity marks.

The heightened interest in unfamiliar female odours may suggest that female recipients are utilising odours within intra-sexual competition. Competitive interactions are common between social female mammals (Stockley and Bro-Jorgensen, 2011) and the banded mongoose is no exception. The threat of infanticide by intra-group females is high (Bell et al., 2012; Gilchrist, 2006) and multiple breeding females can reduce litter survival by increasing competition (Cant et al., 2014). However, why unfamiliar females should pose additional threats is unclear. Although immigrants can usurp females for their natal groups this is an incredibly rare occurrence (Cant et al., 2013). Additionally, the threat of violence from intergroup interactions (IGs) is equal for both male and female intruders who both contribute to

the violence associated with IGIs. Finally, to satisfy the intra-sexual competition hypothesis, one would have expected to see significantly heightened over-marking toward same-sex scents by females. Although unfamiliar female odours receive more marks and over-marking than those of unfamiliar males, there is no third order interaction with recipient sex to suggest this is primarily due to the actions of other females. Nevertheless, female odours receive significantly longer durations of contact and interest than male odours. This would suggest recipients of both sexes are gaining information from such scents that could be used to inform behavioural decisions. Indeed, the composition of anal gland secretions does appear sex-specific. Different suites of compounds are responsible for male and female scent profiles, supporting the observed discrimination of odours based on sex. Furthermore, the composition of the two pregnant odours in this analysis appear to deviate from both non-pregnant females and males. This suggests reproductive state may be detectable via female scent allowing direct assessment of reproductive competition. It would now be useful to consider more targeted research into the reactions of females to same-sex scents (Chapter 4).

Both sexes appear to discriminate familiar odours by relatedness. Males show a significant reduction in contact duration toward odours of increasing relatedness that, in line with initial predictions, would suggest males use scent signals in mate-choice. Considering the evidence for non-random mating with regards to relatedness (Sanderson et al., 2015), this result provides a mechanism for kin discrimination. However, the preference for less-related odours was only present in males as females' refuted predictions and spent longer in contact with more-related, familiar odours. Findings are particularly difficult to interpret due to the non-significance of three-way interactions between donor sex, relatedness and odour sex. Because of this, it is impossible to determine whether males are primarily responding to the odours of unrelated females, which would fully substantiate claims for odour signals to aid mate-choice. This caveat stands when interpreting the female trend to show longer durations of contact when investigating more related odours. Females' longer reactions toward related odours would appear to support previous work suggesting banded mongooses primarily use odour cues for intra-sexual competition (Jordan et al., 2011a; Jordan et al., 2011b). Indeed, the limited dispersal between natal groups means closely related odours likely represent direct breeding competition, if female. Thus, it may benefit females to assess their competitors in line with theories of intra-sexual competition (Stockley et al., 2013). However, as females are not responding exclusively to the odours of other females, it is also possible they use relatedness to assess potential mates.

Mate-choice based upon relatedness would appear important to both sexes because mating with less related individuals provides known benefits to pup fitness (Nichols et al., 2015; Sanderson et al., 2015). Specifically, males should benefit because they are constrained in terms of the number of females they can mate per reproductive bout. Thus selecting a less-related dam should increase their direct fitness by providing more, successful offspring. However, as in most mammals (Clutton-Brock, 2007; Clutton-Brock and Vincent, 1991) female banded mongooses invest heavily in pregnancy and lactation suggesting they should also be choosy regarding their mate. Indeed, females often slip mate-guards to breed elsewhere, however our results do not support this choice being mediated by relatedness. Alternatively, females may be responding to another parameter encoded by these scents (infection status etc.) and thus increased contact toward more related odours may be an artefact of other information involved in mate-choice.

Although I cannot conclusively provide evidence that the detection of relatedness facilitates mate-choice, I have shown that familiar odours are behaviourally discriminated by relatedness. This crucial finding may enlighten other behavioural interactions such as kin

recognition in this system. However, it remains to be seen how such discrimination is possible. Kin-referent phenotype-matching, where kin are used as templates for recognition, is an unlikely explanation. This is because banded mongoose litters are generally of mixed parentage and can contain full siblings as well as completely unrelated individuals. It would thus be unfeasible to use littermates as accurate templates for kin discrimination. Alternatively, self-referent phenotype matching, or "arm-pit effects" could occur (Hauber and Sherman, 2001). Within this mechanism, individuals compare novel odours to their own scent (Hauber and Sherman, 2001; Mateo, 2003; Mateo and Johnston, 2000) making it a feasible explanation for kin-discriminate in natal packs. Indeed in cross-fostering experiments of Belding's ground squirrels (*Urocitellus beldingi*) individuals are able to discriminate true kin from nest-mates in adulthood despite no prior experience of them as juveniles (Mateo, 2010). However, unfamiliar odours were not behaviourally discriminated by relatedness in the banded mongoose. This may be because the unfamiliarity of odours is enough to signal low relatedness. Indeed, in this subset of the population, mean relatedness between familiar odour donors and recipients was 0.189 (range -0.082 to 0.801) whilst unfamiliar odours mean relatedness to recipients was only -0.020 (range -0.0096 to 0.423). As unfamiliar odours are so distantly related, to determine kinship may not require such detailed assessment as do the odours of pack-mates. This would explain the apparent failure of banded mongooses to discriminate relatedness of unfamiliar odours. It does not negate kinship-assessment as an important factor when encountering unfamiliar scents. However, because familiarity co-varies with relatedness so tightly in this dataset, the novelty of an unfamiliar odour may function as a proxy for relatedness.

In summary, banded mongoose odour signals appear to encode sex, familiarity and relatedness. GC-MS data shows male and female gland secretions differ in their chemical composition providing a mechanistic basis for sex-discrimination. However, odour presentation results suggest each sex may utilise odour signals for different purposes. Regardless of recipient sex, female odours received longer periods of interest, and unfamiliar female odours received a higher frequency of marks and over-marks than odours of unfamiliar males. From a female perspective, heightened reactions to same-sex scents could support the intra-sexual competition function of scent marking. Alternatively, male interest in unfamiliar female odours suggests odour signals function within mate-choice. Unfortunately, because the sex of the recipient did not significantly interact with odour-sex and familiarity to influence marking behaviour, these suggestions are difficult to substantiate in the current dataset. The lack of third order interactions also clouds interpretations of how relatedness information may be utilised for specific functions such as mate-choice and intra-sexual competition. Nevertheless, the detection of relatedness within group members via scent is a key novel finding. Targeted studies should now test the ability of banded mongooses to recognise and discriminate kin within natal groups. Apparent sex-differences in the utilisation of odour signals can also be teased apart by considering the potential for odours to encode specific information relating to competition and reproduction.

Chapter 4 Discrimination of female reproductive state via scent

Abstract

In model organisms, odours are known to encode information regarding reproductive state. This remains poorly understood in wild animals and studies tend to focus on the ability of odours to encode oestrus rather than pregnancy. In systems where multiple individuals breed concurrently, detecting pregnancy may be important in terms of both competition and mate-choice. The banded mongoose is a cooperative mammal where female reproductive skew is low but there is intense competition between pregnant dams for access to food and helper resources. Adult males show higher skew, however most will attempt to breed by investing highly in the guarding of females to prevent access by other males. As such, a mechanism to detect pregnancy could benefit both sexes: allowing males to avoid guarding already pregnant dams, and informing females on the competitive landscape of their social group. Here I show, through odour presentation experiments, that pregnancy is discernible via odour cue in the banded mongoose. Males spent more time investigating the odours of non-pregnant females, and deposited more marks around these odours than they did in response to odours of pregnant dams. The female response was more complex with females showing heightened behavioural responses when odours were of the same reproductive state as recipients. These results suggest pregnancy is detectable via scent but that this information may be utilised for sex-specific functions in the banded mongoose. Females appear to rely on odours for competitive cues, whilst males gain information relevant to mate-choice. A mechanism for such discrimination remains elusive; I could not identify convincing chemical differences between the odours of pregnant and non-pregnant females. However, a small-scale proteomic analysis revealed tentative evidence for a shift in protein expression during pregnancy that may underpin behavioural discrimination of female reproductive state.

Introduction

Female odour communication has received significantly less attention than that of males (Stockley et al., 2013). Nevertheless, scent cues are heavily utilised by female animals and appear a reliable method of communicating reproductive information. In ring-tailed lemurs, *Lemur catta* (Crawford et al., 2011), house mice, *Mus musculus domesticus* (Achiraman and Archunan, 2006) and meadow voles, *Microtus pennsylvanicus* (Ferkin et al., 2004) urine and gland secretions advertise the receptivity of their female donors. The semiochemistry of odours can change during oestrus providing a mechanism for detecting receptivity. In house mice, sulphated steroid hormones can be detected by the vemeronasal olfactory sub-system and behavioural research supports the notation that these chemicals facilitate mate attraction during oestrus periods (Achiraman et al., 2010). In ring-tailed lemurs, placing females on an oral contraceptive changes the chemistry of odour signals and males can distinguish between intact and treated females (Crawford et al., 2011). This would appear particularly beneficial considering the lemurs' incredibly short receptive period (Jolly, 1966) where it is imperative males accurately detect receptivity. Finally, asocial meadow voles only come together to breed and thus increased female scent marking during oestrus is thought to facilitate mate attraction (Ferkin et al., 2004). However, females may also benefit from detecting the reproductive state of other breeders allowing them to gauge levels of competition. This appears to be the case in *Eulemur* species where females are dominant over males, show high levels of intra-sexual aggression but also have more chemically elaborate scent cues (delBarco-Trillo et al., 2012). Females may also compete for access to resources (Stockley and Bro-Jorgensen, 2011) such as the male-biased care of callitrichid primates which benefits offspring survival. Here scent

marking rates are higher in females than males and are believed to function within competition between females over access to carers (Heymann, 1998, 2006). Olfactory detection of reproductive state thus appears to inform both mating and competitive interactions.

Considering previous findings the ability of odours to encode female reproductive state have been earmarked as a key area for further empirical attention (Stockley et al., 2013). Several studies now report that receptivity can be detected via scent (Converse et al., 1995; Ferkin et al., 2004; Hudson, 1990; Ziegler et al., 1993). Few, however have focused specifically on the ability of odours to signal pregnancy. This is surprising considering that major and sustained hormonal changes occur during mammalian gestation that may be capable of influencing scent chemistry (Drea, 2011). Indeed Crawford and Drea (2015) show that ring-tailed lemurs can identify pregnant females via scent and that this discrimination may mediate reproductive competition in these social mammals. Such a mechanism may be particularly important in cooperative systems, where females compete for access to resources such as food and care for their offspring. Cooperative breeders are characterised by large social groups and the willingness of non-parent individuals to contribute to offspring care (Faulkes and Bennett, 2001). Reproduction is often constrained to certain group members where the dominant pair monopolise reproduction and subordinates rarely even attempt to breed (Clutton-Brock et al., 2001; Faulkes et al., 1991) . However, in some systems multiple individuals of each sex breed concurrently, creating intense competition and aggression between females. In such situations, the scent marking of oestrus females has been implicated in competitive interactions (Heymann, 1998, 2006; Jordan et al., 2011c). However, reactions to pregnant odours have not been considered beyond the previously mentioned lemur example (Crawford and Drea, 2015). Additionally, in systems where multiple females are concurrently breeding, males may become limited in the number of mates they can monopolise. This means detecting pregnancy, as well as receptivity, may better inform male mate-choice. Thus in cooperative systems with high female competition the communication of pregnancy may benefit both intra-sexual competition and mate-choice.

The banded mongoose is a cooperative system where multiple females within each group breed (Cant et al., 2013). These small mammals represent an ideal target for investigating the ability of scent to encode pregnancy for several reasons. First, the anal gland secretions (AGS) of male and female banded mongooses differ with females producing more chemically complex signals (Jordan et al., 2011a). Second, based on analyses in chapter 3, two odours of pregnant dams appear to differ in terms of their chemical composition from both males and non-pregnant females, suggesting pregnancy may be detectable via scent. Third, pregnant dams represent direct competitive threats to one another. Female reproductive skew is low in the banded mongoose with up to 10 dams breeding per reproductive bout (Cant et al., 2013). However, once over ~8 females are breeding in a single pack, litter success begins to decline due to increased competition for resources including male-biased care (Cant et al., 2014). This heightened reproductive competition has also been revealed as one of the main factors contributing to the probability of eviction, where females are removed from their group by aggressive conspecifics (Gilchrist, 2006). This is because reproductive competition for access to breeding resources increases with the number of females attempting to breed, thus to mediate such competition behaviourally dominant females evict their younger and smaller conspecifics (Thompson et al., 2016). Thus the detection of other pregnancies could provide a mechanism for assessing the competitive landscape of one's group. Finally, the synchronous oestrus of female banded mongooses constrains the number of mates a male can guard. Males thus tend to invest highly in one, or very few mates per reproductive bout, meaning pregnancy detection could be beneficial in dissuading males from guarding already-mated dams.

Here I test the response of males and females to pregnant and non-pregnant odours. I also consider the chemical components of these anal gland secretions to determine whether scents change during pregnancy, thus providing a mechanism for discrimination of pregnant dams. I predict that:

- If females use scent signals within reproductive competition they should show heightened responses to the odours of females representing direct reproductive threats.
- If males use scent signals within mate-choice they should show heightened responses to non-pregnant females.
- If female reproductive state is discernible via scent, the chemistry of anal gland secretions should differ between pregnant and non-pregnant odours.

Methods

Odour collection

Anal gland secretions were collected and stored as per the methods of section 2.5.2. 54 donor females were sampled between 7th April and 29th July 2015. In 20% of cases the same female was sampled twice, once during early pregnancy (Preg) and once when non-pregnant but not during oestrus (Non). Reproductive state was determined by ultrasound scan during captures (see section 2.5) and pregnant females' weight and appearance was then monitored to accurately identify live birth or abortion.

Odour presentations

To test the ability of banded mongooses to discern female reproductive state via scent, male and female recipients were presented with AGS samples from pregnant and non-pregnant females. Female donors and recipients were aged >12 months, thus regarded as adult and sexually mature. Male recipients were aged > 24 months and had shown signs of breeding behaviour including mating and mate-guarding in the months prior to this study. Presentations were conducted from July 3rd to August 14th 2015. Recipient females were presented to within seven days of an ultrasound scan confirming their current reproductive state. Females were not sampled or presented to within 48 hours of giving birth or aborting a litter to avoid the effects of stress linked to these physiological processes. Recipient mongooses were presented with odours from non-neighbouring groups so as not to confound results with the effect of familiarity or previous exposure to the odour.

All odour presentations followed methods outlined in section 2.6.1. Responses were filmed using a handheld camera and three measures of response were scored: "Duration" represented the time before banded mongooses returned to normal foraging behaviour, "Contact" referred to the duration a banded mongoose remained in physical contact with the presented odour and finally the number of scent marks deposited on or around the presentation were counted (see Table 2 in Chapter 2 for full definitions). As females are predicted to utilise scent cues for competitive purposes we considered only the number of over-marks female recipients deposited directly on top of an odour. However, for presentations to male recipients we considered the number of marks deposited within a 30cm radius of the odour as this vicinity marking is thought to function within mate-acquisition rather than competitive interactions (Table 2, Chapter 2).

Statistical analyses

Results were analysed via GLMMs in R version 3.0.2 as outlined in section 2.8. Separate GLMMs were constructed to test the effect of odour pregnancy status (Preg or Non) upon the response

measures of male and female recipients. In all models the identity and social group of both donor and recipient were controlled for by fitting as random effects, and odour familiarity was controlled as all odours were unfamiliar to recipients. Appendix B (Table 1) contains details of all terms included in the following models.

For female data, initial models considered the effect of odour pregnancy status, recipient pregnancy status and their interaction upon marking behaviour. Where significant interactions were detected Tukey post-hoc comparison tests (Hothorn et al., 2008) were used to directly compare response measures between all combinations of presentation types. Female presentations fell into four categories dependant on the reproductive state of donor and recipient (Table 1), presentation type (e.g.: Preg-Preg) was thus the explanatory variable in all post-hoc comparisons.

		State of recipient	
		PREGNANT	NON-PREGNANT
State of donor	PREGNANT	Preg-Preg	Preg-Non
	NON-PREGNANT	Non-Preg	Non-Non

Table 1: Matrix demonstrating the four types of presentation conducted between female mongooses. Presentation format always refers first to the state of the odour-donor, second to the state of the recipient, thus preg-non refers to a trial where a pregnant odour was presented to a non-pregnant female.

The age-linked dominance hierarchy within banded mongoose females (Bell et al., 2012; Cant et al., 2013) suggests their age may influence the response to presented odours. For the female dataset older recipients spent less time in contact with presented odours (LMM: p= 0.003) and deposited fewer scent marks (LMM: p= 0.014). Odour-donor age had no significant impact upon response measures (Appendix A, Table 4). As such, recipient age was retained as a random effect within models testing the effect of female presentation type upon "contact" and "over-marking" but not "duration". For models concerning the response of male recipients, the age of female odour donor was included as a fixed effect. This is due to previous research showing males preferentially mate with older females (Nichols et al., 2010). The effect of donor age may thus be crucial to the interpretation of results with regard to mate choice. Male age was not included as all recipient males were adults aged >24 months and considered sexually mature due to the occurrence of breeding behaviours (mating or mate-guarding) in the three months prior to this study.

Chemical analysis

Anal gland sections (AGS) were analysed via gas chromatography – mass spectrometry (GC-MS) and analysed by non-parametric tests as outlined in section 2.7. I also considered whether proteomic differences in anal gland secretions were apparent during pregnancy by conducting a full proteome digest and analysis of anal gland secretions, for full methods see section 2.7.3. As

protein analysis of scent samples is a relatively new technique, I was guided by my collaborators at the University of Liverpool's centre for proteomics in this analysis.

Results

Female discrimination of reproductive state via odour cue

Initial models tested the effect of odour and recipient pregnancy status (and their interaction) upon responses to presented odours. For both contact and duration there was no significant interaction between the status of odours and recipients to influence response to presented odours (Table 2). However, in both cases pregnant females spent longer in contact with odours (LMM: t = 2.260, p = 0.026, Table 2) and took longer to return to normal foraging behaviour (LMM: t = 2.106, p = 0.038, Table 2) after a presentation than did non-pregnant recipients.

Table 2: Initial LMMs testing the effect of odour and recipient pregnancy status upon response measures to presented odours.

Response measure	Fixed effect	Effect size	Std. error	t value	p value
Duration	Intercept	34.824	7.636		
	Odour pregnant	-8.575	9.263	-0.926	0.357
	Recipient pregnant	**19.544**	**9.279**	**2.106**	**0.038**
	Odour pregnant *recipient pregnant			1.529	0.130
Contact	Intercept	16.583	3.749		
	Odour pregnant	-6.411	4.602	-1.393	0.670
	Recipient pregnant	**10.353**	**4.582**	**2.260**	**0.026**
	Odour pregnant *recipient pregnant			1.622	0.108
Over-marking	Intercept	9.419	1.124		
	Odour pregnant	**-5.330**	**1.658**	**-3.214**	**0.002**
	Recipient pregnant	**-2.947**	**1.720**	**-1.714**	**0.090**
	Odour pregnant *recipient pregnant	**8.412**	**2.388**	**3.522**	**0.007**

Female responses to presented odours varies dependent upon the reproductive state of both donor and recipient. Significant effects highlighted in bold. Analyses based upon the results of 94 presentations to 63 individual mongooses, using 54 female odour donors.

For over-marking frequency there was a significant interaction between odour and recipient pregnancy status (LMM: t = 3.522, p = 0.007, Table 2). As such I remodelled this data so as it was suitable to use a Tukey post-hoc comparative test to visualise how each combination of presentations (non to non, non to preg, preg to preg, preg to non) impacted marking behaviour. Tukey tests reveal that females appear to more heavily over-mark odours of their same reproductive state. Pregnant females scent marked pregnant odours significantly more than did non-pregnant recipients (Tukey: z = 3.338, p = 0.004, Table 3, Figure 1). Additionally, pregnant odours received fewer scent marks from non-pregnant females than did non-pregnant odours (Tukey; t = -2.811, p = 0.025, Table 3, Figure 1). For full Tukey output see Appendix A, Tables 5 and 6).

Table 3: Output of Tukey post-hoc comparison tests used following LMMs testing the effect of presentation type on response measure.

Response measure	Comparison between presentation type	Estimate	Std error	Z value	P value
OVER-MARKING	**preg to preg - preg to non**	**5.423**	**1.601**	**3.338**	**0.004**
	preg to non - non to non	**-4.598**	**1.636**	**-2.811**	**0.025**

Female responses to presented odours varies dependent upon the reproductive state of both donor and recipient. Only significant comparisons are presented, however original models compared all combinations of presentations for each response measure. Analyses based upon the results of 94 presentations to 63 individual mongooses, using 54 female odour donors. Full output is presented in appendix A, Tables 5 and 6).

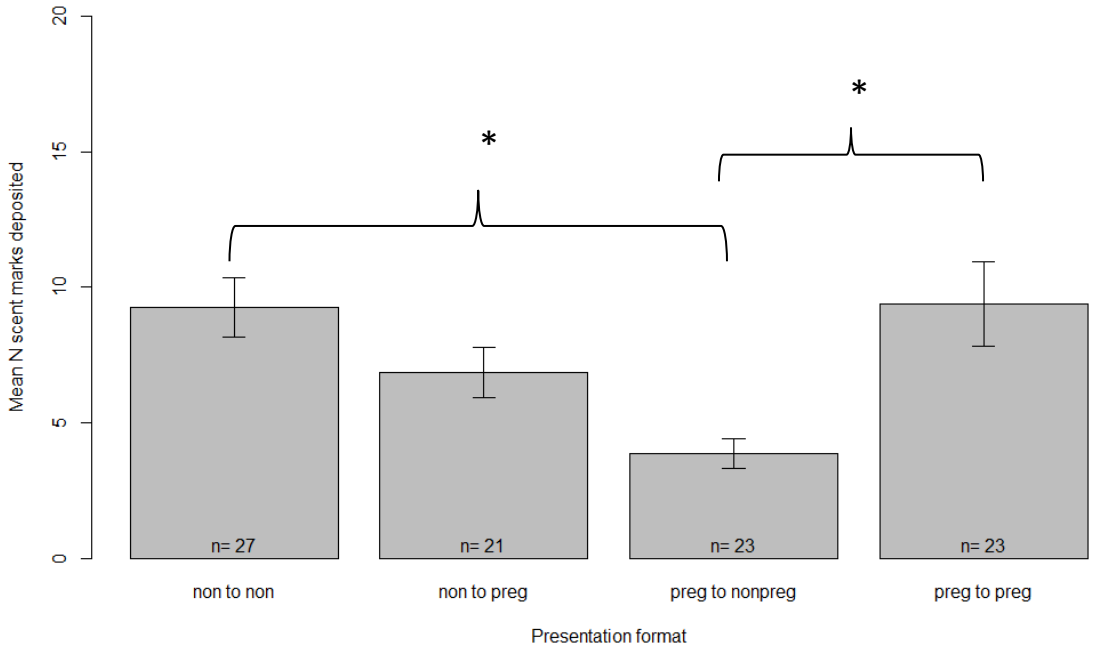


Figure 1: Female banded mongooses deposit significantly more marks over presented odours of their same reproductive state. In all cases presentation format refers first to the state of the odour donor, second to the state of the recipient. Non-pregnant odours provoke more scent marking from non-pregnant recipients than do pregnant odours (far left bar in comparison to second from right). Pregnant odours elicit significantly more scent marking from pregnant than non-pregnant recipients (compare bars on the right). Error bars = standard error.

Male discrimination of reproductive state via odour cue

Males showed significantly heightened responses toward non-pregnant odours, suggesting they are able to discern female reproductive state via scent (Table 4, Figures 2 and 3). Males spent less time in contact with pregnant odours (GLMM: t = -2.253, p= 0.029, Figure 2), and took longer to return to foraging behaviour (GLMM: t = 2.509, p = 0.016, Figure 2). Additionally, males deposited significantly fewer marks near pregnant compared to non-pregnant odours (GLMM: t = -3.109, p = 0.003, Figure 3).

Table 4: Output of GLMMs testing the effect of female reproductive state upon male responses to odour presentations.

Response measure	Fixed effects	Estimate	Standard Error	T value	P value
DURATION BEFORE RETURN TO NORAMAL BEHAVIOUR	Intercept	38.896	3.995		
	Donor age (increasing)	0.001	0.011	0.126	0.900
	Pregnant odour	**-13.625**	**5.431**	**-2.509**	**0.016**
CONTACT	Intercept	19.178	2.738		
	Donor age (increasing)	0.004	0.007	0.554	0.583
	Pregnant odour	**-8.069**	**3.581**	**- 2.253**	**0.029**
VICINITY MARKS	Intercept	8.157	0.787		
	Donor age (increasing)	0.002	0.002	1.142	0.260
	Pregnant odour	**-3.042**	**0.979**	**-3.109**	**0.003**

Results indicate males show heightened responses to the odours of non-pregnant females, remaining interested and in contact with these presentations for longer than those of non-pregnant females. Males also show a significant increase in marking behaviour when presented odours from non-pregnant donors. Analyses based upon the results of 48 presentations to 32 individual males, using 26 female odours. Original models also included the interactions between reproductive state and donor age, however these were sequentially removed due to non-significance (NS). NS fixed effects are retained in the table alongside the p-values upon which they were rejected from the models.

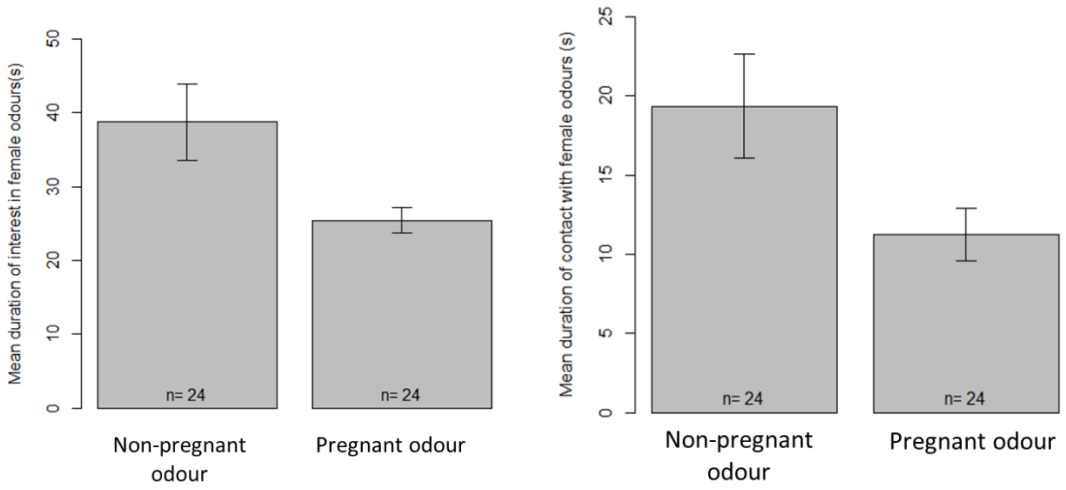


Figure 2: Male duration responses to the presentation of pregnant and non-pregnant odours. Male recipients take longer investigating (left) and in contact with (right) the odours of non-pregnant compared to pregnant females. Error bars = standard error.

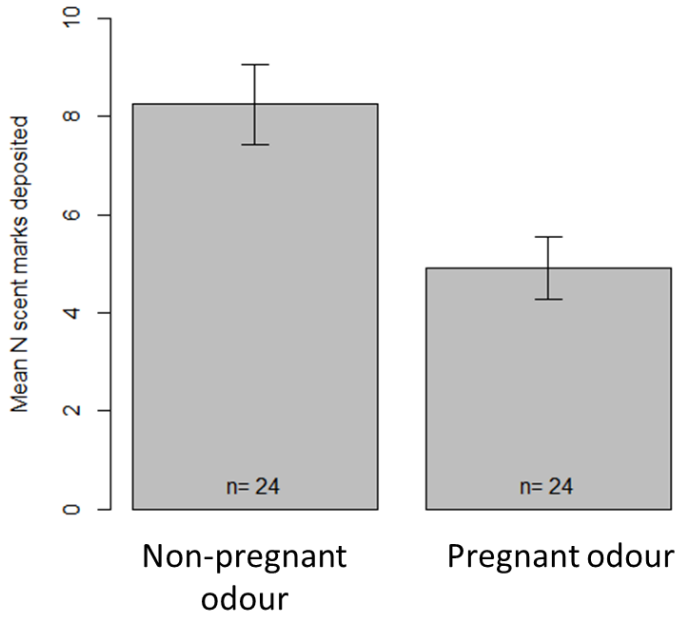
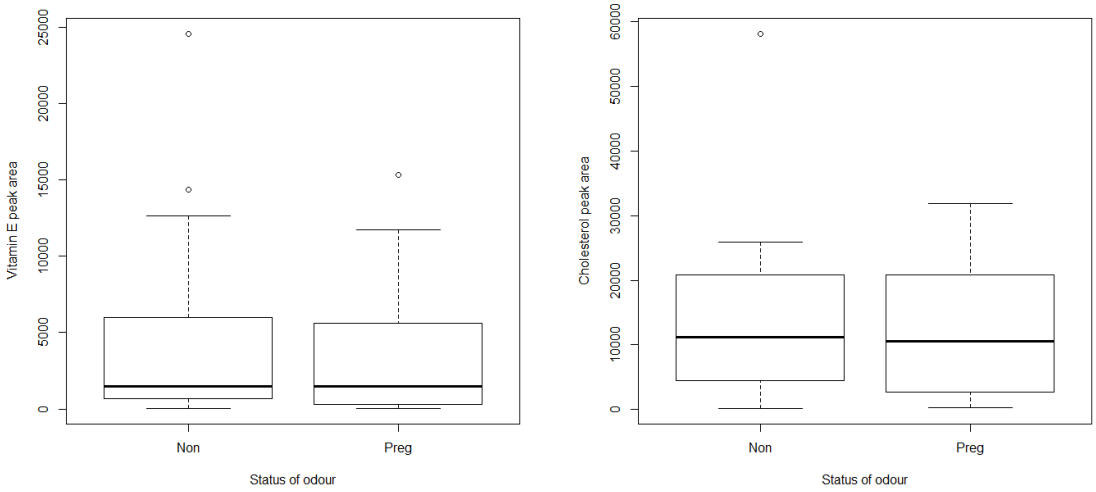


Figure 3: Male vicinity marking responses to pregnant and non-pregnant odours. Male recipients deposit significantly more scent marks around the odours of non-pregnant females compared to odours from pregnant donors. Error bars = standard error.

Chemistry results

The GC-MS analysis for this chapter unfortunately showed that samples had degraded in storage as very few volatile chemicals were present in the resulting chromatograms. This is likely due to the age of the samples as their return to the UK took longer than planned, and lower-weight volatile chemicals could have evaporated during storage and transportation. As such I concentrated my analysis on the two most abundant compounds across all samples, Cholesterol and Vitamin E, which are heavier molecules and thus less likely to have evaporated. In particular, I was interested in whether the peak areas (relative abundance) of each compound differed between pregnant and non-pregnant samples. As neither dataset was distributed normally I used separate Mann-Whitney-Wilcoxon tests run within R version 3.0.2 to compare peak areas based upon pregnancy status. Neither Vitamin E (Wilcoxon test: W = 318, p-value = 0.6379, Figure 4a) nor cholesterol (Wilcoxon test: W = 296, p-value = 0.9761, Figure 4b) peak areas differed in size dependant on the pregnancy status of the odour. This suggests these compounds do not signal pregnancy within banded mongoose anal gland secretions. To test this distinction more specifically, I used the waters offline model builder (Waters UK) to attempt to assign a subset of 10 samples (5 from each state) to their correct reproductive state using only abundance levels of Vitamin E and Cholesterol. In 70% of cases assignment was incorrect again suggesting chemical signatures of pregnancy are not detectable in the current dataset.



Figures 4a and 4b: Boxplots demonstrating that the peak areas (relative abundance) of neither Vitamin E (4a, left) nor cholesterol (4b, right) differed significantly based upon the pregnancy status of the odour donor. Boxplots created with R version 3.0.2 default settings, thick line represents median peak areas.

Protein results

Following GC-MS my collaborating team at Liverpool University's Proteome research centre suggested a protein analysis of banded mongoose AGS. As such, ten samples (five from each reproductive state) were analysed at the proteomic level following the methods described in Chapter 2, section 2.7.3. Progenesis analysis revealed 77 protein groups and 291 peptide sequences of which 120 proteins could be identified (Appendix A, Table 7). Many were lysosomal proteins implicated in cell-signalling and cleavage which, according the expert team at Liverpool, is highly unusual for an animal scent secretion. Two specific proteins (Acid ceramidase OS, Accession N^{o} ASAH1_MOUSE and Arginase-1 OS, Accession N^{o} ARGI1_BOVIN; ARGI1_HUMAN; ARGI1_MOUSE; ARGI1_PIG) were more abundant within non-pregnant females (Table 4). Results must be taken with extreme caution due to the novelty of this protocol and small sample size. Additionally, only one specific peptide sequence for each protein showed significant variation between reproductive states (Table 4). However, this provides tentative evidence for a protein-based scent difference during pregnancy and merits further investigation.

Table 4: Peptide abundances of focal proteins in pregnant and non-pregnant females.

Protein	Peptide	Correlation in abundance between reproductive states	ANOVA	Highest mean abundance	Lowest mean abundance	Neutral mass	Retention time
ARGI1	DIVYIGLR	0.505	0.123	NON	PREG	54831	947.543
	GGVEEGPTVLR	0.859	0.139	NON	PREG	258171.9	112.584
	YFSMTEVDK	**0.863**	**0.011**	**NON**	**PREG**	**113776.6**	**1134.492**
ASAH1	**SGEGCVITR**	**0.87**	**0.0283**	**NON**	**PREG**	**177915.8**	**977.462**
	STYPPSGPTYR	0.87	0.404	NON	PREG	1224.58	17.442

Two proteins showed a difference in abundance between the anal gland secretion samples of non-pregnant and pregnant females. In both cases, it was the relative abundance of a single peptide that underpinned this difference (Highlighted bold). Retention time refers to the amount of time it took each peptide to travel through the mass spectrometer. Neutral mass refers to the mass of the single peptide in laboratory conditions.

Discussion

Pregnancy appears discernible via scent in the banded mongoose. Males spent more time investigating the odours of non-pregnant females, and deposited more marks around them, than they did in response to odours of pregnant dams. The female response however, was more complex with females showing heightened over-marking when odours were of their same reproductive state. In particular, pregnant females displayed more intense reactions to the odours of other pregnant dams, whilst non-pregnant odours received more marks from non-pregnant recipients. These results collectively support pregnancy being detectable via scent, yet a mechanism for such discriminatory behaviour remains elusive as chemical analyses could not detect significant differences in the composition of pregnant verses non-pregnant anal gland secretions (AGS). However, protein signatures in AGS suggest there may be molecular differences facilitating the detection of pregnancy. These results remain in their infancy and thus must be taken with some caution at this stage.

Banded mongoose females show heightened over-marking toward odours of the same reproductive state. This supports intra-sexual competition theories whereby direct over-marking obliterates the scent of one's competitor (Gosling and Roberts, 2001). It also verifies research conducted by Muller et al (2008) and Jordan et al (Jordan et al., 2011c) which suggests competition is an important function of scent communication within the banded mongoose. Females compete intensively within this species for access to breeding opportunities as well as resources for their offspring, including care. Mate-choice occurs in both sexes and thus the extensive over-marking response of non-pregnant females toward non-pregnant odours could function as a mechanism of mate-attraction by covering-up the scents of direct reproductive competitors. For pregnant dams however, one of the most obvious repercussions of intra-sexual competition is the high synchrony of births. In 64% of breeding attempts females give birth on the same night in the same den (Hodge et al., 2009) a technique which successfully minimises infanticide. As this synchrony is so successful, a mechanism to detect pregnancy would appear beneficial and odour-based discrimination of pregnancy could provide this.

However, in order to synchronise births, dams must be aware of the gestational stages of other pregnancies. In the current study, pregnant recipients spent longer in contact with odours and took longer to return to foraging behaviour than did non-pregnant conspecifics. Duration measures are often used as proxies for interest in an odour, suggesting pregnant recipients are extracting valuable information from odours. Although further testing of such a theory is required, it is possible dams are gaining information, such as the gestational timing of the odour-donor, which may influence birth synchrony.

In banded mongooses, birth synchrony enhances litter success but there is a limit to this benefit. Once over ~8 dams are breeding, communal litter success begins to decline (Cant et al., 2010; Cant et al., 2014) as pups compete for access to food and helper resources (Cant et al., 2014; Cant et al., 2013). Thus, pregnant females' interest in odours may allow them to assess how many other breeders are present within their group. This in turn may influence decisions relating to their pregnancy. Abortion and reabsorption do occur in the banded mongoose (Gilchrist, 2006) and, as occurs in other mammals these may be adaptive strategies for mothers who find themselves out-competed or out-of-sync with other breeders (Stockley and Bro-Jorgensen, 2011; Wasser and Barash, 1983). Behaviourally dominant females monopolise reproduction, partially because they are preferred by males (Nichols et al., 2010), but also because they can forcibly evict younger individuals from the social group (Cant et al., 2010; Thompson et al., 2016). Indeed, in both pregnant and non-pregnant females intense reproductive competition is now considered one of the main triggers of eviction (Thompson et al., 2016), a situation which compromises body condition, reproductive output and even survival of the evictee (Cant et al., 2010; Gilchrist, 2006). Top-ranking females regularly evict younger and smaller conspecifics to prevent them becoming pregnant. Pregnant females can also be evicted and this generally results in offspring loss through abortion brought on through stress (Gilchrist, 2006; Thompson et al., 2016). Detecting other pregnancies would thus appear important to dams in terms of both avoiding and instigating eviction.

Male banded mongooses also appear able to discriminate odours on the basis of pregnancy. In support of initial predictions, males spent longer investigating non-pregnant odours and took longer to return to normal foraging following such presentations. Increased duration measures suggest recipients are gaining information from presented scents. Indeed, previous research in the banded mongoose has suggested odours encode oestrus, albeit through same-sex presentations concerning the response of other females (Jordan et al., 2011c). Thus, males may spend more time with non-pregnant odours in order to determine exact receptivity. Males also deposited more vicinity marks around the odours of non-pregnant dams. Vicinity marking is considered to function within mate choice as it maximises the identity of both odours rather than obliterating the original (as direct over-marking does) (Wolff et al., 2002). Combining these results would suggest males are responding to odours for reproductive reasons. However, it must be stressed that non-pregnant odours within this dataset were not taken from females showing oestrus behaviour and thus the discrimination of these odour appears mediated by pregnancy detection. Nevertheless, it would appear intuitive for males to show heightened responses to non-pregnant odours, as these represent potentially receptive mates. In the banded mongoose system, males invest highly in mate-choice by following females and preventing access by other males. This behaviour can prevent them from foraging as efficiently as they must remain vigilant to "pesterer" males who attempt to intercept their female (Cant et al., 2013). Detecting pregnancy via scent should thus be beneficial to males, preventing them from wasting time and resources on already mated dams.

Although I have focused on the potential for female odour cues to function in intra-sexual competition, this does not negate that they also play a role in attracting mates. Both

males and non-pregnant females showed heightened marking toward non-pregnant odours than those of already pregnant dams. Thus although both sexes appear to discriminate reproductive state via scent, non-pregnant females also appear to preferentially over-mark their competitors. This maintenance of olfactory dominance by would suggest female-scent-marking could be implicated in mate-attraction. Few studies have successfully provided evidence for a mechanism of male-mate-choice linked to female receptivity (Edward and Chapman, 2011). In several species of stickleback fish, female nuptial colouration appears to signal readiness to spawn and males preferentially mate colourful females (McLennan, 1995; Rowland et al., 1991). Classic examples also include primate systems such as the mandrill, *Mandrillus sphinx*, where female rump swellings indicate oestrus and allow males to select mates based on receptivity and possibly quality (Setchell et al., 2006). However, regarding olfactory mechanisms, support is more limited. Ring-tailed lemurs can advertise oestrus through scent (Crawford et al., 2011). Yet, in the banded mongoose, previous research suggests scents do not function within mate attraction as females with higher rates of scent marking did not experience more harassment by males (Jordan et al., 2011c). The current study however provides rare evidence that males can discriminate scents based on pregnancy. Thus, although oestrus females with increased marking rates may not attract more male attention, the discriminatory behaviour of males toward pregnant odours could be viewed as a tentative mechanism of male-mate-choice based on female reproductive state.

Exactly how the banded mongoose is able to discriminate pregnancy remains unknown. GC-MS analyses showed no obvious difference in odour chemistry based upon pregnancy. However, AGS samples did contain a high abundance of compounds with high molecular weights, such as cholesterol and vitamin E. In captive ring-tailed lemurs, heavier compounds have been proposed to increase the longevity of genital and brachial secretions (Scordato et al., 2007) by anchoring to the substrate upon which they are deposited. This allows odours to persist over time due to the slower release of volatile compounds that communicate specific information to conspecifics. An exact mechanism for volatile release has not yet been confirmed, however the realisation that banded mongoose odours also contain signalling proteins could provide this mechanism, particularly considering proteolytic functions are recognised to control the release of volatile compounds in rodent odours (Beynon and Hurst, 2004).

Upon the advice of collaborators at Liverpool University, a proteomics analysis of anal gland secretions (AGS) was attempted revealing 201 identified proteins, many of which were lysosomal proteins (Appendix Table 11). Such molecules contribute to cell signalling and proteolytic process by the cleavage of enzymes and other proteins to produce peptides (Braulke and Bonifacino, 2009). In the context of scent-marking such proteins may function in a similar way to major urinary proteins (MUPs) of mice which regulate the release of bound volatiles which communicate information to conspecifics (Beynon and Hurst, 2004). The expert team at Liverpool have pioneered techniques for identifying, quantifying and analysing MUPs within mouse and rat urine (Beynon et al., 2015; Beynon et al., 2014; Beynon and Hurst, 2003; Gomez-Baena et al., 2014; Phelan et al., 2014). These proteins are now recognised to underpin a multitude of behavioural interactions including mate-choice and kin recognition (Beynon and Hurst, 2003) but also to bind molecules implicated in pheromone signalling (Beynon and Hurst, 2004). A similar mechanism may thus exist within banded mongooses considering the wealth of proteins within their AGS. However, proteins themselves may also function as signalling molecules. In captive saddle-back Tamarins (*Saguinus fuscicollis*) two proteins were identified

within urine and glandular secretions, and animals could behaviourally discriminate between scents that held intact and degraded proteins (Belcher et al., 1990). Authors concluded that these proteins were an important component of the scent signal and likely function as carriers and/or reservoirs for the volatiles encoding specific information (Belcher et al., 1990).

Caution is required at this stage as understanding the proteome of banded mongoose AGS requires more detailed research on a larger sample of the population. However, two proteins were seen to be expressed more in non-pregnant samples compared to those of pregnant dams (Acid ceramidase OS, Accession N^{o} ASAH1_MOUSE and Arginase-1 OS, Accession N^{o} ARGI1_BOVIN; ARGI1_HUMAN;ARGI1_MOUSE; ARGI1_PIG). Although only one specific peptide sequence showed significant variation between reproductive states (out of 2 for ASAH1 and 3 for ARGI1) this could be taken as tentative evidence for a protein-based scent difference during pregnancy. This finding is novel and suggests, for the first time, that proteins are implicated in scent signalling in a non-model and wild species. Exactly what information these proteins and their associated compounds encode must now be addressed. It would be useful to consider how protein and chemical components interact to prolong the signal (as speculated above). How such scent-components change throughout the reproductive cycle should also be considered. This could identify potential pheromones linked to pregnancy that may affect behaviour. Indeed, Queen Pheromones control reproductive behaviour across social insects by inhibiting the development of worker ovaries and ensuring Queens remain the only breeders (Gadagkar, 2009; Holman et al., 2010; Kocher and Grozinger, 2011; Peeters and Liebig, 2009). In the banded mongoose, multiple females can breed but to avoid infanticide have developed their rare pattern of synchronous birthing. One avenue for future research would be to test whether dams who scent mark frequently are able to encourage birth-synchrony with their gestational timing. If pheromones linked to pregnancy are identified and synthesised, then their function in birth-synchrony could be tested under controlled conditions. It must be stressed however that research into mammalian oestrus synchrony has been fraught with conflicting results and statistical artefacts (Doty, 2010; Setchell et al., 2011; Wyatt, 2014). Nevertheless, an olfactory mechanism of birth synchrony has not yet been considered and the banded mongoose represents an idea target species due to the common occurrence of synchronous births in this system. Identifying such a mechanism could be particularly beneficial in understanding the population dynamics of other synchronous breeders such as the caribou, *Rangifer tarandus*, (Adams and Dale, 1997) which are believed to birth communally to minimise predation risks.

In summary, banded mongooses appear to discriminate odour cues based on pregnancy status. Females show heightened responses to odours of their same reproductive state suggesting they are utilising odours within the scope of intra-sexual competition. Males however respond more highly to odours of non-pregnant females suggesting these cues aid mate-choice. Such results suggest odour signals may serve sex-specific functions in this species, intra-sexual competition in females and mate-choice in males. Future investigation should now consider the protein and chemical signatures of pregnancy in more detail. Eventually, the synthesis of pheromones linked to pregnancy and receptivity could be used in field-based presentations to assess the ability of odours to influence reproductive decisions including birth synchrony.

Chapter 5 Gastro-intestinal parasites of the banded mongoose: Identification and patterns of variation

Abstract

Parasites may contribute to significant declines in wild mammal populations, carnivores representing a particularly threatened group. Although life-history and ecological factors are known to be influential, few studies have investigated variation in parasite diversity and load in wild carnivores. Here the banded mongoose is used as a model system for carnivore endo-parasitology. Having access to long-term behavioural and life-history data I consider parasitic variation in relation to life-history, social and ecological factors. Results show banded mongooses harbour a diverse endo-parasitic community, which includes taxa known to have fitness implications in other carnivores. Variation in parasite burdens was apparent at both the pack and individual level, with life-history and ecological factors explaining some of this. In particular, body weight had a significant impact on parasite load; heavier individuals were less likely to be infected with certain parasites and when infected, showed lower ova counts and lower overall parasite taxa richness. This suggests heavier individuals have a fitness advantage over lighter conspecifics, which may be particularly relevant to the banded mongooses' breeding ecology where larger individuals of both sexes are more successful breeders. Age and rank also influenced certain parasite burdens and long-term monitoring of the population would now be useful to determine whether age-related immunological changes are responsible for such relationships. Finally, parasite taxa richness of one social group significantly exceeded that of all others, suggesting territory and habitat variation may impact parasite burdens. However, parasite abundance and community composition was similar across social groups, and the same two common parasites appeared responsible for driving within-group similarity and between-group differences in parasitic community composition. Considering these findings, individual, not pack-based variation appears to the main driver of parasite burdens in this banded mongoose population.

Introduction

Parasitic infection is a key factor influencing mammalian population dynamics (Poulin, 1997; Wilson et al., 2002). The exploitative effects of parasites on host health can shape demographic parameters including survival, longevity and fecundity (Anderson and May, 1978; Coltman et al., 1999; Ezenwa et al., 2006; Moore and Wilson, 2002). Carnivores appear particularly at risk as, of the mammal species threatened by parasites (IUCN status), 88% reside within either this taxonomic order or the Artiodactyls (Pedersen et al., 2007). Understanding the dynamics of parasite communities is therefore a key issue in carnivore conservation biology. Unfortunately, despite such threats, the risk-factors associated with carnivore parasites are poorly understood. Meta-analyses suggest that various measures of parasitic infection vary with the host's life-history traits and socio-ecology (Ezenwa et al., 2006; Lindenfors et al., 2007). Indeed, parasites tend not to be randomly distributed but a minority of heavily parasitized host individuals often drive patterns of infection (Hayward, 2013; Poulin and Morand, 2000; Wilson et al., 2002). Understanding the causes of this over-dispersion is key to untangling patterns of parasitic infection in the wild.

Sex differences in susceptibility to parasites are common. Males often harbour higher parasite loads than females (Moore and Wilson, 2002; Scantlebury et al., 2010; Schalk, 1997; Zuk and McKean, 1996), a difference attributed to life-history trade-offs reflected in the higher testosterone levels and larger body sizes of males (Moore and Wilson, 2002; Roberts et al., 2004). Male mammals often enhance their fitness through the solicitation of multiple mates

(Bateman, 1948), subsequently they tend to be the larger, more highly-ornamented and aggressive sex. This may compromise immunity because the production of testosterone, which controls growth and secondary sexual signals and trades-off against immune function thereby increasing male susceptibility to pathogens (Folstad and Karter, 1992; Roberts et al., 2004; Schalk, 1997). However, studies so far have tended to focus on sexually-dimorphic systems where males are significantly larger than females (Coltman et al., 1999; Craig et al., 2008; Harrison et al., 2010; Moore and Wilson, 2002), yet in sexually monomorphic species patterns may be less clear.

Patterns of parasitism relating to body size and host age are less conclusive, with higher burdens often observed at each extreme depending on the host species (Cote and Poulin, 1995; Morand and Poulin, 1998). Reduced body weight is often considered symptomatic of high parasite load due to the costs of fighting resulting infections (Costa and Macedo, 2005). Alternatively weight can be a causal factor; larger individuals are proposed to attract higher levels of pathogens because they can physically harbour more parasites (Hamilton and Zuk, 1982; Poulin, 1997), and because the demands of their larger body size requires ingestions of more food and thus more parasites (Ezenwa et al., 2006; Lindenfors et al., 2007). As previous studies have focused on sexually dimorphic systems, it will be enlightening to investigate size-based parasite variation within the sexually monomorphic banded mongooses particularly as size is a key determinant of reproductive success for both sexes (Cant et al., 2002; Cant et al., 2013; Hodge et al., 2009). Additionally, both parasite load and parasite taxa richness (hereafter PTR) are known to vary with age in mammals (Craig et al., 2008; Hakkarainen et al., 2007; Lucan, 2006; Scantlebury et al., 2010; Smythe and Drea, 2015). However consistent trends are rare as pathogens often show species-specific effects on different age classes. Younger individuals may be more at risk of parasitic infection due to their naive immune systems; alternatively the accumulation of infections over time may lead to higher parasite loads within older individuals (Shanley et al., 2009). Finally, environmental variability and stress can also exacerbate negative effects of chronological ageing on parasite burdens. In feral Soay sheep*, Ovis aries*, faecal parasite burdens increase with age but this increase is steeper if hosts have experienced environmental stressors such as harsh winters (Hayward et al., 2009).

Environmental conditions themselves may influence infection burdens as parasite development is often dictated by conditions external to the host (Turner et al., 2012). Rainfall in particular tends to increase burdens of parasites with free-living larval stages (Turner et al., 2012). Furthermore, environmental, life-history and social conditions can interact, increasing cost of certain pathogens to hosts with particular attributes such as stress, poor body condition or reduced genetic diversity (Coltman et al., 1999; Hayward et al., 2009). This covariation of host and parasite traits and varying effects of parasites across individuals can make it difficult to determine how life-history and environmental factors influence patterns of parasitism. Finally, most studies have tended to focus on a single parasite. This limits the extent to which results can be used to predict fitness repercussions as natural systems tend to harbor multiple infections, often with interacting effects on host fitness (Knowles et al., 2013).

In this study I use the banded mongoose as a model species for investigating parasite dynamics in a social carnivore. As cooperatively breeding and group-living mammals the likelihood of transmitting pathogens between conspecifics, both within and between packs is high. Yet, although the focal population has been studied continuously for 20 years, we know little of their parasitic community. Banded mongoose territories remain largely consistent over time and are defended aggressively from neighbouring groups (Cant et al., 2002; Gilchrist and Otali, 2002; Müller and Manser, 2007). However, boundaries do overlap (Figure 1, Chapter 2) presenting opportunity for inter-group transmission of parasites. Groups also inhabit areas

frequented by other mammals including humans, lions and leopards. It is therefore useful to consider variation in the parasitic communities of each social pack. Individual variance in parasite burdens can also be considered in relation to life-history measures such as age, weight and sex. However, as previous studies have focused on sexually dimorphic systems, it will be enlightening to investigate these relationships in a sexually monomorphic species. Finally, parasite burdens are known to influence behavioural interactions (Poulin, 1994; Poulin, 1995a) and these impacts could be investigated in the banded mongoose system considering the wealth of life-history data available for this population and that mate-choice and competition over reproduction occurs for males and females. In particular, both sexes show preference for older mates (Cant et al., 2002; Nichols et al., 2010) and size is a key determinant of reproductive success (Cant et al., 2002; Cant et al., 2013; Hodge et al., 2009). Therefore understanding how parasite burdens vary with weight and age may aid our understanding of breeding dynamics, including mate-choice, in this system.

Aims

The aims of this study are first, to provide a preliminary overview of banded mongoose endo-parasitology using faecal egg counts (FEC) as a proxy for parasite burden. Second, to discuss how FEC vary in relation to life-history and ecological factors and third, to describe changes in the composition of parasite communities across packs and individuals. This will provide the first investigation into the dynamics of banded mongoose endo-parasitology and allow long term life-history data to be combined with a measurable proxy of parasite loads in a wild mammal on a population-level scale.

Methods

Parasitic analyses

Parasite sample collection, storage and analysis followed protocols outlined in section 2.4. Faecal egg counts (FEC) were used as a proxy for parasite burdens with ova extracted from faecal matter using a modified McMaster technique (see 2.4.2). All ova within the boundaries of the McMaster grid were counted under x 40 magnification and tentatively identified using the veterinary parasitology literature and communication with experts in the field (Bowman, 2014; Leclaire and Faulkner, 2014). Use of FEC has been criticised as its accuracy in predicting internal parasite loads varies (Gillespie, 2006; Hayward, 2013; Poulin and Morand, 2000). Egg-shedding loads may vary with host condition as well as the reproductive stage and fecundity of the parasite and therefore may not reflect true worm burdens (Wood et al., 2013). This is less of a problem for single-celled protozoan parasites such as *Isospora*, where numbers in faeces are a more accurate reflection of internal infections and less dependent on parasite-fecundity. A more reliable method is to dissect hosts and count the number of parasites within the gastrointestinal tract (Poulin and Morand, 2000; Wimmer et al., 2004). However, as with the current system, it is often not possible to dissect study animals, and such methods also rule out longitudinal sampling of individuals. Therefore, FECs are generally regarded as the most practical method for monitoring parasitic communities of wild mammals as long as results are interpreted as a proxy measure (Gillespie, 2006; Rafalinirina et al., 2015).

Individuals sampled

Sampled individuals were aged over twelve months, thus classified as adults (Cant et al., 2013). Female animals were only sampled when non-pregnant and had not given birth or aborted a litter in the week preceding sample collection. Sampled banded mongooses appeared in good health however on two occasions obviously injured animals were excluded from the analysis following snake bites. To consider variation in the parasitic community of this banded mongoose population three measures of parasitic infection were recorded. First, the prevalence

of each commonly seen ova (Table 1) was recorded as either 0 = absent or 1 = present for each faecal sample. Second, parasite taxa richness (PTR) was recorded; this measure refers to the number of taxonomically distinct ova identified in each faecal sample. Finally, the abundance of each parasite was calculated as an egg per gram figure using the standard McMaster equation detailed in section 2.4.2. For each sample, egg-per-gram counts were calculated separately for each of the most commonly recorded ova (Table 1) and were also combined into one measure as a proxy for overall parasite burden.

Statistical analyses

Variation at the individual level

To consider how variation in life history factors affected parasite burdens a series of mixed models were built within R version 3.0.2 (R development core team, 2013) using the lme4 package (Bates et al., 2008). All models included the following explanatory variables: host sex, age in days, age-rank, average weight (in grams on the date of sampling) and rainfall in the month preceding sample collection. Dominance is linked to age in this system but the relationship is not entirely linear (Bell et al., 2012) hence the inclusion of both age and age rank in the models. Regarding age-rank, the oldest individual per group was assigned the rank of 1, the next oldest ranked as 2 and so on following the methods of Nichols et al (2010). For full details of all explanatory terms, calculations and justifications see Table 1 in Appendix B. As random effects, all models included host and pack identity to control for multiple sampling within individuals and social groups across the two-year study period. As sexes differ in their life-history trajectories with females typically reproducing earlier than males, we also included second-order interactions between sex and age or age-rank in the models. Full life-history data were available for 255 samples collected from 93 individuals across five social groups (pack 7a could not be included due to a lack of weights data, see below). The prevalence of each commonly noted ova was recorded as a binomial response variable (1, present or 0, absent) and analysed using a binomial generalized linear mixed effect model with logit link function. With the exception of host sex and age-rank, all explanatory variables were log transformed to improve model convergence. Parasite taxa richness (PTR) followed a normal distribution and so was analysed by a linear mixed effect model fit by maximum likelihood. Again, with the exclusion of sex and age-rank, all explanatory variables were log transformed. For abundance models the response variable was egg-per-gram (epg) load for overall parasite load and each taxa separately. Epg was modelled alongside sex, age, age-rank, weight and rainfall as predictors in generalised linear mixed effect models fit by maximum likelihood with a normal distribution.

Variation at the pack level

The effect of social group upon parasite measures were considered in separate models in order to investigate differences in pack-based parasite burdens, rather than to simply account for variance explained by pack identity. Certain metrics such as weight and age were missing for some individuals, (all of pack 7a lack weights data), as such the pack dataset (358 faecal samples collected from 111 adults across six packs) was larger than that considering individual variation.

Pack-based variation in the prevalence of each common parasite was addressed by binomial linear mixed effects models as described previously. In this case pack was included as the single explanatory variable with individual identity as the random factor. Other metrics (age, sex, weight etc.) were not included as fixed effects as this analysis focused on the specific effect of social group upon parasite burdens. As all packs had similar age structures and sex-ratios (Table 1, chapter 2) the exclusion of these factors is unlikely to skew results. Additionally, as pack is effectively being used as a proxy for geographic location on the field site and territory quality, it was deemed appropriate to leave out such metrics and incorporate pack 7a's data to

maximise sample size. This model was followed by a Tukey post-hoc comparison test (Hothorn et al., 2008) to visualise pair-wise differences in the prevalence of parasites between packs. To consider pack differences in PTR a linear mixed effects model was fit by maximum-likelihood with pack identity as the explanatory variable and individual identity as a random factor. Again, other metrics were not included and significant effects of pack identity were teased apart using Tukey post-hoc comparative tests (Hothorn et al., 2008) to directly compare the PTR of packs on a pairwise basis. Finally, to consider whether social group explained significant variation in banded mongoose parasite communities an analysis of similarities (ANOSIM) was run based upon 9999 permutations of the dataset concerning individual egg per gram counts for the five most commonly identified parasites (Table 1). ANOSIM is a non-parametric permutation test which determines whether there is a significant difference between groups of sampling units (in this case pack) without the need for assumptions concerning data distributions (Clarke and Warwick, 2001). A similarity percentages (SIMPER) analysis followed to assess the percentage similarity between individual parasitic communities based upon social group, and which parasites contributed most to the observed variation. Both ANOSIM and SIMPER analyses were run in Primer E version 7 (Clarke and Warwick, 2001).

Results

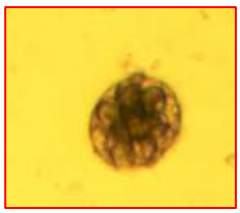
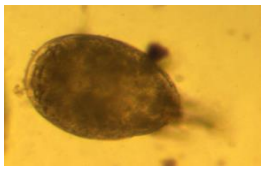
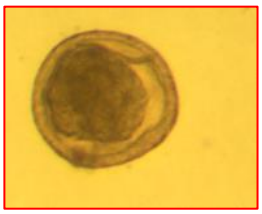
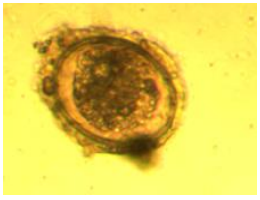
Parasitic identification

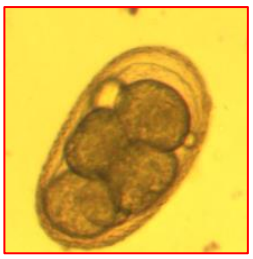
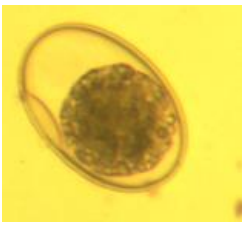
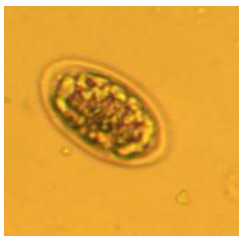
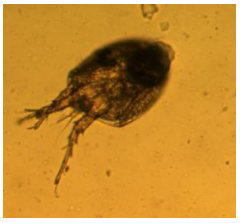
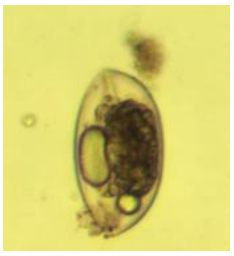
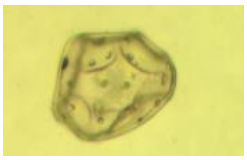
Gastrointestinal parasites were present within all individual banded mongooses sampled across this two-year study period. Multiple parasitic taxa were identified (Table 1) and most individuals were infected with three (median PTR = 2.7) separate intestinal parasites. Four ova (Table 1, outlined in red) were most commonly seen across this population and based on morphology, the large strongyle found in 32% of samples was classified as a hookworm within the *Ancylostomidae* family, which commonly infect canines and other carnivores. Alongside viable ova, hookworms regularly produce immature/unfertilised eggs resembling the small strongyle ova in Table 1, so precise identification pending, these were grouped together in the analyses. Similarly, the ascarid was tentatively identified as a *Toxocara* species due to its morphological similarities to species infecting domestic dogs and cats. *Toxocara* also produce immature ova akin to the smaller ascarid identified within banded mongoose faeces (Table 1). These could represent the same or different species but for analyses reported here, they were counted together.

Single-celled parasites of the subclass Coccidia were identified in 74% of banded mongooses' samples and likely belong to the genus *Isospora*. These are obligate intracellular parasites of the phylum Apicomplexa, which typically infect the cells lining the gut wall of their host. Most *Isospora* are host-specific (Lindsay et al., 1997) and although generally monoxenous, certain species (characterised by doubly-sporulated oocysts, shed in the faeces of their definitive host) are known to infect intermediate hosts (Frenkel and Smith, 2003). In the banded mongoose oocysts were regularly recorded containing up to 4 sporocysts, or being unsporulated, however most common were oocytes with one or two sporocysts (Table 1). Based upon this morphological variation there may be two different species of *Isospora,* or an *Isospora* and *Eimeria* species present in our population (Urquhart et al., 1996). However, as both belong to the same family (*Eimeriidae*) and share similar morphology, genetic identification is required for full classification. For the following analyses coccidian oocytes were therefore considered together as *Isospora*. *Isospora* and *Eimeria* are transmitted directly through contact with infected individuals and/or their faeces.

The fourth ova resembled egg-packets of the canine tapeworm (Dipylidium caninum), however genetic identification is required for classification at the species level. Defining features of this ova included the egg packets containing 8-20 eggs which is typical for Dipylidium caninum, although in the banded mongoose these packets tended to be more spherical in shape than those found in canine faeces. Less common ova observed included a pin worm (Genus Enterobius, present within 4% of samples) and a fluke (Family Fasciolidae present in <1% of samples), an unidentified mite and a large ovum, potentially a mite egg (Full details in Table 1). An artefact likely to be a pollen grain is also reported, as its spherical structure may be easily confused with ova (Table 1).

Table 1: Gastro-intestinal parasites of the banded mongoose.

Phylum	Taxonomic ID	Ova photograph	Key features	% samples present
Apicomplexa (parasitic protists)	Subclass Coccidia Order Eucoccidiorida Genus*: Isospora*		Thin walled, spherical ova 15-40µm in diameter. Often contain two sporocysts but are seen with up to four and also as unsporulated ova. Potentially two different *Isospora* species present.	74
Platyhelminthes (flatworms)	**Order Cyclophyllidea** *Dipylidium* (tapeworm)		Spherical or oval "packet" of 8-20 eggs, 50-80µm in length, 20-50µm in width. Eggs occasionally seen burst from packet in clusters of 3-12.	22
	Order Echinostomida Family *Fasciolidae* (fluke)		Dense ova with thick wall. Oval shaped 80x~40µm	<1
Nematodes (Round worms)	**Order Ascaridida** Likely genus *Toxocara*		Spherical ova with thick, pitted wall. ~70-90µm in diameter. Developing nematode may be visible inside ova.	34
	Order Ascaridida Likely an immature/unfertilised *Toxocara* ova		Smaller ova ~40-60µm in diameter with rough, thick cell wall and densely sporulated centre.	16

	Order Strongylidae Family *Ancylostomatidae* (hookworm)		Large oval shaped ova with thin cell wall and blunt ends, 60-90µm in length and 35-50µ across.	32
	Order Strongylida *Likely an immature/unfertilised example of the above*		Large oval ova with thin cell wall, ~30x60µm in length. Highly sporulated centre	25
	Order *Oxyurida* Likely genus *Enterobius* (pinworm)		Small oval egg ~15µm in diameter by ~30µm in length. Densely sporulated centre.	4
Arthropods	Unidentified mite		Mite with equal length limbs (~35µm) and shorter mandibles (~20µm). Body ~100µm in diameter and roughly spherical.	10
Unknown	*Unidentified at present (possible mite ova)*		Large oval ova (~50x30µm) with slightly pointed ends. Developing larvae often visible inside. Characterised by two green vacuoles.	2
Common artefact	*Pollen grain from unidentified source*		Large spherical structure ~70µm in diameter.	18

Common parasitic ova extracted from banded mongoose faeces using a modified McMaster salt floatation protocol. Identifications based upon morphological features. Column three details notable identification features and the size-range of ova observed.

Variation in the prevalence of specific taxa

Toxocara ova were significantly less prevalent in heavier individuals (GLMM: z= -2.358, p=0.018, Table 2) and following periods of heavy rainfall in the month preceding sampling collection (GLMM: z= -2.766, p=0.006). Isospora prevalence also showed a non-significant decline with weight whilst older individuals were significantly less likely (GLMM: z = -3.601, p=0.0003) to carry this parasite than younger conspecifics. Prevalence declined with age-rank (GLMM: z=-2.468, p=0.014) and as the oldest individuals per group were assigned an age rank of 1, next oldest 2 and so on, this suggests top-ranking and more dominant individuals are more likely to carry Isospora (Table 2). However, considering the effect of age it would also appear likely that dominants who are relatively young for their status are also more likely to be infected. Ecological and life-history variables had no significant effect on the prevalence of any other common ova.

Patterns of taxa richness

Parasite taxa richness (PTR) was significantly lower in heavier banded mongooses (GLMM: t = -2.656, p=0.008, Table 3) suggesting larger individuals harbour fewer taxa (Table 3, Figure 1). No other factors significantly predicted PTR.

Table 2: Factors predicting variation in the prevalence of parasite taxa in the banded mongoose.

Parasite Prevalence	Fixed effect	Effect size	Standard error	Z value	P value
Hookworms	Intercept	-2.281	7.848		
	Host Weight (g)			-0.857	0.391
	Rain			1.423	0.155
	Host Sex (female)			1.540	0.124
	Host Age (days)			1.372	0.170
	Host Agerank			1.432	0.152
Toxocara	Intercept	15.329	6.240		
	Host Agerank			0.932	0.352
	Host Sex (female)			1.243	0.214
	Host Age (days)			1.408	0.159
	Host Weight (g)	**-4.616**	**1.958**	**-2.358**	**0.018**
	Rain	**-0.878**	**0.318**	**-2.766**	**0.006**
Tapeworm	Intercept	-1.126	0.401		
	Rain			0.348	0.728
	Host Age (days)			0.576	0.565
	Host Agerank			-0.310	0.756
	Host Weight (g)			-0.901	0.368
	Host Sex (female)			0.992	0.321
All *Isospora*	Intercept	13.353	3.497		
	Host Sex (female)			-0.920	0.358
	Rain			1.037	0.300
	Host Weight (g)			**-1.901**	**0.057**
	Host Age (days)	**-3.572**	**0.991**	**-3.601**	**0.0003**
	Host Agerank	**-0.325**	**0.132**	**-2.468**	**0.014**

The output of GLMMs on factors predicting the prevalence (1 = present, 0 = not) of common gastrointestinal parasites of the banded mongoose, measured as eggs per gram of faeces. Significant (or borderline significant) effects are presented in bold, and non-significant terms are presented alongside the p values upon which they were sequentially dropped from the models following the backward step-wise simplification procedure. Results are based on analysis of 255 faecal samples collected between July 2013 and August 2015.

Table 3: Life history factors predicting parasite taxa richness in the banded mongoose.

	Fixed effect	Effect size	Standard error	t value	p value
Parasite taxa richness (PTR)	Intercept	11.636	3.562		
	Host Age (days			0.770	0.44
	Rain			-0.852	0.395
	Host Agerank			-0.608	0.946
	Host Sex (female)			-2.413	0.261
	Host Weight (g)	**-2.981**	**1.122**	**-2.656**	**0.008**

Output of GLMM of factors predicting parasite taxa richness in the banded mongoose. Individual weight was the only significant predictor and presented here in bold; all non-significant terms are presented alongside the p values upon which they were sequential removed from the initial model using the stepwise model simplification process. Results are based on analysis of 255 faecal samples collected between July 2013 and August 2015.

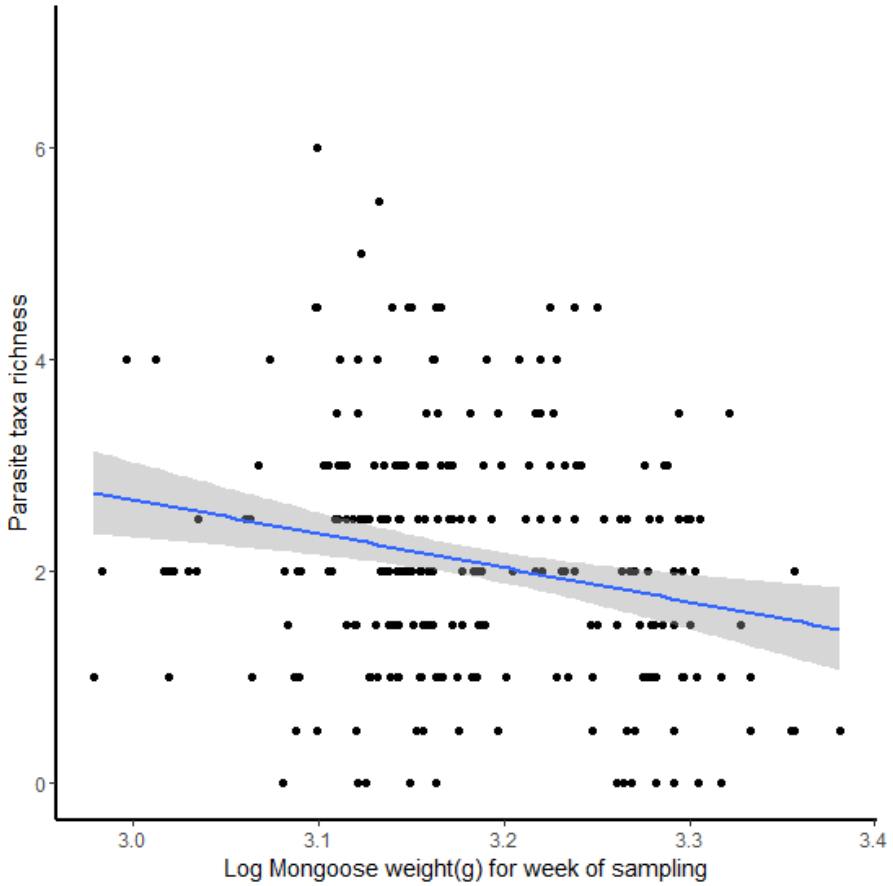


Figure 1: The relationship between banded mongoose weight and gut parasite taxa richness. Heavier individuals have fewer parasite taxa than lighter conspecifics (p =0.008, Table 3). Line represents linear regression of PTR against logged weight with 95% confidence intervals represented by the shaded areas, and points correspond to raw data.

Abundance of specific taxa

Isospora oocytes were less-abundant in females (GLMM: t = 2.191, p= 0.029) whilst hookworm (GLMM: t = -2.490, p=0.013, Table 4) and Toxocara loads (GLMM: t = -2.820, p=0.005) were significantly lower in heavier individuals. Hookworms were significantly more abundant in older individuals (GLMM: t = 2.610, p = 0.010, Table 4) whereas Isospora loads declined with age (GLMM: t=-2.133, p =0.034, Figure 2a). Isospora burdens declined with age-rank (GLMM: t=-2.199, p = 0.029) suggesting top ranking mongooses show higher Isospora burdens than their lower-ranked and younger conspecifics once age has been accounted for (Table 4). However when plotted (Figure 2b) this relationship is not entirely linear. Individuals ranked between 3 and 5 appear to have the highest Isospora loads, but absolutely top ranking individuals do not (trend remains unchanged when fitting age rank as a squared term). There was no significant effect of any life history factors on the abundance of the potential tape worm species.

Table 4: Life history factors predicting variation in the abundance of intestinal parasites in the banded mongoose.

Parasite Abundance	Fixed effect	Effect size	Standard error	t value	p value
Hookworms	Intercept	17335.537	6037.908		
	Rain			0.914	0.362
	Host sex (female)			-0.536	0.593
	Host Agerank			1.104	0.271
	Host Weight (g)	**-10.147**	**4.075**	**-2.490**	**0.013**
	Host Age (days)	**3.401**	**1.303**	**2.610**	**0.010**
Toxocara	Intercept	61533.84	16404.21		
	Host Age (days)			0.087	0.931
	Host Sex (female)			0.236	0.814
	Rain			0.728	0.467
	Host Agerank			-1.700	0.090
	Host Weight (g)	**-29.71**	**10.54**	**-2.820**	**0.005**
Tapeworm	Intercept	954.048	2834.386		
	Rain			-0.185	0.853
	Host Age (days)			-0.789	0.431
	Host Agerank			-0.948	0.344
	Host Sex (female)			1.353	0.177
	Host Weight (g)			1.496	0.136
All *Isospora*	Intercept	5177726.0	1824400.0		
	Rain			0.190	0.849
	Host Weight (g)			-1.903	0.058
	Host Sex (female)	**-1485857.5**	**678137.0**	**-2.191**	**0.029**
	Host Age (days)	**-1319.1**	**618.4**	**-2.133**	**0.034**
	Host Agerank	**-537019.6**	**244164.8**	**-2.199**	**0.029**

Results of GLMMs considering the effect of rainfall in the previous month, host sex, age, age-rank and weight upon the abundance of common gastrointestinal parasites (measured as eggs per gram of faeces). Significant effects are presented here in bold, and non-significant terms are presented alongside the p values upon which they were sequentially dropped from initial models following the step-wise simplification procedure. Results are based on the analysis of 255 faecal samples collected between July 2013 and August 2015.

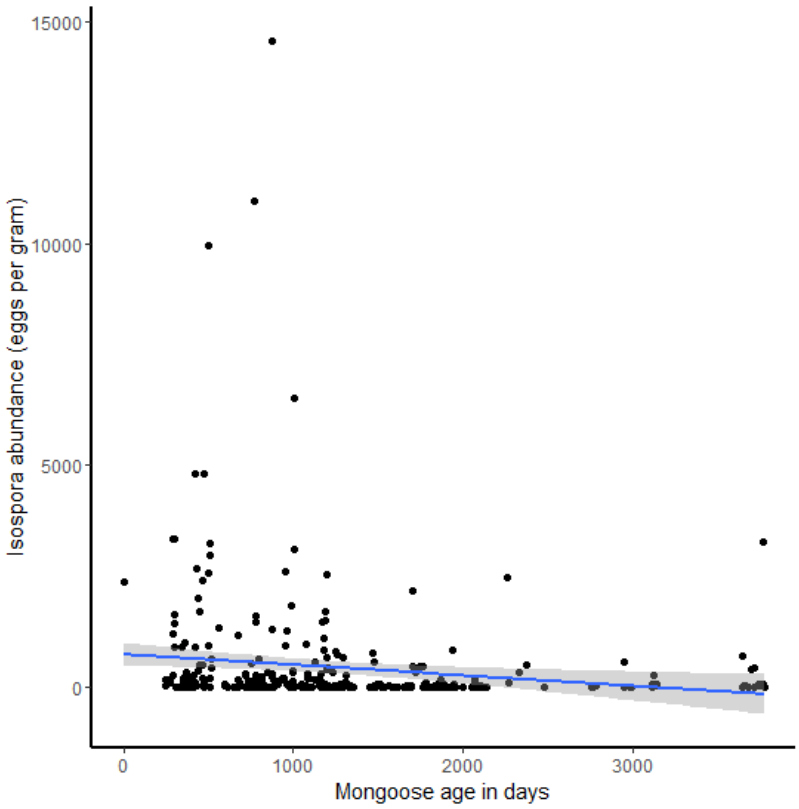


Figure 2a: The effect of age upon Isospora abundance in adult banded mongooses. Age is measured as age in days at the time of faecal sample collection, and Isospora load appears to decline with age.

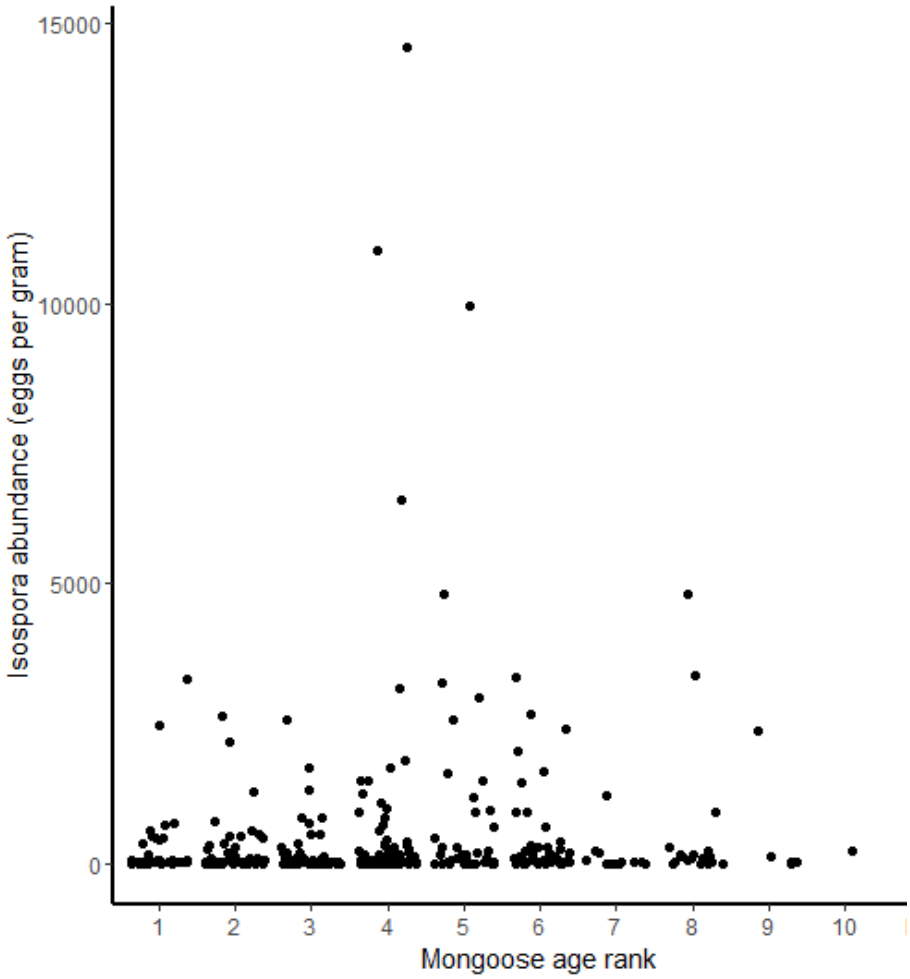


Figure 2b: The relationship between banded mongoose Isospora burden and age rank. The relationship does not appear to be linear. Individuals ranked 4-5 in their group (older but not the oldest members of each sex) have higher Isospora burdens than their highest and lower ranked conspecifics.

Variation at the individual level

In the previous models individual identity was included as a random factor to account for variation between multiple samples collected from the same individual. Individual identity contributed differentially to variation within parasite prevalence and abundance, dependent upon the parasite taxa being examined (Table 5). These results suggest in addition to the life-history and ecological factors considered in this analysis, individual differences such as foraging niche and genetic diversity (Chapter 7) may also influence parasite burdens.

Table 5: The variation in parasite prevalence and abundance explained by individual identity

	Parasite Prevalence		Parasite Abundance	
Parasite taxa	Variance explained by individual	SD	Variance explained by individual	SD
All Isospora	3.126e-01	5.591e-01	0	0
Hookworms	0.1957	0.4423	2.637e+06	5136
Toxocara	0	0	9.565e+07	9780
Tapeworm	5.507e-10	2.347e-05	4.984e+08	22325

Individual identity can explain some variation in parasite prevalence and abundance, dependent upon the parasite taxa in question. Results from GLMM full model outputs considering the effect of aforementioned life-history and ecological factors upon ova burdens, controlling for individual and pack identity.

Individual variation in FEC across time

Temporal variation in parasite burdens and taxa richness were apparent at the individual level. Figures 3 and 4 shows the faecal egg counts and PTR respectively of 3 males and 3 females from pack 1B which were repeatedly sampled across summer 2014. Certain individuals show relatively consistent ova loads (BF561 and BM410) whilst for others this is highly variable (BF484 and BM216). As rainfall was not a significant predictor of parasite load, other factors such as foraging patterns and genetic differences may explain why certain individuals show greater variation in ova burdens than others.

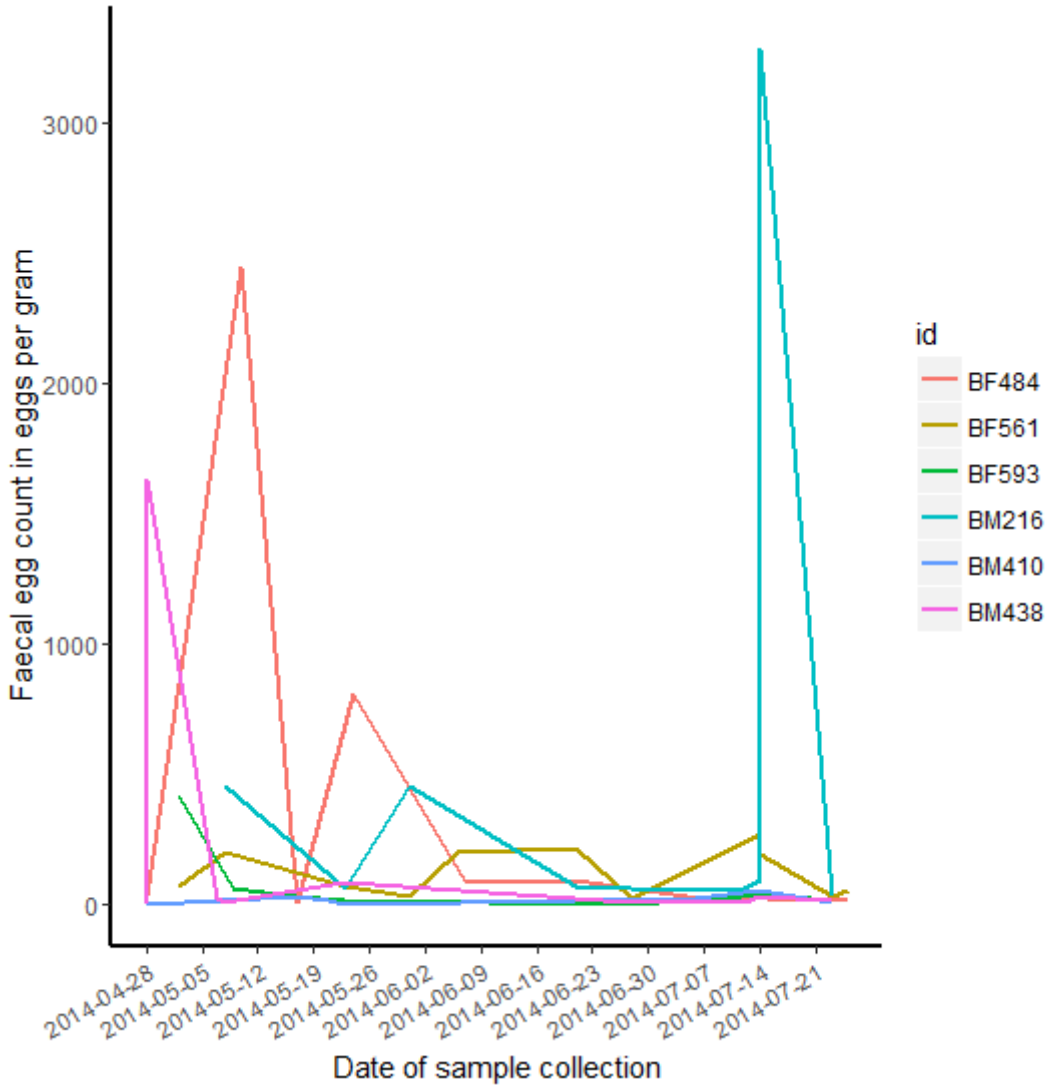


Figure 3: Temporal variation in individual parasite burdens across summer 2014. Raw epg data for six focal individuals within pack 1B collected between April and July 2014. Coloured lines represent each individuals' fluctuating parasite burden. Some individuals (BF561) show relatively repeatable faecal egg counts whereas others are more stochastic (BF484 and BM216).

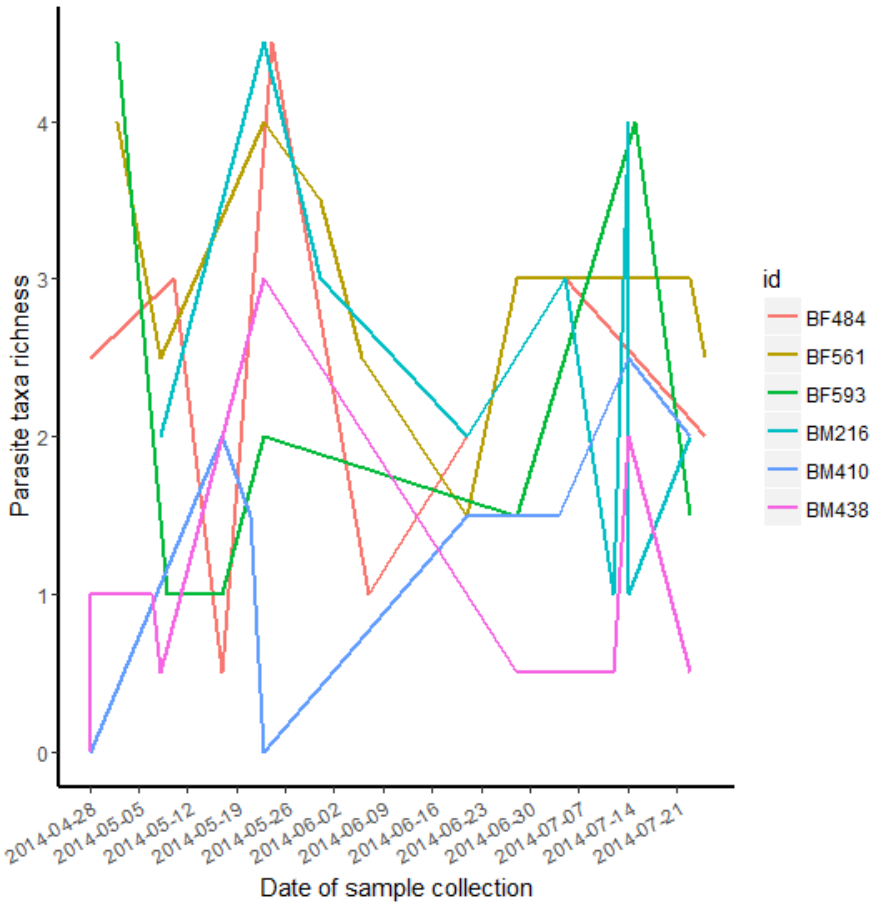


Figure 4: Temporal variation in individual parasite taxa richness (PRT) across summer 2014. Raw PTR data for six focal individuals within pack 1B. Coloured lines represent the number of parasite taxa identified in each sample for each individual between April and July 2014. All six individuals were sampled at least six times over this 3 month period and all show a high degree of variation in terms of the number of parasitic taxa present in each sample.

Variation at the pack level

Regarding the binomial presence data (0 = absent, 1 = present) Isospora prevalence was significantly higher for pack 2 than either 7a or 1H, tapeworm ova were more prevalent within members of pack 11 than all other groups (non-significantly so for packs 2 and 17) and Hookworm ova were significantly more prevalent within pack 7a than any other group save pack 17. Multiple comparisons were significant for Toxocara; these ova were significantly more prevalent within pack 11 compared to packs 7a and 1B, 1H had a higher prevalence than 1B and 7a (for full output of Tukey test results see Appendix B, Table 2). The most significant differences in pack-based parasite prevalence are graphed in Figure 5 considering the percentage of samples infected (Asterisks refer to significant comparisons from Tukey output). Finally, Tukey post-hoc comparative tests showed pack 11 harboured a significantly higher diversity of parasitic species than any other social group (Figure 6). There were no other significant pairwise differences among packs (Appendix B, Tables 3a and 3b).

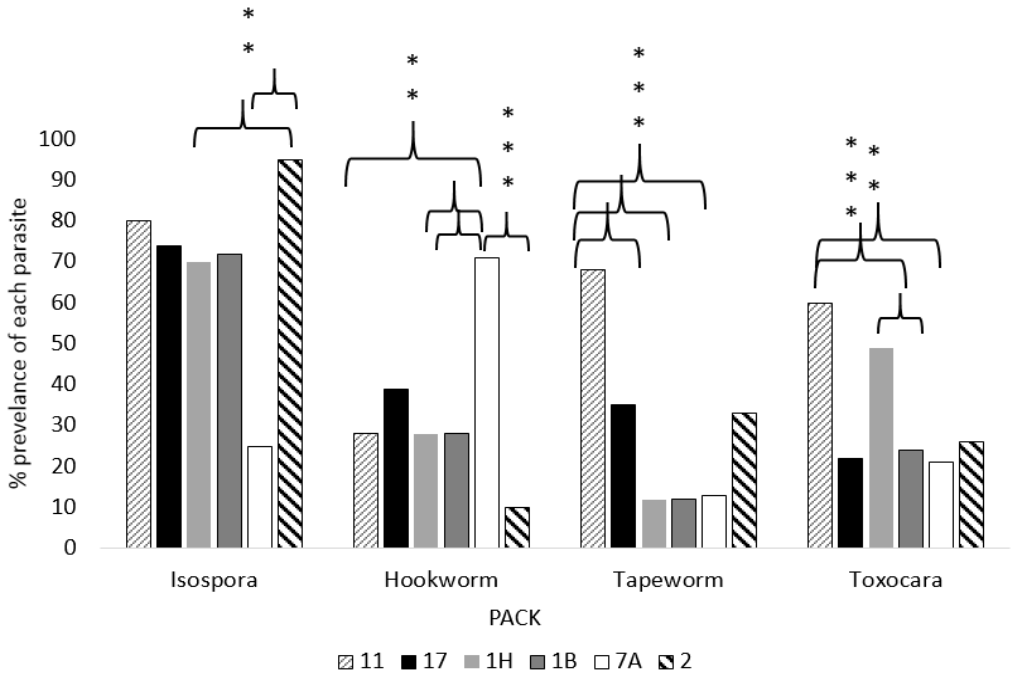


Figure 5: Pack-based variation in the prevalence (% of samples infected) of each commonly seen ova. Asterisks denote significant differences in parasite prevalence as detected by Tukey post-hoc comparisons (full details displayed in Appendix B, Table 2).

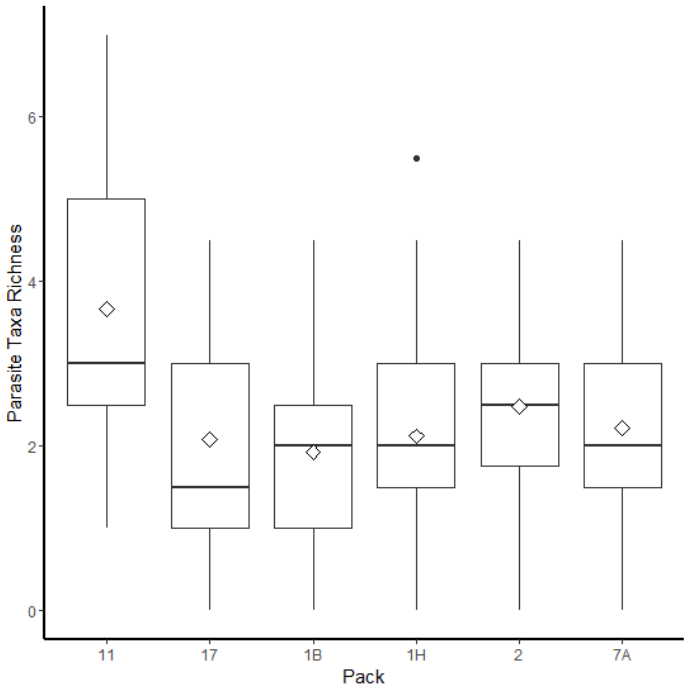


Figure 6: Variation in parasite taxa richness by social group. Pack 11 individuals show significantly higher diversity compared to all other groups and there were no significant differences between other packs. Thick bars represent median taxa richness for each pack and diamond points the mean. Error bars = standard deviation.

ANOSIM results based upon the abundance of the four most common parasites (Table 1, images outlined in red) suggest borderline significant differences between the parasitic communities of each pack (p=0.05). However, the global R value is extremely small (0.034, R values close to zero indicate low separation between packs based on parasite burdens, closer to 1 indicates high separation) suggesting pack explains only a small proportion of overall variation in individual parasite burdens. The breakdown of group comparisons from the ANOSIM shows significant differences ($p > 0.001$) with larger R values when comparing Pack 11's parasitic community to those of packs 17 (R = 0.197), 2 (R = 0.215) and 7a (R = 0.245). For full results see Appendix B, Table 4. These findings suggest pack 11 is different from all other groups, corroborating the GLMM analysis showing that pack 11 had significantly higher parasite taxa richness than any other social group. Members of packs 1B and 1H showed significantly different parasitic abundances as did pack 2 when compared with both packs 17 and 7a. A SIMPER analysis showed that on average, abundance measures for the four most common parasites differed between groups by 57%, whilst similarity within groups averaged 49%. Additionally, SIMPER findings support the low global R values from the ANOSIM as both within-pack similarities and between-pack differences are underpinned by the same two parasites. *Isospora* abundance always accounted for the largest percentage similarities within-groups and the largest percentage differences between-groups' parasitic communities (Appendix B, Tables 4 and 5). *Toxocara* abundances were also important in determining within-group similarity and between-group differences (Appendix B, Tables 4 and 5).

Discussion

Gastrointestinal parasites were present within all individual banded mongooses sampled across this two-year study period. Parasites were identified to family or genus level, with most common ova belonging to the groups *Isospora* (a single-cell coccidian)*, Ancylostomidae* (hookworm)*, Toxocara* (nematode worm) and *Dipylidium* (tapeworm). Other ova were also present albeit at low frequency. Most animals were infected with three different taxa and such multiple infection is typical of a wild mammal (Leclaire and Faulkner, 2014). The banded mongoose population as a whole did not exhibit notable clinical symptoms during the study period which may suggest current infection levels do not have detrimental fitness consequences at the population level. However, as similar parasites have health implications in other mammals (Lee et al., 2010; Lindsay et al., 1997; Urquhart et al., 1996), fitness costs associated with parasitic infection may be apparent at the individual level. Indeed, life-history and environmental variables had some impact upon parasite burdens with body weight, age and age-rank contributing to variation in the prevalence and abundance of certain ova. Parasite taxa richness (PTR) was also higher in lighter banded mongooses. The exact impact of variable parasite burdens upon host fitness in the banded mongoose now merits further investigation. However, the high individual variation in ova counts and PTR emphasize the need to consider faecal egg counts (FEC) as a "snap-shot" of infection status that must be collected on exactly the same temporal scale as associated fitness measures.

Several parasites commonly observed across this banded mongoose population have known health implications. The hookworms of the family *Ancylostomidae* are blood sucking nematodes of the small intestine, causing acute anaemia in young, weak and immuno-compromised hosts. Severe infections reduce appetite, leaving hosts underweight and poorly conditioned (Bowman, 2014; Jex et al., 2009; Urquhart et al., 1996). *Toxocara* nematodes can also cause weight loss and pneumonia during chronic infections in cats and dogs (Lee et al., 2010). The severity of both parasites is greatest in pre-weaned young, likely aided by the larval abilities of both taxa to pass from mother to offspring by migrating to mammary tissues and infecting milk (Jin et al., 2008; Urquhart et al., 1996). *Toxocara* species are also known to cause prenatal infection; larvae present within uterine tissues can migrate to lungs of developing foetuses, causing chronic infection after birth (Urquhart et al., 1996). Such health costs have the potential to severely impact wild populations particularly if dams are infected before and/or during pregnancy. Screening banded mongooses for *toxocara* and hookworm ova during breeding periods could thus be used to assess the impact of the parasites on breeding success and pup survival.

Infection with *Isospora* species is called coccidiosis and again exerts most severe effects upon pre-weaned young (Lindsay et al., 1997; Urquhart et al., 1996). Following ingestion, the sporulated oocytes of coccidians develop within epithelial cells of the intestinal sub-mucosa, rupturing cells as they leave. Severity of damage depends on the intensity of infection and location within the mucosa, but ranges from impairing local nutrient absorption to abdominal pain and diarrhoea, weight loss and full haemorrhage in extreme cases (Urquhart et al., 1996). In some species *Isospora* oocytes have been found in mammary tissues, suggesting they can be passed between mother and offspring (Duszynski and Marquardt, 2006) and potentially impinge on pup survival if the *Isospora* species has a similar transmission mode. Coccidiosis is however generally considered low risk to mature, healthy individuals; impacting hosts only in combination with other pathogenic, viral or immunosuppressive agents (Bowman, 2014; Lindsay et al., 1997). Monitoring coccidian oocyte numbers may therefore be most informative in obviously sick and injured animals as the costs of infection are likely to escalate under these circumstances. As *Isospora* species are highly host-specific (Lindsay et al., 1997) and difficult to identify based on morphology, genetic methods are required to confirm species identity. This would provide more detail on the parasites' host-specificity, transmission mode and life-cycle.

Nevertheless, identifying common canine and feline parasites within banded mongooses is useful considering the study population's close proximity to other carnivores (including lion and leopard). Although *Isospora* infections tend to be host-specific, hookworms, *toxocara* and tapeworms are often able to infect multiple hosts if their diet and/or habitats are similar (Bowman, 2014; Fisher, 2003; Jex et al., 2009; Lee et al., 2010). Additionally, carnivores can acquire *Isospora* infections via ingestion of rodent and avian prey (Urquhart et al., 1996). This may be particularly relevant to the banded mongoose which shows a varied carnivorous diet, but also to the species' preying on the mongooses themselves. Again, assessment of zoonotic transmission and infection risks will require species-level identification of such parasites via genetic methods (Jex et al., 2009) which was beyond the scope of this study.

There were no sex differences in parasite prevalence or PTR (parasite taxa richness) in this banded mongoose population. However as observed across mammals, *Isospora* oocytes were less abundant in female banded mongooses (Moore and Wilson, 2002; Zuk and McKean, 1996). This male-biased parasitism is often attributed to males' investments in size, weaponry, aggression and reproductive behaviours which trade-off against immunity, rendering them more susceptible to infection (Moore and Wilson, 2002; Roberts et al., 2004; Zuk and McKean, 1996). However, in the banded mongoose both sexes engage in mate-choice, intra-sexual competition (Bell et al., 2012; Cant et al., 2010; Cant et al., 2014; Nichols et al., 2010) and both can be forcibly evicted from natal groups (Cant et al., 2010; Thompson et al., 2016). Additionally males and females face similar reproductive costs as, although females invest heavily in pregnancy and lactation, males contribute disproportionately to the care of weaned young (Gilchrist and Russell, 2007; Hodge, 2007). Therefore, trade-offs between immuno-competency and reproductive success may be faced by both sexes, and factors classically used to support male-biased parasite loads may be less relevant to the banded mongoose. This may explain why, beyond *Isospora* abundance, sex-differences in parasite burdens are not apparent in this system. Interestingly, a recent study of the female-dominant meerkat, *Suricata suricatta,* showed that for two nematode species, dominant females harboured higher pathogen loads than subordinates and males (Smythe and Drea, 2015). Dominant meerkat females are hormonally masculinised (Davies et al., 2016 in press) and more behaviourally dominant than males (Clutton-Brock et al., 2001), thus results could be explained by immunological trade-offs exhibited by females. Authors also suggest that female-biased parasite loads may result from the stress associated with dominance acquisition and retention of social status (Smythe and Drea, 2015).

Individual weight was a significant predictor of parasite burden: heavier banded mongooses were less likely to be infected with *Toxocara* (and, non-significantly, *Isospora*), had overall fewer parasite taxa (lower PTR) and, when infected, their hookworm and *Toxocara* epg loads were lower. Other studies have proposed large individuals may have higher loads because they can physically harbour more parasites (Hamilton and Zuk, 1982; Poulin, 1997) or because the demands of their larger body size requires ingestions of more food and thus more parasites (Ezenwa et al., 2006; Lindenfors et al., 2007). Banded mongoose results do not support this idea, however reduced body weight has been suggested to be symptomatic of high parasite load due to the costs of fighting resulting infections (Costa and Macedo, 2005). This theory does appear supported by banded mongoose data; both hookworm and *Toxocara* show reduced abundance in heavier hosts and are known to have costly fitness implications including anaemia and weight loss (Urquhart et al., 1996). Causality in this relationship between weight and parasitic infection remains unknown at present. However, larger individuals may be able to devote more resources to immune function (Rauw, 2012) providing a mechanism to reduce parasite prevalence and abundance. In the banded mongoose system, experimental treatment with anti-parasitic drugs could shed light on whether selective parasite-removal impacts weight.

Not only would this clarify causality in this relationship but would aid understanding as to how each parasite effects health.

Health implications of parasitic diversity (measured in this analysis as parasite taxa richness) remain equally difficult to interpret without experimental intervention. Competition between multiple parasite species (and strains) within the gut can, in some cases, benefit hosts as multiple infections prevent single costly pathogens becoming well-established (Johnson and Buller, 2011; Johnson and Hoverman, 2012). According to this hypothesis a higher PTR may be advantageous (Poulin, 1995b), yet heavier banded mongooses show lower PTR than lighter individuals in this population. Again, experimental manipulations could clarify the relationship between parasite diversity, weight and health as well as interactions between specific parasites. Yet, regardless of the direction of causality, our results suggest that body weight is a reliable indicator of ova burden; an interesting result considering that banded mongooses are sexually monomorphic and size is known to benefit breeding success. Larger males have higher mating success (Cant et al., 2002) and larger females produce heavier pups which in-turn have competitive advantages over lighter conspecifics, and higher survival rates to independence (Hodge et al., 2009). As *Toxocara* and hookworms have most severe effects within pre-weaned young (Eustis and Nelson, 1981; Kirkpatrick, 1998; Lindsay et al., 1997; Mundt et al., 2006; Urquhart et al., 1996), carrying a lower load of such costly parasites would appear beneficial to offspring growth and survival. Thus size-based choice may direct mates to less parasitised individuals considering the negative correlation between weight and both T*oxocara* and hookworm loads. However, although larger individuals are more successful breeders there is no direct evidence that they are preferentially selected as mates.

Mate-choice in the banded mongoose is primarily linked to age because older individuals are more successful and experienced breeders (Nichols et al., 2010). Older males are more successful mate-guards and older females are preferentially guarded by males when receptive (Cant et al., 2002; Nichols et al., 2010). However, if older individuals also have reduced parasite burdens this preference would appear more beneficial. Indeed, *Isospora* were less prevalent in older hosts and abundance declined with increasing host age. Lower prevalence and abundance of *Isospora* infections in older individuals may indicate the acquisition of immunity toward this parasite. In other species, coccidiosis infections can stimulate immunity thus reducing infection prevalence with age (Bowman, 2014; Urquhart et al., 1996). However, they can be more prevalent when hosts are stressed and thus immunocompromised (Bowman, 2014), which may explain why *Isospora* prevalence also varies with age-rank. In contrast, hookworm epg increased with absolute age, whereas rank had no effect. Strongyle ova counts are also higher in older individuals of both the meerkat (Leclaire and Faulkner, 2014) and plains zebra, *Equas quagga* (Fugazzola and Stancampiano, 2012). In general these trends are explained by the process of immunosenescence where an age-related decline in thymic function allows parasites to accumulate in hosts over time, leading to high levels of infection within older individuals (Shanley et al., 2009). Monitoring parasite burdens across host lifespans would allow a better understanding as to whether age-based immunity or immunosenescence occur for any parasite in the banded mongoose. The relationship between rank and *Isospora* load should also be considered in more detail to address the relationship between infections, breeding and social status in the banded mongoose.

Both sexes compete for reproduction in the banded mongoose (Bell et al., 2012; Cant et al., 2013) and age, relative to other group members, is usually a fair predictor of breeding success (Bell et al., 2012; Cant et al., 2014; Cant et al., 2013). Older, top-ranking individuals who breed most regularly may thus be more susceptible to infection due to the stresses of reproduction itself, and maintaining reproductive dominance. This phenomenon is observed in meerkats, *Suricatta suricata*, where dominant females harbour higher nematode loads and

authors attribute this to the cost of maintaining dominance status and monopolising reproduction (Smythe and Drea, 2015). However, when plotted, the relationship between *Isospora* load and rank in banded mongooses did not appear entirely linear. This may reflect the more fluid structure of banded mongoose packs where dominance is not linearly linked to age as it is in meerkats. This warrants the use of both age and age-rank categories which allow models to tease apart general age-related trends but also patterns of parasitism linked to status. In particular, the individuals with very high *Isospora* loads (>5000 epg) are all ranked as 4th or 5th within their pack, yet are actually quite young in terms of absolute age (<1100 days or ~ 3 years old). At this age individuals are beginning to compete with older group-members for breeding opportunities (Cant et al., 2013) and vying for a higher rank. Hence, they may be more at risk of *Isospora* infection due to the pressures of acquiring and maintaining this status at their relatively young age. A similar phenomenon has been observed in the chimpanzee where socially dominant males, and those directly competing for dominance show higher helminth burdens (Muehlenbein and Watts, 2010). Thus although *Isospora* prevalence declines with absolute age in the banded mongoose, the relationship is more complex with respect to rank and must be taken cautiously considering the fluidity of dominance in this system. Nevertheless, it suggests that higher-ranking individuals may incur costs in terms of *Isospora* infection, and the combined effects of age and rank suggest that younger individuals in higher ranking positions may be most at risk.

Due to the seasonality of the field site, variation in parasite abundance and prevalence based upon rainfall is expected. However only *Toxocara* burdens were influenced with ova appearing less prevalent following heavy rain in the month preceding sample collection. Rain is generally associated with higher parasite burdens due to an abundance of insect prey which increases host-contact with larvae as they forage (Turner et al., 2012). However, in adult hosts, *Toxocara* larvae migrate to tissues such as the brain, skeletal muscle, heart, lungs and uterus where they can remain dormant for some time. This study is part of a long term monitoring project which rules out dissections to investigate larval and adult parasite loads throughout the body (Poulin and Morand, 2000). Hence, increases in *Toxocara* abundance following high rainfall could go undetected when using egg-counting methods. Indeed availability of invertebrate prey in this area is known to fluctuate based on rainfall (Nichols et al., 2012a) and, if acting as intermediate hosts, variation in parasite loads generally lag a month behind these fluctuations (Turner et al., 2012). For example a coleopteran beetle is the intermediate host of the meerkat tapeworm (*Pseudandrya suricattae*), beetle numbers fluctuate seasonally and the abundance of tapeworm ova within meerkat faeces increases with rainfall (Leclaire and Faulkner, 2014). However, the banded mongoose tapeworm appears to belong to the genus *Dipylidium* and is likely transmitted by a flea or louse intermediate (Urquhart et al., 1996). These ectoparasites are observed on individuals during routine captures (Jordan et al., 2010), yet there is no obvious seasonality in abundance patters (personal observation). *Toxocara* and the other parasitic taxa identified within banded mongoose faeces are directly transmitted to carnivore hosts. Their reliance on rainfall is therefore limited, beyond it providing stable conditions for sustaining larvae external to the host.

Certain banded mongoose packs have access to human refuse sites which may stabilise their exposure to parasites, regardless of rainfall, due to a consistent supply of moist food items. Larval parasites should thrive in these areas, increasing exposure risks at least to the parasites with direct route of transmission. Between-group variation should thus be important in determining parasite loads. Indeed, packs 2 and 11, which have regular access to refuse sites, do show a higher prevalence of Tapeworms, *Toxocara* (pack 11) and *Isospora* (pack 2) suggesting social factors (i.e. foraging location or interaction frequency) may have a greater bearing on ova burdens than seasonality or weather. Pack 11 also showed significantly higher PTR which is again likely explained by habitat variability and the fact their range overlaps with many other

groups. During the two-year study period, pack 11 patrolled a relatively large area of varied territory including human settlements, refuse sites and "wilder" areas deeper into the national park (Chapter 2, Figure 1). Diverse habitats promote parasitism (Liu et al., 2016) providing a plausible explanation for pack 11's higher PTR. Additionally, their territory regularly overlapped with packs 1B, 2, 1H and 17, whilst the other packs overlapped just one or two territories. This creates a higher potential for inter-group encounters (IGIs) and thus exposure to multiple parasites. IGIs between banded mongoose packs are almost always violent and, where the cause of death is known, IGIs account for 12% of adult and 20% of pup deaths (Cant et al., 2002; Cant et al., 2013; Nichols et al., 2015). Thus if IGIs occur more commonly for pack 11 individuals they may not only risk increased parasitic transmission through direct contact with other groups, but be compromised by physical injuries, rendering them more susceptible to parasitic infection. The imminent threat of violence may also elevate pack 11's parasite burdens compared to other groups, considering that social stressors are known to decrease immune function (Altizer et al., 2003). Finally regardless of the frequency of IGIs, bordering other territories increases the risk of coming into contact with other packs latrine sites (Jordan et al., 2010). Banded mongooses will readily investigate foreign latrines and often over-mark these scents (Jordan et al., 2010; Müller and Manser, 2007) which would represent an ideal opportunity for contracting directly transmitted parasites such as *Isospora, Toxocara* and hookworms.

Despite pack 11's parasitic burden appearing to differ from most other groups, parasite communities across other packs were relatively similar, suggesting individual variation in parasite abundance is greater than pack variation. Indeed, the repeatability of egg counts varied between individuals, meaning factors other than life-history and environmental measures may contribute to ova burdens. A submitted manuscript discussed in Chapter six shows that more homozygous banded mongooses harbour higher overall egg counts (Mitchell et al., 2016 in press). Thus genetic factors, stress and other traits linked to immunity may contribute to individual variation in parasite burdens. Nevertheless, considering their ecology, prevalence across the population and variation in abundance patterns, both *Isospora* and *Toxocara* represent key targets for future research into the costs of parasitic infection in the banded mongoose. *Isospora* prevalence was high: 74% of the population harbours this parasite, which implies tolerance rather than resistance may be at play. Coccidians are generally benign in healthy, mature hosts (Urquhart et al., 1996), but *Isospora* may have subtler implications for health, behaviour and ultimately fitness if it occurs alongside more aggressive parasites or in immunologically compromised hosts. Indeed, high ranking individuals showed higher oocyte burdens which may be linked to the stresses associated with maintaining a dominant breeding position. *Toxocara* also showed variation attributable to life-history, social and ecological factors. Prevalence was higher within individuals of pack 11 than any other social group, heavier hosts showed lower *Toxocara* prevalence and, when infected, had lower *to Toxocara* ova counts than lighter conspecifics. As such, *Toxocara* may have more immediate effects upon fitness, particularly for lighter individuals who appear more susceptible to infection, and have higher ova loads. Chronic infections are known to compromise body condition but the latency of larval stages, which can remain dormant in muscle and other tissue, also means *Toxocara* infections may be difficult to detect via egg-counts alone (Urquhart et al., 1996). Thus both parasites appear key targets to investigate in relation to host-parasite dynamics and fitness implications in future research.

Chapter 6 Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose.

A modified version of this chapter is now published in the Journal of Zoology: Mitchell, J and Vitikainen, EIK and Wells, DA and Cant, MA and Nichols, HJ (2016) *Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose.* Journal of Zoology. ISSN 0952-8369

Abstract

Inbreeding, reproduction between relatives, often impinges on the health and survival of resulting offspring. Such inbreeding depression may manifest itself through immunological costs as inbred individuals suffer increased propensity to disease, infection and parasites compared to outbred conspecifics. Here I assess how the intestinal parasite loads of wild banded mongooses vary with pedigree inbreeding coefficient (ƒ) and standardised multi-locus heterozygosity (sMLH). I find a significant association between increased heterozygosity and lower parasite loads; however, this correlation does not stand when considering ƒ. Such findings may be explained by local genetic effects; linkage between genetic markers and genes influencing parasite burdens. Indeed, I find heterozygosity at certain loci to correlate with parasite load. Although these tentative local effects are lost following multiple-test-correction, they warrant future investigation to determine their strength and impact. I also suggest frequent inbreeding within banded mongooses may mean heterozygosity is a better predictor of inbreeding than pedigree ƒ. This is because inbreeding facilitates linkage disequilibrium, increasing the chances of neutral markers representing genome-wide heterozygosity. Finally, neither ƒ nor heterozygosity had a significant influence on the loads of two specific gastrointestinal parasites. Nevertheless, more heterozygous individuals benefited from reduced overall parasitic infection and genetic diversity appears to explain some variation in parasite burdens in the banded mongoose.

Introduction

Breeding between close relatives often entails a fitness cost termed inbreeding depression, which is thought to result mainly from the unmasking of harmful recessive alleles (Charlesworth and Charlesworth, 1987; Charlesworth and Willis, 2009). The detrimental effects of inbreeding have been documented both in captive (Jimenez et al., 1994; Meagher et al., 2000) and wild vertebrates, where they can lead to a substantial reduction in offspring fitness (Coltman et al., 2001; Ilmonen et al., 2008; Reid et al., 2003; Whiteman et al., 2006).

One mechanism through which inbreeding depression may act is by negatively impacting on immune function. Inbred individuals have been shown to suffer immune-suppression and increased susceptibility to pathogens and disease (Charpentier, 2008b; Coltman et al., 1999; Coltman et al., 2001; Reid et al., 2003). As the immune response to parasitic infection is under genetic control, increased diversity across the genome may correlate with immunity active against a greater diversity of parasites (Reid et al., 2003; Whiteman et al., 2006). Indeed, populations with reduced genetic diversity are more susceptible to disease and parasitism (De Castro and Bolker, 2004). Parasite loads also appear higher in inbred or homozygous individuals compared to outbred conspecifics (Acevedo-Whitehouse et al., 2003; Cassinello et al., 2001; Charpentier, 2008b; Coltman et al., 1999; Coltman et al., 2001). Thus within and between populations, genetic diversity and heterozygosity appear correlated with parasitic infection.

Inbreeding is a growing threat to wildlife, owing to human induced habitat change (Frankham, 2010), and may increase susceptibility to disease outbreak and parasites in small or fragmented populations (De Castro and Bolker, 2004). Understanding how these factors may interact is crucial, yet our knowledge on the relationship between parasites and inbreeding in wild systems is limited. This may reflect well-known problems of using marker heterozygosity to estimate fitness effects of inbreeding, instead of pedigree inbreeding coefficients (*f*). Pedigrees contain ancestral genetic information and have the power to detect inbreeding in previous generations. Thus *f* is considered the most robust and accurate estimate of inbreeding unless a large number of genetic markers are used (Pemberton, 2004; Slate et al., 2004). However, few wild systems have pedigree data and inbreeding is instead estimated by heterozygosity at neutral markers such as microsatellites or SNPs. Correlations between marker heterozygosity and fitness-related traits are termed heterozygosity-fitness-correlations (HFCs hereafter) and require heterozygosity at the neutral marker to correlate with heterozygosity across areas of the genome under selection. This assumption has met wide criticism (Hansson and Westerberg, 2002; Miller and Coltman, 2014; Slate et al., 2004) and the field is receiving renewed interest due to the increased availability of genetic markers for wild populations (Chapman et al., 2009). Two hypotheses are commonly considered to explain HFCs; one possibility is that inbreeding causes associations between neutral markers and genome-wide heterozygosity, termed 'general effects'. Alternatively, HFCs may arise because particular markers are in linkage disequilibrium with non-neutral genes, termed 'local effects' (summarised in Hansson and Westerberg (2002)).

Where comparisons of pedigree *f* and molecular estimates of heterozygosity are both available, their correlation tends to be weak leading to suggestions that heterozygosity does not accurately reflect *f* (Balloux et al., 2004; Pemberton, 2004; Slate et al., 2004). Indeed the rationale in favour of *f* is strong when concerning large, randomly breeding populations (Pemberton, 2004). However, many populations deviate from panmixis, creating situations where marker heterozygosity may actually be a better predictor of inbreeding (Forstmeier et al., 2012; Ruiz-Lopez et al., 2012) . In such cases pedigree *f* and heterozygosity will not be strongly correlated but not necessarily due to the weakness of the molecular markers.

The banded mongoose represents an ideal species for which to investigate the relationship between parasite load and genetic diversity. The focal population is wild and free-ranging but having been studied for ~20 years has a full genetic pedigree, life-history and parasitic data. Faecal analyses show banded mongooses harbour multiple parasite taxa known to have fitness implications in other mammals (Table 1, Chapter 5). Variation in parasite burdens is high across the study population, and there is frequent inbreeding leading to variation in inbreeding coefficients across individuals (Nichols et al., 2014). This provides an ideal setting to investigate whether genetic diversity explains variation in parasite load, and to detect correlations between genetic diversity and fitness related traits (Hansson and Westerberg, 2002). I focus on both overall parasite load, and two taxonomically distinct parasite genera, predicting genetically diverse individuals to show reduced parasite burdens compared to their inbred and more homozygous conspecifics.

Methods

Data were gathered from 5 banded mongoose groups between 21st May and 6th August 2014. The relationship between parasite load and both inbreeding coefficient (*f*) and standardized multi locus heterozygosity (sMLH) was tested for 55 individuals over 6 months old. At this age banded mongooses are foraging independently and samples can be collected routinely. Faecal samples were taken during morning foraging hours (7-11am) and individuals were sampled at least three times during the study period resulting in 185 samples overall.

Parasitology methods

Faeces were collected immediately after deposition, analysed by a modified McMaster technique (Dunn and Keymer, 1986) and identified using the veterinary parasitology literature (Bowman, 2014; Urquhart et al., 1996). Ova counts were converted to an eggs per gram (EPG) value using the standard McMaster (section 2.4.2) and epg values were averaged across each individual for the duration of the study period. Such counts were calculated for overall parasite load and two specific parasite taxa; a coccidian within the genus *Isospora* and a cestode (tapeworm) most likely belonging to the genus *Dipylidium*. These were selected as they occurred in over 30% of individual samples, could be reliably identified at genus level and are known to have negative effects upon host fitness, at least in some carnivore species (Chapter 5). It would also have been desirable to test the relationship between genetic diversity and *Toxocara* load considering the findings of chapter 5. However, for this short time-frame and small sub-sample of the population there was not a high degree of variability in *Toxocara* burdens and a lot of zero values were recorded. As such only the *Isospora* and tapeworm ova were considered.

As discussed in chapter 2, faecal egg counts (FEC) often face criticism regarding their accuracy in quantifying parasite load (Gasso et al., 2015; Villanua et al., 2006). Egg shedding can vary with the life stage of the parasite, co-infection, environmental conditions and host physiology (Dorchies et al., 1997; Raharivololona and Ganzhorn, 2010; Villanua et al., 2006). Thus egg numbers in faeces may not directly represent the parasite community within the host. Nevertheless, FECs remain the best available method for estimating parasite burdens in wild systems. Here it is unfeasible to sacrifice individuals to gain comprehensive adult parasite counts from the gastrointestinal tract (Poulin and Morand, 2000). I aimed to reduce noise resulting from variation in parasite shedding by averaging data for all samples collected per individual. Average egg counts should provide a comparable estimate of individual parasite load across individuals for this short study period as climatic conditions remained consistent (warm with negligible rainfall), all groups retained stable territories and were not subject to large-scale predation or other stressful events. Thus average egg count is unlikely to be skewed by stressors such as weather fluctuations, abnormal foraging patterns or territory shifts. Individuals across the population are also likely to have similar exposure to parasites from the environment due to their similar habitats (Cant, 2000) and preference for foraging in faeces of conspecifics and other mammals.

Genetic methods

Inbreeding coefficients (ƒ) were calculated from a nine-generation-deep pedigree of the study population. Pedigree construction used genetic data from 43 microsatellite markers, along with observational data, for full details Sanderson et al. (2015). The final pedigree used both Masterbayes 2.51 (Hadfield et al., 2006) and COLONY 2.0.5.7 (Jones and Wang, 2010) to infer parentage (1570 maternities and 1476 paternities) at a probability of ≥0.8 across a 14 year period (Sanderson et al., 2015). Although no pedigree collected from the wild can be complete due to the presence of founding members and immigrants, our pedigree has very high coverage of the population (of the 61 individuals sampled for parasite analysis, 55 were assigned both parents and grandparents). Previous research has found evidence of inbreeding depression in certain life-history traits, suggesting the pedigree has adequate power to detect relationships between genetic diversity and fitness-related traits.

The effect of genome-wide heterozygosity upon parasite load was also considered as pedigree inbreeding may not always accurately reflect very deep inbreeding (Keller et al., 2011). This becomes particularly pronounced when the history of founding and immigrant members of a population are unknown (Keller et al., 2011), as is the case for the study population. Thus

standardized multi locus heterozygosity (sMLH) was calculated from raw allele frequencies of the microsatellite markers (Sanderson et al., 2015). In order to gain a comparable assessment of heterozygosity for individuals with parasite data, I removed the loci with less than 90% coverage. Thus sMLH was calculated from the 32 best-amplified loci. I also calculated the parameter g2, a measure of heterozygosity-heterozygosity correlation between loci. If an effect of heterozygosity is due to a genome-wide effect of inbreeding, I would expect the level of heterozygosity at different loci to correlate (g2 > 0). If g2 is exactly zero then local effects are a more plausible explanation and it would not be possible to detect genome-wide effects of heterozygosity using this marker set (David et al., 2007; Szulkin et al., 2010). All analyses involving genetic data used the inbreedR package (Stoffel et al., 2015) within the R statistical framework (R development core team, 2013).

Statistical methods

To investigate whether pedigree ƒ or sMLH affected overall average parasite load a generalized linear mixed effects model was constructed in R version 3.0.2 (R development core team, 2013). I used the MASS package (Venables and Ripley, 2002) to fit the model by penalised-quasi likelihood (glmmPQL) with a negative binomial error distribution due to the over-dispersion of parasite data. The response variable, average eggs per gram parasite load was multiplied by 1000 to generate positive integer values required for a binomially distributed model. Both ƒ and sMLH were included as numerical fixed effects to consider their impact upon egg count. Sex (0 = male, 1 = female) and average age across the study period (in days, continuous numerical value) were included as additional fixed effects because in the closely related meerkat, *Suricata suricatta*, parasite burdens are recognised to vary with sex and age (Leclaire and Faulkner, 2014; Smythe and Drea, 2015). The possibility of sex-specific interactions with genetic variability merit consideration and thus interactions were included in the model. Finally, group identity (a factor with five levels) was fitted as a random factor to account for repeated sampling across social groups. This model was run in full then simplified using a backward stepwise process to sequentially remove each non-significant term. To test the effect of sMLH and inbreeding coefficient upon specific parasite taxa (*Isospora* and *Dipylidium*) I used the lme4 package (Bates et al., 2008) to run linear mixed effects models with Gaussian error distributions. Fixed and random effects remained as specified above.

Results

An average faecal sample harboured 320-40 eggs (mean± SE). Although four samples were devoid of eggs, all 55 individuals were infected with at least with one type of parasite during the study period. For this subset of the banded mongoose population, variance in ƒ calculated directly from the pedigree was 0.007 -0.011 (mean ± SE, range = 0 - 0.28), suggesting there is variability in inbreeding coefficients which should allow heterozygosity-fitness correlations to be detected. Although g2 was not significantly different from zero (g2 = 0.008, SE 0.008, P = 0.101), values >0 suggest markers should have enough power to detect genome-wide effects of heterozygosity (Szulkin et al., 2010).

Table 1: The effect of standardised multi-locus heterozygosity (sMLH) upon average parasite load.

For details please see published text: Mitchell, J and Vitikainen, EIK and Wells, DA and Cant, MA and Nichols, HJ (2016) *Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose.* Journal of Zoology. ISSN 0952-8369

Effect of genetic diversity on overall parasite load

Standardized multi-locus heterozygosity (sMLH) was the only factor to have a significant effect upon parasite load. More heterozygous individuals had lower average parasite loads (Table 1 and Figure 1), while there was no effect of sex, age or inbreeding coefficient (ƒ). To confirm the effects of pedigree ƒ were not masked by collinearity with sMLH (correlation between sMLH and ƒ = 0.437 in the full model), the original model was re-run excluding sMLH. Following model simplification, neither ƒ nor any other fixed effects were significant (effect of ƒ from GLMM: Effect size = 1.822, SE = 1.544, P = 0.244).

The relationship between parasite load and sMLH, but not ƒ suggests local genetic effects may influence parasite burdens. I thus ran negative binomial GLMMs considering the effect of heterozygosity at each locus on average parasite load (Table 2). Separate models were run for each locus with locus heterozygosity (coded 0 or 1) as their fixed effect and pack as a random factor. As before, models were fitted by glmmPQL. P-values were collated for the effect of heterozygosity at each loci and corrected by the Bonferroni multiple test correction (Abdi, 2007). Initially, six loci showed a significant correlation ($P < 0.05$) with parasite load, however, three were only marginally significant ($P > 0.03$) and none remained significant following the correction step (Mon35 P = 0.004, Bonferroni corrected level of acceptance P = 0.0016). Bonferroni corrections are conservative (Moran, 2003; Narum, 2006) meaning loci showing significant relationship with parasite load may be worthy of further empirical attention. Locus Mon35 showed the strongest effect (Table 2), and thus, I excluded it from sMLH calculations and re-ran the original, minimal model. Removing Mon35 from sMLH calculations rendered the effect of heterozygosity on parasite load non-significant (Table 3), suggesting this locus may impact parasite burden.

Figure 1:

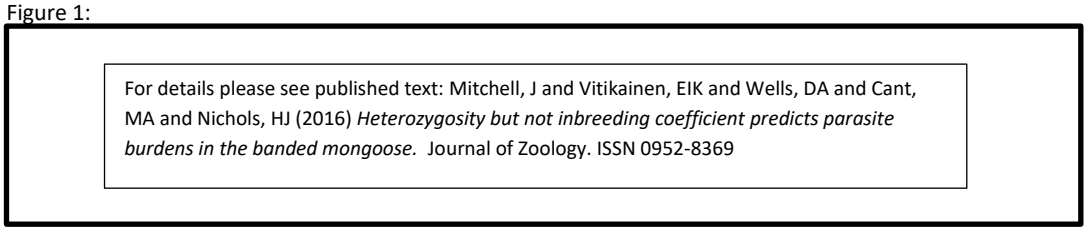
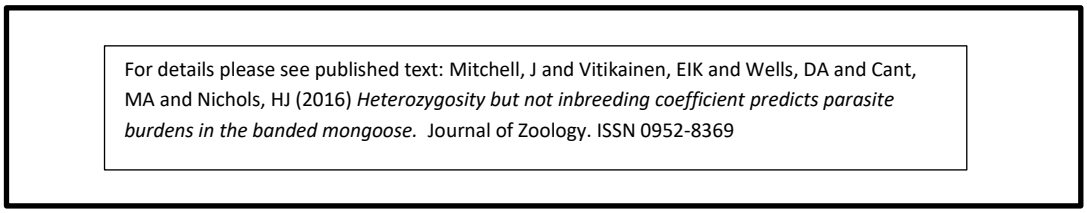


Table 2: Relationship between single-locus heterozygosity and parasite load prior to Bonferroni corrections.



Effect of genetic diversity upon specified parasite taxa

sMLH had no effect on *Isospora* (LMM: Effects size -1.617, SD = 0.932, t-value -1.734, P-value 0.089) or tapeworm loads (LMM: Effect size = -13.51, SD = 14.91, t-value = -0.906, P-values = 0.369). All other fixed effects (age, sex and ƒ) remained non-significant. For full model output see Appendix B, Tables 7 and 8.

Table 3: Output of GLMM testing the effect of sMLH (minus Mon35) upon parasite load.

For details please see published text: Mitchell, J and Vitikainen, EIK and Wells, DA and Cant, MA and Nichols, HJ (2016) *Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose.* Journal of Zoology. ISSN 0952-8369

Discussion

In the banded mongoose, heterozygous individuals (as measured by sMLH) showed significantly lower overall parasite loads than more homozygous conspecifics. Although only marginally significant, this implies genetic diversity may explain some variation in overall parasite burdens across this population. It also suggests an heterozygosity-fitness correlation (HFC) and supports studies in other animals where high pathogen loads correlate with reduced genetic diversity (Coltman et al., 1999; Ilmonen et al., 2008; Luong et al., 2007). There was however, no effect of heterozygosity upon the average load of *Isospora* or tapeworm ova however, measures of individual parasites may be subject to error due to the limitations of FEC discussed previously. In all cases, pedigree inbreeding coefficient (ƒ) failed to explain parasite burdens.

Why heterozygosity but not ƒ correlates with parasite load in the banded mongoose is puzzling. Considering the current pedigree's depth and detail I would predict ƒ to accurately reflect genome-wide heterozygosity. One caveat may be assumptions made during initial pedigree construction. Generally, founding members of a population are assumed unrelated and outbred, yet considering their demography this is unlikely the case for banded mongooses. Groups form via budding dispersal where same-sex coalitions leave (or are forcibly evicted from) natal groups and seek out opposite sex individuals (Nichols et al., 2012b). Such coalitions will often be relatives from the same pack where inbreeding may have been a common occurrence (Nichols et al., 2014; Nichols et al., 2012b). Thus, founding members were likely inbred and related which may have led to biased assessments of ancestral inbreeding. However, I only selected individuals for which all four grandparents were present within the pedigree. This should have successfully removed bias in recent ƒ estimates resulting from assumptions during pedigree construction. Additionally, Keller et al. (2011) showed theoretically that only a small proportion of the variation in ƒ is missed due to ignorance of ancestral inbreeding > five generations ago.

It has been suggested that heterozygosity better reflects inbreeding than pedigree ƒ in certain systems (Ruiz-Lopez et al., 2012). In zebra finches, *Taeniopygia guttata,* just 11 microsatellites generated stronger correlations with phenotypes than did ƒ. Authors attribute this to the high allelic diversity of their microsatellites and that much of the Zebra finch genome is inherited in large blocks which rarely experience cross-over during meiosis (Forstmeier et al., 2012). Data is not currently available regarding segregation and cross-overs in the banded mongoose genome, yet this may provide a feasible explanation as to why heterozygosity provides a better estimate of inbreeding depression. A sequenced genome would also be useful to consider the location of microsatellites, as laying within gene-rich regions would substantially increase their chances of linkage disequilibrium with fitness loci (Hansson and Westerberg, 2002). High rates of linkage disequilibrium can make heterozygosity a better predictor of inbreeding depression than ƒ values (Ruiz-Lopez et al., 2012) and inbreeding is one mechanism recognised to increase levels of linkage disequilibrium (Hansson and Westerberg, 2002; Miller and Coltman, 2014; Slate et al., 2004). Banded mongoose appear to tolerate substantial levels of inbreeding (Nichols et al., 2014), suggesting linkage disequilibrium remains high across the population and heterozygosity may indeed better reflect inbreeding than pedigree ƒ. However,

the current pedigree has successfully identified inbreeding depression within juvenile life-history traits (Sanderson et al., 2015) supporting its power to uncover inbreeding and inbreeding depression.

Alternatively, heterozygosity may reflect local genetic effects rather than a genome-wide association related to inbreeding. Although several loci showed significant correlations with heterozygosity, Bonferroni corrections rendered all non-significant (although one locus only marginally so). This may have occurred because local effects are small and difficult to detect (Hansson and Westerberg, 2002). Although other tests are available to detect the presence of local effects (Szulkin et al., 2010) these lose power once the number of individuals approaches the number of loci in the dataset. This would have been the case for our subset of the banded mongoose population which includes 55 individuals and 32 microsatellite loci. Nevertheless, once parasitic data is available for a larger sample, Szulkin's test could be employed for future consideration of local genetic effects. Secondly, Bonferroni corrections are conservative (Moran, 2003; Narum, 2006) and may dismiss local effects because of their small impact. Indeed, removing the most significant loci (Mon35) from sMLH calculations removed the effect of overall heterozygosity. Mon35 may thus be linked to an important immune-related gene that impacts parasite burden and future research should consider its position within the genome. Alternatively, prior to Bonferroni correction, heterozygosity at loci Mon9 and 41 appears to correlate with increased parasite loads. This opposite effect requires further empirical attention, but competition between multiple parasitic infections can protect hosts from exploitation by single, costly parasites. Thus, heterozygotes with multiple parasites may have a fitness advantage over more homozygous hosts with fewer pathogens.

To summarize, genetic diversity appears to impact overall parasite variation across this banded mongoose population. Heterozygosity correlated with lower overall parasite loads; however, the effect was contingent on one microsatellite marker and ƒ did not show a similar relationship. This implies local effects are at play. Yet, it is possible that frequent inbreeding within this population means linkage disequilibrium is high, leading sMLH to better predict genome-wide heterozygosity than ƒ. To fully understand the relationship between inbreeding and parasite burdens will require further research on a larger subset of the population, ideally using genomic techniques.

Chapter 7 Scent marking advertises parasitic infection status in the banded mongoose.

A modified version of this chapter is now in press at the Journal of Current Zoology.

Abstract

Preference for uninfected mates is presumed beneficial as it minimises one's risk of contracting an infection and infecting one's offspring. In avian systems visual ornaments are often used to indicate parasite burdens and facilitate mate-choice. However, in mammals, olfactory cues have been proposed to act as a mechanism allowing potential mates to be discriminated by infection status. The effect of infection upon mammalian mate-choice is mainly studied in laboratory rodents where experimental trials support preference for the odours of uninfected mates and some data suggest scent-marking is reduced in individuals with high infection burdens. Nevertheless, whether such effects occur in non-model and wild systems remains poorly understood. This is often due to data limitations regarding measures of infection and the potential mechanism for communicating it. However, for the focal population of banded mongooses, *Mungus mungo*, I have both gastro-intestinal parasite data and knowledge of frequent scent-marking behaviour which may act as a mechanism for communicating infection. Here I investigate the interplay between parasite load and scent marking behaviour, focusing on a costly protozoan parasite of the genus *Isospora* and the nematode worm *Toxocara.* I first show that banded mongooses that engage in frequent, intensive scent-marking have lower *Isospora* loads, suggesting marking behaviour itself may be considered an indicator trait regarding infection. I then used odour presentations to demonstrate that both male and female banded mongooses mark less in response to odours of opposite-sexed individuals with high *Isospora* and *Toxocara* loads. As both of these parasites are known to have detrimental effects upon the health of pre-weaned young they would appear key targets to avoid during mate-choice in order to safe-guard offspring fitness. Indeed, parasite detection may be key to mediating mate-choice, which occurs within both sexes of banded mongoose yet neither show obvious visual ornamentation. Results thus provide support for scent to act as an important ornament and mechanism for advertising parasitic infection within wild mammals.

Introduction

One of the major costs of animal social behaviour is the risk of contracting parasitic infections. A variety of behavioural mechanisms have evolved to minimise parasite exposure and to avoid infection (Kavaliers et al., 2005b) and parasites are now considered to play major roles in social organisation including breeding dynamics. Here parasitic infection can be an important influence on sexual selection as infectious pathogens have the potential to affect not only host growth, survival and health (Coltman et al., 1999) but also behaviour (Klein, 2003; Poulin, 1994; Poulin, 1995a). This may in turn affect a hosts' ability to locate, attract and or copulate with potential mates. Therefore, mechanisms to detect and avoid highly parasitized mates are assumed advantageous across species.

In avian systems there is a wealth of research into the ability of bright and conspicuous plumage to advertise health and fitness (Hale et al., 2009; Hamilton and Zuk, 1982; Petrie, 1994). Female-choice based upon such traits select for fitter mates including those of low parasite burden (Buchholz, 2004; Moreno-Rueda and Hoi, 2011; Roulin et al., 2001a). This may benefit females directly by reducing her likelihood of contracting costly parasitic infections (Hamilton and Zuk, 1982; Kavaliers et al., 2005a) but also indirectly by providing offspring with

genes for parasite resistance if this trait is heritable (Boulinier et al., 1997; Moller, 1990). There is also evidence that males prefer visually ornamented females, such as in the barn owl, *Tyto alba*, where spotty plumage indicates lower parasite loads (Roulin et al., 2001b). Mammals tend not to possess such elaborate visual ornaments, although there are exceptions such as secondary sexual colouration of primates which are believed to function in mate-choice (Waitt et al., 2003). However, when tested in mandrills, *Mandrillus sphinx*, neither facial colouration nor rump swellings appeared related to parasitism despite being sexually selected traits (Setchell et al., 2009; Setchell et al., 2006; Setchell et al., 2011). Nevertheless, mate-choice based upon infection status does still occur. Laboratory rodents are consistently observed to avoid mating with infected individuals when concerning parasitic nematodes, viruses and other micro-organisms (Kavaliers et al., 2005a; Penn and Potts, 1998b; Zala, 2004).

In rodents it is olfactory signals that appear to allow mate discrimination on the basis of infection (Arakawa et al., 2011; Ehman and Scott, 2003; Gosling and Roberts, 2001; Kavaliers et al., 2005a; Penn and Potts, 1998a, b). This is unsurprising considering the predominant role of odour signals within mammalian communication (Kavaliers et al., 2005b; Wyatt, 2014). In one specific example Zala (2004) showed that male lab mice infected with *Salmonella enterica* bacteria have reduced marking rates and their scent appears less attractive to females. This suggests that scent-marking behaviour may act as an indicator of health parameters whilst the scent signal also encodes infection status. Indeed, Hamilton and Zuk (1982) initially suggested that animals should benefit from inspecting the odours of a potential mate as an additional way of gauging condition. Even Darwin noted the ability of scent to function within mate-choice stating that elaborate odour glands may function in sexual selection "if the most odiferous males are the most successful in winning the females, and in leaving offspring to inherit their gradually perfected glands and odours" (Darwin, 1871). However, this area received little empirical attention until the past few decades (*as reviewed by Kavaliers et al. (2005b)).* Unfortunately, caveats of previous research include an almost exclusive focus on laboratory rodents with very little consideration of wild systems. Odour presentations also tend to be choice-tests in experimental arenas that may not accurately reflect scent-marking behaviour as it would occur in the wild (Hurst et al., 1994). This makes it difficult to extrapolate findings to natural situations as discussed previously (Chapter 1). Additionally, some frequently cited examples of parasitic avoidance focus on bacteria (Zala, 2004; Zala et al., 2015) or viruses (Penn and Potts, 1998a). Although these organisms may constitute parasites in the broad sense, the mechanisms by which they influence scent composition and marking behaviour will likely differ from gastrointestinal parasites (Kavaliers et al., 2005a, b). This is an important discrimination to make because wild mammals, particularly carnivores, are often heavily infected by gastro-intestinal parasites (Pedersen et al., 2007) suggesting these pathogens could have considerable impacts upon social and sexual behaviour including mate-choice (Poulin, 1994).

I aim to overcome these limitations by investigating whether scent communication can encode parasitic information in the banded mongoose. This cooperative breeder provides a novel opportunity for such research as the wild focal population has been routinely sampled for gastrointestinal parasites and is habituated to human presence allowing targeted odour presentations to be conducted without disturbing natural behaviour. Despite both sexes exhibiting mate-choice (Cant et al., 2013; Nichols et al., 2010), banded mongooses are sexually monomorphic, lack visual ornaments and thus appear limited in terms of cues advertising quality. However both sexes do participate in extensive olfactory marking (Jordan, 2009; Jordan et al., 2010) suggesting odours may encode and/or advertise parasitic infection status. Scent-marking events, such as latrines are common occurrences (Jordan, 2009; Müller and Manser, 2007) and previous research suggests scent is likely utilised for within-group communication (Jordan et al., 2010), particularly intra-sexual competition (Jordan et al., 2011a; Jordan et al.,

2011b; Müller and Manser, 2007; Müller and Manser, 2008). However, it is currently not known whether odour cues may also contain fitness-related-information such as parasitic infection status which could assist in mate-choice.

Here I combine behavioural data with parasitic faecal egg counts (FEC) to assess the ability of odour cues to communicate parasitic infection in the banded mongoose. I specifically focus on two pathogens, a coccidian of the genus *Isospora* and a *Toxocara* nematode species. Although it would have been interesting to consider the other common pathogens (Tapeworm and hookworm), for the time frame of this study (June-August 2014) only 26% of samples contained hookworm ova and 5% Tapeworm ova, hence I was not able to accurately assess their contribution to scent marking behaviour. As such I focused only on *Isospora* and *Toxocara* which are both abundant within the focal mongoose population (*Isospora* present in 100% and Toxocara in 61% of samples collected during this study period and observed respectively in 74% and 34% of samples analysed between 2013 and 2015) and show significant variation with life-history factors (Chapter 5). *Isospora* are spore-forming protozoans of the subclass Coccidia. In other species, their resulting infection (coccidiosis) damages the cells lining the gut wall, leading to diarrhoea and dehydration (Urquhart et al., 1996) which may consequently compromise reproductive success (Hakkarainen et al., 2007; Hill et al., 2005), body condition (Hill et al., 2005), and survival (Alzaga et al., 2007). *Toxocara* are nematode worms which reside in the host's small intestine where they may cause anaemia and malnutrition, however in many host mammals *Toxocara* ova can migrate to other tissues including the lungs, liver and uterus (Urquhart et al., 1996). The latter is particularly problematic for breeding females as ova are able to infect developing foetuses, causing chronic and often fatal infections after birth. Indeed, the most severe effects of both parasites are felt by pre-weaned young (Bowman, 2014; Eustis and Nelson, 1981; Kirkpatrick, 1998; Lindsay et al., 1997; Mundt et al., 2006; Urquhart et al., 1996). Therefore, *Isospora* and *Toxocara* would appear key parasites to avoid during mate-choice in terms of safe-guarding reproductive success and offspring fitness.

To address whether scent-marking behaviour is influenced by parasitic infection in the banded mongoose, I first investigated whether *Isospora* and *Toxocara* FEC predicted scent marking behaviour during natural marking bouts. I then experimentally addressed whether the parasites can be detected via scent by presenting individuals with odours from differentially parasitized opposite-sex group-members. I predict that;

- If parasite load impacts marking behaviour, more heavily infected individuals should engage in fewer social marking bouts and deposit fewer scent marks.
- If parasite burdens are detectable via scent, behavioural aversions should occur in response to the odours of highly parasitized individuals.

Methods

Parasite analysis

Parasitic ova were extracted, identified and counted from faecal samples as detailed in section 2.4. Using a modified MacMaster technique (Dunn and Keymer, 1986), egg-per-gram (epg) counts of *Isospora* oocytes and *Toxocara* ova were obtained for each sample. As discussed previously (see Chapter 5) FEC face criticism as a measure of parasite load due to high variability within individuals sampled (Gasso et al., 2015; Villanua et al., 2006). However the study population could not be dissected for comprehensive adult parasite counts of the gastrointestinal tract (Poulin and Morand, 2000). Instead, to minimise the effect of within-individual variability, 3-6 faecal samples were collected per individual mongoose allowing mean

epg figures to be calculated for both focal parasites. This should provide a comparable estimate of parasite load across individuals for this short-term period (May-August 2014).

Is scent-marking affected by parasite burdens in the banded mongoose?

To investigate whether scent-marking behaviour is affected by parasitic infection I conducted focal observations by filming social marking events within two geographically separated packs (1B and 1H). Marking bouts were filmed as described in section 2.3.3 during two morning foraging sessions per week between 28th May and 31st July 2014. Three key measures of marking behaviour were selected for this analysis (section 2.3.3): (1) The frequency of marking bouts where an individual was present at the marking site but did not deposit a scent mark, (2) the frequency of marking bouts where an individual was actively sniffing other scent marks, and (3) the frequency of marking bouts where an individual deposited five or more scent marks (intensive scent marking). Parasites are known to have variable effects upon host behaviour (Poulin, 1994) and thus the two former parameters (presence and activity at marking bouts) were included to evaluate whether parasitic infection influences general behavioural patterns such as presence at social marking bouts which are regularly attended by all group members. The latter measure was selected as in preliminary observations less than half (31%) of the population were recorded to deposit > 5 marks at more than three different marking bouts. Thus intensive scent marking appears restricted to a sub-set of the population and is likely a sensitive measure of individual variation in terms of behaviour.

The final dataset comprised 102 marking focals in which 40 individuals aged >6 months were present in the morning group composition check (although on some occasions individuals remained at, or returned to, the den to babysit). Female banded mongooses become sexually mature around 7-8 months, first giving birth as early as 9 months old. Yet males have poor reproductive success until around two years of age due to competitive exclusion by older individuals (Nichols et al., 2010), however young males do show interest in oestrus females earlier. We therefore excluded all individuals under 6 months from the analysis as they are unlikely to be using scent to assess potential mates. To accompany behavioural data, weekly faecal parasite samples for each of the 40 individuals were collected during the study period (May – July 2014). Once analysed these samples yielded a mean epg count of I*sospora* oocytes and *Toxocara* ova for each individual within the two focal packs. Note that parasite loads were unknown at the time of video scoring, so all marking data was collected blind to the infection status of individuals.

To test the effect of mean I*sospora* load upon the frequency of marking bouts where an individual was either present, active, or intensely marking, General Linear models (LMs) were constructed in R (version 3.0.2) using the package lme4 (Bates et al., 2008). In all models, sex and pack were included as fixed effects to account for their effects on marking behaviour. As I*sospora* burdens and known to vary between packs and with sex, second order interactions were also included between these factors. Models were fitted in full with a maximum likelihood convergence criteria and Gaussian error distribution then simplified using the step-wise method of sequentially removing each non-significant term (P>0.05). The *Toxocara* data did not conform to normal distributions and thus had to be analysed separately. FECs were multiplied by one thousand and analysed by a model fit by penalised quasi-likelihood (glmPQL) with a negative binomial error distribution, built within the MASS package (Venables and Ripley, 2002) of R version 3.0.2. Again separate models tested the effect of *Toxocara* load upon the three measures of marking behaviour. Alongside *Toxocara* load, sex and pack were fitted as fixed effects and all second order interactions were included in initial models. The backward step-wise simplification method was again used to generate minimal models.

Can parasitic infection be detected via odour cue?

Between 1st June and 2nd August 2014 odour presentations were conducted in the field to test whether banded mongooses discriminate the scent of opposite-sex group-members based upon *Isospora* or *Toxocara* infection. Anal gland secretion samples (AGS) were collected between May 29th and July 31st 2014, following the methods outlined in 2.5.2 and presented to familiar recipients (group members) following the presentation protocol outlined in section 2.6.1. Only familiar odours were used as previous research suggests the discrimination of parasitised odours has a learned component (Choleris et al., 2012; Roberts et al., 2014; Wyatt, 2014). It was thus important to provide odours which recipients had prior experience of.

To assess infection status, faecal samples (3-6 per individual) were collected for each odour donor within a seven-day window either side of odour sample collection. Mean epg *Isospora* and *Toxocara* loads were then calculated for each odour-donor. Parasite loads were assessed upon return from the field and thus were unknown during field-work, which removed the risk of observer and expectation biases. To address whether olfactory cues may encode information pertaining to parasitic infection, three measures of response to odour presentations were considered. As in previous chapters "Duration" represented the time before mongooses returned to normal behaviour (see 2.6.1 for details of normal behaviour), "Contact" referred to the duration a mongoose remained in physical contact with the tile on which the odour was presented. "Vicinity marks" refer to scent marks deposited within 30cm around the odour, but not directly on top (See Chapter 2, Table 2 for full details of response measures). Marking response was categorised this way as previous research suggests that when odours are utilised for mate-choice and self-advertisement, scent marks are placed adjacent to, rather than directly over the original marks (Wolff et al., 2002).

Due to the distribution of average *Isospora* and *Toxocara* load, their effects upon marking behaviour were analysed within models fit by penalised-quasi likelihood (glmPQL) with a negative binomial error distribution built in the MASS package of R version 3.0.2 (Venables and Ripley, 2002). In separate models the epg load of each parasite was multiplied by one thousand (to create full, positive integer values) and fitted as an explanatory variable alongside the age-rank of odour-donors as previous work shows *Isospora* and T*oxocara* loads can vary with both age and rank (Chapter 5). However, in this dataset, odour age and age rank are highly correlated so could not be included in the same model (LMM: t = -14.75, p = 2e-16, Table 1). After running models containing either age or age rank it was decided to use only age rank. This measure produces more powerful models and accounts for age in days as well as social rank within the group, thus providing a well-regarding proxy for dominance status.

Table 1: the correlation between odour-donor's age and age rank for the presentation dataset

	Estimate	Standard Error	t value	p value
intercept	3085.57	114.11	27.04	<2e-16 ***
Correlation between age and rank	-499.04	33.84	-14.75	<2e-16 ***

Results the correlation between age and rank of 21 individual odour donors who provide 53 samples for use in 85 odour presentation trails between June and August 2015. Age and age rank are highly correlated thus the two factors cannot be considered to explain marking behaviour within the same model.

Finally, because relatedness is recognised to influence scent-marking responses toward familiar odours (Chapter 3), pairwise relatedness (calculated as per section 2.8.1) was fitted as a final fixed effect in all models. The identity of the odour donor was included as a random factor

as certain animals yielded larger AGS samples which could be split and used in multiple presentations. Specifics of the recipient did not require inclusion as all were sexually mature adults of opposite sex and familiar to their odour-donors. Initial models included all second order interactions however non-significant terms were removed using the backward step-wise method of model-simplification.

Results

Group marking events occurred on average every 17 minutes in the first two hours of foraging. In 49% of these bouts, every group member over six months of age was present at the marking site and either sniffing or actively scent marking. Previous research has found that sub-adults are more likely to investigate scent marks than adults (Müller and Manser, 2008), but the authors attribute this to a lack of experience rather than an assessment of mating opportunities. During this study only five individuals were classified as sub-adult (between 6-12 months of age) and there was no significant difference in the mean number of marking bouts where they were present (2-sample t-test, t = 1.071, df = 4.67, p = 0.336, Figure 1) or sniffing only (2-sample t-test: t=0.672, df=5.067, p=0.530, Figure 1). There was also no significant difference between the mean number of marking bouts where adults and sub-adults were actively marking (2 sampled t-test: t=-0.882, df= 4.384, p =0.423). However, this behaviour appeared more frequent in adults (Figure 1) suggesting there may be a difference between age groups which could not be detected in the current study due to the small sample of sub-adults. As the current study considered only the behaviour of these focal individuals, which did not differ significantly between age classes, all individuals >6 months of age were included in final analyses.

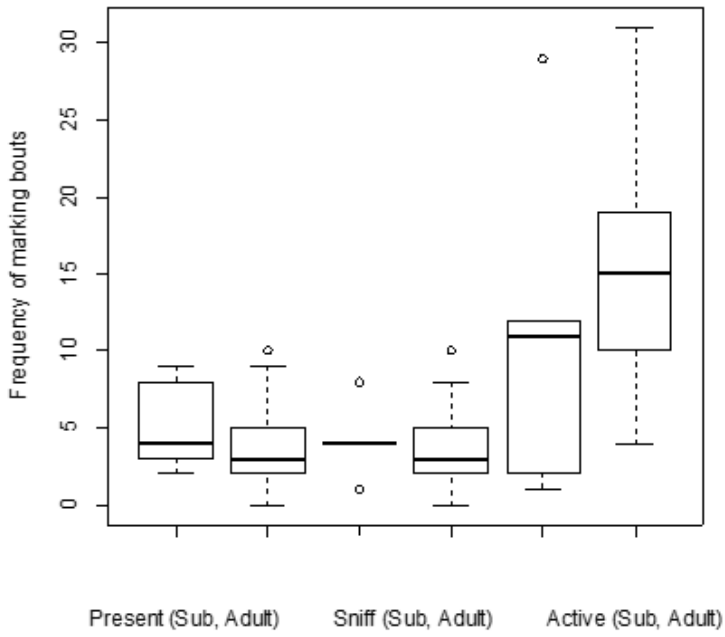


Figure 1: Behavioural differences at scent-marking bouts between adults (>12months) and sub-adults (6-12 months). Results for sub-adults aged 6-12 months are presented first for each category, and then adults aged > 12 months. Error bars represent upper and lower quartile figures and the thick bar represents the means. Sample size for sub-adults during this study period was small (5 sub-adults to 36 adults) but their behaviour does not appear to significantly differ from that of adults >12 months of age.

Is scent-marking affected by parasitic infection in the banded mongoose?

Results do not find a significant relationship between *Isospora* load and presence or activity within marking bouts. However, females were present (but not marking) in significantly more marking bouts than males (LMM: t = 3.335, p = 0.002) whilst males were active in significantly more bouts than females (LMM: t = -3.072, p = 2.0e-4, Table 2). *Isospora* did impact intensive marking behaviour (>5 marks per bout) in interactions with both sex (LMM: t = 2.462, p = 0.019) and pack (LMM: t = 4.203, p = 2.0e-4). In support of our predictions, the frequency of intense marking was significantly higher in individuals of lower *Isospora* load with the exception of female individuals within 1H (Table 2, Figure 2). Regarding *Toxocara* infection, individuals with higher ova burdens were significantly more likely to be present (but not marking) at marking bouts (LMM: t = 2.942, p = 0.003) compared to individuals with low levels of infection. However, although a non-significant trend suggests highly infected individuals are less active in marking bouts, there was no significant difference in marking activity or intense marking relating to *Toxocara* burdens. As before female banded mongooses were present (but not marking) in significantly more bouts than males (LMM: t = 2.895, p = 0.003) whilst males were more likely to be scent marking (LMM: t = -2.912, p = 0.004) and intensively marking (LMM: t= -.3721, p = 2.0e-4) than females.

Table 2: Effect of *Isospora* and *Toxocara* burdens upon marking behaviour at social marking events.

Model testing	Fixed effect	Effect size	Estimate (SD)	t value	p value
Frequency of bouts present	Intercept	2.727	0.481		
	Mongoose sex (female)	**2.323**	**0.697**	**3.335**	**0.002**
	Mongoose pack (1H)			-1.179	0.246
	Isospora load			-0.616	0.542
	Intercept	9.436e-01	1.308e-01		
	Toxocara load	**5.304e-06**	**1.803e-06**	**2.942**	**0.003**
	Mongoose sex (female)	**5.023e-01**	**1.735e-01**	**2.895**	**0.003**
	Mongoose pack (1H)			-0.859	0.390
Frequency of bouts active	Intercept	21.359	1.514		
	Mongoose sex (female)	**-5.464**	**1.779**	**-3.072**	**0.004**
	Mongoose pack (1H)	**-7.172**	**1.779**	**-4.033**	**2.0e-04**
	Isospora load			-0.361	0.720
	Intercept	3.080	0.099		
	Mongoose sex (female)	**-0.361**	**0.124**	**-2.912**	**0.004**
	Mongoose pack (1H)	**-0.473**	**0.123**	**-3.850**	**1.0e-4**
	Toxocara load			-1.879	0.060
Frequency of bouts intensively marking (5+ marks deposited)	Intercept	7.194	0.553		
	***Isospora* load**	**-0.042**	**0.008**	**-5.145**	**1.11e-05**
	Mongoose sex (female)	**-3.042**	**0.717**	**-4.245**	**1.6e-04**
	Mongoose pack (1H)	**-3.980**	**0.694**	**-5.733**	**1.91e-06**
	***Isospora* load: Mongoose sex**	**0.024**	**0.010**	**2.462**	**0.019**
	***Isospora* load:Mongoose pack**	**0.038**	**0.009**	**4.203**	**2.0e-04**
	Intercept	1.727	0.145		
	Mongoose sex (female)	**-0.827**	**0.222**	**-3.721**	**2.0e-04**
	Mongoose pack (1H)	**-0.824**	**0.215**	**-3.833**	**1.27e-04**
	Toxocara load			-1.042	0.298

Results based upon 102 marking bouts within 2 social groups containing 40 individuals over 6 months of age. Full models considered the effect of egg per gram parasite load, sex, pack and all second order interaction between fixed effects. Bold text denotes terms remaining significant within the minimal model. Table details intercept of minimal model and p values upon which fixed effects were sequentially removed from models using the backward step-wise process of model simplification.

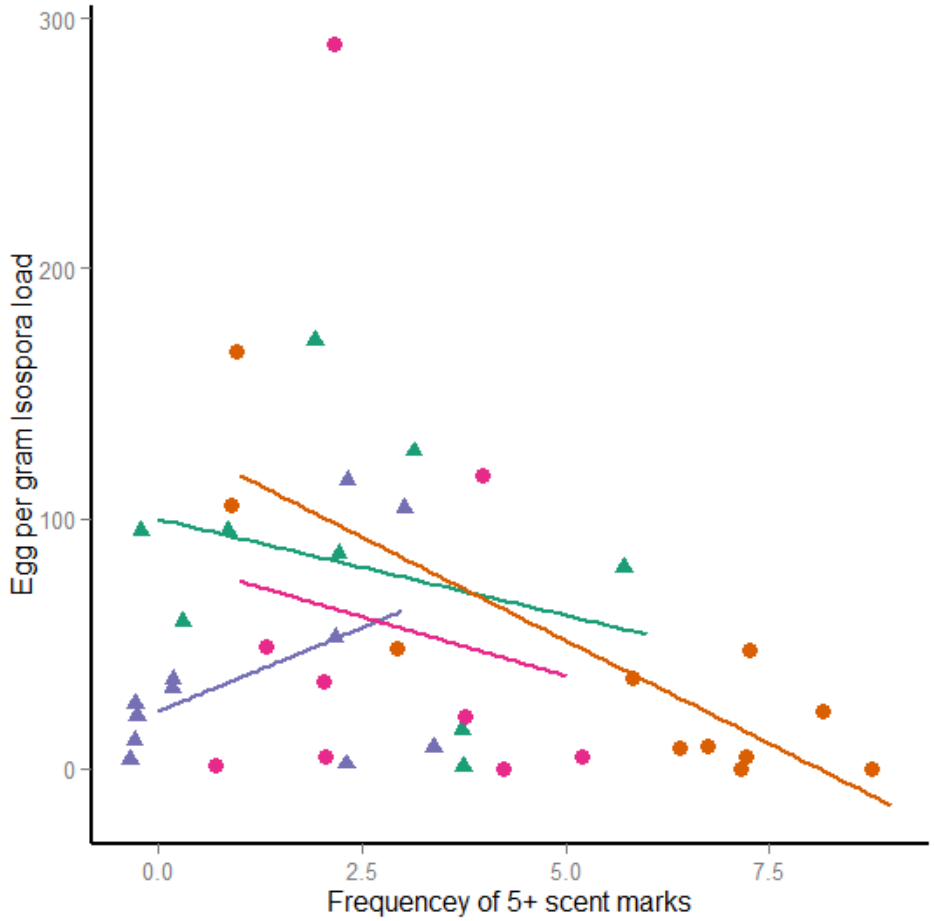


Figure 2: The effect of *Isospora* load upon intensive marking behaviour. Points represent exact data points (circles = male, triangles = female). Lines (green for pack 1B females, orange 1B males, purple for 1H females and pink for 1H males) were calculated by linear regression of *Isospora* load upon the frequency of depositing >5 marks per bout. Results based upon 102 observations of group marking events within two social groups containing 40 individual banded mongooses aged > 6 months. In general, individuals with lower *Isospora* loads engage in intense marking (>5 marks per bout) significantly more frequently than individuals of higher *Isospora* load. The exception to this is females within pack 1H (purple line, triangular points).

Can parasitic infection be detected via odour cue?

Scent marking behaviour in response to opposite-sex odour presentations was impacted by the parasitic burden of the odour-donor. In the *Isospora* dataset trends suggest odours received significantly fewer marks if they came from more heavily infected donors (GLMM: t = -1.924, p = 0.053, Table 2, Figure 3). The sex and *Toxocara* load of odour-donors interacted to influence marking behaviour (GLMM: z = 2.629, p = 0.009, Table 3, Figure 4). Fewer vicinity marks were deposited over male odours (by females) as their *Toxocara* load increased (Figure 4, Black line). This trend was not apparent when considering the response toward female odours (Figure 4, Grey line).

Table 3: Effect of donor *Isospora* and Toxocara burdens upon responses to presented odours.

Model testing	Fixed effects	Effect size	Standard error	Z value	P value
DURATION BEFORE RETURN TO NORMAL BEHAVIOUR	Intercept (*Isospora* model)	2.913	0.146		
	Odour sex (Female)	**0.444**	**0.172**	**2.568**	**0.010**
	Odour *Isospora* count			-0.420	0.674
	Odour age rank (within pack)			0.445	0.657
	Relatedness to odour donor			0.480	0.631
	Intercept (*Toxocara* model)	2.770e+00	2.111e-01		
	Odour sex (female)	**4.895e-01**	**1.885e-01**	**2.598**	**0.009**
	Odour *Toxocara* count			-0.139	0.889
	Odour age rank (within pack)			0.458	0.647
	Relatedness to odour donor			0.478	0.633
CONTACT	Intercept (*Isospora* model)	2.474	0.168		
	Odour sex (female)	**-0.388**	**0.202**	**-1.919**	**0.055**
	Odour *Isospora* count			1.589	0.112
	Odour age rank (within pack)			0.715	0.475
	Relatedness to odour donor			1.220	0.222
	Intercept (*Toxocara* model)	2.107e+00	2.549e-01		
	Odour sex (Female)	-4.066e-01	2.281e-01	-1.783	0.075
	Odour *Toxocara* count			-0.601	0.548
	Odour age rank (within pack)			1.168	0.243
	Relatedness to odour donor			1.031	0.303
TOTAL MARKING	Intercept (*Isospora* model)	2.662e+00	2.390e-01		
	Odour *Isospora* count	**-1.063e-05**	**5.527e-06**	**-1.924**	**0.053**
	Odour sex (female)			-0.684	0.494
	Odour age rank (within pack)	**-2.021e-01**	**6.522e-02**	**-3.099**	**0.002**
	Relatedness to odour donor			-0.173	0.863
	Intercept (*Toxocara* model)	2.041e+00	3.365e-01		
	Odour sex (female)	**1.546e+00**	**5.549e-01**	**2.786**	**0.005**
	Odour *Toxocara* count			-1.619	0.105
	Odour age rank (within pack)			0.103	0.918
	Relatedness to odour donor			-0.242	0.809
	***Toxocara* *Odour sex**	**-7.755e-05**	**2.950e-05**	**-2.629**	**0.009**
	Odour sex * rank	**-4.094e-01**	**1.518e-01**	**-2.697**	**0.007**

Output of GLMMs testing the effect of parasite burden, odour sex, age and age rank upon the response measures of opposite-sexed conspecifics. *Toxocara* results based upon 85 odour presentations to familiar opposite-sex conspecifics. The *Isospora* dataset included 81 presentations as one odour donor, used in four presentations, was excluded from the analysis on the basis of his extremely high *Isospora* burden. All second order interactions were included in original models but if non-significant, they were removed during the backward simplification process. Non-significant fixed effects are presented alongside the p-values upon which they were removed from the models. All intercepts refer to minimal models.

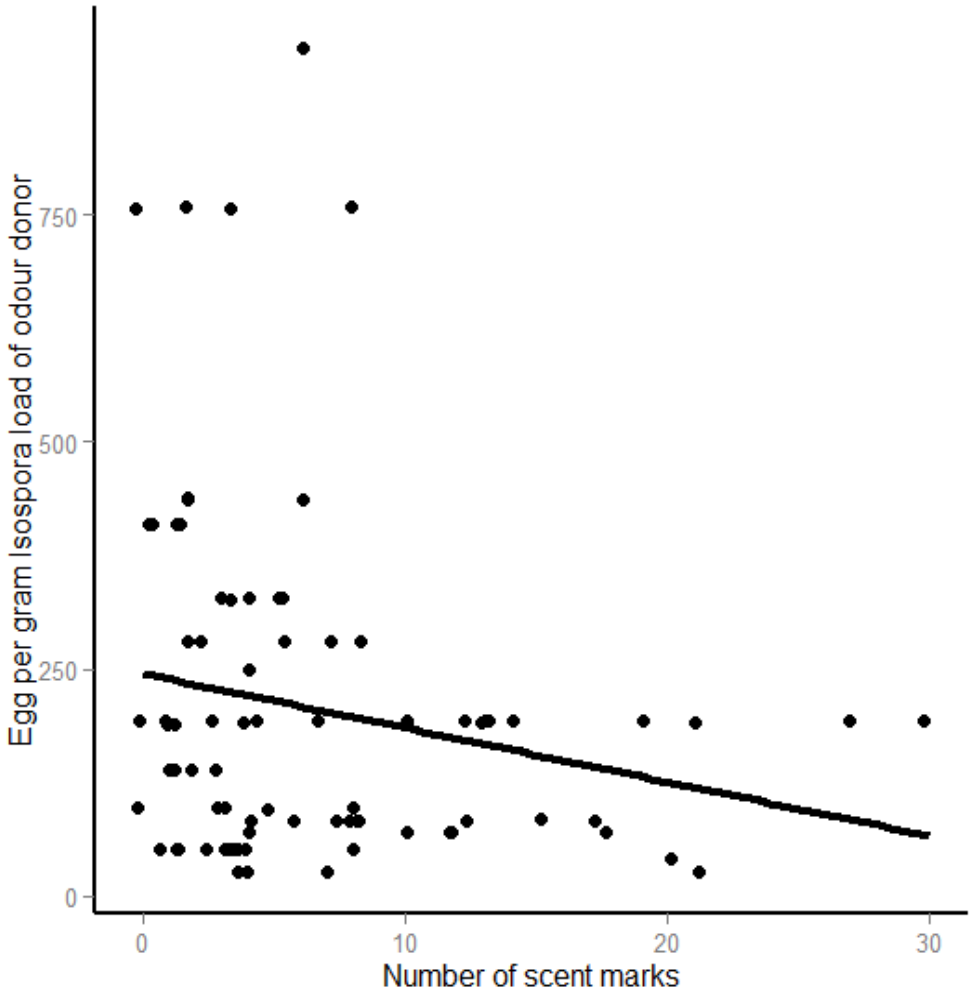


Figure 3: *Isospora* load influences marking behaviour to presented odours. Recipients deposited fewer marks toward opposite sex odours as the *Isospora* load of the odour donor increased. Points represent average egg per gram *Isospora* counts for each odour donor, and lines were fit by linear regression of epg load against scent marking.

In both datasets female odours provoked significantly longer durations of interest (*Isospora* GLMM: t = 2.568, p = 0.010, *Toxocara* GLMM: z = 2.259, p 0.009). However trends show that male odours provoked longer durations of physical contact (*Isospora* GLMM: t = -1.919, p = 0.055, *Toxocara* GLMM: z = -1.783, p 0.075). Odours of top ranked individuals (lower age rank score) provoked significantly more marks in the I*sospora* dataset (GLMM: t = -3.099, p = 0.002) but in the *Toxocara* dataset rank interacted with sex (GLMM: z = 2.697, p = 0.007) such that the odours of more senior ranked females received more marks than those of subordinate females (Figure 5, Grey line). There was no such rank effect regarding the odours of males (Figure 5, Black line).

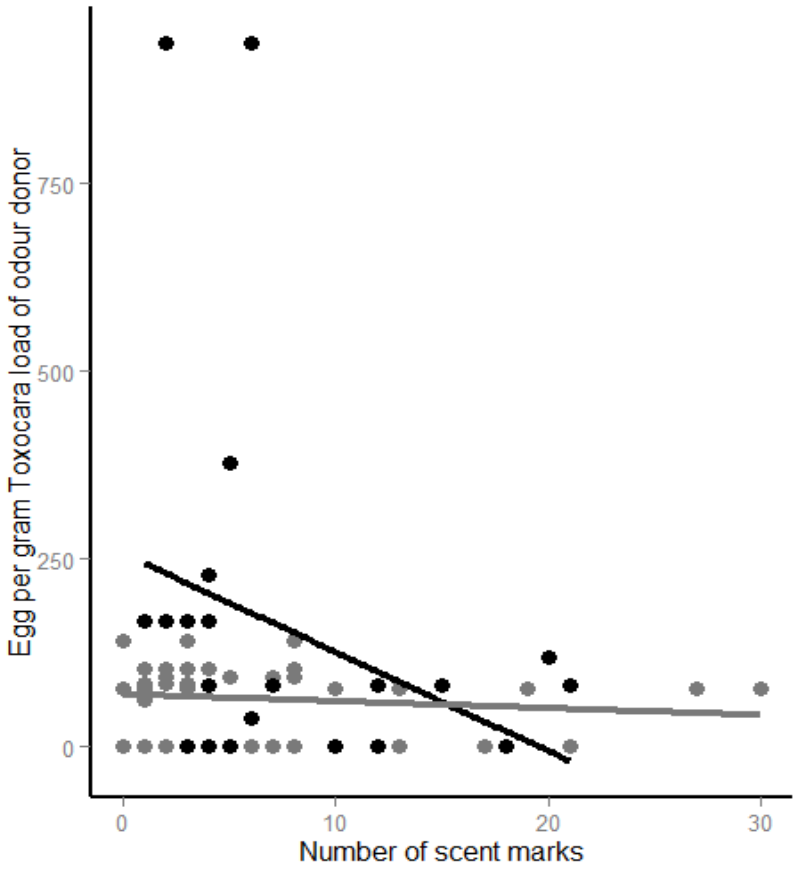


Figure 4: The effect of odour-donor sex and *Toxocara* load upon reactions to opposite-sex presented scents. Recipients deposited more marks toward male odours as the odour's *Toxocara* load decreased (Black points). This trend was not as strong when considering female odours (Grey points). Points represent average egg per gram *Toxocara* counts for each odour donor, lines fit by linear regression of egg load against scent marking to show general trends.

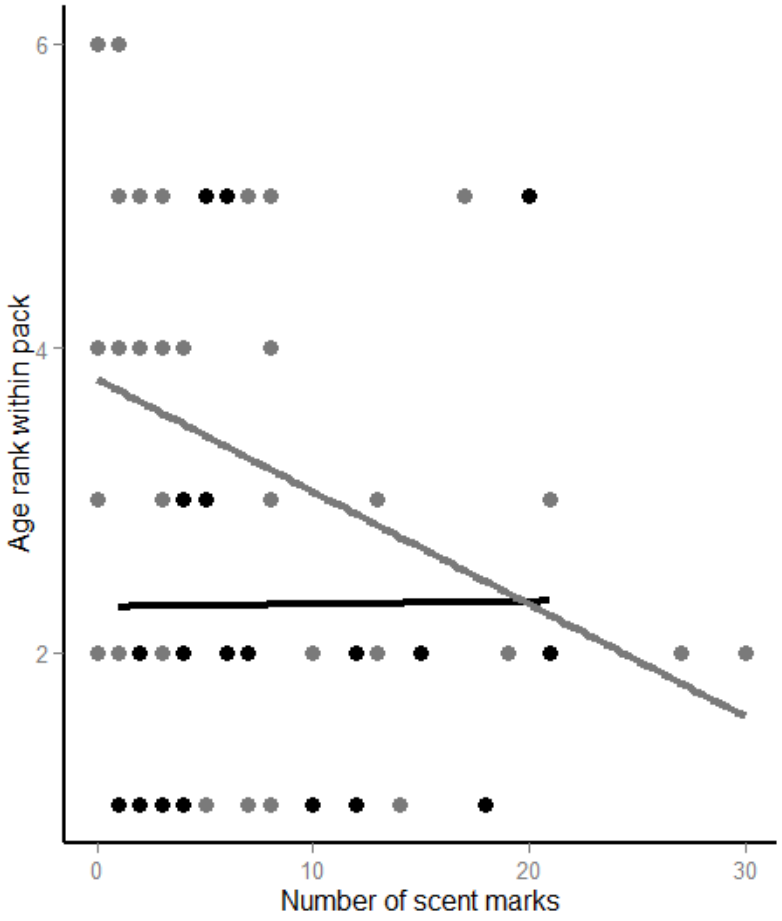


Figure 5: In the *Toxocara* dataset odour sex and rank interacted to influence marking behaviour. As depicted by the grey points and line, female odours received more scent marks as their status in the group increased (lower age rank score). However as per the black line and points there was no such effect of rank upon marking response toward male odours. Points represent the number of scent marks deposited over an odour (grey = female, black = male) with lines fitted by linear regression of age rank against scent marks.

Discussion

Results suggest that in the banded mongoose, scent-marking behaviours are influenced by parasite burden, with highly infected individuals being less likely to mark intensively. In support of initial predictions, banded mongoose odours appear to encode information regarding donor infection status. High infection burdens reduce intensive scent-marking behaviour whilst highly infected odours can be discriminated by opposite-sex conspecifics, suggesting implications for mate-choice. This provides novel evidence of odour-based parasite discrimination in a wild, non-model species.

Banded mongooses that frequently deposited over five scent marks-per-bout (intensive scent-marking) showed significantly lower *Isospora* loads than conspecifics that marked less. This result supports initial predictions and suggests that scent-marking may be considered an indicator trait signalling lower *Isospora* burdens. Olfactory advertisement of quality may function in a similar way to elaborate plumage which often signals parasite resistance in birds (Hale et al., 2009; Petrie, 1994). In house sparrows, females preferentially mate with males who have larger wing-bars. Such males also have larger uropygial glands which are involved in resistance against chewing lice, a common parasite of this species (Moreno-Rueda and Hoi,

2011). Thus choice based on an attractive advert allows females to select better quality mates. Indeed, male banded mongooses who mark most frequently secure more mating opportunities (Jordan, 2009; Jordan et al., 2011a). My results enrich this finding as intense scent-marking appears to act as an indicator trait for reduced *Isospora* infection, thus providing a mechanism by which scent marking can inform mate-choice. However, an exception to this trend were females from pack 1H, stressing the importance of control for social and life-history factors when considering behavioural reactions. Exactly why 1H females showed the opposite trend for more intensive marking as *Isospora* loads increased is unclear. However, these individuals show lower frequencies of intensive marking behaviour compared to other sex-pack cohorts which may also explain their behavioural deviation. Nevertheless, considering the prevailing direction of current results, intensive scent-marking generally appears limited to banded mongooses of low *Isospora* load. This suggests scent-marking may be used to signal low parasitic infection status to potential mates.

In contrast to *Isospora* results, *Toxocara* infection did not appear to influence natural marking behaviour. It may be that this parasite has fewer immediate effects upon host behaviour, indeed *Toxocara* ova can lie dormant in bodily tissues for several years before causing notable health concerns (Urquhart et al., 1996). While *Isospora* load did not affect the frequency with which mongooses attended marking bouts, individuals with higher *Toxocara* burdens were present (but not marking) in significantly more bouts than individuals of lower infection levels, yet activity at marking bouts declined (non-significantly) with increasing *Toxocara* infection. These inconsistent results may occur due to the differing reliability of ova counts to reflect actual parasite burdens. Protozoan parasites, including *Isospora,* shed oocytes consistently and thus ova counts are generally an accurate reflection of infection status. However, the ova of nematode worms may be shed at differing intensities dependant of the life-stage of the parasite and condition of the host (Gasso et al., 2015; Rafalinirina et al., 2015; Villanua et al., 2006). As mentioned *Toxocara* ova can also migrate and mature in other tissues besides the intestine which temporarily decouples ova counts from worm/larvae numbers (Urquhart et al., 1996) meaning ova counts may not be as reliable an indicator of parasitic infection as *Isospora* ooytes. A more controversial explanation would be to suggest that *Toxocara* parasites are able to manipulate host behaviour for their own benefits (Poulin, 1994). Indeed, attending social events such as marking bouts should increase parasite transmission due to contact with multiple individuals. However, under this assumption one would also expect *Toxocara* burdens to be higher in active and intensive scent-markers which is not the case. This does not negate that the two parasites have differing impacts upon marking behaviour, indeed results suggest T*oxocara* impacts upon activity at marking bouts but *Isospora* impacts intensive marking. Once the specific strains of *Isospora* and *Toxocara* have been identified it will be possible to comment in more detail upon the behavioural influence of each parasite. However, at present, the difference in results between parasites appear most likely an artefact of using faecal egg counts as a proxy for parasite load. Unfortunately, this was unavoidable in the current analysis as the research population could not be sacrificed for full worm counts. Nevertheless, the potential for differing impacts of each parasite upon scent-marking is interesting and stresses the need to consider pathogens separately in analyses of how parasite burdens influence behaviour.

The *Isospora* data show that more heavily infected individuals reduce intensive scent-marking behaviour, but still attend marking bouts at the same frequency as less-infected conspecifics. I have suggested that intensive scent-marking functions as an indicator trait advertising low *Isospora* burdens. However, presence or activity at marking bouts are unlikely to function as indicators as in half the observed marking bouts, every group member over six months of age was present at the marking site and either sniffing or actively scent marking.

Indeed, across social mammals including primates and prosimians (Droscher and Kappeler, 2014; Irwin et al., 2004), mustelids (Begg et al., 2003; Clapperton, 1989) and ungulates (Brashares and Arcese, 1999; Gosling, 1987), latrines and social marking events appear to be attended by group members indiscriminately of age, sex or dominance factors. Individual differences only appear when considering the frequency and intensity of specific marking behaviours (Begg et al., 2003; Brashares and Arcese, 1999; Rich and Hurst, 1998, 1999). Considering all group members are able to partake, presence and activity do not appear sensitive enough measures to define individual variation in marking behaviour. When considering intensive scent marking (>5 marks per bout) however, there was much greater individual variability. Although 47% of banded mongooses deposited > 5 scent marks per bout on more than two separate occasions only 31% did so on more than four occasions and only 22% on more than six occasions. This individual variability makes intensive marking an ideal candidate for an indicator trait signalling infection status. However, presence and activity data were still useful measures to consider. In particular, they provide evidence that the natural variation in I*sospora* and *Toxocara* levels do not inhibit social interactions such as attendance at marking bouts.

In support of my second prediction, odour presentation results suggest that banded mongooses are able to discriminate infection status via scent. Recipients significantly reduced vicinity marking around odours of increasing parasite burdens when considering both *Toxocara* and *Isospora* infections. This is an exciting result as, to the best of my knowledge, it provides the first evidence that a wild, non-model mammal can discriminate odours on the basis of infection. For *Isospora*, reduced marking toward highly parasitised odours was evident across the dataset. However, when considering *Toxocara* infection, male odours received significantly fewer vicinity marks as their infection status increased, yet male reactions to female odours did not adhere to this trend. *Toxocara* burdens show greater variability within male donors suggesting female aversion toward higher infection burdens is a biologically relevant response to avoid highly parasitised males. However, the variation in female *Toxocara* counts was not as great suggesting discrimination of female odours on the basis of parasite load may not be behaviourally possible or necessary. Indeed, in most research investigating the ability of scent cues to encode infections, the animals have been experimentally infected (Kavaliers et al., 2005a; Kavaliers et al., 2014; Kavaliers et al., 2003b; Roberts et al., 2014; Zala et al., 2015). This allows greater variation between infected and uninfected individuals meaning any parasite-mediated aversion to their scent cues should be more obvious. However, in wild settings there may not be such variability in infection burdens (Rafalinirina et al., 2015) meaning the discrimination of odours on the basis of infection may not always be a biologically relevant response.

Though the risks of mating an infected individual are likely relevant to both sexes, banded mongooses remain similar to most mammals in that females appear to invest more heavily in offspring (Clutton-Brock, 2007; Clutton-Brock et al., 1981; Clutton-Brock and Iason, 1986; Clutton-Brock and Vincent, 1991; Leimar, 1996). The female costs of pregnancy and lactation suggest that poor mate-choice, and potential offspring loss, is likely to compromise their reproductive success more than that of males. Female aversions toward odours of high *Toxocara* load thus provides a mechanism for such detection and may be a key factor involved in mate-choice. This does not negate that males may also avoid highly infected females; however, the current dataset did not show the same level of variability in female infection burdens to fully confirm this. Nevertheless, results do suggest that banded mongooses are able to detect, and show aversion to, high levels of *Toxocara* and *Isospora* via the odours of potential mates. Where there was greater variation in parasite loads, recipients reduced scent marking around more infected odours.

Avoiding highly parasitized mates should provide direct fitness benefits to banded mongooses. Avoiding parasites minimises one's risk of contracting an infection and transmitting it to offspring. This latter point may be particularly relevant considering that the Coccidosis infection resulting from *Isospora* parasites is most harmful to pre-weaned young (Eustis and Nelson, 1981; Kirkpatrick, 1998; Lindsay et al., 1997; Mundt et al., 2006). Additionally several species of *Toxocara* can lie dormant in uterine tissue and infect offspring during gestation (Urquhart et al., 1996). To avoid contracting this parasite and infecting young, it would thus appear beneficial for females in particular to discriminate the *Toxocara* load of potential mates. There may also be genetic benefits of parasite mediated mate-choice including the production of high-quality offspring (Hamilton and Zuk, 1982). Such benefits are only possible if the genes for parasite resistance are heritable. This area requires more detailed research (Coltman et al., 2001) yet in several systems including feral Soay sheep, *Ovis aries*, (Smith et al., 1999), barn swallows, *Hirundo rustica*, (Moller, 1990) and kittiwakes, *Rissa tridactyla,*(Boulinier et al., 1997) significant heritable variation for parasitic resistance has been shown. With the current, and expanding, genetic pedigree for this banded mongoose population (Sanderson et al., 2015) it may soon be feasible to consider the heritability of endo-parasitic resistance in this species, allowing more detailed commentary on the benefits of choosing less-infected mates. However, current results do provide a mechanism by which individuals can detect costly parasites and discriminate highly infected individuals. This is an important finding considering both sexes in this system engage in mate-choice despite little sexual dimorphism nor obvious visual signals to facilitate choice.

One factor which appears important to banded mongoose mate-choice is age. Older and top-ranking males monopolise breeding success with the majority of pups sired by the three top-ranking males per pack. These individuals are more successful mate-guards particularly concerning the mating of older females (Nichols et al., 2010). Indeed males preferentially guard older, more experienced females whom are more fecund and successful breeders (Nichols et al., 2010). Vicinity marking toward presented odours appears to support these findings. The odours of top-ranking donors (oldest of their sex within their social group) received more marks than lower-ranking scents in the *Isospora* dataset. Investigating rank-biased scent marking was not the main aim of this research, however, preference for higher ranking odours suggests they may encode information relevant to mate-choice. In model systems including ring-tailed lemurs and house mice, odours can encode dominance and social status (Gosling and Roberts, 2001; Rich and Hurst, 1998; Scordato et al., 2007). Dominance in the banded mongoose is less-skewed than in other systems and although closely linked to age this relationship is not entirely linear (Cant et al., 2013). Thus future investigations into the mechanisms underpinning rank-based mate-choice will require some caution and may work best by assigning dominance retrospectively using life-history data regarding breeding success. However, by odour-sampling and presenting to a larger subset of the populations with a wider age-range, it should be possible to investigate whether rank-based odour discrimination does occur.

Finally, there was no significant difference in the duration banded mongooses spent investigating odours based on the *Isospora* or *Toxocara* burden of the donor. As duration measures are considered proxies for interest in olfactory presentations (Hurst and Benyon, 2010) one would perhaps expect individuals to spend less time investigating odours of highly parasitised donors. However, a plausible mechanism exists to explain this observation. In birds, several coccidian parasites are recognised to reduce plasma protein levels and significantly decrease internal pH (Chapman, 2014). Protein and pH differences are likely to alter the chemical profile of odours (Drea et al., 2013) providing a way for individuals to discriminate between donors. However, this change in scent will still require investigation by recipients.

Such a mechanism may explain why it is only scent marking responses which decrease, and not the amount of time banded mongooses spend in contact with the odours of heavily parasitised conspecifics. Similarly, molecular cues of infection have been identified including the formylated peptides released from bacteria which can be detected by the vomeronasal organ of certain rodents (Liberles et al., 2009; Riviere et al., 2009; Wyatt, 2014). Although such molecules may allow identification of infection they still require investigation of the odour. Thus time spent around an odour cue may not be an appropriate measure regarding the discriminate of infection. Considering such information, it is likely that marking response is the best indicator odour discrimination.

Summary

Scent communication is common across mammals yet, with the exception of the laboratory mouse (Beltran-Bech and Richard, 2014; Beynon and Hurst, 2003; Hurst, 2009; Thom et al., 2008; Yamazaki et al., 1979), we know little of how it functions in mate-choice. Here I provide empirical support that banded mongooses can discriminate odours on the basis of common parasitic infections. Additionally, individuals contributing to high levels of marking, within naturally occurring situations, tend to harbour lower loads of *Isospora* oocytes. Although intra-sexual competition appears to be an important function of scent communication in this species (Jordan et al., 2010; Müller and Manser, 2008), I suggest it may also play a role in mate-choice for less infected individuals. Intensive scent-marking by individuals of low parasitic status likely allows advertising to potential mates supporting the function of odour cues in both self-advertisement and mate-choice. Results corroborate previous research showing that regularly refreshed scent marks receive heightened behavioural interest from conspecifics (Jordan et al., 2011a; Jordan et al., 2011b). This ability to mark extensively can now be related to parasite load, suggesting intense scent-marking may act as an indicator trait providing an honest signal of quality in this species. Banded mongooses are also able to discriminate the odours of potential mates based on *Isospora* and *Toxocara* load. In general recipients reduce marking behaviour toward highly-infected opposite-sex odours. These results support predications that, in order to safeguard their own and offspring fitness, individuals should avoid mating with infected conspecifics. Such aversion demonstrates the ability for odour signals to contain information relevant to reproduction and function within mate-choice in the banded mongoose.

Chapter 8 Final discussion and concluding remarks

This thesis adds to a growing body of work regarding mammalian scent communication with the novelty of considering a wild, non-model species. The habituated nature of the focal banded mongoose population has allowed odour presentations to be conducted in the field, mimicking natural scent-investigation behaviour. Results provide support for the ability of banded mongooses to discriminate odours based upon sex, familiarity, relatedness, reproductive state and certain parasitic infections. Coupling such results with new knowledge of the breeding dynamics of this species has allowed the function of scent signals to be re-considered. Previously, intra-sexual competition was assumed a key function of scent-communication, however I propose it may also play a role in mate-choice and self-advertisement. The specifics of discriminatory behaviour, including the mechanisms underlying it, do require more detailed investigation. In particular, certain responses indicate sex specific functions for odour signals. However, this research conclusively shows that multi-modal information can be communicated by odour signals that may underlie behavioural decision-making in the banded mongoose.

The information encoded in banded mongoose odour signals.

In support of previous research (Jordan, 2009; Jordan et al., 2011a) this thesis shows sex is detectable via scent and its discrimination is underpinned by chemical differences in banded mongoose scent profiles. Although the chemical composition of female odours did not differ when pregnant, compared to being non-pregnant, there are suggestions of protein-based differences. The importance of proteins within scent signals has received little attention beyond model systems (Beynon and Hurst, 2003; Beynon et al., 2002; Hurst et al., 1998) where they are believed to prolong the signal and release bound chemicals involved in communication (Hurst et al., 1998; Ziegler et al., 1993). However, as most research focuses on captive and laboratory species the high levels of protein within wild banded mongoose scent samples present a novel opportunity to consider scent proteomics in a wild system. In particular, the suggestion of a protein-based signature of pregnancy is a key progression in deciphering how proteins function within communication.

Both sexes of banded mongooses appear to discriminate female odours by reproductive state. Whist many studies test the discrimination of oestrus females (Achiraman et al., 2010; Crawford et al., 2011; Ferkin et al., 2004; Jordan et al., 2011c) few consider if pregnancy can be detected by scent nor how this could influence behavioural dynamics. Considering the intense female-female competition observed within many mammals (Stockley et al., 2013; Stockley and Bro-Jorgensen, 2011), a mechanism for detecting pregnancy should be beneficial in terms of allowing females to gauge the competitive landscape of their current situation. Indeed, in the banded mongoose the heightened reaction of pregnant females to other pregnant odours would suggest scent provides a way of assessing competitors and may be implicated in intra-sexual competition for breeding resources. This corroborates research on captive lemurs where pregnancy also appears detectable via scent and is assumed to function within competitive interactions (Crawford and Drea, 2015). Identifying the mechanism by which banded mongooses, and potentially other mammals, discern pregnancy can now begin by considering how certain chemicals and or proteins are differentially expressed dependant on reproductive state. If putative pheromones linked to receptivity and pregnancy can be isolated, their effects can be tested through behavioural assays (Wyatt, 2014). For example, the extreme birth synchrony exhibited by banded mongooses is rare in mammals and, although it is recognised to minimise infanticide (Hodge et al., 2009), the mechanisms underpinning synchrony are not yet realised. Because the odours of pregnant females provoke intense reactions from other dams as well as extended periods of interest from females in general, they may encode cues regarding birth synchrony. The banded mongoose thus represents an ideal wild system in which to

identify and test target pheromones linked to gestational timing that may aid the wider understanding of mammalian breeding behaviour.

The mechanism underpinning odour discrimination in the banded mongoose

The availability of chemical and protein data has been invaluable in supporting the observed behavioural discrimination of odours on the basis of sex and pregnancy status. However, previous research into the chemical composition of banded mongoose anal gland secretions has shown no evidence for pack-based scent signatures (Jordan, 2009). In social insects such as the ant, *formica exsecta,* colonies are identifiable by a specific suite of cuticular hydrocarbons whose production appears to be under genetic control (Martin and Drijfhout, 2009; Martin et al., 2008). However, in mammal systems such regimented group-based-signatures are rarely observed. This is likely because mammal odours encode variable parameters such as diet, seasonality, reproductive state and infection (Barnard et al., 1998; Ferkin et al., 1997; Hurst, 2009; Kavaliers et al., 2005b; Sachs, 1997; Scordato and Drea, 2007; Scordato et al., 2007), perhaps explaining why Jordan (2009) could not identify exact chemical differences between banded mongoose packs. Nevertheless, the behavioural discrimination of odours based on familiarity strongly suggests that banded mongooses do recognise the odour of their natal pack, even if this scent is changeable. In chapter three I show how individuals display heightened responses to odours of unfamiliar conspecifics, suggesting the novelty of an odour can be discriminated. Considering that dispersal is limited in both sexes (Cant et al., 2013), banded mongoose packs are made up of close relatives and have likely learned the scent of their pack. Indeed, across model systems we are now aware that scent-discrimination often has a learned component (Hurst et al., 1994; Roberts et al., 2014). In the banded mongoose pack recognition most likely occurs by phenotype-matching where animals learn their own or conspecifics' scents and use these as templates to discriminate unfamiliar individuals (Lacy and Sherman, 1983). If the banded mongoose is to become a model system for scent research, this mechanism must be tested. However, despite the lack of pack-based chemical signatures it appears these wild mammals are capable of detecting familiarity via scent.

Relatedness also appears discernible in odour, yet responses are sex-specific and only demonstrated toward familiar odours. This suggests the novelty of unfamiliar odours may be enough to signal low relatedness, particularly considering that this system experiences limited extra-group mating and immigration. However, when considering familiar odours, recipients modified their behavioural response dependent upon their relatedness to the odour-donor. This is an exciting result as, despite frequent incest, it appears that banded mongooses avoid inbreeding to some degree in their natal packs (Sanderson et al., 2015). Scent could thus provide a mechanism for such discrimination. Indeed, in the meerkat, phenotype matching has been shown to allow females to discriminate unfamiliar male odours on the basis of relatedness (Leclaire et al., 2013). However, the banded mongooses' discrimination of familiar odours with regards to relatedness is likely underpinned by self-referent phenotype matching specifically (Mateo, 2010; Mateo and Johnston, 2000). This is because extreme birth-synchrony produces mixed-parentage litters (Hodge et al., 2009) where litter-mates are of varying relatedness. This makes it difficult to learn the scent of kin at birth, instead banded mongooses likely use their own scent as a template for kin-discrimination (Mateo, 2010). It would now be fruitful to consider how detection of relatedness may function within kin recognition, particularly considering the evidence for non-random mating (Sanderson et al., 2015). Nevertheless, the discrimination of familiar odours by relatedness is valuable to our understanding of mammalian scent communication. Previous studies regarding detection of relatedness and genetic diversity are confined to lab and captive populations yet the banded mongoose results demonstrate such detection can occur in a truly wild system.

The function of odour signals in the banded mongoose system

The wealth of information encoded in banded mongoose scent signals would strongly suggest that scent is used to facilitate important behavioural decisions in this species. As discussed, previous work consistently supports the function of scent within intra-sexual competition (Jordan et al., 2011b; Jordan et al., 2011c; Müller and Manser, 2008). However, throughout this thesis I have provided evidence that odour signals may have other important functions, particularly regarding mate-choice. Chapter three discusses how heightened reactions toward unfamiliar female odours suggests the sexes utilise these odours for different functions, males within mate choice and females within intra-sexual competition. Unfortunately, the non-significance of 3-way interactions considering both odour and donor sex makes it difficult to concussively support this statement as I cannot show that females are preferentially responding same-sex, and males' opposite-sex, odours. However, in chapter four I present more targeted research into the use of female scent-signals. Although male recipients show heightened responses to non-pregnant odours, females scent-mark more and spend longer in contact with odours of their same reproductive state. Female odour communication remains poorly studied compared to that of males (Stockley et al., 2013) however this novel result suggests that a non-model species can detect reproductive state via scent, and that the sexes use female odour cues for different purposes. Males appear to be selecting receptive mates whilst females are responding to direct reproductive threats. Additionally, non-pregnant females also show heightened marking responses to the odours of other non-pregnant females. This satisfies predictions of intra-sexual competition (Gosling and Roberts, 2001) but could also allow dams to advertise themselves to males, hence this chapter also discusses rare evidence for male-mate-choice based upon a female advertisement.

The ability of odour signals to advertise their donor's status is also apparent when considering parasitic infection. Individuals of low *Isospora* load were seen to engage in more intensive scent-marking during natural marking bouts. This suggests intensive scent-marking could be viewed as indicator trait of low parasitic infection, in a similar way to the elaborate plumage of many birds (Hale et al., 2009; Moller, 1990). Additionally, in experimental odour presentations the odours of highly infected donors received fewer scent marks than those of less-infected individuals. The avoidance of parasites is a key dynamic involved in avian mate-choice but research in mammals tends to be confined to laboratory rodents (Kavaliers et al., 2005b; Kavaliers et al., 2003a). In this study I have provided one of the first examples of a wild species using scent to avoid parasitized opposite-sex individuals. It would now be interesting to consider how parasitic infection impacts breeding success, in particular are highly parasitised individuals really discriminated against in terms of mate-choice. Although I have shown behavioural aversions toward parasitised odours, I did not consider actual breeding behaviour. This is an important distinction as in laboratory mice, experimentally infected males can be shunned by females but they do still secure matings where females mate multiply (Zala et al., 2015). Such results demonstrate that odour preferences are proxy measures for mate-choice and it is important to determine how much of this preference is reflected in mating behaviour. To quantify the effects of parasite burdens upon breeding success would be possible in the banded mongoose but as faecal egg counts provide only a snap-shot of infection status, these studies would require constant sampling of individuals throughout the breeding season and gestation periods. The habituated nature of the focal banded mongoose population and the availability of life-history and genetic data would permit this level of data collection and makes them an ideal system in which to investigate parasitic repercussions upon reproduction. In particular it will be useful to understand how parasite-mediated mate-choice occurs in a wild system with natural levels of parasitic variation, as opposed to the experimentally infected laboratory species which are classically used in such research (Kavaliers et al., 2005a).

The future of parasitic research in the banded mongoose

The investigation of gastro-intestinal parasites has provided a novel dataset that will facilitate research into health parameters across this population. I identify a suite of parasites with known fitness costs in other species and show that infection burdens have high individual variability. In the banded mongoose it appears individual parameters contribute most to this variance. In chapter seven, I highlight that heterozygosity at one particular microsatellite locus has a large impact upon the relationship between parasites and genetic diversity. It would now be interesting to consider its location and relationship with other pathogens, as it may be linked to specific immune-related genes. Life-history factors such as weight and age also underpin a limited amount of parasitic variation, whilst certain packs harbour higher burdens than others. However classical trends such as the male-biased parasitism observed in other mammals (Moore and Wilson, 2002) are not well-supported in the banded mongoose. This may reflect their lack of sexual dimorphism and the fact that both sexes invest in mate-choice and intra-sexual competition. Indeed, the female dominant meerkat also refutes these trends and shows female-biased parasitism for certain infections (Smythe and Drea, 2015). Thus long-standing explanations of parasitic variation may merit investigation in more non-model systems.

The impact of identified parasites upon banded mongoose health does require more attention. Firstly, it will be useful to identify these agents at the species level to fully comment on their specificity to the mongoose system and potential for transmission between geographically neighbouring species. This will be particularly useful considering the number of other species inhabiting the Mweya peninsular (Cant, 2000). As many of these are carnivores and ungulates, they may be especially vulnerable to parasitic infection (Pedersen et al., 2007) and understanding the potential threats could guide conservation initiatives. Secondly, parasite removal experiments can be planned to determine exact fitness costs and better understand the causality in the relationship between parasite burdens and weight (Chapter 5). Finally, considering the large genetic pedigree, the heritability of parasitic infection could be addressed for this banded mongoose population. This phenomenon has been considered in very few systems (Boulinier et al., 1997; Moller, 1990; Smith et al., 1999) which makes it difficult to fully evaluate the benefits of parasite-mediated mate-choice. Investigating the heritability of parasitic resistance in the banded mongoose would facilitate such wider knowledge. It would also bolster the data concerning fitness related traits which could then be used to better understand reproductive dynamics in this system. Indeed, my findings that certain parasites are detectable via scent, and that parasitic measures vary with life-history traits, provides a sound basis for investigating how parasitic infection may influence breeding success and mate-choice.

Final conclusion

This thesis presents a detailed assessment of the information encoded in banded mongoose scent signals. Scent communication is common across mammals yet, with the exception of certain model systems (Beltran-Bech and Richard, 2014; Beynon and Hurst, 2003; Charpentier et al., 2010; Drea, 2015; Hurst, 2009; Thom et al., 2008), we understand little of how it functions. In the banded mongoose I demonstrate that anal gland secretions are discriminated on the basis of familiarity, sex, relatedness, female reproductive state and certain measures of parasitic infection. Furthermore, molecular differences between the sexes, and females during pregnancy, support behavioural results. I suggest this multi-modal information allows odour cues to function within both mate-choice and intra-sexual competition in the banded mongoose. However, sex-based interactions with both familiarity and relatedness imply odour signals may serve sex-specific functions. For females, evidence points toward intra-sexual competition, in keeping with previous research on this species (Jordan et al., 2011a; Müller and Manser, 2008). However, males' heightened interest in less-related, unfamiliar and non-pregnant odours suggest scent signals may function within mate-choice.

The discrimination of familiar odours by relatedness is an exciting result as few wild systems have the genetic data to facilitate this type of analyses. Banded mongoose data complement research on model organisms that show the detection of relatedness via scent (Charpentier, 2008a; Cheetham et al., 2007; Hurst and Benyon, 2010; Sherborne et al., 2007). Additionally, this finding could provide a mechanism for the observed non-random mating with respect to relatedness in the banded mongoose (Nichols et al., 2014; Sanderson et al., 2015). Scent signals may also function in mate-choice when considering their ability to communicate parasitic infection. Odour presentations show the banded mongoose is capable of detecting *Isospora* and *Toxocara* parasites. Both infections can be lethal in pre-weaned young and thus appear key targets to avoid during mate-choice. Together results suggest odour-based mate-choice may occur in the banded mongoose and verify existing research on model organisms (Charpentier, 2008a; Charpentier, 2008b; Kavaliers et al., 2005a; Thom et al., 2008).

The banded mongoose represents an exciting model system for future study of functional odour communication. A key next step will be to consider the chemical and protein composition of their anal gland secretions. This could allow detection of molecules encoding measures such as sex, receptivity and infection status, allowing one to understand how discriminatory behaviours are mediated. Any target molecules should be synthesised for use in field-based assays to directly test their effect upon behaviour (Wyatt, 2015). In particular, the ability of odours to enable birth synchrony would be a fruitful avenue of research. The discrimination of familiar odours by relatedness should also be considered in more detail with regards to a mechanism of kin recognition. Finally, the parasitic data should be useful for fitness-related studies in the future, and be of general interest to those studying parasite-mediated-mate-choice. The banded mongoose now represents one of the best-studied wild systems regarding mammalian scent communication. Although further study is required to understand exactly how odour signals function, I have provided evidence that scent contains multi-modal information relevant to reproduction as well as competition. Due to the habituated nature of this population and the wealth of life-history data available, they now represent a novel system for future investigation of mammalian scent communication.

Bibliography

Abdi H, 2007. The bonferoni and sidak corrections for multiple comparisons. Encyclopedia of measurement and statistics Thousand Oaks: Sage. p. 10.13-107.

Acevedo-Whitehouse K, Gulland F, Greig D, Amos W, 2003. Inbreeding: Disease susceptibility in California sea lions. Nature 422:35. doi: 10.1038/422035a.

Achiraman S, Archunan G, 2006. 1-Iodo-2methylundecane, a putative estrus-specific urinary chemo-signal of female mouse (Mus musculus). Theriogenology 66:1913-1920. doi: 10.1016/j.theriogenology.2006.05.010.

Achiraman S, Ponmanickam P, Ganesh DS, Archunan G, 2010. Detection of estrus by male mice: synergistic role of olfactory-vomeronasal system. Neurosci Lett 477:144-148. doi: 10.1016/j.neulet.2010.04.051.

Adams LG, Dale BW, 1997. Timing and synchrony of parturition in Alaskan Caribou. Journal of mammology 79. doi: http://dx.doi.org/10.2307/1382865.

Altizer S, Nunn CL, Thrall PH, Gittleman JL, Antonovics J, Cunningham AA, Dobson AP, Ezenwa V, Jones KE, Pedersen AB, Poss M, Pulliam JRC, 2003. Social Organization and Parasite Risk in Mammals: Integrating Theory and Empirical Studies. Annual Review of Ecology, Evolution, and Systematics 34:517-547. doi: 10.1146/annurev.ecolsys.34.030102.151725.

Alzaga V, Vicente J, Villanua D, Acevedo P, Casas F, Gortazar C, 2007. Body condition and parasite intensity correlates with escape capacity in Iberian hares (Lepus granatensis). Behav Ecol Sociobiol 62:769-775. doi: 10.1007/s00265-007-0502-3.

Anderson RM, May RM, 1978. Regulation and Stability of Host-Parasite Population Interactions: I. Regulatory Processes. Journal of Animal Ecology 47:219-247. doi: 10.2307/3933.

Arakawa H, Cruz S, Deak T, 2011. From models to mechanisms: odorant communication as a key determinant of social behavior in rodents during illness-associated states. Neurosci Biobehav Rev 35:1916-1928. doi: 10.1016/j.neubiorev.2011.03.007.

Balloux F, Amos W, Coulson T, 2004. Does heterozygosity estimate inbreeding in real populations? Mol Ecol 13:3021-3031. doi: 10.1111/j.1365-294X.2004.02318.x.

Barnard CJ, Behnke JM, Gage AR, Brown H, Smithurst PR, 1998. The role of parasite-induced immunodepression. Rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice (Mus musculus). Proceedings of the Royal Society of Biological sciences 265:693-701.

Bateman AJ, 1948. Intra-sexual selection in Drosophila. Heredity 2:349-368.

Bates D, Machler M, Dai B, 2008. Linear mixed-effects models using S4 classess. R package, Version 0999375-28 http://lmer4.r-forge.r-project.org/.

Begg CM, Begg KS, Du Toit JT, Mills MGL, 2003. Scent-marking behaviour of the honey badger, Mellivora capensis (Mustelidae), in the southern Kalahari. Animal Behaviour 66:917-929. doi: 10.1006/anbe.2003.2223.

Belcher AM, Epple g, Greenfield KL, Richards LE, Kuderling I, Smith AB, 1990. Proteins: biologically relevant components of the scent marks of a primate (Saguinus fuscicollis). Chemical Scences 15:431-446.

Bell MB, 2007. Cooperative begging in banded mongoose pups. Curr Biol 17:717-721. doi: 10.1016/j.cub.2007.03.015.

Bell MB, Nichols HJ, Gilchrist JS, Cant MA, Hodge SJ, 2012. The cost of dominance: suppressing subordinate reproduction affects the reproductive success of dominant female banded mongooses. Proc Biol Sci 279:619-624. doi: 10.1098/rspb.2011.1093.

Beltran-Bech S, Richard F-J, 2014. Impact of infection on mate choice. Animal Behaviour 90:159-170. doi: 10.1016/j.anbehav.2014.01.026.

Beynon RJ, Armstrong SD, Claydon AJ, Davidson AJ, Eyers CE, Langridge JI, Gómez-Baena G, Harman VM, Hurst JL, Lee V, McLean L, Pattison R, Roberts SA, Simpson DM, Unsworth J,

Vonderach M, Williams JP, Woolerton YE, 2015. Mass spectrometry for structural analysis and quantification of the Major Urinary Proteins of the house mouse. International Journal of Mass Spectrometry 391:146-156. doi: 10.1016/j.ijms.2015.07.026.

Beynon RJ, Armstrong SD, Gomez-Baena G, Lee V, Simpson D, Unsworth J, Hurst JL, 2014. The complexity of protein semiochemistry in mammals. Biochem Soc Trans 42:837-845. doi: 10.1042/BST20140133.

Beynon RJ, Hurst JL, 2003. Multiple roles of major urinary proteins in the house mouse, Mus domesticus. Biochemical Society Transactions 31:142-146. doi: 10.1042/bst0310142

Beynon RJ, Hurst JL, 2004. Urinary proteins and the modulation of chemical scents in mice and rats. Peptides 25:1553-1563. doi: 10.1016/j.peptides.2003.12.025.

Beynon RJ, Veggerby C, Payne CE, Robertson DH, Gaskell SJ, Humphries RE, Hurst JL, 2002. Polymorphism in major urinary proteins: Molecular heterogeneity in a wild mouse population. Journal of chemical ecology 28:1429-1446.

Boulet M, Charpentier MJ, Drea CM, 2009. Decoding an olfactory mechanism of kin recognition and inbreeding avoidance in a primate. BMC Evol Biol 9:281. doi: 10.1186/1471-2148-9-281.

Boulet M, Crawford JC, Charpentier MJ, Drea CM, 2010. Honest olfactory ornamentation in a female-dominant primate. J Evol Biol 23:1558-1563. doi: 10.1111/j.1420-9101.2010.02007.x.

Boulinier T, Sorci G, Monnat J, Y., Danchin E, 1997. Parent -offspring regression suggests heritable susceptibility to ectoparasites in a natural population of kittiwake Rissa tridactyla. Journal of Evolutionary Biology 10:77-85.

Bowman DD, 2014. Georgis' parasitology for veterinarians, 10 ed. St. Louis, Missouri: Elsevier, Saunders.

Brashares JS, Arcese P, 1999. Scent marking in a territorial african antelope: II the economics of marking with faeces. Animal Behaviour 7:7-11. doi: 10.1006/anbe.1998.0942

Braulke T, Bonifacino JS, 2009. Sorting of lysosomal proteins. Biochim Biophys Acta 1793:605-614. doi: 10.1016/j.bbamcr.2008.10.016.

Buchholz R, 2004. Effects of parasitic infection on mate sampling by female wild turkeys (Meleagris gallopavo): should infected females be more or less choosy? Behavioral Ecology 15:687-694. doi: 10.1093/beheco/arh066.

Cant MA, 2000. Social control of reproduction in banded mongooses. Animal Behaviour 59:147-158. doi: 10.1006/anbe.1999.1279.

Cant MA, 2003. Patterns of helping effort in co-operativley breeding banded mongooses (Mungos mungo). Journal of Zoology:115-121. doi: 10.1017/S0952836902003011.

Cant MA, Hodge SJ, Bell MB, Gilchrist JS, Nichols HJ, 2010. Reproductive control via eviction (but not the threat of eviction) in banded mongooses. Proc Biol Sci 277:2219-2226. doi: 10.1098/rspb.2009.2097.

Cant MA, Nichols HJ, Johnstone RA, Hodge SJ, 2014. Policing of reproduction by hidden threats in a cooperative mammal. Proc Natl Acad Sci U S A 111:326-330. doi: 10.1073/pnas.1312626111.

Cant MA, Otali E, Mwanguhya F, 2002. Fighting and mating between groups in a cooperativley breeding mammal, the banded mongoose. Ethology 108:541-555. doi: 10.1046/j.1439-0310.2002.00795.

Cant MA, Vitikainen E, Nichols HJ, 2013. Demography and Social Evolution of Banded Mongooses. 45:407-445. doi: 10.1016/b978-0-12-407186-5.00006-9.

Cassinello J, Gomendio M, Roldan ERS, 2001. Relationship between coefficient of inbreeding and parasite burden in endangered gazelles. Conservation biology 15:1171-1174. doi: 10.1046/j.1523-1739.2001.0150041171.

Chapman HD, 2014. Milestones in avian coccidiosis research: a review. Poult Sci 93:501-511. doi: 10.3382/ps.2013-03634.
Chapman JR, Nakagawa S, Coltman DW, Slate J, Sheldon BC, 2009. A quantitative review of heterozygosity-fitness correlations in animal populations. Mol Ecol 18:2746-2765. doi: 10.1111/j.1365-294X.2009.04247.x.
Charlesworth D, Charlesworth B, 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology, Evolution and systematics 18:237-268.
Charlesworth D, Willis JH, 2009. The genetics of inbreeding depression. Nat Rev Genet 10:783-796. doi: 10.1038/nrg2664.
Charpentier MJ, Boulet, M., Drea, C. M., 2008a. Smelling right: the scent of male lemurs advertises genetic quality and relatedness. Mol Ecol 17:3225-3233. doi: 10.1111/j.1365-294X.2008.03831.x.
Charpentier MJE, Crawford JC, Boulet M, Drea CM, 2010. Message 'scent': lemurs detect the genetic relatedness and quality of conspecifics via olfactory cues. Animal Behaviour 80:101-108. doi: 10.1016/j.anbehav.2010.04.005.
Charpentier MJE, Williams, C. V., Drea, C. M., 2008b. Inbreeding depression in ring-tailed lemurs (lemur catta): genetic diversity predicts parasitism, immunocompetence and survivorship. Conservation genetics 9:1605-1615. doi: 10.1007/s10592-007-9499-4.
Cheetham SA, Thom MD, Beynon RJ, Hurst JL, 2008. The Effect of Familiarity on Mate Choice. In: Hurst JL, Beynon RJ, Roberts SC, Wyatt TD, editors. Chemical Signals in Vertebrates 11 New York, NY: Springer New York. p. 271-280.
Cheetham SA, Thom MD, Jury F, Ollier WE, Beynon RJ, Hurst JL, 2007. The genetic basis of individual-recognition signals in the mouse. Curr Biol 17:1771-1777. doi: 10.1016/j.cub.2007.10.007.
Choleris E, Clipperton-Allen AE, Phan A, Valsecchi P, Kavaliers M, 2012. Estrogenic involvement in social learning, social recognition and pathogen avoidance. Front Neuroendocrinol 33:140-159. doi: 10.1016/j.yfrne.2012.02.001.
Clapperton BK, 1989. Scent marking behaviour of the ferret, Mustela furo L. Animal Behaviour 38:463-446. doi: 10.1016/S0003-3472(89)80037-5.
Clarke KR, Warwick RM, 2001. Change in marine communities: An approach to statistical analysis and interpretation. Plymouth, UK: PRIMER-E Ltd.
Clutton-Brock TH, 2007. Sexual selection in males and females. Science 318:1882-1885. doi: 10.1126/science.1133311
Clutton-Brock TH, Albon SD, Guiness FE, 1981. Parental investment in male and female offspring in polygynous mammals. Nature 289:487-489. doi: 10.1038/289487a0.
Clutton-Brock TH, Brotherton PN, Russell AF, O'Riain MJ, Gaynor D, Kansky R, Griffin A, Manser M, Sharpe L, McIlrath GM, Small T, Moss A, Monfort S, 2001. Cooperation, control, and concession in meerkat groups. Science 291:478-481. doi: 10.1126/science.291.5503.478.
Clutton-Brock TH, Iason GR, 1986. Sex ratio variation in mammals. The Quarterly Review of Biology 61:339-374.
Clutton-Brock TH, Vincent ACJ, 1991. Sexual selection and the potential reproductive rates of males and females. Letters to Nature 351:58-60. doi: 10.1038/351058a0.
Coltman DW, Pilkington JG, Smith JA, Pemberton JM, 1999. Parasite-mediated selection against inbred soay sheep in a free-living island population. Evolution 53:1259-1267. doi: 10.2307/2640828.
Coltman WD, Pilkington J, Kruuk LEB, Wilson K, Pemberton JM, 2001. Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. Evolution 55:2116-2125.
Converse LJ, Carlson AA, Ziegler TE, Snowdon CT, 1995. Communication of ovulatory state to mates by female pygmy marmosets, Cebuella pygmaea. Animal Behaviour 49:615-621. doi: 10.1016/0003-3472(95)80194-4.

Costa FJV, Macedo RH, 2005. Coccidian oocyst parasitism in the blue-black grassquit: influence on secondary sex ornaments and body condition. Animal Behaviour 70:1401-1409. doi: 10.1016/j.anbehav.2005.03.024.
Cote IM, Poulin R, 1995. Parasitism and group size in social animals: a meta-analysis. Behavioural Ecology 6:159-165. doi: 10.1093/beheco/6.2.159.
Coureaud G, Langlois D, Perrier G, Schaal B, 2003. A single key-odorant accounts for the pheromonal effect of rabbit milk: Further test of the mammary pheromones activity against a wide sample of volatiles from milk. Chemoecology 13:187-192. doi: 10.1007/s00049-003-0249-x.
Craig BH, Tempest LJ, Pilkington JG, Pemberton JM, 2008. Metazoan-protozoan parasite co-infections and host body weight in St Kilda Soay sheep. Parasitology 135:433-441. doi: 10.1017/S0031182008004137.
Crawford JC, Boulet M, Drea CM, 2011. Smelling wrong: hormonal contraception in lemurs alters critical female odour cues. Proc Biol Sci 278:122-130. doi: 10.1098/rspb.2010.1203.
Crawford JC, Drea CM, 2015. Baby on board: olfactory cues indicate pregnancy and fetal sex in a non-human primate. Biol Lett 11:20140831. doi: 10.1098/rsbl.2014.0831.
Crawley MJ, 2012a. Mathematics. The R Book: John Wiley & Sons, Ltd. p. 258-343.
Crawley MJ, 2012b. Mixed-Effects Models. The R Book: John Wiley & Sons, Ltd. p. 681-714.
Cringoli G, Rinaldi L, Veneziano V, Capelli G, Scala A, 2004. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and Dicrocoelium dendriticum in sheep. Vet Parasitol 123:121-131. doi: 10.1016/j.vetpar.2004.05.021.
Darwin C, 1871. The Descent of Man and selection in Relation to Sex. Princeton, N J: Princeton University Press.
David P, Pujol B, Viard F, Castella V, Goudet J, 2007. Reliable selfing rate estimates from imperfect population genetic data. Mol Ecol 16:2474-2487. doi: 10.1111/j.1365-294X.2007.03330.x.
Davies CS, Smythe K, Greene LK, Walsh D, Mitchell J, Manser MB, Clutton-Brock TH, Drea CM, 2016 in press. Exceptional endocrine profiles characterise the Meerkat: sex, status, and reproductive patterns. Science direct.
De Castro F, Bolker B, 2004. Mechanisms of disease-induced extinction. Ecology Letters 8:117-126. doi: 10.1111/j.1461-0248.2004.00693.x.
delBarco-Trillo J, Sacha CR, Dubay GR, Drea CM, 2012. Eulemur, me lemur: the evolution of scent-signal complexity in a primate clade. Philos Trans R Soc Lond B Biol Sci 367:1909-1922. doi: 10.1098/rstb.2011.0225.
Dolan WM, Stevens W, C et al., 1974. The cardiovascular and respiratory effects of isoflurane-nitrous oxide anaesthesia. Canadian Anaesthesia society 21:557-565.
Dorchies P, Bergeaud JP, Van Kahn N, Morand S, 1997. Reduced egg counts in mixed infections with oestrus ovis and haemonchus contortus: influence of eosinophils? Parasitol Res 83:727-730.
Doty RL, 2010. The Great Pheromone Myth. Baltimore MD: Johns Hopkins Univeristy Press.
Drea CM, 2011. Endocrine correlates of pregnancy in the ring-tailed lemur (Lemur catta): implications for the masculinization of daughters. Horm Behav 59:417-427. doi: 10.1016/j.yhbeh.2010.09.011.
Drea CM, 2015. D'scent of man: A comparative survey of primate chemosignaling in relation to sex. Horm Behav 68C:117-133. doi: 10.1016/j.yhbeh.2014.08.001.
Drea CM, Boulet M, Delbarco-Trillo J, Greene LK, Sacha CR, Goodwin TE, Dubay GR, 2013. The "secret" in secretions: methodological considerations in deciphering primate olfactory communication. Am J Primatol 75:621-642. doi: 10.1002/ajp.22143.

Droscher I, Kappeler PM, 2014. Maintenance of familiarity and social bonding via communal latrine use in a solitary primate (). Behav Ecol Sociobiol 68:2043-2058. doi: 10.1007/s00265-014-1810-z.
Dunn A, Keymer A, 1986. Factors affecting the reliability of the McMaster technique. Journal of helminthology 60:260-262.
Duszynski DW, Marquardt WC, 2006. Coccidia in the mammary glands of Shrews (order: Insectivora). The journal of parasitology 89:3. doi: 10.1645/GE-3141RN.
Edward DA, Chapman T, 2011. The evolution and significance of male mate choice. Trends Ecol Evol 26:647-654. doi: 10.1016/j.tree.2011.07.012.
Ehman KD, Scott ME, 2003. Female mice mate preferentially with non-parasitized males. Parasitology 125. doi: 10.1017/s003118200200224x.
Eustis SL, Nelson DT, 1981. Lesions associated with coccidosis in nursing piglets. Vet Parasitol 18:21-28. doi: 10.1177/030098588101800103.
Ezenwa VO, Price SA, Altizer S, Vitone ND, Cook KC, 2006. Host traits and parasite species richness in even and odd-toed hoofed mammals, Artiodactyla and Perissodactyla. Oikos 115:526-536. doi: 10.1111/j.2006.0030-1299.15186.x.
Faulkes CG, Abbott DH, Jarvis JUM, 1991. Social suppression of reproduction in male naked mole-rats, Heterocephalus glaber. Journal of reproduction and fertility 91:593-604. doi: 10.1530/jrf.0.0910593.
Faulkes CG, Bennett NC, 2001. Family values: group dynamics and social control of reproduction in African mole-rats. Trends in Ecology and Evolution 16:184-190. doi: 10.1016/S0169-5347(01)02116-4.
Ferkin MH, Lee DN, Leonard S, T, 2004. The Reproductive State of Female Voles Affects their Scent Marking Behavior and the Responses of Male Conspecifics to Such Marks. Ethology 110:257-275.
Ferkin MH, Sorokin ES, Johnston RE, Lee CJ, 1997. Attractiveness of scents varies with protein content of the diet in meadow voles. Animal Behaviour 53:133-141.
Field CR, Laws RM, 1970. The Distribution of the larger herbivores in the Queen Elizabeth National Park Uganda. Journal of applied ecology 7:273-294.
Fisher M, 2003. Toxocara cati: an underestimated zoonotic agent. Trends in Parasitology 19:167-170. doi: 10.1016/s1471-4922(03)00027-8.
Folstad I, Karter AJ, 1992. Parasites, bright males and the immunocompetence handicap. The American Naturalist 139:603-622. doi: 10.1086/285346.
Forstmeier W, Schielzeth H, Mueller JC, Ellegren H, Kempenaers B, 2012. Heterozygosity-fitness correlations in zebra finches: microsatellite markers can be better than their reputation. Mol Ecol 21:3237-3249. doi: 10.1111/j.1365-294X.2012.05593.x.
Frankham R, 2010. Inbreeding in the wild really does matter. Heredity (Edinb) 104:124. doi: 10.1038/hdy.2009.155.
Frenkel JK, Smith DD, 2003. Determination of the genera of cyst-forming coccidia. Parasitol Res 91:384-389. doi: 10.1007/s00436-003-0969-4.
Fugazzola MC, Stancampiano L, 2012. Host social rank and parasites: plains zebra (Equus quagga) and intestinal helminths in Uganda. Vet Parasitol 188:115-119. doi: 10.1016/j.vetpar.2012.03.019.
Gadagkar R, 2009. Interrogating an insect society. Proc Natl Acad Sci U S A 106:10407-10414. doi: 10.1073/pnas.0904317106.
Gasso D, Feliu C, Ferrer D, Mentaberre G, Casas-Diaz E, Velarde R, Fernandez-Aguilar X, Colom-Cadena A, Navarro-Gonzalez N, Lopez-Olvera JR, Lavin S, Fenandez-Llario P, Segales J, Serrano E, 2015. Uses and limitations of faecal egg count for assessing worm burden in wild boars. Vet Parasitol 209:133-137. doi: 10.1016/j.vetpar.2015.02.006.

Gilchrist JS, 2004. Pup escorting in the communal breeding banded mongoose: behavior, benefits, and maintenance. Behavioral Ecology 15:952-960. doi: 10.1093/beheco/arh071.
Gilchrist JS, 2006. Female eviction, abortion, and infanticide in banded mongooses (Mungos mungo): implications for social control of reproduction and synchronized parturition. Behavioral Ecology 17:664-669. doi: 10.1093/beheco/ark012.
Gilchrist JS, Otali E, 2002. The effects of refuse-feeding on home-range use, group size, and intergroup encounters in the banded mongoose. Canadian Journal of Zoology 80:1795-1802. doi: 10.1139/z02-113.
Gilchrist JS, Russell AF, 2007. Who cares? Individual contributions to pup care by breeders vs non-breeders in the cooperatively breeding banded mongoose (Mungos mungo). Behavioral Ecology and Sociobiology 61:1053-1060. doi: 10.1007/s00265-006-0338-2.
Gillespie TR, 2006. Noninvasive Assessment of Gastrointestinal Parasite Infections in Free-Ranging Primates. International Journal of Primatology 27:1129-1143. doi: 10.1007/s10764-006-9064-x.
Gomez-Baena G, Armstrong SD, Phelan MM, Hurst JL, Beynon RJ, 2014. The major urinary protein system in the rat. Biochem Soc Trans 42:886-892. doi: 10.1042/BST20140083.
Gosling LM, 1987. Scent marking in an antelope lek territory. Animal Behaviour 35:620-622. doi: 10.1016/S0003-3472(87)80298-1.
Gosling LM, Roberts SC, 2001. Scent-marking by male mammals: Cheat-proof signals to competitors and mates. Advances in the study of behaviour 30:169–217. doi: 10.1016/S0065-3454(01)80007-3.
Guillot A, Boulay M, Chambellon E, Gitton C, Monnet V, Juillard V, 2016. Mass Spectrometry Analysis of the Extracellular Peptidome of Lactococcus lactis: Lines of Evidence for the Coexistence of Extracellular Protein Hydrolysis and Intracellular Peptide Excretion. J Proteome Res. doi: 10.1021/acs.jproteome.6b00424.
Hadfield JD, Richardson DS, Burke T, 2006. Towards unbiased parentage assignment: combining genetic, behavioural and spatial data in a Bayesian framework. Mol Ecol 15:3715-3730.
Hakkarainen H, Huhta E, Koskela E, Mappes T, Soveri T, Suorsa P, 2007. Eimeria-parasites are associated with a lowered mother's and offspring's body condition in island and mainland populations of the bank vole. Parasitology 134:23-31. doi: 10.1017/S0031182006001120.
Hale ML, Verduijn MH, Moller AP, Wolff K, Petrie M, 2009. Is the peacock's train an honest signal of genetic quality at the major histocompatibility complex? J Evol Biol 22:1284-1294. doi: 10.1111/j.1420-9101.2009.01746.x.
Hamilton WD, Zuk M, 1982. Heritable true fitness and bright birds: A role for parasites? Science 218:384-387.
Hansson B, Westerberg L, 2002. On the correlation between heterozygosity and fitness in natural populations. Molecular ecology 11:2467-2474.
Harrison A, Scantlebury M, Montgomery WI, 2010. Body mass and sex-biased parasitism in wood mice Apodemus sylvaticus. Oikos 119:1099-1104. doi: 10.1111/j.1600-0706.2009.18072.x.
Hauber ME, Sherman PW, 2001. Self-referent phenotype matching: theoretical considerations and empirical evidence. TRENDS in Neurosciences 21:609-616. doi: 10.1016/S0166-2236(00)01916-0.
Hayter JR, Robertson DH, Gaskell SJ, Beynon RJ, 2003. Proteome analysis of intact proteins in complex mixtures. Mol Cell Proteomics 2:85-95. doi: 10.1074/mcp.M200078-MCP200.
Hayward AD, 2013. Causes and consequences of intra- and inter-host heterogeneity in defence against nematodes. Parasite Immunol. doi: 10.1111/pim.12054.

Hayward AD, Wilson AJ, Pilkington JG, Pemberton JM, Kruuk LE, 2009. Ageing in a variable habitat: environmental stress affects senescence in parasite resistance in St Kilda Soay sheep. Proc Biol Sci 276:3477-3485. doi: 10.1098/rspb.2009.0906.

Hecker E, Butenandt A, 1984. Bombykol revisited—reflections on a pioneering period and on some of its consequences. In: Hummel H, T. M, editors. Techniques in pheromone research Springer series in experimental entomology New York: Springer. p. 1-44.

Heymann EW, 1998. Sex differences in olfactory communication in a primate, the moustached tamarin, Saguinus mystax (Callitrichinae). Behavioural Ecology and sociobiology 43:37-75.

Heymann EW, 2006. Scent marking strategies of New World primates. Am J Primatol 68:650-661. doi: 10.1002/ajp.20258.

Hill GE, Doucet SM, Buchholz R, 2005. The effect of coccidial infection on iridescent plumage coloration in wild turkeys. Animal Behaviour 69:387-394. doi: 10.1016/j.anbehav.2004.03.013.

Hodge SJ, 2005. Helpers benefit offspring in both the short and long-term in the cooperatively breeding banded mongoose. Proc Biol Sci 272:2479-2484. doi: 10.1098/rspb.2005.3255.

Hodge SJ, 2007. Counting the costs: the evolution of male-biased care in the cooperatively breeding banded mongoose. Animal Behaviour 74:911-919. doi: 10.1016/j.anbehav.2006.09.024.

Hodge SJ, Bell MB, Cant MA, 2009. Reproductive competition and the evolution of extreme birth synchrony in a cooperative mammal. Biol Lett 7:54-56. doi: 10.1098/rsbl.2010.0555.

Holman L, Jorgensen CG, Nielsen J, d'Ettorre P, 2010. Identification of an ant queen pheromone regulating worker sterility. Proc Biol Sci 277:3793-3800. doi: 10.1098/rspb.2010.0984.

Hothorn F, Bretz F, Westfall P, 2008. Simultaneous Inference in General Parametric Models. Biometric Journal 50:346-363. doi: 10.1002/bimj.200810425.

Huang da W, Sherman BT, Lempicki RA, 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44-57. doi: 10.1038/nprot.2008.211.

Hudson R, Gonzalez-Mariscal, G., Beyer, C, 1990. Chin marking behaviour, sexual receptivity and pheromone emmission in steriod-treated ovariexctomised rabbits. Horm Behav 24:1-13.

Hurst JL, 2009. Female recognition and assessment of males through scent. Behav Brain Res 200:295-303. doi: 10.1016/j.bbr.2008.12.020.

Hurst JL, Benyon RJ, 2010. Making progression genetic kin recognition among vertebrates. Journal of Biology 9. doi: 10.1186/jbiol221.

Hurst JL, Hayden L, Kingston M, Luck R, Sorensen K, 1994. Response of the aboriginal house mouse, Mus spretus Lataste to tunnels bearing the odours of conspecifics. Animal Behaviour 48:1219-1229. doi: 10.1006/anbe.1994.1354.

Hurst JL, Robertson DH, Tolladay U, Beynon RJ, 1998. Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. Animal Behaviour 55:1289-1297.

Ilmonen P, Penn DJ, Damjanovich K, Clarke J, Lamborn D, Morrison L, Ghotbi L, Potts WK, 2008. Experimental infection magnifies inbreeding depression in house mice. Journal of evolutionary biology 21:834-841. doi: 10.1111/j.1420-9101.2008.01510.x.

Irwin MT, Samonds KE, Raharison J, Wright PC, 2004. Lemur latrines: Observations of latrine behaviour in wild primates and possible ecological significance. Journal of mammalogy 85:420-427. doi: 10.1644/1383937

Jex AR, Waeschenbach A, Hu M, van Wyk JA, Beveridge I, Littlewood DT, Gasser RB, 2009. The mitochondrial genomes of Ancylostoma caninum and Bunostomum phlebotomum - two hookworms of animal health and zoonotic importance. BMC Genomics 10:79. doi: 10.1186/1471-2164-10-79.

Jimenez JA, Hughes KA, Alaks G, Graham L, Lacy RC, 1994. An Experimental study of inbreeding depression in a natural habitat. Science 266:271-273.

Jin Z, Akao N, Ohta N, 2008. Prolactin evokes lactational transmission of larvae in mice infected with Toxocara canis. Parasitol Int 57:495-498. doi: 10.1016/j.parint.2008.06.006.
Johnson PTJ, Buller ID, 2011. Parastie competition hidden by correlated coinfection: Using surveys and experiments to understand parsite interactions. Ecology 92:535-541. doi: 10.1890/10-0570.1.
Johnson PTJ, Hoverman JT, 2012. Parasite diversity and coinfection determine pathogen infection success and host fitness. PNAS 109. doi: 10.1073/pnas.1201790109.
Johnson RP, 1973. Scent marking in mammals. Animal Behaviour 21:521-535.
Jolles AE, Ezenwa VO, Etiene RS, Turner WC, Olff H, 2008. Interactions between macroparasites and microparasites drive infection patters in Free-ranging African Buffalo. Ecology 89:2239-2225.
Jolly A, 1966. Lemur Social Behavior and Primate Intelligence. Science 153:501-506.
Jones OR, Wang J, 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551-555.
Jordan NR, 2009. Scent communication in wild banded mongooses. PhD Thesis University of Cambridge.
Jordan NR, Apps PJ, Golabek KA, McNutt JW, 2014. Top marks from top dogs: tandem marking and pair bond advertisement in African wild dogs. Animal Behaviour 88:211-217. doi: 10.1016/j.anbehav.2013.12.001.
Jordan NR, Manser MB, Mwanguhya F, Kyabulima S, Rüedi P, Cant MA, 2011a. Scent marking in wild banded mongooses: 1. Sex-specific scents and overmarking. Animal Behaviour 81:31-42. doi: 10.1016/j.anbehav.2010.07.010.
Jordan NR, Mwanguhya F, Furrer RD, Kyabulima S, Rüedi P, Cant MA, 2011b. Scent marking in wild banded mongooses: 2. Intrasexual overmarking and competition between males. Animal Behaviour 81:43-50. doi: 10.1016/j.anbehav.2010.07.009.
Jordan NR, Mwanguhya F, Kyabulima S, Cant MA, 2010. Scent marking within and between groups of wild banded mongooses. Journal of Zoology 280:72-83. doi: 10.1111/j.1469-7998.2009.00646.x.
Jordan NR, Mwanguhya F, Kyabulima S, Rüedi P, Hodge SJ, Cant MA, 2011c. Scent marking in wild banded mongooses: 3. Intrasexual overmarking in females. Animal Behaviour 81:51-60. doi: 10.1016/j.anbehav.2010.10.007.
Kappeler PM, 1998. To whom it may concern: the transmission and function of chemical signals in lemur catta. Behavioural Ecology and Sociobiology 42:411-421.
Kavaliers M, Choleris E, Pfaff DW, 2005a. Genes, odours and the recognition of parasitized individuals by rodents. Trends Parasitol 21:423-429. doi: 10.1016/j.pt.2005.07.008.
Kavaliers M, Choleris E, Pfaff DW, 2005b. Recognition and avoidance of the odors of parasitized conspecifics and predators: differential genomic correlates. Neurosci Biobehav Rev 29:1347-1359. doi: 10.1016/j.neubiorev.2005.04.011.
Kavaliers M, Colwell DD, Braun WJ, Choleris E, 2003a. Brief exposure to the odour of a parasitized male alters the subsequent mate odour responses of female mice. Animal Behaviour 65:59-68. doi: 10.1006/anbe.2002.2043.
Kavaliers M, Colwell DD, Cloutier CJ, Ossenkopp K-P, Choleris E, 2014. Pathogen threat and unfamiliar males rapidly bias the social responses of female mice. Animal Behaviour 97:105-111. doi: 10.1016/j.anbehav.2014.09.006.
Kavaliers M, Fudge MA, Colwell DD, Choleris E, 2003b. Aversive and avoidance responses of female mice to the odors of males infected with an ectoparasite and the effects of prior familiarity. Behavioral Ecology and Sociobiology 54:423-430. doi: 10.1007/s00265-003-0631-2.
Keller MC, Visscher PM, Goddard ME, 2011. Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. Genetics 189:237-249. doi: 10.1534/genetics.111.130922.

Kirkpatrick CE, 1998. Epizootiology of endoparasitic infections in pet cats and dogs presented to a veterinary teaching hospital. Vet Parasitol 30:113-124.
Klein SL, 2003. Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. Physiology & Behavior 79:441-449. doi: 10.1016/s0031-9384(03)00163-x.
Knowles SC, Fenton A, Petchey OL, Jones TR, Barber R, Pedersen AB, 2013. Stability of within-host-parasite communities in a wild mammal system. Proc Biol Sci 280:20130598. doi: 10.1098/rspb.2013.0598.
Kocher SD, Grozinger CM, 2011. Cooperation, conflict, and the evolution of queen pheromones. J Chem Ecol 37:1263-1275. doi: 10.1007/s10886-011-0036-z.
Lacy RC, Sherman PW, 1983. Kin recognition by phenotype matching. The American Naturalist 121:489-512.
Leclaire S, Faulkner CT, 2014. Gastrointestinal parasites in relation to host traits and group factors in wild meerkats Suricata suricatta. Parasitology 141:925-933. doi: 10.1017/S0031182013002333.
Leclaire S, Nielsen JF, Thavarajah NK, Manser M, Clutton-Brock TH, 2013. Odour-based kin discrimination in the cooperatively breeding meerkat. Biol Lett 9:20121054. doi: 10.1098/rsbl.2012.1054.
Lee AC, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD, 2010. Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol 26:155-161. doi: 10.1016/j.pt.2010.01.002.
Leimar O, 1996. Life-history analysis of the Tivers and Willard sex-ratio problem. Behavioral Ecology 7:316-325. doi: 10.1093/beheco/7.3.316.
Liberles SD, Horowitz LF, Kuang D, Contos JJ, Wilson KL, Siltberg-Liberles J, Liberles DA, Buck LB, 2009. Formyl peptide receptors are candidate chemosensory receptors in the vomeronasal organ. Proc Natl Acad Sci U S A 106:9842-9847. doi: 10.1073/pnas.0904464106.
Lindenfors P, Nunn CL, Jones KE, Cunningham AA, Sechrest W, Gittleman JL, 2007. Parasite species richness in carnivores: effects of host body mass, latitude, geographical range and population density. Global Ecology and Biogeography 16:496-509. doi: 10.1111/j.1466-8238.2006.00301.x.
Lindsay DS, Dubey JP, Blagburn BL, 1997. Biology of isospora spp. from humans, non-human primates and domestic animals. Clinical microbiology reviews:19-34.
Liu B, Yang L, Yang Y, Lu Y, 2016. Influence of Landscape Diversity and Composition on the Parasitism of Cotton Bollworm Eggs in Maize. PLoS One 11:e0149476. doi: 10.1371/journal.pone.0149476.
Lucan RK, 2006. Relationships between the parasitic mite Spinturnix andegavinus (Acari: Spinturnicidae) and its bat host, Myotis daubentonii (Chiroptera: Vespertilionidae): seasonal, sex- and age-related variation in infestation and possible impact of the parasite on the host condition and roosting behaviour. Folia Parasitologica 53:147-152.
Luong LT, Heath BD, Polak M, 2007. Host inbreeding increases susceptibility to ectoparasitism. Journal of evolutionary biology 20:79-86. doi: 10.1111/j.1420-9101.2006.01226.x.
Lynch M, Ritland K, 1999. Estimate of pairwise relatedness with molecular markers. Genetics 152:1753-1766.
Martin S, Drijfhout F, 2009. A review of ant cuticular hydrocarbons. J Chem Ecol 35:1151-1161. doi: 10.1007/s10886-009-9695-4.
Martin SJ, Vitikainen E, Helantera H, Drijfhout FP, 2008. Chemical basis of nest-mate discrimination in the ant Formica exsecta. Proc Biol Sci 275:1271-1278. doi: 10.1098/rspb.2007.1708.
Mateo JM, 2003. Kin recognition in ground squirrels and other rodents. journal of mammalogy 84:1163-1181. doi: http://dx.doi.org/10.1644/BLe-011.

Mateo JM, 2006. The nature and representation of individual recognition odours in Belding's ground squirrels. Animal Behaviour 71:141-154. doi: 10.1016/j.anbehav.2005.04.006.
Mateo JM, 2010. Self-referent phenotype matching and long-term maintenance of kin recognition. Animal Behaviour 80:929-935. doi: 10.1016/j.anbehav.2010.08.019.
Mateo JM, Johnston RE, 2000. Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. Proc Biol Sci 267. doi: 10.1098/rspb.2000.1058
McLennan D, 1995. Male mate choice based upon female nuptial coloration in the brook stickleback,Culaea inconstans(Kirtland). Animal Behaviour 50:213-221. doi: 10.1006/anbe.1995.0233.
Meagher S, Penn DJ, Potts WK, 2000. Male-male competition magnifies inbreeding depression in wild house mice. Proc Natl Acad Sci U S A 97:3324-3329. doi: 10.1073/pnas.060284797.
Miller JM, Coltman DW, 2014. Assessment of identity disequilibrium and its relation to empirical heterozygosity fitness correlations: a meta-analysis. Mol Ecol 23:1899-1909. doi: 10.1111/mec.12707.
Mitchell J, Vitikainen EI, Wells D, Cant MA, Nichols HJ, 2016 in press. Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose. Journal of Zoology.
Moller AP, 1990. Effects of a Haematophagous Mite on the Barn swallow (Hirundo rustica): A Test of the Hamilton and Zuk Hypothesis. Evolution 44:771-784. doi: 10.2307/2409545.
Moore SL, Wilson K, 2002. Parasites as a Viability Cost of Sexual Selection in natural Populations of Mammals. Science 297:2015-2018. doi: 10.1126/science.1074196
Moran MD, 2003. Arguements for rejecting the sequential Bonferroni in Ecological studies. oikos 100:403-405.
Morand S, Poulin R, 1998. Density, body mass and parasite species richness of terrestrial mammals. Evolutionary Ecology 12:717-727. doi: 10.1023/A:1006537600093.
Moreno-Rueda G, Hoi H, 2011. Female house sparrows prefer big males with a large white wing bar and fewer feather holes caused by chewing lice. Behavioral Ecology 23:271-277. doi: 10.1093/beheco/arr182.
Muehlenbein MP, Watts DP, 2010. The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. BioPsychoSocial Medicine 4:1-12. doi: 10.1186/1751-0759-4-21.
Müller CA, Manser MB, 2007. 'Nasty neighbours' rather than 'dear enemies' in a social carnivore. Proc Biol Sci 274:959-965. doi: 10.1098/rspb.2006.0222.
Müller CA, Manser MB, 2008. Scent-marking and intrasexual competition in a cooperative carnivore with low reproductive skew. Ethology 114:174-185. doi: 10.1111/j.1439-0310.2007.01455.x.
Mundt HC, Joachim A, Becka M, Daugschies A, 2006. Isopspora suis: an experimental model for mammalian intestinal coccidosis. Parasitol Res 98:167-175. doi: 10.1007/s00436-005-0030-x.
Narum SR, 2006. Beyond Bonferroni: Less conservative analyses for conservation genetics. Conservation Genetics 7:783-787. doi: 10.1007/s10592-005-9056-y.
Neal E, 1970. The banded mongoose, Munos Mungo, Gmelin. Journal of Afican Ecology 8:63-71. doi: 10.1111/j.1365-2028.1970.tb00831.x.
Nichols HJ, Amos W, Bell MBV, Mwanguhya F, Kyabulima S, Cant MA, 2012a. Food availability shapes patterns of helping effort in a cooperative mongoose. Animal Behaviour 83:1377-1385. doi: 10.1016/j.anbehav.2012.03.005.
Nichols HJ, Amos W, Cant MA, Bell MBV, Hodge SJ, 2010. Top males gain high reproductive success by guarding more successful females in a cooperatively breeding mongoose. Animal Behaviour 80:649-657. doi: 10.1016/j.anbehav.2010.06.025.
Nichols HJ, Cant MA, Hoffman JI, Sanderson JL, 2014. Evidence for frequent incest in a cooperatively breeding mammal. Biol Lett 10:20140898. doi: 10.1098/rsbl.2014.0898.

Nichols HJ, Cant MA, Sanderson JL, 2015. Adjustment of costly extra-group paternity according to inbreeding risk in a cooperative mammal. Behav Ecol 26:1486-1494. doi: 10.1093/beheco/arv095.
Nichols HJ, Jordan NR, Jamie GA, Cant MA, Hoffman JI, 2012b. Fine-scale spatiotemporal patterns of genetic variation reflect budding dispersal coupled with strong natal philopatry in a cooperatively breeding mammal. Mol Ecol 21:5348-5362. doi: 10.1111/mec.12015.
Pedersen AB, Jones KE, Nunn CL, Altizer S, 2007. Infectious diseases and extinction risk in wild mammals. Conserv Biol 21:1269-1279. doi: 10.1111/j.1523-1739.2007.00776.x.
Peeters C, Liebig J, 2009. Fertility Signaling as a General Mechanism of Regulating Reproductive Division of Labor in Ants. In: Gadau J, Fewell JH, editors. Organization of social insect societies: From genome to sociocomplexity Cambridge, MA: Harvard University Press. p. 220-242.
Pemberton J, 2004. Measuring inbreeding depression in the wild: the old ways are the best. Trends Ecol Evol 19:613-615. doi: 10.1016/j.tree.2004.09.010.
Penn DJ, Potts WK, 1998a. Chemical signals and parasite-mediated sexual selection. Trends in Ecology and Evolution 13:391-396. doi: 10.1016/S0169-5347(98)01473-6.
Penn DJ, Potts WK, 1998b. How Do Major Histocompatibility Complex Genes Influence Odor and Mating Preferences? 69:411-436. doi: 10.1016/s0065-2776(08)60612-4.
Penn DJ, Potts WK, 1999. The evolution of mating preferences and major histocompatibility genes. The American Naturalist 153:145-164.
Petrie M, 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. Letters to Nature 317:598 - 599. doi: 10.1038/371598a0.
Phelan MM, McLean L, Hurst JL, Beynon RJ, Lian LY, 2014. Comparative study of the molecular variation between 'central' and 'peripheral' MUPs and significance for behavioural signalling. Biochem Soc Trans 42:866-872. doi: 10.1042/BST20140082.
Poulin R, 1994. Meta-analysis of parasite-induced behavioural changes. Animal Behaviour 48:137-146. doi: 10.1006/anbe.1994.1220
Poulin R, 1995a. "Adaptive" changes in the behaviuor of parasitized animals: A critical review. International journal of parasitology 25. doi: 10.1016/0020-7519(95)00100-X
Poulin R, 1995b. Phylogeny, Ecology, and the Richness of Parasite Communities in Vertebrates. Ecological Monographs 65:283-302.
Poulin R, 1997. Species richness of parasite assembleges: Evolution and patters Annual review of Ecology and Systematics 28:341-358.
Poulin R, Morand S, 2000. The diversity of parasites. The Quarterly review of biology 75:277-293.
R development core team, 2013. A language and environment for statistical computing. In: team Rdc, editor. R Foundation for Statistical computing Vienna, Austria: http://www.R-project.org.
Rafalinirina HA, Aivelo T, Wright PC, Randrianasy J, 2015. Comparison of parasitic infections and body condition in rufous mouse lemur (microcebus rufus) at Ranomafana National Park, Southeast Madagascar. Madagascar conservation and development 10:6066.
Raharivololona BM, Ganzhorn JU, 2010. Seasonal variations in gastrointestinal parasites excreted by the gray mouse lemur Microcebus murinus in Madagascar. Endangered Species Research 11:113-122. doi: 10.3354/esr00255.
Ralls K, 1971. Mammalian Scent communication. Science 171:443-449.
Rasmussen LEL, Lee TD, Sgabg A, Roelofs WL, Doyle Davies GJ, 1997. Purification, identification, concentration and bioactivity of (Z)-7-Dodecen-1-yl Acetate: Sex pheromone of the female asian elephant (elephas maximus). Chemical Senses 22:417-437.
Rauw WM, 2012. Immune response from a resource allocation perspective. Front Genet 3:267. doi: 10.3389/fgene.2012.00267.

Reid JM, Arcese P, Keller LF, 2003. Inbreeding depresses immune response in song sparrows (Melospiza melodia): direct and inter-generational effects. Proc Biol Sci 270:2151-2157. doi: 10.1098/rspb.2003.2480.
Rich T, Hurst JL, 1998. Scent marks as reliable signals of the competative ability of males. Animal Behaviour 56:727-735.
Rich T, Hurst JL, 1999. The competing countermarks hypothesis: Reliable assessment of competative ability by potential mates. Animal Behaviour 58:1027-1037.
Riviere S, Challet L, Fluegge D, Spehr M, Rodriguez I, 2009. Formyl peptide receptor-like proteins are a novel family of vomeronasal chemosensors. Nature 459:574-577. doi: 10.1038/nature08029.
Roberts ML, Buchanan KL, Evans MR, 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. Animal Behaviour 68:227-239. doi: 10.1016/j.anbehav.2004.05.001.
Roberts SA, Davidson AJ, Beynon RJ, Hurst JL, 2014. Female attraction to male scent and associative learning: the house mouse as a mammalian model. Animal Behaviour 97:313-321. doi: 10.1016/j.anbehav.2014.08.010.
Rood JP, 1975. Population dynamics and food habits of the banded mongooses. Journal of East African Wild life 13:89-111.
Roulin A, Dijkstra C, Riols C, Ducrest A, 2001a. Female and male specific signals of quality in the barn owl. Journal of Evolutionary Biology 14:255-266.
Roulin A, Riols C, Dijkastra C, Ducrest A, 2001b. Female plumage spottiness signals parsite resistance in the barn owl (Tyto alba). Behavioural Ecology 12:103-110.
Rowland WJ, Baube CL, Horan TT, 1991. Signalling of sexual receptivity by pigmentation pattern in female sticklebacks. Animal Behaviour 42:243-249. doi: 10.1016/S0003-3472(05)80555-X.
Ruiz-Lopez MJ, Ganan N, Godoy JA, Del Olmo A, Garge J, Espeso G, Vargas A, Martinez F, 2012. Heterozygosity-Fitness correlations and inbreeding depression in two critically endangered mammals. Conservation biology 26:1121–1129. doi: 10.1111/j.1523-1739.2012.01916.
Sachs BA, 1997. Erection Evoked in Male Rats by Airborne Scent from Estrous Females. Physiology & Behavior 62:921-924.
Sanderson JL, Wang J, Vitikainen EI, Cant MA, Nichols HJ, 2015. Banded mongooses avoid inbreeding when mating with members of the same natal group. Mol Ecol 24:3738-3751. doi: 10.1111/mec.13253.
Scantlebury M, Maher McWilliams M, Marks NJ, Dick JTA, Edgar H, Lutermann H, 2010. Effects of life-history traits on parasite load in grey squirrels. Journal of Zoology 282:246-255. doi: 10.1111/j.1469-7998.2010.00734.x.
Schaal B, Coureaud G, Langlois D, Ginies C, Semon E, Perrier G, 2003. Chemical and behavioural characterization of the rabbit mammary pheromone. Nature 424:68-72. doi: 10.1038/nature01739.
Schalk G, Forbes, M., R, 1997. MAle biases in parasitism of mammals: Effects of study type, host age and parasitism taxon. Oikos 78:67-74.
Scordato ES, Drea CM, 2007. Scents and sensibility: information content of olfactory signals in the ringtailed lemur, Lemur catta. Animal Behaviour 73:301-314. doi: 10.1016/j.anbehav.2006.08.006.
Scordato ES, Dubay G, Drea CM, 2007. Chemical composition of scent marks in the ringtailed lemur (Lemur catta): glandular differences, seasonal variation, and individual signatures. Chem Senses 32:493-504. doi: 10.1093/chemse/bjm018.
Setchell JM, Charpentier MJE, Abbott KM, Wickings EJ, Knapp LA, 2009. Is Brightest Best? Testing the Hamilton-Zuk Hypothesis in Mandrills. International Journal of Primatology 30:825-844. doi: 10.1007/s10764-009-9371-0.

Setchell JM, Charpentier MJE, Bedjabaga I-B, Reed P, Wickings EJ, Knapp LA, 2006. Secondary sexual characters and female quality in primates. Behavioral Ecology and Sociobiology 61:305-315. doi: 10.1007/s00265-006-0260-7.

Setchell JM, Vaglio S, Abbott KM, Moggi-Cecchi J, Boscaro F, Pieraccini G, Knapp LA, 2011. Odour signals major histocompatibility complex genotype in an Old World monkey. Proc Biol Sci 278:274-280. doi: 10.1098/rspb.2010.0571.

Shanley DP, Aw D, Manley NR, Palmer DB, 2009. An evolutionary perspective on the mechanisms of immunosenescence. Trends Immunol 30:374-381. doi: 10.1016/j.it.2009.05.001.

Sherborne AL, Thom MD, Paterson S, Jury F, Ollier WE, Stockley P, Beynon RJ, Hurst JL, 2007. The genetic basis of inbreeding avoidance in house mice. Curr Biol 17:2061-2066. doi: 10.1016/j.cub.2007.10.041.

Slate J, David P, Dodds KG, Veenvliet BA, Glass BC, Broad TE, McEwan JC, 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. Heredity (Edinb) 93:255-265. doi: 10.1038/sj.hdy.6800485.

Smith JA, Wilson K, Pilkington JG, Pemberton JM, 1999. Heritable variation in resistance to gastro-intestinal nematodes in an unmanaged mammal population. Proc R soc Lond B 266:1283-1290.

Smythe K, Drea CM, 2015. Patterns of parastism in the cooperativley breeding Meerkat: a cost of dominance for females. Behavioural ecology 132:1-10. doi: 10.1093/beheco/arv132.

Stockley P, Bottell L, Hurst JL, 2013. Wake up and smell the conflict: odour signals in female competition. Philos Trans R Soc Lond B Biol Sci 368:20130082. doi: 10.1098/rstb.2013.0082.

Stockley P, Bro-Jorgensen J, 2011. Female competition and its evolutionary consequences in mammals. Biol Rev Camb Philos Soc 86:341-366. doi: 10.1111/j.1469-185X.2010.00149.x.

Stoffel MA, Esser M, Nichols HJ, Kardos M, David P, Hoffman JI, 2015. InbreedR: An R package for the analysis of inbreeding based on genetic markers. xx xx:x-xx.

Swaisgood R, Lindburg DG, Zhou X, 1999. Giant pandas discriminate individual differences in conspecific scent. Animal Behaviour 57:1045-1053.

Szulkin M, Bierne N, David P, 2010. Heterozygosity-fitness correlations: a time for reappraisal. Evolution 64:1202-1217. doi: 10.1111/j.1558-5646.2010.00966.x.

Thom MD, Hurst JL, 2004. Individual recognition by scent. Annales Zoologici Fennici 41:765-787.

Thom MD, Stockley P, Jury F, Ollier WE, Beynon RJ, Hurst JL, 2008. The direct assessment of genetic heterozygosity through scent in the mouse. Curr Biol 18:619-623. doi: 10.1016/j.cub.2008.03.056.

Thompson FJ, Marshall HH, Sanderson JL, Vitikainen EI, Nichols HJ, Gilchrist JS, Young AJ, Hodge SJ, Cant MA, 2016. Reproductive competition triggers mass eviction in cooperative banded mongooses. Proc Biol Sci 283. doi: 10.1098/rspb.2015.2607.

Turner WC, Versfeld WD, Kilian JW, Getz WM, 2012. Synergistic effects of seasonal rainfall, parasites and demography on fluctuations in springbok body condition. J Anim Ecol 81:58-69. doi: 10.1111/j.1365-2656.2011.01892.x.

Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW, 1996. Veterinary parasitology, Second ed. Oxford UK: Blackwell Science.

van Zweden JS, d'Ettorre P, 2010. Nestmate recognition in social insects and the role of hydrocarbons. In: Blomquist GJ, Bagnères A-G, editors. Insect Hydrocarbons: Biology, Biochemistry and chemical Ecology Cambridge: Cambridge University press. p. 222-243.

Venables WN, Ripley BD, 2002. Modern applied statistics with R. Fouth Edition. New York: Springer.

Veron G, Colyn M, Dunham AE, Taylor P, Gaubert P, 2004. Molecular systematics and origin of sociality in mongooses (Herpestidae, Carnivora). Molecular Phylogenetics and Evolution 30:582-598. doi: 10.1016/s1055-7903(03)00229-x.

Villanua D, Perez-Rodriguez L, Gortazar C, Hofle U, Vinuela J, 2006. Avoiding bias in parasite excretion estimates: the effect of sampling time and type of faeces. Parasitology 133:251-259. doi: 10.1017/S003118200600031X.

Waitt C, Little AC, Wolfensohn S, Honess P, Brown AP, Buchanan-Smith HM, Perrett DI, 2003. Evidence from rhesus macaques suggests that male coloration plays a role in female primate mate choice. Proc Biol Sci 270 Suppl 2:S144-146. doi: 10.1098/rsbl.2003.0065.

Wasser SK, Barash DP, 1983. Reproductive Suppression Among Female Mammals: Implications for Biomedicine and Sexual Selection Theory. The Quarterly review of biology 58:513-538.

Whiteman NK, Matson KD, Bollmer JL, Parker PG, 2006. Disease ecology in the Galapagos Hawk (Buteo galapagoensis): host genetic diversity, parasite load and natural antibodies. Proc Biol Sci 273:797-804. doi: 10.1098/rspb.2005.3396.

Wickham H, 2009. ggplot2: Elegant Graphics for Data Analysis. Verlag New York: Springer.

Wilson K, Bjornstad ON, Dobson AP, Merler S, Poglayen G, Randolph AF, Skorping K, 2002. Heterogeneities in macroparasite infections: patterns and processes. In: Hudson PJ, Rizzoli A, Greenfell BT, Heesterbeek H, Dobson AP, editors. The Ecology of wildlife diseases New York: Oxford University Press.

Wimmer B, Craig BH, Pilkington JG, Pemberton JM, 2004. Non-invasive assessment of parasitic nematode species diversity in wild Soay sheep using molecular markers. Int J Parasitol 34:625-631. doi: 10.1016/j.ijpara.2003.11.022.

Wolff JO, Mech SG, Thomas SA, 2002. Scent marking in female prairie voles: a test of alternative hypotheses. Ethology 108:483-494.

Wood EL, Matthews JB, Stephenson S, Slote M, Nussey DH, 2013. Variation in fecal egg counts in horses managed for conservation purposes: individual egg shedding consistency, age effects and seasonal variation. Parasitology 140:115-128. doi: 10.1017/S003118201200128X.

Wyatt TD, 2014. Pheromones and animal behaviour. Cambridge: Cambridge University Press.

Wyatt TD, 2015. The search for human pheromones: the lost decades and the necessity of returning to first principles. Proc Biol Sci 282:20142994. doi: 10.1098/rspb.2014.2994.

Yamazaki K, Yamaguchi L, Baranoski L, Bard J, Boyse EA, Thomas L, 1979. Recognition among mice. The Journal of Experimental Medicine 150:755-760.

Zala SM, 2004. Scent-marking displays provide honest signals of health and infection. Behavioral Ecology 15:338-344. doi: 10.1093/beheco/arh022.

Zala SM, Bilak A, Perkins M, Potts WK, Penn DJ, 2015. Female house mice initially shun infected males, but do not avoid mating with them. Behavioral Ecology and Sociobiology 69:715-722. doi: 10.1007/s00265-015-1884-2.

Ziegler TE, Epple g, Snowdon CT, Porter TA, Belcher AM, Kuderling I, 1993. Detection of the chemical signals of ovulation in the cotton-top tamarin, Saguinus oedipus. Animal Behaviour 45:313-322. doi: http://dx.doi.org/10.1006/anbe.1993.1036.

Zuk M, McKean KA, 1996. Sex differences in parasite infections: patterns and processes. Journal of Parasitology 26:1009-1024.

Appendix A: Supporting information for chapters 3 and 4.

Table 1: Common explanatory terms used within models of chapters 3 and 4.

Variable	Unit of measure	Calculations details and justification
Recipient Sex	Categorical (0 = male, 1 = female)	Sex of the banded mongoose receiving the odour presentation. Gender assigned on first capture
Donor sex	Categorical (0 = male, 1 = female)	Sex of the banded mongoose whom the odour was collected. Gender assigned on first capture
Recipient age	Numerical, measured in days	Age of the mongoose receiving the odour presentation. Age calculated by subtracting date of birth from date of faecal sample collection.
Donor age	Numerical, measured in days	Age of the mongoose donating the odour. Age calculated by subtracting date of birth from date of odour sample collection.
Pregnant odour	Categorical (0= non pregnant, 1 = pregnant)	Reproductive state of the female odour donor in chapter 4's odour presentations. Donor pregnancy determined by ultra-sound scan on the date of odour collection. Recipient pregnancy determined by ultra-sound scan <10 days prior to presentation.
Unfamiliar	Factorial with 2 levels, familiar or unfamiliar	The familiarity of the odour donor to the recipient in chapter 3's odour presentations. Familiar trails refer to presentations between animals in the same social group. Unfamiliar presentations between animals from non-neighbouring groups.
Relatedness	Continuous numerical variable ranging between 0 and 1	Calculated from raw allele frequencies of 42 highly polymorphic microsatellite markers (Sanderson et al., 2015) using the Lynch and Ritland method with the InbreedR package (Stoffel et al., 2015)

Table 2: The effect of donor and recipient age upon response to presented odours.

Model testing	Fixed effect	Effect size	SD	t value	p value
DURATION BEFORE RETURN TO NORAMAL BEHAVIOUR	Intercept	30.600	7.6873		
	Recipient Age	-0.006	0.020	-0.30	0.764
	Intercept	34.767	6.636		
	Odour Age	-0.0002	0.0001	-1.567	0.118
CONTACT	Intercept	12.557	5.322		
	Recipient Age	0.011	0.012	0.944	0.346
	Intercept	1.678e+01	4.513e+00		
	Odour Age	-1.019e-04	5.411e-05	-1.883	0.060
TOTAL MARKING	Intercept	4.762	1.529		
	Recipient Age	0.007	0.004	1.863	0.063
	Intercept	6.543e+00	1.265e+00		
	Odour Age	-3.530e-05	1.752e-05	-2.015	0.059

Neither the age of odour-donor nor recipient had a significant effect response measures to presented odours within the mixed-sex dataset utilised for chapter 3's analyses.

Table 3: The chemical compounds identified with banded mongoose anal gland secretions

Jordan Analysis		**Mitchell**

			Analysis
Compound Number	**Retention time (s)**	**Possible ID**	**Mean retention time**
NA			8.63
NA			8.75
c1	10.0–10.5	Phenol	10.48
c2	11.9–13.2	Benzene ethanamine	12.57
NA			14.90
c3	15.8–16.0	1H-indole	
c4	17.1–17.2		
c5	18.1–18.3		
c6	18.4–18.5	Decanedioic acid,didecy lester	
c7	19.1–19.3	2,6-nonadienal,(E,E)-Dodecanoic acid Tridecanol	
c8	19.6–19.9		
c9	20.0–20.1		20.55
c10	20.4–20.8	1-tetradecanol	
c11	20.9–21.1	Triacontane	
c12	21.9–22.5	Propanoic acid, 2-methyl,2-phenylethyl Butanoic acid	22.51
c13	22.7	Acetamide, N-(2-phenylethyl)	
c14	22.8		
c15	23.1–23.5	3-eicosene, E 9-eicosene, E 1-tetracosanal	
c16	23.7–24.0	Hexadecanoic acid	
c17	24.2–24.5	Propanoic acid, 2-methyl,2-phenylethyl Butanoic acid	
c18	24.6–24.9	1, 13-tetradecadiene 1-hexadecanol	
c19	25.0–25.2	Phosphonic acid dioctadecylester	
c20	25.3–25.6	Octadecadienoic acid	
c21	25.7–25.9	9, 12,-octadecadienoic	
c22	26.0–26.3		
c23	26.8–27.1	1-hexadecanol 1-octadecanol	26.93
c24	27.2–28.2	9-octadecen-1-ol, (z)- Tetradecanol	
c25	28.3–28.5	Tricosane	
c26	28.6–28.9	Benzenepropanoic acid, methyl ester	
c27	29.2–29.4		
			30.39
c28	30.8–31.3	Acetamide,N-(2-phenylethyl)	
c29	31.4–31.7	Acetamide,N-(2-phenylethyl)	31.61
c30	32.4–32.6	1-Octadecanol phosphonic acid, dioctadecylester	
c31	33.8–34.1	Acetamide,N-(2-phenylethyl)	34.81
NA			34.56
c32	35.8–36.0	Vitamin E	35.95
c33	36.1–36.3		
c34	36.4–36.6		
c35	36.7–37.0	Cholesterol	37.30
NA		Likely Cholesterol derivatives	37.67
NA		Likely Cholesterol derivatives	38.04

NA		Likely Cholesterol derivatives	39.74
NA		Likely Cholesterol derivatives	40.29
NA		Likely Cholesterol derivatives	38.42
NA		Likely Cholesterol derivatives	40.80
NA		Likely Cholesterol derivatives	38.36
NA		Likely Cholesterol derivatives	41.34
NA		Likely Cholesterol derivatives	41.26
NA		Likely Cholesterol derivatives	41.24
NA		Likely Cholesterol derivatives	41.63
		Likely Cholesterol derivatives	41.77

Compounds identified within the 41 anal gland secretion samples analysed in chapter 3. Results are presented alongside those of Jordan 2010, alongside tentative identification of certain compounds from both analyses.

Table 4: The effect of donor and recipient age upon response to presented female odours.

Model testing	Fixed effect	Effect size	SD	t value	p value

DURATION BEFORE RETURN TO NORAMAL BEHAVIOUR	Intercept	50.914	10.165		
	Recipient Age	-0.012	0.008	-1.514	0.133
	Intercept	40.507	13.987		
	Odour Age	-0.0003581	0.010	-0.037	0.970
CONTACT	Intercept	28.456	4.497		
	Recipient Age	**-0.011**	**0.004**	**-3.041**	**0.003**
	Intercept	19.405	7.095		
	Odour Age	-0.001	0.005	-0.213	0.832
OVERMARKING	Intercept	9.830	1.540		
	Recipient Age	**-0.002**	**0.001**	**-2.510**	**0.014**
	Intercept	7.714	2.107		
	Odour Age	-0.0002	0.001	-0.159	0.874

In female-female odour presentations, recipients did not change marking response based upon the age of the odour donor however older recipients deposited fewer marks and spent less time in contact with presented odours than their younger conspecifics. Bold text denote significant effects. All models in chapter 4 concerning contact duration and marking behaviour thus control for recipient age.

Table 5: Initial analysis of the effect of presentation type upon over-marking response to presented odours between females.

Fixed effect	Estimate	Std error	t value	P value
Intercept	11.173	1.289		
Type: non to preg	-2.006	1.560	-1.286	0.202
Type: preg to non	**-4.598**	**1.636**	**-2.811**	**0.006**
Type: preg to preg	0.826	1.590	0.520	0.604
Recipient age	**-0.002**	**9.0e04**	**-2.428**	**0.017**

Full output of LMM testing the effect of presentation type upon females' contact duration with presented odours. All comparisons of fixed effects are made in reference to non-non presentations. Tukey post-hoc tests were then run on this model to determine how the state of odour donor and recipient interact to scent marking.

Table 6: Full output of Tukey post-hoc comparison determining the effect of presentation type upon females' over-marking response to presented odours

Comparison	Estimate	Std. Error	z value	Pr(>|z|)
preg to non - non to non	**-4.598**	**1.636**	**-2.811**	**0.025**
preg to preg - preg to non	**5.423**	**1.601**	**3.338**	**0.004**
non to preg - non to non	-2.006	1.560	-1.286	0.571
preg to preg - non to non	0.826	1.590	0.520	0.954
preg to non - non to preg	-2.592	1.754	-1.478	0.450
preg to preg - non to preg	2.832	1.716	1.650	0.349

Full output of Tukey post-hoc comparisons shows non-pregnant females deposit significantly more marks over non-pregnant odours than they do to pregnant odours. Additionally, pregnant odours receive more marks from pregnant rather than non-pregnant recipients. All other comparisons remain non-significant for this response measure.

Table 7: Protein content of banded mongoose anal gland secretions (AGS).

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
3	1	P04264|K2C1_HUMAN	277.50	27	19	7	Y	66039	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
7	4	P13645|K1C10_HUMAN	276.68	22	15	3	Y	58827	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6
6	7	Q6EIZ0|K1C10_CANLF	272.25	22	14	2	Y	57711	Keratin, type I cytoskeletal 10 OS=Canis lupus familiaris GN=KRT10 PE=2 SV=1
11	3	Q6EIY9|K2C1_CANLF	259.99	20	13	2	Y	63790	Keratin, type II cytoskeletal 1 OS=Canis lupus familiaris GN=KRT1 PE=2 SV=1
5	5	P49064|ALBU_FELCA	252.82	21	14	1	Y	68660	Serum albumin OS=Felis catus GN=ALB PE=1 SV=1
9	9	P25473|CLUS_CANLF	240.78	20	11	7	Y	51790	Clusterin OS=Canis lupus familiaris GN=CLU PE=2 SV=1
14	6	P35908|K22E_HUMAN	236.08	17	11	3	Y	65433	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2
17	8	P49822|ALBU_CANLF	220.60	13	10	1	Y	68605	Serum albumin OS=Canis lupus familiaris GN=ALB PE=1 SV=3
38	22	P07602|SAP_HUMAN	210.67	11	6	2	Y	58113	Prosaposin OS=Homo sapiens GN=PSAP PE=1 SV=2
10	67	P00761|TRYP_PIG	199.82	25	4	3	Y	24409	Trypsin OS=Sus scrofa PE=1 SV=1
28	53	P07339|CATD_HUMAN	194.73	14	5	1	Y	44552	Cathepsin D OS=Homo sapiens GN=CTSD PE=1 SV=1
18	10	P35527|K1C9_HUMAN	191.99	13	8	6	N	62064	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3
23	19	P14639|ALBU_SHEEP	191.23	9	7	1	Y	69188	Serum albumin OS=Ovis aries GN=ALB PE=1 SV=1
97	27	P26779|SAP_BOVIN	186.19	18	7	4	Y	58051	Prosaposin OS=Bos taurus GN=PSAP PE=1 SV=3
43	66	Q4LAL9|CATD_CANLF	184.77	14	4	1	Y	44320	Cathepsin D OS=Canis lupus familiaris GN=CTSD PE=2 SV=1
30	15	Q6IG01|K2C1B_RAT	183.71	10	6	2	Y	57255	Keratin, type II cytoskeletal 1b OS=Rattus norvegi

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
									cus GN=Krt77 PE=3 SV=1
16	49	Q2T9M7|P20D1_BOVIN	183.22	10	5	2	Y	55785	Probable carboxypeptidase PM20D1 OS=Bos taurus GN=PM20D1 PE=2 SV=1
82	68	A1E295|CATB_PIG	182.18	16	5	3	Y	36901	Cathepsin B OS=Sus scrofa GN=CTSB PE=1 SV=1
32	30	P30740|ILEU_HUMAN	174.48	10	5	1	N	42742	Leukocyte elastase inhibitor OS=Homo sapiens GN =SERPINB1 PE=1 SV=1
67	38	Q18835|BGLR_CANLF	172.26	8	6	1	Y	74433	Beta-glucuronidase OS=Canis lupus familiaris GN= GUSB PE=1 SV=1
52	34	Q5XLE4|ALBU_EQUAS	170.49	10	6	1	Y	68539	Serum albumin OS=Equus asinus GN=ALB PE=1 S V=1
60	129	Q9MZS8|CATD_SHEEP	168.97	14	3	1	Y	39815	Cathepsin D (Fragment) OS=Ovis aries GN=CTSD PE=1 SV=1
54	29	Q7Z794|K2C1B_HUMAN	168.85	8	5	3	Y	61901	Keratin, type II cytoskeletal 1b OS=Homo sapiens GN=KRT77 PE=2 SV=3
281	124	Q28262|PAFA_CANLF	132.04	9	4	2	Y	50136	Platelet-activating factor acetylhydrolase OS=Canis lupus familiaris GN=PLA2G7 PE=2 SV=1
578	287	Q8WNN6|SODC_CANLF	130.95	17	2	1	N	15921	Superoxide dismutase [Cu-Zn] OS=Canis lupus fa miliaris GN=SOD1 PE=2 SV=1
78	168	P81265|PIGR_BOVIN	128.80	3	2	1	Y	82435	Polymeric immunoglobulin receptor OS=Bos taurus GN=PIGR PE=2 SV=1
102	147	Q5RDA4|LG3BP_PONAB	128.01	5	3	1	Y	65267	Galectin-3-binding protein OS=Pongo abelii GN=LG ALS3BP PE=1 SV=1
102	148	Q08380|LG3BP_HUMAN	128.01	5	3	1	Y	65331	Galectin-3-binding protein OS=Homo sapiens GN= LGALS3BP PE=1 SV=1
92	443	P37153|APOD_RABIT	127.68	20	3	2	Y	21478	Apolipoprotein D OS=Oryctolagus cuniculus GN=AP OD PE=2 SV=1
643	299	Q13231|CHIT1_HUMAN	123.34	7	3	3	Y	51681	Chitotriosidase-1 OS=Homo sapiens GN=CHIT1 PE =1 SV=1
34	271	Q9BQS7|HEPH_HUMAN	118.16	2	3	2	Y	130449	Hephaestin OS=Homo sapiens GN=HEPH PE=2 SV

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
									=3
1738	822	P20757|ANGT_SHEEP	116.04	6	2	2	Y	51304	Angiotensinogen OS=Ovis aries GN=AGT PE=1 SV=2
2739	833	P01620|KV302_HUMAN	112.31	15	1	1	N	11775	Ig kappa chain V-III region SIE OS=Homo sapiens PE=1 SV=1
2739	831	P01623|KV305_HUMAN	112.31	15	1	1	N	11746	Ig kappa chain V-III region WOL OS=Homo sapiens PE=1 SV=1
2739	835	P18135|KV312_HUMAN	112.31	12	1	1	N	14073	Ig kappa chain V-III region HAH OS=Homo sapiens PE=2 SV=1
2739	834	P18136|KV313_HUMAN	112.31	12	1	1	N	14089	Ig kappa chain V-III region HIC OS=Homo sapiens PE=1 SV=2
216	89	P62976|UBIQP_CRIGR	111.21	5	3	3	N	73950	Polyubiquitin OS=Cricetulus griseus PE=2 SV=2
153	149	Q9WV54|ASAH1_MOUSE	109.21	5	2	1	Y	44670	Acid ceramidase OS=Mus musculus GN=Asah1 PE=1 SV=1
398	84	Q08DD1|ARSA_BOVIN	108.91	4	2	1	Y	53807	Arylsulfatase A OS=Bos taurus GN=ARSA PE=2 SV=1
863	123	P05154|IPSP_HUMAN	106.40	4	2	2	N	45675	Plasma serine protease inhibitor OS=Homo sapiens GN=SERPINA5 PE=1 SV=3
218	122	P61917|NPC2_PANTR	106.26	17	3	2	Y	16570	Epididymal secretory protein E1 OS=Pan troglodytes GN=NPC2 PE=2 SV=1
218	121	P61916|NPC2_HUMAN	106.26	17	3	2	Y	16570	Epididymal secretory protein E1 OS=Homo sapiens GN=NPC2 PE=1 SV=1
218	120	P61918|NPC2_MACFA	106.26	17	3	2	Y	16570	Epididymal secretory protein E1 OS=Macaca fascicularis GN=NPC2 PE=2 SV=1
248	649	P29700|FETUA_PIG	104.57	6	2	1	Y	38425	Alpha-2-HS-glycoprotein (Fragment) OS=Sus scrofa GN=AHSG PE=1 SV=1
577	256	Q5R6D1|CATB_PONAB	104.56	8	2	1	Y	37821	Cathepsin B OS=Pongo abelii GN=CTSB PE=2 SV=1
577	255	Q4R5M2|CATB_MACFA	104.56	8	2	1	Y	37777	Cathepsin B OS=Macaca fascicularis GN=CTSB PE=

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
									2 SV=1
58	958	P51909|APOD_CAVPO	103.72	10	2	1	Y	21610	Apolipoprotein D OS=Cavia porcellus GN=APOD PE =2 SV=1
531	220	Q29545|ICA_PIG	103.31	2	2	2	N	77634	Inhibitor of carbonic anhydrase OS=Sus scrofa GN =ICA PE=1 SV=1
66	378	P05090|APOD_HUMAN	103.27	7	2	1	Y	21276	Apolipoprotein D OS=Homo sapiens GN=APOD PE= 1 SV=1
2149	219	P62805|H4_HUMAN	97.09	17	2	2	N	11367	Histone H4 OS=Homo sapiens GN=HIST1H4A PE= 1 SV=2
2149	218	P62803|H4_BOVIN	97.09	17	2	2	N	11367	Histone H4 OS=Bos taurus PE=1 SV=2
2149	217	Q4R362|H4_MACFA	97.09	17	2	2	N	11367	Histone H4 OS=Macaca fascicularis GN=QtsA-1932 7 PE=3 SV=1
2149	216	P62802|H4_PIG	97.09	17	2	2	N	11367	Histone H4 OS=Sus scrofa PE=1 SV=2
2149	215	P62806|H4_MOUSE	97.09	17	2	2	N	11367	Histone H4 OS=Mus musculus GN=Hist1h4a PE=1 SV=2
2149	214	P62804|H4_RAT	97.09	17	2	2	N	11367	Histone H4 OS=Rattus norvegicus GN=Hist1h4b PE =1 SV=2
2149	213	Q5RCS7|H4_PONAB	97.09	17	2	2	N	11367	Histone H4 OS=Pongo abelii PE=3 SV=1
644	142	P50428|ARSA_MOUSE	94.95	3	2	1	N	53748	Arylsulfatase A OS=Mus musculus GN=Arsa PE=1 SV=2
1018	140	Q0V8R6|HEXA_BOVIN	92.54	3	2	2	N	60353	Beta-hexosaminidase subunit alpha OS=Bos taurus GN=HEXA PE=2 SV=1
532	915	P11936|DNAS1_PIG	92.11	6	2	2	Y	31626	Deoxyribonuclease-1 OS=Sus scrofa GN=DNASE1 PE=1 SV=2
1740	826	Q9D7X8|GGCT_MOUSE	91.40	7	1	1	N	21166	Gamma-glutamylcyclotransferase OS=Mus musculu s GN=Ggct PE=1 SV=1
314	847	P20758|IGHA1_GORGO	90.19	3	1	1	N	37756	Ig alpha-1 chain C region OS=Gorilla gorilla gorilla GN=IGHA1 PE=1 SV=1
314	846	P01876|IGHA1_HUMAN	90.19	3	1	1	N	37655	Ig alpha-1 chain C region OS=Homo sapiens GN=I

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
									GHA1 PE=1 SV=2
864	167	O19113|CTGF_PIG	89.83	7	2	1	Y	38007	Connective tissue growth factor OS=Sus scrofa GN =CTGF PE=2 SV=1
1466	840	P01810|HVM40_MOUSE	87.96	10	2	2	Y	13240	Ig heavy chain V region J539 OS=Mus musculus PE =1 SV=1
2175	843	Q52RN5|SODC_BOSMU	85.25	7	1	1	Y	15658	Superoxide dismutase [Cu-Zn] OS=Bos mutus gru nniens GN=SOD1 PE=2 SV=3
2175	842	P00442|SODC_BOVIN	85.25	7	1	1	Y	15683	Superoxide dismutase [Cu-Zn] OS=Bos taurus GN =SOD1 PE=1 SV=2
29	373	Q32KY0|APOD_BOVIN	85.23	10	2	1	Y	21402	Apolipoprotein D OS=Bos taurus GN=APOD PE=2 S V=1
1734	223	Q8N1N4|K2C78_HUMAN	84.94	4	2	1	Y	56866	Keratin, type II cytoskeletal 78 OS=Homo sapiens GN=KRT78 PE=2 SV=2
2155	635	P03988|IGHM_RABIT	83.23	5	2	1	Y	49897	Ig mu chain C region secreted form OS=Oryctolagu s cuniculus PE=2 SV=1
2155	636	P04221|MUCM_RABIT	83.23	5	2	1	Y	52351	Ig mu chain C region membrane-bound form OS=O ryctolagus cuniculus PE=2 SV=2
1026	422	O08841|QSOX1_CAVPO	82.92	1	1	1	N	68595	Sulfhydryl oxidase 1 OS=Cavia porcellus GN=QSOX 1 PE=2 SV=2
757	361	Q9XSB8|TPP1_CANLF	81.12	4	2	1	N	61362	Tripeptidyl-peptidase 1 OS=Canis lupus familiaris G N=TPP1 PE=3 SV=1
189	562	P25311|ZA2G_HUMAN	79.34	6	2	2	Y	34259	Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=A ZGP1 PE=1 SV=2
3478	184	Q08188|TGM3_HUMAN	78.61	3	2	2	Y	76632	Protein-glutamine gamma-glutamyltransferase E O S=Homo sapiens GN=TGM3 PE=1 SV=4
665	795	Q8WNR9|CYTA_FELCA	74.99	12	1	1	N	11041	Cystatin-A OS=Felis catus GN=CSTA PE=1 SV=1
4599	779	P01784|HV01_CANLF	74.05	17	1	1	N	12430	Ig heavy chain V region GOM OS=Canis lupus famil iaris PE=1 SV=1
1435	584	Q9N0C7|EPDR1_MACFA	71.54	5	1	1	Y	25532	Mammalian ependymin-related protein 1 OS=Maca

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
									ca fascicularis GN=EPDR1 PE=2 SV=3
1033	884	Q9UHL4|DPP2_HUMAN	71.50	2	1	1	N	54342	Dipeptidyl peptidase 2 OS=Homo sapiens GN=DPP 7 PE=1 SV=3
1241	848	Q9XT56|JAM1_BOVIN	70.67	4	1	1	Y	32456	Junctional adhesion molecule A OS=Bos taurus GN =F11R PE=2 SV=1
6309	852	P81605|DCD_HUMAN	65.69	10	1	1	N	11284	Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2
3479	245	P15586|GNS_HUMAN	63.31	2	1	1	N	62082	N-acetylglucosamine-6-sulfatase OS=Homo sapien s GN=GNS PE=1 SV=3
1448	555	P19957|ELAF_HUMAN	59.43	8	1	1	Y	12270	Elafin OS=Homo sapiens GN=PI3 PE=1 SV=3
1239	812	Q6NXT2|H3C_HUMAN	57.85	5	1	1	N	15214	Histone H3.3C OS=Homo sapiens GN=H3F3C PE=1 SV=3
880	565	Q9HCJ5|ZSWM6_HUMAN	57.70	1	1	1	N	133470	Zinc finger SWIM domain-containing protein 6 OS= Homo sapiens GN=ZSWIM6 PE=1 SV=2
2744	855	P79105|S10AC_BOVIN	57.15	10	1	1	N	10685	Protein S100-A12 OS=Bos taurus GN=S100A12 PE =1 SV=3
670	638	Q3T0I2|CATH_BOVIN	56.35	2	1	1	N	37352	Pro-cathepsin H OS=Bos taurus GN=CTSH PE=2 S V=1
1465	858	P01790|HVM21_MOUSE	56.34	16	1	1	Y	13652	Ig heavy chain V region M511 OS=Mus musculus P E=1 SV=1
1465	857	P01789|HVM20_MOUSE	56.34	16	1	1	Y	13626	Ig heavy chain V region M603 OS=Mus musculus P E=1 SV=1
1465	864	P01787|HVM18_MOUSE	56.34	15	1	1	Y	13777	Ig heavy chain V regions TEPC 15/S107/HPCM1/HP CM2/HPCM3 OS=Mus musculus PE=1 SV=1
1465	863	P01792|HVM23_MOUSE	56.34	15	1	1	Y	13880	Ig heavy chain V region HPCG8 OS=Mus musculus PE=1 SV=1
1465	862	P01791|HVM22_MOUSE	56.34	15	1	1	Y	13895	Ig heavy chain V region HPCM6 OS=Mus musculus PE=1 SV=1
1465	861	P01794|HVM25_MOUSE	56.34	15	1	1	Y	13807	Ig heavy chain V region HPCG14 OS=Mus musculus PE=1 SV=1

Protein Group	Protein ID	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	PTM	Avg. Mass	Description
1465	860	P01793|HVM24_MOUSE	56.34	15	1	1	Y	13808	Ig heavy chain V region HPCG13 OS=Mus musculus PE=1 SV=1
1465	859	P01788|HVM19_MOUSE	56.34	15	1	1	Y	13805	Ig heavy chain V region H8 OS=Mus musculus PE=1 SV=1
1153	2951	Q5T8P6|RBM26_HUMAN	56.22	0	1	1	N	113597	RNA-binding protein 26 OS=Homo sapiens GN=RBM26 PE=1 SV=3
1153	2970	Q6NZN0|RBM26_MOUSE	56.22	0	1	1	N	114143	RNA-binding protein 26 OS=Mus musculus GN=Rbm26 PE=1 SV=2
1759	595	Q3MI05|PPGB_BOVIN	55.23	2	1	1	N	53980	Lysosomal protective protein OS=Bos taurus GN=CTSA PE=2 SV=1
2745	951	A6QP57|TGM3_BOVIN	52.14	1	1	1	N	76791	Protein-glutamine gamma-glutamyltransferase E OS=Bos taurus GN=TGM3 PE=2 SV=1
3481	666	Q32LE4|GGCT_BOVIN	51.57	5	1	1	N	21177	Gamma-glutamylcyclotransferase OS=Bos taurus GN=GGCT PE=2 SV=1
6310	877	Q96P63|SPB12_HUMAN	49.39	2	1	1	N	46276	Serpin B12 OS=Homo sapiens GN=SERPINB12 PE=1 SV=1
1069	963	P01888|B2MG_BOVIN	45.12	5	1	1	N	13677	Beta-2-microglobulin OS=Bos taurus GN=B2M PE=1 SV=2
1069	964	O77526|B2MG_CALPP	45.12	5	1	1	N	13729	Beta-2-microglobulin OS=Callicebus personatus personatus GN=B2M PE=3 SV=1
total 102 proteins									

Appendix B: Appendices for Chapters 5 to 7

Table 1: Explanatory variables included in models of chapters 5 to 7.

Variable	Unit of measure	Calculations details and justification
Sex	Categorical (0 = male, 1 = female)	Gender assigned on first capture
Age	Numerical, measure in days	Age on date of faecal sample collection (based on DOB in life-history records)
Weight	Numerical, measured in grams	Average value of morning weights for week preceding sample collection. Banded mongooses are weighed morning and evening; however only morning weights were included in this analysis to exclude the effects of particularly fruitful or challenging foraging days
Age rank	Continuous numerical rank	Following methods of methods of Nichols et al (2010) Individuals were ranked in age order within their social group and sex. For example; oldest female and male each received a rank of 1, the next oldest female and male, 2 and so on. As multiple individuals are born within the same litter, same-sex individuals could be assigned the same rank within a group and as such age and rank are not linearly correlated. This measure is a reliable way of encapsulating dominance information into the model as dominance for each sex is closely linked to age but, again, not in an entirely linear fashion (Bell et al., 2012; Cant et al., 2013)
Rainfall	Numerical, measure in mm	Total rainfall in mm during the 30-day period prior to sample collection 30-day timeframe deemed appropriate as peaks in parasitism tend to lag one month behind rainfall (Turner et al. 2012)
sMLH	Numerical measure (rage: -1 to 1)	Calculated from raw allele frequencies using the inbreedR package for analyses in chapter 6 only.
Inbreeding coefficient	Numerical measure (range: 0 to 1)	Calculated from the nine-generation-deep pedigree of the study population for use in analyses in chapter 6 only.

Details of explanatory variables use in mixed models to how parasite burdens vary with social, life-history and ecological factors. Unless specified, all models included individual and pack ID as random factors to control for multiple sampling of the same individuals and packs across the study period.

Table 2: Differences in the prevalence of specific parasites based on social group.

Parasite	Pack comparison	Estimate	Std. Error	z value	Pr(>|z|)
Isospora	17-11	0.37461	0.76571	-0.489	0.9961
	1B-11	-0.43039	0.59804	-0.720	0.9774
	1H-11	-0.60920	0.59906	-1.017	0.9042
	2-11	1.59504	0.92187	1.730	0.4888
	7a-11	-0.81567	0.68091	-1.198	0.8248
	1B-17	-0.05579	0.59846	-0.093	1.0000
	1H-17	-0.23459	0.59656	-0.393	0.9986
	2-17	1.96965	0.92305	2.134	0.2511
	7a-17	-0.44107	0.67833	-0.650	0.9856
	1H-1B	-0.17880	0.36228	-0.494	0.9960
	2-1B	2.02543	0.78732	2.573	0.0946
	7a-1B	-0.38528	0.48606	-0.793	0.9655
	2-1H	**2.20424**	**0.79142**	**2.785**	**0.0542**
	7a-1H	-0.20648	0.47986	-0.430	0.9979
	7a-2	**-2.41071**	**0.85552**	**-2.818**	**0.0495**
Toxocara	17-11	-1.68640	0.64978	-2.595	0.09115
	1B-11	**-1.54903**	**0.46058**	**-3.363**	**0.00920**
	1H-11	-0.45857	0.44954	-1.020	0.90435
	2-11	-1.47018	0.54877	-2.679	0.07337
	7a-11	**-1.72722**	**0.57008**	**-3.030**	**0.02711**
	1B-17	0.13737	0.54866	0.250	0.99985
	1H-17	1.22782	0.53942	2.276	0.19092
	2-17	0.21622	0.62453	0.346	0.99928
	7a-17	-0.04082	0.64334	-0.063	1.00000
	1H-1B	**1.09045**	**0.28442**	**3.834**	**0.00165**
	2-1B	0.07885	0.42421	0.186	0.99997
	7a-1B	-0.17819	0.45145	-0.395	0.99864
	2-1H	-1.01160	0.41220	-2.454	0.12832
	7a-1H	**-1.26865**	**0.44018**	**-2.882**	**0.04186**
	7a-2	-0.25704	0.54112	-0.475	0.99670
Hookworm	17-11	0.54545	0.66533	0.820	0.961
	1B-11	0.02008	0.52445	0.038	1.000
	1H-11	0.04779	0.52717	0.091	1.000
	2-11	-1.26095	0.71901	-1.754	0.478
	7a-11	**1.94940**	**0.62619**	**3.113**	**0.021**
	1B-17	-0.52537	0.52232	-1.006	0.910
	1H-17	-0.49766	0.52303	-0.952	0.928
	2-17	-1.80641	0.72078	-2.506	0.114
	7a-17	1.40395	0.61553	2.281	0.190
	1H-1B	0.02771	0.32924	0.084	1.000
	2-1B	-1.28103	0.59069	-2.169	0.238
	7a-1B	**1.92932**	**0.46982**	**4.106**	**<0.001**
	2-1H	-1.30874	0.59452	-2.201	0.223
	7a-1H	**1.90162**	**0.46717**	**4.070**	**<0.001**
	7a-2	**3.21036**	**0.68900**	**4.659**	**<0.001**
Tapeworm	17-11	-1.38238	0.61277	-2.256	0.2048
	1B-11	**-2.19299**	**0.48746**	**-4.499**	**<0.001**
	1H-11	**-2.79399**	**0.52034**	**-5.370**	**<0.001**
	2-11	**-1.44692**	**0.54700**	**-2.645**	**0.0829**
	7a-11	**-2.64084**	**0.64353**	**-4.104**	**<0.001**
	1B-17	-0.81061	0.49543	-1.636	0.5640
	1H-17	**-1.41161**	**0.52782**	**-2.674**	**0.0771**
	2-17	-0.06454	0.55412	-0.116	1.0000
	7a-17	-1.25846	0.64959	-1.937	0.3691
	1H-1B	-0.60100	0.37511	-1.602	0.5868
	2-1B	0.74607	0.41131	1.814	0.4458

7a-1B	-0.44785	0.53300	-0.840	0.9581
2-1H	**1.34707**	**0.44979**	**2.995**	**0.0314**
7a-1H	0.15315	0.56323	0.272	0.9998
7a-2	-1.19392	0.58795	-2.031	0.3152

Results based upon Tukey post-hoc comparisons following an LMM to test the effect of pack upon the prevalence (1 = present, 0 = absent) of each parasite. Analysis considered 358 faecal samples collected from 111 individuals across 5 social groups between July 2013 and August 2015.

Table 3a: Differences in parasite taxa richness (PTR) between pack 11 and all other social groups.

Fixed effects	Effect size	Standard error	T value	P value
Intercept	3.675	0.248		
Pack 17	-1.578	0.374	-4.223	3e-05
Pack 1B	-1.770	0.285	-6.204	5e-09
Pack 1H	-1.574	0.285	-5.518	7e-08
Pack 2	-1.191	0.319	3.738	2e-03
Pack 7A	-1.434	0.338	4.249	3e-05

Results of GLMM testing the effect of social group on parasite taxa richness (PTR) for 358 faecal samples from 111 adult individuals across 5 social groups. Figures compare each listed group's PTR to that of pack 11.

Table 3b: Comparison of parasite taxa richness between social groups.

Pack comparison	Estimate	Std. Error	z value	Pr(>|z|)
17 – 11	**-1.578**	**0.373**	**-4.223**	**<3e-03**
1B -11	**-1.770**	**-6.204**	**0.285**	**< 1e-04**
1H – 11	**-1.574**	**0.285**	**-5.518**	**< 1e-04**
2 -11	**-1.191**	**0.319**	**-3.738**	**0.002**
7A - 11	**-1.434**	**0.337**	**-4.249**	**<3e-03**
1B – 17	-0.192	0.313	-0.614	0.990
1H - 17	0.003	0.313	0.010	1.00
2 - 17	0.386	0.344	1.123	0.867
7A - 17	0. 143	0.361	0.396	0.999
1H – 1B	0.196	0.200	0.978	0.922
2 – 1B	0.578	0.245	2.358	0.164
7A - 1B	0.335	0.269	1.245	0.807
2 – 1H	0.383	0.245	1.561	0.614
7A – 1H	0.140	0.269	0.519	0.995
7A - 2	-0.243	0.304	-0.798	0.966

Output of Tukey multiple comparison test performed upon the GLMM testing the effect of social group upon PTR (Table 6, full text). Results based upon 358 faecal samples from 111 individuals across 5 social groups, all groups show significantly lower PTR than pack 11, no other pairwise comparisons are significant.

Table 4: Parasitic abundance values (epg) contributing most to within-pack similarities.

Pack	Average similarity between group members	Parasite	Average abundance per group member	Average similarity in abundance between group members	% contribution to group similarity	Cumulative contribution to group similarity
11	54.93	*Isospora*	5.30	32.93	59.95	59.95
		Toxocara	3.01	14.21	25.87	85.82
1H	42.80	*Isospora*	3.64	29.95	69.99	69.99
		Toxocara	1.60	10.67	24.92	94.91
2	62.30	*Isospora*	5.88	58.00	93.10	93.10
17	46.40	*Isospora*	3.54	36.99	79.72	79.72
		Toxocara	1.39	5.43	11.70	91.42
7a	46.25	*Isospora*	2.96	20.63	44.61	44.61
		Toxocara	1.68	13.59	29.39	74.00
1B	39.38	*Isospora*	3.01	35.29	89.62	89.62
		Toxocara	0.79	3.15	7.99	97.61

Output of SIMPER, similarity percentages analysis to determine the parasites whose abundance values contribute most to within-group similarities in parasitic community composition. All abundance measures refer to egg-per-gram (epg) counts.

Table 5: Parasitic abundance values (epg) contributing most to differences between packs.

Packs	Average dissimilarity between group members	Parasite	Average dissimilarity in abundance between groups	% contribution to group dissimilarity	Cumulative contribution to group dissimilarity
11&1H	56.34	*Isospora*	18.97	33.67	33.67
		Toxocara	12.96	23.00	56.67
11&2	48.16	*Isospora*	13.33	27.68	27.68
		Toxocara	3.01	26.32	54.00
1H&2	53.33	*Isospora*	26.76	50.19	50.19
		Toxocara	12.06	22.61	72.79
11&17	56.69	*Isospora*	17.08	30.12	30.12
		Toxocara	13.69	24.14	54.26
1H&17	55.87	*Isospora*	22.61	40.47	40.47
		Toxocara	14.08	25.21	65.67
2&17	51.09	*Isospora*	24.55	48.06	48.06
		Toxocara	14.08	20.66	68.72

11&7a	59.42	*Isospora*	18.13	30.52	30.52
		Toxocara	11.24	18.92	49.44
1H&7a	58.01	*Isospora*	20.53	35.39	35.39
		Hookworm	13.23	22.80	58.20
		Toxocara	12.37	21.32	79.51
2&7a	59.03	*Isospora*	25.11	42.54	42.54
		Hookworm	11.71	19.84	62.38
		Toxocara	11.20	18.97	81.35
17&7a	56.51	*Isospora*	18.52	32.77	32.77
		Hookworm	13.23	23.40	56.17
		Toxocara	12.69	22.46	78.64
11&1B	64.27	*Isospora*	23.01	35.81	35.81
		Toxocara	16.60	25.83	61.64
1H&1B	60.99	*Isospora*	29.54	48.43	48.43
		Toxocara	16.49	27.04	75.47
2&1B	55.90	*Isospora*	34.48	61.69	61.69
		Toxocara	10.69	19.13	80.81
17&1B	57.77	*Isospora*	27.58	47.74	47.74
		Toxocara	13.35	23.11	70.85
7a&1B	63.98	*Isospora*	23.18	36.23	36.23
		Hookworm	15.51	24.24	70.85
		Toxocara	15.15	15.56	84.16

Output of SIMPER, similarity percentages analysis to determine the parasites whose abundances contribute most to between-group differences in parasitic community composition. All abundance measures refer to egg-per-gram (epg) counts.

Table 6: The effect of inbreeding coefficient upon parasite load (sMLH not included in model).

Fixed effect	Effect size	Standard error	t-value	p-value
Intercept	10.215	0.167		
Inbreeding coefficient	1.822	1.544	1.180	0.244
Age (in days)	0.0001	0.0002	0.377	0.707
Sex (female)	-0.053	0.267	-0.199	0.843

GLMM fit by penalised-quasi likelihood, testing the effect of age, sex and inbreeding coefficient upon average ova load for the study period. sMLH removed from model to show NS effect of inbreeding coefficient and negligible collinearity between these two genetic variables for the study population. Original model included all second order interactions but these were sequentially removed due to non-significance. All fixed effects had non-significant (NS) effects on parasite load and are presented here alongside the p-values upon which they were sequentially rejected from the model.

Table 7: The effect of sMLH and inbreeding coefficient on average *Isospora* load.

Fixed effect	Effect size	Standard error	t-value	p-value
Intercept	3.751	0.949		
sMLH	1.618	0.932	-1.734	0.089
Age (in days)	-0.0002	<0.001	-1.050	0.298
Sex (female)	-0.447	0.365	-1.225	0.226
Inbreeding coefficient	1.196	2.310	0.518	0.607

Output of LMM testing effect of sMLH and inbreeding coefficient upon average isospora load. Second order interactions were included in original model but sequentially dropped due to non-significance. Fixed effects are presented alongside the p-values upon which they were dropped from the model, no terms remained significant following model simplification.

Table 8: The effect of sMLH and inbreeding coefficient on average tape worm load.

Fixed effect	Effect size	Standard error	t-value	p-value
Intercept	23.37	15.18		
sMLH	-13.51	14.91	-0.906	0.369
Sex (female)	3.996	5.830	0.685	0.496
Age (in days)	0.002	0.004	0.662	0.511
Inbreeding coefficient	11.512	37.365	0.308	0.759

Output of LMM testing effect of sMLH and inbreeding coefficient upon average tapeworm load. Second order interactions were included in original model but sequentially dropped due to non-significance. All fixed effects are presented alongside the p-values upon which they were dropped from the model, no terms remained significant following model simplification.