Bidirectional Interactions between Behavior and Disease in Banded Mongooses (*mungos mungo*) Infected with *Mycobacterium mungi*

Bonnie Marie Fairbanks

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Dana M. Hawley
Kathleen A. Alexander
William A. Hopkins
Ignacio T. Moore
Jeffery R. Walters

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ABSTRACT

Behavior and disease interact bidirectionally and on multiple levels of host organization, and these interactions can have important consequences for population-level disease dynamics. I explored how behavior can both influence and respond to infectious disease in a banded mongoose population experiencing epidemics of tuberculosis (TB) caused by the bacterial pathogen *Mycobacterium mungi* in the *M. tuberculosis* complex (Alexander et al. 2010). Banded mongooses are highly social carnivores that live in troops of 5 to 65 individuals. *Mycobacterium mungi* appears to be primarily environmentally transmitted, but direct horizontal transmission cannot be ruled out. Approximately 10-20% of mongooses become diseased with TB each year in the study population in and around Chobe National Park, Botswana, and all mongooses with clinical signs of TB die within months. Characteristics of both banded mongooses and clinical TB provided a productive study system for exploring interactions between behavior and disease: first, free-living mongooses can be habituated and directly observed; second, the clinical signs of TB can be visually assessed non-invasively; and third, the mongooses' high sociality and egalitarianism provide a unique and ecologically relevant host social system for examining bidirectional interactions between behavior and infectious disease.

I found that banded mongooses influenced and responded to disease through their behavior at both the individual and troop level, with possible implications for banded mongoose population and disease dynamics. Due to the environmental transmission of *M. mungi*, which appears to invade mongooses through breaks in the skin and nasal planum (Alexander et al. 2010), I focused on aggressive interactions as a potential risk factor for acquiring TB in this system. Troops with higher levels of aggression had more injuries, and at the individual level, injuries were a strong predictor of TB, suggesting that aggression may increase risk of disease by creating potential invasion sites for the pathogen. Troops were more aggressive when they foraged in garbage than when they foraged in other habitats, presumably due to the concentration of resources at this highly modified habitat. Overall, my results on how behavior can influence disease in this system suggest that anthropogenic supplementation of food, albeit inadvertent in this system, augments aggression levels in banded mongooses and may in turn lead to a higher incidence of TB.

Second, I examined how behavior responds to disease in banded mongooses. Diseased individuals showed significantly lower activity and alertness, but intriguingly, did not show a reduction in overall social behaviors. Diseased individuals were less likely to disperse than healthy individuals, and healthy individuals with diseased troopmates may have been more likely to disperse than individuals without diseased troopmates. Despite this latter possible increase in dispersal in the presence of diseased conspecifics, diseased individuals were not avoided by their troopmates in daily social interactions. For example, diseased individuals were allogroomed at a higher than expected rate even though their reciprocation during allogrooming was approximately half that of healthy individuals.

These interactions between behavior and disease have implications for banded mongoose troop and population dynamics, via changes in dispersal behavior and mortality, and can also affect disease dynamics, such as transmission rate. For example, changes to dispersal may affect the amount of inbreeding and outbreeding that occurs in this normally inbred species, and disease might be amplified in areas where aggression is increased by resource augmentation from humans. Additionally, the role that garbage plays in mongoose aggression suggests that humans may be inadvertently increasing disease incidence in this system, as well as in other taxa for which anthropogenic food augmentation may alter disease dynamics via changes in intraspecific aggression. This research sheds light on ways that behavior can influence and respond to disease that are often overlooked in disease ecology.

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Table of contents

List of Table

List of Figures

Chapter 1
Introduction: Interactions Between Host Social Behavior, Physiology, and Disease Susceptibility: The Role of Dominance Status and Social Context

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Introduction

Parasites and pathogens are often considered the bane of social animals (Alexander, 1974; Møller et al., 1993; Loehle, 1995; Altizer et al., 2003; Nunn and Altizer, 2006). Both within and among species, group sizes positively predict parasite and pathogen prevalence for a suite of vertebrates (e.g., Brown and Brown, 1986; Moore et al., 1988; Davies et al., 1991; Cote and Poulin, 1995; Nunn et al., 2003; Ezenwa, 2004) (but see Godfrey et al., 2006; Chapman et al., 2009; Woodroffe et al., 2009). These correlative patterns indicate that group living indeed carries costs in terms of increased transmission of infectious organisms, particularly those with a narrow host range (Loehle, 1995; Ezenwa, 2004). Less well understood are the mechanisms that contribute to the striking variation in infection outcomes within social groups (Hausfater and Watson, 1976; Hawley et al., 2007). Variation in infection outcome within groups is likely the rule; in some cases, only 10% to 40% of closely interacting group members become infected with a directly transmitted parasite or pathogen (Sydenstricker et al., 2006). This heterogeneity likely relates directly to the costs and benefits of group living, which distribute factors important to both exposure and susceptibility (i.e., resources, stress, aggression) disproportionately across group members. Surprisingly few studies have examined the sources of variation in exposure and resistance within social groups, despite the fact that parasites and pathogens represent one of the key costs to group living, and therefore are an important ecological factor mediating the evolution of sociality and the costs and benefits of behavioral strategies within groups (Moller et al., 1993).

Social behavior can lead to variation in pathogen susceptibility and exposure among individuals in a group via two broad proximate pathways. First, social behavior may alter individual susceptibility to infectious organisms via changes in resource access that mediate individual condition and energetics (resource-mediated pathway; Lochmiller, 1996). In addition to influencing immunity, variable resource access directly influences exposure to ingested or environmentally transmitted pathogens. Second, aggressive behavioral interactions may alter immunity via neuroendocrine changes such as circulating hormone levels, a mechanism demonstrated in numerous studies of laboratory vertebrates (e.g., Gross and Siegel, 1973; Barnard et al., 1998; de Groot et al., 1999). Aggressive interactions are also likely to have direct impacts on contact rate and therefore influence exposure to directly transmitted pathogens. Resource- and aggression-mediated pathways rarely act independently to alter individual infection outcomes because condition and aggression are likely to covary both among and within groups—that is, when resources are sparse, competitive behavioral interactions are more frequent. Furthermore, subordinate animals in a group may be both highly susceptible to infection due to poor condition or high levels of received aggression, and have the highest rates of exposure to infectious organisms because they are forced to feed in non-optimal, contaminated

habitats. The nature and extent of these types of patterns for group-living vertebrates—whereby certain group members are more susceptible and/or more exposed—remain largely unknown, and a number of patterns are possible. For example, subordinate group members may have higher rates of exposure to environmentally transmitted organisms if dominant individuals choose resources based on parasite load, or subordinates may have lower exposure to these types of pathogens if these pathogens are present at higher, infectious doses in the most valued resources. Additionally, dominant group members may in some cases have higher exposure to directly transmitted pathogens if they have more social contacts than subordinates; however, if subordinates receive wounds from aggressive encounters with dominants, this may be a pathway for exposure to directly transmitted organisms. Because these patterns and their physiological and behavioral mediators are likely to vary across host social contexts and transmission mode of the infectious organism, they are ripe for exploration in group-living vertebrates.

Scaling up to population-level pathogen dynamics: does individual behavior matter?

Historically, variation among individuals has been largely ignored by population-level disease studies, which generally assume that all individuals in a population have equivalent susceptibility and exposure to pathogens. However, empirical disease studies suggest that individual variation is the rule rather than the exception. In a U.S. gonorrhea outbreak, 60% of infections were caused by only 2% of individuals (Bansal et al., 2007), and in wild mouse populations, 80% of macroparasitic worms are transmitted by less than 20% of individuals (Ferrari et al., 2004). These key hosts are referred to as "superspreaders," or individuals who contribute disproportionately to pathogen transmission (Lloyd-Smith et al., 2005). The most infamous superspreader was Typhoid Mary, an asymptomatic cook who infected more than 54 individuals with Salmonella enterica. Although rarely that extreme, key hosts represent a broader phenomenon whereby only a subset of individuals in a population contribute most significantly to pathogen epidemics. The population-level consequences of key hosts are striking: models reveal that individual variation results in more severe pathogen outbreaks (Lloyd-Smith et al., 2005; Bansal et al., 2007), and furthermore, social hierarchies alone can affect the prevalence and persistence of disease within a group (Davidson et al., 2008). Because key hosts are defined by their contributions to secondary pathogen transmission, or R0 (a pathogen's basic reproduction number), two variables largely characterize these hosts: (1) higher or longer infectiousness due to differences in susceptibility or immune strategies, and (2) more frequent contacts with susceptible conspecifics (Temime et al., 2009). For example, a suite of recent studies identified vertebrate males as key hosts for infectious parasites and pathogens (Perkins et al., 2003; Ferrari et al.,2004; Clay et al., 2009). However, all of the studies were performed on territorial, non-social mammal species. Given the physiological and behavioral suites of changes associated with group living, social factors such as rank may be strong predictors of key hosts in group-living organisms.

In this review, we synthesize the potential pathways by which social status and/or aggressive behavior may influence both susceptibility to disease and exposure to infectious agents. It is particularly difficult to tease apart effects of variables such as social rank on individual susceptibility versus exposure in free-living systems. For example, higher parasite loads are often observed in large, sexually mature (Ferrari et al., 2004) or territorial males (Ezenwa, 2004), which may result from higher exposure, higher susceptibility, or both. We discuss both types of mechanisms, and emphasize the need for more experimental studies that begin to tease apart the role of exposure versus susceptibility. Finally, we emphasize the critical role of social and ecological context on the nature and extent of relationships between dominance status and

disease outcomes. Although studies to date suggest that correlations between dominance status and disease outcomes abound, these relationships are likely to vary both within and among species. We propose that the effect of dominance status on disease outcomes, much like previously described patterns of dominance and stress hormones, is largely context-dependent. In particular, effects of dominance on disease outcome may be a function of intraspecific aggression experienced and the stability of the social group. However, significantly more studies are needed to test these predictions across a suite of vertebrate taxa.

We divide our review into three sections. First, we discuss the causes and effects of dominance status in vertebrates, dividing the literature synthesis largely into behavioral versus physiological effects of dominance. Second, we synthesize and discuss the few studies that have examined the implications of behavioral and physiological correlates of dominance on health outcomes. Third, we discuss exciting topics and challenges for future research.

Causes and Effects of Dominance Status

Why create a social hierarchy?

Animals that live in groups can benefit from the presence of their group-mates in a multitude of ways, including but not limited to increased predator detection and/or avoidance (Pulliam, 1973), increased success in foraging or hunting, and increased access to mates. However, group living also has costs, such as increased competition for food or mates and increased exposure to parasites and pathogens (Alexander, 1974). Social structures such as dominance relationships or ranks may produce disparities across individuals in the way that the costs and benefits of group living are distributed. For example, dominant individuals may have more access to food or mates, but may also have increased exposure to disease because of increased contact rates with conspecifics. Dominance relationships are hypothesized to be useful to both the dominant and subordinate individuals, because it saves both types of individuals the time, energy, and risk of injury associated with repeated conflicts (Creel, 2001). Overall, dominance may be a high-cost, high-benefit strategy, while subordinancy may be a low-cost, low-benefit strategy (Sands and Creel, 2004), allowing all individuals to retain some benefits of group living.

Definitions of dominance

The study of dominance relationships, though fruitful, has some recurring problems. There are varied definitions of dominance, and researchers often fail to explicitly define the term. Because others have made extensive and important arguments for the need for definitions (Bernstein, 1981; Hand, 1986; Drews, 1993), here we briefly make our own definition and encourage investigators to read the papers cited above to understand the importance of properly defining dominance and rank, and the reasons behind the definitions given below.

Most simply, dominance is a relationship between two individuals. Although it may depend on the attributes of each individual, dominance is not an attribute of the individuals themselves. In other words, it is incorrect to call a single individual either dominant or subordinate; these terms must be used in relation to another individual. As a result, selection can act on attributes that affect dominance, such as aggression, but selection cannot act on dominance itself, as this is a relationship that exists between individuals. Although several have been offered, there is one definition of dominance that we find particularly compelling: the dominance relationship is a relationship between two individuals in which there is a consistent winner and loser of agonistic encounters when this winning is based on the outcome(s) of previous encounter(s) (Bernstein, 1981; Hand, 1986; Drews, 1993).

The presence of dominance relationships often leads to the establishment of a stable social hierarchy in animal groups, whereby animals are observed and assigned ranks based on relative wins and losses. Bernstein (1981) points out that, in many cases, there may be little biological significance to rank. An animal may know to whom it is dominant and to whom it is subordinate, but being 6th versus 8th in a group of 20 may not have meaningful biological significance. It is telling that in some studies a significant correlation between a given variable and rank is not found, but when animals are divided into dominant and subordinate groups rather than ranked, relationships between dominance and the given variable begin to appear (e.g., De Luca and Ginsberg, 2001). This distinction suggests that there are likely to be differences between an animal that is dominant in most of its relationships versus one that is subordinate in most of its relationships, but one must use caution when drawing inference between animals of close rank.

Genetic causes and effects of dominance

There are many behavioral, physiological, and genetic attributes that may contribute to an individual's ability to become socially dominant over others. Although genetic correlates of dominance have been characterized in only a single system to date (Tiira et al., 2006), aggressive and social behaviors more broadly have been linked with levels of genetic variation in free-living female rhesus macaques (Charpentier et al., 2008) and levels of inbreeding in mice, Mus musculus (Eklund, 1996; Meagher et al., 2000). Genetic effects can also act to modify the effects of social status; in female rhesus monkeys, Jarrell and coworkers (2008) demonstrated that genetic polymorphism at the serotonin uptake transporter gene interacts with social status to influence physiological changes such as serum leptin levels under high-stress social contexts. These genotype-by-environment interactions that result from social status underscore the potential complexities in dissecting the causes and consequences of dominance. As next-generation sequencing continues to increase the ease of genetic characterization of non-model organisms, we expect a suite of exciting studies at the interface of vertebrate genetics, behavior, and physiology that will begin to tease these complexities apart. Although the current chapter focuses on the effects rather than the causes of dominance, genetic components of rank are important to keep in mind as they may result in genotype-by-environment effects on the rank-related consequences of interest here.

The effects of dominance status on physiology, behavior, and disease is an enormous topic. Therefore, we limit this chapter to a review and synthesis of the literature that correlate behavior and/or physiology to social status, with an emphasis of potential mechanisms by which dominance may affect health via exposure or susceptibility. A suite of studies has looked at correlations between behavior and/or physiology and dominance, but fewer have made strong links between dominance, its health consequences, and the behavior or physiology that mediates this link through exposure to pathogens and/or susceptibility to disease.

Behavioral correlates of dominance

Aggression

Dominance status often correlates with the amount of aggression both given and received. For example, subordinates had more aggression directed toward them in captive male cynomolgus monkeys (Cohen et al., 1997) and male dwarf mongooses during the mating season (Creel et al., 1992). Several studies show dominant individuals initiating more aggression than subordinates (woodland caribou, Rangifer tarandus caribou: Barrette and Vandal, 1986; African wild dogs during the mating season: Creel et al., 1997; dwarf mongoose: Creel and Sands, 2003; captive

female mountain gorillas, Gorilla gorilla beringei: Scott and Lockard, 2006). Bite wounds from aggression increase with rank in spotted hyenas (East et al., 2001), indicating that individuals of higher rank are presumably involved in more costly fights. In some cases, relationships between dominance and aggression are sex-specific: in Nubian ibexes (Capra nubiana), the dominant female initiated and was involved in more aggressive encounters than subordinate females (Shargal et al., 2008). In contrast, the dominant male, which is more solitary in this species, was involved in fewer encounters than subordinate males, although the dominant male initiated most of the aggressive interactions in which he was involved. Finally, in some group-living species such as wolves (Canis lupus), the number of aggressive encounters is unrelated to dominance rank (Sands and Creel, 2004). The above studies represent a small subset of the literature that relates aggression to dominance, but illustrate the widespread but not universal trend whereby dominant individuals typically initiate more aggressive interactions while subordinate individuals have more aggression directed toward them. Several features of aggression, both given and received, have important effects on hormone levels and immunity that will be discussed later in the chapter.

Access to resources

Access to resources is often highly skewed in dominance hierarchies (e.g., Barrette and Vandal, 1986; Scott and Lockard, 2006; Hogstad and Pedersen, 2007; Atwood and Gese, 2008). In fact, the near ubiquity of differential access to resources is the reason that dominance status is sometimes defined and/or measured by it (e.g., Metcalfe et al., 1995) Dominant animals may obtain the best food (Scott and Lockard, 2006), preferred territories or locations in the group (Rubenstein and Hohmann, 1989), or mating privileges (Zine and Krausman, 2000). Their differential resource access means that dominant individuals may in some systems be more or less likely to forage in infectious or contaminated habitats. The role of social status in foraging in parasite-rich versus -poor areas has not been examined to date, but represents a key area of future study given that selective foraging in parasite-free areas has been documented for several vertebrates (Ezenwa, 2004; Hutchings et al., 2007). Overall, the effects of differential access to resources on health may accrue through nutritional effects on immunity, or in some cases, through variable exposure via habitat use.

In summary, both aggression and access to resources are generally correlated with dominance rank. Interestingly, both of these traits can have dual effects on individual disease susceptibility by altering both exposure and immunity. While high aggression levels may help to maintain dominance status, they can also lead to higher exposure to parasites or pathogens through wounds and/or contact with infected individuals. Similarly, higher resource access for dominants can lead to stronger immunity and resistance due to nutritional benefits, but may also result in higher exposure to pathogens and parasites through preferred food sources or locations. The outcome of these dual effects will depend both upon the transmission biology of the pathogen/parasite of interest and the social context of the host under study, both of which are discussed below.

Physiological correlates of dominance

Extensive work has been done on physiological correlates of dominance (Table 1.1). We divide these into three categories: hormones, energetics, and immunocompetence.

Stress hormones

Glucocorticoids are the most commonly measured hormonal stress response. When an animal experiences an acute stressor, the hypothalamic-pituitary-adrenal (HPA) axis responds with a cascade of secretions, ultimately producing glucocorticoids. Glucocorticoids are hormones that signal the body to stop functions unnecessary for immediate survival (i.e., growth, reproduction, digestion) and mobilize energy to areas used to avoid or eliminate the stressor (Sapolsky, 2005). This response is adaptive for short-term stressors, such as fleeing a predator or chasing prey, but long-term activation can be damaging. A healthy stress response typically includes low basal glucorticoid levels and a fast, high pulse of glucorticoids in the presence of an immediate stressor. In contrast, long-term activation of the stress response causes high basal glucorticoid levels and a sluggish glucorticoid response to an acute stressor (Sapolsky, 2005).

A cursory scan of the literature reveals apparent inconsistency among studies of stress hormones and dominance (particularly when dominance is loosely defined by researchers). Social rank and glucorticoids can have positive, negative, or no correlation (Table 1.1), depending on the species, the season, and/or whether the animal is in captivity or the wild. However, patterns have begun to emerge that explain some of this variation. Many early studies were conducted in captivity, and most captive studies show that subordinates have higher circulating basal glucorticoid levels. This has been credited to the fact that subordinates in captivity have limited ability to avoid dominant individuals and therefore experience more harassment and fighting than animals would in the wild (Creel, 2001; Sands and Creel, 2004). However, this does not explain all of the variation in glucorticoids and rank.

Recent reviews and a meta-analysis reveal that social context is key to understanding the direction of relationships between glucocorticoids and dominance status. Sapolsky (2005) reviews several types of primate societies and concludes that when dominants must repeatedly physically assert their rank, experience times of rank instability, or are cooperative breeders, glucorticoid levels tend to be higher in dominants than subordinates. Alternatively, subordinates generally show higher basal glucorticoid levels in societies where there is non-physical rank maintenance within a stable hierarchy, and subordinates are exposed to frequent social stress with little social support. A meta-analysis by Abbott and coworkers (2003) of several primates reveals similar results, concluding that subordinates will have higher basal glucorticoid levels when exposed to higher stressor rates than dominants and have low levels of social support. Finally, reviews of birds and mammals by Creel (2001) and of cooperative breeding mammals by Creel and Sands (2003) further confirm that social context is crucial to understanding trends in glucorticoids. In cooperative breeders, in which only dominants are guaranteed reproduction and subordinates help to raise young, dominants generally have a higher level of basal glucorticoids. Furthermore, it is rare in permanent groups for subordinates to be more stressed than dominants. Together, the above studies indicate that the effects of social rank on stress hormones are strongly context-dependent, with important implications for rank-related immunity and disease susceptibility.

Sex hormones

Because dominants typically have higher aggression rates, many researchers have considered whether the sex steroid testosterone correlates with social dominance. Some studies reveal a positive correlation between testosterone and rank in males (cichlids: Aubin-Horth et al., 2007; Iberian wolves: Barja et al., 2008; wild African wild dogs: Creel et al., 1997; willow tits: Hogstad and Pedersen, 2007; captive African wild dogs: Johnston et al., 2007; Nubian ibexes: Shargal et al., 2008). Interestingly, most of these are cooperative breeders, except Nubian ibexes

and willow tits, although dwarf mongooses are cooperative breeders and do not show a positive relationship between testosterone and rank (Creel et al., 1992). Another study showed a correlation between dominance and testosterone only seasonally in animals that are not cooperative breeders (tropical hair rams: Aguirre et al., 2007), and still other studies show no correlation between testosterone levels and dominance (chickens: Gauly et al., 2007; captive tammar wallabies: Hynes et al., 2005; young white-tailed deer: Taillon and Cote, 2008; Ursine colobus monkeys: Teichroeb and Sicotte, 2008). Beehner and coworkers (2006) show a particularly interesting trend in male chacma baboons: testosterone was not significantly correlated with current rank, but predicted rank several months into the future, as well as future reproduction. Taken together, these studies suggest that in some social contexts, males exhibit elevated levels of testosterone during times of high aggression, competition, or reproduction, but testosterone does not universally correlate with current dominance rank or aggression.

Less research regarding sex hormones and rank has been done with females, and research on testosterone and dominance in females is particularly rare. However, in species where females tend to be dominant to males and/or "masculinized," testosterone or its precursors are often high in females (Aubin-Horth et al., 2007; Drea, 2007; Malaivijitnond et al., 2007). Although most work on testosterone in females has been done in species with masculinized females, differences in testosterone according to rank also occur in species without female masculinization. For example, dominant Nubian ibex females have higher testosterone levels than subordinate females and equal testosterone levels to subordinate males (Shargal et al., 2008).

In females, there are some cases where sex hormones other than testosterone or its precursors vary according to rank. In female dwarf mongooses, the dominant female has higher baseline and peak urinary estrogen conjugates during the mating season (Creel et al., 1992). Similarly, in Iberian wolves, the dominant female has higher levels of fecal progesterone and estradiol compared to subordinates (Barja et al., 2008). In African wild dogs, the dominant female in the group is the only female with a hormone cycle that facilitates reproduction (Creel et al., 1997). The above studies were all conducted on cooperative breeders, whereby the dominant female is often or always the only female in a group that reproduces. Overall, it seems that when a female holds a position of dominance that confers reproductive rights, she has a different hormone profile than subordinates in her group. More studies are needed to see if this holds only across similar social contexts, or if it is a common trend for female hormones to correlate with rank in all vertebrate societies.

Energetics: metabolic rate

Most studies of energetics and dominance have been performed on birds, making this area of research taxonomically narrow. One exception is a study in Atlantic salmon by Metcalfe and coworkers (1995), which showed that metabolic rate was established before hatching, and that those fish with higher metabolic rates were dominant (i.e., claim better feeding positions and obtain desirable food resources), thus suggesting that metabolic rate is one of the causes of dominance in this species. However, in several studies of birds where metabolic rate was correlated with dominance, it appears that metabolic rate is a consequence rather than cause of dominance. For example, in willow tits, metabolic rate positively correlated with dominance, and removing the alpha male caused the beta male to rise to alpha position, and his metabolic rate increased to a rate close to the former alpha (Hogstad, 1987). On the other hand, in dark-eyed juncos, although there was no correlation between rank and metabolic rate before treatment, a dominant male that was moved into a new group and subsequently decreased in rank showed an

increase in metabolic rate (Cristol, 1995). These results suggest that a change in dominance is stressful, resulting in increased metabolic rate, but glucocorticoids were not measured in the former study, and did not change significantly in the latter. Alternatively, Hogstad (1987) suggests that the higher metabolic rate of alpha-male willow tits may result from higher activity levels.

In other studies of metabolic rate, dominance was not manipulated, but metabolic rate was sometimes positively correlated with rank (dippers, basal metabolic rate: Bryant and Newton, 1994; pied flycatchers and great tits, resting metabolic rate: Roskaft et al., 1986). However, not all studies measured the same kind of metabolic rate (e.g., resting metabolic rate, basal metabolic rate). The importance of this was highlighted by Senar and coworkers (2000), who measured both resting metabolic rate (oxygen consumption in the dark for a few hours) and active metabolic rate (oxygen consumption in the light). They found a negative correlation between active metabolic rate and rank, but no correlation between resting metabolic rate and rank. They suggest that subordinates are more susceptible to stress, causing the increase in metabolic rate only when subordinate birds could see their unusual surroundings during the measurement. Another study of dark-eyed juncos found no correlation between resting metabolic rate and rank (Vezina and Thomas, 2000). No study has been able to measure metabolic rate via oxygen consumption during normal activity, because subjects must be in a box or wearing a mask to measure this variable. Thus far, the paucity of studies and inconsistent measurements hinder synthesis regarding metabolic rate and dominance. Future studies should measure both resting/basal metabolic rate according to rank (indicating a physiological difference in ranks) and active metabolic rate during normal social activity (indicating an activity difference in ranks or stress levels due to social encounters). It is now possible to attach small heart-rate and/or temperature monitors to animals to collect data from which metabolic rate can be inferred (Cooke et al., 2004). In addition, a variety of taxa need to be studied, as bird flocks and pairs of salmon represent a small subset of the variety of social contexts that exist.

Energetics: body condition

Measures of body condition are often positively correlated with rank (captive male cynomolgus monkeys, weight: Cohen et al., 1997; captive male tammar wallabies, weight: Hynes et al., 2005; white-throated sparrows, fat storage: Piper and Wiley, 1990; great tits, heart weight: Roskaft et al., 1986; siskins, body mass: Senar et al., 2000). This is interesting in light of the fact that metabolic rate is often also positively correlated with dominance. Since energy that is acquired will either be used (metabolic rate) or stored (body condition), we might expect to see a trade-off wherein dominants with a high metabolic rate do not store as much energy, and vice versa. However, because both increase with rank (although not all studies compared both factors), we can conclude that dominant individuals must have a high energy intake to maintain both a high metabolic rate and a high body condition. Thus, in general, dominance does seem to have a positive influence on food resource acquisition, which in turn affects health.

Immunocompetence: immune parameters

Of the few experiments that have related immunocompetence to social rank, most show a positive correlation between dominance and immunity. Cohen and coworkers (1997) created groups of male cynomolgus monkeys and kept half stable for 15 months, while the other half were rearranged every month, creating instability. All individuals were exposed to a common-cold–like virus, and regardless of social stability, dominants had significantly lower infection

rates than subordinates. Interestingly, this was independent of glucocorticoid levels. In Lindström's (2004) study on greenfinches (Carduelis chloris), animals were grouped by similar plumage color, body mass, and age. Dominance was determined and then all were infected with Sindbis virus. The dominant animals had higher blood virus titers in the initial days of infection but cleared the virus more quickly than subordinate individuals. Together, these two studies suggest that dominants have lower susceptibility to and faster recovery from virus infection. Perhaps more interestingly, Lindström's (2004) results suggest that, in some systems, dominant and subordinate animals may have distinct resistance strategies, with dominant animals investing early in pathogen clearance. The role of social status on infection clearance (i.e., reduction of pathogen load) versus tolerance (i.e., the maintenance of individual fitness in the presence of a given pathogen load; Read et al., 2009) would be particularly interesting to examine, as suggested by this study. To date, no studies have considered how social context and social rank might influence immune strategies within and among groups.

Three other studies investigated specific immune responses to challenges based on dominance. In one of the few studies to manipulate rank and test for an effect, Hawley (2006) found that in house finches (Carpodacus mexicanus), forcing a decrease in rank for initially dominant birds caused a proportional decrease in antibody response to sheep red blood cells. This effect was likely mediated through a change in the extent of social defeat. No change in antibody response was seen in initially subordinate birds that changed rank. Additionally, Hawley and coworkers (2007) found a positive correlation between dominance and cell-mediated immunity, but a negative correlation between dominance and antibody response, suggesting that rank may mediate important trade-offs within the immune system. Dominant males also showed less severe symptoms and a shorter time symptomatic when inoculated with Mycoplasma gallisepticum (a naturally occurring bacterial pathogen in house finches) than subordinates, but dominant females did not show this difference. Finally, Zuk and Johnsen's (2000) study in male red jungle fowl (Gallus gallus) measured immune parameters before and after combining males into groups composed of two males and three females. They found that dominant males generally had higher overall immunocompetence both before and after flock formation based on four measures of immunocompetence, although the difference was not significant before flock formation. This result intriguingly suggests that social context can exacerbate preexisting, potentially minimal, differences in immunity, thereby magnifying phenotypic variation in disease susceptibility within social groups.

Immunocompetence: sickness behaviors

A number of recent studies have examined the influence of social status on the expression of sickness behaviors, a constellation of behavioral changes that occur early in infection as part of the inflammatory response. These behaviors are thought to be adaptive by directing energy away from temporally unnecessary social behaviors and toward recovery and fever in order to clear infection (Hart, 1988). As such, the relative expression of sickness behaviors is predicted to vary with the ability of an individual to direct energy away from key social behaviors. For example, Cohn and Sa-Rocha (2006) found that subordinate mice injected with lipopolysaccharide (LPS), a component of the bacterial cell wall that stimulates sickness behaviors, did not express any signs of sickness behavior and were indistinguishable from control animals. Dominant mice, on the other hand, expressed sickness behavior within the social context. Cohn and Sa-Rocha (2006) hypothesized that subordinate mice were more likely to prioritize defense behaviors over recovery in a social context. Again, these results suggest that dominant and subordinate animals

may exert distinct resistance strategies in response to a parasite or pathogen challenge: dominant animals may be more likely to devote available resources toward rapid clearance, while subordinate animals may be more likely to exhibit tolerance strategies in order to prioritize social behaviors in the presence of aggressive conspecifics.

Cohn and Sa-Rocha (2009) took their prior results a step further by testing how dominant versus subordinate mice treated with LPS responded to an intruder in their home cage. In each treatment, either the dominant or subordinate of a pair was injected with LPS; in the control treatment, both dominant and subordinate were injected with saline. They hypothesized that responses of the dominant and subordinate to an intruder would not only differ by rank but would respond to the expression of conspecific sickness behaviors. Consistent with their prior results, dominant animals spent more time expressing sickness behaviors in the social context. Intriguingly, subordinate animals increased their social behaviors when the co-housed dominant was expressing sickness behavior. This suggests that the social context not only influences sickness behavior across rank, but can influence the behavior of non-"infected" individuals in the group.

Overall, results to date, though limited to a few systems, suggest that dominant individuals generally have stronger immune responses, and this may be both a cause and effect of dominance. Individuals in higher overall condition as a result of genetic and non-genetic factors are likely to have a stronger immune system, and are also more likely to become dominant upon meeting a rival. Interpreting the results of studies that show an effect of changing dominance status or social context on immunocompetence is more difficult. Why does a drop in dominance rank result in decreased immune response in male house finches, while an increase in rank has no effect (Hawley, 2006)? In this study, it appeared that losing fights rather than resource access was the mechanism causing the degree of the decrease in immune response: the number of defeats experienced by dominant house finch males in their new social environment directly predicted the extent of immunosuppression. Similarly, in the Zuk and Johnsen (2000) study, differences in resource access or mass did not explain the socially induced differences in immunocompetence. Instead, social interactions, perhaps via changes in hormone levels, appear to mediate rank-related immune competence in a social context. Taken together, these studies suggest that neuroendocrine changes and social interactions, particularly aggression, likely underlie the patterns detected to date in terms of dominance and immune competence.

Table 1.1. Behavioral and Physiological Correlates of Dominance.

Variable	Correlation w/Dominance	Possible Interactions	Potential Health Effects of Dominance	References
Aggression given	Positive	Testosterone	Increased exposure to disease through intraspecific contact and wounds.	Barrette and Vandal, 1986; Creel et al., 1997; East et al., 2001; Creel and Sands, 2003; Scott & Lockard, 2006; Shargal et al., 2008
Aggression received	Negative	Stress hormones	Increased exposure to disease through intraspecific contact and wounds. Increased susceptibility through increased stress	Creel et al., 1992; Cohen et al., 1997

			response.	
Access to resources	Positive	Body condition	Decreased susceptibility due to lack of nutritional stress. Increased or decreased exposure through access to preferred food/sites/mates (depending on parasite/pathogen distribution).	Barrette & Vandal, 1986; Rubenstein & Hohmann, 1989; Zine & Krausman, 2000; Scott & Lockard, 2006; Hogstad & Pedersen, 2007; Atwood & Gese 2008
Stress hormones	Various, depending on social context	Immuno-competence	See reviews by Sapolsky (2004, 2005)	Creel, 2001; Abbott et al., 2003; Creel & Sands, 2003 Sands & Creel, 2004; Sapolsky, 2005
Testosterone	Positive or none	Aggression; immuno-competence	Increased susceptibility due to decreased immunity. Increased exposure through aggression.	Positive correlation: Creel et al., 1997; Beehner et al., 2006; Aguirre et al., 2007; Aubin-Horth et al. 2007; Drea, 2007; Hogstad & Pedersen, 2007; Johnston et al., 2007; Malaivijitnond et al., 2007; Barja et al., 2008; Shargal et al., 2008. No correlation: Hynes et al., 2005; Gauly et al., 2007; Taillon & Cote, 2008; Teichroeb & Sicotte, 2008
Female reproductive hormones	Positive	Immuno-competence	Increased susceptibility through nutritional stress and increased immunity during pregnancy	Creel et al., 1992; Creel et al., 1997; Barja et al., 2008
Metabolic rate	Positive	Body condition	Increased exposure through nutritional stress	Roskaft et al., 1986; Hogstad, 1987; Bryant & Newton, 1994; Cristol, 1995; Metcalfe et al., 1995; Senar et al., 2000; Vezina & Thomas, 2000
Body condition	Positive	Metabolic rate; immuno-competence	Decreased susceptibility to disease due to energy storage	Roskaft et al., 1986; Wiley, 1990; Cohen et al., 1997; Piper and Senar et al., 2000; Hynes et al., 2005
Immuno-competence	Positive	Sex hormones; stress	Decreased susceptibility to disease due to increased defenses	Cohen et al., 1997; Zuk & Johnsen 2000; Lindstrom, 2004; Hawley, 2006;

		hormones; body condition		Hawley et al., 2006, 2007
Sickness behavior	Positive	Conspecific sickness behavior	Decreased susceptibility due to induction of fever and behavioral changes associated with innate immunity	Cohn and Sa-Rocha, 2006, 2009

Information for each correlate includes the direction of its relationship with dominance (e.g., positive = the correlate increases as dominance increases; causation is not inferred here), possible interactions with other correlates of dominance, possible health effects of the correlate, and references.

Health Consequences of Dominance

Physiological and behavioral correlates of dominance have diverse and complex interactions with one another. For example, dominance and aggression may affect testosterone levels, which interact bidirectionally with glucocorticoid levels (Sapolsky, 1985; Ketterson et al., 1991), and all of these can in turn affect immunity and responses to parasites and pathogens. This section will explore some of the many connections between dominance behavior and physiology, susceptibility, and exposure to pathogens.

Levels of aggression

Higher rates of aggression, whether associated with testosterone or not, are often correlated with dominance rank (see Behavioral Correlates of Dominance section), and increased aggression can have major health implications. Aggression is particularly interesting and important because it can have dual effects on both exposure and susceptibility. First, aggression may increase contact with conspecifics and cause wounds, both of which lead to higher rates of infection through exposure to pathogens. Second, given or received aggression causes rapid neuroendocrine responses and heightened energy expenditure, both of which can decrease the strength of the immune response and, consequently, the ability to resist a parasite or pathogen. An example of the first mechanism occurs for Serengeti spotted hyenas, where, as previously mentioned, dominants experience more bite wounds than subordinates (East et al., 2001). Interestingly, East and coworkers found a positive correlation between dominance rank and rabies virus exposure, which they hypothesized was spread by the higher incidence of bite wounds in dominants and the submissive act of subordinates licking the open mouths of dominants. Although the rabies virus is asymptomatic in this species, making the fitness consequences difficult to interpret, this study provides a rare example from a free-living system of the potential health costs of aggression for dominant individuals.

The physiological effects of aggression on immunity have been well documented in numerous laboratory and domesticated vertebrates (Gross and Siegel, 1973; Barnard et al., 1998) and can vary with social status (Stefanski, 1998). Interestingly, in some cases a single social defeat can cause a constellation of physiological changes and alterations to the HPA axis that ultimately affect immunity (de Groot et al., 1999; Goncalves Carobrez et al., 2002). Consistent with these studies, Hawley (2006) directly linked immunosuppression in male house finches with the extent of social defeat experienced in a new social context. These data suggest a strong role for neuroendocrine changes associated with defeat on the immune system. The higher aggression often received by subordinates and their lower average immunocompetence in the studies reviewed herein are consistent with a strong role for aggression in mediating immune

competence and pathogen clearance in vertebrates. Overall, the studies to date highlight the potential health implications of dominance status, and spur exciting questions about physiological and behavioral mechanisms.

Exposure via resource access or territory defense

One of the less-studied consequences of dominance status on disease outcome is the cost of access to preferred resources, which may inadvertently increase the exposure of dominant individuals to infectious agents. Halvorsen (1986) showed that calves of dominant female reindeer had better access to favored food as well as a higher rate of infection with a nematode parasite, most likely because the gastropod host of the nematode used the reindeer's favored food source. Hausfater and Watson (1976) found higher parasite ova emission in dominant male baboons, and speculated that it could be due to food access, though they did not test this. Hogstad and Pedersen (2007) found that dominant willow tits had greater food access as well as higher cadmium levels in the liver. Plowright and coworkers (2008) found that Hendra virus infection in little red flying foxes increased with body size. They speculated this trend occurred because body size correlates with dominance, and dominant males have large harems and defend food sources, both of which cause high conspecific contact rates. Finally, Ezenwa (2004) showed that territorial male gazelle have higher intensities of gastrointestinal parasites than bachelor males, juvenile males, or females. The examples above are case-specific, and there may be times where the less-favored resources that subordinates are forced to use expose them to more pathogens, a possibility that has not yet been empirically demonstrated. Additionally, when dominants do have higher exposure to pathogens through resource access, it may be outweighed by the nutritional or other benefits gained (e.g., higher weight in dominants: Halvorsen, 1986; higher feather regrowth in dominants: Hogstad and Pedersen, 2007).

The higher prevalence of parasites found in dominant animals in several of the studies summarized above (Hausfater and Watson, 1976) appear to contradict the results of captive experimental inoculation studies, whereby dominant individuals show decreased susceptibility to disease and/or faster clearance of infectious organisms (Cohen et al., 1997; Lindstrom, 2004; Hawley et al., 2007). These conflicting patterns in captivity, whereby exposure was held constant, versus the wild indicate that heightened immunity of dominants, if present, may be insufficient to overcome greater exposure to pathogens through increased conspecific contact, increased food demand, and increased access to resources that may contain pathogens. On the other hand, dominant animals may, in some cases, show higher parasite loads or prevalence because they are practicing immune strategies such as pathogen tolerance by investing energy into territory defense or reproduction, rather than pathogen clearance, in order to maintain fitness. Finally, the costs and benefits of higher resource access may be context-dependent, whereby dominant animals are more exposed in some social systems or contexts than others.

Behavioral avoidance/preference of conspecifics

Selection should favor individuals who can recognize and avoid infected conspecifics in order to reduce their own risk of infection, particularly for directly transmitted pathogens with a limited host species range (Loehle, 1995). For many of these types of pathogens, evidence is accumulating that healthy animals actively avoid infected conspecifics (Kiesecker et al., 1999; Behringer et al., 2006; Kavaliers et al., 2006; but see Bouwman and Hawley, 2010), presumably to reduce their own risk of infection. The role of social status in mediating avoidance of infected conspecifics remains unknown, but partner choice is affected by social hierarchy for a suite of

vertebrates (Cheney et al., 1986; Dugatkin and Sih, 1995; Schino, 2001; Smith et al., 2007). If dominants tend to be less infected or infectious, social partner choice may represent a critical mechanism by which dominant individuals, via selective access to "desirable" conspecifics, alter their probability of exposure. However, Rubenstein and Hohmann (1989) found that in island feral horses, dominant females prefer to be near the most dominant animal, which is the stallion. This preference increases the parasite load of the dominant females because the stallion attracts more flies than females, and the flies he attracts also bite the dominant females close to him. Thus, dominant animals may make partner choices based on factors other than infectiousness and thereby increase their exposure.

Hormone-mediated pathways

The health consequences of glucocorticoid levels, and stress more broadly, are now widely accepted (Sapolsky, 2004, 2005) but can result in immunostimulation or suppression depending on the stressor and the timing (Sapolsky 2005). Similarly controversial are the immunosuppressive effects of testosterone. Mixed support has been found that testosterone may suppress the immune system (reviewed for birds in Owen-Ashley et al., 2004) and increases the rate of ectoparasites, at least in some species (Grossman, 1985; Roberts et al., 2004). Testosterone is also often associated with increased aggression (Balthazart, 1983; Baum, 1992), although many other factors may influence the expression of aggressive behavior, especially in primates (see Booth et al., 2006, for the effects of testosterone in humans). Dominant animals with increased testosterone levels may also have higher contact rates with conspecifics, not only through aggressive interactions but also through courtship and mating. In an experimental testosterone-implant study with wild male white-footed mice, Grear and coworkers (2009) documented a significant increase in contact rates for testosterone-treated individuals and predicted a two-fold increase in parasite transmission on plots where testosterone levels were elevated. Interestingly, no wildlife study to date has directly examined how dominance rank or testosterone levels correlate with conspecific contact rate, particularly between infectious and susceptible individuals, the key variable driving the transmission of directly transmitted pathogens.

Metabolic rate

If an individual's rank is associated with a high metabolic rate for an extended period of time, its nutritional intake or energy reserves must be able to supply that metabolic demand. Since dominants usually have greater access to resources, it is likely that they can sustain a higher metabolic rate with less risk to health, as shown by their ability to also sustain higher body condition. However, the higher nutritional demand is not without possible costs; Hogstad and Pedersen (2007) found that dominant male willow tits had higher testosterone levels, more food access, and higher cadmium levels than subordinates. Earlier work (Hogstad, 1987) showed that dominants had higher metabolic rates, so it is possible that the higher metabolic rate led to higher food demand, which they were able to obtain, but which exposed dominants to greater amounts of environmental pollutants. The role of rank-related differences in metabolic rate on infection outcomes has not been examined but represents an important possible mechanism by which individuals within a group may show diverse infection outcomes.

Context-dependent physiological costs of dominance

Creel and Sand's (2003) suggestion that dominance may be a high-risk, high-yield strategy and subordinancy a low-risk, low-yield strategy seems borne out by the research summarized above; in general, dominant animals appear to have higher immune competence (Hawley, 2006) and resistance to experimental infection (Lindstrom, 2004; Hawley et al., 2007) in stable social groups. However, when group membership changes (Cristol, 1995; Hawley, 2006), social dominance may carry physiological costs such as elevated metabolic rate and immune suppression. The effect of social stability on rank-related immune competence within or among species has not been directly examined, but Hawley and coworkers (2006) manipulated the extent of group-level aggression in captive house finches by providing 4 versus 16 feeder-access points for flocks of 8 finches. The results indicate that the extent of aggression in the social group can mediate the relative costs and benefits of dominance: dominant house finches had higher immune competence in low-competition flocks (16 feeder-access points), and aggression rates decreased over time in these groups as membership stabilized. However, in high-competition groups (4 feeder-access points), high levels of aggressive interaction were maintained throughout the experiment. In this treatment, dominant individuals did not have higher immune competence. Interestingly, several of the high-ranking individuals in each flock did not mount an immune response when aggressive interactions were high. The discrepancy at high versus low social aggression corroborates results obtained in the previously discussed meta-analyses of social rank and glucocorticoid levels, whereby social stability is a strong determinant of rank-related costs and benefits (Creel, 2001; Creel and Sands, 2003; Sapolsky, 2005). Although it is difficult to make generalizations with the limited number of studies available, we hypothesize that relationships between dominance and pathogen susceptibility will reflect the extent of stability and aggression in a social group. During times of the year where membership changes occur and/or aggression levels are enhanced, the physiological costs of dominance in terms of increased pathogen susceptibility will likely outweigh the benefits detected in previous studies. These situations may be particularly important for population-level spread of directly transmitted diseases, particularly if dominant animals have higher exposure to these infectious organisms during periods of intense intraspecific aggression and contact. Context-dependent costs of dominance under particular social contexts may result in positive covariation between exposure and susceptibility within dominant individuals, leading to key hosts and a high potential for epidemics (Hawley et al., 2011). The role of social context in driving phenotypic covariation in pathogen-relevant traits is particularly important to understand because it is non-heritable, resulting in variation in disease dynamics with no potential for directional selection for pathogen resistance.

Challenges and Suggestions for Future Research

Considering our current understanding of how social rank mediates pathogen exposure and susceptibility, how should future research be directed? One of the most pressing needs in the field of dominance more generally is to design experiments that reveal cause and effect. Correlations abound, but a clear understanding of how dominance results in behavioral or physiological traits will remain elusive until manipulative studies are performed. Below we discuss a few of the challenges for future research and potential exciting directions for elucidating how social context influences disease outcomes.

Dissecting effects of dominance on susceptibility versus exposure

One of the key challenges in understanding how social status influences health is to dissect the relative influence of dominance interactions on exposure to infectious agents versus responses to infection once exposed. Correlative patterns, whereby dominant animals may have lower or higher pathogen prevalence, are the outcome of potentially complex interactions whereby dominant animals may be more frequently or less frequently exposed to an infectious organism, but may also clear that organism more quickly or slowly. Knowledge of pathogen exposure is therefore critical to ask whether variation in infection outcomes results from underlying immune variation, behavioral processes that control transmission, or both. Based on the results discussed above, we predict that dominant animals will generally have higher exposure to directly transmitted organisms due to higher contact rates with conspecifics, but the type of conspecific with which dominant animals primarily interact will be critical (see the section on infection-induced changes). Dominant animals with larger home ranges should also have higher exposure to pathogens that are environmentally transmitted, as suggested by the research above. On the other hand, subordinate animals may be more likely to be exposed to vector-transmitted parasites and pathogens due to less-preferred positions with the group, but the sole study to examine ectoparasite prevalence by rank found the opposite result, whereby the dominant stallions were most heavily infested (Rubenstein and Hohmann, 1989).

Teasing apart the relative role of susceptibility versus exposure on rank-related infection dynamics is particularly challenging because resistance and exposure may covary within individuals. In one of the few studies to date to explicitly examine covariation between host susceptibility and exposure, sheep that were genetically resistant to gastrointestinal helminths also avoided foraging in parasite-rich areas of habitat more effectively (Hutchings et al., 2007). Whether this type of covariation is present for a particular species or under particular social contexts may determine the extent to which individuals of a given rank serve as key hosts for secondary pathogen transmission. Although genetic covariation remains challenging to study in non-model organisms, understanding whether pathogen exposure and resistance phenotypically covary across social rank, and if so, whether that covariation is negative or positive, is both feasible and particularly important to consider given the population-level impacts of this covariation on infection dynamics (Hawley et al., 2011).

Only a handful of studies have attempted to tease apart the role of variation in susceptibility versus exposure on rank-related health. The simplest approach is to use novel immune antigen injections (as per Zuk and Johnsen, 2000), to which no individuals have been previously exposed, in order to determine how rank affects a measure of susceptibility (i.e., immune competence) without confounding effects of exposure or secondary transmission among group members. A second option is to experimentally eliminate variation in the timing and dose of exposure to a pathogen across social rank by simultaneously inoculating naïve individuals with identical doses of an ecologically relevant pathogen (as per Lindstrom 2004; Hawley et al., 2007). This approach allows the investigator to isolate the influence of social rank on recovery per se while ignoring the confounding effects of rank-related variation in exposure. In some cases, independent experimental manipulation of behavioral and/or immune phenotypes may be possible. Mougeot and coworkers (2005) directly measured the influence of testosterone on host immunity versus behavior by implanting male red grouse (Lagopus scoticus scoticus) with testosterone while simultaneously blocking androgen receptors, thus eliminating behavioral responses. Enhanced susceptibility of testosterone-implanted males to parasites was still detected, suggesting an immunological cause for sex differences in infection. These types of

manipulations have not been performed across individuals of varying social rank, but would be particularly useful in elucidating behavioral versus physiological mechanisms.

The role of social rank in driving variation in exposure to pathogens is unusually challenging to study because measurements of exposure in free-living populations are tightly linked with immune responses. For example, seroconversion may indicate recent exposure to a pathogen to which specific antibodies are mounted; however, the use of seroconversion is limited to pathogens that cause measurable immunity, and measures of seroconversion can have high measurement errors, rendering interpretation difficult at the level of individuals. More refined techniques for measuring individual variation in exposure are needed to document how dominance influences exposure across a suite of social contexts (i.e., high vs. low aggression or stability). Social networks are a useful tool for quantifying variation in conspecific contact rate and types of interactions among group members, and network measures such as individual connectivity predict parasite prevalence for a suite of systems to date (e.g., Bansal et al., 2007; Perkins et al., 2009). Although social networks are increasingly being applied to understand disease transmission (Corner et al., 2003; Bansal et al., 2007; Godfrey et al., 2009; Perkins et al., 2009), they have not yet been used to examine how rank-related network characteristics influence parasite and pathogen exposure in social groups.

Creative solutions to the experimental quantification of transmission are beginning to be developed. Naug (2008) used non-pathogenic, intestinal microbeads to map how social networks in honeybees influence the spread of a pseudo-intestinal pathogen throughout the social group. Kulkarni and Heeb (2007) used a non-pathogenic soil bacterium, Bacillus licheniformes, as a model for bacterial transmission in zebra finches. They inoculated the feathers of paired male zebra finches with this "bacterial tracer" and found very high rates of allo-infection (~80%) in the gut of the co-housed individuals only 24 hours after treatment (Kulkarni and Heeb, 2007). They hypothesized that transmission resulted from allo-preening, but the exact mechanism was unknown. Similar studies could be conducted on paired males of known social rank for a variety of vertebrate species. Depending on the transmission mode of the pathogen of interest (a.k.a. environmental vs. direct, and if direct, sexual vs. non-sexual), experimental models of transmission may be a productive and informative way of examining how social context and dominance status influence the likelihood of exposure to parasites and pathogens of varying life-histories and transmission modes.

Infection-induced behavioral changes and social rank

For directly transmitted pathogens, the behavior of individuals once infected is the key determinant of a resulting pathogen epidemic (Lloyd-Smith et al., 2004). Therefore, while understanding how individual behavior might influence the probability of infection is useful for understanding the costs and benefits of behavioral strategies and group living, behaviors prior to infection are unlikely to be relevant to resulting population-level spread. There is a need to understand how aggressive behavior and social rank influence infection-induced behavioral changes such as sickness behavior in order to ultimately understand how social structure might affect broad-scale disease dynamics (Lloyd-Smith et al., 2004; Funk et al., 2009).

Evidence to date suggests that infection-induced changes in behavior can directly influence the spread of directly transmitted pathogens. In laboratory rats, healthy individuals actively avoid bedding of conspecifics that were injected with the bacterial mimic LPS (Arakawa et al., 2010), suggesting that olfactory cues are sufficient to alter contact rates between healthy and susceptible individuals in this species, and potentially in other vertebrates that utilize olfactory signals for

conspecific communication. If rats, like mice (Cohn and Sa-Rocha, 2006), show status-related differences in sickness behavior expression, contact rates with infected dominants may be lower than with infected subordinates. The relative infectiousness of individuals across status would then determine resulting effects on transmission. In birds, which are thought to rely less heavily on olfactory cues than mammals, behavioral changes involved in the sickness response may alter contact rates. In male house finches, sickness behaviors resulting from infection with the naturally occurring bacterium Mycoplasma gallisepticum significantly attract healthy conspecifics seeking to avoid behavioral aggression at feeders (Bouwman and Hawley, 2010). These changes will likely increase contact rates between healthy finches and infected males, and hence may facilitate the transmission of M. gallisepticum in wild house finch populations.

The proximate mechanisms that underlie infection-induced behavioral changes comprise a network of neuroendocrine changes, which include glucocorticoid and testosterone levels as well as the expression of pro-inflammatory cytokines in the brain (Besedovsky and del Ray, 2001; Dantzer, 2004). Interestingly, these neuroendocrine pathways are known to respond to social context. Weil and coworkers (2006) challenged adult male rats with LPS under three different social contexts: housed alone, with a single juvenile male, or with an adult female. The presence of the juvenile male, but especially the adult female, significantly increased gene expression of two pro-inflammatory cytokines, TNFα and IL-1β, although behavioral differences were not noted. In all three treatments, LPS-injected males showed strong decreases in testosterone, a characteristic of the sickness response that appears to occur after antigen injection for a suite of vertebrates (Boonekamp et al., 2008). In vitro experiments indicate that the pro-inflammatory cytokines IL-1α (e.g., Hales 1992) and IL-1β (e.g., Lister and van der Kraak, 2002), released during an inflammation response in vertebrates, suppress testicular steroid production. However, no study to date has examined whether male vertebrates alter their testosterone levels when infected with an ecologically relevant pathogen, and furthermore, how decreases in testosterone vary with social status and ultimately alter contact rates with susceptible individuals, the most important component for emergent population-level transmission (Swinton et al., 2002).

The study of conserved vertebrate signaling molecules such as hormones or cytokines, both of which can have dual effects on host exposure and susceptibility, have the potential to help tease apart the mechanisms underlying rank-related variation in disease outcomes (Hawley and Altizer 2011). As described above, the steroid hormone testosterone can increase transmission-relevant behaviors such as contact rate (Grear et al., 2009) and has also been implicated in immunosuppression or immunomodulation across a range of taxa. Similarly, pro-inflammatory cytokines released during the acute phase response influence both within-host immune processes and, for the limited species of mammals and birds studied to date, behaviors relevant to transmission (e.g., sickness behavior and attraction or aversion of healthy conspecifics). If immunological and behavioral effects of rank positively co-occur during vertebrate infection (Hawley et al., 2011), key hormones such as testosterone may serve as common drivers of key hosts, broadly linking within-host (e.g., infectiousness) and among-host (e.g., contact rate) processes across individuals of varying social status. Their functional conservation across diverse vertebrate taxa makes these signaling molecules even more compelling candidates for considering mechanistic associations between dominance behavior, susceptibility, and transmission.

Overall, more mechanistic and integrative studies are needed on infection-induced behavioral changes in group-living organisms, as well as the influence of dominance status and aggression

on these changes. Ultimately, these data will reveal whether common traits (i.e., dominance or high aggression) characterize the individuals that largely contribute to transmission in a group.

Going beyond immune competence and pathogen resistance

The studies to date concerning how dominance rank influences immunity and disease have almost universally assumed that higher responses are "better" (i.e., higher antibody titers reflect stronger resistance and higher fitness). There are multiple problems with this assumption that may limit our conclusions about the costs of benefits of dominance status. First, strong immune responses can have substantial fitness costs, including immunopathology (Graham, 2002). Second, traditional measures of immune competence are often not very predictive of outcomes to particular infections (Adamo, 2004). Finally, a "competent" immune system may employ tolerance strategies (e.g., the maintenance of host fitness in the presence of a pathogen load; Read et al., 2009), which is difficult to assess via infection outcome or antibody production alone.

The fitness consequences of immune investment and pathogen burden provide a critical context for understanding observed outcomes but are often overlooked (Baucom and DeRoode, 2011; Graham et al., 2011). For example, dominant individuals that invest in costly defenses in the absence of exposure to disease-causing agents could be making a flawed investment that will ultimately lead to lower fitness, but such hosts might readily be deemed "immune competent" by traditional eco-immunology assays (e.g., immune cell recruitment, parasite agglutination and/or lysis, and antibody production). Though challenging, we suggest that future studies incorporate fitness outcomes to the extent possible in order to provide context to results obtained (Graham et al., 2011). It may be that dominant and subordinate individuals dynamically employ different strategies of clearance versus tolerance across varied social contexts, depending on the context-dependent fitness outcomes that result. The use of varying immune strategies (e.g., tolerance vs. clearance) by dominant individuals across social contexts is an area of exciting and unexplored territory.

Summary

The dynamic role of social context on immunity has long been of interest for closely housed domesticated species (Gross and Siegel, 1973; Morrow-Tesch et al., 1994; Tuchscherer et al., 1998; De Groot et al., 2001). In contrast, eco-immunology investigators are just beginning to appreciate the way in which social context and individual dominance rank mediate both immunity and behaviors relevant to exposure for group-living vertebrates. The limited studies to date on dominance status and disease susceptibility indicate that social status has contrasting and context-dependent effects on health; in some cases, dominant animals have higher exposure due to greater resource access or wounds, while in other species, dominant individuals appear to have higher immune competence and lower disease susceptibility. Further work is sorely needed to determine whether characteristics of social groups and/or the transmission mode of parasites and pathogens might help to parse out this variability in relationships between social status and disease susceptibility. The complexity of interactions between behavior, hormones, and immunity provide a significant challenge to understanding relationships between social rank and disease. However, the extensive work done on how social rank mediates stress hormones provides a testable framework for how social rank may also modulate immune responses and pathogen susceptibility. Variation in susceptibility via host immunity must then be incorporated with variation in exposure across rank to provide a complete understanding of how parasites and

pathogens influence the costs and benefits of dominance and the evolution of sociality. Dissecting the mechanisms that contribute to the substantial heterogeneity in disease within and among social groups is critical to understand in order to predict the potential for disease outbreaks and the appropriateness of disease management strategies for socially structured vertebrate populations.

Attribution

B.M. Fairbanks was the main contributor to this chapter. D.M. Hawley contributed with substantial editing and contribution of immunological concepts and text.

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Chapter 2

Do not feed the wildlife: Behaviour and disease consequences of foraging in garbage for banded mongooses (*Mungos mungo*)

Bonnie M. Fairbanks[1]*, Dana M. Hawley[1], Kathleen A. Alexander[2.3]

[1] Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA
[2] Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, VA, USA
[3] Center for African Resource: Animals, Communities, and Land use (CARACAL), Kasane, Botswana
* Corresponding author: bonniemf@vt.edu

Abstract

Urbanization may indirectly affect disease dynamics by altering host behaviour in ways that influence transmission. Few opportunities arise to investigate behaviourally-mediated effects of urbanization in natural host-pathogen systems, but we provide a unique example of this phenomenon in banded mongooses (*Mungos mungo*), a social mammal. Our banded mongoose study population in Botswana is affected by tuberculosis (TB) caused by a novel pathogen, *Mycobacterium mungi* that primarily invades the host via breaks in the skin. Urban banded mongooses in our study site had significantly higher within-troop aggression levels when foraging in garbage compared to other foraging habitats. Second, monthly rates of aggression were a significant predictor of monthly number of injuries in troops. Finally, injured individuals had a 75% TB incidence compared to a 0% incidence in visibly uninjured mongooses. Thus, our data suggest that urban mongoose troops that forage in garbage are at greater risk of acquiring TB by incurring injuries that may allow for pathogen invasion. We highlight the potential for urbanization to impact disease transmission, potentially influencing wildlife health and increasing human exposure to wildlife-associated zoonotic diseases. As urban areas expand along with garbage and urbanized wildlife, our findings demonstrate the need for improved waste management in these systems.

Introduction

Urbanization is increasing worldwide, generating a need to understand the direct and indirect influence of urban environments on disease emergence and dynamics in humans and wildlife (Bradley and Altizer 2007). Urbanization can cause a number of changes in the population density, behaviour, or physiology of pathogens, vectors, and hosts important for disease dynamics. Sometimes it is simply a "numbers game": urbanization can alter the number of species potentially involved in pathogen dilution or transmission (Keesing et al. 2010) or the population sizes or densities of competent hosts or vectors, which leads to a concomitant change in contact rates and pathogen transmission potential (Acosta-Jamett et al. 2011; Bradley and Altizer 2007; Johnson et al. 2013; Shapiro et al. 2012). Alteration of wildlife host behaviour is a less considered but likely widespread effect of urbanization on disease dynamics that is particularly interesting due to the potential for behavior to influence both host exposure to pathogens and/or susceptibility to disease (Hawley et al. 2011; Riordan et al. 2011; Wright and Gompper 2005). These behavioural changes add a layer of complexity to the effects of

urbanization on disease dynamics because they involve a multitude of possible indirect effects which have not yet been explored in free-living host-pathogen systems.

One widespread cause of changes in host behaviour in urban environments is resource alteration and concentration. Humans often discard plant material, animal carcasses, and food waste at garbage sites, which can become a predictable, non-seasonal, and highly concentrated source of food for wildlife. Thus, garbage sites are a specific component of urbanization that might affect disease dynamics through changes in host behaviour, even in the absence of garbage-induced changes in host density or population size. For example, when two similarly-sized populations of raccoons were supplemented with equal amounts but different distributions of food, raccoons aggregated at clumped food resources but did not aggregate at dispersed food resources (Wright and Gompper 2005). Furthermore, though the raccoon populations had similar endoparasite prevalence and abundance before supplementation, both parasite metrics increased in the population with clumped supplemental food resources, but not in the population with dispersed food resources, due to increased contact rates among individuals (Wright and Gompper 2005). Population size did not change with resource clumping, indicating that behavioural changes per se were responsible for altering parasite dynamics in this system. Human-augmented food sources for wildlife have been shown to alter wildlife behavior (e.g. Prange et al. 2004; Yirga et al. 2012), increase contact rates within and between species (e.g. Campbell et al. 2013; Totton et al. 2002), and have been linked to altered disease outbreaks in a number of systems (Cross et al. 2007; Hosseini et al. 2004; e.g. Totton et al. 2002). Though not all were conducted in an urban setting, these studies demonstrate how food augmentation (whether purposeful or unintentional) in urban areas might influence host behaviour, impacting disease dynamics. However, in these previous studies, the mechanism linking shifts in behaviour with altered disease dynamics was an increase in disease transmission at sites of human-augmented resources. Urban resource augmentation may also cause behaviourally-induced changes that indirectly alter disease dynamics, but these indirect mechanisms have not been previously explored, as we do here.

We examined how altered food resources associated with urbanization influence behaviour and disease dynamics in a mammal that is currently affected by an observable disease, and that readily uses both natural and human-modified habitats for foraging: banded mongooses (*Mungos mungo*). Banded mongooses are diurnal, highly social, fossorial carnivores that eat invertebrates (e.g., arthropods and worms) and small vertebrates (e.g., amphibians and small mammals) in their natural habitat, but at garbage in urban areas, forage predominately on discarded human food. A novel *Mycobacterial tuberculosis* complex (TB) pathogen, *Mycobacerium mungi*, has been identified among banded mongooses at our study site (Alexander et al. 2010). Direct, respiratory transmission is typical of *M. tuberculosis* and other organisms in the complex, but *M. mungi* appears to invade the mongoose host through cuts or abrasions on the skin or nasal planum from environmental sources with only one case of primary respiratory transmission identified (Alexander et al. 2010 and unpublished data). Thus, behavioural interactions such as aggression that result in cuts or abrasions (i.e., injuries) may be particularly important to disease dynamics in this system. Although aggressive interactions among group members are rare in this low-skew, communal species due to a relaxed or absent dominance structure (Gilchrist 2006, Muller et al. 2008), substantial levels of within-group aggression appeared to occur at refuse sites. We therefore quantified 1) whether aggression during foraging differed across five types of foraging habitat with varying degrees of human modification, from refuse sites to natural, unmodified environments, 2) whether higher rates of

within-troop aggression were associated with increased rates of injury, and 3) whether higher injury incidence was associated with increased incidence of clinical TB.

Methods

General Observation Methods

Tracking and Identification. We studied four banded mongoose troops in and around Chobe National Park, Botswana from March to November 2011. Observed troops ranged in size from 11 to 56 individuals. One or two animals in each troop were radio-collared as described elsewhere (Laver, 2013). Individual mongooses are virtually indistinguishable from one another by appearance, regardless of sex. Therefore, ear tags were used to mark up to six additional animals in the troop. Some animals were also identifiable by natural scars or injuries. Thus, taken together at least 10-33% and at most 100% of the individuals within each troop were identifiable. This study was conducted under a permit from the Botswana Ministry of Environment, Wildlife, and Tourism and approval of the Virginia Tech's Institutional Animal Care and Use Committee (Protocol number 07-146-FIW).

Behavioural Observations. Two datasets were used in this analysis. The first is a 9-month general behavioural dataset, composed of scan and focal samples of four mongoose troops. The second is a short-term (3 month) data set designed specifically to measure differences in aggression at distinct foraging areas. All data were collected by one observer (BF).

In order to collect both data sets, mongoose troops were followed on foot or by car. Two troops were intensively observed each week for five days per week, alternating troops in the morning and afternoon (e.g. Monday AM: troop A, Monday PM: troop B; Tuesday AM: troop B, Tuesday PM: troop A, etc.). The following week, the other two troops were followed. This arrangement meant that a total of 5 mornings and 5 afternoons per month were spent with each troop. The schedule was occasionally disrupted by weather or inability to find or follow a troop.

Scan and Focal Sampling Methods

While following a troop, both focal and scan samples (Altmann 1974) were collected when at least half of the troop was active, i.e. at least half of the individuals were engaged in behaviour other than lying down or sitting still. Scan samples, which capture a snapshot of the behaviour of all individuals in the troop, were collected at least 20 minutes apart, and were recorded by speaking the behaviour (according to a pre-determined ethogram) of each visible individual into a voice recorder, along with the time and location of the scan. Focal samples (intensive observations of single individuals) of identifiable individuals were conducted between scan samples. Data were collected on a smartphone (Motorola Q9h (Motorola Mobility Inc., Libertyville, IL, USA) running Windows Mobile 6 (Microsoft, Redmond, WA, USA)) using the program PhoneRecord (W. Tietjen, Bellarmine University, Louisville KY), which time stamps (to the second) each key pressed on the smartphone, each of which represents a behaviour from a pre-determined ethogram. The order by which individuals were focal sampled was determined both randomly and opportunistically: once a month, a randomized list was made of all identifiable individuals in the troop and the first four individuals on the list were searched for. The first of those four individuals to be found was focal sampled. The next individual in the list was added to the search, the sampled individual removed from the list, and this continued until as many animals as possible were sampled. If all the animals in the randomized list were sampled, sampling would start again from the beginning of the list, allowing at least one hour to elapse between an individual's focal samples. Each focal sample was between 1.5 and 10 minutes long.

If an animal went out of sight during a sample, the sample was re-commenced when the animal came back into sight as long as the entire sample fell within 10 minutes. If an animal was not in sight for a minimum of 1.5 minutes, the focal sample was discarded. During focal and scan samples, a behaviour was scored as aggression if individuals made agonistic physical contact with one another, including lunging contact, biting, scratching, or deliberate and forceful body contact.

Foraging Aggression Methods

To collect targeted data on aggression in different types of foraging areas, we counted the number of aggressive sounds emitted each minute while a troop was foraging. Aggressive sounds were measured rather than aggressive behaviours because all animals in a group cannot be seen simultaneously (particularly when they forage in garbage, where garbage containers and garbage itself can obstruct observation), but all can usually be heard. During scan and focal samples, we observed that nearly all incidents of aggression between mongooses were accompanied by an aggressive sound. Aggressive sounds, therefore, serve as a reasonable index for aggressive behaviours. Aggressive sound data were collected on one or two days of each of three months (Aug, Sept, Oct) within the intensive observation schedule described above (under *Behavioural Observations*).

We measured aggressive sounds during banded mongoose foraging at five types of foraging locations: 1) at garbage, including household garbage bins, larger garbage receptacles, or areas of ground where humans discarded garbage; 2) under outdoor lights, which attract insects at night that might die or burrow into the soil under the lights, allowing mongooses to forage on them during the day; 3) on lawns, which might draw mongooses' natural prey due to daily watering and thick grass cover (in contrast to the surrounding dry natural landscape during the study period); 4) in human-modified areas that do not have predictable sources of food for mongooses, such as roads and paths (hereafter called "other modified areas"); and 5) in their natural habitat. The "other modified" areas served as a type of control because it separated the effects of resource clumping found at garbage from other effects of habitat modification.

Disease and Injury Observations

In addition to the behavioural observations described above, signs of TB and injury were assessed each day by observing as many individuals as possible through binoculars and/or at close range. Clinical signs of TB include cachexia, lethargy, lagging, hunching, discoloured fur, sneezing, wet eyes/nose, swollen or distorted nose, drooping or enlarged testicles, and fearlessness. Signs were graded on a scale from 1 to 10, with 10 being completely healthy and 1 being the most severe clinical presentation. For data analysis, we defined an individual as diseased if, during the last month it was observed, it displayed at least 3 clinical signs rated as 8 or less. The start month for each disease case was defined as the first month that a diseased individual displayed two signs rated 9 or less.

We defined injury as any break in the skin, swelling, or limping, persisting for more than 5 days. Injuries were described and if possible, scores were given on the same type of scale as clinical signs (e.g., limping was a common injury and could be scored from a very mild, weight-bearing limp, 9, to the animal dragging the limb and bearing no weight on it, 1).

Data Analysis: Is aggression higher in human-modified foraging habitats?

Using the foraging aggression dataset described above, a generalized linear mixed model (GLMM) was used to determine if mongooses utilizing the different foraging area types differed by the number of aggressive sounds they emitted. The number of aggressive sounds during a foraging bout was the response variable and area type was the fixed effect. Because the number of observation minutes and troop size varied by troop and foraging bout, we used log (minutes*troop size) as an offset in the model. For our data, this offset, a statistical tool used to account for unequal explanatory variables (such as observation time) across subjects, is akin to analysing on a "per minute per individual" basis. Troop was included as a random effect. We used a negative binomial distribution with a log link function, and the Satterthwaite method to determine denominator degrees of freedom. Because of overdispersion (χ^2/DF=3.41), an R-side scale parameter was used (Bolker et al. 2009). The Tukey-Kramer adjustment was used as a post-hoc test to determine if different areas had significantly different levels of aggression from one another.

Data Analysis: Does aggression predict injury?

We tested to see if troop-level rates of aggression explained the number of injured animals in a troop. We used monthly totals per troop in order to account for the non-independence of observations made on the same troop during the same month. Because of the limited time frame (three months) of the aggressive sound observations, we instead used our more extensive focal sample dataset to determine if aggression had an effect on the number of injuries in a troop over a longer period of time. Both scan and focal samples can estimate the proportion of time animals spend in particular activities (Altmann 1974). However, scan samples are records of states not events (Altmann 1974), and aggression tends to be very brief in mongoose troops (rarely lasting more than one second according to our focal sample data), making it an event rather than a state. Therefore, we used focal samples for this analysis because they reliably capture these brief events. To analyse the data we totalled the number of injuries, the number of aggressive behaviours observed during focal samples, and the total number of minutes of focal samples (i.e. observation time) per month for each troop. We performed a GLMM with number of injuries as the response variable, and aggression per minute of observation, troop size, and month (class variable) as fixed effects (no 2-way interactions were significant, so we removed them from the final model). We considered using troop size as an offset for this analysis, but when we tested for a relationship between troop size and number of injuries in this dataset, troop size was not a strong predictor of number of injuries (GLMM with negative binomial distribution, troop size as the only fixed effect, troop as a random effect. Troop size: F=1.94, P=0.06).Thus it was not appropriate to use an offset, but we used it as a fixed effect to determine if troop size was significant in light of other factors. We expected auto-correlation in number of injuries from one month to the next, so we used first-order auto-regression (AR(1)) to account for auto-correlation between months within troops. Troop was a random effect. We used a negative binomial distribution with a log link function, and there was only slight overdispersion (χ^2/df=1.05). We used the Satterthwaite method to determine denominator degrees of freedom.

Data Analysis: Does injury predict tuberculosis?

To determine if injured animals are more likely to show clinical signs of TB than uninjured animals, we compared the proportion of visibly uninjured mongooses that showed clinical signs of TB across all observed troops with the proportion of injured mongooses that

progressed to clinical TB using a likelihood ratio Chi-square test. Because a robust estimate of incubation period of the pathogen is lacking, we only included injured animals that we were able to observe for at least 5 months past the first observation of injury to ensure that enough time was allowed from injury to infection to clinical signs of disease. This precaution ensured that assumptions were not made about infection status in injured individuals that disappeared or died within a few months after injury, when the infection may not have had time to progress to observable clinical signs. All analyses were performed using SAS 9.3 (Cary, NC, USA).

Results

Is aggression higher in human-modified foraging habitats?

The extent of aggression varied significantly by area type ($F_{4, 51}$=10.63, P<0.0001, Fig. 2.1), with the highest levels of aggression at garbage sites. The parameter estimate for aggression at garbage was significantly different from zero (t=2.93, P<0.0001) and the least squares (LS) mean of garbage was significantly greater than lawn, natural, and other modified, and nearly significantly different from under lights (Table 2.1). The parameter estimate for under lights was significantly different from zero (t=1.47, P=0.02), but its LS mean was not significantly different from the other areas (Table 2.1).The parameter estimates for lawn, other modified areas, and natural areas were not significantly different from zero, and their LS means were not significantly different from one other (Table 2.1).

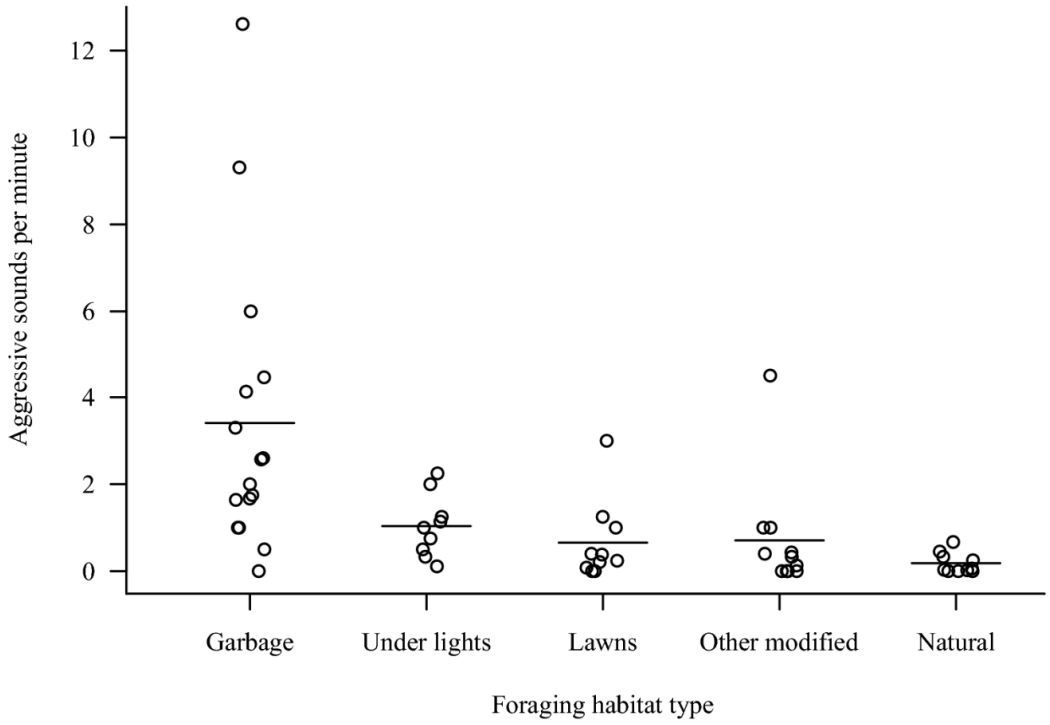

Figure 2.1. The number of aggressive sounds emitted by banded mongooses per minute during each foraging bout, by area type. Horizontal lines are means for each foraging habitat type, and types are ordered by degree of human modification, decreasing from right to left.

Table 2.1. Parameter estimates and differences of each area type. LS means comparisons have the Tukey-Kramer adjustment for multiple comparisons.

			t-values (P-values) for Differences of LS Means				
Area	**Parameter Estimate (SE)**	**t-value (P-value) for parameter difference from zero**	**Garbage**	**Light**	**Lawn**	**Other modified**	**Natural**
Garbage	2.92(0.52)	5.61 (<0.0001)		2.66 (0.08)	4.34 (<0.01)*	4.11 (<0.01)*	5.61 (<0.0001)*
Under Lights	1.47 (0.60)	2.46 (0.01)			0.96 (0.87)	1.37 (0.65)	2.46 (0.12)
Lawn	0.89 (0.58)	1.54 (0.13)				0.48 (0.99)	1.54 (0.54)
Other modified	0.60 (0.63)	0.94 (0.35)					0.94 (0.88)
Natural	0†	.					

†In proc GLIMMIX, one parameter estimate (here, natural) is set to zero to estimate the other parameters.
* indicates significant differences.

Aggression and Injury

Using the dataset of focal samples across 9 months of observation, troop-level aggression significantly predicted injury counts ($F_{1, 20.18}$=13.06, P=0.002), as did month ($F_{8,19.36}$=8.11, P<0.0001), while troop size did not ($F_{1,19.45}$=2.84, P=0.11).

Injury and TB

Of the injured animals that did not die or disappear within a day or two of injury (n=29), 12 were observable for at least five months after injury. Of these 12, nine advanced to clinical TB within five months (75%, 95%CI=50-100%) a proportion significantly higher than the proportion of clinical TB cases identified in visibly uninjured mongooses in 2011 (0%, n=126; LR χ^2=53.04, P<0.0001; Fig. 2.2). Injuries associated with progression to TB disease presented as persistent, non-healing lesions and/or limping for greater than two months duration.

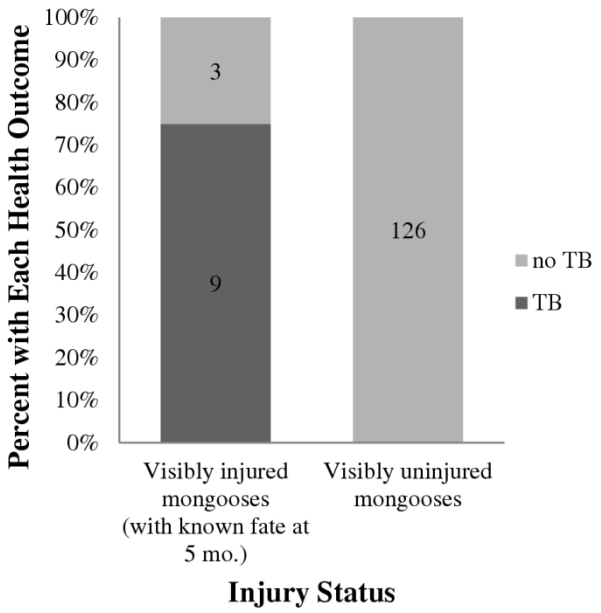

Figure 2.2. Percentage of tuberculosis in injured and uninjured mongooses. Numbers in bars indicate count of individuals with a given injury and tuberculosis outcome.

Discussion

Here we show that banded mongooses (*Mungos mungo*) exhibited significantly increased rates of within-troop aggression while foraging in garbage, where resources are clumped and likely of high value. Furthermore, troops with higher rates of aggression had higher numbers of injuries and clinical TB. Our research indicates that augmented food resources, a common feature of urbanization (Shochat et al. 2006), can cause changes in wildlife behaviour in ways that can indirectly affect disease dynamics. Our results are unique in that the increased contact at garbage does not appear to result in increased direct horizontal transmission of TB at the food resource, as in some studies of clumped augmented resources (Wright and Gompper 2005), nor is the garbage itself the source of infection as in baboons that became infected with bovine TB (Sapolsky and Share 2004). Instead, in our system, increased aggression at garbage has an indirect effect on disease dynamics by creating injuries that allow apparent environmental transmission to occur.

The significantly higher levels of aggressive behaviour that we observed in banded mongooses foraging at garbage (Fig. 2.1, Table 2.1) are most likely due to the highly clumped and presumably "valuable" nature of food resources there. Although we could not identify every food item that led to aggression, aggression appeared to occur most often in response to the discovery of large food items regardless of foraging habitat type. However, if mongooses were dispersed enough to allow the individual that found the item to remain undetected by the troop, then aggression was less likely. Thus, aggression during foraging is likely due to an interaction between item size (perceived "value") and resource distribution. Garbage sites provide a more clumped food resource and may have more large food items than the other foraging habitats. Our "other modified" habitats, such as paths that infrequently have large food items, served as a control for the possibility that human modification itself, rather than resource clumping or food

item size, alters banded mongoose aggression. That aggression levels in the "other modified" habitats were similar to those observed in natural habitats supports the interpretation that variation in resource clumping and/or food item size at garbage is the likely mechanism driving our results. There was also a non-significant trend for mongooses to be somewhat more aggressive while foraging for insects under lights than while foraging on lawns, other modified areas, or their natural habitat (Fig. 2.1). While insects are a natural part of the mongooses' diet, it appears that they are a more highly clumped resource below lights than in their natural environment. Further work should measure the extent to which banded mongoose resources are clumped and perceived as valuable on lawns, under lights, and at garbage, in order to confirm the role of resource concentration versus food item value in driving the detected results. More broadly, these results suggest that researchers should consider multiple forms of human modification that result from urbanization when investigating its effects on wildlife.

Aggression associated with urbanization is particularly important in understanding disease transmission dynamics as it can influence both pathogen exposure and susceptibility (Fairbanks and Hawley 2012). Not only can wounds be a potential site for exposure to pathogens in the environment, as is likely the case in our study system, but pathogens can also be directly transmitted during aggressive behaviour (e.g. McCallum et al. 2007). In addition, aggression can cause neuroendocrine responses, such as changes in glucocorticoid (a.k.a. stress hormone) levels, that lead to changes in immunity (e.g. de Groot et al. 2001). While glucocorticoid levels may influence susceptibility to or progression of tuberculosis in our system especially via nutritional and reproductive stressors (Laver et al. 2012), aggression was not related to glucocorticoid levels in this dataset, as measured by faecal glucocorticoid metabolite analysis (unpublished data). This lack of relationship between glucocorticoids and aggression suggests that exposure may be the most important mechanism linking aggression and disease via injury in banded mongooses. However, changes in aggression due to urbanization may affect both exposure and susceptibility simultaneously in some systems, causing substantial alterations to disease spread.

Our research underscores the need to examine effects of urbanization on disease dynamics beyond changes to population sizes of pathogens, vectors, and hosts. Behavioural changes may work synergistically with (e.g. Plowright et al. 2011), antagonistically to (Page et al. 2008), or in lieu of (Wright and Gompper 2005) changes in population characteristics that lead to changes in disease dynamics. These interactions have important applied implications, because culling or otherwise reducing wildlife populations is frequently considered as an option for disease control. Reducing host population size may not have the desired disease-reducing effect if disease dynamics are heavily influenced by behaviours that are not density dependent. When trying to understand or mitigate changes to disease dynamics caused by urbanization, studies should consider behavioural or physiological changes alongside the better studied population-level parameters such as host density. As urbanization increases around the world, disease research and management in urban areas must not ignore the behavioural effects of urbanization on wildlife species and consequent impacts on host – pathogen interactions. Urban planners should be aware that these effects of urbanization pose a risk not only to wildlife health, but also to human health in the case of zoonotic disease in urbanized wildlife. Our study specifically indicates that property managers have the opportunity to reduce their impact on urban wildlife diseases using relatively simple practices, such as excluding wildlife from garbage at residences and businesses.

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Attribution

B.M. Fairbanks was the main contributor to this chapter, providing the intellectual impetus, and performing data collection, data analysis, writing and substantial grant writing that supported the work. D.M. Hawley and K.A. Alexander contributed with substantial editing, guidance of ideas and research, and funding. K.A. Alexander initiated and maintains the banded mongoose project in Botswana.

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Chapter 3

The Impact of Health Status on Dispersal Behavior in Banded Mongooses (*Mungos mungo*)

Bonnie M. Fairbanks[1]*, Dana M. Hawley[1], Kathleen A. Alexander[2.3]

[1] Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA
[2] Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, VA, USA
[3] Center for African Resource: Animals, Communities, and Land use (CARACAL), Kasane, Botswana
* Corresponding author: bonniemf@vt.edu

Abstract
While disease and injury have obvious impacts on mortality, they can have less understood non-lethal impacts on individual behavior, with important consequences for population-level disease dynamics. Here we discuss two questions about how health status affects dispersal behavior: Do unhealthy individuals disperse at a different rate than healthy individuals, and does the presence of unhealthy individuals within a social group impact the dispersal rate within the group as a whole? To explore these questions, we use our dataset of dispersal in banded mongooses (*Mungos mungo*), a cooperative social mammal, in the Chobe district of Botswana. Banded mongooses in this region are infected by a novel tuberculosis pathogen in the *Mycobacterium tuberculosis* complex, *M. mungi*, and this pathogen's impacts on mongoose behavior and social dynamics are not yet known. In our study, we opportunistically observed dispersal events and found that diseased and/or injured banded mongooses were significantly less likely to disperse than healthy individuals. Additionally, we found a non-significant positive relationship between the numbers of diseased and/or injured individuals in the troop and the proportion of individuals that dispersed from the troop, possibly suggesting that the presence of disease in a troop can affect dispersal behavior of many individuals within the troop regardless of their own health status. Our results highlight the importance of identifying behavioral differences between diseased and healthy individuals because these differences can strongly influence population-level disease dynamics.

Introduction

Disease and injury are major threats to survival and reproduction, demonstrated by the presence of immune defenses in all organisms (Cooper 2010; Playfair and Chain 2009). Although disease-associated morbidity and mortality are well documented, the non-lethal impacts of disease on individual behavior are less well-understood. Just as the non-lethal effects of predation via behavioral changes in prey were found to be as great, or greater than its lethal effect on prey population size (e.g. Peacor and Werner 2001), the non-lethal effects of disease and injury on behavior may have a major impact on individuals, social groups, and populations. Additionally, the way animals behave when infected with a directly-transmitted pathogen will have a significant influence on resulting transmission dynamics (Hawley and Altizer 2011). As reviewed by Moore (2002), some parasites and pathogens actively control or redirect host behavior in order to facilitate transmission (e.g. Klein 2003; Lagrue and Kaldonski 2007; McCurdy et al. 1999). However, other behavioral changes during infection may be the host's response to disease, which are usually adaptive for the host (Johnson, 2002). Regardless of

whether behavioral changes are driven by pathogen "manipulation" or host pathology, the behavior of infected individuals has the potential to greatly influence the population dynamics of directly-transmitted pathogens.

One component of behavior that is likely to both be influenced by disease status and have a large impact on infectious disease transmission, is dispersal, or permanent movement from one territory or social group to another. An individual's decision to disperse or not can be dependent on many interacting social, physiological, and ecological factors. Considerable research has been performed on phenotype dependent dispersal, such as the effect of energy stores, sex, or age (Clobert et al. 2009; Ims and Hjermann 2001). Health status, including both disease and injury, may also be important in dispersal decisions given the energy requirements of immune functions (e.g. Eraud et al. 2005) and potential behavioral changes caused by disease (Adelman and Martin 2009), but has rarely been examined. Similarly, while the effect of host dispersal on disease spread has been studied in several species (Clements et al. 2011; Tuyttens et al. 2009), little research exists on the opposite phenomenon, the influence of disease on host dispersal decisions. Disease and dispersal may have important feedbacks on one another that have not yet been explored.

Dispersal is a prime candidate for a behavior that may change based on health status, because dispersal is not an obligatory behavior in many species. For example, Lachish and co-workers (2011) showed that female Tasmanian devils (*Sarcophilus harrisii*) have a lower dispersal rate and shorter dispersal distances after the onset of an epidemic of devil facial tumor disease. This was a population-wide effect, and the researchers concluded that females moved shorter distances and less frequently because the decline in conspecifics led to an increase in resources and consequently lower motivation to move. However, they did not identify at the individual level whether diseased devils were more or less likely to disperse than healthy devils. When culling was evaluated as a tuberculosis control approach in Britain, researchers found that European badgers (*Meles meles*) infected with the bovine tuberculosis pathogen *Mycobacterium bovis* move farther than uninfected individuals (Pope et al. 2007). However the authors did not distinguish between dispersal and non-dispersal movements in the analysis, and thus could not conclude whether infected badgers dispersed more often or further than uninfected badgers (i.e. the longer movements by infected badgers may or may not have been permanent movements out of their social group) (Pope et al. 2007). These studies indicate that disease can have an effect on movement behavior, but do not address whether a diseased individual is more or less likely to disperse than a healthy individual. If infected individuals make different dispersal decisions than uninfected individuals, it could have a significant impact on social group structure, population dynamics, and pathogen transmission.

Two complementary but distinct questions lack study within both the dispersal and disease ecology literature: First are diseased individuals more or less likely to disperse than healthy individuals? Secondly, within a social group, does the presence of diseased individuals influence the rate of dispersal in the group as a whole? While a change in the dispersal rate of diseased individuals themselves may lead to a change in overall dispersal rate, the presence of diseased group mates may influence the dispersal rate of healthy group members as well. This dispersal strategy could be a behavioral adaptation to avoid infection (Behringer et al. 2006b), or a habitat selection strategy that uses the health of group mates as an indicator of habitat quality (for example, habitat quality can influence health status via resource abundance if fewer food resources lead to decreased immunity (Lochmiller and Deerenberg 2000)).

In 2000, a novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi,* was identified among banded mongooses (*Mungos mungo*) in Chobe District, Botswana (Alexander et al. 2010). A long-term study was initiated to understand this emerging host-pathogen system. Mortality associated with *M. mungi* ranges from 10-15% per annum in our study population. Invasion by the pathogen appears to occur primarily through breaks in the skin, thus injury is highly associated with subsequent onset of clinical signs of the disease (Fairbanks et al.). After the onset of clinical signs, diseased mongooses normally die within 2 to 10 months (Alexander et al., 2010 and KAA unpublished data). *M. mungi* infection is associated with easily observable clinical signs, and mongooses are social animals that engage in dispersal behavior from troops. Therefore, this system provides a rare opportunity to collect behavioral observations of both diseased and healthy individuals in order to evaluate the impact of infectious disease on the behavioral ecology of a host population, including whether health status affects individual dispersal behavior.

Though observations of dispersals were limited, they presented an opportunity to explore these previously unexplored questions about health and dispersal. First, are diseased or injured mongooses more or less likely to disperse than healthy mongooses? We hypothesized that diseased or injured mongooses would be less likely to voluntarily disperse, because dispersal and disease or injury both entail high energetic requirements, making it difficult for an individual to meet the requirements of both simultaneously. Alternatively, diseased or injured individuals may be harassed and forcibly evicted from the group, or may disperse voluntarily in order to avoid directed aggression from healthy group members. Eviction of unhealthy group members may be an adaptive strategy by healthy individuals to avoid infection or attracting predators. Banded mongooses sometimes evict group members ((Cant et al. 2001) and see details in methods), so we alternatively hypothesized that diseased and/or injured individuals may be more likely to disperse due to harassment or eviction than healthy individuals. Second, we asked whether mongooses are more or less likely to disperse when their troop contains diseased or injured conspecifics. We hypothesized that as the number of diseased or injured individuals increased within a troop, the proportion of dispersal would increase within the troop as a whole due to increased dispersal of healthy troop members. Here we investigate both questions using data from opportunistically observed dispersal events in our study population.

Methods

Ethics Statement: Animals were radio-collared or ear-tagged by trapping and anaesthetizing animals as previously published (Cant 2000) and all efforts were made to minimize pain and suffering. During 2010-2011, 5-12 individuals were radio-collared at any single point in time with a total of 21 individuals collared in all troops combined over that time period. This research was conducted under permit from the Botswana Ministry of Environment, Wildlife, and Tourism and with approval of the Virginia Tech's Institutional Animal Care and Use Committee (Protocol number 07-146-FIW). Observations were conducted with permission on both private and public lands.

Eight banded mongoose troops were monitored regularly in the study area along the Chobe River in northeastern Botswana, within the townships of Kasane and Kazungula and the Chobe National Park. These eight troops had 1-2 radio-collared individuals to allow reliable observation of the troops. Other troops existed within the study area and were monitored opportunistically. Observations were conducted by two observers. One observer conducted frequent but short observations on the eight radio-collared troops (observing each troop up to one

hour, two times a day, 3 to 6 times per week, for three years). The other observer performed longer and more detailed but less frequent observations on four of the eight radio-collared troops (observing one or two troops per week, 3-12 hours per day depending on weather and mongoose visibility, 5 days per week, for three years). In the four intensively observed troops, up to 6 individuals per troop were marked with ear tags to facilitate discrimination among individuals (see below). Both observers recorded location, weather, number of individuals (including sex and ages when possible), clinical signs of TB, and behavior (e.g. activity budgets and social interactions) using both scan and focal sampling (Altmann 1974).

Dispersal events were unpredictable, and were thus observed opportunistically. Dispersals typically occur from September to November towards the end of the dry season (April-October) and the beginning of the wet season (November-March). Dispersal events in our study population in Botswana showed more plasticity than that reported in Uganda banded mongoose troops (Cant et al. 2001). In Uganda, emigration occurs in same-sex groups that are usually forced to disperse (Cant et al. 2001). In contrast, we observed at least 2 instances of an individual emigrating alone, and we observed 3 voluntary, mixed-sex emigrations, 1 forced same-sex emigration, and 2 same-sex emigrations with unknown forced/voluntary status (where we determined forced/voluntary status from extensive, directed aggression within the troop within the week before the dispersal). In each case, these dispersals were confirmed by observation of the group of dispersers apart from the natal troop at least once (thus, these were not simply disappearances). In this paper, we focus on the six dispersal events where more than one individual was observed to permanently leave the troop (Table 3.1), because solitary dispersal is very rare in banded mongooses and may involve different factors than group dispersal. When these group dispersal events occurred, to the extent possible, we recorded number, age, sex, and health classification of dispersers (see below for classifications).

In the absence of ear tags or radio collars, individual mongooses are virtually indistinguishable from one another. However, individuals are readily identifiable via natural scars, injuries, or disease. Therefore, all diseased and/or injured animals in a troop were individually identifiable at the time of dispersal events (0-12 diseased and/or injured individuals per dispersal event, a total of 21 over all dispersal events; Table 3.1). In addition, a minimum of one and a maximum of seven identifiable healthy mongooses were identifiable per dispersal event via scars, radio collars, or ear tags (a total of 29 identifiable healthy individuals over all dispersal events). Age class and sex (the latter distinguishable only by genitalia) were known for all identifiable individuals. Exact ages were unknown for most of the individuals in the study, but where possible, were categorized by size (classifying "young" mongooses as those approximately 1.5 years or younger and "old" mongooses as those older than 1.5 years). Additionally, minimum ages of identifiable individuals observed for several years were known (e.g. an identifiable individual that was fully grown (e.g. at least 1.5 to 2 years old) in 2008 would be at least 5 years old in 2011).

We classified health status of an individual as either healthy or diseased and/or injured. Briefly, animals presenting with clinical signs consistent with *M. mungi* infection were classified into the diseased and/or injured group. The initial and most common clinical presentation for *M. mungi* is progressive weight loss (Alexander et al. 2010). As this disease advances, clinical signs can also include cachexia, lethargy, lagging, hunching, discolored fur, sneezing, wet eyes/nose, swollen or distorted nose, drooping or enlarged testicles, and fearlessness. An individual was considered diseased if, during the last month it was observed, 3 or more of these clinical signs were rated as 8 or less on a scale of 1 to 10 (10 being completely healthy, 1 being severely

disordered). Observations of this clinical presentation in mongooses have only been linked to *M. mungi* infection (K. A. Alexander, unpublished data). We defined injured animals as any individual presenting with a persistent (greater than 5 days) break in the skin, persistent unusual swelling of any extremity (including the nose), and/or persistent limping.

We combined injured animals with diseased individuals rather than keeping them in separate classifications in our analysis for three reasons. First, due to the apparent transmission of *M. mungi* through breaks in the skin, injured animals may be more susceptible to *M. mungi* invasion (Alexander et al. 2010). Thus, the potential impacts of dispersal on disease transmission in this system would apply similarly to actively diseased animals and injured animals that are highly likely to become actively diseased within a few months of injury. Second, the clinical signs of a chronic injury and the early clinical signs of TB infection are similar (e.g. lethargy, lagging behind troop, reduced foraging, visible weight reduction) and difficult to distinguish. Therefore, we could not accurately identify a specific time point that an animal moved from a classification of injured into a classification of diseased. Third, injured animals that became infected with TB did not show resolution of their injury; thus many individuals were classified as both injured and diseased until death or disappearance, precluding us from categorizing them separately.

Question 1: Are diseased and/or injured mongooses more or less likely to disperse than healthy mongooses?

To determine if an individual's health status affected whether or not it dispersed, we analyzed data from dispersal events that occurred when troops contained diseased and/or injured individuals (n = 177 individuals spread over 4 dispersal events). We performed a generalized linear mixed model (GLMM) using the events per trial format for the response variable, where events were dispersed individuals within a given health status, and the trials were the number of individuals in that health status for a given dispersal (i.e. the response variable was the proportion of individuals that dispersed with a given health status during a given dispersal). When using this format in the SAS GLIMMIX procedure, the binomial distribution and logit link function are used, inducing a logistic regression that can have random intercepts. We used troop as a random effect. Health status and troop size were fixed effects (the interaction between these two effects was not significant so was removed from the model). An R-side scale parameter was used to account for underdispersion (χ^2/DF=0.49 without this scale parameter).

Question 2: Are mongooses more or less likely to disperse when their troop contains diseased and/or injured conspecifics?

We determined if the health status of group mates affected the proportion of individuals that dispersed using all six dispersal events (n=237 individuals spread over 6 events). A similar GLMM was performed as for Question 1 (i.e., events/trials format in proc GLIMMIX, binomial distribution, logit link function). However, for this analysis, the response variable was the proportion of individuals in the troop that dispersed (number of individuals that dispersed / troop size). The number of diseased and/or injured individuals in the troop at the time of dispersal was the single fixed effect. Troop size was not used as a fixed effect in this analysis because it is the denominator of the response variable. We again used a random intercept with troop as the subject. An R-side scale parameter was used to account for overdispersion (χ^2/DF=1.33 without this scale parameter).

Both GLMM analyses were performed using SAS (version 9.3; SAS Institute, Cary, NC; 2010) with α=0.05.

Age and Sex Biases

If sex or age biases exist in dispersal rates or disease and/or injury rates, these biases could be confounding factors when analyzing for the effects of disease and/or injury on dispersal. For example, if young individuals are more likely to disperse than older individuals, but most diseased and/or injured individuals are old, it would appear that disease and/or injury leads to a lower likelihood of dispersal, while in fact the true factor affecting dispersal is age. Because of their large troop sizes, similarity in appearance, and high activity level, it is extremely difficult to get an accurate count of a banded mongoose troop that includes sex and/or age of all individuals. We therefore were unable to include sex and age in our statistical models of dispersal. However, because we had complete information on age and sex of all diseased and/or injured individuals, we could examine whether a bias existed with respect to the sex and age ratio of all diseased and/or injured individuals alive at the time of the observed dispersal events.

First, we examined the potential for age to be a confounding factor in our data set. Among the diseased and/or injured individuals available for dispersal, there was a biased age ratio (4 young, 16 old). We used a Fisher's exact test to determine if this age distribution of diseased and/or injured individuals was similar to that present in the three troops with observed dispersal events. Age ratios for each troop immediately before dispersal were not available, however we did have age ratios for these three troops in the dry season of 2010, two to six months before the 2010 dispersals occurs (no births and few deaths occur during the dry season). To further test whether dispersed individuals were a biased subset of a troop's age distribution, we included in the Fisher's exact test the age ratio of all identifiable animals (regardless of health status) observed to disperse. Thus, this Fisher's exact test determined if the age ratio was the same among three groups: pre-dispersal diseased and/or injured individuals, pre-dispersal troops, and recognizable dispersers.

Second, we determined if sex was a confounding factor in our analyses. We had no fine-grained information on sex ratios in these troops, so we could not perform a test similar to the age test described above. Among the diseased and/or injured individuals, there was no bias in the sex ratio (11 males, 10 females). Thus, with regard to sex, there was an unbiased pool of diseased and/or injured individuals available for dispersal. However, if troops have strongly uneven sex ratios overall, and/or dispersal is sex biased, there could appear to be different dispersal rates between the two health statuses that are actually due to differing sex ratios. We knew the exact dispersal rate for each sex within each dispersal event for diseased and/or injured individuals. For each dispersal event, we calculated the minimum possible dispersal rate for each sex for healthy individuals by determining the minimum possible number of each sex, as well as the maximum number of dispersers of each sex. A one-tailed Wilcoxon signed rank test was used to test whether the sex dispersal rates for diseased and/or injured individuals was significantly less than the minimum sex dispersal rates for the healthy individuals.

Sex and age ratio analyses were performed with SAS JMP Pro (version 10.0.0; SAS Institute, Cary, NC; 2012) and α=0.05.

Results

Question 1: Are diseased and/or injured mongooses more or less likely to disperse than healthy mongooses?

Dispersal of diseased and/or injured individuals was rare during the four dispersal events that occurred while diseased and/or injured individuals were in a troop (Table 3.1). Health status and troop size were both significant predictors of dispersal ($F_{1,3}$=153.71, P=0.001; $F_{1,3}$=128.04, P=0.002 respectively; Fig. 3.1). The estimate for troop size (-0.7624, SE= 0.06738) indicated that troop size had a negative relationship with proportion of individuals dispersing, however the range of troop sizes in these four dispersal events was small (40-50 individuals per troop).

Table 3.1. Summary of the observed dispersal events in our study population.

Troop ID	Number of diseased/ injured in troop	Month and year	Troop size (pre-dispersal)	Number and sex of dispersers	Number and sex of diseased/injured dispersers	Forced or voluntary dispersal
CCH	3	Nov 2010	42	10 males	0	Unknown
CCH	12	Oct 2011	40	19 mixed sex	2 males	Voluntary
CGL	1	Oct 2011	50	13 males	0	Unknown
CSL	5	Sept 2011	45	30 mixed sex	1 male	Voluntary
CSL	0	Sept 2010	35	10 mixed sex	n/a	Voluntary
CSL	0	Oct 2010	25	10 females	n/a	Forced

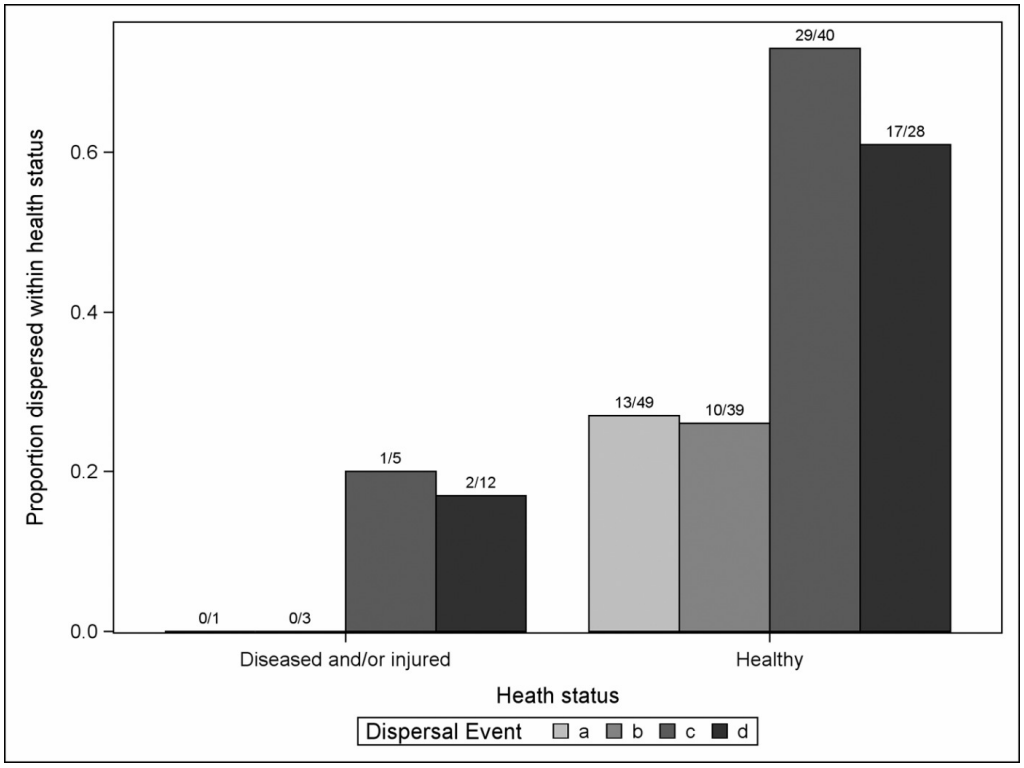

Figure 3.1. The percentage of individuals that dispersed within each health classification during three dispersal events. Fractions above bars indicate the number that dispersed out of the number of individuals in each health classification at the time of each dispersal event. In troops that contained diseased and/or injured individuals, health status was a significant predictor of dispersal ($F_{1,3}$=153.71, P=0.0011), with a significantly greater proportion of healthy individuals dispersing than diseased and/or injured individuals.

Question 2: Are mongooses more or less likely to disperse when their troop contains diseased and/or injured conspecifics?

In addition to the four dispersal events above, two dispersal events occurred when all individuals in a troop were healthy. When all six dispersal events were examined, there was a non-significant positive relationship between the number of diseased and/or injured individuals in the troop and the proportion of the troop that dispersed ($F_{1,2}$=7.52, P=0.11; Fig. 3.2).

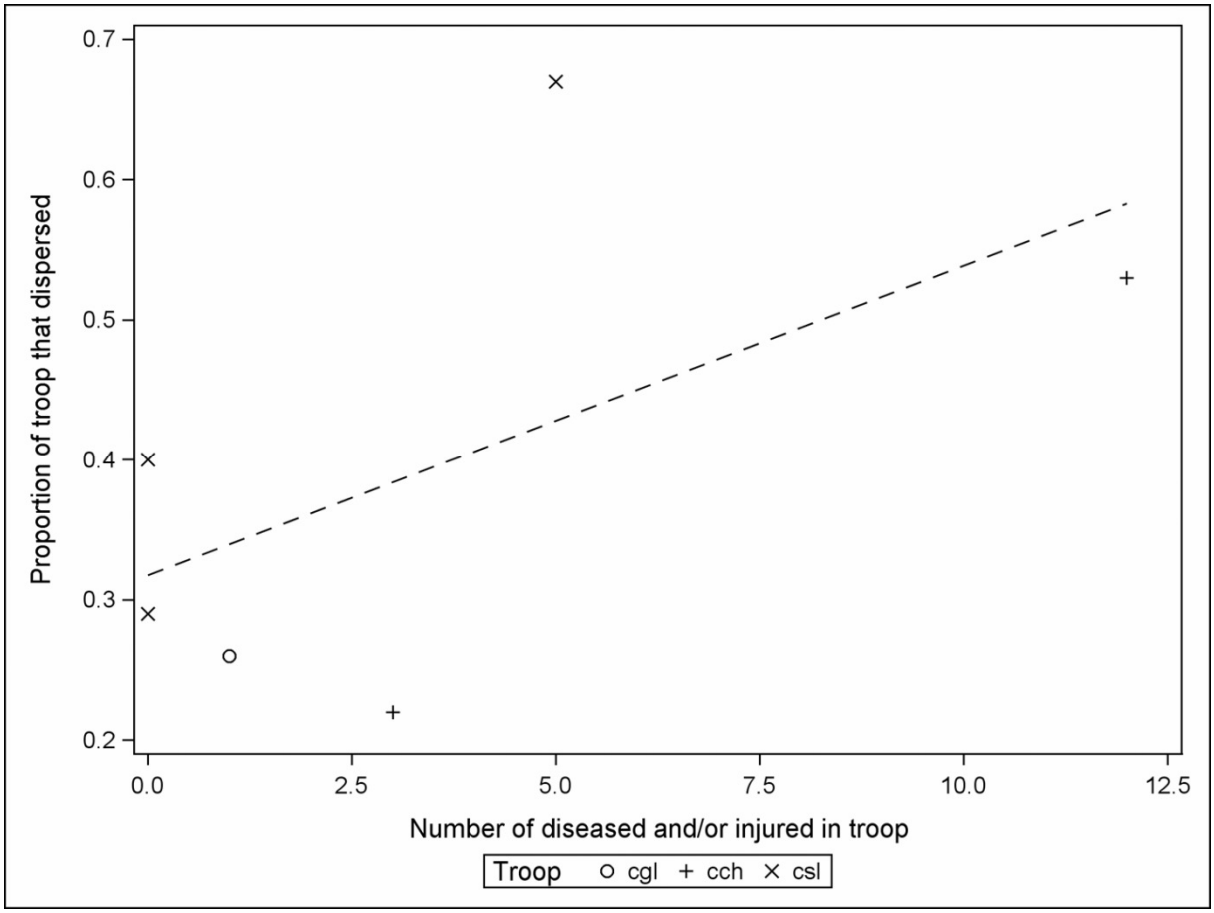

Figure 3.2. Proportion of individuals that dispersed *vs*. proportion of diseased and/or injured individuals present in troop. Dotted line indicates the non-significant positive relationship ($F_{1,2}$=7.52, P=0.11).

Age and Sex Bias

We observed six dispersal events wherein a group of individuals permanently left a troop (Table 3.1). The age bias present in diseased and/or injured individuals alive at the time of a dispersal event (20% under 1.5 years) was not significantly different from the age bias present in

the troops during the 2010 dry season (18.2-36.4%), nor was it significantly different from the age bias in identifiable animals that dispersed (33.3%; Fisher's exact test=0.0002, P=0.35).

The dispersal rate of each sex was always lower in the diseased and/or injured group, except for two dispersal events where no females dispersed regardless of health status. This difference in sex dispersal rates was significant (1-tailed Wilcoxon signed rank test: P=0.31).

Conclusions

Our results show that diseased and/or injured individuals are significantly less likely to disperse than healthy individuals. These results indicate that studies of disease transmission and dynamics should consider differences in individual behavior between healthy and diseased individuals, and that studies of phenotype dependent dispersal must consider the health status of potential dispersers. We considered sex and age biases as possible confounding factors for these results. There was no significant difference in the age ratio among diseased and/or injured individuals, a "typical" troop during the dry season, and identifiable animals that dispersed, indicating that age, as we have categorized it, is not a factor that affects dispersal or disease and/or injury in our dataset. However, because we had only two broad categories for age, the possibility remains that age could be a factor in dispersal when categorized more precisely. Furthermore, within each dispersal event, we found dispersal rates of each sex were lower for diseased and/or injured individuals than healthy individuals, indicating that the lower overall dispersal rate of diseased and/or injured individuals was not a result of differences in the sex ratios of the two health classes. Overall it appears that sex and age biases did not strongly affect our test of whether health status influences the probability of dispersal in banded mongooses.

Our results support the hypothesis that diseased and/or injured individuals are less likely to disperse than healthy individuals, possibly due to energetic or locomotor constraints. The decreased dispersal of diseased and/or injured individuals could be driven by the energy demands of immune functions during disease and injury (e.g. Eraud et al. 2005), and/or the behavioral changes induced by sickness behavior (Adelman and Martin 2009; Hawley et al. 2007) or injury (e.g. pain while walking may discourage locomotion). Energy demands or behavioral changes due to sickness and injury could not only reduce an individual's ability to cover the distances needed for some dispersals, but also lead to an inability to keep up with other, healthy dispersers, leaving a diseased and/or injured individual vulnerable to predation. Our observations were not consistent with our alternative hypothesis that diseased and/or injured animals are forced to disperse, or that they disperse voluntarily due to increased aggression directed toward them. The only two diseased and/or injured individuals that were confirmed as dispersers were part of a voluntary dispersal event. Additionally, the only confirmed forced dispersal occurred in a troop with no diseased and/or injured individuals. Anecdotally, we observed no harassment or avoidance of diseased and/or injured individuals by healthy individuals, and diseased or injured mongooses were often allo-groomed at a normal or higher rate than conspecifics.

Decreased dispersal of diseased individuals would be expected to dampen among-group transmission of directly transmitted pathogens, provided individuals are not infectious prior to developing clinical disease. Our results therefore highlight the need for models of infectious disease to incorporate parameters that reflect variation in individual health status (Wendland et al. 2010). Most current models disregard the influence of disease status on dispersal and the effect this may have on disease transmission dynamics (but see Castillo-Chavez and Yakubu 2001; Wang and Mulone 2003). For example, Nunn et al. [23] developed a model to understand

how disease mortality within a social group affects dispersal out of that social group, and thus spread of disease through the population (using chimpanzees (*Pan troglodytes*) and gorillas (*Gorilla gorilla)* to parameterize the model). However, they assumed that healthy and diseased individuals disperse equally and that an individual disperses because its mating partner dies. Using average dispersal rates of a population to predict disease dynamics might be misleading if infectious individuals have higher or lower dispersal rates than healthy individuals. Modeling dispersal and disease with bi-directional influences on one another has the potential to increase the accuracy of predictions about disease dynamics. However, in order to accurately predict the influence of health status on population-level disease dynamics, it will be necessary to identify the relationship between pathogen transmission potential and stage of disease.

We also hypothesized that healthy individuals may use the health status of group mates when making dispersal decisions similar to the way that individuals use the reproductive or foraging success of others as "public information" to select habitat (Danchin et al. 2004; Rieucau and Giraldeau 2011). "Informed dispersal" (Clobert et al. 2009) using public information was found to occur in the collared flycatcher (*Ficedula albicollis*), with immigrants using high quantity of conspecifics' offspring in an area as a cue for settlement, and residents using both low offspring quantity and quality as a cue to emigrate (Doligez et al. 2002). In our study, we hypothesized that individuals might use the health status of group mates as a cue to emigrate, because group mates' health status might provide information about disease risk and/or habitat quality. Although we found a non-significant positive relationship between troop dispersal and the presence of diseased/injured troop mates (Fig. 3.2), we were not able to conclusively demonstrate that healthy individuals base dispersal decisions on the health status of troop mates. However, due to the small number of observed dispersal events, further study is needed in order to fully address this question.

Our study used data from six opportunistically observed dispersal events in order to examine relationships between dispersal and disease status. Because our inferences are based on a limited number of events, the patterns we found must be taken with some caution. However, these data, though limited, are particularly valuable because of the difficulty in observing dispersal in banded mongooses and other species (rather than inferring dispersal from the disappearance of individuals, for example). Our aim with the present study is to use our unique observations to generate hypotheses that disease ecologists can incorporate into their future research and refine with larger datasets. Our results provide an interesting glimpse into the complex influence of infectious disease on host behavioral ecology. Importantly, the interaction identified here between dispersal and disease is potentially bidirectional and might create as yet unstudied feedback loops, whereby disease affects dispersal behaviors that then directly influence disease transmission. Differences in dispersal and migration behavior of healthy, diseased, and immune individuals can have a significant impact on pathogen transmission and persistence dynamics across the landscape (Altizer et al. 2011; Plowright et al. 2011; Vial and Donnelly 2012; Wang and Mulone 2003) and must be considered as we seek to understand and manage infectious diseases of concern to human, domestic animal, and wildlife health.

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Attribution

B.M. Fairbanks was the main contributor to this chapter, providing the intellectual impetus, and performing data collection, data analysis, writing, and substantial grant writing that supported the work. D.M. Hawley and K.A. Alexander contributed with substantial editing, guidance of ideas and research, and funding. K.A. Alexander initiated and maintains the banded mongoose project in Botswana.

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Chapter 4

Infectious disease and behavior in banded mongooses: no evidence for avoidance of visibly diseased conspecifics in a highly social species

Bonnie M. Fairbanks[1]*, Dana M. Hawley[1], Kathleen A. Alexander[2.3]

[1] Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA
[2] Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, VA, USA
[3] Center for African Resource: Animals, Communities, and Land use (CARACAL), Kasane, Botswana
* Corresponding author: bonniemf@vt.edu

Abstract

Host individuals may behaviorally respond to infectious disease in ways that can alter population-level disease dynamics. A novel pathogen in the *Mycobacterium tuberculosis* complex, *M. mungi*, has emerged among banded mongooses (*Mungos mungo*) in Northeastern Botswana. This host – pathogen system provides an opportunity to study how individual behavior and social interactions in a group-living species might respond to infectious disease. We used repeated focal observations of known individuals to identify differences in behavior and social interactions between healthy individuals and those with clinical signs of tuberculosis (TB). Clinically diseased banded mongooses exhibited a significantly smaller proportion of time active and alert, a larger proportion of time resting, and a slower behavioral transition rate compared to healthy individuals. They also showed lower reciprocation of allogrooming than healthy individuals, reciprocating in approximately half of allogrooming events whereas healthy individuals reciprocated in nearly all allogrooming events. In contrast to prior studies of other species, we found no evidence for avoidance of clinically diseased mongooses by healthy individuals or vice-versa: clinically diseased individuals did not have lower levels of social behaviors than healthy individuals and clinically diseased mongooses were allogroomed at the expected level despite their decreased reciprocation. Our results show that although behavior (and other traits) of clinically diseased individuals may differ from that of healthy individuals, avoidance of diseased conspecifics did not occur in this highly social species. We discuss hypotheses for this lack of avoidance and implications for pathogen transmission.

Introduction

Disease can affect the behavior of both infected and healthy individuals, for example by altering the activity level of diseased individuals (e.g. Martin et al. 2007) and by inducing avoidance behavior in healthy individuals (e.g. Behringer et al. 2006b). Both types of behavioral changes can influence the spread of directly-transmitted parasites and pathogens by reducing opportunities for interactions between infectious and susceptible individuals (Hawley et al. 2011). Researchers have long hypothesized that there should be strong selection for non-diseased individuals to recognize and avoid diseased conspecifics (Kiesecker et al. 1999), yet few studies have tested this idea. These few studies, performed on various taxa, have generally found that healthy individuals can recognize diseased individuals and typically do avoid them (Arakawa, Arakawa, & Deak, 2010; Behringer et al., 2006b; Edwards, 1988; Kavaliers et al., 2003; Kiesecker et al., 1999; Tobler & Schlupp, 2006; Zylberberg, Klasing, & Hahn, 2013, but see

Bouwman & Hawley, 2010; Table 4.1). However, all but one of these studies (Behringer et al. 2006a) were performed only in laboratory settings. Furthermore, all of the taxa studied to date live in fluid rather than stable social groups (Table 4.1).

Table 4.1. Disease avoidance behavior in various species.

Species	**Detection method**	**Social group type**	**Notes**	**References**
Bullfrog tadpoles *(Rana catesbeiana)*	Chemosensory (confirmed)	Temporary[1], fluid[2], voluntary[3]		(Kiesecker et al. 1999)
Caribbean spiny lobsters *(Panulirus argus)*	Chemosensory (probable)	Temporary, fluid, voluntary		(Behringer et al. 2006b)
Lab mice (*Mus Musculus*)	Chemosensory (confirmed)	Semi-stable[4], voluntary	Disease can be detected in an individual or their waste	(Edwards 1988; Kavaliers et al. 2003)
Lab rats (*Rattus norvegicus*)	Chemosensory (confirmed)	Semi-stable, voluntary	Disease can be detected in an individual or their waste	(Arakawa et al. 2010)
House finches (*Carpodacus mexicanus*)	Visual (probable)	Temporary, fluid, voluntary	General avoidance observed, but healthy males prefer to feed near diseased conspecifics due to reduced aggression	(Bouwman and Hawley 2010; Zylberberg et al. 2013)
Western mosquitofish (*Gambusia affinis*)	Not reported	Temporary, fluid, voluntary	Not only are diseased individuals avoided, diseased individuals avoid shoaling	(Tobler and Schlupp 2006)

[1] Temporary = groups form and disband frequently (e.g. foraging flocks in birds), rather than being permanent.
[2] Fluid = Individuals come and go from the group.
[3] Voluntary = An individual can either join a group or live alone. It may do both at different times in life.
[4] Semi-stable = Groups may be fairly long lasting (several days or months) and have some change in members, but they are unlikely to be permanent throughout an individual's life.

The predictions for avoidance of infected conspecifics may differ for highly social species that live in stable and permanent social groups, such as banded mongooses (*Mungos mungo*). For example, Hart (1990) suggests that helping diseased or injured group members may be adaptive for all group members in highly social species because of the strong benefits of large social groups in these types of societies. Loehle (1995) similarly suggests that if exposure is likely to

be equal for all group members regardless of social contact, continuing beneficial social behaviors may be more adaptive for all group members than avoidance. Alternatively, he suggests that diseased individuals living in groups with kin, such as banded mongooses, may avoid healthy group mates to increase inclusive fitness. While it is unclear how social structure may influence avoidance behavior, it is clearly an important factor that has the potential to modify both pathogen transmission dynamics and the costs and benefits of conspecific avoidance.

Here we studied the behavioral differences between healthy mongooses and those exhibiting clinical signs of infection in a group-living and free-living host, the banded mongoose. This system is particularly amenable to non-invasive behavioral sampling due to the visible clinical signs of tuberculosis caused by a novel pathogen, *Mycobacterium mungi,* affecting banded mongooses in northeastern Botswana (Alexander et al. 2010). While most pathogens in the Mycobacterium tuberculosis complex are directly transmitted, our findings thus far suggest that horizontal transmission of *M. mungi* occurs only infrequently and that the pathogen is primarily environmentally transmitted through breaks in the nasal planum and/or the skin of the mongoose host (Alexander et al. 2010 and KAA unpublished data). Annual variation in infection rates exists, but typically between 10-20% of individuals show clinical signs of TB each year, and all that have shown clinical signs have died within 2-10 month of disease onset (Alexander et al. 2010 and KAA unpublished data). Banded mongooses are semi-fossorial carnivores that live in stable groups, referred to as troops, with a low-skew communal breeding system and a loose or non-existent dominance hierarchy (Bell et al. 2012; Fischbacher 1993; Gilchrist 2006; De Luca and Ginsberg 2001). Within a troop, some individuals are kin while others are not, and there is no evidence for inbreeding avoidance (Gilchrist 2006; Waldwick et al. 2003).

In order for avoidance (or helping) of diseased conspecifics to occur, healthy individuals must be able to accurately detect the presence of disease in others. Chemical cues have been shown to be a method of disease detection in bullfrog tadpoles, laboratory mice and laboratory rats, and are thought to be the most likely cues for spiny lobsters (Arakawa et al., 2010; Behringer et al., 2006a; Edwards, 1988; Kavaliers et al., 2003; Kiesecker et al., 1999; Table 4.1). Visual behavioral cues from diseased conspecifics are thought to be the cue used for alteration of conspecific behavior in house finches (Bouwman and Hawley 2010). Banded mongooses have an acute sense of smell (Neal 1970) and can identify troops and probably individuals by scent (Muller et al. 2008), and they also have keen vision (Rood 1975) and commonly respond to visually-perceived behavior of conspecifics (e.g. Cant et al. 2002 and pers. obs.). While scent and non-behavioral visual cues such as poor coat quality may be indicators of disease to healthy individuals, any behavioral differences between clinically diseased individuals and healthy individuals, such as reduced activity, may be additional cues for recognition of diseased individuals. Here we examined differences in behavior between diseased and healthy individuals using an extensive ethogram of 29 behaviors (Table 4.2).

In order to determine whether healthy mongooses avoid diseased conspecifics, we examined a suite of social behaviors including play and allogrooming. While aggressive behavior has frequently been found to be lower in diseased individuals (e.g. Bouwman and Hawley 2010; Owen-Ashley et al. 2006), this result may be primarily due to a reduction in activity (Adelman and Martin 2009) and alone provides little information on disease avoidance behavior. Furthermore, whereas aggressive behavior is an agonistic and active social behavior, play and allogrooming are both affiliative social behaviors, with play being an active behavior and allogrooming being a resting behavior for mongooses (we consider allogrooming to be

resting because it typically occurs in brief bouts while mongooses are laying down together). These different kinds of social behavior should give a more comprehensive look at how diseased individuals' social behavior differs from that of healthy individuals, if at all, and if diseased individuals are avoided by healthy individuals (or vice versa) in any particular social behavior. We predict that if avoidance is occurring, social behaviors in general and affiliative social behaviors, such as play and allogrooming, will be lower for diseased individuals than for healthy individuals, whereas agonistic social behaviors may not differ, or may even increase if diseased individuals are harassed by healthy individuals. Alternatively, if no avoidance occurs but the behavior of diseased individuals is influenced by disease in ways typical of sickness behavior (Johnson 2002), then we expect a reduction of social behaviors that are active, but either no change or an increase in social behaviors that are restive. Here, we explore the behavioral differences between clinically diseased and healthy individuals, and determine whether diseased individuals are avoided in banded mongooses.

Methods

Troop, Individual, and Disease Identification

A total of six banded mongoose troops in and around Chobe National Park, Botswana were studied for at least six months in 2009, 2010, and 2011 (June-December, 2009; May-December, 2010; March-November, 2011). Due to two troops merging in 2009 and the addition of two troops to the observation schedule in 2010, the identity of troops observed in each year changed from 2009 to 2010, but four troops were observed in each study year. Observed troops varied in size over time and ranged from 5 to 65 individuals.

One or two animals in each troop were radio-collared as previously described (Laver 2013) so that troops could be located at any time. Troops were observed five days a week by locating a troop via radio tracking and conducting observations on foot or from a vehicle. In 2009, one troop was observed each week, 5-10 hours per day, with a weekly rotation of troops so that each of four troops was observed within a month. In 2010 and 2011, two troops were studied each week, alternating mornings and afternoons. The following week, the other two troops were observed. This schedule allowed for observation of each troop for five mornings and five afternoons per month, thus the same total amount of time per month as in 2009, but more frequently. Occasional disruptions to observations occurred because of weather or inability to locate or follow a troop.

Individual mongooses are usually indistinguishable from one another by appearance because of their similarity in size and pelage, regardless of sex. Radio-collared individuals were identifiable, and we used small, silver ear tags to identify up to six more individuals in a troop by varying the location of the tags among individuals. Some individuals were also recognizable by natural scars or injuries. Thus, at least 10-33% of each troop were identifiable (in the troop of five individuals in 2010, all were identifiable).

We determined disease status (i.e. healthy or clinically diseased) by assessing clinical signs associated with TB infection each day, observing individuals through binoculars and/or at close range. Clinical signs include cachexia, hunching, discolored fur, sneezing, wet eyes/nose, swollen or distorted nose, drooping or enlarged testicles, lethargy, lagging, and fearlessness. This clinical presentation has only been associated with TB disease. The latter three clinical signs are behavioral, and so were not included in disease assessments in the current analyses to avoid the circular reasoning of using a behavior to diagnose disease and then analyzing the effect of disease on behavior. These behavioral signs are never the initial signs of disease, so excluding

these behavioral signs did not change the estimate of the onset of disease, nor did it change categorization of disease status for any individual. Observed clinical signs were graded on a scale from 1 to 10, with 10 being healthy and 1 being severely disordered. We categorized an individual as clinically diseased if it displayed at least three clinical signs rated as 8 or less during the last month it was observed. We determined the month that clinical disease began as the first month that a diseased individual displayed two signs rated 9 or less. We characterize healthy individuals as those animals without clinical signs of TB disease.

Focal Samples

Focal samples (Altmann 1974) were collected when at least half of the troop was active, defined as at least half of the individuals engaging in behavior other than lying down or sitting or standing still. Focal sample data were collected using the program PhoneRecord (W. Teitjen, Bellarmin Unveristy, Louisville, KY) on a smartphone (Motorola Q9h (Motorola Mobility, Inc., Libertyville, IL, USA) running Windows Mobile 6 (Microsoft, Redmond, WA, USA)). This program time stamps to the second each key pressed, with each key representing a behavior from the predetermined ethogram (Table 4.2). Only one behavior was scored at a time. Occasionally, vigilance occurred while another behavior was being performed, in which case the other behavior was scored. The order of individuals focal sampled was determined both randomly and opportunistically: a randomized list of identifiable individuals in the troop was created for each troop in each month. The first four individuals on the list were searched for, and the first of these four individuals found was focal sampled. Then, the sampled individual was dropped from the search and next individual in the list was added to the search. This system continued until as many individuals as possible were sampled. If all individuals from the list were sampled, the sampling would begin again from the beginning of the list, allowing at least one hour to elapse between an individual's focal samples. Each focal sample was between 1.5 and 10 minutes long. If the focal individual went out of sight during a sample, "out of sight" was indicated in the focal sample. If the individual came back into sight, the sample was re-commenced as long as the entire sample fell within 10 minutes. Focal samples of less than 1.5 minutes within sight were discarded.

Table 4.2. Ethogram and categorization of behaviors. Italics indicate social behaviors.

Category	**Behavior**	**Description**
Active behaviors: Behaviors involving movement.	Forage	Digging for food item, manipulating food item, chewing.
	Locomotion	Walking, trotting, or running.
	Play (alone)	Manipulating non-food object, usually interspersed with bouncing and play calls.
	Contact garbage or human food	Contact with garbage or human food, primarily while foraging in it.
	Scent mark	Rubbing ground or object with anal glands.
	Emerge	Emerging from den or other structure.
	Excavate	Digging at a den site. Differs from digging for food in that both front paws are usually used synchronously for excavating, the depth is usually greater than for foraging, and no food items are eaten.
	Smell	Smelling the ground or an object.

	Social play	Rough and tumble contact or chasing interspersed with bouncing and play calls.
	Aggression/defense	Agonistic contact with at least one other mongoose. Distinguished from play by aggressive sounds, lack of bouncing, and more forceful motions that may cause injury (e.g. forceful scratching and biting rather than pawing and mouthing).
	Social scent mark	Rubbing another mongoose with anal glands.
	Social smell	Smelling another mongoose.
	Copulate	Mating.
	Allofeed	Feeding another mongoose, typically a pup.
	Jerky greeting	A greeting where two animals jerk their heads back and forth while approaching each other, culminating in bodily contact with continued jerking of the head.
	Clasp	One mongoose clasping another with front paws, similar to a mating position, but without intromission. Often performed during play.
	Beg	Performed by pups and subadults. Following and sometimes touching another mongoose while emitting begging calls.
	Lead	An individual incites the group to move from the current location with contact calls that are louder and faster than usual while walking or trotting away from current location.
	Follow	Walking or trotting in direction that leading animal is moving. Usually accompanied by mimicking the leading call.
Resting behaviors: Behaviors involving little to no movement.	Lying down	Lying down with very little movement, whether asleep or awake.
	Defecate/urinate	Excretion. Categorized as resting because movement behavior must be paused.
	Sit or stand still	Sitting or standing with little to no movement. Head is below horizontal or oriented toward another mongoose.
	Groom	Scratching, gnawing, or licking oneself. Counted as resting behavior because generally interspersed with lying down or sitting during periods when the troop is resting.
	Allogroom	Licking or gnawing another mongoose. Typically performed during a troop's resting periods.
	Social rest	Lying while in physical contact with at least one other mongoose.
	Social sit or stand	Sitting or standing while in physical contact at least one other mongoose.
Alert behaviors: Behaviors that	Vigilance	Head is horizontal or above horizontal, but not oriented to another mongoose. Head-bobbing may

are anti-predatory in nature.		occur. Gaze may be directed toward a perceived threat, or may shift over the landscape.
	Flee	Trotting or running from a perceived threat.
	Alarm call	Any of several calls used when a perceived threat is present. Often accompanied by vigilance or fleeing.

Behavioral Categorization

For analysis, behaviors from the ethogram were first categorized into three, non-overlapping activity categories: active, resting, and alert (Table 4.2). Briefly, active behaviors involve movement, resting behaviors involve lack of movement, and alert behaviors are those typically found to reduce predation risk (Lima and Dill 1990).

Behaviors were also categorized as either social or non-social. These two categories constitute a subset of the three previous categories (Table 4.2). Social behaviors were defined based on involving physical contact with at least one other mongoose, with the exception of leading and following. These two behaviors were categorized as social rather than as normal locomotion because leading differed from normal travel in that a leader was near the front of the troop and used a specific call type and frequency that appeared to induce a change in direction and/or pace in the rest of the troop, which we termed following. These two behaviors constitute a very small proportion of all behaviors (~0.002% of recorded behaviors). For each focal sample, the proportion of time spent in each behavioral category was determined by summing the amount of time spent in each category's behaviors and dividing by the total length of the focal sample (any time spent out of sight of the observer was removed from the length of the focal sample). We also quantified the rate at which mongooses transitioned between different behaviors during a focal sample, divided by the length of the focal sample, as a measure of behavioral pace.

Statistical Analysis: behavioral differences between healthy and diseased individuals

The behavioral differences in activity categories between healthy and diseased individuals and the rate of behavioral transition were analyzed using Generalized Linear Mixed Models (GLMMs; SAS proc GLIMMIX). Because proportions or the transition rate were the response variable for the GLMMs, the binomial distribution was used. Fixed factors in the GLMM were disease status, length of focal sample, year, day of year, and time of day. Day of year and time of day were both tested for quadratic effects in addition to their linear effects. If they did not display a significant quadratic effect, the quadratic effect was removed from the model. Interactions between fixed effects were analyzed, and removed if they were not significant. Main (linear) effects were not removed regardless of significance. The hierarchical nature of the effects of individual identity and troop identity were captured by nesting individuals within troops as hierarchical random effects. In some models, the variance of the random effect for one or more troops was equal to zero (i.e. the estimated G matrix was not positive definite). However, because it reflects the structure of mongoose sociality and the design of the data collection, troop was always left in the model as a random block effect (Kiernan et al. 2012). In SAS, proc GLIMMIX returns the same results for the model whether or not the zeroed random effect is used (Kiernan et al. 2012). All of the models were underdispersed (χ^2/df = 0.04-0.33), so an R-side scale parameter was used. We used the Satterthwaite method to determine denominator degrees of freedom. Because of the large number of fixed effects when interactions were tested, some of the models required relaxed convergence criteria. Criteria were relaxed by increasing the number of iterations allowed from 20 to 100 (MAXOPT=100) and/or by increasing the

parameter estimate convergence criterion from the default $1e^{-8}$ to no larger than 0.0001 (PCONV=0.0001). These relaxed criteria were usually only needed while all interactions were present, and were converted back to the default values or to the strictest possible that allowed convergence when the model was reduced. Least squares means were estimated for healthy and clinically diseased individuals and the Tukey-Kramer adjustment was used to determine significant differences between these LS means.

We individually analyzed three behaviors particularly relevant to our assessment of behavior and disease: Allogrooming, play, and aggression/defense (hereafter, agonistic encounters). These behaviors were relatively rare events (i.e. zero-inflated), so GLMMs could not be used to analyze them. Instead, we determined if the distribution of each of these behaviors for healthy and clinically diseased individuals corresponded to the expected distribution based on the amount of time each disease status was observed (e.g. if healthy individuals were observed for 70% of the total observation time, we expect them to be responsible for 70% of the allogrooming that was observed). For each behavior, we used a chi-square test to determine if the observed distribution of the behavior matched the expected distribution. For these analyses, durations of behaviors were used for allogrooming and play whereas counts were used for agonistic encounters because they are typically very short events (~1 second). These analyses were performed in SAS 9.3 (Cary, NC, USA).

In 2011 only, we noted whether allogrooming during focal samples was reciprocal (both individuals groom each other, typically simultaneously, occasionally sequentially) or uni-directional (only one individual grooms the other). We could not perform complex GLMMs because of the small sample size (103 occurrences of allogrooming in 47 focal samples from 16 individuals). Instead, we used a Fisher's Exact Test (FET) of a 2x2 contingency table to determine if the proportion of allogrooming reciprocation between healthy and clinically diseased individuals was different. We tested in two ways. First, we used all 104 occurrences as the sample unit in the test. However, there can be several occurrences per focal sample and these occurrences might not be independent, which violates the assumption of independence for the FET. Therefore, we also ran the test using the focal sample as the sample unit. In all but one case, an individual either always reciprocated or never reciprocated within a focal sample, so its reciprocation could be scored as one or the other. In the exceptional case, allogrooming occurred three times, so the behavior that occurred two of the three times was used as the individual's reciprocation behavior for that focal sample. These analyses were performed in SAS JMP Pro (version 10.0.0; SAS Institute, Cary, NC; 2012).

Results

Disease Status, Behavioral Categories, and Transition Rates

We collected 1528 focal samples from 68 individuals in six troops over three years. Disease status had a significant effect on the proportion of time spent active ($F=14.34$, $P=0.0004$; Table 4.3), with diseased individuals significantly less active than healthy individuals ($t=-3.79$, $P=0.0004$; Fig. 4.1a). Proportion of time resting was also affected by disease ($F=32.12$, $P<0.0001$; Table 4.3), with diseased individuals spending a significantly greater proportion of time resting than healthy individuals ($t=5.67$, $P<0.0001$; Fig. 4.1b). Disease status affected the proportion of time alert ($F=6.98$, $P=0.01$; Table 4.3), with diseased individuals significantly less alert than healthy ones ($t=-2.64$, $P=0.01$; Fig. 4.1c). Also, the transition rate between behaviors was affected by disease status ($F=35.21$, $P<0.0001$; Table 4.4), with diseased individuals transitioning at a significantly slower rate ($t=-5.93$, $P<0.0001$; Fig. 4.1e). However, the

proportion of time spent in social behaviors was not affected by disease status (F=1.74, P=0.19; Table 4.4; Fig. 4.1d).

Table 4.3. Type III tests of fixed effects for GLMMs for proportions of time active, resting, and alert.

	Proportion of Time Active, χ^2=0.31				Proportion of Time Resting, χ^2=0.32				Proportion of Time Alert, χ^2=0.15			
Effect	**Num DF**	**Den DF**	**F-value**	**P-value**	**Num DF**	**Den DF**	**F-value**	**P-value**	**Num DF**	**Den DF**	**F-value**	**P-value**
Length of focal sample	1	1490	15.59	<.0001	1	1481	20.39	<.0001	1	1480	4.16	0.04
Disease status	1	59.66	14.34	0.0004	1	43.33	32.12	<.0001	1	74.17	6.98	0.01
Year	2	179.5	5.75	0.004	2	1447	3.21	0.04	2	160.9	13.86	<.0001
Time	1	1482	9.02	0.003	1	1486	11.67	0.0007	1	1492	4.46	0.03
Day of year	1	1485	19.02	<.0001	1	1494	21.63	<.0001	1	1399	14.7	0.0001
Time^2									1	1492	4.19	0.04
Day of year^2	1	1488	26.66	<.0001	1	1478	18.54	<.0001	1	1410	17.04	<.0001
Length * time	1	1487	7	0.008	1	1480	11.21	0.0008				
Time * day of year	1	1480	10.38	0.001	1	1481	12.74	0.0004				
Time * day of year^2	1	1480	14.44	0.0002	1	1480	18.24	<.0001				
Year * day of year					2	1410	3.43	0.03				
Year * day of year^2					2	1375	3.23	0.04				

Table 4.4. Type III tests of fixed effects for GLMMs for proportion of time social and behavioral transition rate.

	Proportion of Time Social, χ^2=0.33				Transition Rate, χ^2=0.04			
Effect	**Num DF**	**Den DF**	**F-value**	**P-value**	**Num DF**	**Den DF**	**F-value**	**P-value**
Length of focal sample	1	1484	11.79	0.0006	1	1509	150.14	<.0001
Disease status	1	1399	1.74	0.19	1	87.36	35.21	<.0001
Year	2	220	3.46	0.03	2	723.3	11.77	<.0001
Time	1	1491	5.08	0.02	1	1505	21.21	<.0001
Day of year	1	1424	0.73	0.39	1	1292	7.36	0.007
Time^2	1	1488	6.41	0.01				
Length * time	1	1454	5.15	0.02				
Disease status * time	1	1369	5.66	0.02				
Disease status * year	2	16.63	4.32	0.03				

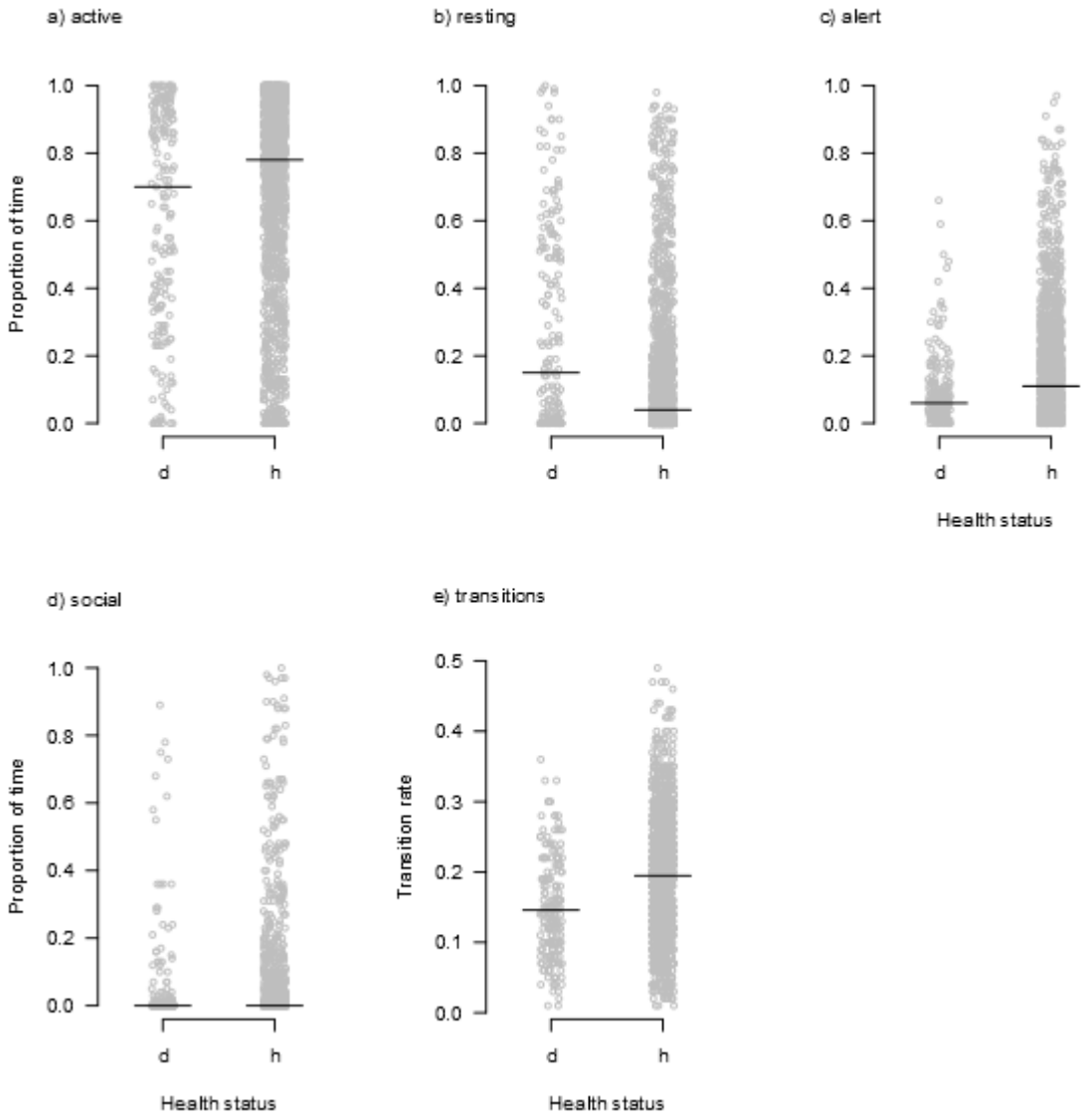

Figure 4.1. Data by disease status for behavioral categories and behavioral transition rate. Gray circles indicate data points for focal observations and horizontal bars indicate median for disease status, where "d" represents clinically diseased and "h" represents healthy.

Disease Status and Specific Behaviors

For rare behaviors, we determined if the distribution of observations of a particular behavior varied from the distribution of the total amount of time we observed healthy and diseased individuals. Observations of healthy individuals accounted for 87% of total observation time, and observation of diseased individuals accounted for 13% of total observation time (thus, this distribution is the expected distribution for the following tests; Fig. 4.2). The observed distribution of agonistic encounters did not vary from what was expected based on the distribution of the amount of time they were observed (Fisher's exact test (FET), P=0.54), where diseased individuals accounted for 11% (asymptotic standard error (ASE)=0.03) of agonistic encounters (Fig. 4.2). However, play behavior and allogrooming deviated from the expected

distributions (FET, P<0.0001 and P=0.0003, respectively). Diseased individuals participated in play less than expected, accounting for 1% (ASE=0.002) of observed play (Fig. 4.2). In contrast, diseased individuals participated in allogrooming more than expected, accounting for 16% (ASE=0.007) of observed allogrooming (Fig. 4.2).

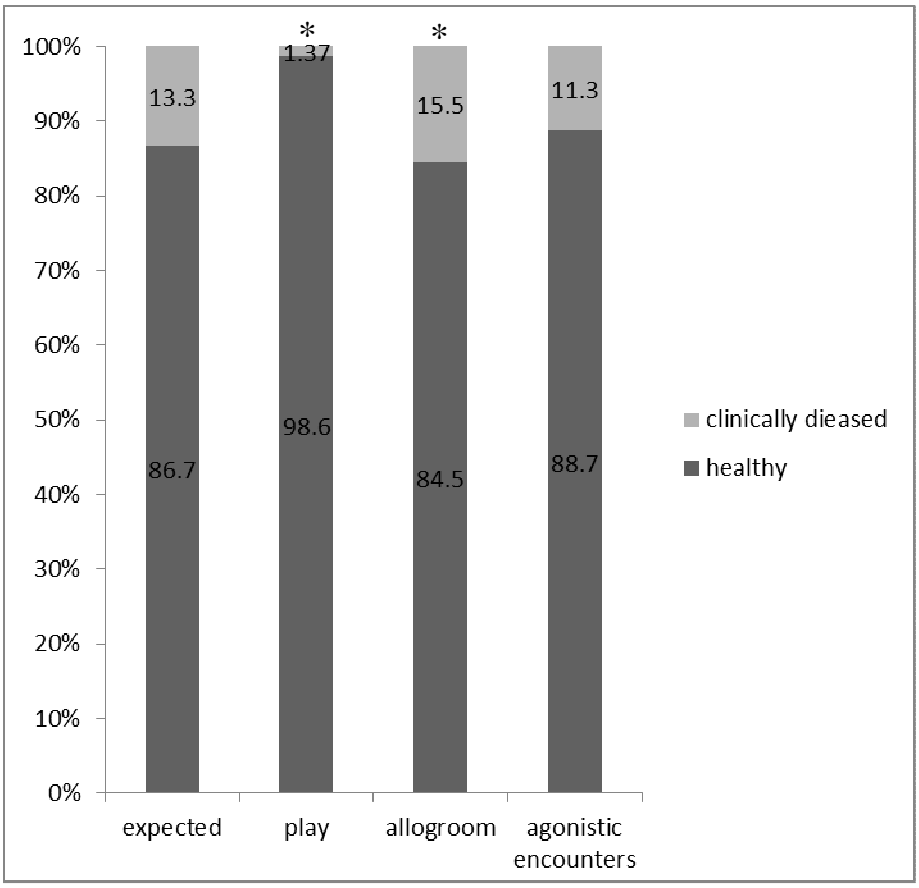

Figure 4.2. Expected and observed distributions of specific behaviors. Significant differences from expectation indicated by *.

In 2011, we recorded reciprocation of allogrooming, observing 103 occurrences of allogrooming in 47 focal samples from 16 individuals. When each occurrence of allogrooming was the sample unit, we found that diseased mongooses reciprocated significantly less than healthy individuals did (FET, P<0.0001), with healthy individuals reciprocating in 99% of occurrences and diseased individuals reciprocating in 44% of occurrences. Similarly, when the focal sample was the sample unit, healthy individuals reciprocated 100% of the time, whereas diseased individuals reciprocated 54% of the time, a significant difference (FET, P<0.0002).

Discussion

Differences in behavior between diseased and healthy individuals can have important implications for disease transmission dynamics (Behringer et al. 2008; Hawley et al. 2011; Lloyd-Smith et al. 2004), as can avoidance of diseased individuals by healthy individuals (Behringer et al. 2006a). Our results show that there are a number of significant behavioral differences between clinically diseased and healthy banded mongooses, but unlike previous

studies, we did not find evidence to support that avoidance of diseased conspecifics occurs in this system.

Clinically diseased banded mongooses exhibited significantly smaller proportions of time active and alert, a larger proportion of time resting, and a slower behavioral transition rate compared to healthy individuals. While this behavioral transition rate measured the rate of change from one behavior to another, we did not measure speed at which a particular behavior might be undertaken. Therefore, the behavioral differences in activity may be more pronounced than indicated by the models because diseased and healthy individuals may also perform each type of behavior at different speeds, e.g. a diseased individual may have a slower walking pace than a healthy individual. Furthermore, severity of disease is very likely to have an effect on the behaviors we measured, with individuals in end stage disease exhibiting the most extreme changes. Thus our analysis of behavioral differences between diseased and healthy individuals is likely to be conservative, especially for advanced disease.

Overall, social behaviors took up a relatively small proportion of time in our observations because we conducted observations only when at last half of the troop was active. Banded mongooses spend most of their active periods in non-social behavior (i.e. they interact relatively little, though they are usually within sight of each other), but spend most of their inactive periods in social behaviors (nearly all individuals are socially resting during inactive periods). Because there is almost no individual variation in behavior during troops' inactive periods, only observing troops' active periods allowed us to capture the period where the most behavioral variation occurred. Observing the inactive period would likely have greatly increased the amount of social behavior observed (specifically social resting), but would not have been informative because of the lack of individual variation in behavior.

Despite the significant differences in behavior of diseased mongooses, we found no evidence for avoidance of clinically diseased mongooses by healthy individuals or vice-versa: clinically diseased individuals did not have lower levels of social behaviors than healthy individuals, and our analysis of three specific social behaviors did not indicate avoidance. First, diseased mongooses were not involved in either more or fewer agonistic encounters than expected. This result suggests that diseased mongooses are not the focus of increased harassment from troop mates but neither are they spared from agonistic encounters. Overall, agonistic encounters are rare and brief within troops (Gilchrist et al. 2004 and pers. obs.), so these occasional agonistic encounters might not have a substantial effect on diseased individuals. In contrast, other studies have found fewer aggressive interactions in diseased individuals than in healthy individuals in other species (e.g. Owen-Ashley et al. 2006).

Second, diseased mongooses were involved in very little play behavior, accounting for only about 1% of observed play. Banded mongoose play bouts are typically energetic and relatively long, lasting from 5 minutes up to half an hour or more. Diseased individuals might abstain from this behavior as a way to reduce activity levels and direct energy to the immune system, as diseased individuals of other species have often been found to do with active behaviors (Adelman & Martin, 2009; Johnson, 2002). Alternatively, healthy individuals may be rejecting them as play partners as a disease avoidance measure, but this explanation is not consistent with the measures of overall social behaviors, aggression, or allogrooming (below). To our knowledge, play behavior has not been studied in diseased animals, although it has been recommended for study as a behavior that might change in response to disease (Weary et al. 2009). The adaptive benefits of play behavior has been an enduring question in behavioral ecology (Fagen 1981; Graham and Burghardt 2010). A recent definition of play requires that

behavior meet five criteria to be considered play, one of which is that it is initiated in the absence of severe or chronic stress, i.e. in a "relaxed field" (Burghardt 2005). Assuming that disease is considered severe or chronic stress (because activation of the immune system results in activation of the hypothalamic-pituitary-adrenal axis (Rook et al. 1994)), this criterion suggests that individuals in advanced stages of disease will not play. Related to this criterion, the surplus energy theory posits that play occurs only when an individual has more than enough energy available (Burghardt 2005). This theory has been empirically supported (e.g. Sharpe et al. 2002), and our study also lends some support to this theory, because both the lethargic behavior and the severe weight loss observed in diseased mongooses point to a lack of sufficient calories. The study of the costs and benefits of play behavior may be enriched from research on individuals that do not play in normally playful species such as banded mongooses.

The third social behavior that indicated a lack of avoidance of diseased conspecifics was allogrooming. In our study, diseased individuals were involved in more allogrooming than expected despite a 50% lower rate of reciprocation. This higher-than-expected allogrooming in diseased individuals cannot be explained by diseased individuals frequently grooming one another, because we observed only one instance of allogrooming where both individuals were diseased. These allogrooming results are not only a strong indication that diseased individuals are not avoided, they provide some evidence, though limited, that healthy individuals might help diseased individuals, as hypothesized by Loehle (1995) and Hart (1990). To our knowledge, these helping hypotheses have not been tested in any species and has only been supported with a few published observations (e.g. Rasa 1983), so we suggest controlled experiments and/or extensive observations to this end. Helping diseased group mates may be adaptive for the helper if the helper benefits from maintaining the group size (Hart 1990) or from positive social interactions, as has been hypothesized for highly social species (Loehle 1995). Grooming diseased troopmates may be an especially beneficial behavior for the healthy groomer, because reducing ecto-parasites on diseased troopmates may prevent parasitism for all individuals including the groomer, especially when diseased individuals have high ecto-parasite loads, as is often the case in diseased free-ranging wildlife (KAA, unpublished data). However, helping diseased troopmates may also come at the cost of possible infection, though for pathogens such as *M. mungi* that appear to be transmitted primarily through an environmental route, the cost of helping a diseased conspecific may less significant. But even for horizontally transmitted pathogens, if exposure is equal among troop members regardless of social interactions, helping diseased individuals may be a useful strategy in highly social species.

Overall, in contrast to previous research on avoidance of diseased conspecifics (Table 4.1), we did not find evidence that healthy mongooses avoid diseased individuals despite the significantly lower activity of diseased individuals. Avoidance behavior (or lack thereof) may affect disease dynamics due to its implications for pathogen transmission (Behringer et al. 2006b; Bouwman and Hawley 2010). For directly transmitted diseases, avoidance behavior is adaptive for a potential host individual only if it reduces the likelihood of infection. The observed lack of avoidance in our study has at least three possible explanations. First, lack of avoidance may be due to the relatively short time that the pathogen has been infecting this host species. The pathogen is thought to have emerged in 2001 with the first cases identified, and this may not have been enough time for avoidance to evolve in this system. Second, avoidance may not occur due to the apparently rare occurrence of horizontal transmission of this pathogen. However, if there were any previous horizontally-transmitted pathogens infecting banded mongooses for which avoidance was adaptive, an avoidance strategy could have evolved that

would respond to general signs of disease (e.g. lethargy, poor coat quality, or altered scent) that are also present for this TB disease. Additionally, because diseased individuals of many species often tend to have more ecto-parasites (Hart, 1988), avoidance may be beneficial even in the absence of horizontal transmission of the pathogen itself. Alternatively, it is possible that increased allogrooming of diseased individuals rather than avoidance of them is an adaptive strategy against an environmentally transmitted pathogen that results in increased ecto-parasites on diseased individuals. However, if horizontal transmission occurs even rarely, avoidance of diseased individuals could be adaptive in this system if it reduces the likelihood of infection, because this disease is always fatal in banded mongooses.

The third possible explanation for lack of avoidance behavior in banded mongooses is that reducing transmission by avoidance is ineffective in these stable, long-term, and highly interactive social groups. Heterogeneity in pathogen exposure or the ability to induce this heterogeneity via avoidance behavior is a requirement for avoidance behavior to evolve or be maintained in a host species. The species previously studied for avoidance behavior typically live in temporary, fluid, and voluntary social groups, such as a foraging flock of birds (Table 1), where it may be possible for a healthy bird to completely avoid contact with a diseased individual by increasing its distance from the diseased individual or by moving to another flock. In contrast, banded mongooses have closely-knit social groups with stable membership: some individuals remain in their natal group for their entire lives, and troopmates frequently have physical contact with one another, especially while sleeping. Banded mongooses regularly rest in direct contact with six to eight troopmates during the day, and the entire troop sleeps together in a small, enclosed den at night. Because of this type of social system, it may be unlikely that avoidance of diseased individuals during active periods would effectively reduce exposure to pathogens, even for those that are horizontally transmitted. Thus, pathogen exposure might be equal among group mates in some highly social species, regardless of avoidance behavior.

However, if exposure is not equal and horizontal transmission of *M. mungi* occurs, the social behaviors we analyzed may play a key role in transmission. We found that diseased individuals were not avoided in aggression and were allogroomed more than the expected amount, and these two behaviors were both shown to be important to transmission of bovine TB (caused by *Mycobacterium bovis*) in meerkats (Drewe 2010). If horizontal transmission can occur in our system and allogrooming changes the exposure of those that perform it, it is possible that the high level of allogrooming of diseased individuals could amplify transmission among banded mongooses. While this scenario is possible, it is more probably that horizontal transmission is rare (based on the pattern of the TB epidemic; Alexander et al. 2010) and that exposure to *M. mungi* is equal or nearly equal among troopmates (based on the high sociality of this host species). Thus, the contrast between our findings and those of other studies of avoidance of diseased conspecifics may be due to the differences between the sociality of banded mongooses and that of the other species under study (Table 1), and/or due to the primarily environmental transmission of the pathogen. Our unique findings indicate that future studies of avoidance behavior should focus on host species with diverse levels of sociality and pathogens with diverse transmission modes in order to shed light on the conditions that shape the costs and benefits of avoidance behavior in social hosts.

Attribution

B.M. Fairbanks was the main contributor to this chapter, providing the intellectual impetus, and performing data collection, data analysis, writing, and substantial grant writing that supported the

work. D.M. Hawley and K.A. Alexander contributed with substantial editing, guidance of ideas and research, and funding. K.A. Alexander initiated and maintains the banded mongoose project in Botswana.

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Chapter 5

Synthesis and suggestions for future research

Behavior can influence and respond to disease at multiple levels of host organization. I found that behavior influenced disease at the troop level in banded mongooses. Mongoose troops were more aggressive when foraging in garbage than when foraging in any other habitat. Troops that had higher levels of aggression also had higher levels of injury. Injury was a strong predictor of disease in individuals, indicating that individuals who were behaviorally prone to injury, for example through aggression at garbage, were at risk for TB. Thus, urbanization may alter disease dynamics in this system, because troops that use garbage as a resource may have higher risk for and incidence of TB. These results present a unique example of how urbanization can alter disease dynamics, because while other species have shown altered disease dynamics due to feeding on garbage, in those cases either the garbage itself was the source of infection (e.g. Sapolsky and Share 2004) or garbage caused an increase in horizontal transmission due to increased contact among conspecifics at clumped resources (e.g. Wright and Gompper 2005). In contrast, my research indicates an indirect effect, where garbage causes an increase in aggressive interactions, which leads to injuries, which then allow pathogen invasion, probably from a source other than garbage or a conspecific. The unusual egalitarian social system of banded mongooses may contribute to the increase in aggression at garbage, because in societies with stricter dominance hierarchies, dominants may typically control food resources without the use of aggression (Hand 1986). Since egalitarianism is relatively rare in social vertebrates (at least in those that have been extensively studied), this dramatic increase in aggression between individuals at garbage may actually be more likely to occur in solitary species when conspecifics with unresolved social relationships (Hand 1986) encounter one another at a desirable resource. In these species, fights may be necessary to acquire the resource if no pre-determined hierarchy exists.

Other sources of aggression not examined in my work may also have an effect on TB incidence. I observed three important natural sources of aggression: mating, large natural food items (e.g. rodents, birds, or reptiles), and encounters with neighboring troops. All of these events are rare, but any injuries produced by them may provide a point of invasion for this pathogen. Of these sources of aggression, encounters with other troops seem to cause the most injuries, followed by mating. Inter-troop encounters are unpredictable, but may be the most important natural source of injuries, and therefore have the largest impact on TB infection for troops that live away from human-modified areas. To study the effect of inter-troop aggression on TB disease dynamics, it may be necessary to identify troops that tend to have frequent encounters of this kind, as some troops seem to have more inter-troop encounters than others (e.g. the Chobe Safari Lodge troop has many more inter-troop encounters than the Chobe Game Lodge troop). I predict that troops with many aggressive inter-troop encounters will have a higher TB incidence than troops with few encounters. Also, troops that encounter similarly sized troops may have more injuries and thus higher TB incidence, because when troops of vastly different sizes meet, the smaller troop tends to flee when the larger troop is in sight rather than engage in a fight that they are likely to lose.

Although fights within a troop during mating can cause injuries, there is variation in how intense these fights are. In some troops, especially smaller troops, there tends to be little physical contact during mating disputes, with disputes being settled by posturing and calls rather than

outright aggression. However, for troops in which fights for mates are intense, mating may be a somewhat predictable source of injuries that could be studied for its effect on TB invasion. In these troops, TB infections may be predicted to appear a few weeks to months after mating occurs. Overall, the rarity of and variation in natural aggression among banded mongooses may aid in studying the link between aggression and TB incidence if surveillance for aggression can be intense enough to accurately measure the variations in aggression across different groups and even different individuals.

Not only did behavior influence disease, but also the converse occurred. Individual behavior responded to disease in both predictable and unpredictable ways. First, I found that banded mongooses clinically diseased with TB had low activity and alertness, high resting behavior, and a slow behavioral transition rate. Second, diseased individuals were less likely to disperse than healthy individuals were. The detected differences in behavior are likely very conservative, because severity of disease may affect these behaviors. Indeed, individuals in the initial stages of disease seem to behave similarly to healthy individuals, whereas individuals in the end stage of the disease have extreme behavioral differences from healthy individuals. For example, the two unhealthy individuals that dispersed had relatively old injuries and were either not yet showing signs of disease or only in the very early stage of disease (the transition between injury and disease is difficult to identify). No individuals in or near end-stage disease dispersed, and based on the extremely lethargic behavior of these individuals, it seems unlikely that they would be able to disperse. In the future, if a particular behavior is being tested for its response to TB, severity of disease should be taken into account, and the complexity of this analysis is likely to require a large sample size of diseased individuals.

A third and unexpected response to disease was a lack of avoidance of diseased individuals by healthy individuals. Avoidance of diseased individuals has been studied and confirmed in six species to date (Arakawa et al. 2010; Behringer et al. 2006b; Edwards 1988; Kavaliers et al. 2003; Kiesecker et al. 1999; Zylberberg et al. 2013). Additionally, avoidance of healthy kin by diseased individuals has been predicted due to the possible inclusive fitness benefits associated with this behavior (Loehle 1995). Despite these predictions, I did not find avoidance of diseased individuals by healthy individuals or vice versa in banded mongooses when I analyzed play, agonistic encounters, allogrooming, and all social behaviors combined. In fact, healthy mongooses may have even "helped" diseased individuals by allogrooming them at a higher than expected rate despite a 50% reduction in reciprocation on the part of diseased individuals. These results may constitute evidence supporting hypotheses that predict helping of diseased group mates in highly social species (Hart 1990; Loehle 1995).

To more rigorously test these helping hypotheses, cooperative and helping behaviors should be studied in more detail. Experiments could help distinguish between three possibilities that exist: 1) diseased individuals are helped less than healthy individuals; 2) diseased individuals are targeted for extra help compared to healthy individuals; or 3) diseased individuals are not targeted for more help than healthy individuals, but rather they need more help (e.g., have more ecto-parasites), and receive help in accordance with their need just as a healthy individual would. One way to test this would be to create treatment groups of diseased and healthy individuals that are treated for ecto-parasites and observe if allogrooming differs among treated healthy, treated diseased, control diseased, and control healthy individuals. Reciprocation should also be measured in these interactions, as it may have an effect on the amount of allogrooming received and can be used as a co-factor in the analysis. In addition to allogrooming and its reciprocation, responses to diseased individuals' distress calls, such as lost calls, should be

compared to responses to distress calls of healthy individuals. This research could be done using playback experiments, measuring the intensity of the troop's response, as well as the diminution of the response with repeated playback from the same individual. Response seems to diminish in natural situations where an individual (whether diseased or healthy) is repeatedly separated from the group. If the response to diseased individuals' lost calls is less intense and diminishes faster than the response to healthy individuals' calls, then diseased individuals may be helped less than healthy individuals are. More intense responses and slower diminishing responses toward diseased individuals than toward healthy individuals would indicate targeted helping behavior toward diseased individuals. Equal intensity and diminution of response would indicate neither targeting nor withholding of helping behavior for diseased mongooses, but that they simply receive help in accordance with their need. Very little research has been performed to explore helping behavior toward diseased conspecifics and its effects on disease dynamics, and banded mongooses may be the ideal host species for this undertaking.

Aside from the implications for disease ecology, the behavioral differences between diseased and healthy individuals inform several aspects of behavioral ecology. Ideas about the evolutionary basis and maintenance of many behaviors are usually deduced by study of only healthy individuals. Observing diseased individuals' behavior and how healthy individuals interact with diseased individuals may modify these ideas. For example, observation of allogrooming in only healthy banded mongooses would suggest that this behavior works on a tit-for-tat rule, because healthy individuals almost always reciprocate with one another. However, diseased individuals reciprocate in only about 50% of allogrooming, and yet still receive allogrooming. This suggests that allogrooming is not tit-for-tat in this species. Allogrooming may be maintained in banded mongooses because of its potential benefit to the groomer as well as the groomed (reduced ecto-parasites on both individuals), or allogrooming may be based on kinship or the history of the relationship between the individuals. This latter possibility was suggested by observations of one particular diseased individual, who was quite old (at least six years old) when he became clinically diseased. All diseased individuals that I observed were groomed at seemingly normal or slightly higher than normal rates, but this particular individual seemed to be groomed a very high rate (at a higher rate than he was groomed while he was healthy). Due to his age, he likely had time to develop long-term relationships within the troop and the apparent increased allogrooming could have been based on these relationships. Alternatively, he could have had a particularly high ecto-parasite load that required extra allogrooming. More research should be conducted on the evolution and maintenance of allogrooming and other social behaviors using individuals that are outside the range of "normal" behaviors, such as diseased individuals. Similarly, play behavior presents a long standing enigma in behavioral ecology. Dozens of hypotheses have been proposed for the evolution and maintenance of play behavior (Baldwin and Baldwin 1977), many of which could be tested through the study of diseased or otherwise physically impaired individuals.

Despite the lack of avoidance behavior within social interactions, I found a possible trend for higher dispersal of individuals that had diseased troopmates, indicating that disease of one individual may affect the dispersal behavior of another individual and lead to higher dispersal rates from troops with diseased members. Dispersal may be one way to avoid diseased individuals, especially if avoidance of diseased individuals in troop social interactions is ineffective or has yet to evolve. If this trend holds true, then TB may have an important impact on banded mongoose population dynamics. For example, an increase in dispersal could lead to greater outbreeding as a greater number of dispersers join pre-existing troops or dispersers from

different natal troops form new troops. Increased outbreeding of this typically inbred species (i.e. mating with relatives is not avoided and there is high relatedness within a group; Waldwick et al. 2003) may have important implications for banded mongoose sociality if high relatedness has influenced the evolution and maintenance of the banded mongoose social system (Kokko and Ots 2006; Waldwick et al. 2003). To test if outbreeding is increasing, the level of inbreeding could be tracked over the coming years using previously characterized polymorphic microsatellite loci (Waldwick et al. 2003). Increased dispersal may also lead to an overall decrease in troop size due to higher emigration from troops with infection, and to an increase in the number of troops within the area as dispersers form new troops. Although emigration and troop size in troops that are currently being observed can be tracked with relative ease, identifying newly formed troops is difficult, especially in areas with limited road access. In my estimation, the intensity of surveillance necessary to accurately measure formation of new troops is not feasible for this study site.

A number of important questions remain about this system. For example, while environmental transmission seems to be the primary mechanism in this system, it is unknown if diseased individuals shed the pathogen into the environment, either while alive or after death. Shedding may be an important factor in disease dynamics, because diseased individuals may cause increased transmission within their troop's territory even if no direct transmission occurs. A behavioral question of interest is the interaction between diseased individuals and pups. I observed pups make frequent contact with diseased individuals and diseased individuals also were likely to remain at the den in a babysitting capacity (a babysitter stays with the pups and defend the young if attacked) when pups were too young to travel with the troop (although their ability to babysit effectively is questionable, and often a healthy individuals would also remain at the den). If horizontal transmission is possible and/or if diseased individuals shed the pathogen, this contact seems particularly risky, however I only observed one diseased pup (the pup's disappearance meant that it could not be tested for positive identification of the pathogen, but clinical signs matched those of TB). More extensive behavioral observations of diseased individuals' contact with pups and juveniles would be of interest, especially in conjunction with testing these young for exposure to the pathogen. The low disease incidence in young individuals coupled with high contact with diseased individuals suggests several possibilities. It may be that horizontal transmission and/or shedding are very rare or absent. It may be that contact with diseased individuals exposes young to low doses of the pathogen that could be protective for a short time, or perhaps young are rarely injured and thus do not have the necessary portal of entry for the pathogen to invade.

Overall, these results represent examples of interactions between behavior and disease that may affect group and population dynamics of the host as well as the transmission dynamics of the pathogen. Many of the questions I pursued were previously little-explored, and many of the results were unexpected. Thus, this research introduces ideas that require further study in other disease-host systems to ascertain whether these interactions between behavior and disease are unique to the TB-banded mongoose system, or are broader characteristics of various host-pathogen systems. My impression is that this system presents a fairly unusual situation due to unusual characteristics of both the pathogen and the host: an environmentally transmitted pathogen that invades through breaks in the skin, and a highly social, egalitarian, low-skew communal host. However, the uniqueness in no way diminishes the importance of these findings, because it is often unusual situations that give us a fuller understanding of more typical conditions and of the true selective pressures involved when multiple hypotheses can explain the

typical patterns. For example, the lack of avoidance of diseased individuals in banded mongooses reveals that avoidance behavior may be dependent on the sociality of the host species. However, one way in which this system is not particularly unique is in the way that human activity attracts some wildlife species to human-occupied areas and simultaneously alters wildlife disease dynamics (Bradley and Altizer 2007). Understanding interactions between behavior and disease in these circumstances is paramount, especially considering the risk to human health from zoonotic diseases (Daszak et al. 2001), because these interactions can influence disease dynamics. Few models of disease dynamics take into account these interactions between behavior and disease. Incorporating knowledge of the ways that behavior influences and responds to disease will allow for better prediction, management, and general understanding of wildlife disease.

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