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Intrinsic and extrinsic factors affecting group decision-making

Benjamin Cobb

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Abstract

To stay cohesive and benefit from group-living, members of social species must frequently make decisions together. Variation in intrinsic characteristics, such as dominance and sex, leads to conflicts of interest. But extrinsic factors, such as intergroup conflict, are also likely to affect group decisions. This thesis investigates how both factors influence group decision-making by using two complementary study systems: wild dwarf mongooses (*Helogale parvula*) and captive colonies of the monogamous ant *Temnothorax nylanderi*. Using long-term behavioural and GPS data from dwarf mongooses, Chapter 2 shows that dominants and males are most likely to lead group movements from a morning sleeping burrow, especially in the breeding season. Dominant males led more the day after an intergroup interaction in the non-breeding season, and male leadership increased in territory areas overlapping with neighbouring groups. Chapter 3 provides experimental evidence that acoustic movement signals in dwarf mongooses attract followers. Whilst group members are equally likely to respond to the movement calls of dominants and subordinates while foraging, a simulated rival group did not impact follower responses. The remaining data chapters are based on an experiment where *Temnothorax nylanderi* colonies were split and recombined as a host (with a nest) or an invader, to investigate how the presence of queens affects colony fusion. Chapter 4 presents the development and testing of an automated image-processing script used to generate data from the experimental photographs. Chapter 5 uses those data to show that fusion is less likely when both colonies contain a queen, and post-fusion dynamics are affected by the presence of at least one queen. For example, the presence of a queen meant colonies remained separated within the nest even after fusion, suggesting colonies may be waiting to split once again. Overall, this thesis demonstrates that both intrinsic and extrinsic factors, particularly conflict, affect group decisions.

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Authors declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Chapter 1

General Introduction

1.1 Group living

Across the animal kingdom, individuals come together to form groups. These range from simple or temporary aggregations (Klok and Chown, 1999) to stable, socially complex groups where individuals display apparently altruistic behaviour (Bourke and Franks, 1995; Hamilton, 1964; Krause and Ruxton, 2002). For example, fruit flies (*Drosophila melanogaster*) temporarily aggregate when foraging (Lihoreau et al., 2016), whilst vast flocks of birds and shoals of fish occur from simple interaction rules when foraging or moving (Couzin and Krause, 2003). In cooperatively breeding birds and mammals, such as pied babblers (*Turdoides bicolor*) and meerkats (*Suricata suricatta*), individuals provide care to the offspring of breeding individuals (Radford and Ridley, 2006; Russell et al., 2003), while worker castes of the eusocial Hymenoptera sacrifice reproduction to raise the brood of queens (Bourke, 2011; Queller and Strassmann, 1998). Group formation is a prerequisite to the evolution of social groups, providing benefits such as a reduced predation risk (Bourke, 2011; Heg et al., 2004) or protection against social parasites such as cuckoos (Canestrari et al., 2009). But group-living also has associated costs; for example, individuals may experience increased competition for food, conflict over reproductive opportunities and increases in disease transmission (Krause and Ruxton, 2002). Overall, for social groups to form and remain stable, the fitness advantages to individuals must outweigh the costs.

There are many benefits to group-living. One that has received much attention is protection from predators (Krause and Ruxton, 2002). Individuals may be less vulnerable, for example, because there is a reduced risk of any given individual being targeted by a predator, or because group members can use other individuals as cover (Hamilton, 1971; Quinn and Cresswell, 2006). Use of modern technology, such as virtual groups of prey with live predators, has allowed insights into how spatial positioning affects predation while accounting for confounding effects. For instance, the number of nearby groupmates may decrease risk (Lambert et al., 2021), while individuals at the front of groups may suffer an increased risk compared to those further back (Ioannou et al., 2019). Group-living also gives rise to cooperative predator defence, such as the mobbing behaviour of meerkats in which individuals confront and attack a potential predator (Graw and Manser, 2007). Moreover, individuals in groups benefit from greater anti-predator vigilance than those living alone. The larger a group, the more individuals a predator must avoid detection from, and individuals can also spend more time foraging due to the vigilance of other group members contributing to the overall time of 'scanning' behaviour (Beauchamp, 2003). Once an individual detects a predator, it can alert others using warning signals, thus reducing the predation risk of all (Treherne and Foster, 1981). In some species, group members even take turns as 'sentinels', remaining in a highly vigilant

state to keep watch while others can continue to forage. These sentinels are usually the first to detect an incoming predator and can alert the rest of the group (Bednekoff, 2015).

Group-living species may also benefit from greater foraging efficiency, protection against environmental factors and the opportunity for social learning. In terms of foraging, naked mole-rats (*Heterocephalus glaber*) and Damaraland mole-rats (*Fukomys damarensis*) cooperatively search for food (Jarvis et al., 1994), while social spiders and some carnivores that hunt cooperatively are able to target larger prey otherwise unattainable by single individuals (Creel and Creel, 1995; Stander, 1992; Yip et al., 2008). Social foraging, in which individuals rely on informed individuals who know the location of food, is important for some species such as sea birds or vultures where food sources are scarce and widely scattered (Harel et al., 2017; Ward and Zahavi, 1973). By foraging as part of a group, individuals may increase their intake despite local competition. To survive the extreme cold in the Antarctic winter, emperor penguins (*Aptenodytes forsteri*) huddle together and thus raise the ambient temperature that they experience (Gilbert et al., 2006). Group-living also enables social learning, whereby individuals learn from others. For example, meerkat pups improve their use of the distinct anti-predator vocalisations for terrestrial and aerial predators by observing adult behaviour (Hollén et al., 2008), and are taught better foraging strategies by older group members (Thornton and Clutton-Brock, 2011).

While there are many benefits to group-living, there are also associated costs. Being in a group generally reduces predation risk for individuals, but groups may be more likely to be attacked than solitary individuals as the former are more conspicuous (Botham et al., 2005). In addition, competition over resources such as food is likely, with larger groups being more likely to deplete food resources, lowering the amount available to individuals (Markham and Gesquiere, 2017). Certain group members, such as more dominant individuals, may monopolise food resources while others tend to suffer relatively greater costs (Papageorgiou and Farine, 2020). The same may apply to reproduction with, for example, infanticide of subordinate young in mammals or policing of worker eggs in the Hymenoptera (Clutton-Brock et al., 1998; Ratnieks and Visscher, 1989). Groups may also be more susceptible to certain diseases; for instance, group size is positively correlated with the prevalence of parasites (Patterson and Ruckstuhl, 2013).

To maintain stable groups, within-group mechanisms have evolved to reduce the costs of group-living. For example, pied babblers produce “close calls” that help to maintain distance between foragers, thereby reducing the costs of local competition (Radford and Ridley, 2008), while dominance hierarchies may reduce within-group conflict escalating over resources (Tibbetts et al., 2022). Group-making decisions may also prevent certain costs from becoming too great; for

example, if a dominant individual monopolises a particular food resource, subordinates may decide to move to another foraging area and force movement elsewhere (Papageorgiou and Farine, 2020). In the social Hymenoptera, social immunity has evolved to reduce the impact of pathogens and parasites. This involves behavioural, organisational and physiological adaptations (Cremer et al., 2007, 2018), ranging from parasite avoidance and grooming, older individuals tending to forage more (exposing the most expendable group members to a higher risk of pathogens) and biochemical immune responses (Cremer et al., 2007; Siva-Jothy et al., 2005). While these mechanisms reduce the costs of group-living, there are still likely to be conflicts of interest over various decisions because social groups are comprised of heterogenous individuals (see **1.2 Remaining cohesive**).

Stable groups also face a variety of threats from conspecific outsiders; individuals or groups seeking to obtain resources (e.g., food, territory, reproductive opportunities; Christensen et al., 2016; Lemoine et al., 2020). This outgroup conflict can lead to physical contests (with the risk of injury or death), and the cumulative threat can generate further fitness consequences through elevated stress, resulting in a significant selection pressure (Braga Goncalves et al., 2022; Lemoine et al., 2020; Morris-Drake et al., 2022). Individuals should also preferentially direct helping behaviour toward kin for inclusive fitness gains, which is facilitated by the recognition of group members versus outsiders (Sturgis and Gordon, 2012). Many species ranging from invertebrates to mammals are known to hold and defend stable territories against rival groups (Bateman et al., 2015; Kesler and Haig, 2007; Newey et al., 2010). Research has tended to focus on who is involved in conflict or the immediate behavioural consequences but recently studies have begun to investigate the indirect effects of conflict and its longer-term impacts (see **1.3 Outside threats**).

1.2 Remaining cohesive

Within groups, individuals vary in their physiology and behaviour. As a result, the motivations of group members may contrast with one another at any given time, which leads to within-group conflict (Hardy and Briffa, 2013)**.** For groups to remain stable, individuals must overcome these conflicts. Even within the eusocial insects, which display the most extreme form of cooperation by sacrificing reproduction to raise their closely related queen's offspring, within-group conflict is common (Ratnieks and Visscher, 1989; Stroeymeyt et al., 2007)**.** For example, in many ants and honeybees, workers still retain the ability to produce male offspring (Heinze, 2005), which they are more related to than their brothers. Workers may therefore make direct fitness gains by raising their own offspring over brothers (Bourke, 2011; Heinze, 2005)**.** Because workers are less closely related to their nephews, and queens are more related to their own daughters, selfish worker egg-laying

comes at a fitness cost to the rest of the colony (Heinze, 2005)**.** As a result, mechanisms have evolved to control this behaviour, including queens punishing workers or using pheromones to stop reproduction of workers, and worker control through egg removal or cannibalism (Heinze, 2005; Ratnieks and Visscher, 1989; Wenseleers and Ratnieks, 2006). Within-group conflict also occurs over many other aspects of group-living, such as group defence and resources. For example, female vervet monkeys (*Chlorocebus pygerythrus*) appear to punish males that do not engage in intergroup conflict (Arseneau-Robar et al., 2016), and in many species, dominants are able to monopolise food or mates at the expense of lower-ranking individuals (Barton et al., 1996; Clutton-Brock and Huchard, 2013; King et al., 2008; Papageorgiou and Farine, 2020).

One common occurrence of within-group conflict concerns the need for group members to make collective movement decisions. For groups comprised of individuals that largely interact on a local scale, simple interaction rules with a neighbour can lead to self-organisation and collective behaviours across the whole group (Couzin et al., 2005; Couzin and Krause, 2003; King et al., 2009; King and Cowlishaw, 2009). For example, in a theoretical game with two players in which individuals can either forage or rest, the hungriest individual will tend to lead because they want to forage sooner, and the other individual will follow because they likely gain benefits from cohesion (Rands et al., 2003). In bird flocks and fish shoals, for example, whole group movements are thought to be influenced by interactions between group members and their immediate neighbours (Herbert-Read et al., 2011; Pettit et al., 2013). Couzin and Krause (2003) suggest that, generally, models of self-organisation from local interaction rules apply best to groups with a strong common goal.

By contrast, in more heterogenous groups, including those with more stable membership, there is greater variation in the influence and motivation of groupmates, and therefore conflicts of interest over movement decisions are common (Conradt and Roper, 2009). Individuals should, for the most part, avoid becoming isolated to retain the benefits of group-living. This is highlighted by the short-term costs associated with dispersal, such as a reduction in body mass and an increase in stress hormone levels (Bonte et al., 2012; Maag et al., 2019). Conflicts over group-movement decisions could relate to the timing, direction, speed and final location. Where interests don't align, individuals may suffer consensus costs if a decision competes with their preference (Conradt and Roper, 2005). For example, when Bechstein's bats (*Myotis bechsteinii*) are deciding where to sleep, perceived costs associated with a given location for only certain individuals may result in them splitting from the rest of the group and forming subgroups (Fleischmann et al., 2013; Fleischmann and Kerth, 2014). Forming subgroups may alleviate the full costs of becoming isolated, while avoiding individual costs of collective decisions.

The costs of collective movement decisions may also be reduced if group members are able to influence the decision. Communication appears to be particularly important in relation to decisions about the timing or direction of movements (Bradbury and Vehrencamp, 2011; Sperber et al., 2017). Individuals may be able to "vote" on movement decisions, signalling their readiness or preference (Bousquet et al., 2011; Sperber et al., 2017; Walker et al., 2017). For example, red-fronted lemurs (*Eulemur rufifrons*) increase their vocalisation rate prior to departure (Sperber et al., 2017), and recent experimental work in jackdaws (*Corvus monedula*) has shown that vocal signalling can lead to faster group departures (Dibnah et al., 2022). In this way, collective movements may benefit the majority of group members, reducing the overall consensus costs. While there has been a large amount of work on collective decision-making and group movements, there has been far less experimental work investigating signalling relating to movements, particularly in vertebrates, many of which have complex communication systems. This could be, in part, due to the difficulty both practically and ethically of manipulating wild animal groups in this context. Despite these challenges, vocal social species provide an interesting way to study how communication is used to coordinate movements. For example, using movement signals from different individuals (Gall et al., 2017) can allow us to investigate how group members respond, based on intrinsic characteristics.

Leaders and followers may also emerge during within-group decisions; a single individual may exert more influence over a group movement, and the remaining group follow them (Brent et al., 2015; King et al., 2009). Leaders may be able to pursue their own selfish motivations; for example, they may gain from being the first to arrive at a foraging patch, and may be able to consume more food (Björnsson et al., 2018; Jolles et al., 2017; King et al., 2008). However, leaders may also suffer fitness costs such as increased energetic rate or threat of predation (Ioannou et al., 2019). If followers can avoid these costs, and harness the information of more experienced (e.g., older or trained) group members (Björnsson et al., 2018; Hall et al., 2017; Leblond and Reebs, 2006; Pillot et al., 2010), then following may provide many benefits to individuals. In addition, leadership could perhaps increase the speed of group decisions or help to overcome conflicts of interest. For example, in an experiment with chicks (*Gallus domesticus*) given a warm lamp to move towards, removing the typical leader prior to departure led to groups taking longer to reach their target (Collias, 2000).

Intrinsic characteristics, such as dominance status and sex, shape how individuals can influence movement decisions (Brent et al., 2015; King et al., 2008; Turbé, 2006). Pregnant females, for example, may have a higher motivation to forage and want to leave a resting position sooner than non-pregnant females or male group members (Fischhoff et al., 2007; Turbé, 2006). Dominant individuals may exert more influence on decisions due to age, experience or status (Brent et al.,

2015; King et al., 2008; McComb et al., 2011). However, even for decisions that appear to be made by a single dominant individual without an obvious form of communication, individuals may still be able to influence a decision, or even subsequently force group movements elsewhere if the costs are too severe. For example, in vulturine guineafowl (*Acryllium vulturinum*), when dominants monopolise a food patch, subordinates can initiate a group movement and force the dominant to abandon the food patch (Papageorgiou and Farine, 2020). This highlights that potential followers can still impact movements (Bourjade and Sueur, 2010; Petit and Bon, 2010). For instance, an individual may attempt to lead the group, but may fail to attract followers if their motivation does not align with the other group members, and they may have to return to the group.

Extrinsic factors are also likely to have a strong influence on collective movement decisions but have been given far less attention. Predation, for example, is known to be a strong selective pressure on individuals to form groups, and the response to predators can vary across individuals (Abbey-Lee et al., 2016; Carter et al., 2012; Tuliozi et al., 2021), yet how it affects leadership decisions is largely unknown. In a study of pairs of house sparrows (*Passer domesticus*), Tuliozi et al. (2021) showed that individuals who tended to lead when exploring a novel environment were less likely to do so when faced with a simulated predator. This may relate to individual variation in risk-taking behaviour, with potential benefits arising from the following of more risk-averse individuals (Tuliozi et al., 2021). Research using predators attacking visually simulated prey has shown that leadership at the front of the group may entail costs through increased attacks (Ioannou et al., 2019). Similarly, conflict with conspecifics is a significant extrinsic selection pressure (see **1.3 Outside threats**), and has been shown to affect the behaviour, physiology and fitness of individuals (Braga Goncalves et al., 2022; Lemoine et al., 2020; Morris-Drake et al., 2019, 2022). In particular, both physical cohesion (proximity to each other) and social cohesion (e.g., grooming levels) are known to increase after outgroup conflict (Morris-Drake et al., 2019; Thompson et al., 2020). It is plausible that increased cohesion may translate to changes in collective movement and leadership patterns. A recent comparative review suggests that females tend to lead collective movements while males tend to engage more in fighting (Smith et al., 2022). In banded mongooses (*Mungos mungo)*, males that tend to follow females into contests with rival groups suffer increased mortality costs, while females can benefit from extra-group matings (Johnstone et al., 2020). However, there has been little work investigating how the threat of outgroup conflict affects subsequent decisions, such as which group member to follow.

1.3 Outside threats

Across taxa, social groups often interact with outsiders, both individuals and groups. While interactions can be peaceful (Fruth and Hohmann, 2018), they often involve conflict, as outsiders can pose significant threats to individuals and the stability of groups. Outgroup conflict has been shown to be a significant selection pressure, with immediate consequences involving injuries and death of adults or infants (Braga Goncalves et al., 2022; Dyble et al., 2019; Hrdy, 1974; Morris-Drake et al., 2022). Other, often longer-term consequences involve changes to group structure (e.g., due to usurpation or immigration; Schneider-Crease et al., 2020; Strätz et al., 2002), subsequent movement patterns or space use (Christensen et al., 2016; Dyble et al., 2019; Radford and Fawcett, 2014) and changes to within-group behaviour such as affiliative interactions and vigilance (Morris-Drake et al., 2019; Thompson et al., 2020).

Traditionally, research has focused on the immediate behaviour and interactions of outgroup conflict. For example, investigating who is more likely to participate in contests depending on intrinsic characteristics, as variation amongst group members means conflict can provide varying levels of costs and opportunities to different individuals (Arseneau-Robar et al., 2016; Braga Goncalves and Radford, 2019; Kitchen and Beehner, 2007). As a specific example, an outside male attempting to usurp the current dominant male may be able to gain direct fitness benefits through reproduction, which poses significant fitness costs to the existing dominant particularly if infanticide is a possibility (Hrdy, 1974). Because costs are highest for the current dominant male compared to other group members, they may invest more than females in repelling potential usurpers (Mares et al., 2012). Intergroup interactions (outgroup conflict specifically entailing conflict between rival groups) also result in different benefits and costs to different group members. For instance, in banded mongooses, a dominant female may gain from a conflict by mating with members of another group, whilst males suffer reproductive costs along with physical injuries (Johnstone et al., 2020).

In some ant species, queen usurpation or nest takeover by an alien queen or colony poses a significant cost to colony inclusive fitness, as workers will end up raising a non-related brood (Buschinger, 2009; Foitzik and Heinze, 1998; Rudolph and McEntee, 2016). Of over 12,000 ant species, around 250 are “slave-makers” that either temporarily or permanently force a different host ant species to care for their own brood, eventually resulting in the death of the host colony (Buschinger, 2009). Workers of some species cannot even feed themselves, instead being highly specialised for fighting, and are completely reliant on the host colony that the queen first infiltrates and parasitises. Some work has shown that the nearby presence of slave-making ants leads to higher

aggression from the host species (D'Ettorre et al., 2004), an example of the "nasty neighbour" phenomenon in which nearby groups pose a greater threat than those from further afield (Christensen and Radford, 2018).

Researchers have also studied how factors like group size affect the assessment of rival groups, mortality rates and the likelihood of fighting success (Adams, 2016; Lanchester, 1916; McComb et al., 1994). Larger groups are generally expected to pose a greater threat (McComb et al., 1994), though group members may be less likely to participate in intergroup contests (Crofoot and Gilby, 2012) and so smaller groups may be able to compensate and still win (Crofoot et al., 2008). Contest avoidance has likely evolved to reduce costs of physical fighting, with many species using signals to assess other groups or individuals. Founding queens of *Lasius niger* ants use chemical signals to decide whether to engage in fights (Berthelot et al., 2017), while green woodhoopoes (*Phoeniculus purpureus*) engage in vocal displays with rival groups that can last up to 45 minutes (Radford, 2003). Signalling to assess rivals may itself be costly, but likely less so than enduring physical injuries, and group members can also glean information on rival group membership.

In the last decade, there has been a shift in research focus toward the consequences of conflict, particularly in terms of the subsequent behaviour of individuals or groups. Interactions with outsiders can lead to group members becoming more cohesive, potentially providing social benefits, a reduced personal risk and priming groups for future conflict (Birch et al., 2019; Morris-Drake et al., 2019). For example, within-group affiliation (e.g., grooming of others) may increase after conflict; this could be a response to heightened stress levels, or a way to increase participation in future conflicts (Radford, 2008a). In vervet monkeys, females selectively groom males that engage in fights with rivals while at the same time attacking males that do not engage, leading to increased participation of males in subsequent intergroup conflict (Arseneau-Robar et al., 2016). Group members may also be more vigilant after conflict (Morris-Drake et al., 2019), which may have knock-on fitness implications if the group is more likely to spot predators (Morris-Drake et al., in revision). Conflict has also been shown to affect movement decisions of groups. After green woodhoopoes engage in vocal contests with rivals, groups are more likely to roost near their territorial boundaries than on days where no such contests took place; roosting near the boundary is also more likely if contests with rivals are longer (Radford and Fawcett, 2014). Contest outcome has also been shown to affect subsequent movement patterns; for example, losing groups of white-faced capuchins (*Cebus capucinus*) travel further and are more likely to change sleeping sites than those that won an intergroup contest (Crofoot, 2013).

As well as directly affecting behaviour in the short and long term, intergroup conflict is also likely to affect behaviour indirectly; for example, through cues from outsiders such as faeces (Christensen et al., 2016). For territory-holding species, certain locations may be more valuable if they contain greater resources, or if groups can forage more efficiently in their core territory due to experience (Crofoot et al., 2008). Groups may therefore be more likely to defend certain areas (Crofoot et al., 2008). We might expect greater levels of vigilance or different movement patterns in these areas, as well as in areas where intergroup interactions are more common, such as the periphery of territories. As well as greater threat levels of conflict, rival territory may provide opportunities for expansion or mating opportunities (Mayer et al., 2017). Whilst conflict is a significant extrinsic factor affecting both immediate and longer-lasting within-group behaviours, how it interacts with intrinsic characteristics of groups to affect decision-making is less known.

1.4 Study species

1.4.1 Dwarf mongooses (*Helogale parvula*)

Dwarf mongooses are a cooperatively breeding carnivore found widely distributed in Africa (Creel, 2013). They are part of the Herpestidae family along with 33 other species of mongooses, which are distributed across Africa, the Middle East and Asia (Veron et al., 2022). Of the 34 species, 23 are solitary and 11 are group-living (Veron et al., 2022). The latter include the well-studied meerkats and banded mongooses. Dwarf mongooses are the smallest of the mongooses, with adults weighing around 200–300 g. They mostly feed on invertebrates, but their diet also includes small mammals, amphibians and reptiles (Rasa, 1987). Groups of two to 30 individuals comprise a dominant breeding pair, who monopolise the majority of reproduction, and subordinate helpers of both sexes who provide care for pups through feeding and protection (Rasa, 1987). They are a highly vocal species with a large repertoire, including close calls to maintain contact, a “watchman’s song” while on sentinel duty, “lost” calls when individuals become isolated, snake mob calls and both aerial and terrestrial predator alarm calls (Collier et al., 2020; Kern and Radford, 2013, 2016; Morris-Drake et al., 2017; Rubow et al., 2017). Individuals can gleam information on individual identity through vocal information alone (Kern and Radford, 2016; Sharpe et al., 2013).

Being diurnal, groups sleep overnight; mostly in termite mounds, but also in trees and rock crevices (Hoffmann et al., 2014). Groups emerge around sunrise and groom one another before leaving the sleeping burrow to forage. Most of the day is spent moving and foraging as a group, though in the summer months groups rest during the middle of the day in shade (Rasa, 1987).

Groups maintain territories that can overlap with neighbours, regularly encountering cues such as faecal deposits (Christensen et al., 2016), and intergroup interactions between both neighbouring and non-neighbouring groups take place relatively frequently. These involve high levels of vigilance, vocalisations and sometimes physical fighting (Rasa, 1987). Though these encounters are not typically as violent as species such as meerkats or banded mongooses, injuries and mortality do still occur.

The Dwarf Mongoose Research Project (DMRP) was founded in 2011 and is a year-round research project that tracks the behaviour of wild, habituated dwarf mongooses on Sorabi Rock Lodge Reserve, Limpopo Province, South Africa (24° 11'S, 30° 46'E). Around eight groups of dwarf mongooses are habituated to human presence (<5 m) at any one time, with individuals recognisable through unique blond-hair-dye marks applied by researchers (Kern and Radford, 2013). Each group is visited by the field team around once a week for ~3 days at a time; this ensures frequent behavioural and life-history data are collected, but also minimises the contact time with humans. Predation by birds and feline species has been directly observed, so the impact of humans appears minimal. A long-term dataset on both behaviour and life-history of all habituated individuals, from pup to adult, has been maintained since 2012. Previous work on the DMRP population has included research on vocalisations such as their watchman's song and alarm calls, the effect of anthropogenic noise on information use and behaviour, and how conflict affects within-group behaviour (Kern et al., 2017; Kern and Radford, 2013; Morris-Drake et al., 2017, 2019).

Observers first find a group by traversing its known territory and frequently visited sleeping burrows, or by returning to a burrow where the group were known to have slept the night before. Study individuals are trained to climb onto a balance scale for a small food reward (boiled egg), and groups are weighed three times a day: in the morning before the group has left the sleeping burrow, 3–4 hours later after a foraging session, and again when the group has returned to their sleeping burrow. Prior to the group leaving, individuals tend to close call, with the overall group close call rate increasing prior to departure. When the group leaves the sleeping burrow to start foraging, the leader of the group is recorded – who leads is dependent on various factors (see **Chapter 2**). While groups are out foraging, they may slowly traverse an area, but often an individual may attempt to move elsewhere and signal to other individuals in an apparent attempt to attract followers (see **Chapter 3**). Throughout the day, the observer collects data on mongoose sentinel behaviour, proximity to one another while foraging, latrine behaviour and intergroup interactions. These data are then entered into the project database by the observer, which is checked (e.g., for invalid identity codes and typos) and maintained by the Project Manager.

1.4.2 *Temnothorax* ants

Temnothorax nylanderi is a monogamous ant, found across central Europe (Foitzik and Heinze, 1998). Colonies tend to be small, comprised of several dozen workers, and the queen is singly mated (Foitzik et al., 1997; Foitzik and Heinze, 2000; Heinze et al., 1996). Microsatellite analysis shows that male production by workers appears to be rare (Foitzik et al., 1997; Foitzik and Heinze, 2001). Unlike some species, *T. nylanderi* do not build their own nests, but inhabit vegetation such as rotten twigs, hollow acorns and grass stems. The availability of these nesting materials changes during the year due to decay, with numerous nest sites available early in the year, which then decline over time and become rare in spring and summer (Foitzik and Heinze, 1998).

When nest sites are available, colonies split and inhabit them - colony removals from nests led to other colonies rapidly migrating into the empty nest sites (Foitzik and Heinze, 1998). This is known as seasonal polydomy, and could provide some security to colonies in the likely event that one of their nests later becomes uninhabitable (Foitzik and Heinze, 2001). When nest sites decline and become limited, there are no longer enough nest sites for all sub-colonies. As a result, colony fusions and takeovers by unrelated queens occur (Foitzik and Heinze, 1998; Strätz et al., 2002). These fusions or takeovers can lead to colonies with two queens, one of which dies within several months (Foitzik and Heinze, 1998; Strätz et al., 2002). Experimental work has shown that, given the choice, founding queens prefer to join queenless colonies in a nest over an empty nest (Foitzik and Heinze, 1998). Fusion between colonies leads to aggression between workers, though this is usually ineffective at keeping the outside colony from entering the host nest site, and colonies end up co-habiting (Foitzik and Heinze, 1998). Genetic analysis has shown that, despite being monogamous, colony fusions and takeovers lead to higher heterogeneity amongst nests than expected, with a quarter of nests sampled containing more than two worker lineages (Foitzik and Heinze, 2001).

Temnothorax colonies used for an experiment by Nathalie Stroeymeyt (see **Chapters 4** and **5**) were collected in March and June 2011 from a study site in Germany (previously reported on by Foitzik and Heinze, 2000). The site is an open pine-oak forest near Sommerhausen, 15 km south of Würzburg (10°02'–10°03' E, 49°42'–49°43' N), that contains a dense population of *T. nylanderi*. Colonies were contained in controlled laboratory conditions (14L:10D cycle, 25°C, 55% RH) within plastic boxes (155 x 135 x 50 mm) that had Fluon-coated walls, which prevented ants from escaping. Colonies were at first contained within the twigs that they were collected from, but then moved to artificial nests. These were comprised of a cardboard perimeter wedged between two glass slides, which equated to a nest cavity of 36 x 48 x 12 mm, with an entrance of 8 x 2 mm. Colonies were fed

a 10% honey solution weekly, along with ad libitum water. Prior to the experiment, colonies remained in the laboratory for a mean ± SE of 80 ± 3 days.

1.5 Thesis aims

This thesis uses a long-term dataset and experimental work to investigate how within-group characteristics and intergroup conflict affect group decisions in two social species: dwarf mongooses and the eusocial ant *Temnothorax nylanderi*. Using habituated wild dwarf mongooses allows us to glean ecologically valid insights into how intrinsic characteristics of group members (dominance, sex) and extrinsic factors (season, intergroup conflict) affect group movement decisions. Using a laboratory based *Temnothorax* experiment provided tightly controlled conditions, allowing us to investigate how colony fusion dynamics between rival colonies was affected by the presence or absence of queens. On starting my PhD in September 2019, the initial plan was to work solely on the dwarf mongooses. However, during my first field season at the DMRP, the COVID-19 pandemic took hold. I therefore stayed for an extended field season to perform additional experimental work. But, thereafter there was a lot of uncertainty about future travel, so we collaborated with Nathalie Stroeymeyt on experimental work that she had previously undertaken on *T. nylanderi*. After 3 years of the PhD, I accepted a job with NHS Digital and carried on with the PhD part-time.

Chapter 2 uses the long-term dataset of the DMRP to investigate how leadership is affected by dominance and sex, season (comparing breeding and non-breeding periods) and intergroup conflict – both the immediate impact of direct conflict and an increased threat level associated with different areas of territory. **Chapter 3** takes an experimental approach, focussing on follower decisions: it investigates the response of dwarf mongooses to hearing the movement call of a group member, whether individuals prefer to follow dominants over subordinates, and whether the simulated threat of intergroup conflict affects this decision. **Chapter 4** is a methodological one, describing the development and validation of an image-processing programme to extract automated data from photographs of interactions between ant colonies; those images were from an experiment carried out by Nathalie Stroeymeyt. The data generated are used in **Chapter 5** to study how the presence or absence of queens affects fusion dynamics between *Temnothorax* colonies. Finally, **Chapter 6** brings this work together and discusses how research in this area could move forward.

Chapter 2

Intrinsic and Extrinsic Factors Influence Leadership of Group Movements in Cooperatively Breeding Dwarf Mongooses

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BC co-developed the initial idea for analysis, collated and cleaned the data, carried out most of the data analysis, interpreted the results and drafted the manuscript. PK suggested analyses, helped interpret the results and provided comments on the manuscript; AMD managed the Dwarf Mongoose Research Project (DMRP) and long-term data collection, and provided comments on the manuscript; JMK set-up the DMRP, managed the DMRP and long-term data collection, and provided comments on the manuscript; ML managed the DMRP and long-term data collection, and provided comments on the manuscript; JA provided comments on the manuscript and carried out the permutation analysis; ANR co-developed the initial idea for analysis, helped with data analysis and interpreting the results, and provided substantial comments on the manuscript.

2.1 Abstract

In social species, individuals must coordinate their behaviour to perform collective actions and thus maintain the benefits of group-living. When a group moves location, there may often be a single leader that is followed by others. Both intrinsic (e.g., dominance status and sex) and extrinsic (e.g., season and intergroup conflict) factors are predicted to affect the likelihood of leadership, yet the latter have received less empirical attention. To address this, we analysed long-term leadership data collected from wild groups of dwarf mongooses (*Helogale parvula*), a territorial cooperative breeder. We found that dominant individuals (the breeding pair) and males were more likely to lead group movements from morning sleeping burrows than subordinates and females, with these differences being stronger in the breeding compared to the non-breeding season. Dominants are generally older individuals and may therefore have a greater knowledge of the territory, which could confer advantages when protecting vulnerable young. Males may have a greater motivation to lead because they are more likely than females to disperse and to seek extra-group mating opportunities. Intergroup conflict also affected leadership patterns: dominant males led more the day after an intergroup interaction in the non-breeding season, whilst male leadership increased in the breeding season when groups were in territory areas that overlapped with those of rivals and thus the likelihood of an intergroup interactions was greater. Overall, we have expanded our understanding of how extrinsic factors can interact with intrinsic factors in determining leadership patterns in social species.

2.2 Introduction

Animals living in groups must often coordinate their behaviour to achieve collective action. Collective movements can ensure cohesion and thus preserve the benefits of group-living, such as reduced predation risk and better resource defence (Krause and Ruxton, 2002). When a group moves, there is often a discernible 'leader'; an individual at the front being followed by group members. In some contexts, this leader could be acting on collective information pooled from the rest of the group (Conradt and Roper, 2005; Seeley and Buhrman, 1999); alternatively, leaders may be exerting more influence by making individual movement decisions (Conradt and Roper, 2005). Where the interests of group members align, followers can benefit from a knowledgeable leader and avoid potential costs of leadership, such as an increased predation risk (Ioannou et al., 2019). Even in cases where accepting a leader is costly for some group members (e.g., due to differences in motivation), the benefits of cohesion are likely to outweigh the costs of leaving a group (Johnstone et al., 2020), though group splits can occur where collective action fails (Fleischmann et al., 2013). Whilst much research has considered how leadership is influenced by intrinsic factors, less attention has been paid to the importance of extrinsic factors, especially intergroup conflict.

Leadership of group movements is known to be affected by intrinsic factors such as dominance status (King et al., 2008) and sex (Barelli et al., 2008; Lee and Teichroeb, 2016). Dominant or higher-ranking individuals may lead more than subordinates in certain contexts, as shown in green woodhoopoes (*Phoeniculus purpureus*), chacma baboons (*Papio ursinus*) and free-ranging dogs (*Canis lupus familiaris*) (Bonanni et al., 2010; King et al., 2008; Radford, 2004). Dominant individuals may be more likely to lead if, for example, they are more experienced or knowledgeable of foraging locations (McComb et al., 2001; Radford, 2004) or because they are able to monopolise potential resources (King et al., 2008). Similarly, leadership may be affected by the sex of individuals. In many species, including plains zebras (*Equus burchellii*), white-handed gibbons (*Hylobates lar*) and meerkats (*Suricata suricatta*), females lead more than males (Barelli et al., 2008; Fischhoff et al., 2007; Turbé, 2006). More frequent leadership by females is likely a result of increased energetic demands associated with pregnancy and lactation (Furrer et al., 2012; Turbé, 2006); by leading, females may gain better or quicker access to resources such as food or water (Barelli et al., 2008; Fischhoff et al., 2007). But there are also examples of male-biased leadership: in vervet monkeys (*Chlorocebus pygerythrus*), for instance, males appear to lead more when groups leave sleeping sites, possibly in an attempt to reach food before others (Lee and Teichroeb, 2016). The importance of intrinsic factors in determining leadership may also be modified by extrinsic factors. Season is one such factor that has received attention – for example, female leadership in meerkats increases in the

breeding cf. nonbreeding season (Turbé, 2006) – but other extrinsic factors have been less well-studied in this regard.

Intergroup conflict occurs throughout the animal kingdom, with rival groups competing over resources such as food, territory and mating opportunities (Johnstone et al., 2020; Wilson and Wrangham, 2003). Contests between rival groups can be costly in terms of time and energy, and can also result in a variety of immediate, delayed and knock-on negative fitness consequences (Braga Goncalves et al. 2022). On the other hand, conflict can also provide opportunities: individuals can attempt to mate with rival group members or seek to disperse and thus avoid inbreeding (Johnstone et al., 2020; Nelson-Flower et al., 2012; Nichols et al., 2015). Behaviour can be influenced by both intergroup interactions (IGIs) and the threat of intergroup conflict; rival-group cues such as scent-markings might indicate the likelihood of an imminent IGI, and such cues (e.g., at latrine sites) might be more prevalent in territorial areas that border those of neighbours (Christensen et al., 2016; Rosell et al., 1998). For example, within-group affiliation may increase and groups may preferentially avoid or return to sites of conflict in the aftermath of IGIs (Radford, 2008b; Radford and Fawcett, 2014; Yi et al., 2020), whilst encountering rival-group faeces can lead to increases in vigilance, greater group cohesion and altered movement decisions (Christensen et al., 2016; Morris-Drake et al., 2019). Intergroup conflict therefore has the potential to affect leadership of group movements.

Intergroup conflict results in different costs and benefits to different group members, with participation and behaviour surrounding IGIs varying depending on, for example, dominance status and sex (Braga Goncalves and Radford, 2019; Kitchen and Beehner, 2007; Morris-Drake et al., 2022). Dominants who hold a breeding position could face the highest costs of being usurped or losing out on mating opportunities, and thus may invest more in defensive actions (Gavrilets and Fortunato, 2014; Mares et al., 2012). Dominants could also attempt to encourage participation from subordinates in future contests, as suggested to occur in green woodhoopoes (Radford, 2011). Behaviour in relation to IGIs may also differ between the sexes. For example, male Javan gibbons (*Hylobates moloch*) contribute more than females in IGIs, and sleep further away from previous areas of conflict (Yi et al., 2020). In vervet monkeys, females appear to encourage male aggression through affiliative grooming during breaks in contests (Arseneau-Robar et al., 2016). Despite these clear interindividual differences in IGI participation, little work has investigated how leadership patterns among group members are influenced by intergroup conflict. An exception is the study of banded mongooses (*Mungos mungo*) which shows that females lead more than males in the periphery of the territory (Preston, 2020); females lead groups into IGIs to gain extra-pair matings, whilst male followers suffer injuries and mortality from fighting (Johnstone et al., 2020).

Dwarf mongooses (*Helogale parvula*) provide an excellent opportunity to investigate how leadership of group movements is affected by both intrinsic and extrinsic factors. They are cooperative breeders, with groups comprising a dominant breeding pair that are assisted by subordinate helpers in the rearing of up to three litters per breeding season (Kern and Radford, 2013; Rood, 1980). Group members generally move as a cohesive whole around their territory (Cobb et al., 2022), which they defend year-round by scent-marking at communal latrines and by engaging in interactions with rivals when they are encountered (Christensen et al., 2016; Morris-Drake et al., 2019). All group members sleep together in a burrow each night (Rasa, 1987), collectively leaving to start foraging the following morning; on such occasions, one individual adopts a leadership role (i.e., is followed by the other group members). Using long-term data, we investigated how dominance status, sex, season and intergroup conflict affect leadership of group movements from a communal sleeping burrow. We predicted that dominant individuals would lead more due to their generally greater experience, but that dominant females would be particularly prevalent in a leadership role during the breeding season due to increased energetic needs. We also predicted that the recent occurrence of an IGI and the threat of a likely IGI (when the group was in an overlapping compared to core territorial area) would result in an even stronger likelihood of leadership by males, due to potential mating or dispersal opportunities (Mayer et al., 2017).

2.3 Methods

2.3.1 Study site and population

We collated long-term observational data from the Dwarf Mongoose Research Project (DMRP) in Limpopo Province, South Africa (24° 11'S, 30° 46'E); details about the study site can be found in Kern and Radford (2013). Work was conducted under permission from the Limpopo Department of Economic Development, Environment and Tourism (permit number: 001-CPM403-00013), and the relevant Ethical Review Groups of the University of Bristol, UK (University Investigator Numbers: UB11/038, UB/14/044, UIN/17/074) and Pretoria University, South Africa (Animal Use and Care Committee number: EC057–11). Data were collected throughout the year between December 2012 and March 2021, from 13 wild but habituated groups, each comprising 2–14 adults (individuals >1 year old). Groups are habituated to the nearby presence of humans (<5 m), with each individual identifiable through unique blond-hair-dye marks (Kern and Radford, 2013). Each study group was generally visited every 3–4 days to maintain habituation and to collect behavioural and life-history data. If a group's sleeping burrow was known from the previous evening, an observer arrived at the burrow the next morning before the group emerged. Once emerged, mongoose individuals groom

one another in the immediate vicinity of the burrow (Morris-Drake et al., 2019). When an individual attempts to leave the burrow area and is followed by the rest of the group, this individual is considered the leader of a group movement and a leadership event is recorded, with only one event recorded per morning. Group compositions are taken each morning at the sleeping burrow, providing the identity of all followers. Individuals sometimes attempt to leave and are not followed (returning to the rest of the group on such occasions); data on this aspect of 'failed' leadership are not routinely collected. We excluded pups from the analyses because individuals less than 1 year old rarely lead the group (Cobb et al., 2022). Our overall dataset comprises 551 leadership events, but subsets were used for different questions and so sample sizes vary between models (see later).

The long-term DMRP database is initially populated by field team members who have collected the relevant data from their focal group that day. Each field team member receives the same standardised training and visits all the groups being studied during their time at the field site. Databases are subjected to a sequence of rigorous checks. A field manager scans the data monthly and amends any obvious mistakes, such as incorrect ID or sex codes and group sizes, before sending the data to a data manager. The data manager goes through each spreadsheet in detail, raising any queries that need input from field researchers and amending any mistakes as they arise (e.g., typographical errors, incorrect data placement, duplicate entries, contradictions in data entry and general anomalies).

2.3.2 Dominance status, sex and season

Within the DMRP population, the dominance status (dominant = breeding pair; subordinate = all other adults) and sex of all individuals is known through observations of aggressive interactions and ano-genital grooming (Kern and Radford, 2013, 2016). Groups produce up to three litters per breeding season, which coincides with increased rainfall (Rood, 1980). We considered the breeding season to start when the first dominant female in the population was in oestrus (when males start initiating mating attempts) and to end when the last litter of pups in the population first emerged from the breeding burrow (Morris-Drake et al. In revision). Thus, the dates for breeding seasons differed between years (average breeding season range from 2nd September to 31st March). The remainder of each year was designated as the non-breeding season.

2.3.3 Intergroup conflict

Dwarf mongoose groups maintain territories throughout the year, periodically encountering neighbouring groups. IGIs are recorded when two groups show signs of being aware of each other's presence; this usually involves increased vigilance and vocalising, with some interactions escalating to physical fighting (Christensen et al., 2016; Morris-Drake et al., 2019). As well as assessing the direct effect of IGIs on leadership, we investigated how leadership is affected by the threat of likely IGIs. As a proxy for IGI threat level, we used territory location where the leadership event occurred: core areas were classified as low threat, whilst areas where there was overlapping usage with a neighbouring group were classified as high threat.

To construct territory maps, we used GPS data. Once a group leaves its sleeping burrow in the morning, waypoints are recorded every 15 minutes on a handheld GPS device (Garmin eTrex 10; Garmin Europe Ltd, Southampton, UK) and later saved as a Garmin MapSource (GDB) file, with the filename labelled with the group, date and observer. We extracted waypoints from GDB files using the opensource software GPSBabel v. 1.7 (Lipe, 2022). We cleaned data in R v. 4.1.1 (R Core Team, 2022) using the *tidyverse* package (Wickham et al., 2019). Coordinates (longitude and latitude) were extracted and overlaid on a map of the DMRP using *ggmap* (Kahle and Wickham, 2013) for outlier checking. Outliers (coordinates furthest from the average coordinate location) were visually checked one group at a time, and their corresponding information from the original filename compared to the DMRP Diary of Group Visits (DOGV), in which group observation sessions are recorded. Outliers were either (i) verified as correct where a DOGV entry matched the date, group and observer of a given GDB filename, (ii) corrected to the appropriate group where a file was mislabelled, or (iii) removed for ambiguous cases (e.g., where no corresponding DOGV entry for that day and observer existed).

The kernel utilisation distribution (UD) was used to determine territory areas, from the R package *adehabitatHR* (Calenge, 2006); this calculates the minimum area within which a group has a given probability of being located (Worton, 1989; Calenge, 2006). To account for changes in territories across time, we constructed both breeding and non-breeding territories for each group for each year. Territories from the 2013 non-breeding season to the 2021 breeding season were used; GPS data before 2013 were incomplete and thus were excluded. We used the R package *sf* (Pebesma, 2018) to classify leadership events as being in the core territory (within the group's 50% UD) or overlapping territory (within an area in which at least two groups' 95% UD contours overlap).

2.3.4 Statistical analysis

We performed all analysis using R v.4.0.3 (R Core Team, 2022). For our main analyses, we used an information theoretic (IT) approach (Burnham and Anderson, 2002), because our data are purely observational and stepwise-deletion methods for such datasets have been criticised in recent years (Grueber et al., 2011; Tredennick et al. 2021; Whittingham et al., 2006). The IT approach allows comparison and inclusion of multiple models starting from an initial global model, which accounts for model uncertainty if there are several similar-fitting models. We performed selection using the corrected form of Akaike's Information Criterion (AICc), which compensates for smaller sample sizes and is recommended by Burnham and Anderson (2002).

We built generalised linear mixed models (GLMMs) using *lme4* (Bates et al., 2015). As sample sizes differ for each model, we carried out the model selection process on different global models, with fixed effects and interactions based on *a priori* hypotheses. The R package *MuMIn* (Bartoń, 2020) was used for model comparison and selection. Different model structures were ranked by AICc; those within six AICc of the best-fitting model were included in the model set (Harrison et al., 2018). We also applied the nesting rule to avoid inclusion of overly complex models (Arnold, 2010; Harrison et al., 2018; Richards, 2008): if a given model has the same fixed effects as another model, but with any additional fixed effects, their AICc values are compared; if the more complex model has a higher AICc (less support), then that model is removed.

The response term for all models was whether an individual led or followed during a leadership event, and thus we used a binomial error family. The link *cloglog* was used for all models, which is recommended for datasets in which there is an imbalance in the binomial response (Thomas, 2021); in our data, there is a mean of 5.8 followers for every leader. We also compared model fits for each binomial link (*logit*, *probit and cloglog*), with none providing noticeable improvements in fit. All models included the random term 'leadership event' nested within 'group identity', to account for each leadership event being a unique observation, with repeated sampling of each group. We initially included individual as a crossed random term, to account for repeated sampling of individuals, but these models failed to converge and so the random term was dropped. Group size (individuals observed as present that morning) was included as a fixed effect in all models.

We checked global models for multicollinearity using the R package *performance* (Lüdecke et al., 2021), which provides a variation inflation factor (VIF) for each fixed effect in the model (interaction terms were excluded to avoid VIF inflation; Lüdecke et al., 2021).There was no indication of multicollinearity. Once a global model was built, we standardised continuous variables (Grueber

et al., 2011) and assessed the model fit (Burnham and Anderson, 2002; Harrison et al., 2018), both by checking that the residual deviance was less than the residual degrees of freedom and by visually inspecting a histogram of deviance residuals and a plot of binned residuals (Gelman and Hill, 2006). After model selection, we visualised interactions using the packages *ggplot2* and *cowplot*, presenting back-transformed predicted model values to compare leadership probabilities. Model selection tables including nested models removed for analysis are presented (where applicable) in **2.6 Appendix 1** (see end of chapter).

We first investigated whether leadership is affected by dominance status, sex and season. These factors were included as a three-way interaction in our first model. Because the interaction was important (see Results), we split our initial dataset by season into two subsets: non-breeding season and breeding season. We used these subsets for the remainder of our analyses. We then investigated how intergroup conflict affected leadership in two ways. Our first models assessed the direct effect of IGIs, including whether a group was involved in one the day before a leadership event (yes or no) in a three-way interaction with dominance status and sex. If the group was not observed the day before a leadership event, that event was excluded from the dataset for this model. A second set of models examined whether the threat level of intergroup conflict affected leadership. Location of leadership event (core or overlapping territory area) was included as a three-way interaction with dominance status and sex. For both sets of intergroup conflict models, non-breeding and breeding season data were analysed separately.

In addition to the model-selection analysis, we conducted permutation tests to verify the relationships seen between leading and social class, season and/or intergroup threat. For each leading event, one individual was randomly selected as the leader, thus meaning there was one data point per event. We repeated this process 1000 times to generate a distribution of the expected number of leading events for each social class (dominant female, dominant male, subordinate female, subordinate male), under the null assumption that all individuals were equally likely to lead. These distributions were then compared to the observed number of lead events for each class. We undertook this process on the subsets of the data that were found to have important predictors of interest in the main analyses (see **2.4 Results**): breeding vs non-breeding seasons, IGI day before vs no IGI day before (non-breeding season only), and core territory vs overlapping territory areas (breeding season only). To compare across classes, we calculated and standardised the likelihood of leading compared to random ('effect sizes')—which accounts for group size and composition—such that a value of 1.5 denotes that an individual is 50% more likely to lead than random. This enables direct comparison between the likelihood that a given individual of a certain class led an event, as for most events there are multiple subordinate females and males but only one dominant of each

sex. Standardised point estimates for likelihood of leadership are taken from the number of observed lead events divided by the median number of permuted (expected) lead events, whilst 95% confidence intervals are calculated from the observed number of lead events divided by 0.025 and 0.975 quantiles of permuted events. Permuted data plots are presented in **2.6 Appendix 1** (**Figures A1.1**–**A1.3**) with qualitatively the same findings as presented in the Results section below.

2.4 Results

2.4.1 How is leadership affected by dominance status, sex and season?

In an initial analysis of leadership, we found support for an effect of the three-way interaction between dominance status, sex and season (present in the only candidate model; **Table 2.1**). To explore this interaction in more detail, we split our data by season (non-breeding and breeding) and ran analyses examining the effect of dominance status and sex on each data subset separately.

In the non-breeding season analysis, we found some support for an effect of the interaction between dominance status and sex (present in the top candidate model, but not in subsequent models; **Table 2.2a**). Sex was present in all three models and dominance status was in the top two models (**Table 2.2a**). Overall, males were more likely to lead group movements than were females, and a given dominant individual was more likely to lead than a given subordinate; there was a greater sex difference among subordinates than dominants (**Figure 2.1a**). In the breeding season, the interaction between dominance status and sex was slightly more important (present in the top of two candidate models), with dominance status and sex included in both models (**Table 2.2b**). As in the non-breeding season, males led group movements more than did females, especially among subordinates, but there was a larger difference between dominants and subordinates: a given dominant was much more likely to lead than a given subordinate (**Figure 2.1b**).

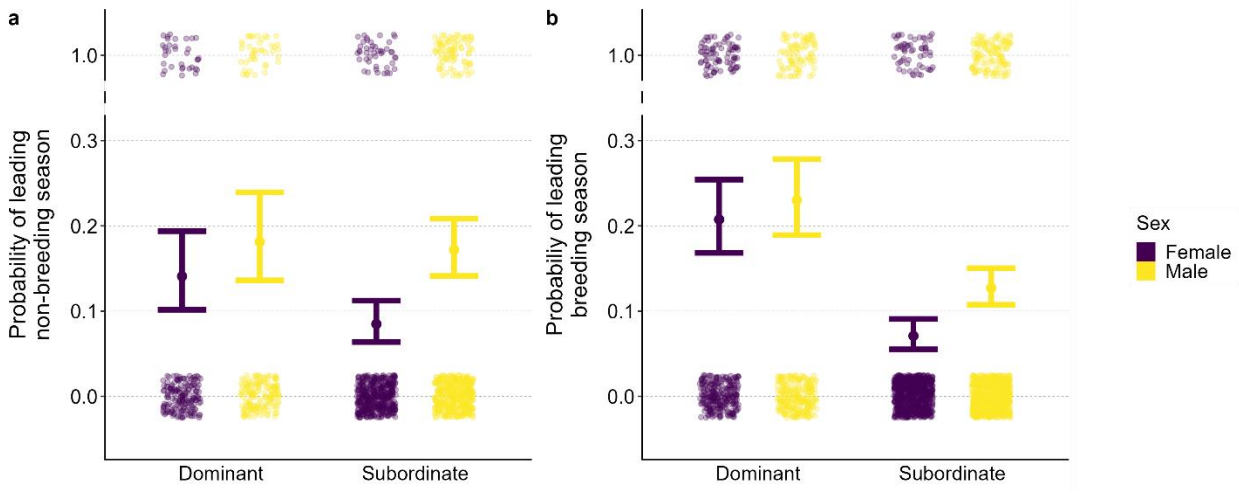

Figure 2.1. The effect of a two-way interaction between dominance status and sex on the probability of leading a group movement in the (a) non-breeding (*N* = 207 leadership events) and (b) breeding (*N* = 344 leadership events) season. Large points show probability of leadership by a given individual ± SE bars; small, clustered points show the response variable as jittered raw data, with 'lead' and 'follow' being centred at 1 and 0 respectively. Dashed line indicates a break in the y axis scale.

2.4.2 How does intergroup conflict influence leadership patterns?

We found an influence of interactions with rival groups (IGIs) on leadership in the non-breeding season but not the breeding season. In the non-breeding season, there was support for an influence of the three-way interaction between dominance status, sex and an IGI the day before a leadership event (present in the top of two candidate models; **Table 2.3a**). Sex and dominance were included in both models (**Table 2.3a**). Overall, males led group movements more than females and a given dominant was more like to lead than a given subordinate, but dominant males were especially likely to lead when there had been an IGI the day before (**Figure 2.2**). In the breeding season, there was no support for an influence of the interaction between dominance status, sex and having an IGI the day before a leadership event (not present in the one candidate model; **Table 2.3b**). Dominance status and sex were both in that top candidate model (**Table 2.3b**), with the same directions of effects as in earlier analyses.

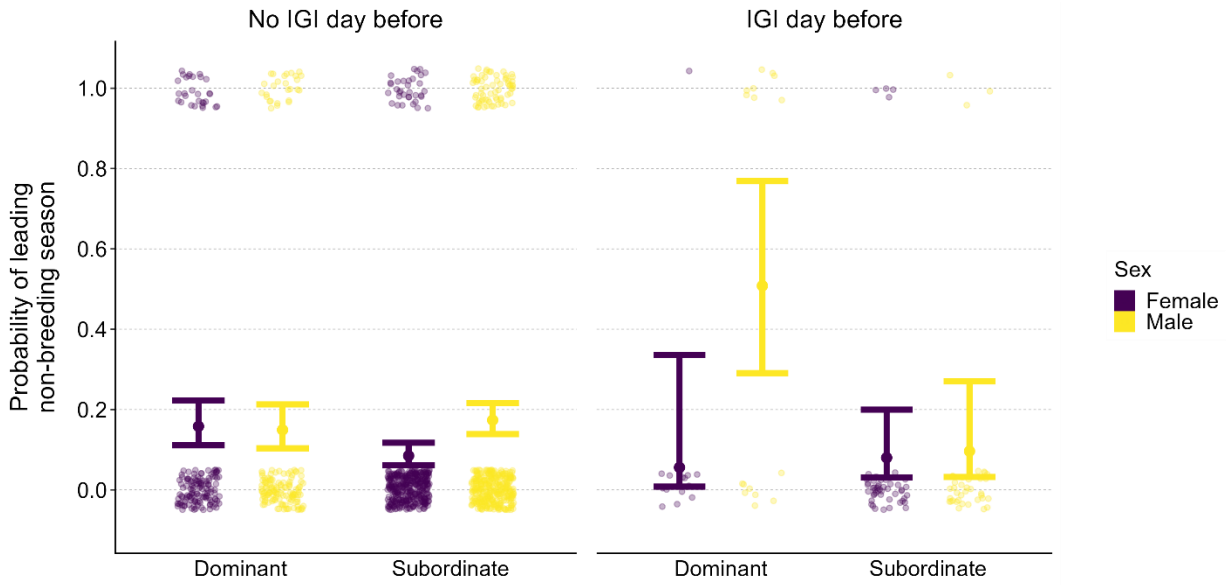

Figure 2.2. The effect of a three-way interaction between dominance status, sex and having an IGI the day before on the probability of leading a group movement in the non-breeding season. Large points show probability of leadership by a given individual ± SE bars; small, clustered points show the response variable as jittered raw data, with 'lead' and 'follow' being centred at 1 and 0 respectively. *N* = 172 leadership events.

We found an influence of intergroup threat on leadership in the breeding but not the non-breeding season. In the non-breeding season analysis, the three-way interaction between dominance status, sex and territory area (core or overlap) was not present in the one candidate model (**Table 2.4a**). Sex was found to be important, with males being more likely to lead a group movement than were females (**Table 2.4a**); this was the case in both core and overlapping areas. By contrast, there was some support for an influence of the interaction between dominance status, sex and territory area in the breeding season (present in the top of two candidate models; **Table 2.4b**). Dominance status and sex were present in both models (**Table 2.4b**). Overall, in the breeding season, there was a larger sex difference in overlapping compared to core territory areas; males were more likely to lead than females, and this was particularly apparent for subordinate males cf. subordinate females (**Figure 2.3**).

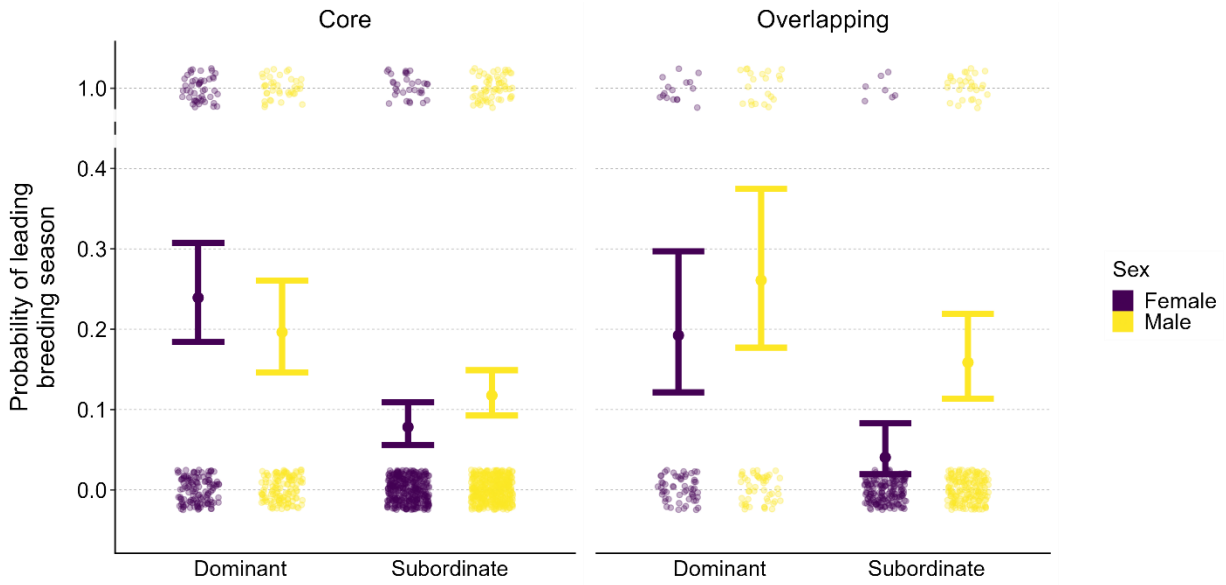

Figure 2.3. The effect of a three-way interaction between dominance status, sex and territory area on the probability of leading a group movement in the breeding season. Large points show probability of leadership by a given individual ± SE bars; small, clustered points show the response variable as jittered raw data, with 'lead' and 'follow' being centred at 1 and 0 respectively. Dashed line indicates a break in the y axis scale. *N* = 254 leadership events.

Table 2.1. Model selection table investigating the effects of dominance status, sex and season on leadership. Models within six AICc of the top model are included. Values for predictor variables indicate coefficients (± standard errors) and ✓ indicates inclusion of an interaction in the model. D = dominant, S = subordinate, NB = non-breeding, B = breeding, M = male and F = female. Delta value (Δ) indicates the difference in AICc between the given model and the top candidate model. Weight refers to the likelihood of the model being the best model, given the other models in the set. *N* = 551 leadership events.

Intercept	Dominance (D > S)	Season (NB > B)	Sex (M > F)	Group size	Dom: Sex: Season	df	AICc	Δ	Weight
-2.65 ± 0.32	-0.08 ± 0.19	0.30 ± 0.14	0.75 ± 0.18	-0.72 ± 0.10	✓	11	3005.80	0.00	0.99

Table 2.2. Model selection table investigating the effects of dominance and sex on leadership in the (a) non-breeding and (b) breeding season. Models within six AICc of the top model are included. Values for predictor variables indicate coefficients (± standard errors) and ✓ indicates inclusion of an interaction in a model. D = dominant, S = subordinate, M = male and F = female. Delta value (Δ) indicates the difference in AICc between the given model and the top candidate model. Weight refers to the likelihood of the model being the best model, given the other models in the set. *N* values refer to the number of leadership events.

Intercept	Dominance (D > S)	Sex (M > F)	Group size	Dom: Sex	df	AICc	Δ	Weight
(a) Non-breeding (*N* = 207)								
-1.89 ± 0.18	-0.54 ± 0.23	0.28 ± 0.23	-0.82 ± 0.17	✓	7	1141.50	0.00	0.45
-2.06 ± 0.15	-0.25 ± 0.15	0.57 ± 0.14	-0.81 ± 0.17		6	1142.10	0.62	0.33
-2.24 ± 0.12		0.58 ± 0.14	-0.86 ± 0.16		5	1142.90	1.38	0.22
(b) Breeding (*N* = 344)								
-1.46 ± 0.12	-1.15 ± 0.18	0.12 ± 0.16	-0.66 ± 0.12	✓	7	1869.60	0.00	0.82
-1.6 ± 0.11	-0.85 ± 0.11	0.37 ± 0.11	-0.66 ± 0.12		6	1872.60	3.00	0.18

Table 2.3. Model selection table investigating the effects of having an IGI the day before, dominance status and sex on leadership in the (a) non-breeding and (b) breeding season. Models within six AICc of the top model are included (excluding nested models for (b); see **Table A1.1**). Values for predictor variables indicate coefficients (± standard errors) and ✓ indicates inclusion of an interaction in a model. D = dominant, S = subordinate, N = no, Y = yes, M = male and F = female. Delta value (Δ) indicates the difference in AICc between the given model and the top candidate model. Weight refers to the likelihood of the model being the best model, given the other models in the set. *N* values refer to the number of leadership events.

Intercept	Dominance (D > S)	IGI day before (N > Y)	Sex (M > F)	Group size	Dom: IGI: Sex	df	AICc	Δ	Weight
(a) Non-breeding (*N* = 172)									
0.10 ± 1.32	-1.95 ± 0.69	-0.63 ± 0.59	0.19 ± 0.77	-0.80 ± 0.18	✓	11.00	951.70	0.00	0.87
-2.01 ± 0.17	-0.29 ± 0.16		0.52 ± 0.16	-0.78 ± 0.18		6.00	957.20	5.46	0.06
(b) Breeding (*N* = 320)									
-1.65 ± 0.11	-0.79 ± 0.12		0.40 ± 0.11	-0.67 ± 0.13		6.00	1749.4	0.00	0.57

Table 2.4. Model selection table investigating the effects of dominance status, sex and territory area on leadership in the (a) non-breeding and (b) breeding season. Models within six AICc of the top model are included (excluding nested models for (a); see **Table A1.2**). Values for predictor variables indicate coefficients (± standard errors) and ✓ indicates inclusion of an interaction in a model. D = dominant, S = subordinate, M = male, F = female, O = overlapping territory, C = core territory. Delta value (Δ) indicates the difference in AICc between the given model and the top candidate model. Weight refers to the likelihood of the model being the best model, given the other models in the set. *N* values refer to the number of leadership events.

Intercept	Dominance (D > S)	Sex (M > F)	Territory (O > C)	Group size	Dom: Sex: Territory	df	AICc	Δ	Weight
(a) Non-breeding (*N* = 165)									
-2.26 ± 0.13		0.64 ± 0.16		-0.9 ± 0.18		5	905.70	0.00	0.44
(b) Breeding (*N* = 254)									
-2.95 ± 0.59	-0.56 ± 0.29	1.43 ± 0.42	0.32 ± 0.22	-0.68 ± 0.15	✓	11	1376.60	0.00	0.89
-1.55 ± 0.12	-0.87 ± 0.13	0.31 ± 0.13		-0.68 ± 0.15		6	1381.70	5.17	0.07

2.5 Discussion

We found evidence that both intrinsic and extrinsic factors affect leadership of dwarf mongoose group movements from a communal sleeping burrow in the morning. Overall, a given dominant individual was more likely to lead than a given subordinate, and males led group movements more than females. In general, dominant dwarf mongooses are older than subordinates and thus it is possible that they lead more due to greater experience of the territory and resources within it. The sex difference in leadership could be because males are more likely than females to disperse (Kern and Radford, 2017) or to seek extra-group mating opportunities; if leading the group allows an individual to influence travel direction, males could build their territory knowledge and gather information on rival groups. The extent of the dominance and sex differences in leadership was, however, dependent both on the season and on intergroup conflict.

Dominants were even more likely than subordinates to lead in the breeding season compared to the non-breeding season. If dominants, due to their age and experience, have better knowledge about safer areas of the territory or where to hide from predators (Rasa, 1987), they may increase their leading in the breeding season when vulnerable offspring are present (Rood, 1978). Similarly, if knowledgeable dominants can lead the group to better foraging patches than subordinates, this may be particularly important at times when there is increased energy expenditure associated with communal pup care (e.g., babysitting); energetic needs are likely greatest first thing in the morning too. Unlike some species where food resources can be monopolised by a dominant individual, which creates a conflict of interest (King et al., 2008; Papageorgiou and Farine, 2020), dwarf mongooses forage on loosely scattered, mostly invertebrate, prey (Rasa, 1987) and thus consensus costs may be low for followers (Conradt and Roper, 2005). Previous work on meerkats has found that it is dominant females in particular that are more likely to lead in the breeding season (Turbé, 2006), likely due to increased energetic needs (Conradt et al., 2009; Fischhoff et al., 2007; Rands et al., 2003). However, we found that both dominant females and males led more in the breeding season. This is perhaps because there is no obvious dominance hierarchy within the breeding pair (personal observation). Moreover, as subordinate males occasionally engage in sneaky matings with females in other groups, dominant males could face the greatest consequences from encountering rivals at this time of year, so may be attempting to avoid this threat by leading the group. Alternatively, because there are potential predation costs of leading at the front (Ioannou et al., 2019), pregnant dominant females might benefit from others taking leadership when they may be less able to avoid predation. A study of captive house sparrows (*Passer domesticus*) showed that individuals who normally led turned to following behaviour under the

simulated threat of a predator, indicating flexibility in response to extrinsic factors (Tuliozi et al., 2021). If both dominant male and female dwarf mongooses are similarly knowledgeable of good foraging locations, then the dominant female may not face a substantial cost by following her breeding partner even when her nutritional needs are greatest.

In both the non-breeding and breeding season, subordinate males were more likely than subordinate females to lead group movements away from the sleeping burrow. One possible explanation is that subordinate males are trying to influence travel direction, visiting certain locations or rival boundaries to build 'cognitive maps' of territories (Spencer, 2012) without sacrificing the benefits of group cohesion (Krause and Ruxton, 2002). In dwarf mongooses, subordinate males disperse more commonly than subordinate females (Kern and Radford, 2017), so such information may be particularly important to them. Whilst further work would be needed to confirm this, 'dispersal forays' have been observed both in other mammals (Debeffe et al., 2013; Mayer et al., 2017) and other taxa, including birds (Kesler and Haig, 2007). Subordinate Eurasian beavers (*Castor fiber*), for example, often enter rival group territories in the non-breeding season, and move faster and visit more rival-group territories than do dominants, suggesting they could be gathering information to assess the likelihood of dispersal success (Mayer et al., 2017). Whilst prospecting alone is possible, it likely carries high costs (Ridley et al., 2008; Young and Monfort, 2009), so the ideal for subordinates is to gather valuable information whilst still moving as a group.

With respect to intergroup conflict, we found some evidence that the occurrence of an IGI the day before can affect leadership in the non-breeding season, with dominant male leadership more likely the next day. This cannot be due to extra-pair mating attempts given the time of year. Instead, dominant males may have an increased motivation to lead for several other reasons. If they face higher costs from IGIs than other individuals (e.g., if they engage more than groupmates in physical fights with rivals and if male takeovers are more common than female equivalents), they may try and avoid further contests with rival groups by leading the group elsewhere. For example, in dyadic interactions between lizards establishing territories, physical contests often lead to future conflict avoidance (Stamps and Krishnan, 1998), and male Javan gibbons, who invest more in conflict than females, often rest further away from previous zones of conflict (Yi et al., 2020). Alternatively, given that serious injuries or fatalities are rare in dwarf mongoose IGIs (DMRP unpub. data), it could be that dominant males want to lead the group into zones of conflict to encourage territory defence. Dominant males are often the largest and oldest males too, so may have the greatest fighting ability. In support of this, previous work in dwarf mongooses found that, after presentations of rival-group faeces, groups moved less and stayed in the area longer, likely to watch out for rivals and thus defend the territory (Christensen et al., 2016). Similarly, green woodhoopoe groups return to the

site of morning IGIs later that evening, potentially to protect resources (Radford and Fawcett, 2014). We found no influence of IGIs on leadership in the breeding season, which could be because IGIs present different opportunities and costs at different times of the year. In cooperatively breeding pied babblers (*Turdoides bicolor*), lower food availability and energetic reserves in the non-breeding season likely explains lower investment in contests with rival groups (Golabek et al., 2012). If both dwarf mongoose dominants invested more into conflict during the breeding season, their similar motivations may have led to the similar frequencies of leading that we found. To understand fully how IGIs affect leadership, future work should investigate in detail the intensity of IGIs, who participates in contests and the direction and characteristics of movement patterns depending on who leads the group.

When considering the potential threat of intergroup conflict, we found that territory location influenced the dominance and sex patterns of leadership in the breeding but not the non-breeding season. In areas where territories of rival groups overlapped, both dominant and subordinate males increased their leadership slightly cf. core areas, whilst female dominants and subordinates decreased leadership slightly. Given this occurred in the breeding season, it lends support to the idea that male leadership is at least partially driven by the opportunities for extra-pair paternity. By contrast, in banded mongooses it is females that tend to lead more than males in peripheral territory areas, which may allow females to gain extra-pair matings (Johnstone et al., 2020; Preston, 2020). Male-biased dispersal in dwarf mongooses (Kern and Radford, 2017) cf. to no sex bias in dispersal of banded mongooses (Cant et al., 2013) could partly explain the difference, though more work would be needed to investigate extra-pair matings in dwarf mongooses to disentangle the interspecific differences.

We have focused on factors affecting which individuals lead group movements under different circumstances. Leadership may reflect individual decision-making or collation of information from others. For example, prior to group movements from a sleeping burrow, there is a gradual build-up of close calls, until the leader initiates collective action by rapidly moving in a given direction, usually whilst emitting 'movement calls' (Cobb et al., 2022). This build-up of close calls could be a form of 'voting' on the final outcome as seen in other taxa, such as sneezing in wild dogs (*Lycaon pictus*) and vocalisations in white-faced capuchins (*Cebus capucinus*), jackdaws (*Corvus monedula*) and meerkats (Boinski, 1993; Bousquet et al., 2011; Dibnah et al. 2022; Walker et al., 2017). It is also possible that group movements may be influenced by those in spatial positions other than the front (Pyritz et al., 2011). Future work is needed to investigate these aspects of leadership; for example, through the use of experimental playbacks to simulate 'voting' (as in Dibnah et al., 2022), combined with supplemental feeding of individuals to increase differences in motivation (as

in Arbon et al., 2020). What our current work has done is to expand our understanding of how extrinsic factors (season and intergroup conflict) can affect how intrinsic factors (dominance status and sex) drive leadership patterns in social species.

2.6 Appendix 1

2.6.1 Supplementary Tables

Table A1.1. Model selection table investigating the effects of having an IGI the day before, dominance status and sex on leadership in the breeding season; equivalent to main paper Results, **Table 2.3b**, but including nested models. Models within six AICc of the top model are presented. Values for predictor variables indicate coefficients (± standard errors) and ✓ indicates inclusion of a categorical fixed effect or interaction in a model. Delta value (Δ) indicates the difference in AICc between the given model and the top candidate model. Weight refers to the likelihood of the model being the best model, given the other models in the set. *N* = 320 leadership events.

Intercept	Dominance	IGI day before	Sex	Group size	Dom: IGI: Sex	df	AICc	Δ	Weight
-1.66	✓		✓	-0.67		6	1750.50	0.00	0.59
-1.66	✓	✓	✓	-0.67		7	1752.50	2.01	0.22
-1.62	✓	✓	✓	-0.67	✓	11	1752.70	2.25	0.19

Table A1.2. Model selection table investigating the effects of dominance status, sex and territory area on leadership in the non-breeding season; equivalent to main paper Results, **Table 2.4a**, but including nested models. Models within six AICc of the top model are presented. Values for predictor variables indicate coefficients (± standard errors) and ✓ indicates inclusion of a categorical fixed effect or interaction in a model. Delta value (Δ) indicates the difference in AICc between the given model and the top candidate model. Weight refers to the likelihood of the model being the best model, given the other models in the set. *N* = 165 leadership events.

Intercept	Dominance	Sex	Territory	Group size	Dom: Sex: Territory	df	AICc	Δ	Weight
-2.26		✓		-0.90		5	905.70	0.00	0.44
-2.13	✓	✓		-0.86		6	906.60	0.85	0.29
-2.27		✓	✓	-0.90		6	907.70	2.00	0.16
-2.14	✓	✓	✓	-0.86		7	908.60	2.85	0.11

2.6.2 Supplementary Figures

Results generated from permutation tests described in the main paper.

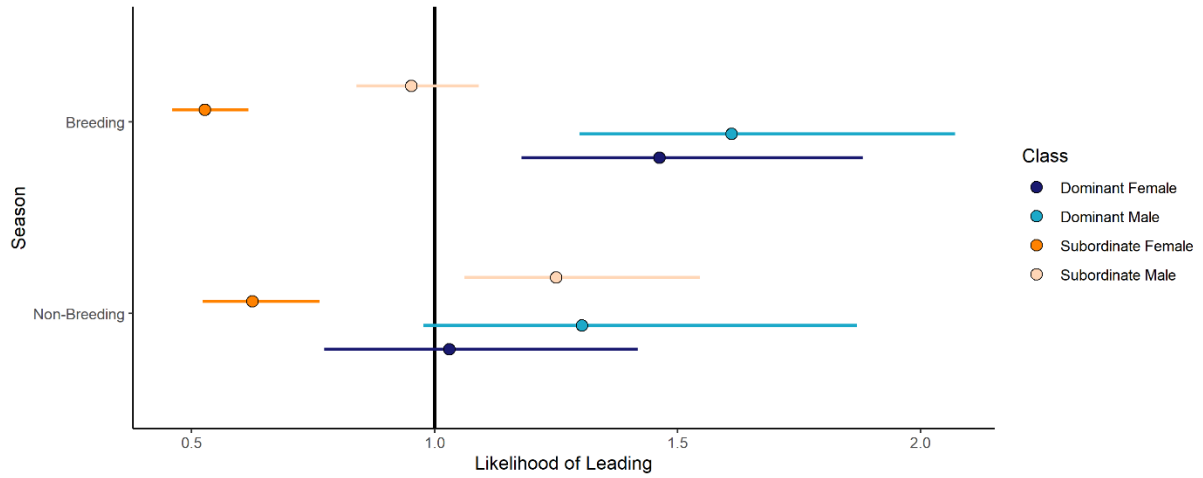

Figure A1.1. Estimated likelihood of leading an event across seasons for different classes of individual (see also main text **Figure 2.1**). Overall, dominants were more likely to lead than subordinates and males were more likely to lead than females; dominants of both sexes led more than expected in the breeding season, whilst subordinate males were the only class to lead more than expected in the non-breeding season. Subordinate females led less than expected under random leadership, and less than all other classes in both seasons. Point estimates denote observed/median permuted value, 95% CIs median/0.025 and 0.975 permuted quantiles.

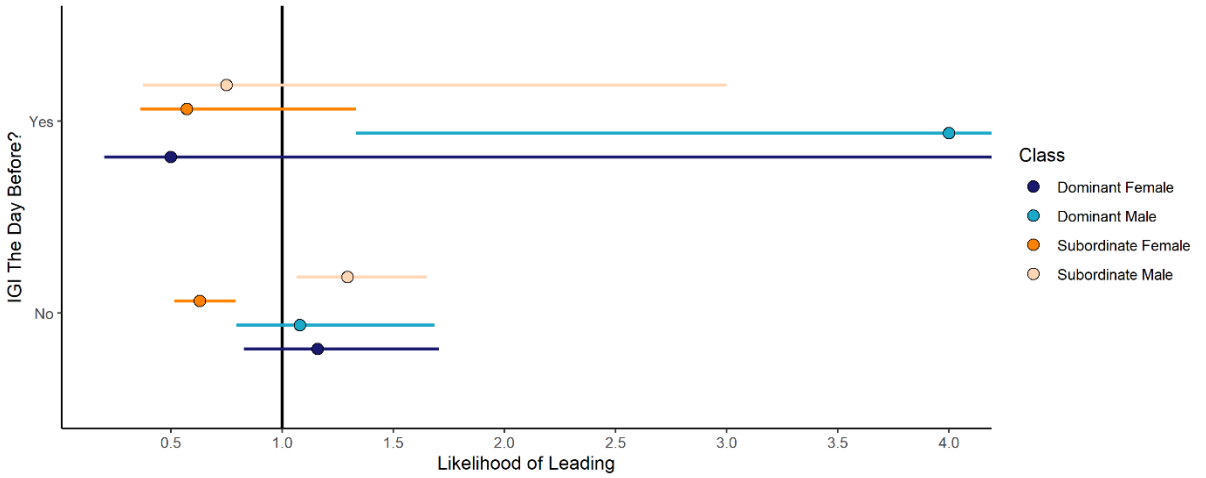

Figure A1.2. Estimated likelihood of leading an event relative to the occurrence of an intergroup interaction (IGI) the day before, for different classes of individual in the non-breeding season (see also main text **Figure 2.2**). Dominant males were more likely to lead than expected following an IGI relative to all other classes. Point estimates denote observed /median permuted value, 95% CIs median/0.025 and 0.975 permuted quantiles.

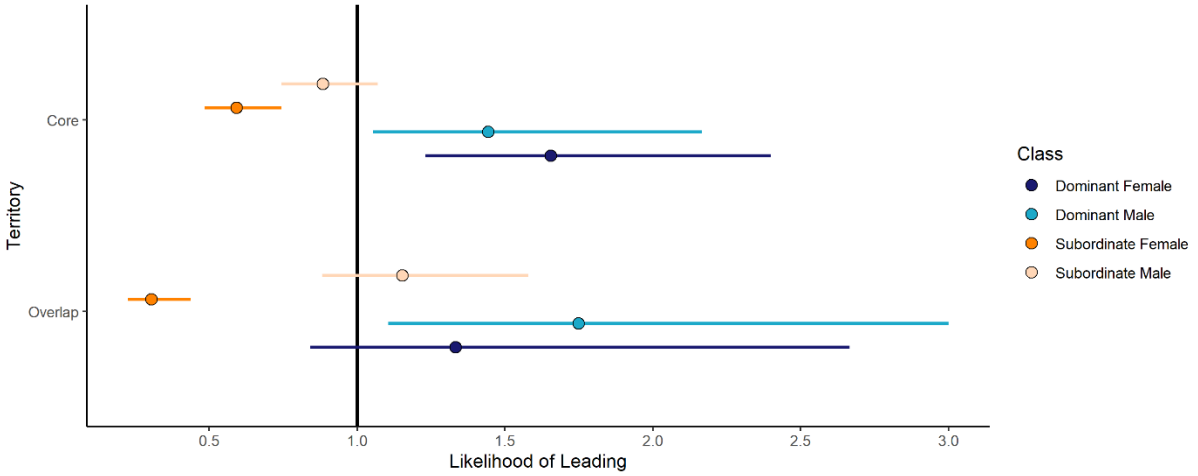

Figure A1.3. Estimated likelihood of leading an event depending on territory area, for different classes of individual in the breeding season (see also main **Figure 2.3**). Dominant individuals were more likely to lead in general, but dominant males in particular were most likely to lead in overlap areas. In both areas, subordinate females were the least likely to lead of all classes, leading less than expected by chance. Point estimates denote observed/median permuted value, 95% CIs median/0.025 and 0.975 permuted quantiles.

Chapter 3

Factors Affecting Follower Responses to Movement Calls in Cooperatively Breeding Dwarf Mongooses

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Chapter 3 has been published in Animal Behaviour.

BC co-designed the experiments, carried out experimental work, analysed the data, interpreted the results and wrote the original draft. AMD co-designed the experiments, provided supervision and comments on the manuscript, and managed the project. PK advised on analysis and provided comments on the manuscript. ML helped carry out experimental work, managed the project, and provided comments on the manuscript. JMK provided comments on the manuscript and originally setup the project. ANR co-designed the experiments, advised on analysis, provided substantial comments on the manuscript and provided supervision.

3.1 Abstract

In social species, individuals maximise the benefits of group living by remaining cohesive and coordinating their actions. Communication is key to collective action, including ensuring that group members move together; individuals often produce signals when attempting to lead a group to a new area. However, the function of these signals, and how responses to them are affected by intrinsic characteristics of the caller and extrinsic factors, has rarely been experimentally tested. We conducted a series of field-based playback experiments with habituated wild dwarf mongooses (*Helogale parvula*), a cooperatively breeding and territorial species, to investigate follower responses to movement calls. In our first experiment, we found that focal individuals were more likely to respond to playback of 'movement calls' than control 'close calls', indicating movement calls function as recruitment signals. In a second experiment, we found that focal individuals responded similarly to the movement calls of dominant and subordinate groupmates, suggesting that dominance status (an intrinsic factor) does not influence receiver responses. In a final experiment, we found that individuals responded to the simulated presence of a rival group, but that this outgroup conflict (an extrinsic factor) did not affect responses to movement calls compared to a control situation. This may be because attention is instead focused on the potential presence of an imminent threat. By using playbacks to isolate the acoustic signal from physical movement cues, our results provide experimental evidence of how movement calls help leaders to attract followers and thus adds to our understanding of recruitment signals more generally.

3.2 Introduction

To maximise the benefits of group living (e.g., resource defence and reduced predation risk), group members need to act collectively; they must remain cohesive and coordinate with one another (Conradt and Roper, 2005; Ioannou et al., 2019; Krause and Ruxton, 2002). Since groups are composed of a heterogenous mix of individuals whose interests do not perfectly align (Conradt and Roper, 2005), communication is often crucial to ensure collective action (Bradbury and Vehrencamp, 2011). Signals relating to collective movement can be produced at two stages of the process, which are not necessarily mutually exclusive. Individuals may produce a signal to indicate their readiness to move and/or when they attempt to initiate group movement, either following earlier signals of readiness or independently (Bousquet et al., 2011; Sperber et al., 2017; Turbé, 2006). For instance, in wild dogs (*Lycaon pictus*), observational work indicates that a threshold of 'sneezing' individuals is needed to initiate group movements from a resting period (Walker et al., 2017), while 'moving calls' from several individuals are similarly required in meerkats (*Suricata suricatta)* for the group to change from one foraging patch to another (Bousquet et al., 2011). In some species, or certain contexts, a single individual may attempt to move elsewhere; attracting followers will avoid them becoming isolated and thus putative leaders may use movement signals to enhance the likelihood that they are joined. For example, meerkats also produce a distinct 'lead call', which is used when a potential leader attempts to initiate movement from a sleeping burrow to start foraging (Turbé, 2006). In white-faced capuchins (*Cebus capucinus*), backward glances seem to be important in recruiting others when shifting from resting to foraging, as the number of followers increases after a glance from a moving individual (Meunier et al., 2008). The faster 'grunt' rates of leaders compared to followers in redfronted lemurs (*Eulemur rufifrons*) when moving throughout the day suggests that this call may function as a movement signal (Sperber et al., 2017), and vocalising when leaving the group increases the chances of an individual green woodhoopoe (*Phoeniculus purpureus*) being followed by its groupmates when changing foraging patches (Radford, 2004). While movement signals appear to be important in coordinating the actions of group members, there has been little experimental testing of the proposed function to recruit followers (for an exception, see Teixidor and Byrne, 1999), or of how follower responses differ depending on intrinsic characteristics of the signaller (e.g., their identity; but see Preston, 2020) and on extrinsic factors (e.g., the level of outgroup threat).

On hearing a movement signal, individuals might use information about the dominance status of the leader when deciding whether to follow. In principle, dominant individuals could be more likely to be followed if subordinates gain some benefit from doing so; for instance, if following

increases future social tolerance or social-bonding opportunities (King et al., 2008; Smith et al., 2015). Dominant individuals could also be considered more reliable sources of information. For example, if they have greater knowledge of the environment, they may be more likely to lead individuals to better foraging patches (Brent et al., 2015; McComb et al., 2001). Alternatively, if group decisions are more evenly distributed across group members (Leca et al., 2003), then both dominants and subordinates could elicit similar responses from followers (Jacobs et al., 2011; Leca et al., 2003; Wang et al., 2016). Most work to date has investigated how dominance status affects the likelihood of leading. For example, in chacma baboons (*Papio ursinus*), the dominant individual tends to arrive at experimental food patches first, with subordinates following behind (King et al., 2008), while observations of Tibetan macaques (*Macaca thibetana*) suggest that dominance rank does not affect who leads the group away from depleted foraging patches (Wang et al., 2016). Far less work has examined how individuals respond to movement signals depending on the rank of the caller. One exception is an observational study of meerkats showing that dominant females producing a 'lead call' were more likely to be followed by group members than dominant males or subordinates producing the same call (Turbé, 2006), but experimental tests are needed.

Extrinsic factors can also affect follower decisions – for instance, simulated predator attacks on captive house sparrows (*Passer domesticus)* have been shown to reverse leader–follower positions relative to an exploratory context (Tuliozi et al., 2021) – but the influence of outgroup conflict in this regard has been little considered. Members of social species often interact with outside groups or individuals, which can pose a threat. For example, rival groups may be attempting to steal territory or resources (Dyble et al., 2019; Kelly, 2005), while individual outsiders may be seeking mating opportunities or a breeding position (Braga Goncalves and Radford, 2019; Mares et al., 2012). Contests with outsiders can have immediate consequences, such as physical injury or death (Dyble et al., 2019; Morris-Drake et al., 2022), while the threat of outgroup conflict can cause significant changes to within-group behaviour, including elevated levels of grooming, contact or aggression (Arseneau-Robar et al., 2018; Birch et al., 2019; Radford, 2008a). Subsequent movement patterns and collective decision making have also been shown to be influenced by outgroup conflict (Christensen et al., 2016; Dyble et al., 2019; Morris-Drake et al., 2021a; Radford and Fawcett, 2014). Deciding to follow another individual under conflict scenarios could have significant fitness implications; for instance, banded mongoose (*Mungos mungo*) males that follow a dominant female into violent contests suffer an increased mortality cost (Johnstone et al., 2020). When there is the prospect of an imminent outgroup contest, group members may want to stay more cohesive due to heightened anxiety or to prime for battle (Birch et al., 2019; Morris-Drake et al., 2019), and thus could be more receptive to movement signals from leaders.

Dwarf mongooses (*Helogale parvula*) are an ideal species in which to investigate experimentally the responses of group members to movement calls. They live in cooperatively breeding groups that each defend a year-round territory (Rasa, 1987), with group members spending most of the day foraging together throughout their territory before returning to a communal burrow to sleep (Rasa, 1987). Dwarf mongooses are highly vocal, maintaining contact during foraging by producing sporadic 'close' calls (Rasa, 1987). When departing or returning to a sleeping burrow, and when moving from one foraging patch to another, individuals move cohesively at a heightened pace, usually following a leader that has initiated the movement while producing a 'movement call' – a fast burst of multiple close calls. Prior to movement from a resting position (e.g., from a sleeping burrow) there is also a gradual increase in the frequency of close calls, which may indicate an increasing willingness to move (Sperber et al., 2017). By contrast, when dwarf mongoose groups move from one foraging patch to another, there is no obvious predeparture behaviour; instead, an individual attempts to initiate group movement by moving at pace while producing a movement call. We focus on the latter behaviour in this paper.

Dwarf mongoose groups comprise a dominant breeding pair and subordinate helpers (all other adults); group members can obtain information about dominance status and individual identity from various calls (Kern et al., 2016; Morris-Drake et al., 2021b; Sharpe et al., 2013). Previous work reported that dwarf mongoose movement decisions are despotic in nature, with the dominant female always leading the group (Rasa, 1987), but recent observations show that over half of group movements are led by subordinates (Cobb et al., 2022). Groups come into conflict with conspecific rivals, both neighbours and those from further afield (Christensen et al., 2016; Rasa, 1987), on average once every 2 weeks in the study population (DMRP unpub. data); groups encounter faecal deposits of rival groups much more regularly (Christensen et al., 2016). Intergroup interactions (IGIs) involve a combination of group members looking at each other, vocalising and, on some occasions, escalation to physical fights (Rasa, 1987). Individuals forage closer to their nearest neighbour after the simulated threat of a rival group (Morris-Drake et al., 2019), which could proximately be a response to heightened anxiety about imminent conflict (Radford et al., 2016), and ultimately represent priming behaviour to ensure the most collective response to outsiders (Birch et al., 2019; Radford, 2011).

We investigated subordinate group member responses to dwarf mongoose movement calls in three related field experiments. First, we tested whether the call functions to attract followers. We predicted that, compared to control close calls, movement calls would elicit a 'follow' response, with the focal individual becoming more vigilant, vocalising and moving towards the loudspeaker. Second, we tested whether individuals respond differently to movement calls from dominant and

subordinate group members, predicting either a stronger response to movement calls from dominant individuals, or for there to be no clear difference in response to movement calls from dominant versus subordinate individuals. Third, we tested how the threat of a nearby rival group affects the response to movement calls. We predicted that, compared to a control stimulus, the simulation of an intergroup threat would result in heightened responses to movement calls, such that the group would remain cohesive in case a contest occurred imminently.

3.3 Methods

3.3.1 Study site and population

We carried out the research at the Dwarf Mongoose Research Project (DMRP) in Limpopo Province, South Africa (24°11'S, 30°46'E); see Kern and Radford (2013) for more details. Eight wild but habituated groups, each comprising 4–12 adults (individuals >1 year old), were used in experiments during the study period (April–August 2020). Groups are habituated to close human presence (<5 m) and individuals are uniquely dye-marked (Kern and Radford, 2013). The dominance status (dominant or subordinate; identifiable from the outcome of aggressive interactions such as foraging displacements) and sex (identifiable from anogenital grooming bouts) of all individuals is known from the long-term observations (Kern and Radford, 2013, 2016). We considered only adults for playback experiments because individuals less than 1 year old rarely lead the group (DMRP unpub. data).

3.3.2 Experimental overview

We conducted three playback experiments to investigate the responses of focal subordinate individuals to the movement call of another group member. In experiment 1 (10 April – 8 June 2020), we determined the baseline responses to the movement call of a dominant individual by comparing them to the responses elicited by close calls (given while foraging) of the same dominant group member. In experiment 2 (27 April – 25 June 2020), we tested whether responses differed depending on the dominance status of the caller, comparing those elicited by movement calls of dominant and subordinate group members of the same sex (the focal individual was not necessarily sex-matched to the signallers). In experiment 3 (10 July – 16 August 2020), we tested how the simulated presence of a rival group affected responses to movement calls. Experiment 3 involved two parts: an initial playback of close calls and 'lost' calls (high-pitched vocalisations usually produced while foraging, particularly when an individual becomes isolated) from a non-neighbouring

rival group or control herbivore sounds, and then playback of the same movement call of a dominant group member. All three experiments had matched-pairs designs, with each focal subordinate in an experiment receiving two treatments in a counterbalanced order (*N* = 18 individuals from six groups for experiment 1 and 2; *N* = 16 individuals from eight groups for experiment 3).

3.3.3 Recordings and playback tracks

We recorded calls ad libitum within 3 m of an individual in calm conditions, using a Marantz PMD661MKII solid-state recorder (Marantz, Kanagawa, Japan) and a Sennheiser MKE600 shotgun microphone (Sennheiser, Wedemark, Germany) coupled with a Rycote Softie windshield (Rycote Microphone Windshields, Stroud, Gloucestershire, U.K.). As all groups are well habituated to close human presence, the behaviour and vocalisations of individuals were not impacted during recordings. We recorded individual close and lost calls while groups were foraging throughout the day, and we recorded individual movement calls when a group moved collectively (sometimes excluding individuals such as babysitters; personal observation) from a sleeping burrow to a foraging site, from one foraging patch to another, or to a sleeping burrow before. Collective group movements are initiated by one individual moving quickly away from the group while producing a movement call; those following often produce movement calls too.

To construct playback tracks, we used Audacity 2.3.3. For all tracks, we superimposed good-quality recordings of calls (e.g., no overlapping sounds such as conspecific calls) onto recordings of ambient sound recorded in calm conditions in the centre of a group's territory when no dwarf mongooses were present. We used a HandyMAN TEK 1345 sound meter (Metrel UK Ltd; Epsom, Surrey, U.K.) to standardise playback volume of calls to match natural vocalisations, as well as amplifying calls in Audacity where needed. We applied a high-pass filter (filtering out frequencies below 300 Hz) in all tracks to improve signal-to-noise ratio and to standardise background sound. The same ambient-sound recording was used for both playbacks within a pair (i.e., the two treatments to a focal individual in a given experiment). Movement calls, which are composed of fast-repeating close call elements, are often preceded by infrequent close calls (Maier et al., 1983). To replicate this combination and to standardise track length, movement call tracks for all three experiments consisted of 25 s of ambient sound, with two close calls (one at 2 s and one at 8 s after the start of the track) followed by a movement call commencing 14 s from the start of the track (**Figure 3.1**, bottom). We standardised movement calls to be 10 close call elements within 6–7 s based on early analysis of a subset of recordings during the field season (mean ± SE call rate = 1.5 ± 0.1 close call elements/s, range 0.4–3.6); thus, the movement call playback rate ranged from 1.4 to

1.6 close call elements/s. For all experiments, both female and male vocalisations were used for playbacks. The same calls were sometimes used across experiments.

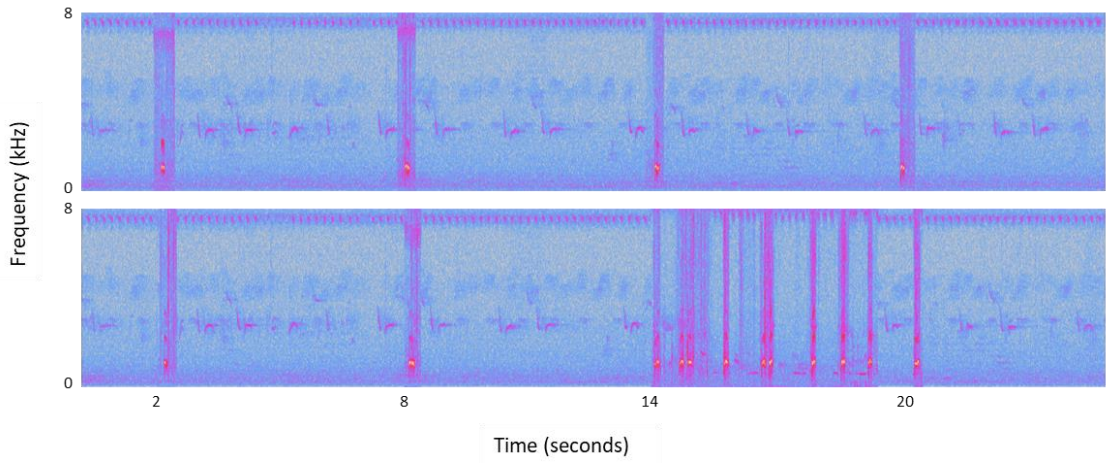

Figure 3.1. Spectrogram of close call control track (top) and movement call track (bottom). Blue indicates low-amplitude noise; red indicates higher-amplitude noise. Taken and adapted from Audacity 2.3.3.

In experiment 1, we compared responses to movement call and control tracks from the same dominant individual. Control tracks comprised 25 s of ambient sound with four close calls at 2, 8, 14 and 20 s from the start of the track (**Figure 3.1**, top). We standardised both close calls and movements calls to 50–55 dB from 1 m. Within the experiment, a given individual was used as a source of calls no more than three times (mean = 1.8), and a given call was only used once in playback tracks.

In experiment 2, we compared responses to movement call tracks from a dominant and subordinate individual. A given individual was used as a source of calls no more than three times (mean = 1.4). We standardised calls to 50–55 dB from 1 m, and used a given call once within the experiment. The two playbacks to a focal individual were of calls from individuals of the same sex as each other (e.g., a dominant male and a subordinate male) to ensure the sex of the caller had no effect on responses.

Experiment 3 involved two parts. For part 1 (the rival group or herbivore control playback), we created tracks using similar methodology to Morris-Drake et al. (2019); call rates within tracks matched those heard naturally. Herbivore tracks were made up of herbivore feeding sounds available from previous work, and included plains zebras (*Equus quagga*), blue wildebeest (*Connochaetes taurinus*), giraffe (*Giraffa camelopardalis giraffe*) and waterbuck (*Kobus*

ellipsiprymnus). We pasted four herbivore sounds onto 12 s of ambient sound, to create four different sequences. We then pasted these sequences into a 1 min track (one sequence being used twice) in a random order, which we duplicated to make a 2 min herbivore track. Rival group tracks each contained calls from a single other group: close calls from four individuals, including at least one dominant, and lost calls from two individuals. We inserted four close calls (one from each individual) into a 3 s sequence. Four sequences were constructed, each with a randomised order of caller. We then inserted these four sequences into 12 s blocks of ambient sound, to make five 12 s blocks, with each block having a randomised sequence order. These blocks were then combined to make a 1 min track, and five calls were removed at random to create a call rate of 75/min, as per the natural call rate of a foraging group and in line with previous experimental work (Morris-Drake et al., 2019; Sharpe et al., 2013). In this 1 min segment, four lost calls from two individuals (two each) were then inserted into the track at random time stamps within the first 30 s, alternating between individuals. As lost calls are difficult to predict and record, some recordings from previous field seasons from individuals no longer in the group were used. As we were playing back calls from non-neighbouring groups, we did not expect this to affect responses of the focal group. We then duplicated each 1 min track to make 2 min tracks. We faded rival group tracks so that the maximum amplitude (50–55 dB at 1 m for close calls and 60–65 dB at 1 m for lost calls) was reached at 1 min, to simulate a rival group approach. Previous work has shown that individuals are able to distinguish between calls of their own group and those of a rival group (Morris-Drake et al., 2019).

Some close calls and herbivore sounds were used more than once within part 1 of the experiment, but the component parts of each track were arranged randomly in a different order to generate unique tracks. We used the same group for playback construction no more than four times (mean = 2.3), with a maximum of three focal individuals per group receiving playbacks (mean = 2). The same rival group was used for playback on a maximum of two focal individuals from the same group. As rival tracks were from non-neighbouring groups (and thus all rivals were unknown outsiders from the perspective of a focal group), it is unlikely that group identity affected focal responses, and a 2-week gap was left between trials on different individuals within the same group to avoid habituation to the calls (see 'Experimental protocol' below for further details).

For part 2 (the movement call playback), a given individual was used as a source of call no more than twice (mean = 1.2), with different calls used for different focal individuals. Calls were standardised to 50–55 dB from 1 m. After receiving the playback track in part 1, a focal individual received a movement call track from a given dominant individual within its group. The same movement call track was used following a herbivore or rival group track to ensure differences in movement calls had no effect on responses.

3.3.4 Experimental protocol

For all three experiments, we conducted trials during the day when the group was foraging, in calm weather conditions and at least 10 min after a group movement, latrine behaviour, snake mob or other disturbance. If an IGI occurred, at least 30 min was left before running a trial in experiments 1 and 2; for experiment 3, trials were carried out on a different day to IGIs. We started trials when the focal individual was foraging at least 2 m from other individuals.

We carried out experiments 1 and 2 using a similar experimental protocol. We placed a loudspeaker (Rokono B10 or Rokono BASS+ Mini, Boundless Technology Limited, Devon, U.K.) connected to an MP3 device (either a Moto G 5 phone; Motorolo Inc, Chicago, IL, U.S.A., or a Kubik Evo; Kubik Digital Electronics) 3 m perpendicular from the focal individual (chosen randomly before visiting the group), hidden in vegetation. Trials to the same individual were separated by at least 1 day and performed at a similar time of day. Within a group, at least 30 min was left between trials on different individuals. If a trial was disturbed (e.g., due to conspecific alarm calls or the focal individual moving into vegetation and out of view), it was abandoned (experiment 1: $N = 4$; experiment 2: $N = 7$) and repeated that day or at a later date, but with the order of the treatments reversed. The playback track in the abandoned trial was therefore not used more than once on the same day, to avoid habituation.

For experiment 3, we used two loudspeakers, one for each part. To avoid disturbing the focal individual during loudspeaker set-up, a small amount of egg was used to attract it to an area where the two loudspeakers were already positioned. When playback started, the focal individual was thus 5 m from the first loudspeaker (used to broadcast either the rival group or herbivore track) (Morris-Drake et al., 2019). The second loudspeaker (used for the movement call playback) was placed diagonally ca. 3 m from the first loudspeaker so that, if the focal individual approached the first loudspeaker, the second loudspeaker would be positioned to one side of the individual. Following initial playback of a rival group or herbivore track, the movement call track was started at least 30 s, and no more than 5 min, later. Variation in time between playbacks was due to individuals moving out of view, for example into dense vegetation, before the movement call track could be started, but there was no difference between treatments (mean ± SE time after a rival group track = 110 ± 22 s, herbivore track = 112 ± 21 s). Trials to the same focal individual were separated by at least 1 day, and at least 2 weeks were left before conducting trials on another individual in the same group, to avoid habituation. Trials abandoned due to disturbances (e.g., alarm calls or the focal individual going out of view) were repeated with different rival group or herbivore tracks at least 2 days later ($N = 7$).

For all experiments, we recorded the following responses to movement calls (and close calls in experiment 1): (1) whether the focal individual looked (head raised and directed towards the loudspeaker), orientated (whole body turned to face the loudspeaker) and/or approached (after orientating, moved at least 50 cm towards the loudspeaker); (2) whether they vocalised (gave either close calls and/or movement calls); (3) the rate and proportion of time spent vigilant (head raised). These responses were collected from 14 s after the start of the playback (i.e., once the movement call period had commenced; see **3.3.3 Recordings and playback tracks**), and focal individuals were observed for a minimum of 25 s after the playback finished. We analysed data for a maximum of 60 s response time, as we assumed that individuals would not be responding to movement calls after this point. Chi-square tests were performed to show that there were no differences between treatments in the response time analysed: experiment 1 ($\chi^2_1 = 0$, $P = 1$), experiment 2 ($\chi^2_1 = 1.45$, $P = 0.229$) and experiment 3 ($\chi^2_1 = 0$, $P = 1$). For part 1 of experiment 3 (the rival group or herbivore playback), we recorded whether the individual looked, orientated and approached the loudspeaker during the 2 min playback period, to ensure individuals were responding to rival group calls as expected from Morris-Drake et al. (2019). All trials were filmed using a GoPro Hero 7 strapped to the head of the observer, who also narrated responses into a Dictaphone (Sony ICD-PX370) while standing ~3 m away from the focal individual and loudspeaker to avoid disturbances.

3.3.5 Ethical note

All work was conducted with permission from the Limpopo Department of Economic Development, Environment and Tourism (permit number: 001-CPM403-00013), the Ethical Committee of the University of Pretoria, South Africa and the Ethical Review Group of the University of Bristol, U.K. (University Investigator Number: UIN/17/074). Only those individuals comfortable with close presence of experimenters were included in the study. To minimise anxiety, rival group playbacks were limited to a maximum of three focal individuals per group.

3.3.6 Statistical analysis

We extracted data using Boris 7.9.19 (Friard and Gamba, 2016). Video footage from GoPro recordings was used where quality was sufficient, but where recordings failed, or quality was poor (e.g., due to dense vegetation), only Dictaphone audio was used for both treatments in a pair. We used R v.4.0.3 for statistical analyses (R Core Team, 2020) and *ggplot2* to construct figures (Wickham, 2016). McNemar tests (with continuity corrections) were used for paired responses with a binary outcome. Paired *t* tests were used for continuous response variables where assumptions

were met (paired differences and residuals being normally distributed, checked visually with histograms and *Q*–*Q* plots). Where assumptions were violated, Wilcoxon signed-rank exact tests were performed. To compensate for an increased likelihood of Type I error due to multiple testing, we used sequential Bonferroni corrections (Rice, 1989) for tests within three grouped response variables for each experiment: (1) physical response (look, orientate, approach); (2) vocal response (close call, movement call) and (3) vigilance response (proportion of time vigilant, vigilance rate). Adjusted α levels are given within each grouping where at least one significant result is reported.

3.4 Results

3.4.1 Experiment 1

In response to movement call playback, focal individuals were significantly more likely to look (McNemar's test: $\chi^2_1 = 12.07$, $P < 0.001$, adjusted $\alpha = 0.017$; **Figure 3.2a**) and approach ($\chi^2_1 = 6.13$, $P = 0.013$, $\alpha = 0.025$; **Figure 3.2b**), but not orientate ($\chi^2_1 = 2.29$, $P = 0.131$; **Figure 3.2c**), towards the loudspeaker than in close call (control) trials. There was no significant difference between treatments in the number of individuals that gave movement calls ($\chi^2_1 = 2.25$, $P = 0.137$; **Figure 3.2d**). Individuals were more likely to give close calls in response to movement call playbacks than in response to close call playbacks, but this was not significant after Bonferroni correction ($\chi^2_1 = 4.92$, $P = 0.027$, $\alpha = 0.025$; **Figure 3.2e**). Movement call playback resulted in significantly greater vigilance than in control trials (paired *t* test, proportion of time spent vigilant: $t_{17} = 3.39$, $P = 0.004$, $\alpha = 0.025$, mean difference = 0.14, 95% CIs = 0.05–0.22; **Figure 3.2f**; vigilance rate: $t_{17} = 2.24$, $P = 0.039$, $\alpha = 0.05$, mean difference = 2.18 look-ups per min, 95% CIs: 0.13–4.24; **Figure 3.2g**).

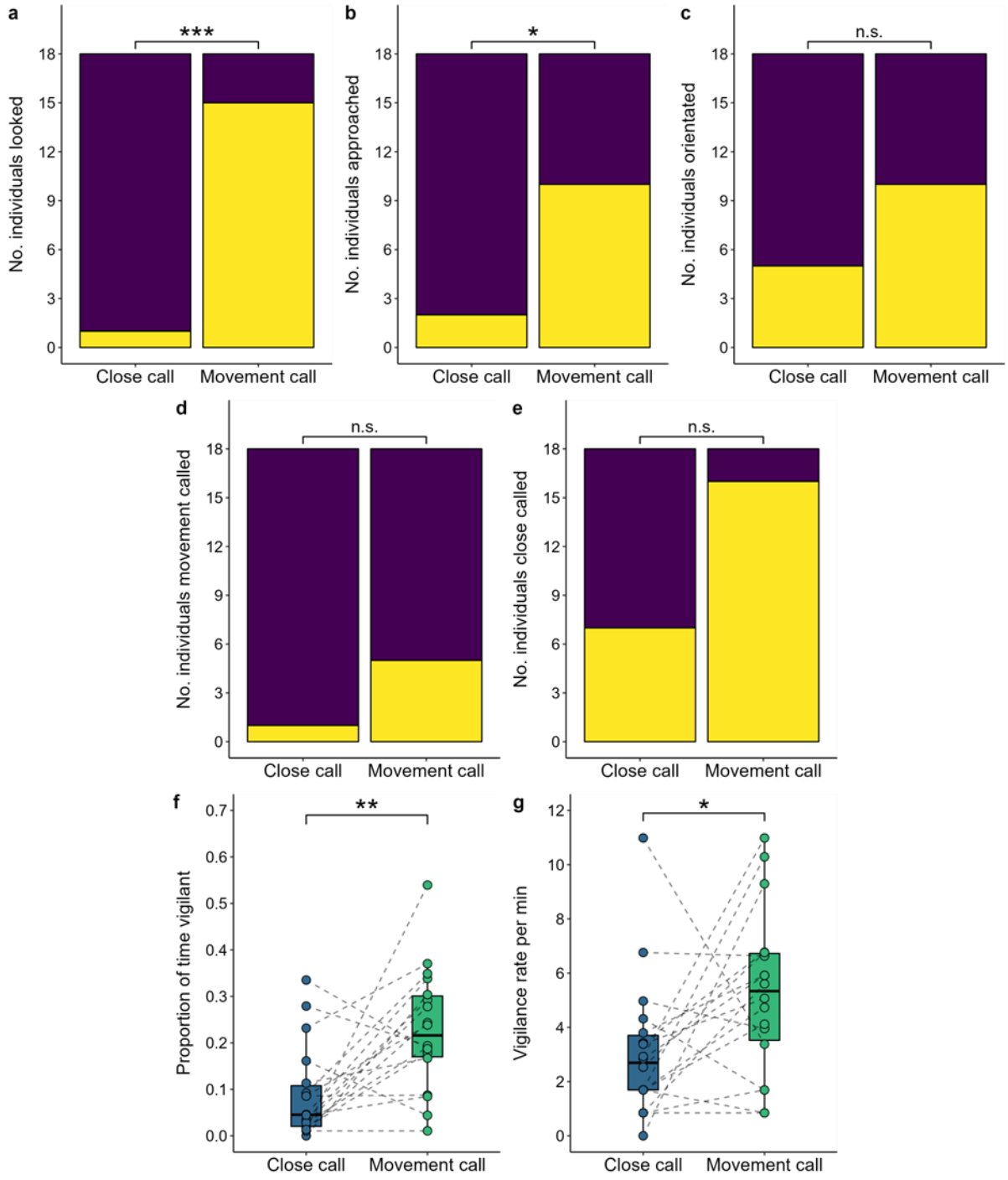

Figure 3.2. Number of individuals that (a) looked, (b) approached and (c) orientated towards the loudspeaker, and that gave (d) movement calls and (e) close calls in response to playback of close calls and movement calls. Purple bars indicate no response, yellow bars show a positive response. (f) Proportion of time spent vigilant and (g) vigilance rate in response to playback of close calls and movement calls. Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range). Dotted lines link data points from the same individuals in the two treatments (circles). *$P < 0.05$; ***$P < 0.001$. $N = 18$ individuals receiving paired trials.

3.4.2 Experiment 2

There was no significant difference in the number of individuals that looked (McNemar's test: χ^2_1 = 0.13, *P* = 0.724; **3.6 Appendix 2**, **Figure A2.1a**), orientated (χ^2_1 = 1.5, *P* = 0.221; **3.6 Appendix 2**, **Figure A2.1b**) or approached (χ^2_1 = 0, *P* = 1; **Figure 3.3a**) towards the loudspeaker in response to playback of movement calls from dominant versus subordinate group members. There was also no significant treatment difference in the number of individuals that gave movement calls (χ^2_1 = 0.8, *P* = 0.371; **3.6 Appendix 2**, **Figure A2.1c**) or close calls (χ^2_1 = 0.17, *P* = 0.683; **Figure 3.3b**). Finally, neither the proportion of time spent vigilant (paired *t* test: t_{17} = 0.22, *P* = 0.827, mean difference = 0.02, 95% CIs: -0.14–0.17; **3.6 Appendix 2**, **Figure A2.1d**) nor the vigilance rate (t_{17} = 0.12, *P* = 0.903, mean difference = 0.14 look-ups per min, 95% CIs: -2.23–2.51; **Figure 3.3c**) differed significantly between treatments.

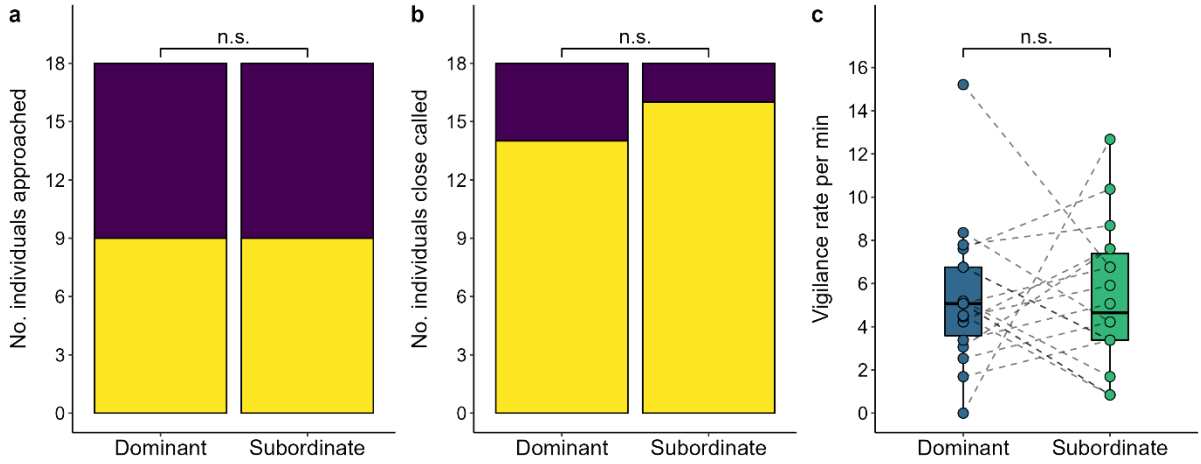

Figure 3.3. Number of individuals that (a) approached and (b) gave close calls, and (c) the vigilance rate of individuals in response to playback of dominant and subordinate movement calls. For (a) and (b), purple bars indicate no response, yellow bars show a positive response. For (c), box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range) and dotted lines link data points from the same individuals in the two treatments (circles). *N* = 18 individuals receiving paired trials.

3.4.3 Experiment 3

In part 1 of experiment 3, individuals were significantly more likely to look (McNemar's test: χ^2_1 = 6.13, *P* = 0.013, α = 0.017; **Figure 3.4a**), orientate (χ^2_1 = 4.9, *P* = 0.027, α = 0.05; **Figure 3.4b**) and approach (χ^2_1 = 5.82, *P* = 0.016, α = 0.025; **Figure 3.4c**) towards the loudspeaker in response to rival group playback than in response to playback of herbivore control sounds.

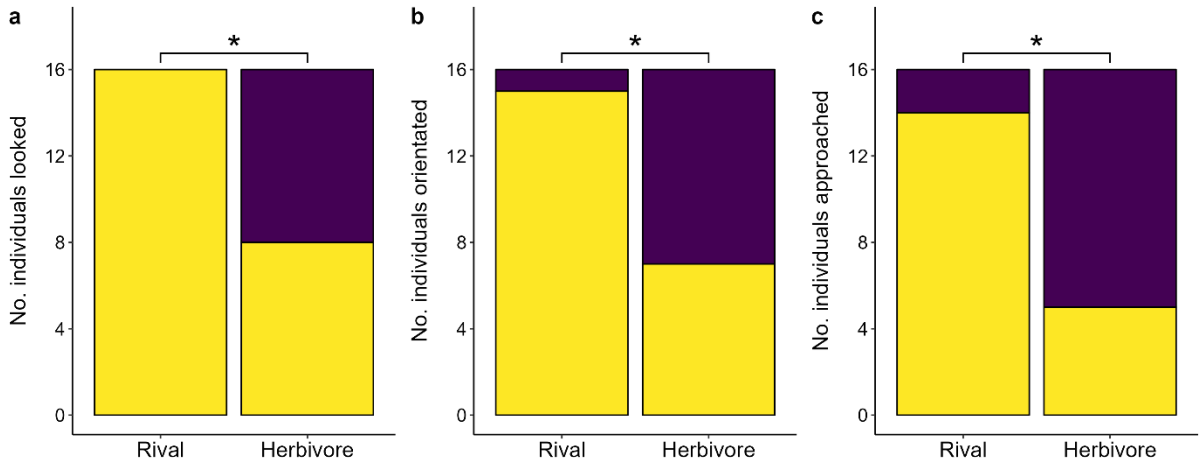

Figure 3.4. Number of individuals that (a) looked, (b) orientated and (c) approached towards the loudspeaker in response to playback of rival group or herbivore sounds. Purple bars indicate no response, yellow bars show a positive response. *$P < 0.05$. $N = 16$ individuals receiving paired trials.

During part 2 (playback of a movement call), there was no significant difference between treatments (following either rival group or herbivore playback) in the number of focal individuals that looked (McNemar's test: $\chi^2_1 = 0$, $P = 1$; **3.6 Appendix 2**, **Figure A2.2a**), orientated ($\chi^2_1 = 1.5$, $P = 0.221$; **3.6 Appendix 2**, **Figure A2.2b**) or approached ($\chi^2_1 = 0.13$, $P = 0.724$; **Figure 3.5a**) towards the loudspeaker. Similarly, there was no significant difference between treatments in the number of individuals that gave movement calls ($\chi^2_1 = 0$, $P = 1$; **3.6 Appendix 2**, **Figure A2.2c**) or close calls ($\chi^2_1 = 1.5$, $P = 0.221$; **Figure 3.5b**). There was also no significant treatment difference in the proportion of time spent vigilant (Wilcoxon signed-rank exact test with continuity correction: $V = 93$, $N = 16$, $P = 0.211$; **3.6 Appendix 2**, **Figure A2.2d)** or in vigilance rate of individuals ($V = 46.5$, $N = 12$, $P = 0.583$; **Fig. 3.5c**).

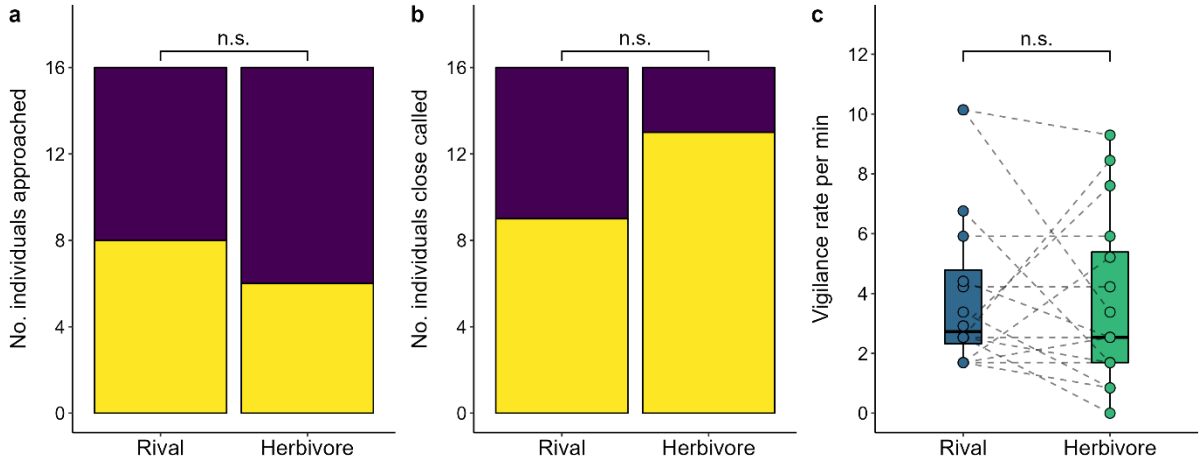

Figure 3.5. Number of individuals that (a) approached and (b) gave close calls, and (c) the vigilance rate of individuals in response to playback of movement calls following playback of either rival group or herbivore sounds. For (a) and (b), purple bars indicate no response, yellow bars show a positive response. For (c), box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range) and dotted lines link data points from the same individuals in the two treatments (circles). *N* = 16 individuals receiving paired trials.

3.5 Discussion

In response to movement call playbacks compared to control playbacks, dwarf mongoose individuals were more likely to look and approach the loudspeaker and were more vigilant (experiment 1), suggesting movement calls function as recruitment calls. Focal subordinates responded similarly to playbacks of movement calls from dominants and subordinates (experiment 2), suggesting that the dominance rank of the caller (an intrinsic factor) may not influence a decision on whether to follow another individual. The playback of a rival group caused individuals to look, orientate and approach the loudspeaker more than when played a control herbivore track, but this heightened outgroup conflict (an extrinsic factor) did not translate into a difference in response to movement calls (experiment 3). Using playback experiments allowed us to eliminate confounding factors, such as physical movement cues, and thus isolate the importance of the acoustic movement call in follower decision-making.

Much observational work suggests that signals are important in coordinating group movements in a variety of taxa (Conradt and Roper, 2005; Sperber et al., 2017). Here, we have shown experimentally that a movement call alone is sufficient to elicit a movement response in a nearby group member. While foraging for prey, dwarf mongooses spend the majority of their time with their heads down (Rasa, 1989), and vegetation can be dense, meaning that purely visual cues of

a lead attempt may be obscured or missed. Thus, a salient acoustic signal is likely useful in attracting the attention of other group members and increasing the likelihood of recruiting followers so that the putative leader is not left isolated. Similar vocalisations have been observed in other species and may be important for both recruiting followers and in coordinating movement among group members (Sperber et al., 2017); distinct vocalisations may exist for these somewhat different functions. In meerkats, for example, a 'lead call' is produced by a potential leader seemingly to attract followers (Bousquet et al., 2011); this is similar in context to the dwarf mongoose movement call that we studied. Meerkats also exhibit predeparture behaviour when changing foraging patches, with several group members giving 'moving calls', possibly to ensure a foraging patch is depleted before leaving (Bousquet et al., 2011). In dwarf mongooses, any potential 'voting' process, whereby individuals contribute to a group decision, is perhaps more likely to occur when changing activities, rather than when moving during foraging (the context that we investigated): prior to leaving a sleeping burrow or returning in the evening, there is a gradual increase in the frequency of close calls before an individual first produces a movement call and moves off (personal observation). In our first experiment, there was a nonsignificant tendency for individuals to produce close calls more in response to movement call playbacks than in response to close call playbacks. This might be an indication that followers are signalling to the leader their intention to follow, although individuals did not produce movement calls more in response to movement call playbacks than in response to close call playbacks. The lack of a strong vocal response might perhaps be due to the use of a static loudspeaker in our experiment, which likely represents a weaker stimulus than a natural lead event involving a physical cue too; future experimental work could use a moving loudspeaker (Gall and Manser, 2017). Interactive playbacks (King, 2015) could also help our understanding of how followers and leaders vocally interact with one another to coordinate movements; for example, whether vocal feedback from followers is required to initiate a group movement (Bousquet et al., 2011).

In experiment 2, we found no significant differences in response to dominant versus subordinate movement calls, but responses for both were similar to those in the movement call treatment of experiment 1. In principle, one explanation could be that movement calls do not convey information on individual identity or dominance status. However, previous work on dwarf mongooses has shown that individuals respond differently to sentinel calls depending on the dominance status of the caller (Kern et al., 2016). Furthermore, Sharpe et al. (2013) showed that, in response to close calls of higher-ranked versus lower-ranked individuals of similar ages, focal individuals with a food item were more vigilant, suggesting discrimination based on social rank. We therefore suggest that individuals were still responding to movement calls, but with no preference in

following individuals of different dominance status. Where within-group conflict is frequent, such as in chacma baboons, dominant leadership patterns have been observed, and following a dominant and maintaining social bonds with them could ease anxiety or reduce the chance of receiving aggression (Kalbitzer et al., 2015; King et al., 2008). In dwarf mongooses, there are relatively low levels of within-group conflict, perhaps in part because aggressors receive less grooming at the evening sleeping burrow (Morris-Drake et al., 2021b). Rather than dominance status per se, other factors such as nutritional requirements may be more important (Sueur et al., 2013). If movement calls are a form of honest signal, in that they are often produced by individuals with the highest needs (Conradt et al., 2009; Rands et al., 2003), then other group members could respond to them regardless of the relative social rank of the caller due to inclusive fitness benefits (Hamilton, 1964). As playbacks were conducted while foraging, the experiments could mimic a situation whereby the caller is motivated to move to another foraging patch due to the current one being depleted. If the receiver's foraging success was low at the time, it could also be in their best interest to respond to movement calls, in anticipation of a richer foraging patch. Alternatively, other individual attributes regardless of status could be important. For example, individuals could be more likely to respond to those groupmates to whom they are more strongly bonded, as previous work in dwarf mongooses has demonstrated for snake mob calls (Kern and Radford, 2016).

For our final experiment, which entailed an initial playback of either a rival group track or control herbivore track, we found a stronger response towards the former in line with previous work (Morris-Drake et al., 2019). But, we found no difference in response towards a subsequent dominant movement call, in contrast to our prediction of a heightened response. One explanation is that there could be no increase in response towards a movement call after simulated rival group presence due to heightened anxiety and alertness for rivals; rather than being more likely to respond to a movement call, the immediate threat of a rival group demands more attention from a given individual and thus movement calls might not elicit a different response, or even a weaker response. It would be interesting to conduct similar experiments during the breeding season, in which we might expect a stronger response to rival group calls. In pied babblers (*Turdoides bicolor*), for example, groups respond to rival group calls more strongly in the breeding season, likely due to increased food availability and having more energy to invest (Golabek et al., 2012). However, the lack of difference between treatments in our experiment could also be due to methodological reasons. In contrast to experiments 1 and 2, movement call playback in our control treatment elicited a weaker response. This could be due to the use of egg prior to playback to get focal individuals into position – it is possible individuals were less likely to respond to a movement call in both treatments if they anticipated more food in the area. The presence of a rival group would

clearly demand more immediate responses from individuals despite the presence of food, which we found, but responses to a subsequent movement call may have been subdued. We also found no difference in vigilance levels during the movement call playback, despite previous work showing increased vigilance following rival group playback (Morris-Drake et al., 2019). As we gave egg to a single individual, rather than to the whole group as in Morris-Drake et al. (2019), the incentive for food may have been larger in our study and affected behaviour more. Conflict has previously been shown to affect movement decisions across taxa, with groups or individuals either staying in an area to defend their territory, or moving elsewhere to avoid any further costly contests (Christensen et al., 2016; Descovich et al., 2012; Radford and Fawcett, 2014; Yi et al., 2020). As costs and opportunities of contests differ between group members, conflict is likely to affect leaders and followers differently (Johnstone et al., 2020). Further work should look to use these conflicts of interests to investigate variation in responses to movement signals, and communication more generally, while under threat.

Our current work has focused on movement decisions, but recruitment signals are widespread in the animal kingdom and occur in a variety of contexts. In dwarf mongooses alone, three different recruitment signals exist: in addition to the movement call investigated here, there is a lost call and a snake mob call (Kern and Radford, 2016; Rubow et al., 2017). Different calls likely exist because different responses are required from the receivers in each context. Across species, there are a variety of other contexts in which recruitment signals may be produced, such as attracting groupmates to foraging patches (Hauser et al., 1993; Radford and Ridley, 2006). Similar or different intrinsic and extrinsic factors could affect how individuals respond to different recruitment signals. As we learn more about recruitment signals and follower responses, comparative studies will allow us to investigate this variety in more detail.

3.6 Appendix 2

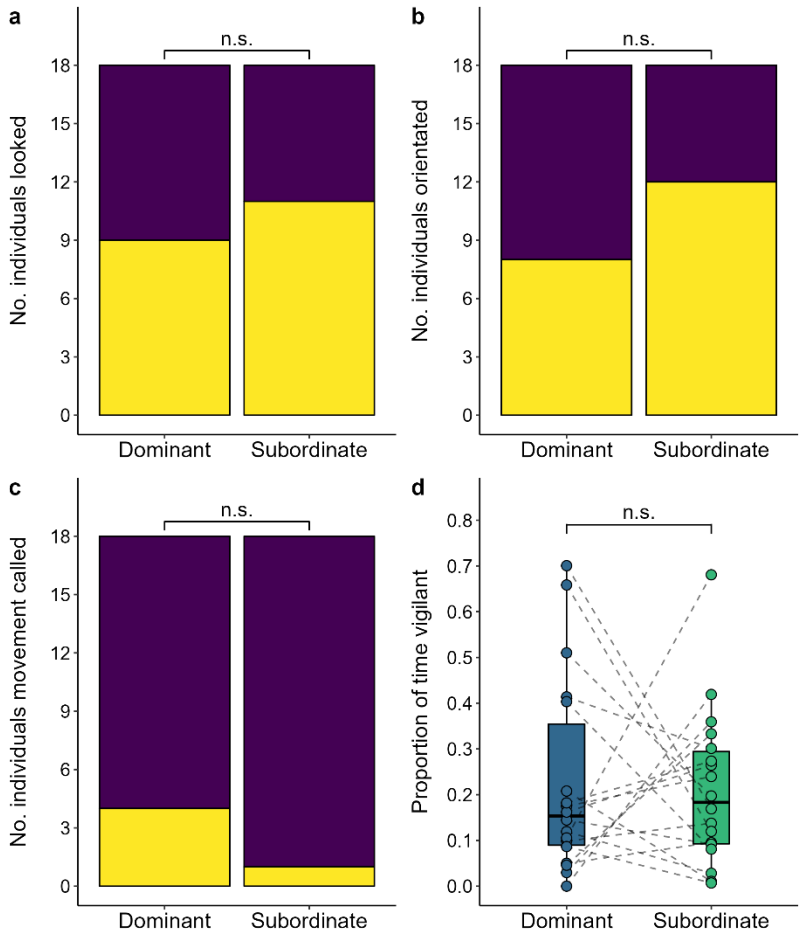

Figure A2.1. Number of individuals that (a) looked and (b) orientated towards the loudspeaker and (c) that gave a movement call in response to playback of dominant and subordinate movement calls. Purple bars indicate no response, yellow bars show a positive response. (d) Proportion of time spent vigilant in response to playback of close calls and movement calls. Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range). Dotted lines link data points from the same individuals in the two treatments (circles). *N* = 18 individuals receiving paired trials.

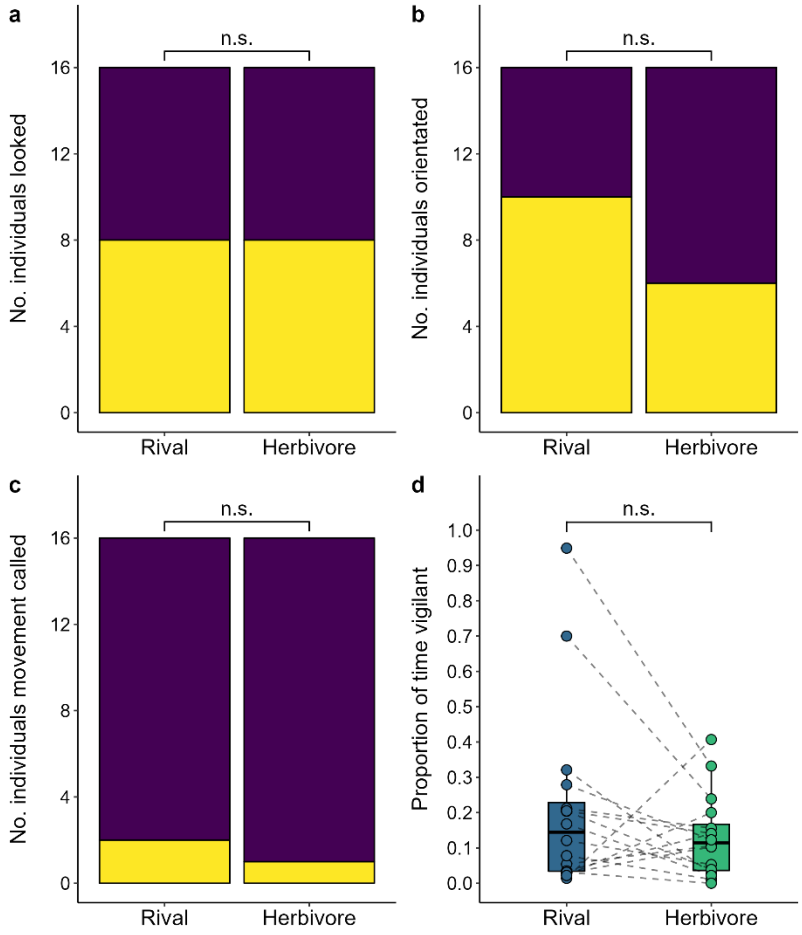

Figure A2.2. Number of individuals that (a) looked and (b) orientated towards the loudspeaker, and (c) that gave a movement call in response to playback of movement calls following playback of either rival group or herbivore sounds. Purple bars indicate no response, yellow bars show a positive response. (d) Proportion of time spent vigilant in response to playback of movement calls following playback of either rival group or herbivore sounds Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range). Dotted lines link data points from the same individuals in the two treatments (circles). *N* = 16 individuals receiving paired trials.

Chapter 4

Image Processing Methodology

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Thank you to Jitte Groothuis for the photo.

Chapter 4 will be combined with Chapter 5 for publication.

BC created and error-checked the image-processing script and drafted the manuscript; NS designed and carried out the experimental work, provided guidance on error-checking and made comments on the manuscript; PK provided comments on the manuscript; and ANR provided guidance throughout and substantial comments on the manuscript.

4.1 Abstract

As the scientific world extracts and develops bigger datasets, it has become unfeasible to process some of these manually. To tackle such huge datasets, automated processing can be used as it is much more efficient and can also remove observer biases. Analysis of the resulting data can then allow scientists to glean otherwise elusive insights into biological systems. However, automated processing can itself introduce errors, including false positives and false negatives, so some form of manual checking is needed to ensure that errors only occur at an acceptable level. In this chapter, we detail the development of an automated image-processing script, used to extract coordinates of colour-painted *Temnothorax* ants from thousands of images. Coordinate extraction first involved segmentation of the images, in which we isolated ants from the background, before detecting the locations of paint marks. We then manually extracted coordinates for a subset of images to check the performance of the script. Calculation of F1 scores, which quantify the method's overall accuracy based on false positives and missed detections, showed that it performed well. The image-processing script therefore provided a large dataset available for testing of biological questions in Chapter 5.

4.2 Introduction

As technology advances, so does our ability to acquire larger and more complex data (Myers, 2012). For instance, northern sky surveys that took over 10 years using telescopes in the 1990s amounted to around 3000 gigabytes of data, while future surveys of the entire sky are expected to exceed 4 billion gigabytes of data (Zhang and Zhao, 2015). The human genome originally took over 10 years to sequence, but now it takes less than a day due to better sequencing methods, leading to much larger volumes of data available for analysis (Navarro et al., 2019). Manual processing of such large datasets can be unfeasible, and therefore automated computer systems are increasingly employed to process data. In biological image processing, scientists often use automated systems to deal with large datasets such as microscopy images of tissues or cells, x-rays of patients or samples of invertebrate specimens (Ärje et al., 2020; Bégin et al., 2014; Mehdy et al., 2017). Automated techniques can remove observer biases, and even perform better than manual image processing if sufficiently reliable (Uchida, 2013), generating potentially vast datasets that can provide valuable insights into biological processes.

One of the main aims of image processing is to detect or classify certain objects of interest (Uchida, 2013), such as plant diseases or cancer cells (Ärje et al., 2020; Mohanty et al., 2016). To detect objects of interest accurately, image manipulation is usually performed (Gonzalez et al., 2009). For example, the contrast can be adjusted, noise (e.g., a grainy appearance) can be removed and 'segmentation' (partitioning an image into regions or objects) can be completed in an attempt to remove as much background as possible (Gonzalez et al., 2009; Uchida, 2013). These image-manipulation stages aim to improve the final detection of objects of interest. However, automated image processing is not infallible. Facial recognition software, for example, may produce racial or sex-biased results and thus generate considerable ethical concerns (Libby and Ehrenfeld, 2021). Verification of the performance accuracy of automated image processing is therefore a vital step in any study. One way in which performance can be assessed is by calculation of the number of false positives (incorrect detections of something else as the object of interest) and false negatives (missed detections of the object of interest) relative to the number of true positives, in which the object of interest is correctly identified (Wirth, 2005). An overall score, such as an F1 value (Hripcsak and Rothschild, 2005), can then be calculated to indicate the level of image-processing performance. In addition to assessing the process performance, it is also important to ensure that no biases are introduced at the data-analysis stage. For example, if processing images in an experiment with multiple treatments, performance should be similar across treatments. These checks are essential to ensure that analysis and interpretation of image datasets is reliable and robust.

Animals are often marked as part of behavioural studies to allow, for example, determination of the number and location of individuals in an area, as well as information about their interactions. For instance, identifiers such as barcodes or paint marks have been used on invertebrates in the laboratory to investigate interactions between different individuals and group spatial dynamics (Richardson et al., 2021; Stroeymeyt et al., 2018). As a specific example, an experiment was carried out by Nathalie Stroeymeyt in 2011 to investigate the dynamics of colony interactions in the ant species *Temnothorax nylanderi*. Colonies of *T. nylanderi* were split into two fragments. Interactions were experimentally induced by placing a nest-less colony fragment (the invader) outside the nest of another colony fragment (the host). Fragments were either queenless or queenright (i.e., still had their original queen); the experiment aimed to elucidate how queen presence affected interactions, the likelihood of colony fusion and spatial dynamics within and between members of the two colony fragments (full details of the experiment are available in **Chapter 5**). Host and invader worker ants were painted different colours on their gasters (abdomens), as were queen ants, and photographs of the nest arena were taken over a 2-week period. In total (see **4.3.1 Image acquisition** for details), there were nearly 140,000 images generated. Here, we describe the creation of an automated image-processing script with the aim of extracting the spatial coordinates of ants from both colony fragments in each image of each trial, and perform error-checking to ensure that the final script performed reliably.

4.3 Image-processing methodology

4.3.1 Image acquisition

The experiment involved five treatments: a control (C; invader and host from the same colony); worker–worker (WW; no queens in either fragment); queenright–worker (QW; queenright invader and queenless host); worker–queenright (WQ; queenless invader and queenright host); and queenright–queenright (QQ; queenright invader and host). Prior to the start of the experiment, host and invader workers and queens were marked on their gaster with different coloured paints (i.e., host workers were painted one colour and invader workers were painted another, with the queen in each fragment painted a different colour from her workers). Seven colours were used across trials: green, light blue, dark blue, red, orange, pink and yellow. The same brand and model of paint (Pactra R/C acrylic paint) was used for all colours. Originally, 16 trials per treatment were performed, but due to the use of incorrect ant species or data errors, seven trials were excluded. This resulted in 73 trials for image processing (14 trials each for control and WQ treatments, 15 trials each for WW, QW and QQ treatments). After the host colony was introduced to its nest, JPEG photos of the nest were

taken every 15 minutes for 2 weeks. The invader colony was introduced outside the host's nest (and outside the camera frame) after a mean ± SD of 50.2 ± 1.6 hours. After the first 10 trials were conducted, the frequency of photographing was increased to one every 0.5 minute after the invader colony was introduced, for a 2-hour period, to obtain more detailed information on the spatial dynamics at this crucial stage. For all 73 trials, the mean ± SD number of images per trial was 1905 ± 171.

4.3.2 Extraction of coordinate data from images

For image processing, we created a script (see **4.5 Appendix 3**) in MATLAB 2020b (Massachusetts, 2020). The process involved two main parts: segmenting ants from the background and detecting paint marks in the image. Due to variation across trials (e.g., in the lighting or the combination of ant colours), the detection of paint marks could differ in accuracy across trials. To compensate for this, parameters used in the script could be manually changed between experimental trials and colours to improve segmentation and detection of paint marks; a complete list of parameters is presented in **Table 4.1** and indicated in the main text in italics. We prioritised minimising false positives in coordinate extraction at the expense of more false negatives, as the former introduced noise into the data while the latter introduced missing data.

For segmentation, the aim was to eliminate as much background as possible and thus isolate the ants in the image. This is a common stage in image processing (Branson et al., 2009; Gonzalez et al., 2009). First, to allow adaptive image thresholding (Bradley and Roth, 2007), we converted an image from the RGB (red, green, blue) colour space into grayscale. This thresholding process first calculates for each pixel the mean intensity of the surrounding area. We used the default setting of the MATLAB function to calculate the size of the surrounding area, which takes the height and width of the image (2736 by 3648 pixels), divides both values by 16, rounds down, then multiplies the values by 2 and finally adds 1 to both values. This resulted in a surrounding area of 343 by 457 pixels for a given pixel. Next, using the mean intensity of the surrounding area, a threshold value was automatically determined. Pixels below this threshold are turned white and pixels above this threshold are turned black, which preserved high contrast areas (e.g., ant bodies on a grey background), while removing low contrast areas (the grey background). We could adjust the sensitivity of this adaptive threshold to make the process more lenient, for example if too many ant bodies were being excluded (**Table 4.1**; *Threshold sensitivity*). This process resulted in a black-and-white image which more clearly separates ants from the background; ants are black and most of the background is white (**Figure 4.1**).

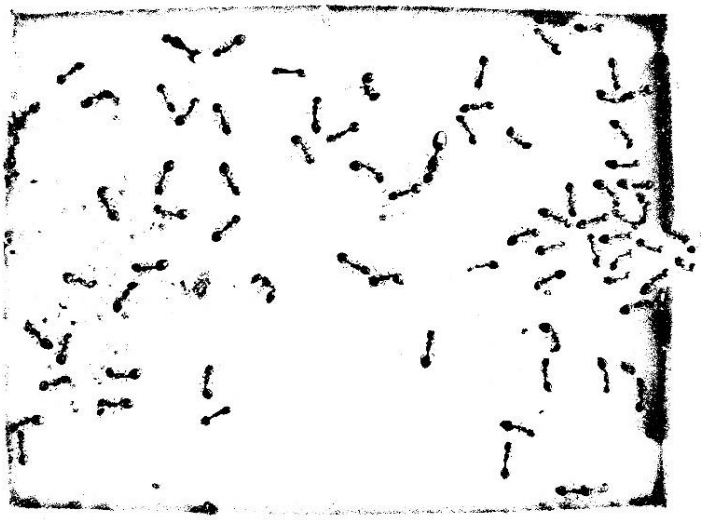

Figure 4.1. Example of black-and-white image produced from the image-thresholding process.

From this black-and-white image, we then attempted to isolate ants from the background further, by removing unwanted black objects and ensuring ants were not split into fragments. We first removed any objects typically smaller than ant bodies, such as bits of background that remained as black in the image – any connected pixels that were below a certain size (**Table 4.1**; *Minimum object size S1*) were excluded. This is known as image 'opening' and is frequently used in object-detection methods (e.g., Gal et al., 2020). As certain paint colours (e.g., pink and light blue) blended in with the background more than the body of ants, which were more distinct, paint marks could sometimes be erroneously removed. To account for this, the image was dilated after image opening, where existing black areas of the image that remained (e.g., ant bodies) were expanded outwards using a disk shape of a certain size (**Table 4.1**; *Dilation disk size S1*), before any holes within objects were filled in. This resulted in a final black-and-white image, similar to **Figure 4.1**, where much of the background was white and ants were black objects. This resulting image was then overlayed onto the original RGB image to remove the unwanted background (**Figure 4.2a**). As this process was not perfect, we performed further processing (see below).

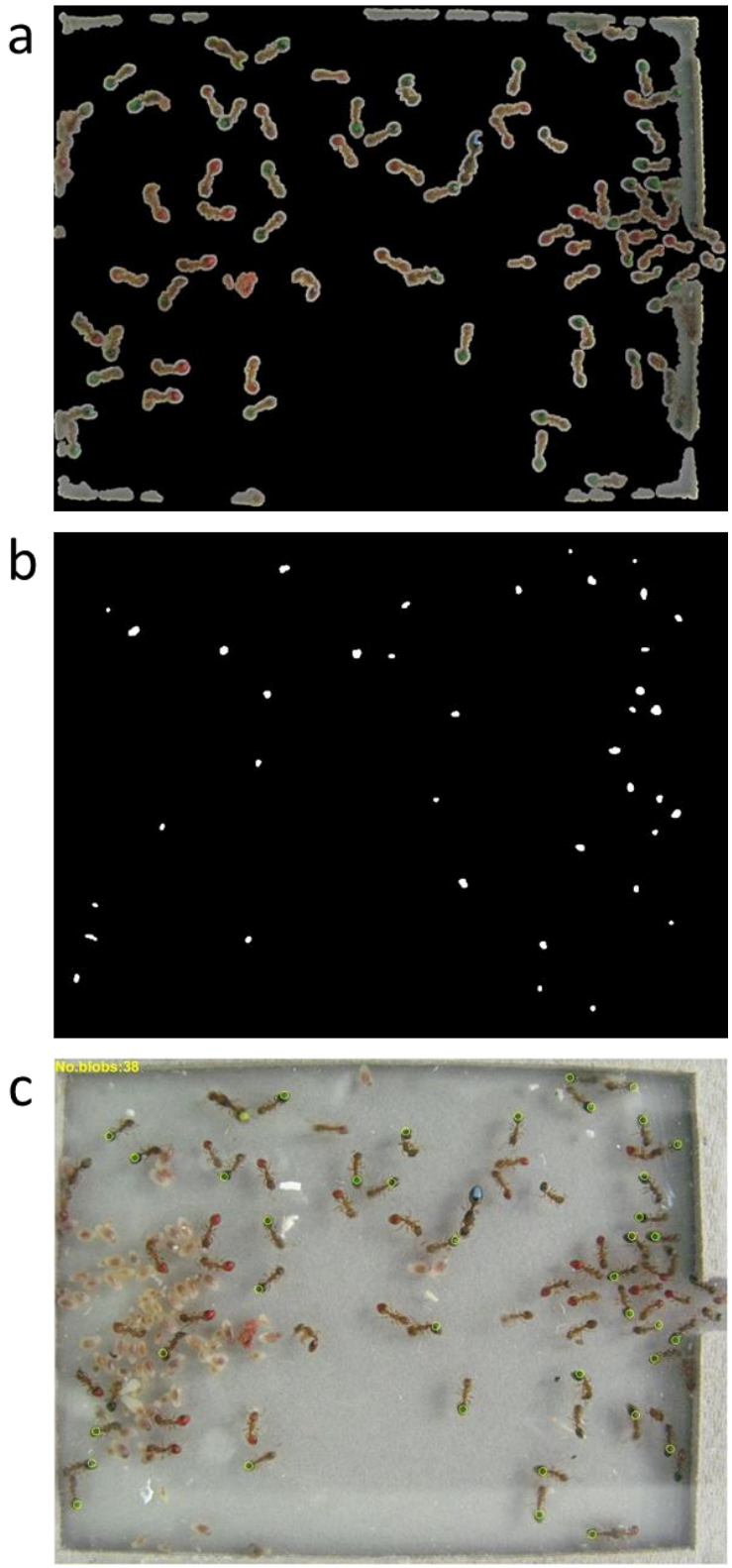

Figure 4.2. Example of green worker detection in an image: (a) shows image after segmentation, (b) shows the final image from which coordinates of objects are extracted and (c) shows these coordinates displayed on the original image with yellow circles.

The next part involved detecting paint marks in the image. First, we blurred the final black-and-white image – this helped to remove remaining paint marks that had rubbed off ants onto the nest (by effectively reducing their size), and reduced jagged edges of objects (**Table 4.1**; *Gaussian filter value*). We then converted the image to the L*a*b* colour space, which comprises lightness (L*), red-green (a*) and yellow-blue (b*) channels, and is more colour accurate than RGB (Gonzalez et al., 2009). We also expected the lightness channel would be useful in separating cases where similar paint colours were used, but where the lightness differed between the colours (e.g., orange and red). Early comparisons between L*a*b* and the HSV (hue, saturation, value) colour space, which is also commonly used for image processing (Uchida, 2013), indicated that the former performed better for paint-mark detection in our images. To detect ant paint marks, we first needed a reference paint mark for each colour for comparison. To create a reference paint mark, we drew a polygon around three paint marks of a given colour, to extract the mean a* and b* values, which were saved into a text file to be used in the main image-processing script (**Table 4.1**; *mean a** and *mean *b value*). These three regions were chosen to account for differences in colour or lighting; three paint marks that varied in appearance (e.g., due to lighting across the nest) were selected. We then used a threshold to retain pixels of similar a* and b* values to this given reference colour (i.e., filtering the image to keep only similar colours to the reference paint mark). The threshold could be made more or less strict (**Table 4.1**; *Colour threshold value*), which was used to reduce false positives where colours could have similar a* and b* mean values and were sometimes misclassified (e.g., red and orange). For a given colour, the same reference a* and b* values were used across most trials, but were sometimes redrawn if paint marks or lighting varied between trials. This was done if a visual assessment of the programme showed poor performance. In addition, filters could be included for each channel to help with similar colours overlapping (**Table 4.1**; *Min and max luminance*, *a* and b**). We then, once again, filtered remaining objects in the image by size to remove any small or large objects (**Table 4.1**; *Min and max object size*). For example, light blue paint marks were a similar colour to the light blue reflections in the nest which were often large objects, and small paint marks were often paint marks rubbed off on the nest.

We also used certain properties of the objects detected to reduce the occurrence of false positives (where an object was incorrectly detected as a paint mark, or a single paint mark was detected as multiple marks). During preliminary checking of the script, we extracted the circularity (the roundness) and the extent (the total pixel area of an object divided by the area of the smallest possible box that can fit around the object, known as the bounding box) of detected objects. Both these measures proved useful in excluding certain objects (**Table 4.1**; *Min circularity size*, *min and max extent*), the main one being light shadows of ants that were essentially the same colour as light

blue paint marks. These shadows tended to have lower circularity and lower extent values than actual paint marks, and so could be filtered out. However, for non-blue paint marks which didn't have this issue, we could disable this parameter to avoid removing paint marks that were oddly shaped and happened to have low circularity values.

We then dilated the image again to prevent false positives due to duplicate detections (**Table 4.1**; *Dilation disk size S2*). For example, where one ant had two paint marks on their body (e.g., due to another ant grooming the middle section of the paint mark off) or cases where there was variation in the colour of the paint mark across the gaster and so two marks instead of one were detected. Dilation attempted to join these into one single mark. Of the remaining objects in the image, we could then apply a filter to retain only the x largest objects detected (**Table 4.1;** *Max number of objects*). For example, if 10 objects were in the image, we could filter it to retain only five objects with the largest areas. In practice, this parameter was sometimes useful for queen detection, where there should only have been one queen of a given colour per image, and often this was a large paint mark. If there were any false positives from similar-coloured workers, there would be multiple objects. By setting a filter to retain only the single largest object, false positives (worker detections) were removed. Next, we removed any large objects that remained in the image that were unlikely to be paint marks, such as large blue reflections or the reddish colour of brood. To do so, objects above a certain number of pixels (**Table 4.1**; *Max object size*) were removed.

The coordinates of the remaining objects in the final image (**Figure 4.2b**) were extracted into a text file. These coordinates were then overlayed as circles on the original image and exported as JPEG files (an example is shown in **Figure 4.2c**). This process was repeated for a given caste (worker or queen) and colour, to process every image across trials containing the relevant painted ants.

Table 4.1. Parameters of image-processing script that could be tweaked to improve ant paint-mark detection.

Parameter	Description
Stage 1	
Threshold sensitivity	Value used to adjust how strict adaptive image thresholding was in removing background. Higher value = more lenient filter
Min object size S1	Size of smallest object to keep in image; used to exclude small regions of background
Dilation disk size S1	Size of disk used to dilate the image; used to ensure paint marks not excluded
Stage 2	
Gaussian filter value	Value used to blur image to reduce jagged edges and small paint marks in nest
Mean a* value	Average value in the a* (red–green) channel for a region drawn by the observer, used as a reference to match similar colours
Mean b* value	Average value in the b* (yellow–blue) channel for a region drawn by the observer, used as a reference to match similar colours
Colour distance value	Value used in colour thresholding to retain similar colours to reference paint mark. Higher value = more lenient filter.
Min and max luminance	Minimum and maximum values of luminance, used to prevent false positives due to similar colours
Min and max value of a*	Minimum and maximum values of a* channel, used to prevent false positives due to similar colours
Min and max value of b*	Minimum and maximum values of b* channel, used to prevent false positives due to similar colours
Min and max object size	Filter for objects of certain size, used to remove small paint marks in the nest or large blue reflections
Min circularity size	Minimum value of object circularity; used to exclude blue shadows
Min extent value	Minimum value of object extent; used to exclude blue shadows
Dilation disk size S2	Size of disk used to dilate the image; used to avoid duplicate detections
Max number of objects	Value used to filter the largest N objects detected in the image, for queen detection
Max object size	Maximum value of final object size to be retained in image; used to exclude brood and shadows

4.3.3 Error checking

4.3.3.1 Performance of colour programme

To quantify the performance of the programme, we manually checked a subset of photographs from 25 of the 73 trials. For each worker paint colour, five trials were selected randomly from both host and invader colonies, one from each treatment. For each trial, four photos were checked (image 250, 400, 800, 1200; numbered from the start of the trial), as performance could change over time with changes in lighting or ant movements. This ensured a minimum of 20 images per worker colour was checked; we then checked the same images for the opposing colony colour. For example, if we originally checked green host workers in a trial, we then also checked the detection of invader

workers (which were a different colour) for those same images. For treatments in which at least one queen was present in a colony, we also checked the detection performance of all queens in the images across both host and invader colonies. To check images manually, we created a script in MATLAB to improve efficiency and reduce errors. The observer clicked on all ants of a given colour and caste, and each click was recorded as coordinates. A circle appeared after clicking each ant (**Figure 4.3**), to minimise human error in missing ants or recording duplicates. The image could be zoomed in to improve accuracy (**Figure 4.3**), and circles/coordinates could also be removed while processing the image if incorrectly clicked. After finishing an image, coordinates were exported into a text file. The coordinates from these images were then compared to the coordinates extracted by the automatic programme (**Table 4.2**).

In testing for differences between colours, we found that the proportion of detections that were false positives did not significantly differ between worker colours (Kruskal-Wallis test: X^2 = 6.90, df = 4, P = 0.140). We did not analyse queen false positives as there were none in any of the images we checked (**Table 4.2**). We did find that that colour had a significant effect on the proportion of missed worker ants (one-way ANOVA: $F_{4,180}$ = 2.82, P = 0.027); post-hoc tests showed that the script missed more light blue ants than green ants (mean difference = 0.11 ± 0.03 SE, t test: t = 3.16, df = 180, P = 0.018). This was primarily due to optimising the process to exclude false positives from blue shadows, leading to more missed light blue ants. All other pairwise comparisons of colours were non-significant (all t < 2.06, all P > 0.202). For queens, we also found that colour affected the proportion of queens missed (Kruskal-Wallis test: X^2 = 25.92, df = 5, P < 0.001). This was driven by the detection of red queens being significantly (or close to significantly) worse than detection of queens of other colours (Dunn multiple comparisons: red–yellow, Z = 4.90, P < 0.001; red–dark blue, Z = 4.40, P < 0.001; red–green, Z = 4.40, P < 0.001; red–light blue, Z = 3.47, P = 0.002; red–pink, Z = 2.30, P = 0.065). No other pairwise comparisons were significantly different (all Z < 1.45, all P > 0.366).

Despite the lower performance of light blue worker detection and red queen detection, the use of colours was balanced in the experiment and did not differ between treatments for workers (Chi square test: X^2 = 6.27, df = 16, P = 0.98) nor for queens (X^2 = 17.18, df = 15, P = 0.31). Therefore, no consistent colour treatment bias was introduced.

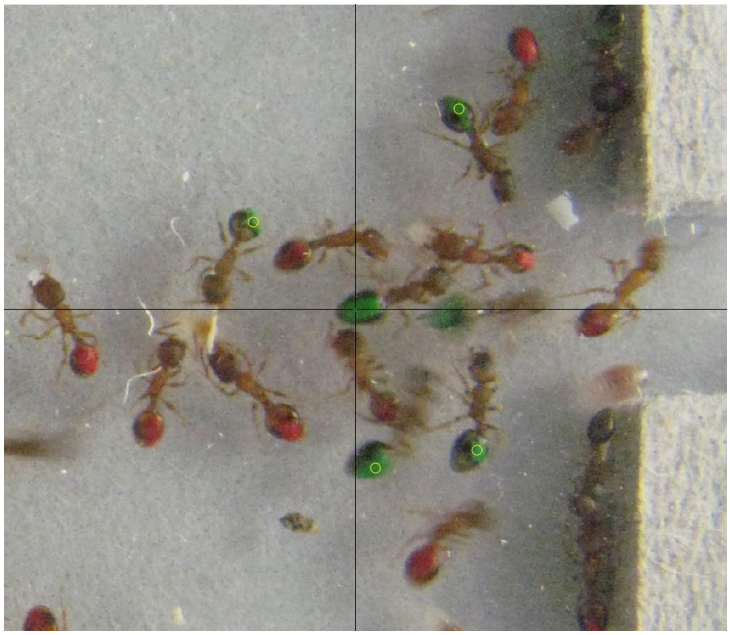

Figure 4.3. Manual image-checking process, whereby the observer is shown an image and can zoom in and click on all workers of a given colour (previous clicks indicated by yellow circles). The cursor position is shown by the intersection of vertical and horizontal lines.

As an additional assessment of the performance of the image-processing programme, we calculated F1 scores, which are commonly used in machine learning applications to assess the overall performance of a programme (Hripcsak and Rothschild, 2005; Van Rijsbergen, 1979). F1 scores are calculated by weighing the 'precision' of the programme against the 'recall' of the programme. The precision was calculated by taking the number of true positives (correct paint-mark detections) and dividing it by the sum of the number of true positives and the number of false positives (incorrect detection of paint marks). The recall score was similarly calculated, but using the number of missed detections instead of false positives. Finally, the F1 score was calculated by multiplying the precision and recall scores, dividing this by the sum of recall and precision scores, and then multiplying this value by 2 (**Table 4.3**). This gives an overall measure from 0 to 1, based on precision and recall, of how well the programme is performing; a score of 1 indicates perfect performance. F1 scores were greater than 0.9 for all worker and queen colours, except for red queens (**Table 4.3**).

Table 4.2. The performance of the paint-detection script for (a) workers and (b) queens. As queens were sometimes absent from the nest, the sum of correctly identified and missed queens does not equal the number of images checked.

	Number correctly identified	Number missed	% missed	Number of false positives	% false positives	Number of images checked
(a) Worker colour						
Green	1263	122	8.8	31	2.5	60
Light blue	751	155	17.1	7	0.9	40
Orange	662	91	12.1	21	3.2	28
Pink	940	140	13.0	17	1.8	40
Red	492	74	13.0	10	2.0	32
Total	4108	582	12.4	86	2.1	200
(b) Queen colour						
Dark blue	11	0	0	0	0	16
Green	11	0	0	0	0	20
Light blue	9	1	10	0	0	16
Pink	1	0	0	0	0	4
Red	2	2	50	0	0	12
Yellow	40	0	0	0	0	52
Total	74	3	3.9	0	0	120

Table 4.3. F1 scores showing performance of paint-detection script for (a) workers and (b) queens. Values range from 0 to 1, with 1 indicating perfect performance.

	Precision	Recall	F1 score
(a) Worker colour			
Green	0.98	0.91	0.94
Light blue	0.99	0.83	0.90
Orange	0.97	0.88	0.92
Pink	0.98	0.87	0.92
Red	0.98	0.87	0.92
(b) Queen colour			
Green	1.00	1.00	1.00
Light blue	1.00	0.90	0.95
Dark blue	1.00	1.00	1.00
Pink	1.00	1.00	1.00
Red	1.00	0.50	0.67
Yellow	1.00	1.00	1.00

4.4 Discussion

To analyse photographic data collected from an experiment investigating how the presence of queens affected *Temnothorax nylanderi* colony interactions, we designed an automated image-processing script to process nearly 140,000 images. We verified that the script performed reliably in detecting individual paint-marked ants, and that there was no major bias between colours. We could therefore be confident in using coordinate data for analysis in **Chapter 5.**

To assess the performance of the image-processing script, we manually checked 200 images and compared them to automatically processed images. For workers, an average of 12.4% painted ants were missed, and 2.1% of detections were false positives. For queens, 3.9% were missed and there were zero false positives. This performance is similar to or better than other research; for instance, Ulrich et al. (2018) tracked painted ants using images and found that, on average, 22.9% of ants in images were missed and 5.6% of ants were assigned to the wrong colour. As an overall assessment of the performance, we then calculated F1 scores, which are affected by both false positives and false negatives; a score of 1 indicates perfect performance. The mean F1 score was 0.92 for workers and 0.94 for queens, indicating our methodology performed reliably. As F1 scores vary between applications and are typically used in other disciplines (Hripcsak and Rothschild, 2005), we found no image-processing methodology similar to here in which F1 scores are used; thus, we could not compare F1 scores to other work. However, future work using similar image-processing methodology could report F1 scores as a standardised way of comparing performance.

While the overall performance of the image-processing script was good, we did find some variation in performance across colours. Light blue workers were missed more than green workers, which was due to the shadows of ants and light reflections that were a similar colour to light blue paint marks. A trade-off meant that by reducing the number of false positives, we also increased the number of missed light blue ants. By contrast, green worker detection had few issues with false positives; more lenient parameters could be used to improve detection and reduce the number of green workers missed. We also found that the detection of red queens was worse than that of other queen colours. This was due to the similarity of red queens to orange workers and ant bodies in some images, so parameters were tweaked to prevent false positives, leading to more missed red queen. We prioritised reducing false positives over missed detections as these would have introduced more noise into response variables (see **Chapter 5**) through incorrect coordinates—for example, paint marks in the corner of the nest increasing nearest-neighbour distances—while missed detections were less likely to have a major effect on our response metrics. Overall, errors in image processing are expected; it is difficult to make a script perform perfectly. Even if we had

performed manual image-processing checking, it is likely that human error would have had an impact on performance. There was also a trade-off with improving detection slightly but at the cost of time. Ultimately what matters is that the colours used to paint ants were not biased across treatments, so variation in paint loss and colour detection should have had little effect on the conclusions that can be drawn from analysis based on automated image scoring. Moreover, the reliability of response variables do not differ between treatments (see analysis in **Chapter 5**).

Automatic image processing allowed us to use the full extent of the dataset; manual coordinate extraction of all images would have been unfeasible. Manual processing of images with an average of 30 ants per image took over 2 minutes per image. For an image with 48 ants (the overall mean number of ants in an image across the experiment), it would take approximately 200 seconds, which equates to a total of ca. 330 days to process every image in the experiment. An alternative option would have been to use a smaller number of images (a subset from each trial), assessed manually, but this would have greatly reduced the temporal resolution and fine-scale nature of the data. For example, from when invaders were first introduced (around 3000 minutes into the experiment) to the first 5000 minutes when invader and host numbers plateaued, we had a mean of 560 images per successful fusion. This allowed us to ask questions about rapid changes in both invader and host worker numbers in the nest across time, in addition to providing enough data for analysis of the extent of colony integration at two separate time points (see **Chapter 5**). Automatic processing also removed the possibility of certain observer biases, such as differences in observer detection ability depending on fatigue (Krupinski et al., 2017). The large number of images, combined with the reliable performance of the programme, should also have reduced the effect of image-processing errors relative to correct detections. This meant that we could be more confident in the results from statistical analyses and thus make stronger conclusions. By using the full dataset provided by automatic image processing, we could investigate multiple metrics to answer biological questions.

The main image-processing script is available in **4.5 Appendix 3**. This reads in parameter files and processes a given caste and colour for all relevant trials. Ideally, other researchers would have been able to use the script in processing their own image datasets; for example, scientists tracking marked ant movements on a 2D plane (Mersch et al., 2013; Ulrich et al., 2018). However, MATLAB is not opensource so would not be accessible to all researchers, whereas there are other opensource options that have subsequently become available, such as anTraX (Gal et al., 2020). These opensource options also allow users to track movements of painted insects down to the individual-level in contrast to our script.

In this chapter, we have described the steps in building an image-processing script to extract coordinate data and the associated error checking to verify the performance of the script. Newer methods to track ant movements include the use of barcodes glued to ants, which can provide greater temporal resolution and detection accuracy (Mersch et al., 2013; Stroeymeyt et al., 2018). However, these techniques may require high-resolution cameras and be time-consuming. The use of paint marks remains a cheaper alternative and still provides reliable data. Future use of paint marks should aim to use multiple distinct colours on individual ants, to compensate for lost paint marks (e.g., see Richardson et al., 2021). In the next chapter (**Chapter 5**), we take the coordinate data extracted from our automated image-processing script and use them to investigate colony fusion dynamics.

4.5 Appendix 3

4.5.1 Main script

```
% Clear workspace and command window
clear
clc

% Select input and output folders
% Note personal computer file paths have been removed
prompt_message = "Do you want to specify folder inputs/outputs manually, or are you working
from uni or home?";
answers = questdlg(prompt_message,"Pick an
                   option","Manually","Home","University","University");
% Handle response
switch answers
    case 'Manually'
        getInputFolder = uigetdir([],"Choose Input Folder");
        getOutputFolder = uigetdir([],"Choose Output Folder");
        getParametersFolder = uigetdir([],"Choose Parameters Folder");
    case 'Home'
        getInputFolder = "Removed file path";
        getOutputFolder = "Removed file path";
        getParametersFolder = "Removed file path";
        getTrialsProcessedFolder = "Removed file path";
    case 'University'
        getInputFolder = "Removed file path";
        getOutputFolder = "Removed file path";
        getParametersFolder = "Removed file path";
        getTrialsProcessedFolder = "Removed file path";
    otherwise
        errorMessage = sprintf("%s","Error: No folders chosen");
        uiwait(warndlg(errorMessage));
        return
end

% Check if all folders exist
if ~isfolder(getInputFolder)
    errorMessage = sprintf('Error: The following folder does not exist:\n%s\nCheck your
folder paths again', getInputFolder);
    uiwait(warndlg(errorMessage));
    return
end
if ~isfolder(getOutputFolder)
    errorMessage = sprintf('Error: The following folder does not exist:\n%s\nCheck your
folder paths again', getOutputFolder);
    uiwait(warndlg(errorMessage));
    return
end
if ~isfolder(getParametersFolder)
    errorMessage = sprintf('Error: The following folder does not exist:\n%s\nCheck your
folder paths again', getParametersFolder);
    uiwait(warndlg(errorMessage));
    return
end
if ~isfolder(getTrialsProcessedFolder)
```

```
    errorMessage = sprintf('Error: The following folder does not exist:\n%s\nCheck your
folder paths again', getTrialsProcessedFolder);
    uiwait(warndlg(errorMessage));
    return
end

% Create path for txtfile with list of trials with the invader name, in
% order to later skip images before the invader is introduced
Invader_trials_path = sprintf("%s",getParametersFolder,"\Invader_trials_list.txt");
if ~isfile(Invader_trials_path)
    errorMessage = sprintf("Error: The list of invader trials doesn't exist!");
    uiwait(warndlg(errorMessage));
    return
else
    % Read trials to be processsed if they do exist
    Invader_trials_to_process = readtable(Invader_trials_path,"Delimiter","tab");
    Invader_trials_to_process = table2array(Invader_trials_to_process);
    fprintf("%s\n","Successfully found text file with list of invader folders")
end

% Get directory of folders
InputFolders = dir(getInputFolder);
OutputFolders = dir(getOutputFolder);
ParametersFolder = getParametersFolder; % don't need directory yet

% Specify worker colour using UI input with list of options
list = {'darkblue','lightblue','green','yellow','red','orange','pink'};
[indx,tf] = listdlg('ListString',list,"SelectionMode","Single");

prompt_message = "Do you want to process worker or queen images";
answers = questdlg(prompt_message,"Pick an option","Worker","Queen","Queen");
% Handle response
switch answers
    case 'Worker'
        ant_colour = sprintf("%s","Workers_",list{indx});
    case 'Queen'
        ant_colour = sprintf("%s","Queen_",list{indx});
    otherwise
        return
end
%
% Creat txt file name for storing txtfile with list of trials that have
% been processed
txtfile_name_trials_processed = sprintf("%s",
getTrialsProcessedFolder,"\",ant_colour,"_Trials processed.txt");
if ~isfile(txtfile_name_trials_processed)
    writematrix(["Trials processed"],txtfile_name_trials_processed,"Delimiter","tab");
else
    fprintf("%s\n","Trials processed txt file already exists, NOT overwriting")
end

% Remove . and .. from folder.name (this refers to parent folders, not needed)
InputFolders = InputFolders(~ismember({InputFolders.name}, {'.', '..'}));
OutputFolders = OutputFolders(~ismember({OutputFolders.name}, {'.', '..'}));

% Sort folder order correctly with a file exchange function nasortfiles
[~,ndx] = natsortfiles({InputFolders.name});
InputFolders = InputFolders(ndx);
```

```
[~,ndx] = natsortfiles({OutputFolders.name});

% Check if folder length is equal
if isequal(length(InputFolders),length(OutputFolders))
    fprintf("Length of input and output folders match
(%s)\n",num2str(length(InputFolders)));
else
    error("Folder length does not match - check directories again!")
end

tic % start timer

for k = 1:length(InputFolders)

    % Generate folder paths
    Input = sprintf("%s",InputFolders(k).folder,"\",InputFolders(k).name);
    Output = sprintf("%s",OutputFolders(k).folder,"\",InputFolders(k).name,"\");
    Current_folder = InputFolders(k).name;

    % Get a list of all JPG files in the folder
    filePattern = fullfile(Input, '*.JPG');
    jpgFiles = dir(filePattern);

    % Sort jpg files using nasortfules function, from filexchange
    [~,ndx] = natsortfiles({jpgFiles.name});
    jpgFiles = jpgFiles(ndx);

    % Get a list of txt files in parameters folder
    filePatternTxt = fullfile(ParametersFolder,"*.txt"');
    txtFiles = dir(filePatternTxt);

    [~,ndx] = natsortfiles({txtFiles.name});
    txtFiles = txtFiles(ndx);

    if  isfile(sprintf("%s",ParametersFolder,"\",InputFolders(k).name,"_",
     ant_colour,"_ROI.txt")) && ...
           isfile(sprintf("%s",ParametersFolder,"\",InputFolders(k).name,
            "_",ant_colour,".txt"))
       fprintf("\rParameters and ROI files found for %s%s%s%s  \n",
     InputFolders(k).name,"_",ant_colour, ", processing...");

        % Read in ROI values from pre-existing txt file
        ROI_path = sprintf("%s",ParametersFolder,"\",
          InputFolders(k).name,"_",ant_colour,"_ROI.txt");
        ROI_table = readtable(ROI_path);
        ROI_table_values = ROI_table(:,2);
        ROI_table_values = table2cell(ROI_table_values);
        aMean = ROI_table_values{1}; % Mean a* value
        bMean = ROI_table_values{2}; % Mean b* value

        % Read in parameters from pre-existing txt file
        % Where possible, the same parameters were used for a given colour
        % But for some trials, tweaks were made to improve detection
        Parameters_path = sprintf("%s",ParametersFolder,
        "\",InputFolders(k).name,"_",ant_colour,".txt");
        Parameters_table = readtable(Parameters_path);
        table_values = Parameters_table(:,3);    % index the third column
        table_values = table2cell(table_values); % convert to cells, so don't have to index
```

```
both row and column


% Index parameter values from the txt file
F1Parameters.adaptedThresholdValue = table_values{1}; % Threshold sensitivity
F1Parameters.BWopenValue = table_values{2}; % Min object size S1
F1Parameters.seDiskValue = table_values{3}; % Dilation disk size S1
F2Parameters.distLab_ThresholdValue = table_values{4}; % Colour distance value
F2Parameters.seDiskValue = table_values{5}; % Dilation disk size S2
F2Parameters.smallestPixelSize = table_values{6}; % Min object size
F2Parameters.largestPixelSize = table_values{7}; % Max object size
F2Parameters.maxNumberofAnts = table_values{8}; % Max number of objects
F2Parameters.circularity_min_size = table_values{9}; % Min circularity size
F2Parameters.luminanceMax = table_values{10}; % Max luminance
F2Parameters.luminanceMin = table_values{11}; % Min luminance
F2Parameters.AChannelMax  = table_values{12}; % Max value of a*
F2Parameters.AChannelMin = table_values{13}; % Min value of a*
F2Parameters.BChannelMin = table_values{14}; % Min value of b*
F2Parameters.BChannelMax = table_values{15}; % Max value of b*
F2Parameters.gaussian_filterValue = table_values{16}; % Gaussian filter value
F2Parameters.MinExtent = table_values{17}; % Min extent value
F2Parameters.MaxExtent = table_values{18}; % Max extent value (not used in end)
F2Parameters.FinalMaxPixelSize = table_values{19}; % Max object size

% create name values for output files, may not need all
txtfile_name = sprintf("%s",Output,InputFolders(k).name,"_",ant_colour);
txtfile_name_parameters = sprintf("%s",txtfile_name,".txt");
txtfile_name_ROI = sprintf("%s",txtfile_name,"_","ROI.txt");
txtfile_name_coords = sprintf("%s",txtfile_name,"_","coordinates.txt");

% Append trials processed txt file, with current folder

writematrix([InputFolders(k).name],txtfile_name_trials_processed,"WriteMode",
"append",  "Delimiter","tab");


% Try and write txt file of coordinates at default location - if
% file is locked, then use alternative txt file name to avoid
% script stopping
try
    if ~isfile(txtfile_name_coords)
% create txt file with headers to be appended later in loop, storing image
name, coordinates, and area of blob
writematrix(["Image","X","Y","Area","Circularity","Extent","Height","Width"],
,txtfile_name_coords,"Delimiter","tab");
    else
        fprintf("Txtfile with coords already exists, not overwriting\n")
    end
catch
    fprintf("Coordinate txt file locked, CHECK TRIAL! \n")
    writematrix([InputFolders(k).name, "ERROR - Coord file COULD BE LOCKED.
    Check  trial!"],txtfile_name_trials_processed,"WriteMode","append",
    "Delimiter","tab");c
    continue
end
```

```
        % Run loop function
        ProcessAntImages(jpgFiles,F1Parameters,F2Parameters, aMean, bMean,
        Output,txtfile_name_coords,ant_colour, Current_folder, Invader_trials_to_process,
       ,txtfile_name);

    else fprintf("Parameter and ROI files not found for %s%s%s%s
    \n",InputFolders(k).name,"_",ant_colour,", skipping folder");

    end
end
toc % end timer

% Single_folder_loop placed into function
function [] = ProcessAntImages(
 jpgFiles,F1Parameters,F2Parameters, aMean, bMean,
 Output,txtfile_name_coords,ant_colour,Current_folder,
 Invader_trials_to_process,txtfile_name
)

% If trial is invader colour/folder, skip first 150 images as invaders not yet introduced
Trial_name = sprintf("%s",Current_folder,"_",ant_colour);
%
if contains(Trial_name,Invader_trials_to_process) == 1
    fprintf("Ants from this trial are invaders, starting from image 150... \n");
    firstimage = 150;
else
    fprintf("Ants from this trial are hosts, starting from the first image... \n");
    firstimage = 1;
end

% Read in existing coordinate table, so you can check if an image has
% already been processed in the for loop below
check_if_processed_table =
readtable(txtfile_name_coords,"Delimiter","tab","Format","%s%*s%*s%*s%*s%*s%*s%*s"); % %s
specifies text, * doesn't read a given column - only need first column here
check_if_processed_array = table2array(check_if_processed_table);

for k = firstimage:length(jpgFiles)

    baseFileName = jpgFiles(k).name;
    fullFileName = fullfile(jpgFiles(k).folder, baseFileName);

    % create filename for final output path
    filename = sprintf("%s", baseFileName); % Naming the file output
    finalfile = sprintf("%s",Output,filename); % combine output path + file name
    [~,name,~] = fileparts(finalfile);
    append_end_image = sprintf("%s",Output,ant_colour,"_",name,".jpg");


    if ~isfile(append_end_image) % If image does not exist:
        if ~isempty(check_if_processed_array) % if coordinate txt file is not empty
            % if coordinates text file contains the current image name, don't process
            if any(contains(check_if_processed_array,filename,"IgnoreCase",true))
                continue % end current iteration of loop, do not process image
            end
        fprintf("NOTE: Processing image not found in text file...\n")
            % read image into ant variable
            ant = imread(fullFileName);
```

```
% create burned image with function 1
burnedAnt = createAntSegmentation(ant,F1Parameters);

% create final image with function 2
[centroids, area_of_centroid, circularity, extent] = createantmask(
   burnedAnt,aMean,bMean,F2Parameters
 );

% write Image with centroids, to check accuracy, if at least one centroid
if centroids >= 1
    cla reset;
    close
    figure("Visible","off"), imshow(ant); % plot the figure without displaying
    hold on; % hold figure
    plot(centroids(:,1),centroids(:,2),"yo","markersize",10,"linewidth",1);
    hold on
    text(1,40,sprintf("No.blobs:%s",num2str(height(centroids))),'FontSize', 20,
    'FontWeight','Bold',"Color","Yellow") % add text of number of blob
    hold off % end figure display
    exportgraphics(gca,append_end_image);  % export current plot to file
end

% Take height and width of image in case needed
[imageheight, imagewidth, ~] = size(ant);

% concatenate filename of image and centroids, so there is one row per
% image and centroid. If no centroids detected, ensure a blank row
% is created for the image anyway by changing 0 to 1
row_length = height(centroids); % get the number of rows in centroids
row_length(row_length < 1) = 1; % and if less than 1, change it to one
filename_repeated = repelem(filename,row_length); % repeat the filename for
every row (every time a centroid detected)
filename_column = filename_repeated(:); % turn this vector into one single
column, so you can concatenate it with the correct number of centroids

height_repeated = repelem(imageheight,row_length);
width_repeated = repelem(imagewidth,row_length);
height_column = height_repeated(:);
width_column = width_repeated(:);


% write matrix to txt file.
try
    if centroids >= 1 % Append, don't overwrite
        writematrix([filename_column, centroids, area_of_centroid,
        circularity, extent, height_column, width_column],
        txtfile_name_coords, "WriteMode","append", "Delimiter","tab");
    else % Write txtfile with image name, and NAs if no ants detected
        writematrix([filename_column, "NA", "NA", "NA",
        "NA","NA","NA","NA"],txtfile_name_coords,"WriteMode","append",
        "Delimiter","tab");
    end
catch
    fprintf("%s\n","Could not write coordinate text file,
    file could be locked,  so writing file to alternative txtfile name")
end
```

```
    else
      % do nothing if image file already exists. Avoids unncesary text lines,
      reduces time as not overwriting images
    end
  end
  end
```

4.5.2 Function 1 – segment ants

```
function burnedAnt = createAntSegmentation(ant,F1Parameters)

greyant = rgb2gray(ant); % convert image to grayscale
adaptedAnt = adaptthresh(greyant,F1Parameters.adaptedThresholdValue,
  "ForegroundPolarity","dark"); % threshold image (Threshold sensitivity)

BW = imbinarize(greyant,adaptedAnt); % binarize ant image, using the above threshold
BWopen = bwareaopen(~BW,F1Parameters.BWopenValue); % exclude small pixels (Min obj size S1)

se = strel("disk",F1Parameters.seDiskValue); % for dilating image (Dilation disk size S1)
BWdilate = imdilate(BWopen,se); % dilate image, expand white pixels to not cut off paint

BWfilled = imfill(BWdilate,"holes"); % fill in any holes
burnedAnt = imoverlay(ant,~BWfilled,"k"); % final image, mask burned onto original image

end
```

4.5.3 Function 2 – colour detection

```
function [centroids, area_of_centroid, circularity, extent] = createantmask(burnedAnt, aMean,
bMean, F2Parameters)
% outputs the final image, centroid x and y, and the area of each centroid

% introduce gaussian filter, to avoid jagged edges and get rid of paint on arena (Gaussian
% filter value)
burnedAnt = imgaussfilt(burnedAnt, F2Parameters.gaussian_filterValue); %

antLAB = rgb2lab(burnedAnt); % convert RGB to LAB colour space
antL = antLAB(:,:,1);        % luminance channel
antA = antLAB(:,:,2);        % red-green channel
antB = antLAB(:,:,3);        % blue-yellow channel
distLab = sqrt((antA - aMean).^2 + (antB - bMean).^2);
% distance matrix, difference between average colour and every pixel

% threshold the distance matrix and use colour channel min and max filters
% (Colour distance value, Min amd max lumiannce, Min and max value of a*, Min and max value
% of b*)
mask = distLab < F2Parameters.distLab_ThresholdValue & antL < F2Parameters.luminanceMax...
    & antL > F2Parameters.luminanceMin & antA < F2Parameters.AChannelMax & antA >
F2Parameters.AChannelMin & antB < F2Parameters.BChannelMax & antB > F2Parameters.BChannelMin;
```

```
% Pixel size filter (Min and max object size)
maskcleaned = bwareafilt(mask,[F2Parameters.smallestPixelSize,
F2Parameters.largestPixelSize]);

% remove areas with low circularity (Min circularity size)
connected_BW = bwconncomp(maskcleaned);
stats = regionprops(connected_BW,"Circularity");
filter_areas = find([stats.Circularity] > F2Parameters.circularity_min_size);
maskcleaned_shadows = ismember(labelmatrix(connected_BW),filter_areas);

% remove areas with low extent (Min extent value; the maximum value was not needed to filter
% objects)
maskcleaned_extent = bwpropfilt(maskcleaned_shadows,"Extent",[F2Parameters.MinExtent,
F2Parameters.MaxExtent]);

% dilate image (Dilation disk size S2)
se = strel("disk",F2Parameters.seDiskValue); % create disk shaped area for dilation
dilatedant = imdilate(maskcleaned_extent,se); % dilate image
maskfilledholes = imfill(dilatedant,"holes");  % fill in holes within a blob

% filter for largest X blobs - sometimes used for queen detection (Max number of objects)
mask_maxnumberants =
bwpropfilt(maskfilledholes,"area",F2Parameters.maxNumberofAnts,"largest");

% final pixel size filter - to remove very large blobs e.g. brood (Max object size)
finalantimage = bwareafilt(mask_maxnumberants,[1, F2Parameters.FinalMaxPixelSize]);

% find coordinates of centroids and extract area, circularity and extent of objects
antmeasurements = regionprops(finalantimage, "centroid","area","circularity","extent");
centroids = cat(1,antmeasurements.Centroid); % turn centroids into two columns (x and y)
area_of_centroid = [antmeasurements.Area]';  % extract area of each blob into new variable
circularity = [antmeasurements.Circularity]'; % extract circularity
extent = [antmeasurements.Extent]'; % extract extent of objects
end
```

Chapter 5

The Presence of Queens Affects Fusion Dynamics in *Temnothorax* Ants

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Thank you to Jitte Groothuis for the photo.

Chapter 5 will be combined with further analysis for future publication.

BC carried out data extraction and analysis, interpreted the results and drafted the manuscript; ANR helped with data analysis, interpreting the results and provided substantial comments on the manuscript; PK helped with data analysis, interpreting the results and provided comments on the manuscript; NS ran the original experiment, helped with data analysis and interpreting the results, and provided comments on the manuscript.

5.1 Abstract

Hamilton's theory of kin selection explains that altruistic behaviour in social Hymenoptera can evolve through the helping of relatives. As a result, colonies should avoid integrating with non-kin, with conflict both between species and within species being widespread. However, fusions of non-related colonies do occur, including in the monogamous ant *Temnothorax nylanderi*, with colonies facing high competition for nest sites. This is surprising given the associated fitness costs, particularly as only one queen of a given colony tends to survive fusion. We investigated whether the presence of queens affects fusion dynamics in *T. nylanderi*, using experimental data in which an 'invader' colony (either with or without a queen) was placed outside the nest of a queenright or queenless 'host' colony. Overall, we found that the presence of at least one queen affected fusion dynamics. For treatments where both colonies had a queen (in which most fighting was predicted due to the costs of losing a queen), mortality of workers was higher and fusion was less likely to occur. For colonies that successfully fused, invader workers entered the nest at a slower rate compared to the control (in which the same colony was split and reunited again). Furthermore, analysis of nearest-neighbour distances and overlap between colonies that fused suggested greater separation toward the start of the experiment than in the control treatment. Our results suggest that fusions are most costly for queenright colonies, and that queenright colonies may attempt to avoid fusion by fighting more. If they do fuse, they remain somewhat separated, perhaps to retain the option for migration to a new nest site if that opportunity arises later. Our work contributes to knowledge on fusions between colonies in which we would expect conflict over cooperation. Further work could investigate the costs of failing to fuse, and the mechanisms of how queen presence modifies worker behaviour.

5.2 Introduction

In the social Hymenoptera, workers often display apparently altruistic behaviour by sacrificing their own reproductive output and instead caring for the offspring of a queen. This can be explained through Hamilton's theory of kin selection, in which altruistic behaviour can evolve by helping other carriers of the same genes (Hamilton, 1964; Kay et al., 2020). To direct altruistic behaviour towards kin, groups should associate with relatives while simultaneously excluding outsiders. This is enabled by colony odours, which are based primarily on cuticular hydrocarbons, allowing discrimination between nestmates and outsiders (Sturgis and Gordon, 2012). Conflict with rival colonies over resources or to maintain nests and territory is common, with the intensity of fighting varying widely across species and within species (Adams, 2016).

While on one hand conflict is widespread, unrelated colonies are known to come together on some occasions. For instance, 'supercolonies' in unicolonial ants, where there is little aggression between colonies often over large distances (Bourke and Franks, 1995; Helanterä, 2022). Because this leads to low relatedness amongst nestmates, supercolonies seem to be an evolutionary paradox to kin selection (Bourke and Franks, 1995; Helanterä, 2022), and could even be evolutionary 'dead-ends' (Helanterä et al., 2009); this may partly explain why it remains a rare phenomenon (Helanterä, 2022). Another example of unrelated colonies coming together occurs in those species where there are queen takeovers and colony fusions (Foitzik and Heinze, 1998, 2001; Rudolph and McEntee, 2016). As with supercolonies, these events appear to pose significant fitness costs for the usurped colony, as workers from the usurped colony may end up rearing individuals to which they are not highly related (Foitzik and Heinze, 1998). We would therefore expect to observe high levels of conflict between two fusing colonies. Previous examples of colonies raising unrelated kin typically come from the slave-making ants, in which host workers are taken from their nests and made to raise the parasitic ant's brood (Rudolph and McEntee, 2016), though fusions between non-slave-making ants have also been observed (Foitzik and Heinze, 1998; Rudolph and McEntee, 2016). Why these fusions occur, and the mechanisms behind them, are of interest to researchers as we would expect conflict over cooperation due to high fitness costs (Foitzik and Heinze, 2001; Rudolph and McEntee, 2016).

To investigate fusion dynamics between colonies, we studied a monogamous ant found across central Europe (Foitzik and Heinze, 1998), *Temnothorax nylanderi*. These ants do not construct nests, but inhabit sites such as rotting twigs, grass stems and hollow acorns (Foitzik and Heinze, 1998). As such, they are reliant on the availability of nest sites in the environment. In early spring, when there is an abundance of available nest sites due to their accumulation over winter

(Foitzik and Heinze, 1998, 2001), colonies often split, resulting in both queenright (a queen is present) and queenless colony fragments (Foitzik and Heinze, 1998). As nest-site opportunities decline over the summer, competition between neighbouring colonies increases, leading to nest takeovers by unrelated colonies or queens and colony fusions. This is thought to increase genetic heterogeneity in nest sites (Foitzik and Heinze, 1998, 2001). While the usurping colony gains a nest site, the current resident would be expected to defend their nest and prevent the takeover to avoid a decrease in inclusive fitness that would result from raising unrelated brood instead of their own. Previous work has demonstrated that conflict does occur when an invader colony is introduced to a host nest, and that the relative size of colonies may impact whether invaders can successfully overcome the host and enter the host nest (Foitzik and Heinze, 1998). While both queenright and queenless colonies are expected to defend their colony, queenright colonies may have more to lose as the death of their queen would cease production of highly related kin, comparable to the difference in fitness costs that predator and prey face in a chase (Dawkins et al., 1997). Because of this difference, we would expect the presence of queens to affect the motivation and intensity of conflict between colonies. In particular, the greatest levels of conflict are expected in queenright–queenright fusions, as generally only one queen will survive (Strätz et al., 2002).

An experiment carried out by Nathalie Stroeymeyt in 2011 investigated how the presence or absence of queens affected rival colony integration in *Temnothorax* ants. Colonies were split into either queenright or queenless fragments and then paired with another fragment. One fragment was placed inside a nest and became the 'host' colony, while the other fragment had their nest destroyed and was placed outside the nest of the host colony; this became the 'invader' colony. We were interested in whether queen presence in both the host and invader fragments affected the likelihood of colony fusions, and for successful fusions, the subsequent interactions between workers from different colonies. We expected that colonies would be less likely to fuse in the presence of a queen and that, for successful fusions, integration amongst host and invader workers in the nest would be lower in treatments with at least one queen.

5.3 Methods

5.3.1 Experimental design

Temnothorax colonies were collected for the experiment in March and June 2011 from an existing study site in Germany (Foitzik and Heinze, 2000) – an open pine-oak forest near Sommerhausen, 15 km south of Würzburg (10°02'–10°03' E, 49°42'–49°43' N). Colonies were maintained as described in Stroeymeyt et al. (2017); they were kept in controlled laboratory conditions (14 L : 10D cycle, 25°C, 55% RH) within plastic boxes (155 x 135 x 50 mm) that had Fluon-coated walls, which prevented ants from escaping. Initially, colonies were housed within the twigs from which they were collected, before being transferred to artificial nests made up of a cardboard perimeter wedged between two glass slides (resulting in a nest cavity of 36 x 48 x 12 mm, with an entrance of 8 x 2 mm). A 10% honey solution along with ad libitum water was fed to colonies weekly. Colonies were kept in the laboratory prior to the experiment for a mean ± SE of 80 ± 3 days.

Prior to the experiment, colonies were split into two fragments. Within a given fragment, workers were painted on their gasters the same colour as their nestmates, though any queen in a colony fragment was painted a different colour to her workers. Each fragment was painted with different colours. Seven colours were used across trials—green, light blue, dark blue, red, orange, pink and yellow—with colour use balanced across treatments. An experimental arena comprised an oval area (152 mm x 114 mm), within which was a rectangular nest container (outer wall = 76 x 50 mm, inner wall = 48 x 36 mm, entrance width = 3 mm, entrance length = 8 mm). At the start of an experimental trial, a colony fragment was placed in a nest inside the arena and became the 'host' (mean ± SD host size = 50 ± 23 individuals). After ca. 50 hours (during which the host colony had time to settle and acclimatise to the new nest and arena), another colony fragment was introduced outside the nest (within the arena) and became the 'invader' (mean ± SD invader size = 70 ± 31 individuals). The ratio of host-to-invader workers was tightly controlled across replicates. Above each nest, a camera (Canon Powershot G7) was held in place by two clamps (height above nest for camera body = 8.5 cm, height of lens = 3.5 cm). The entire nest was in view, but the remainder of the arena (where the invaders were introduced) was not in view. Photographs were taken at a resolution of 3648 x 2736 pixels in macro mode with no flash, with autofocus on, in JPEG format.

There were five different treatments: the control (invader and host belonged to the same original colony); worker–worker (WW; queenless invader and queenless host); queen–worker (QW; queenright invader, queenless host); worker–queen (WQ; queenless invader and queenright host); and queen–queen (QQ; queenright invader and queenright host). For the control treatment, the queen was alternated between host and invader for each replicate. Sixteen replicates were

performed per treatment. Photographs were taken every 15 minutes for 2 weeks, from when the host was introduced to the nest. After the first 10 trials were completed, photos were taken at a greater frequency (every 0.5 minutes) for the 2-hour period after the invaders were introduced, to ensure that more detail on the spatial dynamics was extracted at this important stage. Data corruption and the use of incorrect ant species meant that seven trials were excluded. This resulted in 73 trials for image processing (14 trials each for control and WQ treatments, 15 trials each for WW, QW and QQ treatments). The number of dead workers at the end of the experiment was counted, which we used for analysis of the proportion of host and invader workers that died across all trials (*N* = 73). We then performed an analysis on the likelihood of fusion using all trials (*N* = 73). Colony fusion was deemed successful if, by the end of the experiment, both colonies lived together in the nest. We then investigated measures of integration between colonies using those trials in which a successful fusion occurred (*N* = 50).

5.3.2 Coordinate extraction

To assess how treatment affected integration of successfully fused colonies, we required spatial coordinates of invader and host ants in the images. To extract coordinate data, we built an image-processing script (see **Chapter 4**). Overall, the script performed well, though we found that more light blue workers were missed compared to green workers, and more red queens were missed compared to other queen colours (see **Chapter 4**). However, as colour use was balanced across treatments (see **Chapter 4**), no treatment bias was introduced. We also calculated F1 scores (which range from 0 to 1, with 1 representing perfect performance) based on false positive and missed detections. F1 scores were at least 0.9 for all worker colours (**Chapter 4; Table 4.3**), so we were confident in using the coordinate data generated by the image-processing script for analysis. We used coordinate data to extract a count of host and invader numbers in the images, and for calculation of nearest-neighbour distances and a measure of colony overlap (details below).

5.3.3 Integration between fused colonies

5.3.3.1 Proportion of invader workers in the nest

We investigated whether the proportion of invader workers in the nest increased more slowly and plateaued at a lower level in treatments where at least one of the invader and host had a queen; we expected the slowest increase and lowest plateau when both invader and host had a queen. We used a proportion, rather than an absolute count, as the starting number of invader workers differed

between trials. As invader worker numbers in the nest rapidly increased and plateaued within the first 5000 minutes in successfully fused trials (**Figure 5.1)**, we used the R package *growthcurver* (Sprouffske, 2020) to fit logistic growth curves to each trial for this period (**Figure 5.2**). We extracted both the r (rate of growth) and K (carrying capacity) values from the curve generated for each trial (*N* = 50).

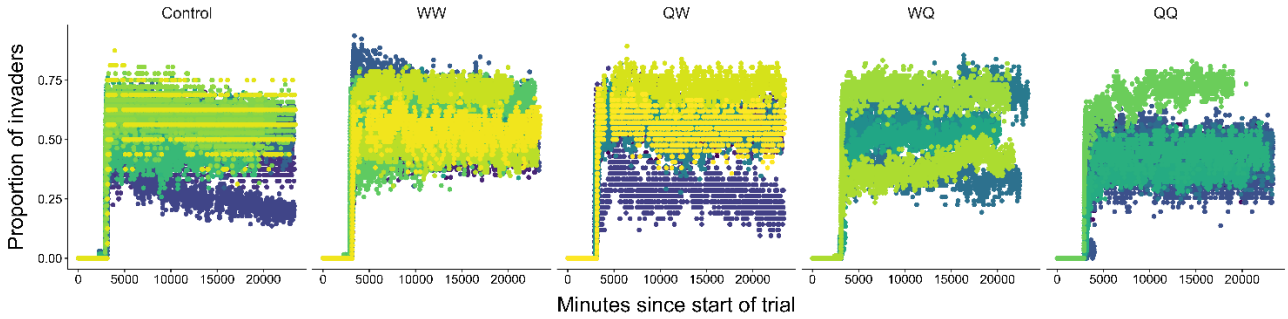

Figure 5.1. The proportion of invader workers detected in the nest across the entire trial. Per treatment, colour denotes trial ID. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen. Total successful fusion trials *N* = 50.

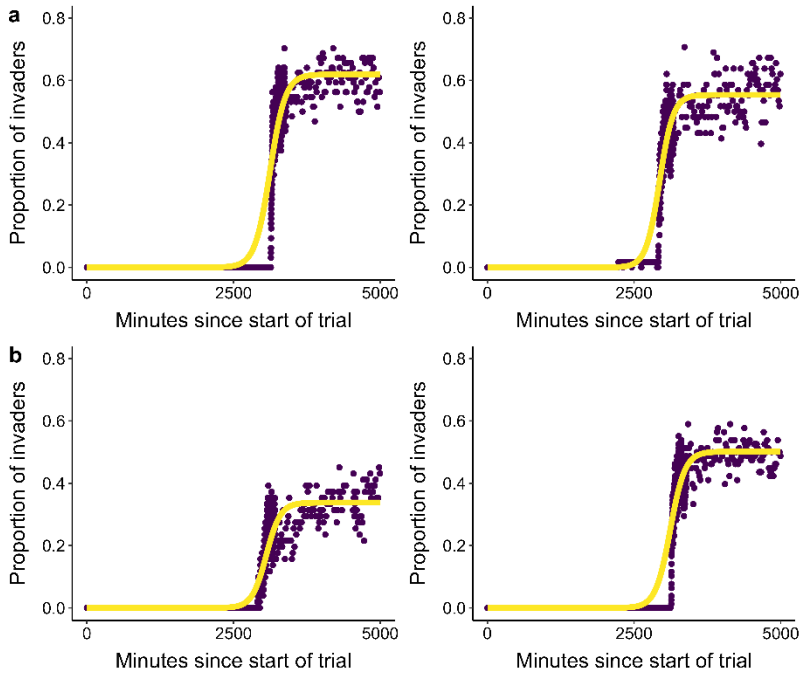

Figure 5.2. Examples of fitted logistic curves (in yellow) on the proportion of invader workers (purple dots are raw data) in the nest during the first 5000 minutes in (a) control trials and (b) queen–queen trials.

5.3.3.2 Proportion of host workers in the nest

We investigated whether more host workers left the nest immediately after introduction of the invaders in treatments where at least one of the invader and host had a queen; we expected more host workers to leave when both the invader and host had a queen. We used proportional data due to varying host numbers, and again examined the period within the first 5000 minutes of a trial. The number of host workers in the nest was generally lower than the maximum starting number for a trial. This was due to some ants losing paint marks due to grooming and missed detections of ants (see **Chapter 4**). So, to calculate the proportion of host workers in the nest, for each trial we divided the number in the nest for a given image by the maximum number of host workers detected in the nest during the first 5000 minutes. To assess the decrease in the proportion of host workers in the nest once the invader colony was introduced, we categorised the data for a given trial into pre-invader introduction and post-invader introduction periods. For each period, we fitted a linear model with proportion of host inside the nest as our response variable and minutes since the start of trial as the predictor. For each of the two models, we calculated the predicted proportion of host workers inside the nest at the point of invader introduction using the model coefficients (proportion of host workers = intercept + time of invader introduction * slope). We then took the difference in these values, resulting in a difference in the predicted proportion of host workers in the nest for each trial (**Figure 5.3**).

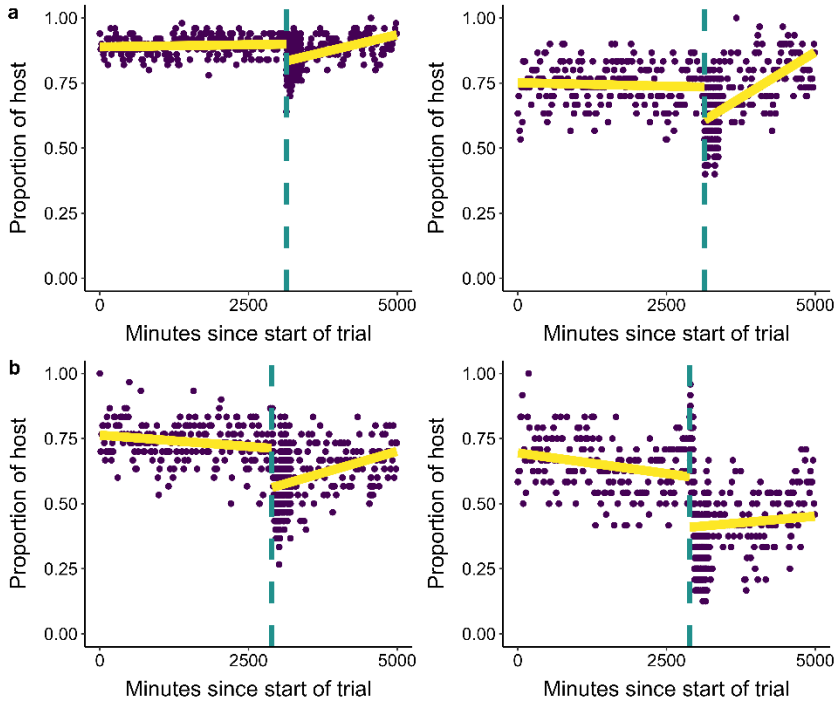

Figure 5.3. Proportion of host workers detected in the nest for (a) two control trials and (b) two queen–queen trials. Linear models (in yellow) were fitted for the pre-invader introduction period and the post-invader introduction period (purple dots are raw data), and the difference in the fitted Y value was taken at the point of invader introduction (denoted by vertical dashed lines).

5.3.3.3 Nearest-neighbour distances

After successful fusion, we expected there to be less integration between colony fragments in treatments where at least one of the invader and host had a queen; we anticipated the least integration where both invader and host had a queen. To assess colony integration, we used the R package *spatstat* (Baddeley et al., 2015) to extract nearest-neighbour distances between host and invader workers. For a given image, a distance was calculated from every individual worker to every worker from the rival colony. For each ant, a mean value of distances to rival group workers was then calculated. Finally, a mean across all ants was taken so that, for a given image, we had a single observed host–invader worker distance. We only used images with at least five host and five invader workers to calculate nearest-neighbour distances, to reduce the effect of any false positives.

As an additional check on the reliability of our automated image-processing script (see **Chapter 4**), we compared nearest-neighbour distances calculated from both manually and automatically extracted coordinates. Originally, we manually extracted coordinates for 100 images, but due to some images having zero invader workers, 83 images across 25 trials were used for

comparison (20 for control, 17 for WW and QW, 14 for WQ and 15 for QQ). We found no significant effect of treatment on the difference between manual and automated distance calculations (linear mixed model [LMM]: $F_{4,16.4} = 0.53$, $P = 0.717$; **Figure 5.4**), and thus no treatment bias from using our automated image-processing script.

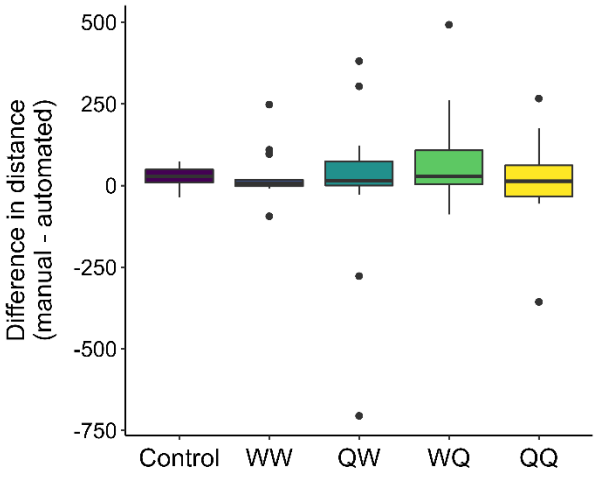

Figure 5.4. Difference in mean host–invader nearest-neighbour distances between manually and automatically processed images. Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range), black points show outliers. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen. *N* = 25 trials.

We then compared the observed nearest-neighbour distances to randomised host–invader worker distances. To calculate a random distribution for a given image, the labels of coordinates (i.e., invader or host) were randomly shuffled, and we repeated the above process of nearest-neighbour calculations for 100 randomisations of each image. Each randomisation generated a mean host–invader worker distance for the image. The mean value of the 100 randomised distances was then taken, resulting in a single mean randomised host–invader distance per image. For our analysis of nearest-neighbour distances, we used the difference between the observed and mean randomised values for each image as our response variable. These differences show how far observed nearest-neighbour distances deviate from random expectations. A small difference indicates that distances between host and invader workers closely match a random distribution (i.e., they are scattered throughout the nest and are integrated). Conversely, a larger difference indicates greater distances between host and invader ants relative to random (i.e., there is more colony separation and spatial segregation between colonies).

5.3.3.4 Overlap of host and invader colonies

As a second measure of the post-fusion level of integration, we calculated the amount of overlap in space-use between host and invader colonies, expecting that overlap would be lower in treatments where at least one of the invader and host had a queen; we expected the lowest overlap when both invader and host had a queen. To do so, we used methods typically used for home-range analysis. There are two main methods to analyse home-range data – using geometric or statistical techniques (Fleming et al., 2015). Geometric techniques include, for example, calculating a minimum convex polygon to generate the smallest shape possible around a set of coordinates. While this is simple, it often leads to overestimations of home ranges (Baíllo and Chacón, 2020). Instead, we used statistical techniques, relying on kernel density estimates (KDE). Specifically, we used the R package *adehabitatHR* to calculate the utilisation distribution overlap index (UDOI) between 50% core areas. This gave an idea of the size of overlap between colonies based on their coordinate locations across images. We used this metric as it is recommended for assessing how space is shared between home ranges, while some other metrics (e.g., volume of intersection or Bhattacharyya's affinity) are better for assessing the similarity between two home ranges (Fieberg and Kochanny, 2005). A minimum of five coordinates was required to calculate home ranges; thus, images with fewer than five host or invader workers were excluded.

Similar to our nearest-neighbour analysis, we also checked whether the UDOI between host and invader colonies differed between manually and automatically extracted coordinates. As some of the 100 images we manually extracted coordinates for in **Chapter 4** had fewer than five host or invader individuals, 58 images across 17 trials were used for comparison (20 for control, 11 for WW, 12 for QW, 3 for WQ and 12 for QQ). We found no significant effect of treatment on the difference in UDOI (LMM: $F_{4,47} = 0.64$, $P = 0.635$; **Figure 5.5**), and thus no treatment bias from using our automated image-processing script.

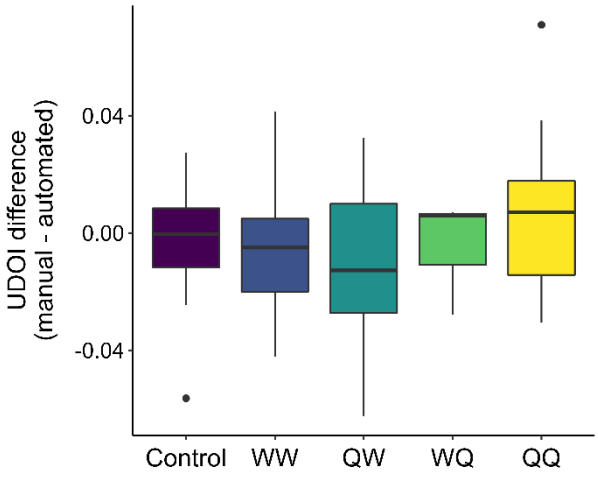

Figure 5.5. Difference in UDOI between manually and automatically checked images. Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range) and black points shower outliers. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen. *N* = 17 trials.

5.3.5 Statistical analysis

All analysis was conducted in R (R Core Team, 2022). Assumptions of parametric statistical testing were checked by visually inspecting residuals. Where assumptions were violated, the non-parametric equivalent was used. For data manipulation and cleaning, we used the *tidyverse* package (Wickham et al., 2019). Figures were created using *ggplot2* and *cowplot* (Wickham, 2016; Wilke, 2020), and colour palettes used were from the colour-blind friendly *viridis* package (Garnier et al., 2021).

For mortality analysis, we performed one-way ANOVAs on the proportion of host and invader workers that died across all trials (*N* = 73), with treatment as the only predictor variable. For analysis of the number of successful fusions across treatments (*N* = 73), we used a Fisher's exact test, as the expected frequencies from using a chi-square text were below 5 for at least one category. Post hoc comparisons for this test were performed using the R package *rcompanion* (Mangiafico, 2015). To reduce Type I errors (i.e., false positives), Benjamini-Hochberg corrections to *P* values were made (Benjamini and Hochberg, 1995). Subsequent analyses were performed on trials where there had been a successful fusion (*N* = 50). For the proportion of invaders and host workers in the nest, there was a single datapoint per trial for each response variable, so we performed one-way ANOVAs with treatment as the single predictor variable. For nearest-neighbour distances and space overlap, we analysed data in two periods, as we expected colonies could integrate more as

time progressed. The first was a 48-hour period starting 24 hours post-fusion, once workers had stopped fighting; the second period was the final 48 hours of each trial. The mean number of images per trial for each treatment was similar for both periods (188–192 images across treatments in period 1 and 181–192 images for period 2). As there were multiple images per trial, we fitted LMMs using the R package *lme4* (Bates et al., 2015), with trial ID as a random intercept and treatment as a fixed effect. For nearest-neighbour distance analysis, data were log transformed to improve model fits. For significant and marginally non-significant effects (i.e., *P* values within 0.001 of an adjusted α of 0.05), we performed post hoc comparisons with the R package *emmeans* (Lenth, 2022). To avoid Type II errors (i.e., false negatives), post hoc tests did not include every possible comparison; instead, we compared the control treatment to every other treatment, and the QQ treatment against remaining treatments, as we expected this would show the largest differences. To reduce Type I errors (i.e., false positives), Benjamini-Hochberg corrections to *P* values were made (Benjamini and Hochberg, 1995).

5.4 Results

5.4.1 Mortality of invader and host workers

The proportion of invader workers that died across all trials was significantly affected by treatment (one-way ANOVA: $F_{4,68} = 5.59$, $P < 0.001$). Post-hoc tests showed that invader worker mortality was significantly higher in the QQ treatment than in the control, WW and QW treatments (**Table 5.1**). Mortality was also significantly higher in the WQ treatment than in the control (**Table 5.1**). Similarly, the proportion of host workers that died was significantly affected by treatment ($F_{4,68} = 3.56$, $P = 0.011$); post hoc tests revealed that host mortality in all treatments where at least one colony had a queen was significantly higher than in the control (**Table 5.2**).

Table 5.1. Post hoc comparisons of treatments with respect to the proportion of invader workers that died during the experiment. *P* values are adjusted for multiple testing (Benjamini-Hochberg procedure), with significant comparisons shown in bold. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen. *N* = 73.

	Estimate ± SE	***t***	***P***
Control – WW	-0.08±0.06	-1.30	0.279
Control – QW	-0.06±0.06	-0.97	0.391
Control – WQ	**-0.21±0.06**	**-3.52**	**0.003**
Control – QQ	**-0.22±0.06**	**-3.79**	**0.002**
QQ – WW	**0.14±0.06**	**2.53**	**0.024**
QQ – QW	**0.16±0.06**	**2.87**	**0.013**
QQ – WQ	0.01±0.06	0.21	0.836

Table 5.2. Post hoc comparisons of treatments with respect to the proportion of host workers that died during the experiment. *P* values are adjusted for multiple testing (Benjamini-Hochberg procedure), with significant comparisons shown in bold. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen. *N* = 73.

	Estimate ± SE	***t***	***P***
Control – WW	-0.13±0.07	-1.91	0.106
Control – QW	**-0.17±0.07**	**-2.59**	**0.028**
Control – WQ	**-0.22±0.07**	**-3.15**	**0.009**
Control – QQ	**-0.22±0.07**	**-3.29**	**0.009**
QQ – WW	0.09±0.07	1.40	0.231
QQ – QW	0.05±0.07	0.71	0.559
QQ – WQ	0.01±0.07	0.09	0.933

5.4.2 Number of successful fusions

Treatment significantly affected the number of successful fusions (Fisher's exact test: *P* = 0.002; **Figure 5.6**). Post hoc tests showed that, compared to the control treatment, colonies in the QQ (*P* = 0.003) and WQ (*P* = 0.019) treatments were significantly less likely to fuse, whilst there was a strong but statistically non-significant trend for colonies in the QW treatment to be less likely to fuse (*P* = 0.052). In addition, there was a strong but statistically non-significant trend for colonies in the WQ treatment to be less likely to fuse than those in the WW treatment (*P* = 0.052).

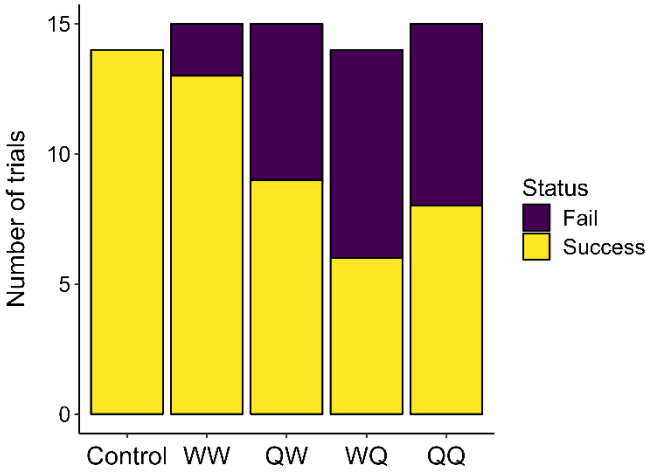

Figure 5.6. The number of failed and successful fusions in the five different treatments. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen. *N* = 73.

5.4.3 Proportion of invader workers in the nest

Treatment significantly affected the r value (rate of growth) of the proportion of invader workers in the nest (one-way ANOVA: $F_{4,45} = 4.14$, $P = 0.006$; **Figure 5.7a**), but had no significant effect on the K value (carrying capacity; $F_{4,45} = 2.16$, $P = 0.089$; **Figure 5.7b**). Post-hoc comparisons showed that the r value for the QQ treatment was significantly lower than that for the control, QW and WW treatments (**Table 5.3, Figure 5.7a**). None of the other paired comparisons were significant (**Table 5.3**).

Table 5.3. Post-hoc comparisons on the effect of treatment on the r value of logistic curves. *P* values are adjusted for multiple testing (Benjamini-Hochberg procedure), with significant comparisons shown in bold. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen.

	Estimate ± SE	***t***	***P***
Control – WW	-0.0001 ± 0.0005	-0.12	0.904
Control – QW	0.0004 ± 0.0006	0.69	0.578
Control – WQ	0.0009 ± 0.0006	1.35	0.258
Control – QQ	**0.0021 ± 0.0006**	**3.58**	**0.003**
QQ – WW	**-0.0021 ± 0.0006**	**-3.63**	**0.003**
QQ – QW	**-0.0017 ± 0.0006**	**-2.66**	**0.025**
QQ – WQ	-0.0012 ± 0.0007	-1.72	0.163

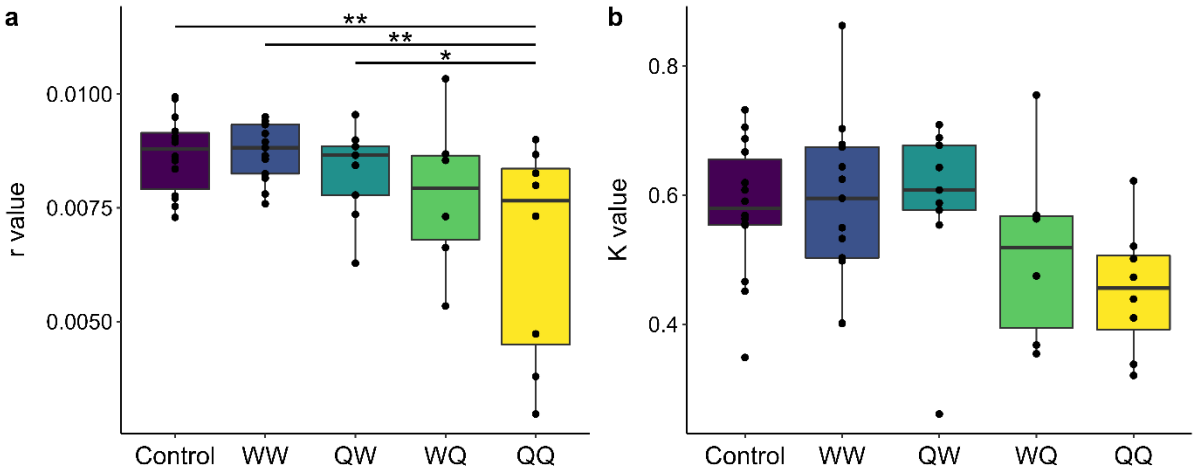

Figure 5.7. (a) r values and (b) K values from logistic curves fitted on trials where there was successful fusion between colonies. Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range). Raw data displayed as black points. * $P < 0.05$, ** $P < 0.01$, $N = 50$ trials. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen.

5.4.4 Proportion of host workers in the nest

Treatment had a near-significant effect on the proportion of host workers leaving the nest after invaders were introduced (one-way ANOVA: $F_{4,45} = 2.58$, $P = 0.050$). As such, we performed post hoc tests. Though no post-hoc comparisons were significant, there were strong trends for the proportion of host workers leaving the nest in the WQ and WW treatments to be higher than in the control treatment (**Table 5.4; Figure 5.8**).

Table 5.4. Post-hoc comparisons on the effect of treatment on the difference in predicted proportions of host workers in the nest before and after invader introduction. *P* values are adjusted for multiple testing (Benjamini-Hochberg procedure). WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen.

	Estimate ± SE	*t*	*P*
Control – WW	-0.11 ± 0.05	-2.47	0.062
Control – QW	-0.07 ± 0.05	-1.46	0.264
Control – WQ	-0.16 ± 0.06	-2.80	0.053
Control – QQ	-0.09 ± 0.05	-1.79	0.188
QQ – WW	-0.02 ± 0.05	-0.35	0.732
QQ – QW	0.02 ± 0.06	0.35	0.732
QQ – WQ	-0.07 ± 0.06	-1.06	0.413

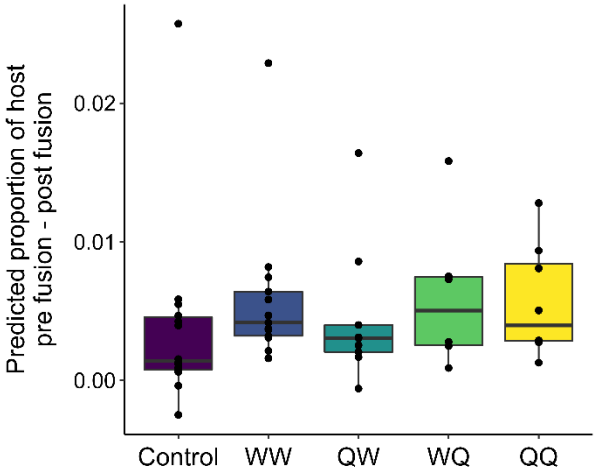

Figure 5.8. The proportion of host workers leaving the nest when invaders were introduced, calculated from the difference in predicted y values from two linear regressions for the pre-introduction and post-introduction periods. Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range). Raw data displayed as black points. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen. *N* = 50 trials.

5.4.5 Nearest-neighbour distances

In period 1 (soon after fusion), treatment significantly affected the difference between randomised and observed nearest-neighbour distances (LMM: $F_{4,45}$ = 2.84, P = 0.035). Treatment also had a borderline significant effect on the difference in period 2 (at the end of the trial; $F_{4,45.03}$ = 2.57, P = 0.050). As such, we performed post hoc tests on both periods, but as no comparisons were close to significantly different for period 2 (all P > 0.15), we only present comparisons for period 1 (**Table 5.5**). Differences between randomised and observed nearest-neighbour distances indicate how far distances deviate from random; a larger difference indicates more spatial segregation between colonies. Only the QQ treatment was significantly higher than the control treatment, indicating that host and invader workers were more segregated in the QQ than the control treatment. In addition, both the QW and WQ treatments were close to significantly higher than the control, but no other comparisons were significant (**Table 5.5; Figure 5.9a**).

Table 5.5. Post-hoc comparisons on the effect of treatment on the log-transformed difference between observed and randomised nearest-neighbour distances. *P* values are adjusted for multiple testing (Benjamini-Hochberg procedure), with significant differences shown in bold. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen.

Contrast	Estimate ± SE	*Z* ratio	*P*
Control – WW	-0.11 ± 0.09	-1.16	0.343
Control – QW	-0.22 ± 0.1	-2.15	0.082
Control – WQ	-0.24 ± 0.11	-2.11	0.082
Control – QQ	**-0.31 ± 0.1**	**-2.96**	**0.022**
QQ – WW	0.20 ± 0.11	1.92	0.096
QQ – QW	0.09 ± 0.11	0.80	0.492
QQ – WQ	0.07 ± 0.13	0.52	0.600

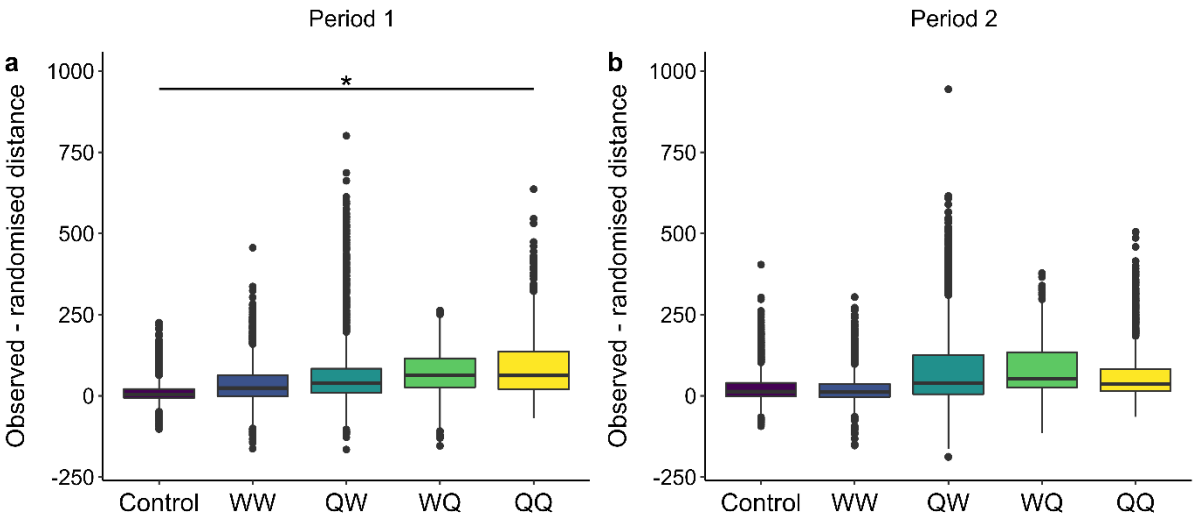

Figure 5.9. Difference (in pixels) between observed nearest-neighbour distances and randomised nearest-neighbour distances following successful fusions, in (a) the 48 h after colonies fused and (b) the final 48 h of the trial. Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range). Raw data displayed as black points. * $P < 0.05$. $N = 50$ trials. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen.

5.4.6 Spatial overlap

Treatment had a significant effect on the UDOI (level of overlap) between host and invader colonies in period 1 (soon after fusion; LMM: $F_{4,45} = 3.27$, $P = 0.020$), but not in period 2 (at the end of the trial; $F_{4,45.02} = 1.62$, $P = 0.186$). Post hoc tests showed that all treatments with a queen present had significantly lower overlap between host and invader colonies than the control, with all differences showing similar effect sizes (**Table 5.6; Figure 5.10**). In other words, host and invader colonies remained more segregated from each other in treatments with a queen. No other comparisons were significant.

Table 5.6. Post-hoc comparisons on the effect of treatment on the space overlap (UDOI) between host and invader workers. *P* values are adjusted for multiple testing (Benjamini-Hochberg procedure), with significant differences shown in bold. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen.

Contrast	Estimate ± SE	*Z* ratio	*P*
Control – WW	0.02 ± 0.01	1.56	0.179
Control – QW	**0.04 ± 0.02**	**2.46**	**0.033**
Control – WQ	**0.05 ± 0.02**	**2.69**	**0.025**
Control – QQ	**0.05 ± 0.02**	**2.90**	**0.025**
QQ – WW	-0.03 ± 0.02	-1.52	0.179
QQ – QW	-0.01 ± 0.02	-0.48	0.734
QQ – WQ	0.00 ± 0.02	0.05	0.959

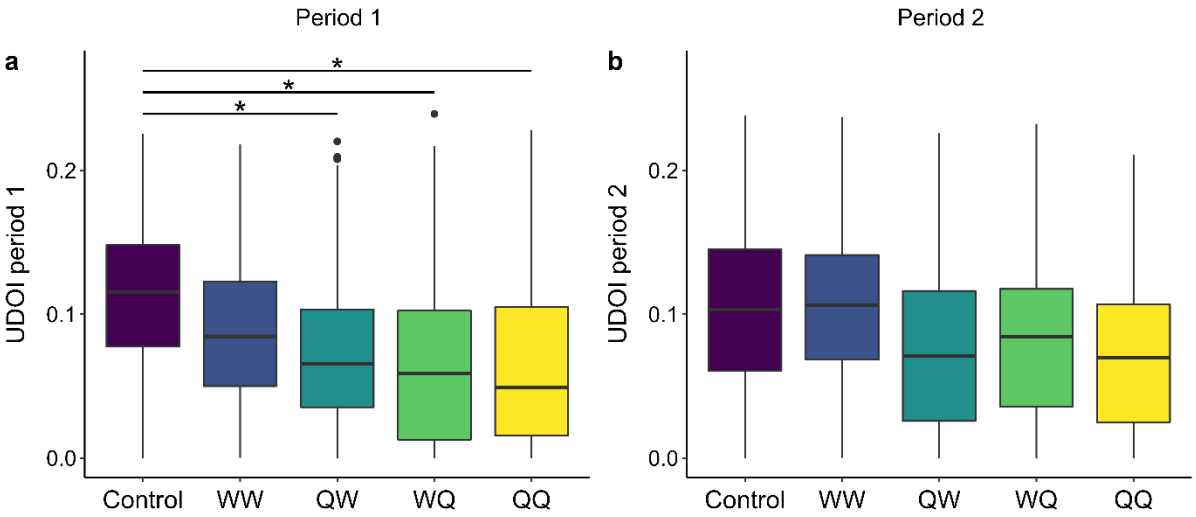

Figure 5.10. UDOI of host and invader workers for (a) the 48 h after colonies fused and (b) the final 48 h of the trial. Box plots show medians and quartiles, whiskers show upper and lower quartiles (± 1.5 times the interquartile range). Raw data displayed as black points. * $P < 0.05$. $N = 50$ trials. WW = invader worker–host worker, QW = invader queen–host worker, WQ = invader worker–host queen, QQ = invader queen–host queen.

5.5 Discussion

Our experiment showed that the presence of queens affected the mortality of workers and the likelihood of fusion between host and invader *Temnothorax nylanderi* colonies, as well as the subsequent integration of those colonies that did fuse. Invader worker mortality was higher where both colonies had a queen compared to the control (the reuniting of two parts of the same original colony) and treatments in which colonies had no queens (WW) and there was an invader queen and no host queen (QW). Invader mortality was also higher in treatments with no invader queen and a host queen (WQ) than the control. Host worker mortality was higher in all treatments with a queen compared to the control. Fusion was less likely where one or both colonies had a queen than in treatments where neither colony had a queen (WW) or the control. Subsequent analyses of integration in successful fusions found that invader workers entered the nest at a slower rate where both colonies had a queen (QQ) compared to all other treatments except that with a queenless invader and queenright host (WQ); a similar trend was observed for the proportion of host ants leaving the nest after fusion. We also found that, near the start of the experiment, nearest-neighbour distances between host and invaders in the QQ treatment differed more from random than control treatments, suggesting more separation between colonies. In addition, there was lower overlap of host and invader colonies where at least one queen was present compared to control

colonies. These differences in integration were no longer present toward the end of the experiment. Fusion and integration dynamics may be affected by the presence of queens for several reasons, which are discussed below.

Fusion was less likely in treatments with at least one queen, and mortality of host or invader workers was higher in all treatments with a queen compared to the control. This may suggest a greater motivation to avoid fusion through increased fighting. As only one queen survives colony fusion (Foitzik and Heinze, 1998), workers should be able to maximise their inclusive fitness gains by raising their current brood and ensuring future reproduction of their queen in the following year. Previous work found that introducing an invader colony outside a host's nest led to fighting between workers, including the host attempting to remove invader workers (Foitzik and Heinze, 1998). If the invader colony was large enough, they continued to enter the nest and also transported their own brood in; invaders failed to enter in cases where the host was relatively large to the invader (Foitzik and Heinze, 1998). In *Formica selysi* ants, acceptance of an alien queen was more likely if she was accompanied by workers than if she was alone, possibly due to an increased risk of fighting associated with additional rival workers (De Gasperin et al., 2021). For our experiment, it could be that failed fusions arise when one colony is able to repel the rival successfully, whilst fusions occur where fighting forces are similarly matched; for example, in terms of the size or fighting ability of colonies, which may affect the overall outcome of conflict (Lanchester, 1916; Plowes and Adams, 2005). There may be a point during conflict at which colonies assess that the cost of coexisting with a rival colony is lower than the potential or realised costs associated with conflict, including mortality, increased energy expenditure and lack of future defensive ability (Green et al., 2021; Rudolph and McEntee, 2016). Ants have been shown to modulate their aggressiveness based on a number of factors, including the size and behaviour of opponents (Adams, 2016; Tanner and Adler, 2009), though future work should examine how workers assess a rival colony with a queen present compared to one with no queen. For example, the presence of a queen itself may be enough to indicate that rival workers are more willing to defend their colony, or a queen pheromone could attract workers and lead to more cohesive colonies less likely to fuse.

For successfully fused colonies, the rate at which invader workers entered the host's nest was slower when both colonies had a queen (QQ) compared to the control, WW and QW (invader queenright – host queenless colonies), and a similar (nonsignificant) trend was observed for the number at which invaders plateau in the nest. This could be a result of increased worker mortality; increased fighting outside the nest between queenright colonies prior to fusion may have prevented invaders from entering as quickly, in addition to a reduced number of workers being able to enter. While we didn't find that more host workers left the nest after invader introduction in the QQ

treatment, multiple invader workers could be engaged with fighting fewer host workers. Alternatively, workers may stay closer to their queen to protect her, in which case we might expect a slower rate of entry of invaders and no clear increase in host numbers leaving the nest in the QQ treatment, due to the presence of queens in both colonies. After fusion, the QQ treatment had a lower number of invaders in the nest, with a similar trend overall being apparent for the WQ treatment. By contrast, for queenright invaders entering a queenless nest (QW), the rate at which they entered was similar to control and WW treatments, and higher than QQ fusions, which may indicate a queenless host nest is easier to overthrow, for example due to lower motivation or ability to fight.

The costs of fusions could be partly mitigated by workers hedging their bets. Foitzik and Heinze (1998) argue that workers should still want to avoid fusion or takeovers as they can raise their existing brood without a queen. They therefore classify nest takeovers as a form of parasitism, which is somewhat similar to that of avian brood parasitism (Davies and Brooke, 1988). However, even after fusions, workers may still be able to produce sons and gain direct fitness benefits, though worker-produced males appear to be uncommon (Foitzik and Heinze, 2001). In certain species in which workers can become reproductive, such as some termites, workers may benefit from fusions as the chance of them becoming reproductive increases after the death of a queen (Johns et al., 2009; Kellner et al., 2010; Korb and Roux, 2012).

We found that, immediately after fusion, nearest-neighbour distances between host and invaders in the QQ treatment differed more from random than control treatments, indicating more separation between colonies. In addition, we found that overlap of host and invader colonies was lower in all treatments with at least one queen compared to control colonies. By the end of the experiment, colonies were more integrated; we found no treatment differences in nearest-neighbour distances or overlap, although there was still a non-significant pattern that all treatments with a queen had lower levels of overlap compared to control and WW fusions. This could suggest there may be a window of opportunity post-fusion for both colonies to regain colony integrity by splitting again if other nest sites become available. By remaining segregated even after fusion, colonies may be able to disperse more quickly. Previous work has shown that, after removing *Temnothorax* colonies from an area, neighbouring colonies rapidly migrate into the area to fill empty nest sites (Foitzik and Heinze, 1998). The ability to migrate quickly could be particularly important for QQ fusions, which showed the greatest segregation, as generally only one queen survives until the next season (Strätz et al., 2002). Over time, the chance of a new nest site may have diminished, or colonies may integrate more due to a loss in ability to discriminate between nestmates and non-nestmates. In *Temnothorax nylanderi*, environmental odour cues are important in nestmate

recognition (Foitzik et al., 2007)**;** given both host and invader are in the same nest, their colony odour may become indistinguishable. Previous observations of alien queen introductions to orphaned worker colonies showed that workers were aggressive towards a new queen, but that this decreased with time (Strätz et al., 2002). Workers could perhaps avoid antagonism at first by remaining further away from the core area of rival workers. However, further work would be needed to confirm the reasons for integration over time; for example, by comparing the speed of nest site relocation at different points post-fusion, as well as testing whether colony discrimination declines over time in fused colonies.

While fusions are expected to be costly, particularly for the usurped colony, there may be some benefits to colony fusion after fighting has taken place, such as improved foraging efficiency and greater division of labour due to a greater workforce (De Gasperin et al., 2021; Ulrich et al., 2018). In acacia ants (*Crematogaster mimosa*e), conflict over acacia trees can result in high mortality, and victors often fuse with workers and brood from the losing colony, potentially as a way of boosting colony size to protect the colony better from competition or herbivores feeding on acacia (Rudolph and McEntee, 2016). It may get to the point that continued fighting is more costly than fusion itself, and fusion may help to recuperate lost worker numbers. This may avoid a higher vulnerability of colonies and help to ensure future foraging success and future colony defence in *Temnothorax* ants, in which high densities of colonies is common (Foitzik and Heinze, 1998). Alternatively, the fusion of colonies with unrelated queens could perhaps be due to homogenisation of colony odours or habituation to rival odour (Rudolph and McEntee, 2016; Stroeymeyt et al., 2010). Previous work has shown the nesting material of colonies is important in nestmate recognition, with more aggression directed toward ants from different nesting material, even conspecifics from the same nest experimentally split into different nests (Heinze et al., 1996). While a rapid change in the discrimination of odours between colonies is possible, this is perhaps unlikely here given that we found colonies still showed segregation after fighting had stopped, suggesting that recognition systems were still intact. In both single-queen colonies of ants that show exchange of workers and in supercolonies, studies have shown that workers are still able to discriminate between nestmates and other colonies despite the high level of integration (Holzer et al., 2006; Steiner et al., 2007).

Colony fusions have been observed in several species including ants and termites, typically where competition for limited resources is high (Foitzik and Heinze, 2000; Johns et al., 2009; Kellner et al., 2010; Korb and Roux, 2012; Rudolph and McEntee, 2016). Across taxa, fission–fusion dynamics are found in some social vertebrates, in which space use or limiting resources may also be an important factor in determining social grouping (Baden et al., 2020). Fusions in Hymenoptera could

be a form of intraspecific parasitism, with colonies making the best out of a bad situation, or a result of inadequate recognition systems (Davies and Brooke, 1988; Foitzik and Heinze, 1998; van Wilgenburg et al., 2006). We have shown that queenright colonies are less likely to fuse and that they appear to be more motivated to fight, evidenced by higher worker mortality. Costly fusions may be unavoidable in densely populated habitats, though some mechanisms may exist to reduce these costs, such as remaining segregated in the hope of a new nest site. There could be other benefits of fusions such as reduced future conflict over limited resources, rapid recovery from conflict and larger colony size resulting in greater competitive ability (Johns et al., 2009; Kellner et al., 2010; Rudolph and McEntee, 2016; van Wilgenburg et al., 2006). More work should be done to characterise the costs of failing to fuse and the role of queen fertility signals in affecting worker fighting behaviour, to understand fully the motivations of workers and the mechanisms behind fusions.

Chapter 6

General Discussion

6.1 Synthesis

Group living is ubiquitous in animal taxa and arises due to the fitness benefits that individuals gain (Krause and Ruxton, 2002). But there is much variation amongst group members, leading to differing motivations. This can cause conflict over group decisions, including those relating to movement (Conradt and Roper, 2009). Intrinsic characteristics, such as sex and age, may influence movement decisions and make certain individuals more likely to lead or to follow (Fischhoff et al., 2007; Furrer et al., 2012; King et al., 2008). The presence of key individuals, such as matriarchs or social insect queens, may also have a large influence on within-group behaviour and group decisions (Brent et al., 2015; Conte and Hefetz, 2008). As well as intrinsic characteristic of group members, extrinsic factors, such as intergroup conflict, also have significant effects on the behaviour of groups, in relation to movement decisions, group defence and space use (Christensen et al., 2016; Radford, 2004; Radford and Fawcett, 2014; Rudolph and McEntee, 2016). There has been a small amount of work investigating how intrinsic characteristics of groups interact with extrinsic factors in influencing group behaviour (e.g., see Johnstone et al., 2020), but further work is needed.

This thesis uses two model systems to investigate how both intrinsic and extrinsic factors affect group decision-making about movement and space use. Long-term observational data on dwarf mongooses (*Helogale parvula*) were used in **Chapter 2** to show that dominants and males were the most likely to lead from the morning sleeping burrow, particularly in the breeding season, and also the day after an intergroup interaction (IGI). In addition, male leadership increased in the breeding season when the group was in territorial areas that overlapped with usage by rival groups. In **Chapter 3**, experimental playbacks were used to investigate followership in response to a movement call during foraging. Movement calls are reported widely in social species, but their function has rarely been explicitly tested (Gall and Manser, 2017; Sperber et al., 2017). A first experiment confirmed the function of movement calls in dwarf mongooses; subsequent experiments showed that individuals respond similarly to both dominants and subordinates giving this call type, but that the threat of intergroup conflict did not affect responses. In subsequent chapters, data from a laboratory experiment on the ant *Temnothorax nylanderi*, allowing tightly controlled conditions, were used to examine how the presence or absence or queens affects the fusion of colonies. **Chapter 4** presents the methodology developed to allow automated processing of images taken of fusions between colonies, and **Chapter 5** presents the results of the experiment. The presence of at least one queen led to higher worker mortality and meant fusions were less likely. Of those that successfully fused, colonies with at least one queen were more separated in terms of their nearest-neighbour distances and overlapping areas. These results suggest that fusion is most costly for

queenright colonies and that they may attempt to stay separate even when fused, in case they can migrate to a new nest site subsequently. The following sections discuss these findings in the broader context.

Intrinsic and extrinsic characteristics affect within-group dynamics and interactions, and subsequently group decisions. In a review article, Farine et al. (2015) highlight the importance of considering "group phenotypic compositions" on the outcomes of group behaviour, and how this ultimately influences individual selection. **Chapter 3** demonstrated that movement decisions are affected by dominance status, while **Chapters 4 and 5** showed that colony fusions occur between non-related *Temnothroax nylanderi* groups, but that queen presence increases mortality, makes fusion less likely and colonies that do fuse appear to remain segregated for some time. What is unknown is the long-term fitness consequences of these outcomes. For instance, dominant leadership from the sleeping burrow in dwarf mongooses could lead to fitness gains through better foraging ability of the group, or fused ant colonies with a non-related queen might perform worse in collective tasks such as nest defence. Alternatively, if ant colonies are larger, they may gain some advantages over smaller colonies (Rudolph and McEntee, 2016). The fitness consequences of mongoose movement decisions could be studied using body-mass data as a proxy for foraging success; the habituated study population are trained to climb onto a balance scale for a small food reward, and are weighed up to three times per day. For example, it would be possible to assess whether groups collectively consume more food when a dominant individual leads. Further laboratory work on ants would provide an ideal setup for experimental manipulations into the fitness consequences of queen presence (see **6.2 Moving forward**).

Existing research has shown that movement patterns and space use of species change post-conflict. For example, green woodhoopoes (*Phoeniculus purpureus*) and the seed-harvesting ant (*Messor andrei*) later return to the site of conflict (Brown and Gordon, 2000; Radford and Fawcett, 2014), dwarf mongooses spend longer in the area after encountering rival cues (Christensen et al., 2016), and male red foxes (*Vulpes vulpes*) move their home range towards the site of rival cues (Arnold et al., 2011). The reasons for changes in movement patterns are speculated to include territory defence, conflict avoidance or the gathering of information. However, we don't know whether movements in social species within this context are driven by a single leader or shared amongst group members. In addition, little work has directly investigated how leadership and followership is affected by conflict. One exception is research into banded mongooses (*Mungos mungo*) showing that dominant females appear to lead groups into conflict at the cost of males (Johnstone et al., 2020). The analysis in **Chapter 2** showed that conflict interacts with intrinsic characteristics of group members when determining leadership, while there was no evidence in

Chapter 3 that conflict affects responses to movement calls. Further work is needed to understand how conflict affects leader and followership, which could in turn help our understanding of the mechanisms behind changes to group movement patterns. More observational work of who leads and who follows directly after intergroup conflicts (or experimental cues) could suggest which individuals are more influential in directing movements post-conflict.

Chapter 2 and **3** also showed that the timing and context of movement may affect leadership and followership decisions. For example, whilst dominants led more group movements from the sleeping burrow (**Chapter 2**), there was no difference in response to experimental playback of dominant and subordinate movement calls while foraging (**Chapter 3**). However, further work would be needed to compare different contexts directly; for instance, comparing responses to movement calls from a sleeping burrow and during foraging. Work on meerkats (*Suricata suricatta*), for example, suggests that both "lead" calls (when a single individual initiates movement and signals) and "move" calls (where multiple individuals signal to move while foraging) are important in group movements (Gall et al., 2017), and that the frequency of these vocalisations depends on the context, including time until sunset and distance to the sleeping burrow (Gall et al., 2017). While both dominant and subordinate meerkats vocalised at a similar rate in this context, when departing from a sleeping burrow, dominant females in the breeding season vocalised more and led more (Turbé, 2006). With the dwarf mongooses, we might expect different responses depending on the time of day. In the morning, group members are not satiated, so following a dominant might provide the best chance of eating sooner if they are more experienced. Alternatively, movements usually occur after bouts of grooming, which could also influence leader–follower decisions if grooming garners social support or strengthens social bonds (Kern and Radford, 2018; King et al., 2008). By contrast, once the group has foraged for several hours, dominants (who sometimes displace subordinates from food items) might be satiated and want to move elsewhere (e.g., to communal latrine sites). If some group members are still not satiated, they may prefer to remain in the same foraging patch and be less likely to respond differently to a movement attempt by a dominant.

There has recently been an increase in interest in how intergroup conflict affects the longer-term behaviour of groups (Bateman et al., 2015; Braga Goncalves et al., 2022, 2022; Kranstauber et al., 2020; Lemoine et al., 2020; Morris-Drake et al., 2021a). For example, previous work in dwarf mongooses shows that grooming increased the day after simulated group intrusions, and that multiple intrusions over several days caused groups to forage less and to become more cohesive (Morris-Drake et al., 2021a). Similarly, **Chapter 2** showed that leadership of dwarf mongoose groups is affected the day after conflict, and **Chapter 5** indicated that the effects of *Temnothorax* fusions can last over the subsequent two weeks. As leaders will influence where social groups move, conflict

is also likely to also have long-term effects on territory boundaries. In meerkats, for example, there is some evidence that territory shifts could be caused by frequent IGIs (Kranstauber et al., 2020). It would be interesting to know, both in the meerkats and other social species, which direction groups move in after conflict, who leads and how this could influence territory shifts. Fusions between invertebrate colonies also appear to have long-term impacts on behaviour. For example, in fusions between colonies of the termite *Cryptotermes secundus*, workers may be waiting to become sexual and gain delayed benefits from fusions (Korb and Roux, 2012), so some behavioural changes may not be observed immediately. In the Tem*nothorax* experiment, colonies with a queen still showed a tendency to be separated even after two weeks (**Chapter 5**), similarly suggesting long-term impacts of conflict and rival colony presence.

Analyses in **Chapters 2** and **5** used coordinates from GPS devices and from images of an experimental arena to analyse space use. Use of GPS data, video recording and tracking technology in the last decade has allowed new insights into animal behaviour (Christensen et al., 2016; Couzin and Heins, 2023; Ioannou et al., 2019; Lambert et al., 2021; Stroeymeyt et al., 2018). For example, GPS data can be combined with satellite images to examine how extrinsic factors, such as habitat structure, affect movement decisions (Strandburg-Peshkin et al., 2017). While certain technologies like drones can be valuable in tracking movement of some species, they are still limited in the use of mammals such as dwarf mongooses that remain vigilant for aerial predators. To understand fully leader and follower dynamics in dwarf mongooses, one possibility is using individual GPS trackers to determine, for example, whether the leader consistently remains the leader during movements, and whether other followers can influence direction too. In flocks of pigeons for example, use of individual GPS trackers enabled researchers to show that there is a leader–follower hierarchy (Nagy et al., 2010), and GPS collars have been used to investigate how intrinsic characteristics affect group movement direction and speed in meerkats (Averly et al., 2022). The use of GPS trackers combined with monitoring of vocalisations from multiple individuals could provide fascinating insights into collective movements.

6.2 Moving forward

Chapter 2 identified some intrinsic characteristics affecting leadership, so the next step would be to determine reasons why certain individuals would lead more under different contexts. For example, female leadership is often attributed to increased energetic requirements due to pregnancy (Fischhoff et al., 2007; Furrer et al., 2012; Turbé, 2006), because leading individuals may benefit from quicker access to resources (Fischhoff et al., 2007). Dominants might lead more due to greater

knowledge about profitable foraging patches. The habituated nature of the dwarf mongoose population means that it is feasible to collect accurate measures of food intake, as well as regular body-mass measurements, so differences depending on the identity of the leader could be assessed. Another possible reason for differences in leadership is that individuals could be acting selfishly, with the hungriest leading (Furrer et al., 2012); again, body-mass measures could help to determine the likelihood of this explanation. It would also be valuable for more research to evaluate the costs and benefits of leadership and followership. For example, leaders may be able to benefit from being the first to arrive at a food patch (Björnsson et al., 2018; Jolles et al., 2017; King et al., 2008), but also may suffer an increased predation risk (Ioannou et al., 2019). A simulated aerial predator could be used during a group movement, which should cause the group to suddenly perform evasive behaviours, moving to safe areas such as termite holes or tree and rock crevices (Rasa, 1987). Comparing the time taken to get to safety between leaders and followers could provide a proxy of likelihood of being predated depending on their role and spatial positioning. In addition, we could test the idea that experienced individuals (Rasa, 1987), such as dominants, might have better knowledge of hiding spots by assessing the average time taken for the group to get into cover, depending on who is leading.

Chapter 2 also showed that dominant male leadership increased the day after an IGI in the non-breeding season. To understand whether this was due to conflict avoidance (e.g., due to high costs of conflict; Stamps and Krishnan, 1998; Yi et al., 2020) or a desire to encourage territory defence, we would need to characterise IGIs better in dwarf mongooses. For example, recording whether the dominant male engages more in physical fights during IGIs and likely faces higher costs, as is the case in banded mongooses (Johnstone et al., 2020). Such data collection is inherently difficult because IGIs can be chaotic events, with much activity of fast-moving individuals obscured by vegetation. In addition, further work should look to use GPS or observational data to determine where the group goes after the dominant male leads the group. For instance, if the group returns to the site of conflict (Radford and Fawcett, 2014), then it may support the idea that males want to encourage territory defence. Conflict is already known to affect space use in dwarf mongooses following conflict (Christensen et al., 2016), as is the case for other taxa such as birds and invertebrates (Brown and Gordon, 2000; Radford and Fawcett, 2014). While we can assume that leadership decisions may impact subsequent movement patterns, linking leadership to these movement patterns would help fully understand how conflict affects space use.

Playback experiments have been widely used to investigate behavioural responses of animals, yet have been underused in the study of movement decisions. This could be because collective movement does not always require active communication but, in many species, vocal

repertoires include signals used to coordinate movement (Sperber et al., 2017). **Chapter 3** provided an example of one way to test the functionality of movement calls, but further work could use playbacks to test whether quorum thresholds exist in movement decisions (e.g., see Dibnah et al., 2022). Interactive playbacks (King, 2015) could also provide an important avenue of research. **Chapter 3** included a non-significant tendency for individuals to respond to movement calls with close calls. Interactive playbacks could help determine whether feedback from group members increases the likelihood of vocal responses to movement calls. It would also be interesting to generate conflicts of interests between group members by playing movement calls from different individuals at a similar time, to see if this helps elucidate how intrinsic characteristics affect preferences of group members. In meerkats, when a conflict of interest over which direction to travel was introduced (by training individuals to associate different locations of food rewards), Bousquet and Manser (2011) found that individuals chose group cohesion over their motivation for food.

Segregation between rival *Temnothorax* colonies in **Chapter 5** could be an adaptation to wait for future nest sites to become available again, which would demonstrate that colonies are planning for the future. Providing empty nest sites post-fusion would help confirm this hypothesis. Given that fusion of non-related colonies should be costly, a greater understanding of the costs and benefits of colony fusions is needed. In acacia ants, after experimentally induced conflicts, colonies suffered a drop in defence against herbivores, but colony fusions may provide a quick way to increase collective defence (Rudolph and McEntee, 2016). In the termite *Zootermopsis nevadensis*, colonies live in densely populated damp wood, and fights can cause the death of their kings and queens (Johns et al., 2009). Unlike some ant species, after the death of their king and queen, individuals can reproduce in the future and so may gain future benefits from fusion if they are able to reproduce. Similar to the acacia ants, larger colonies also have a better chance of defence and survival against future conflict (Johns et al., 2009; Thorne et al., 2003). Further laboratory work could compare the defensive, anti-predator and foraging abilities of fused vs non-fused colonies, to help us understand why colony fusions occur. For example, a rival colony (with its own nest to avoid further fusion) could be placed nearby a fused and non-fused colony, with limited food resources in between the colonies. Measures such as mortality could be recorded to determine whether fused or non-fused colonies fare better against a rival.

6.3 Conclusion

Overall, this thesis has considered how intrinsic and extrinsic factors affect within-group decisions in social species, both the immediate and longer-term impacts. **Chapters 2** and **3** combine observational and experimental work to study leader and followership in two different contexts, demonstrating that both intrinsic and extrinsic factors influence who leads, and confirm that movement signals are important in coordinating movement. **Chapters 4** and **5** use image processing of a laboratory experiment to show that queen presence affects colony fusions and may have long-lasting effects. Future work should aim to understand the fitness consequences of how intrinsic and extrinsic factors affect decision making in social species, combining the use of observational, in situ and laboratory experimental work.

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