The evolution of intergroup discrimination and colony fusion in the primitive termite, *Zootermopsis angusticollis*

Submitted by

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to the University of Exeter as a thesis for the degree of Masters by Research in Biological Sciences, September 2020.

I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Signed: R. Padget

Abstract

Groups of animals interact in a variety of different ways, from lethal conflict to cooperation and fusion. While intergroup conflict is relatively widely studied, the mechanisms and selective forces underpinning cooperation and group fusion are less well understood. The lower termites are a group of eusocial insects that displays a spectrum of intergroup relationships from violent battles to avoidance to colony fusion, making them a valuable model system with which to study intergroup interactions. In Chapter 2, I present a trial of a novel method of marking termites (Visible Implant Elastomer), finding that it causes slight changes to survival and behaviour associated with reproductive disinhibition. In the following chapters, I investigate two aspects of intergroup interactions in a single-piece nesting species of lower termite, *Zootermopsis angusticollis*: nestmate recognition and soldier caste ratio. In Chapter 3, I investigate the implications of colonies sharing similar nesting material on the ability of *Z. angusticollis* pseudergates to discriminate between members of their own and a different colony. I find that *Z. angusticollis* pseudergates discriminate between nestmates and non-nestmates but that this does not appear to be dependent on whether they encounter a non-nestmate raised on the same or a different wood type. Contrary to prediction, I also find that non-nestmates experience the same levels of cooperative allogrooming as nestmates. In Chapter 4, I use a theoretical model to examine the consequences of colony fusion on the sterile caste, the soldiers, at the

colony level. This model predicts that the reported increase in soldier number from some studies is supported under a narrow range of cost and benefit parameters, and that termites can benefit from fusion at the colony level under two scenarios: when fusion results in higher net benefit from soldiers, or when a colony can take advantage of the other's soldiers. This suggests two pathways to the evolution of colony fusion, which might be applicable to lower termites of different ecological habits. Evidence from both Chapter 3 and Chapter 4 suggest that colony fusion in the lower termites could be driven by selection on pseudergates, which can gain direct fitness benefits from fusion. I suggest that this might be a general phenomenon and that the presence of conflict within a group can facilitate cooperation between groups across taxa.

Acknowledgements

I would firstly like to thank Mike Cant and Faye Thompson for letting me loose on the termites and for all their support this past year. Thank you for being so welcoming, and for all the opportunities you have given me – I learned so much from you both! Thank you also to Bram Kuijper and Rufus Johnstone for all your help and insight into the world of theoretical modelling. I would also like to thank the whole MongWasp group for valuable comments, suggestions and general social evolution chat.

My thanks also go to everyone who helped in the termite lab, especially Tom Collins who was always there to help keep the termites happy and put the world to rights!

Thank you to my family: mum and dad, for your support and encouragement, and Ollie and Emma for always cheering me on. To Liv, for always being there. And finally, Jordan – thanks for all the gin.

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Declaration

All chapters in this thesis were written by Rebecca Padget with comments from Professor Michael Cant and Dr Faye Thompson, who also provided guidance throughout.

Termite stock colonies were collected by Professor Cant and Dr Thompson under permit from East Bay Regional Parks and the California Department of Fish and Wildlife and imported under license from the UK Animal and Plant Health Agency. The laboratory population was maintained by Dr Thompson and a team of undergraduate and postgraduate volunteers. Additional termite colonies were provided by Professor Rebeca Rosengaus, Northeastern University.

The experiment in Chapter 2 was conducted by Rebecca Padget and Dr Thompson. The experiment in Chapter 3 was conducted by Rebecca Padget with help from Megan Nicholl and Annie Santamaria. Dr Abraham Kuijper, and Professor Rufus Johnstone at the University of Cambridge provided additional guidance for the development of the theoretical model in Chapter 4 and Dr Kuijper also provided comments on this chapter.

All research adhered to the Guidelines for the Treatment of Animals in Behavioural Research and Teaching set out by the Association for the Study of Animal Behaviour, and was approved by the Ethical Review Committee of the University of Exeter.

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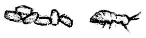
Chapter 1

General introduction



Cooperation, conflict and sociality

All animals face dangers and trials throughout their lives. From predation to parasitism, founding a family to finding food, life is a challenge. Many elaborate adaptations have evolved that enable their bearers to navigate these difficulties, but arguably the most interesting of these is sociality. When individuals begin to interact in groups, their actions begin to have fitness consequences for others: interactions can be cooperative, exploitative or helpful, and this has resulted in a vast array of social systems across the animal kingdom (Bourke, 2011). Just as individuals interact, so too do the groups that they form. While much of the emphasis of research into intergroup interactions has focused on conflict and its consequences, violence and aggression are not the only outcomes of intergroup interactions. Tolerance, cooperation and even permanent fusion are also important outcomes of intergroup interactions in many species, that could have both short- and long-term fitness consequences for individuals in the groups involved (Robinson and Barker, 2017).

In many social animals, which form permanent social groups, the benefits of group living come from not just the presence of others, but from cooperative and altruistic interactions (Franz et al., 2013). In some species, this can extend to reproduction such that some individuals help to raise offspring that are not their own as in cooperative breeding and eusocial societies (Cant, 2012). Eusocial (‘truly’ social; coined by Batra, 1966) and cooperative breeding societies are similar in that only some individuals reproduce. Eusocial and cooperative breeding societies differ (under most definitions, but see Gadagkar, 1994) because the helpers of eusocial groups become irreversibly sterile, and will never reproduce (Crespi and Yanega, 1995). They therefore rely on the indirect fitness benefits that they gain by helping their kin to breed in contrast to some cooperative breeding helpers, which can gain

Table 1.1: **Comparison of cooperative breeding and eusocial societies.** Both societal structures share the characteristics of alloparental care and limited dispersal, but eusociality represents an extreme version of cooperative breeding whereby helping individuals never disperse and remain lifelong sterile.

Cooperative breeding	**Eusociality**
Alloparental care	Alloparental care
Temporary reproductive division of labour	Permanent reproductive division of labour
Delayed dispersal	Eliminated dispersal
Kinship not necessary	Kinship necessary
Selection acts on individuals with conflicting reproductive interests	Individuals share a common reproductive interest

direct benefits to future fitness through helping behaviour (West-Eberhard, 1975; Cockburn, 1998; Riehl, 2013; e.g. Koenig, 1981; Shreeves et al., 2003; Table 1.1).

The fitness interests of individuals in cooperative breeding and eusocial societies are typically aligned by kinship (though not always; Thompson et al., 2017a), which is usually high within groups and lower between groups, particularly in viscous populations in which there is limited dispersal between groups (Queller, 1994). This means that while there can be high levels of within-group cooperation and altruism, there is often between-group conflict, and the evolution of the two often go hand-in-hand (Choi and Bowles, 2007). Intergroup conflict can occur over limited resources such as food and access to mates, and can take different forms, from scramble competition to aggressive (even lethal) contest (e.g. Wilson and Wrangham, 2003; Harris, 2010). However, conflict is not the only possible outcome of an intergroup interaction. Just as individuals can benefit from cooperation with others within a group, there can also be benefits to cooperation between groups (Robinson and Barker, 2017).

Groups can benefit each other by exchanging resources such as food or mating opportunities (e.g. Ellis et al., 2014; Lucchesi et al., 2020), and relationships between groups can be temporary or permanent and often depend on ecological factors such as food availability or disease prevalence (e.g. Lehmann et al., 2007; Smith et al., 2008; Kashima et al., 2013; Lucchesi et al., 2020). In many cases, groups remain distinct even within a larger aggregation, however permanent group fusion can also occur, notably in social insects (e.g. Deheer and Vargo, 2004; Korb and Schneider, 2007; Vásquez and Silverman, 2008; Howard et al., 2013).

In social insects, most individuals in a colony are functionally sterile and rely on indirect fitness obtained via altruistic care of siblings. It might therefore be expected that sterile individuals would show high levels of aggression, not cooperation, toward members of other groups to ensure that the benefits of altruistic brood care and collective foraging behaviour are not misdirected towards non-kin. However, both termites and ants are known to regularly form fused groups with workers apparently providing altruistic help to individuals that are distantly or not at all related (Kronauer et al., 2010; Howard et al., 2013; Rudolph and McEntee, 2016), and thus appear to present a scenario of genetic altruism without manipulation amongst non-kin, which cannot evolve (Hamilton, 1964; Bourke, 2011).

The evolutionary principles that govern social evolution act at many very different levels of organisation, from genes in genomes to animals in societies (Bourke, 2011). These principles can also be adapted to understand the evolution of interactions at the intergroup level. The phylogenetically basal termites offer an opportunity to study selection at different levels, not only because they can be readily cultured and manipulated experimentally, but also because of their unique social biology.

Termite sociality

Termite sociality evolved on Pangea, under the feet of the ancestors of whales (Thorne et al., 2000). Fossil evidence from the Cretaceous period and more recent genetic analyses reveal that the Isoptera, a monophyletic infraorder, diverged from the ancestors of cockroaches (Order: Blattodea) in the late Jurassic period (Thorne et al., 2000; Inward et al., 2007). Termite social systems soon diversified from small simple families, and groups increased in size and complexity, becoming the earliest examples of eusocial animal societies (Martinez-Delclos and Martinell, 1995; Thorne et al., 2000; Engel et al., 2016). The combination of ancestral monogamy and wood-dwelling ecology is thought to have been an important driver of the evolution of eusociality in the termites (West-Eberhard, 1975; Nalepa et al., 2001; Boomsma, 2009; Nalepa, 2015). Monogamy acts as a genetic bottleneck through which all individuals in the group must pass, ensuring their close relatedness (Boomsma, 2009), while wood dwelling provided a stable food source, but required vertically transmitted gut microbial symbionts, providing conditions that promoted delayed dispersal of offspring (Nalepa et al., 2001; Nalepa, 2015).

All extant termites are considered eusocial, but there remains a diversity in colony organisation and in particular, a distinction between the ‘higher’ and ‘lower’ termites. The higher termites (those of the Termitidae family), are thought to display more derived social and morphological features and have extensive within-colony specialisation (Gerber et al., 1988; Van Quang et al., 2018). The lower termite families, however, are basal in phylogeny, morphology and behaviour, and live in colonies that primarily comprise pseudergates (Noirot and Pasteels, 1987; Thompson et al., 2000). Pseudergates (‘false workers’) differ from ‘true workers’ in that they are developmentally totipotent juvenile helpers that can differentiate into alates, the dispersing adult reproductives, or soldiers, the juvenile, but irreversibly sterile defence caste. Alternatively, under certain conditions, pseudergates can develop gonads and become neotenic reproductives that remain in the colony as secondary reproductives to the king and queen (Greenberg and Stuart, 1982).

The capacity for pseudergates to become reproductive throughout life is in contrast to the early specialisation shown in the (true) workers of higher termite species and means that the societies of the lower termites sit on the borderline between a cooperative breeding system and eusociality. In the lower termites, pseudergates retain the ability to become reproductive and can do so given favourable environmental conditions, as in a cooperative breeding society (Emlen, 1982; Korb, 2007a; e.g. Bergmüller et al., 2005). However, once a pseudergate has differentiated into a soldier, it is irreversibly sterile, as in more typical eusocial societies. Termite ecology and nesting behaviour is considered an important driver of the evolution of sterile worker castes (Nalepa, 2015; Boomsma, 2009).

Termite nesting behaviour can be broadly categorised into single-piece (also referred to as one-piece) and multiple-piece (or separate-piece) nesting types (Eggleton and Tayasu, 2001). Multiple-piece nesting species can be central-place foragers or nomadic, and find food sources outside of their nest, with foraging territories that are often quite large (e.g. Haverty et al., 1975; Su et al., 1993). In contrast, single-piece nesting colonies are founded on a single piece of dead wood, which is then both the food source and nesting material for the expanding colony until the wood is exhausted, at which point pseudergates differentiate en masse into alates, and the colony 'dies'. Because they spend their entire lives in a single piece of wood, colonies of single-piece nesting termites often live at relatively higher densities than multiple-piece nesting termites, unable to re-locate to avoid competition or unfavourable environmental conditions. This results in nests that can be poorer quality and harder to defend, and expansion into the substrate leads to frequent colony-colony interactions (Howard et al., 2013). The outcomes of these interactions can vary from avoidance (often maintained by the building of 'faecal fortresses'; Thorne quoted in Bordereau and Pasteels, 2010) to the opposite extreme: group fusion. Group fusion is thought typically to follow aggression but ultimately results in two once-separate colonies occupying the same large nest and behaving as a single colony (Johns et al., 2009; Howard et al., 2013).

Group fusion is a particularly puzzling outcome of colony-colony interactions as most termites in a colony – pseudergates and soldiers – rely on inclusive fitness maintained through a high average relatedness to the group (West-Eberhard, 1975). Because termites are diploid and colonies comprise simple (or extended but inbred) family groups headed by a monogamous pair, their average relatedness is 0.5 (theoretically Boomsma, 2009; and empirically Howard et al., 2013). This means that termites are equally related to their sibling (and inbred niece and nephew) nestmates as they are to their potential future offspring, meaning that providing only a small benefit to the reproductive success of their colony results in a higher inclusive fitness than dispersing to reproduce, facilitating the evolution of these large societies of sterile helpers (Boomsma, 2009; Box 1).

During group fusion, not only is there a sudden drop in relatedness, but the early stages are also often characterised by aggression, particularly towards reproductives, which are often killed (Johns et al., 2009). This means that pseudergates and soldiers might find themselves orphaned in a group to which they are now less related than they are to potential future offspring (around 0.3; Howard et al., 2013). This could create an environment that favours selfish, rather than altruistic behaviour. This leads to three predictions about termite behaviour following a group fusion event: 1) helpers preferentially provide help to kin; 2) pseudergates differentiate into reproductives, either dispersing to found a new colony as alates or mating within the new group as neotenic reproductives; 3) no new soldiers differentiate as there is now insufficient benefit from this altruistic behaviour. Of these, one is supported empirically – some pseudergates develop into neotenic reproductives following fusion, as might be anticipated (Johns et al., 2009; Howard et al., 2013). However, alates do not proliferate, care given by helpers in a fused group does not appear discriminatory, and soldiers do not stop developing in fused groups. This raises two questions: what mechanism, if any, do termites use to discriminate between conspecifics? And why do altruistic soldiers continue to differentiate in a group in which relatedness is so low?

Box 1: Hamilton's rule and eusociality in diploid organisms

Altruism cannot evolve unless altruistic behaviour results in the allele(s) conferring altruism being more likely to enter the next generation than other alleles. This means that when establishing whether altruism will evolve, the relatedness of an actor to both the beneficiary of its actions (r_b) and its own potential offspring (r_c) must be taken into account, as in the version of Hamilton's rule below:

$$r_c c < r_b b.$$

In termites, $r_c = r_b = 0.5$, essentially 'cancelling out' the effect of relatedness so that the inequality becomes $c < b$. This means that a helper need only provide a small increase to its parents' and siblings' reproductive output to facilitate the evolution of eliminated dispersal in a simple (or extended but inbred) diploid family group (Queller and Strassmann, 1998; Boomsma, 2009).

Nestmate recognition

Nestmate recognition is the ability of social insects to identify members of their own colony (or ‘nest’), and allows colonies to evict conspecific intruders, ensuring that altruism is directed only towards members of the group (D’Ettorre and Lenoir, 2010). Nestmate recognition differs from kin recognition because kin recognition relies on genetically encoded cues that allow an individual to approximately assess its relatedness to another (Grafen, 1990; D’Ettorre and Lenoir, 2010). However, nestmate recognition cannot be used to distinguish individuals of different levels of relatedness within the same group (e.g. van Zweden et al., 2010). Nestmate or, more generally, group-member recognition can be favoured by natural selection over kin recognition if inappropriate rejection of kin is more costly than accurate recognition is beneficial (Duncan et al., 2019; Faria and Gardner, 2020). This is typically the case in groups in which average relatedness is high, as in termite and other social insect colonies.

Nestmate recognition, like kin recognition, has three components: the expression of a cue, its perception and the consequent response (Halpin, 1991; van Zweden and d’Ettorre, 2010). Perceived cues are compared against a template (a neurological representation of an individual’s own cue; van Zweden and d’Ettorre, 2010) and some threshold level of deviation from this template triggers a response that is appropriate for either a nestmate (if it is below the threshold), or a non-nestmate (if it is above the threshold; Reeve, 1989; van Zweden and d’Ettorre, 2010). An individual must therefore display a reliable and persistent cue so as not to trigger a false rejection by its nestmates (Reeve, 1989). Also important is that a cue is sufficiently different from neighbouring colonies that a false acceptance error is not made, as this could leave a colony vulnerable to social parasitism (Reeve, 1989). In termites, two distinct lines of evidence have emerged from mechanistic studies of nestmate recognition, suggesting the importance of olfactory cues from one or a combination of cuticular compounds and the gut microbiome.

Mechanisms of nestmate recognition

Cuticular compounds represent an array of chemicals, largely hydrocarbons, present on the surface cuticle of insects, and are important in insect communication, for example, as sex pheromones and dominance signals (e.g. Tregenza and Wedell, 1997; Roux et al., 2002). In social insects they can maintain social cohesion, for example through fertility signalling and suppression (D'Ettorre et al., 2004; Steitz and Ayasse, 2020). Some studies in termites find that nestmates with manipulated cuticular compounds are treated with uncharacteristic aggression (e.g. Aguilera-Olivares et al., 2016), and cuticular hydrocarbons in particular have heritable and nest-specific components (e.g. Dronnet et al., 2006; Kaib et al., 2004), suggesting that they might be used in nestmate recognition. However, other studies show that levels of aggression do not necessarily correlate with cuticular compound phenotype (e.g. Chouvenc and Su, 2017). Where aggression does correlate with cuticular compounds, this is not necessarily well correlated with colony membership and individuals from two different colonies with similar cuticular compounds can show no aggression (Haverty and Thorne, 1989; Kaib et al., 2002).

Further nestmate recognition studies in termites have found a link between diet and nestmate recognition. For example, Florane et al. (2004) showed that *Coptotermes formosanus* colonies fed on the same type of wood became less aggressive towards each other than towards colonies fed on a different type of wood. Studies in some ants have shown that dietary hydrocarbons from insect prey could affect cuticular compound composition (Liang and Silverman, 2000). Termites eat primarily wood, so it is possible that diet might impact cuticular compounds in more indirect and variable ways. The apparent role of diet in termite nestmate recognition could instead be indicative that cues from the gut microbiome play a role.

The microbiome refers to the microbial community that lives on and inside multicellular animals. The gut microbiome is an important and well-characterised aspect of termite biology as termites feed largely on wood comprising hard-to-digest lignocellulose, and rely on mutualistic symbioses to provide sufficient levels of vital nutrients (Higashi et al., 1992; Brune, 2014). These symbiotic mutualisms are particularly important for the single-piece nesting termites, which have more limited dietary options than separate-piece species and so have evolved complex stable associations with symbiotic bacteria and protists to obtain sufficient dietary nutrients (Higashi et al., 1992; Noda et al., 2007).

The gut microbiome is thought to be nest-specific (Minkley et al., 2006), and direct evidence for a role of the gut microbiome in nestmate recognition comes from Matsuura (2001), who found that manipulating the gut microbiome of *Reticulitermes speratus* individuals resulted in an uncharacteristic, aggressive response towards nestmates. Similarly, Kirchner and Minkley (2003), found that gut flora – populated via trophallaxis – was an important determinant in nestmate discrimination in *Hodotermes mossambicus*. Further suggestion that nestmate recognition is mediated by the gut microbiome comes from studies that show that diet can influence nestmate recognition (Florane et al., 2004; Pan et al., 2006), as there is a direct link between diet and the gut microbiome (Pan et al., 2006; and reviewed in Dillon and Dillon, 2004). However, these studies have been carried out in termites that forage away from the nest, whereas nestmate recognition studies in single-piece nesting termites have focused on cuticular compounds.

In single-piece nesting termites, the gut microbiome is likely to be more similar amongst interacting colonies because they share the same type of wood diet, which could affect the gut microbiome and thus the ability to discriminate nestmates from non-nestmates. If the gut microbiome does affect nestmate recognition in single-piece nesting species, it could be that dietary mediation acts at a finer scale than diet type, because single-piece nesting termites only ever encounter other colonies that live in and feed on the same piece of wood. Nevertheless, we might predict that if the gut microbiome was being used as a cue that non-nestmates with a more distinct gut microbiome (for example those feeding on a different wood type) would elicit a more aggressive response than those with a more similar gut microbiome. However, an alternative hypothesis is that there is very little selection pressure for single-piece nesting termites to respond differently to non-nestmates raised on a different food source as they never meet in nature. This hypothesis makes a different prediction, that there will be no difference in response to non-nestmates raised on different wood type. In Chapter 3, I describe an experiment in which we investigated whether nestmate recognition in the single-piece nesting termite, *Zootermopsis angusticollis*, might be constrained by broad-scale diet type.

The soldier caste in termites

The soldier caste in termites is the most morphologically distinctive, and the only caste that is irreversibly sterile in all species. Specialised for colony defence, soldiers have highly sclerotised mandibles, which are enlarged and can take different shapes depending on the threats that a species faces (e.g. Kuan et al., 2020). Soldiers are thought to be the first caste to have evolved in termites, and evidence for this comes from an intermediate caste found in the lower termites: reproductive soldiers (Thorne et al., 2003).

Reproductive soldiers are morphologically similar to sterile soldiers, including en-

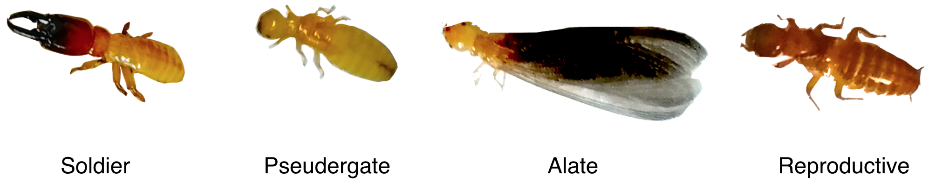


Figure 1.1: **Morphologies of the four main castes in *Zootermopsis angusticollis*.**

larged defensive mandibles (but to a lesser extent). However, they also have developed gonads, and are behaviourally similar to the primary reproductives (Thorne et al., 2003). These "monstrosities" were originally considered a rare developmental abnormality, a result of unusual dietary conditions in large colonies (Heath, 1907 quoted in Myles, 1986). However, it became apparent that reproductive soldiers are widespread both numerically and taxonomically in the lower termites (Myles, 1986). Reproductive soldiers emerge frequently as a result of intraspecific threat, and are aggressive during colony-colony encounters (Thorne et al., 2003). Because they have defensive weaponry, but retain the ability to reproduce, reproductive soldiers are hypothesised to represent the evolutionary transition to the presence of a permanent soldier caste (Myles, 1986; Queller and Strassmann, 1998).

Sterile soldiers (herein, 'soldiers') differentiate from pseudergates via a 'pre-soldier' stage (Park and Raina, 2003). Soldier numbers fluctuate seasonally (Waller and La Fage, 1988; Liu et al., 2005), and appear to self-regulate via a negative feedback loop whereby a high number of soldiers prevents the differentiation of further soldiers (Mao et al., 2005; Watanabe et al., 2011). Proximately, this is likely as a result of pheromones emitted by soldiers that prevent or promote differentiation onto different pathways by pseudergates (Mao et al., 2005). Ultimately, these patterns are likely to follow fluctuations in the costs and benefits of soldier presence, as the seasonal peak in soldier number typically coincides with alate emergence, when colony defence is likely most vital (Waller and La Fage, 1988; Liu et al., 2005).

Caste ratio as a collective investment problem

Considering group-level costs and benefits can be a useful simplification in studies of intergroup interactions, in particular for eusocial species where there is such group cohesion that a eusocial colony is considered to represent a major transition in the evolution of the individual (Maynard Smith and Szathmáry, 1995; Bourke, 2011; West et al., 2015). Considering the soldier caste at the colony level in termites, the more soldiers a colony produces, the fewer dispersing reproductives (alates) it can maximally produce. However, if a colony produces too few soldiers then the alates that it does produce could be less successful, meaning that each soldier was a less efficient investment. Additionally, fewer alates are produced overall in colonies with removed soldiers (Roux and Korb, 2004). Soldier differentiation can therefore be modelled as an optimal investment problem at the colony level.

When two colonies fuse, as they do regularly in the lower termites (Perdereau et al., 2010; Howard et al., 2013; Luchetti et al., 2013), relatedness between individuals within the group is no longer guaranteed, and this introduces greater potential for conflict. Two unrelated colonies fusing results in a potential conflict over how colonies share the cost of soldiers, from which both colonies benefit. This conflict has the potential to reduce fitness in one or both interacting colonies. However, the prevalence of group fusion in nature suggests that it can also have fitness benefits (Johns et al., 2009; Howard et al., 2013). These benefits could arise through either selfish exploitation of one colony by another, or by cooperation between the two colonies. To investigate the dynamics of soldier investment following a colony fusion event, in Chapter 4 I present a model, based on the collective investment game, that describes how colonies might alter their soldier investment following fusion with another group.

Termites as a model organism

Termites are an easily-cultured insect and this has led to their extensive use as a model organism. They have been used to study a variety of biological questions from the benefits of sociality (Rosengaus and Traniello, 2001) to cellulose metabolism in animals (Watanabe et al., 1998), and have even inspired robots that demonstrate collective behaviour (Werfel et al., 2014). The potential of termites as a model for studying social evolution is becoming more evident as the significance of details of their societal structure and its interaction with ecology become better understood. Their convergence with the eusocial Hymenoptera in many aspects of their social structure, despite ancestral and genetic dissimilarity, means that termites provide an opportunity for comparative studies that can shed further light on the evolution of eusociality (e.g. Thorne and Traniello, 2003).

The utility of termites as a model organism depends on developing reliable methods of culture, manipulation and observation that do not affect their individual or social behaviour. In Chapter 2, we evaluate subcuticular Visible Implant Elastomer (Northwest Marine Technologies) injections for use as an individual marker in termites, which are prone to losing internal and external marks (Thorne et al., 1996).

With their broad range of social structures in extant species, termites represent a unique opportunity to investigate the ecological and social drivers underpinning a number of interesting phenomena, such as those I have mentioned here – chemical or microbial communication and fusion of social groups – and many more. The use of individual identifiers could facilitate research into various previously inaccessible areas such as dominance structures, individual variation in helping behaviour and longitudinal studies, which could prove fruitful areas for further investigation, allowing us to combine theoretical and empirical evidence to better understand aspects of social evolution.

Thesis aims

In this thesis, I will begin with a trial of a novel method of individually marking termites to evaluate its potential for use in behavioural studies. I will then address questions relating to the causes and consequences of an interesting phenomenon that is common across the lower termites: group fusion. I will do this by investigating two aspects of termite social behaviour: nestmate recognition and soldier differentiation. Specifically, I will investigate whether pseudergates of the lower termite, *Zootermopsis angusticollis*, identify and discriminate between nestmates and non-nestmates, and whether this ability is diminished by living within a similar wood substrate. I will then use a theoretical model to investigate cooperation between colonies following group fusion, specifically with regards to investment in the defensive soldier caste. Finally, I will discuss the implications of my findings in relation to group fusion in the lower termites, and within the more general topic of cooperation between animal groups.

Chapter 2

Trialling Visible Implant Elastomer in a small hemimetabolous insect

Abstract

Advances in individually marking animals have facilitated detailed studies of animal populations and behaviour as they allow tracking of individuals through time and space. Hemimetabolous insects, representing a wide range of commonly-used model organisms, present a unique challenge to individual marking as they are not only generally small-bodied, but also moult throughout development, meaning that traditional surface marks are not persistent. Visible Implant Elastomer offers a potential solution as small amounts of the inert polymer can be implanted under the skin or cuticle of an animal. Visible Implant Elastomer has proved useful for individually marking fish, crustaceans and amphibians in both field and laboratory studies, and has recently been successfully trialled in laboratory populations of worms and fly larvae. We trialled Visible Implant Elastomer in the single-piece nesting termite *Zootermopsis angusticollis*, a small hemimetabolous insect. We found that marks persisted following moulting but that there was a small effect of Visible Implant Elastomer on survival and butting, a behaviour associated with reproductive development in lower termites. This suggests that Visible Implant Elastomer could be an appropriate technique to use for marking hemimetabolous insects, but that caution should be taken, particularly when behavioural observation is important.

Introduction

Individually marking animals is a useful tool in the study of animal populations, ecology and behaviour in both field and laboratory research (e.g. Hjort and Lindholm, 1978). Persistent marks are particularly useful for animals that are difficult to track by an experimenter, for example because they are regularly obscured by the environment, live in inaccessible areas or because animals look similar to each other. These difficulties necessitate the use of identifying marks that are reliably persistent, but marks must also be non-invasive such that they do not impact individuals' survival or behaviour (Batsleer et al., 2020).

The trade off between a mark that is persistent but non-invasive manifests differently in different animals, and hemimetabolous insects present a unique combination of challenges. Hemimetabolous insects moult during development, meaning that surface marks widely used on other insects – such as paint or correction fluid – only persist until the next moult, which is often difficult to predict in standard natural or laboratory populations. This makes medium- or long-term studies of individuals challenging as surface marks would require regular re-application, and it could be difficult or impossible to re-identify individuals that have lost their marks.

To combat this issue, oil-soluble dyes that collect in insect soft tissues have been used for internally marking insects (Hagler and Jackson, 2001; e.g. in Vilarinho et al., 2006). These dyes can be persistent, though their efficacy can be dependent on environmental conditions like diet (Thorne et al., 1996). Internal dyes can also be transferred amongst individuals both vertically and horizontally via trophallaxis and other social interactions, which means that they cannot be used to reliably mark individuals, particularly of social species. Some of these dyes also require specialist equipment like UV lights, while others rely on dissecting the insects to see the colour internally, eliminating the possibility of continued observation (Schroeder and Mitchell, 1981).

Hemimetabolous insects are also generally small-bodied animals, meaning that even a small mark can be relatively invasive, affecting survival and behaviour of individuals and having both ethical and scientific implications (Batsleer et al., 2020; e.g. De Souza et al., 2012). Some marks, like surface paints, could also affect behaviour due to the volatile chemicals that they contain, as volatiles can interfere with chemical communication across insect species, affecting behaviour (e.g. Wang et al., 2016; Jürgens and Bischoff, 2017). It is therefore a challenge to mark small insects in a way that is both reliably long-lasting and non-invasive.

Visible Implant Elastomer (VIE; Northwest Marine Technology) is a system used to mark individuals by injecting a small bead of inert, coloured polymer under the skin or cuticle. VIE has been widely demonstrated in both natural and laboratory populations of reptiles, amphibians and fish (e.g. Penney et al., 2001; Bushon et al., 2007; Bainbridge et al., 2015). VIE has also been trialled in blow flies and earth worms, with results suggesting no impact on survival or development (Butt and Lowe 2007; Moffatt 2013). Because VIE is implanted under the cuticle, it should persist through the moults of hemimetabolous insects, and should not disrupt chemical communication. Additionally, small, controlled amounts of the polymer can be injected with a micro needle, meaning that even small individuals can be marked.

We tested VIE in a randomised trial in the basal termite, *Zootermopsis angusticollis* (Termopsidae), a small, hemimetabolous insect. We censused small groups of termites that had been injected with VIE alongside un-injected control groups and conducted behavioural assays to establish whether VIE affected the survival or behaviour of laboratory-cultured termites.

Methods

Stock colony collection and maintenance

Thirteen stock colonies of *Zootermopsis angusticollis* termites were created by collecting whole natural colonies, including nesting material, from Redwoods Regional

Park, California, USA (37.813556°N, 122.165917°W) under permit from East Bay Regional Parks and the California Department of Fish and Wildlife, and imported under license from the UK Animal and Plant Health Agency. Colonies were collected in 2018 and 2019 and were maintained in 35 litre plastic boxes containing a mixture of California redwood (*Sequoia*) and birch (*Betula*) in a dark room at 22°C and 85% humidity. Humidity was maintained by spraying the colonies with distilled water twice weekly. Trials were carried out in the Centre for Ecology and Conservation, University of Exeter, UK between January and March 2020.

Experimental design

We took ten termites from each stock colony, totalling 130 individuals. We weighed each individual and placed it into an 'arena', such that each of 26 arenas contained five termites. Termites weighed between 0.0168g and 0.1096g (overall mean$\pm$SD $=$ 0.0531 ± 0.0207; see Appendix A.2), and were a combination of pseudergates ($n = 70$) and nymphs ($n = 60$). Arenas comprised a single 3mm-thick piece of balsa cut to $\sim$120mm square with a 45mm-diameter circle cut out of the centre. The balsa was then placed between two sheets of 2mm clear, colourless perspex (also cut to $\sim$120mm square) with a sheet of moistened 50mm-diameter cellulose filter paper; the construction was fastened using bulldog clips (Figure 2.1). For the duration of the trial, termite arenas were stored in a single layer in flat eight liter plastic boxes lined with moistened paper towel, and sprayed twice weekly to maintain moisture. The boxes were kept in a controlled environment in the dark at 22°C and 85% humidity. If termites began to tunnel towards the edge of the arena, a small amount of metal gauze was clipped to the open edge to prevent escape.

Termites were left to acclimatise to their arenas overnight before being assigned to either the treatment group (to be injected with Visible Implant Elastomer) or the

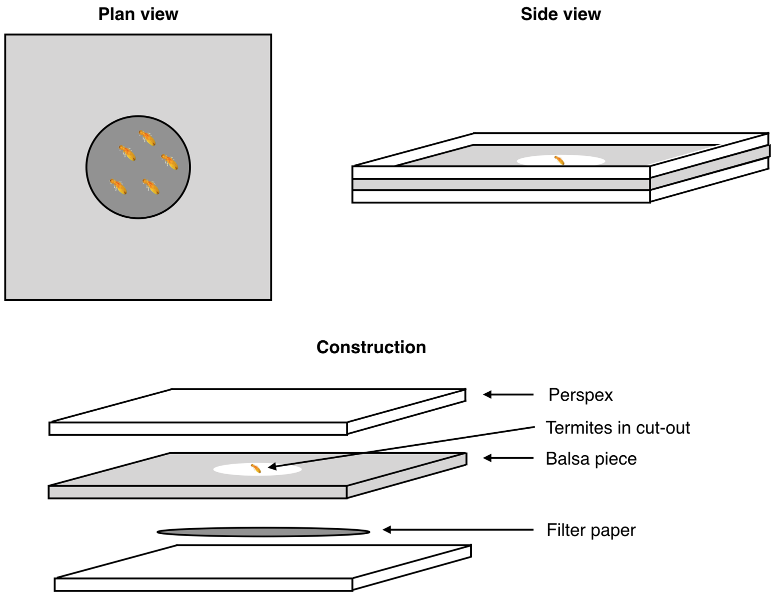


Figure 2.1: **Schematic of balsa arena setup.** Balsa was placed in between two perspex sheets and the termites placed in a cut-out circle in the middle of the wood.

control group (no injection). All termites in an arena were assigned to the same treatment to ensure that any behavioural changes caused by the VIE would not affect control individuals. Both treatment and control individuals were cold-immobilised and the control group was handled for the time that it took to inject VIE into a treatment individual (approximately 20 seconds) to ensure that any effects we observed were a result of the injection and implant, not disturbance or handling. The treatment group was injected with a small amount of green VIE, prepared by mixing a drop of setting agent with $\sim$0.2ml of the colour component in the syringe. A 29 gauge (0.3366mm outer diameter) needle was used and the VIE was injected under the cuticle on the upper abdomen (Figure 2.2). During injection, termites were held securely between the first two fingers and thumb to prevent movement and provide access to the upper abdomen.

Figure 2.2: **Visible Implant Elastomer was placed under the cuticle on the upper abdomen.** This photo shows the placement of the VIE (green mark) on the upper abdomen of a termite nymph.

Data collection

Termites were kept in their arenas and censused daily for 35 days, following which they were censused weekly for a further four weeks, totalling a 63-day trial period. Any termites that died during this period were left in arenas to avoid differences in disturbance levels amongst groups.

To collect behavioural data, termites were filmed for fifteen minutes on days 2, 4 and 10 after treatment (the day of treatment being day 0). If termites had created tunnels which would have allowed them to remain unseen in the video, the top wood of this tunnel was cut away with scissors, otherwise they remained undisturbed. Termites were filmed in their arenas under red light using a Sony HDR-CX240E video camera. Videos were then coded at 4x speed by focal sampling each living termite using Behavioural Observation Research Interactive Software (BORIS; Friard and Gamba, 2016). Data were collected from the last ten minutes of each videoing period to give the termites a five-minute settling time so that behaviour that we observed was not affected by the disturbance of being moved from their storage boxes.

We measured seven behaviours: allogrooming, antennation, biting, butting, self-grooming, trophallaxis and any interaction with the environment – i.e. the wood, filter paper or faeces in the arena (Table 2.1). For allogrooming, antennation, trophallaxis and interaction with the environment, we measured the total time that each behaviour was carried out; for biting, butting and self-grooming, the number of occurrences was counted.

From videos, we identified recently moulted individuals by their paler heads and mandibles. If it was a VIE-injected individual, we noted whether the mark was still present on the termite.

Statistical analyses

Survival analysis

A total of 130 termites were initially extracted from stock colonies but one control was injured during transfer to the arena on day 0 and so was removed from analysis, leaving 64 control individuals and 65 VIE-injected individuals housed in 26 groups of five. To determine whether the VIE injection had an effect on survival of the termites, we recorded the number of termites alive in the treatment and control groups on each census day. To model survival over time, we used these data to generate Kaplan-Meier survival curves, and performed a log-rank test to evaluate whether survivorship was different between the treatment and control groups. We did this over the entire 63-day sampling period and over the first ten days to identify any short-term effects that might have been lost over a longer time frame. To investigate factors that affected the hazard (the instantaneous probability of death) of termites in each group, we used a mixed effects Cox proportional hazards model with treatment group as a fixed effect and colony of origin as a random effect. The mixed effects

Table 2.1: **Behaviours measured during video observation.** References: 1 - Korb (2008), 2 - Korb et al. (2012), 3 - Zhukovskaya et al. (2013), 4 - Ishikawa and Miura (2012), 5 - Böröczky et al. (2013), 6 - Korb (2007b), 7 - Crosland et al. (1997), 7 - Korb and Schmidinger (2004).

Behaviour	**Description**	**Data type**	**Reference**
Allogrooming	Focal individual uses mandibles on other termite's body or head such as not to cause injury or flee response from the recipient.	Total time performing behaviour.	1, 2, 3
Antennation	Focal individual moves antennae over other termite's head, body or antennae.	Total time performing behaviour.	2
Biting	Focal individual uses mandibles on another termite's head or body and causes damage (e.g. leaking from body) or fleeing response.	Count.	4
Butting	Quick vibrations of the whole body by focal individual.	Count.	2
Interaction with environment	Focal individual uses mandibles to chew filter paper or wood, or move pieces of filter paper, wood or faeces around the arena.	Total time performing behaviour.	-
Self-grooming	Focal individual pulls antenna forward to mandibles.	Count	5
Trophallaxis	Focal individual has mouth to mouth or mouth to anus contact with another termite.	Total time performing behaviour.	6 (but see 7)

Cox proportional hazards model assumes a time-constant hazard function given in the form:

$$h(t) = h_0(t)e^{X\beta+Zb}.$$

The hazard function ($h(t)$) gives the probability of a subject dying at a given time point, t, given the level of a fixed variable, X, and its coefficient, β, and a random variable, Z, and its coefficient, b. The exponential of a coefficient gives the hazard ratio of that variable; the hazard ratio is the factor change in survival for a unit increase in the variable (the effect size). Here, we report the estimated coefficient, the hazard ratio (HR) and the 95% confidence interval of the hazard ratio along with the p-value.

We fitted a Cox proportional hazards model to the data over the entire 63-day census period. To validate our model fit, we simulated time until death data for 130 individuals in 1,000 simulated trials using the Cox model coefficient calculated from our data, and deaths per day per individual (as calculated from the data) as a basic linear hazard function (code in Appendix A.1). We then qualitatively compared the survival curves produced from these simulated data to our experimental data.

Analyses were carried out in R 3.6.1 using the 'survival' and 'survminer' packages (Therneau and Grambsch, 2000; Kassambara et al., 2019).

Behavioural analysis

All behaviours measured were recorded sufficiently for analysis, though data for all behaviours were zero-inflated. Principal component analysis was conducted to see whether the seven behaviours measured could be reduced to fewer underlying behavioural patterns, and to identify any clustering in the data by either treatment (VIE-injected or control) or day after treatment (2, 4 or 10, as factors). Pairwise Pearson correlation coefficients were also used to identify any correlations between behaviours.

Due to zero-inflation of the data, the assumptions of standard linear models were violated for all behaviours and this was not solved by transformation of the data. We therefore used a Bayesian framework to fit generalised linear mixed effects models (GLMMs) to data for each behaviour with Markov chain Monte Carlo (MCMC) and weakly informative priors ($\beta_i \sim \mathcal{N}(0, 2.5)$). We fitted models to untransformed data, assuming a zero-inflated beta distribution for the total time data (allogrooming, antennation, environment, trophallaxis) and a negative binomial for the count data (butting and self-grooming). We included treatment (VIE-injected or control) and day after treatment (2, 4 or 10, as factors) and their interaction as fixed effects terms, and colony of origin as a random effect. Models were fitted to a total of 369 individual observation sessions in 78 videos over the three videoing days. On day 2, 124 individuals were sampled (treatment: $n = 60$, control: $n = 64$) due to six deaths since treatment; on day 4, 123 individuals were sampled (treatment: $n = 59$, control: $n = 64$) and on day 10, 122 individuals were sampled (treatment: $n = 58$, control: $n = 64$) due to additional deaths.

To test for an effect of treatment, we calculated the Bayes factor – the posterior probability ratio – for the full model over a reduced model that excluded the treatment and interaction terms. We interpret Bayes factors according to (Lee and Wagenmakers, 2014) (Table 2.2). To provide additional support for our findings, we also report the point estimate and the 95% credible interval for each coefficient. The estimate given is the median of the posterior probability distribution for the coefficient, and the 95% credible interval represents the region within which there is a 95% probability that the coefficient lies, giving a measure of uncertainty in the estimate.

Analyses were carried out in R 3.6.1. We used the ‘brms’ package for the zero-inflated beta models (Bürkner, 2018), the ‘rstanarm’ package for the negative binomial models (Brilleman et al., 2018) and ‘gpplot2’ and ‘bayesplot’ to create figures (Wickham, 2016; Gabry et al., 2019).

Table 2.2: **Common interpretations for the Bayes factor - the odds ratio of the alternative over the null (or reduced) model.** Adapted from Lee and Wagenmakers (2014).

Bayes factor	Interpretation
$>$ 100	Extreme evidence for alternative.
30 - 100	Very strong evidence for alternative.
10 - 30	Strong evidence for alternative.
3 - 10	Moderate evidence for alternative.
1 - 3	Weak evidence for alternative.
1	No evidence for either model over the other.
1/3 - 1	Weak evidence for null.
1/10 - 1/3	Moderate evidence for null.
1/30 - 1/10	Strong evidence for null.
1/100 - 1/30	Very strong evidence for null.
$<$ 1/100	Extreme evidence for null.

Results

Survival analysis

Of the 64 control individuals, 47 (73%) survived until the last census day (day 63). Of the 65 VIE-injected individuals, 42 (65%) survived until the last census day. The mean number alive in each arena fell from five to 3.77 for the controls and 3.21 for the VIE-injected groups (Figure 2.3; for full list see Appendix A.2). Of the 26 arenas in each group, individuals died from five arenas in the control group and ten in the treatment group, suggesting non-independence amongst individuals that lived in the same arena.

Survivorship of the treatment and control groups was not found to be significantly different by log-rank tests of the Kaplan-Meier survival curves generated from the data; this was true over the full 63 days (log-rank test: $p = 0.367$; Figure 2.4a), and

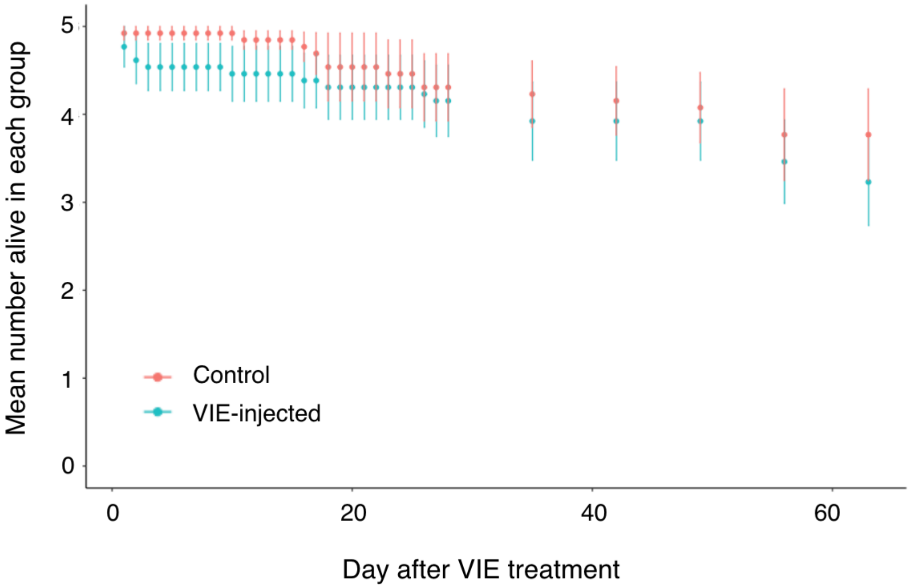


Figure 2.3: **Mean number of termites alive in each arena on each census day.** Control is shown in red and treatment in blue. Vertical lines show standard error.

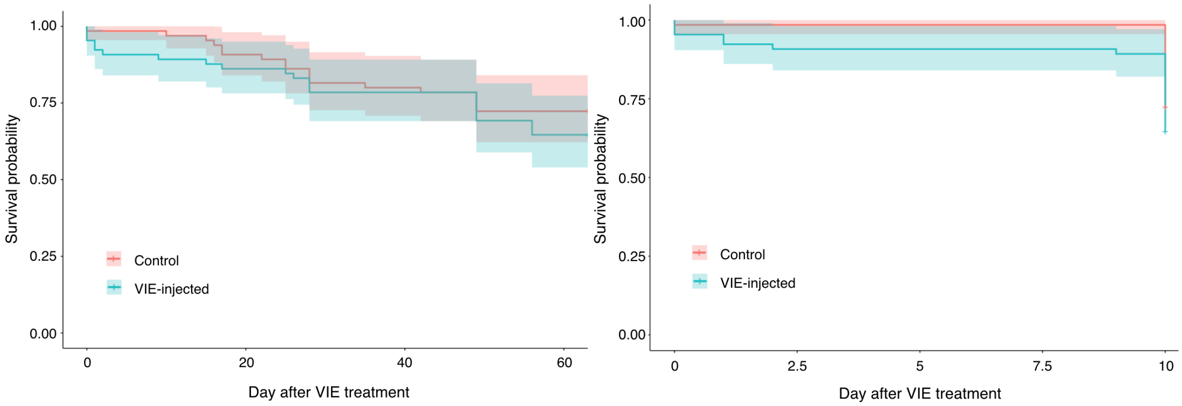


Figure 2.4: **There was no difference in survival curves of treatment and control groups over either 63 or ten days.** Plots show Kaplan-Meier survival curves and confidence intervals for treatment (blue) and control (red) groups over a) the entire 63-day census period, and b) the first ten days of censusing.

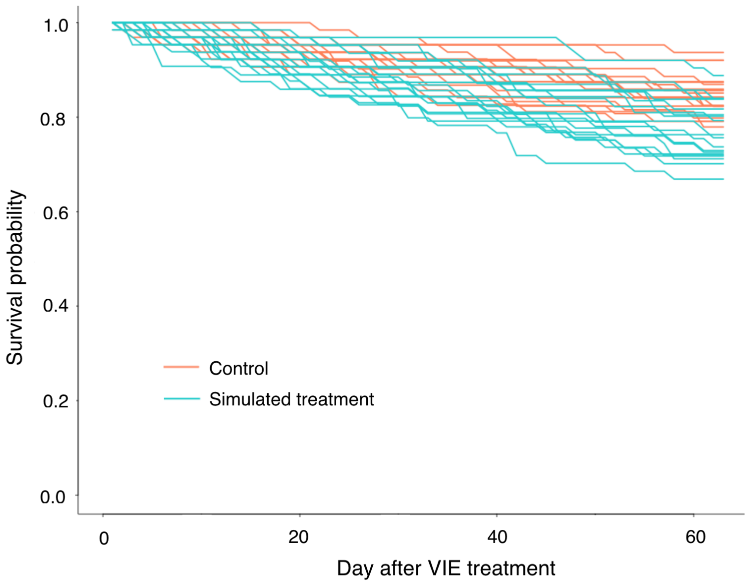


Figure 2.5: **Simulated survival curves are qualitatively similar to that produced from the real data.** Plot shows simulated survival curves for a random sample of 20 simulated trials to illustrate that the survival curves simulated using the Cox model coefficients were similar to that for the real data, suggesting that the Cox model captured the important predictors of survival.

over the first ten days (log-rank test: $p = 0.235$; Figure 2.4b).

The mixed effects Cox proportional hazards model showed that there was no significant difference in hazard between treatment and control groups in the study ($\beta = 0.272$, HR $= 1.31$, 95% CI $= 0.679, 2.54$, $p = 0.42$). The coefficient estimate suggested that the VIE implant caused a 1.31-fold increase in hazard in injected compared to control individuals, however the 95% confidence interval was wide, likely due to the low overall number of deaths we observed. Simulations showed that a trial of 130 individuals will find a significant difference in survivorship roughly 10% (104 out of 1000 simulations) of the time, given the Cox model coefficient of 0.272 and an alpha value 0.05. That a significant difference is found in more than 1 in 20 trials, and the estimated hazard ratio of 1.31, together suggest that VIE has a small effect on termite survival. Based on qualitative comparisons of survivorship curves from the simulated and real data, the Cox model appeared to capture the important effects in the data, as survival curves showed similar trends (Figure 2.5).

Behavioural analysis

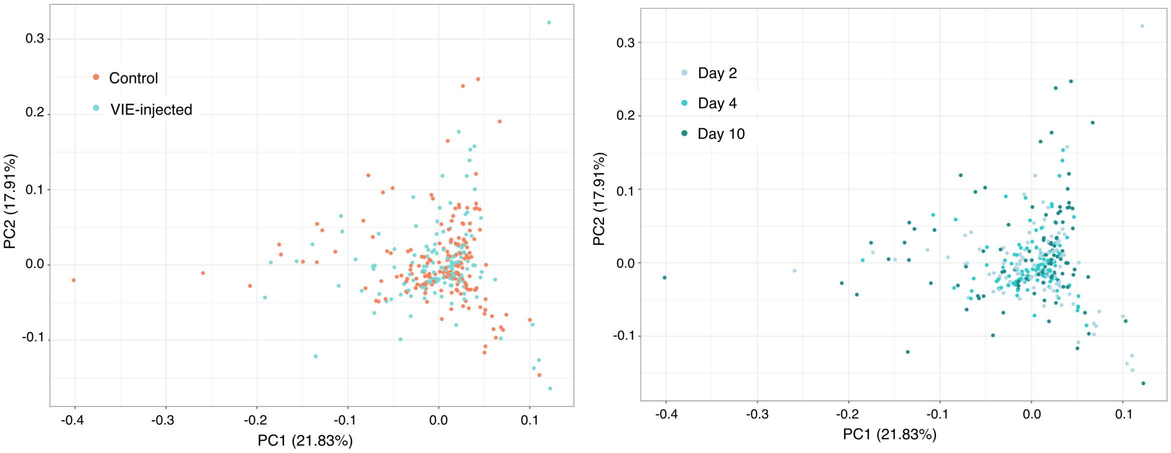


Figure 2.6: **Principal component analysis showed that behaviours did not cluster by either treatment (a) or day (b).** Principal components also did not account well for variation across behaviours, and the first two principal components explained only 21.82% and 17.91% of the variation suggesting no common underlying behavioural drivers.

Principal component analysis showed that the observed levels of different behaviours did not cluster by treatment or by day after treatment, suggesting that neither treatment nor day caused large differences in any of the behaviours (Figure 2.6). The first two principal components explained only 21.83% and 17.91% of the variation, suggesting that there were no common drivers underlying any of the behaviours. There were significant but weak positive correlations between allogrooming and antennation ($\rho = 0.131$, $p = 0.0118$), and allogrooming and trophallaxis ($\rho = 0.180$, $p = 0.000528$) but only the correlation between allogrooming and trophallaxis was significant after Bonferroni correction (with an alpha value of 0.003); see Appendix A.3 for full correlation matrix. We therefore continued with analysis using data for all

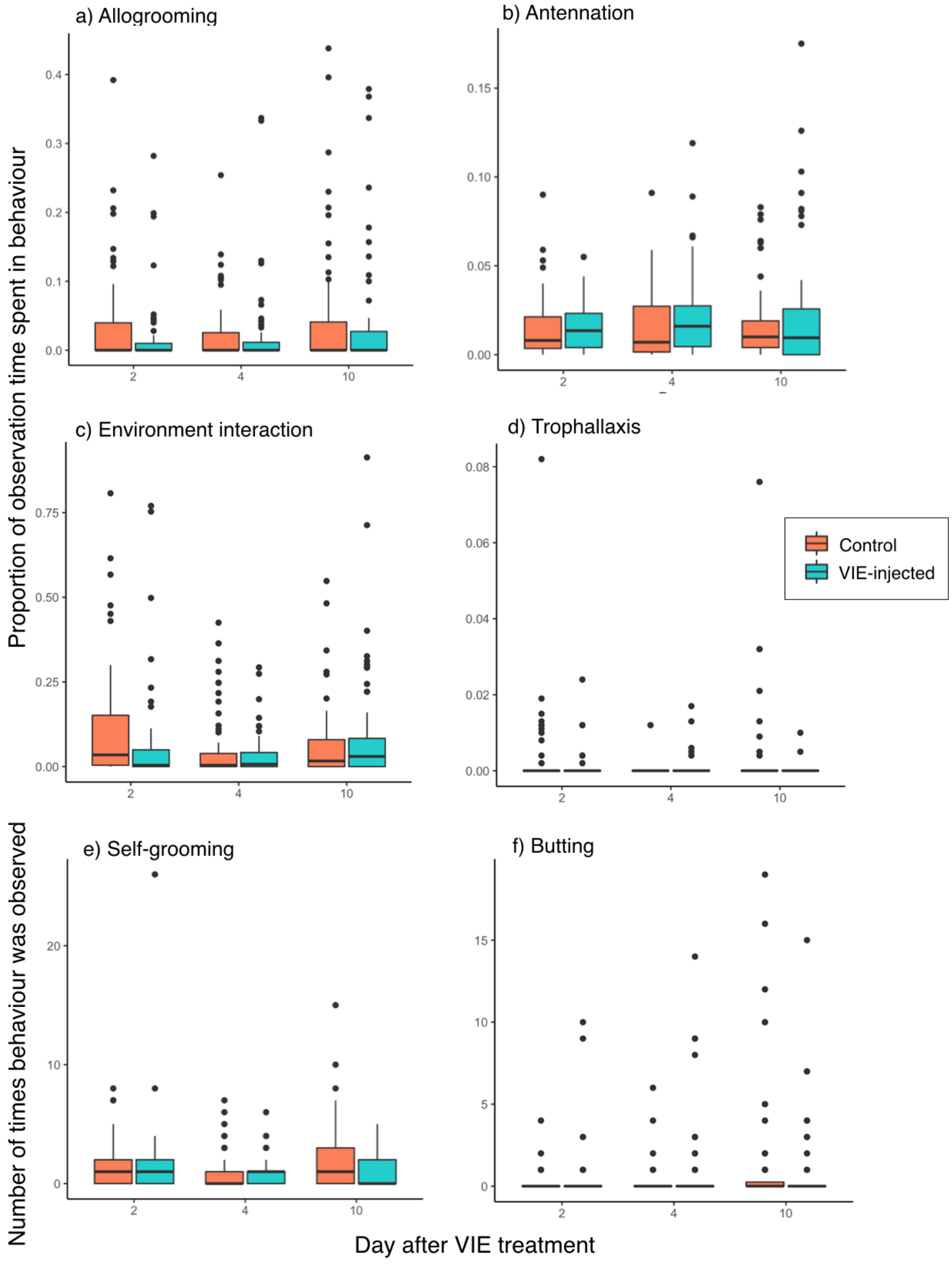


Figure 2.7: **Raw data for all behaviours measured.** Raw data shown for a) allogrooming, b) antennation, c) interaction with the environment, d) trophallaxis, e) self-grooming, and f) butting for each of days 2, 4 and 10 that they were videoed after Visible Implant Elastomer treatment for control (red) and treatment (blue). Boxes show interquartile ranges of the data with the median shown by the horizontal black line in each box. Vertical lines show the range of the data and points show potential outliers.

Table 2.3: **GLMM output from models exploring the effect of VIE treatment.** Coefficient estimate, 95% credible interval boundaries and Bayes factor for the full model over the reduced for the treatment term in the model for each behaviour.

	Estimate	**95% CI**	**BF$_{10}$**
Allogrooming	-0.368	-0.853, 0.102	0.0364
Antennation	0.107	-0.188, 0.402	0.00383
Butting	0.764	-0.383, 1.887	0.339
Environment	-0.122	-0.573, 0.303	0.00141
Self-grooming	0.176	-0.315, 0.677	0.0267
Trophallaxis	-0.0836	-1.15, 1.10	0.0237

seven behaviours (Figure 2.7).

Bayes factor analysis of GLMMs for all behaviours found moderate to extreme support for the reduced model over the full (Lee and Wagenmakers, 2014), suggesting that treatment did not affect any of the behaviours overall (Table 2.3). There was however, a slight interaction between treatment and day whereby control individuals performed increased butting on day 10, and treatment individuals did not (day 10 control: estimate $= 2.13$, 95% CI $= 1.00, 3.28$; day 10 treatment: estimate $= -1.07$, 95% CI $= -2.56, 0.50$). See Appendix A.4.

By day 2 (the first day of filming), two treatment but no control individuals had moulted. Recently moulted termites can be identified by their paler heads and mandibles, and we identified that the VIE mark remained visible on both individuals (Figure 2.8).

Discussion

We investigated the effect of Visible Implant Elastomer (VIE) injections on the survival and behaviour of *Zootermopsis angusticollis* to evaluate aspects of the suitability of VIE for use as an individual marker in small, hemimetabolous insects. In a study

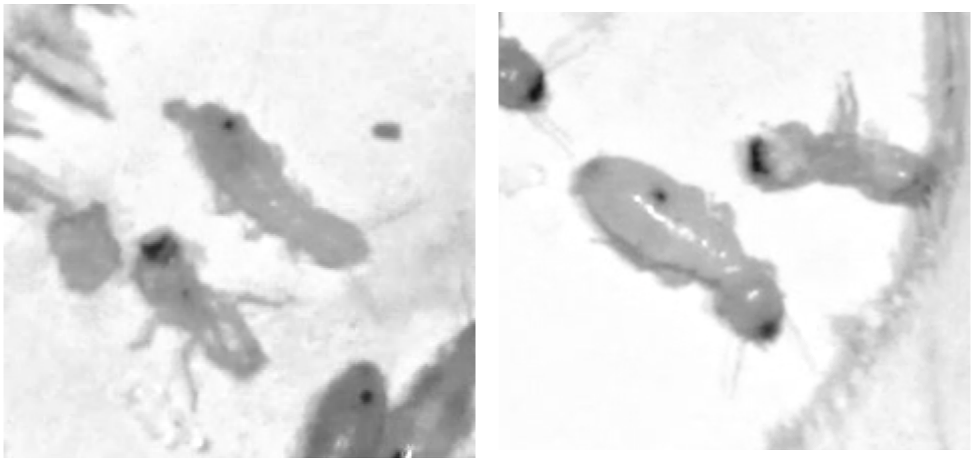


Figure 2.8: **In both individuals that had moulted, the Visible Implant Elastomer-mark was still visible on videos.** Photos show a marked individual next to its moulted exoskeleton.

of 129 individuals, we found a non-significant effect of VIE injection on the survival of termites, and an effect on butting but no other behaviour. However, we also found that the marks persist through moulting, suggesting that, with appropriate caution, VIE could be used as a method to individually mark small insects like termites.

We observed no significant difference in survival between the treatment and the control group, likely due to the small effect and sample sizes as the coefficient of the treatment term in the Cox proportional hazards model suggested that there was an increase in hazard for VIE-injected individuals. All methods of marking insects are likely to cause some disturbance (Hagler and Jackson, 2001), and the increase in hazard that is deemed acceptable for a given study will depend on both ethical and practical considerations of specific research questions. It is important that harm is limited to ensure that methods do not inappropriately affect the outcome of manipulations, and to maintain both ethical standards and public support (Freelance, 2019; Drinkwater et al., 2019).

The effect that we found on butting behaviour suggests that care should be taken if VIE is used in behavioural or developmental studies of insects. Butting behaviour in lower termites is associated with reproductive disinhibition, particularly in orphaned colonies in which pseudergates (totipotent termite helpers) can differentiate into a reproductive form (Johns et al., 2009; Hoffmann and Korb, 2011; Penick et al., 2013). Our data suggest that this might be delayed or inhibited in VIE-injected pseudergates. However, because we only monitored behaviour for ten days following treatment, it is not possible to say whether VIE-injected pseudergates were delayed or inhibited from undergoing differentiation into reproductives, and this effect was small. A longer-term behavioural study of the effects of VIE is needed to establish whether there are permanent behavioural differences caused by the VIE injection.

The rates of survival that we report here are lower than those of previous VIE studies on other insects (e.g. in blow fly larvae Moffatt, 2013 and earthworms Butt and Lowe, 2007), for both the treatment and the control group, suggesting that termites might be sensitive to disturbance in general. Behavioural changes in termites have been reported following disturbance and periods in laboratory culture, and behaviour can differ in tests of similar phenomena (e.g. nestmate recognition) depending on assay design (Cornelius and Osbrink, 2009). Similarly, behavioural changes are reported following separation of pseudergates from reproductives (Penick et al., 2013), but effects of separation from reproductives on survival are unknown. Taken together, this suggests that termites are sensitive to disturbance and that caution should be taken when drawing general conclusions about behaviour and causes of death in studies that disrupt termites, for example by moving them into an unfamiliar arena away from their natal colony.

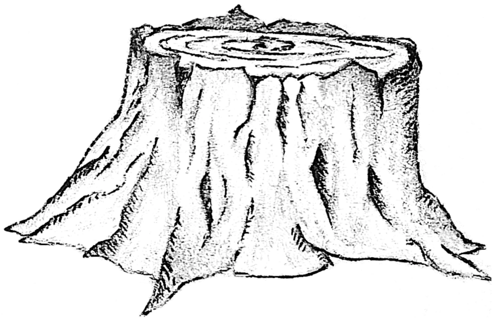
Conclusions

Our finding that VIE affects both termite survival and behaviour suggests that caution should be taken if this method of marking is to be used in experimental studies.

However, we found that the changes in survival and behaviour were small, suggesting that VIE might be appropriate in some research settings given careful application, particularly as the marks appear to be persistent through moults. Extensions of trials similar to this would facilitate better understanding of the long-term and physiological effects of VIE on marked individuals, but these results suggest that VIE could be of some use for the study of hemimetabolous insects, given that care is taken to minimise other sources of disturbance.

Chapter 3

No evidence that broad-scale diet type mediates nestmate recognition in a single-piece nesting termite (Isoptera: Termopsidae)



Abstract

Recognition of group members is an important adaptation in social animals, and mechanisms underpinning this ability vary greatly amongst taxa. Nestmate recognition in social insects commonly relies on cues that are mediated by environmental factors such as food source. However, the influence of environmental factors on nestmate recognition in single-piece nesting termites remains unclear because interacting groups share nesting material and diet – wood substrate serves as both. In this study, we raised incipient colonies of a single-piece nesting termite, *Zootermopsis angusticollis*, on two different wood types and conducted behavioural assays of staged introductions to determine whether nestmate recognition is constrained by shared environmental conditions amongst interacting colonies. We found that non-nestmates elicited more behaviour associated with identity-checking and defence than did nestmate controls, but that there was no effect of wood type on the behavioural response of termites to non-nestmates. Interestingly, we also found that a cooperative behaviour (allogrooming) was performed equally towards both nestmates and non-nestmates. We conclude that single-piece nesting termites discriminate non-nestmates and that this ability is not diminished when non-nestmates share the same broad-scale diet. We suggest that recognition cues in single-piece nesting termites might have a strong non-environmental component, or that environmental influences occur at the microclimate scale.

Introduction

In social animals, the ability to recognise members of the group is important because it enables helping behaviour to be directed towards individuals who either are likely to reciprocate, or are kin. In social insects, nestmate recognition – a distinction between members of the same nest and 'outsiders' – enables sterile workers to direct their altruistic helping behaviour towards colony members, who are typically close kin (D'Ettorre and Lenoir, 2010). Accurate nestmate recognition could be particularly important for species in which colonies often meet as a high error rate under these circumstances could lead to high inclusive fitness costs (Faria and Gardner, 2020). Single-piece nesting termites often not only live at high colony densities, but are also confined to live within the same log in which the colony was founded, increasing competition for nesting space between colonies. A high level of competition combined with a high frequency of colony-colony interactions means that nestmate recognition is likely to play an important role in the evolution of sociality in the lower termites.

Nestmate recognition is influenced by a number of environmental and genetic factors, and cross-fostering experiments have shown that both manipulation of cuticular compounds and diet-mediated changes to the gut microbiome can alter the responses of termites to nestmates and non-nestmates (Matsuura, 2001; Kirchner and Minkley, 2003; Aguilera-Olivares et al., 2016). Environmental mediation of nestmate recognition cues is of particular interest in single-piece nesting termites, whose entire colony life cycle is completed within a single piece of dead wood. Confinement to a single home means that interacting groups share a common environment, relying on the same nesting material and food source. This limits the extent to which environmental factors can cause nestmate recognition cues to diverge amongst colonies.

Despite this potential constraint on nestmate recognition, laboratory studies of a single-piece nesting species (*Zootermopsis nevadensis*) report targeted aggression towards non-nestmates when two colonies meet (Johns et al., 2009). This suggests that they can distinguish between nestmates and non-nestmates, but it is unclear whether environmental homogeneity constrains this ability. To test this, we manipulated the environment of incipient colonies of the single-piece nesting termites *Zootermopsis angusticollis* by raising colonies on two different types of wood substrate before conducting assays of the behavioural responses of pseudergates to non-nestmates. Wood substrate serves as both the food source and nesting material for single-piece nesting termites, and nestmate recognition cues that are strongly affected by environmental conditions are likely to show a greater difference amongst individuals raised on a different substrate.

Zootermopsis termites show a more aggressive response to heterospecifics than to non-nestmate conspecifics (Haverty and Thorne, 1989), suggesting that strength of behavioural response is reflective of their ability to classify individuals as a member of a different group. We therefore predicted that if being raised on the same wood type constrained nestmate recognition, a more aggressive response would be shown towards non-nestmates raised on a different wood type compared to non-nestmates raised on the same wood type.

Methods

Termite collection and maintenance

To establish stock colonies, whole natural *Zootermopsis angusticollis* colonies were collected from Redwoods Regional Park, California, USA (37.813556°N, 122.165917°W) under permit from East Bay Regional Parks and the California Department of Fish and Wildlife, and imported under license from the UK Animal and Plant Health Agency. Each stock colony was housed in a 35 litre plastic box containing a mixture of their native wood (California redwood, *Sequoia*) and birch (*Betula*) (Cole et al., 2018; Cole and Rosengaus, 2019). Incipient colonies were founded from large stock colonies by pairing de-winged virgin alates collected and weighed during dispersal, and allowing them to raise a colony separately in a 60mm by 15mm round petri dish provided with moistened cellulose filter paper and some small pieces of birch wood (as described in Cole et al., 2018 and Cole and Rosengaus, 2019). When incipient colonies had developed ∼5 larvae and a soldier, they were transferred to a larger (100mm), square Petri dish with their current wood, and some additional wood. A total of 40 incipient colonies were used in trials. 35 of these were founded between 2018 and 2019 collected from R. Rosengaus' laboratory at Northeastern University, USA; the other five were established in August 2019 at the Centre for Ecology and Conservation, University of Exeter, UK.

Wood substrate was provided *ad libitum* to incipient colonies. For 25 of the colonies, we continued to provide birch throughout development, and 15 were transferred onto sycamore (*Acer*) at least one month prior to trials. Termites were kept in the dark in a controlled environment at 22℃ and 85% humidity. Moisture was introduced by initially soaking the wood substrate and a small amount of cellulose filter paper (∼2cm x 3cm) in distilled water, and was maintained by spraying colonies with distilled water approximately twice weekly.

Of the 40 colonies used in the trial, 24 were founded by inbreeding alates from the same colony, 8 by outbreeding alates from different colonies and 8 were founded by reproductives of unknown origin. Colonies were between 7 and 19 months old and contained at least 7 individuals, including at least one soldier and two reproductives; maximum colony size was ∼50 individuals. None of the colonies contained secondary reproductives or reproductive soldiers.

Behavioural assays

Experiments were carried out in February and March 2020 at the Centre for Ecology and Conservation, University of Exeter, UK. Trials were conducted in batches of three replicates at a time. For each replicate, three termite workers from an incipient colony were placed into an uncovered 50mm Petri dish arena containing a cut-to-size circle of dry filter paper. These groups were left for two minutes under red light to settle after the disturbance before a fourth worker was introduced and allowed to interact for three minutes. The introduced individual was one of: a non-nestmate raised on the same type of wood ($n = 20$), a non-nestmate raised on a different type of wood ($n = 17$) or a nestmate control ($n = 17$; Figure 3.1). Individuals were not marked. Videos were recorded for the whole five minute trial time using a Sony HDR-CX240E video camera. Of the 40 unique colonies that were used in the trials, 16 were used twice. On each occasion, workers were collected at random, meaning that some workers may have been used in two trials. If a colony's workers were used twice, the trials were carried out at least 14 days apart.

Due to sample size limitations, termites were not size-matched, so relative size of the introduced individual was included in the analysis. Relative size was calculated as the ratio of the introduced individual's length over the mean of the length of the interacting group (excluding the introduced individual), measured in millimetres from the videos; this was log-transformed to ensure linearity. Videos were coded using Behavioural Observation Research Interactive Software (BORIS; Friard and Gamba, 2016) and each video was coded blind to treatment. To account for small discrepancies in introduction and filming times, data were collected from the last two and a half minutes of each 5 minute video period. We recorded the proportion of time or the number of times individuals performed behaviours associated with cooperation, aggression or defence towards the introduced individual. Behaviours of interest were:

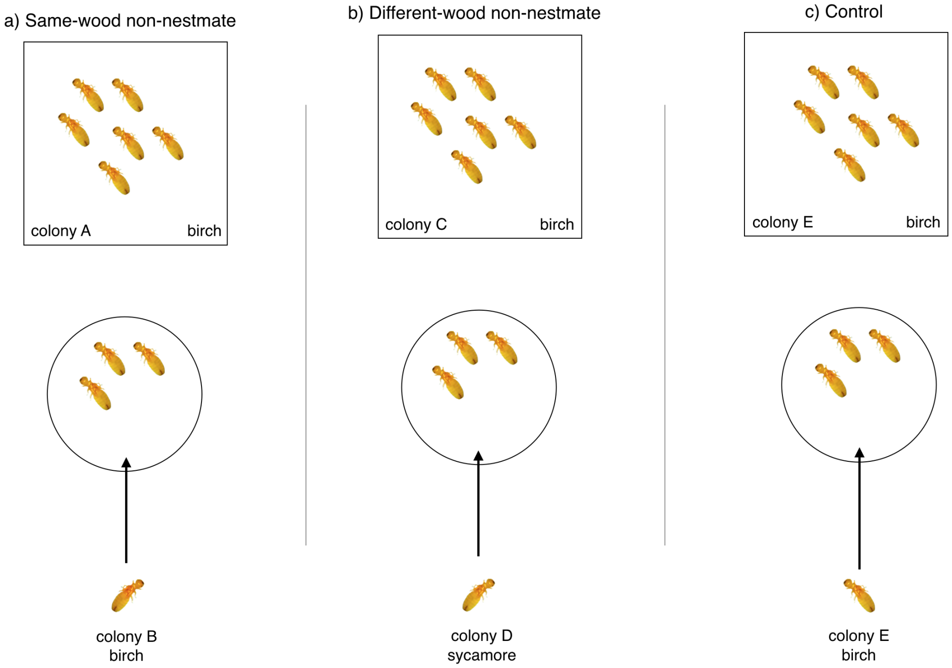


Figure 3.1: **Schematic of an example trial set up.** A new individual was introduced to an arena containing a group of three workers from a colony. This introduced individual was one of: a) a non-nestmate raised on the same wood type, b) a non-nestmate raised on a different wood type or c) a nestmate. This schematic shows example wood type combinations, but other combinations of wood type were used (e.g. birch-raised individual introduced to sycamore-raised group).

allogrooming, antennation, biting, butting, recoiling and trophallaxis (Table 3.1).

Statistical analysis

Of the behaviours measured, only three: allogrooming, antennation and recoiling occurred sufficiently for statistical analysis. Trophallaxis involving the introduced individual occurred in four replicates; biting of the introduced individual was recorded in one replicate (biting by the introduced individual was recorded in one other replicate); butting was never recorded. Trophallaxis, biting and butting were therefore excluded from statistical analyses.

Table 3.1: **Behaviours measured during staged interactions.** In this study, we observed only allogrooming, antennation and recoiling sufficiently for statistical analysis. References: 1 - Korb (2008), 2 - Korb et al. (2012), 3 - Zhukovskaya et al. (2013), 4 - Ishikawa and Miura (2012), 5 - Šobotník et al. (2008), 6 - Korb (2007b), 7 - Crosland et al. (1997), 8 - Korb and Schmidinger (2004).

Behaviour	**Description**	**Data type**	**Reference**
Allogrooming	Cooperative. Uses mandibles on other termite's body or head such as not to cause injury or flee response from the recipient.	Proportion time introduced individual was allogroomed.	1, 2, 3
Antennation	Communicative. Moves antennae over other termite's head, body or antennae.	Proportion time introduced individual was antennated towards.	2
Recoiling	Defensive. Sudden jump back from another termite, often following short antennation period.	Number of times resident individuals performed behaviour towards introduced individual.	5
Biting	Aggressive. Uses mandibles on another termite's head or body and causes damage (e.g. leaking from body) or fleeing response.	Number of times introduced individual was bitten.	4
Butting	Alarm/aggression. Quick vibrations of the whole body.	Number of times resident individuals performed behaviour after arrival of the introduced individual.	2
Trophallaxis	Cooperative. Mouth to mouth or mouth to anus contact with another termite.	Proportion time introduced individual gave or received feeding.	6 (but see 8)

For allogrooming and antennation, the proportion of the two and a half minute data collection period that the introduced individual was a recipient of each of these behaviours was recorded; for recoiling, counts were recorded (Table 3.1). Data for all behaviours contained a high proportion of zeroes, and assumptions of normality and homoscedasticity of residuals for standard linear models were violated, even after transformation of the data. Therefore, we used a Bayesian framework with Markov chain Monte Carlo (MCMC) and scale-adjusted, weakly informative priors ($\beta_i \sim \mathcal{N}(0, 2.5)$) to fit generalized linear mixed effects models (GLMMs) to the raw (untransformed) data for allogrooming, antennation and recoiling separately. We assumed a zero-inflated beta distribution for the data in the allogrooming and antennation models (following Douma and Weedon, 2019), and a negative binomial distribution in the model of recoiling. Introduced individual identity (nestmate or non-nestmate) and its interaction with wood type (same wood or different wood) were fixed effects in the models, along with relative size, to account for being unable to size-match termites. Resident colony ID and filming batch were included as random effects.

To investigate the effect of each covariate on the response, we calculated Bayes factors. The Bayes factor is the posterior probability ratio between the full model and a reduced model (i.e. that excludes the covariate of interest). This means that the Bayes factor quantifies the support for a particular covariate in the model. We have interpreted Bayes factors according to Lee and Wagenmakers's 2014 classification (which follows Jeffreys, 1961; Table 3.2). The point estimate for the model coefficient, median absolute deviation (MAD) and 95% credible intervals (CI) of the posterior parameter distributions from 4000 draws are also reported. This point estimate is the median of the posterior distribution of estimates of the model coefficient for that covariate, and the MAD and 95% CI describe the variation, giving a measure of uncertainty in the estimate. The 95% credible interval is the region within which the model predicts there to be a 95% probability that the value of the coefficient

Table 3.2: **Common interpretations for the Bayes factor - the odds ratio of the alternative over the null (or reduced) model.** Adapted from Lee and Wagenmakers (2014).

Bayes factor	Interpretation
> 100	Extreme evidence for alternative.
30 - 100	Very strong evidence for alternative.
10 - 30	Strong evidence for alternative.
3 - 10	Moderate evidence for alternative.
1 - 3	Weak evidence for alternative.
1	No evidence for either model over the other.
1/3 - 1	Weak evidence for null.
1/10 - 1/3	Moderate evidence for null.
1/30 - 1/10	Strong evidence for null.
1/100 - 1/30	Very strong evidence for null.
$<$ 1/100	Extreme evidence for null.

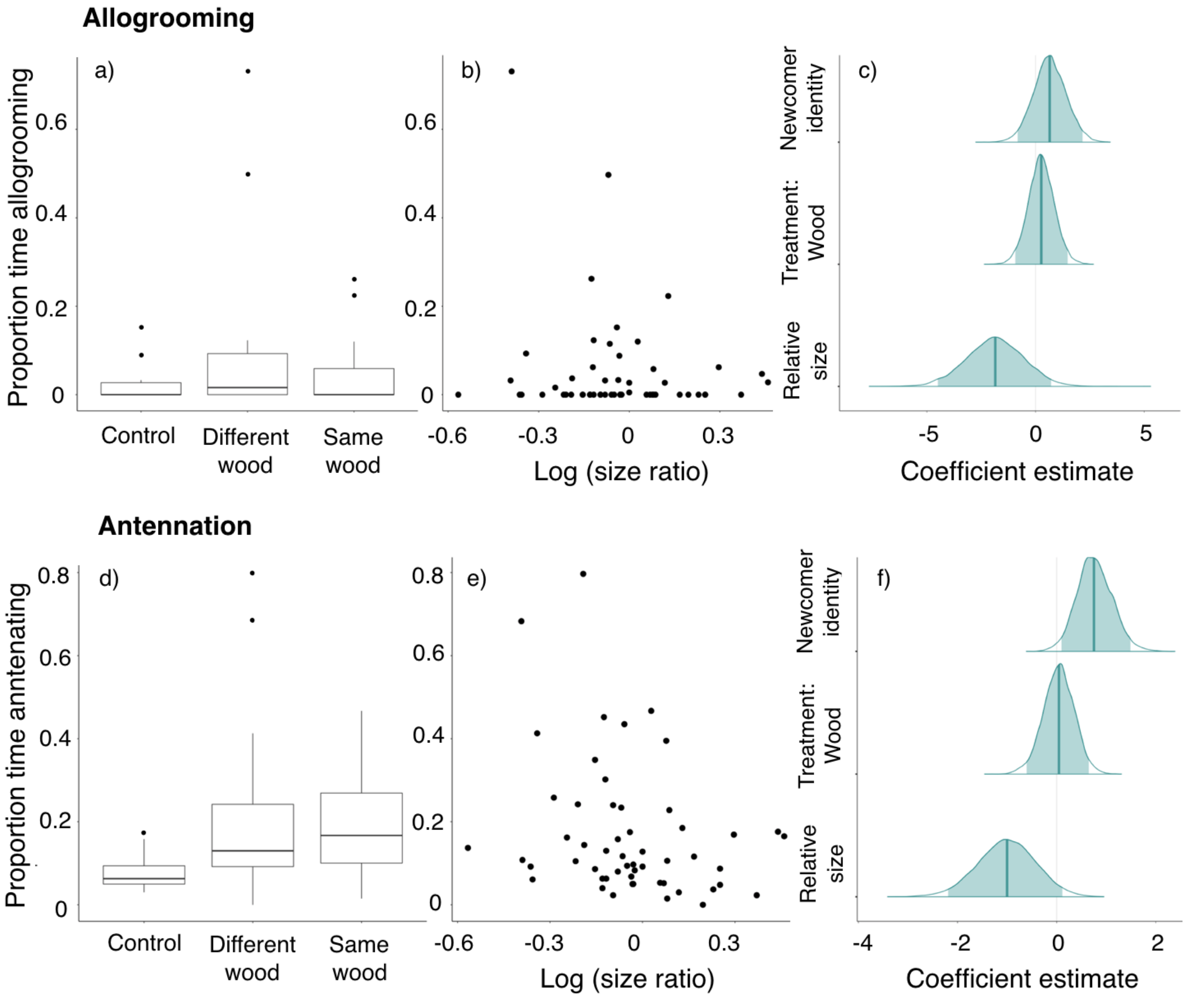
lies (McElreath, 2020). Therefore if zero lies within the credible interval for a given coefficient, this indicates a chance that the value of the coefficient is zero (and thus that the covariate does not help to explain the response). Here, we do not use a threshold credible interval to determine what we consider to be a significant effect, but instead use Bayes factor hypothesis testing and report 95% credible intervals to highlight variation across the coefficient estimates.

Analyses were carried out in R3.6.1 (R Core Team, 2019) using the ‘brms’ library (Bürkner, 2018) for the zero-inflated beta models, and the ‘rstanarm’ library (Brilleman et al., 2018) for the negative binomial models. Plots were generated using bayesplot (Gabry et al., 2019) and ggplot2 (Wickham, 2016).

Results

Allogrooming

Analyses of allogrooming data (Figure A.1a, b, c) suggested that wood type had no effect on allogrooming towards non-nestmates, and that nestmates and non-nestmates received equal levels of allogrooming (wood type: BF_{10}=0.198, estimate =



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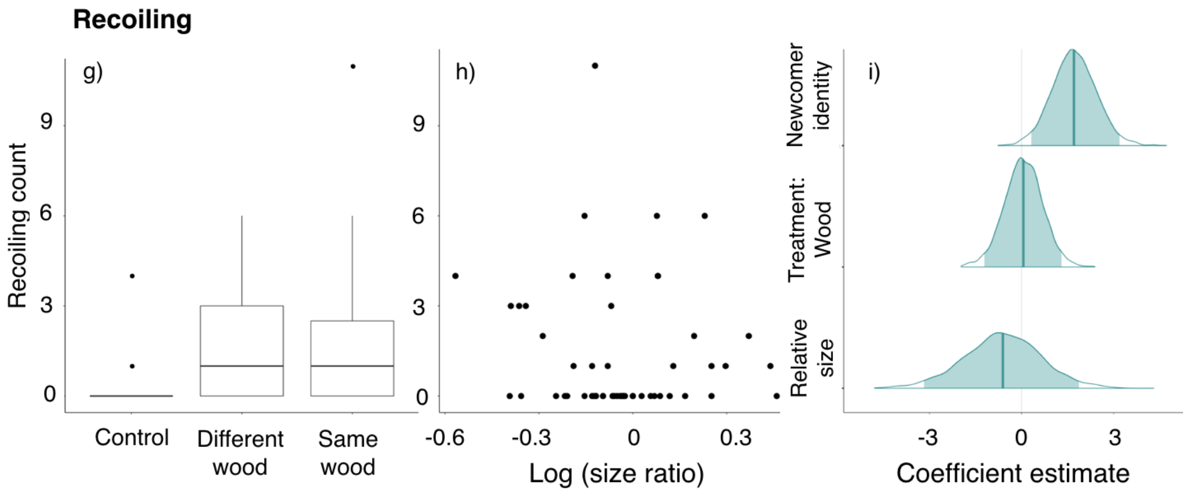


Figure 3.2: **Raw data (a, b, d, e, g, h) and posterior distributions of coefficient estimates (c, f, i) for allogrooming, antennation and recoiling.** Boxes in a), d), and g) show the inter-quartile range, with middle line representing the median; whiskers show the range of the data and points represent potential outliers. Posterior distributions of the coefficient estimates shown in c), f), and i) are for nestmate identity (top), treatment:wood type interaction (middle), and relative size (bottom) in the GLMM. Shaded regions show the 95% credible intervals; the blue vertical lines show the medians of the distributions.

0.261, MAD = 0.587, 95% CI = -0.932, 1.48; identity: BF_{10}=0.742, estimate = 0.649, MAD = 0.735, 95% CI = -0.824, 2.16; Figure A.1a, c). There was however an effect of size: termites allogroomed relatively smaller introduced individuals for longer than larger introduced individuals (BF_{10}=6.40, estimate = -1.85, MAD = 1.31, 95% CI = -4.50, 0.714; Figure A.1b, c).

Antennation

Wood type had no effect on antennation towards non-nestmates; the Bayes factor showed moderate support for the reduced model and a near-zero coefficient estimate (BF_{10}=0.139; estimate = 0.0460, MAD = 0.311, 95% CI = -0.606, 0.649; Figure A.1d, f). The Bayes factor showed moderate support for the model including introduced termite identity, suggesting that non-nestmates were antennated towards for longer than nestmates (BF_{10}=4.10, estimate = 0.753, MAD = 0.350, 95% CI = 0.0965, 1.49; Figure A.1d, f). Size had no effect on antennation (BF_{10}=0.106; estimate = -1.00, MAD = 0.581, 95% CI = -2.19, 0.114; Figure A.1e, f).

Recoiling

Non-nestmates triggered more recoiling behaviour than nestmates, but this did not depend on wood type or relative size of the introduced individual (Figure A.1). This is demonstrated by the Bayes factor hypothesis test for the effect of wood type, which showed weak support for the reduced model; the coefficient estimate for the interaction term in the full model was also close to zero (BF_{10}=0.242; estimate = 0.494, MAD = 0.593, 95% CI = -1.11, 1.21; Figure A.1g, i). The Bayes factor showed moderate support for the model including introduced termite identity (BF_{10}=7.46; estimate = 1.63, MAD = 0.666, 95% CI = 0.245, 3.01; Figure A.1g, i), however there was weak evidence against an effect of size on the response (BF_{10}=0.117; estimate = -0.605, MAD = 1.30, 95% CI = -3.15, 1.90; Figure A.1h, i).

Discussion

If nestmate recognition in single-piece nesting termites is constrained by the shared environment of interacting groups, we expected that non-nestmates raised on a different type of wood would elicit a more aggressive response than those raised on the same type of wood. In contrast, we found that termite pseudergates anntenated towards and recoiled from non-nestmates more than they did the nestmate control, and that this was not dependent on dietary treatment. Taken together, this difference in behavioural response suggests that *Zootermopsis angusticollis* pseudergates can discriminate between nestmates and non-nestmates, and that this is not constrained by sharing nesting material and diet with interacting colonies. We also found that pseudergates allogroomed equally towards nestmates and non-nestmates, suggesting that pseudergates might benefit from cooperating with (rather than evicting) non-nestmates.

We predicted that pseudergates would respond more aggressively to non-nestmates raised on a different wood compared to non-nestmates raised on the same type of wood because there is evidence that cues involved in nestmate recognition – from cuticular compounds or the gut microbiome – are affected by diet in some species (Liang and Silverman, 2000; Pan et al., 2006). However, there is evidence that key components of the gut microbiome are stable to dietary perturbations in some cockroaches and termites, suggesting that a change in broad-scale diet might not affect any potential gut microbial nestmate recognition cues (Boucias et al., 2013; Tinker and Ottesen, 2016). The influence of diet on cuticular compounds is reported at the species level in insectivorous species whose dietary hydrocarbons act as precursors in endogenous hydrocarbon biosynthesis (Blomquist and Jackson, 1973; Blomquist et al., 1987; Liang and Silverman, 2000). Single-piece nesting termites have a limited diet of woody plant matter meaning that dietary mediation of cuticular compounds, if present, is likely to be more indirect and potentially more variable.

Alternatively, nestmate recognition cues might be dependent on environmental mediation at a finer scale. The gut microbiome is dependent on microenvironmental heterogeneity in some higher termites (Mikaelyan et al., 2017), and could be similarly regulated in the lower termites. Fine-scale variation is likely to be higher within a wood type than between different wood types (Shellman-Reeve, 1994), and this could explain why termites responded similarly to non-nestmates raised on a different wood type.

The equal levels of cooperative allogrooming towards non-nestmates that we saw in these assays contradicted our prediction that there would be increased aggression towards non-nestmates. This could be because pseudergates of the basal termites can gain fitness advantages from cooperating with non-nestmates during colony fusion, after which pseudergates can gain the opportunity to become reproductive (Johns et al., 2009; Korb and Roux, 2012; Howard et al., 2013). However, the lack of aggression that we observed could have been influenced by experimental design. Petri dish experiments tend to report lower levels of aggression towards non-nestmates than experiments that allow termites to meet in connecting tubes, which are more similar to the natural galleries in which they encounter non-nestmates in nature (Messenger and Su, 2005; Cornelius and Osbrink, 2009 e.g. Haverty and Thorne, 1989; Delphia et al., 2003; Cooney et al., 2016; Thorne, 1982; Johns et al., 2009; Howard et al., 2013).

Termites are also sensitive to disturbance, showing behavioural changes for a period afterwards (Matsuura and Nishida, 2001). It is possible, therefore, that transferring termites from their natal nest environment into a Petri dish could lead to a reduction in sensitivity to differences amongst non-nestmates as they acclimatise to their new environment and recover from the disturbance. This might result in the uniform response to non-nestmates that we see here where a distinction between same- and different-wood non-nestmates might be found in an experiment more reflective of natural colony-colony encounters. Experiments in which interactions are facilitated within the nest environment, for example by connecting groups with tubing, as in Thorne et al. (2002) and Cornelius and Osbrink (2009), simulate natural encounters more closely and behaviour displayed by termites under these conditions might give a more accurate reflection of real-world behaviour.

One unanticipated finding was that introduced individuals that were smaller than the resident group were allogroomed at a higher rate than relatively larger introduced individuals. Selective allogrooming of smaller individuals might be expected to evolve in termite colonies if allogrooming is costly to the groomer and has higher benefits to smaller recipients. One selection pressure that might cause size-based variability in recipient benefit is disease. The smaller workers in the study are likely to have been younger termites and it has been shown that younger termites are at increased risk of disease compared to older termites (Rosengaus and Traniello, 2001). Allogrooming reduces risk of disease, and younger termites are shown to be less at-risk when in the presence of older individuals (Rosengaus et al., 1998; Rosengaus and Traniello, 2001). This suggests that the increased allogrooming towards smaller individuals could have been targeted cooperation towards younger, more disease-prone termites. To test whether allogrooming is selectively targeted towards younger, more at-risk individuals, an experiment in which termites of different ages are infected with a pathogen and allowed to interact with uninfected nestmates could reveal this selectivity in allogrooming if it is present.

Conclusions

The great variation in termites' responses to non-nestmates throughout the literature suggests that termites might use a combination of cues in nestmate recognition, and that the relative importance of these cues could differ between species with varying ecology. The results presented here demonstrate that nestmate recognition is not constrained by shared broad-scale environmental conditions in single-piece nesting termites. This suggests that environmentally-mediated recognition signals are either less important in single-piece nesting termites, or that environmental influences act on a finer scale. Cue transfer experiments, combined with studies of the biochemical pathways that link environmental factors with potential nestmate recognition cues are needed to provide more precise details of this important aspect of termite behaviour.

Chapter 4

Consequences of colony fusion for intergroup cooperation in a lower termite: a theoretical model

Abstract

Relationships between animal societies vary greatly from lethal conflict to cooperation and fusion. In the lower termites, colonies often meet and are regularly known to fuse, which presents an evolutionary puzzle as sterile individuals then provide help in a group to which they could be largely unrelated. We present a game theoretical model of collective investment – whereby two players contribute to investment in a common good – and apply this to predict soldier production in a fused group of termites. Fused termite groups comprise two unrelated colonies, each with the option to contribute to a public good – the defensive soldier caste. This introduces the potential for the exploitation by one or other of the colonies in the group. We re-derive the collective investment game for a termite colony that can adjust its level of investment based on its representation in a fused group and the relative cost and benefit of soldier production. Our model predicts that soldier investment will typically decrease following colony fusion. However, the reported increase in post-fusion soldier investment from experimental studies could be explained if the cost/benefit ratio of soldier production falls following fusion. The model predicts that the number of successfully dispersed alates will also typically fall for a colony in a fused group, unless per capita efficiency is increased through fusion, or the cost/benefit ratio of soldiers decreases sufficiently. We suggest that these results demonstrate two potential pathways to the evolution of group fusion in the lower termites, but discuss within-group conflict as a potential reason for discrepancies between this model and empirical reports.

Introduction

The optimal allocation of resources is a fundamental aspect of life history for all animals, both solitary and social, and can greatly impact development, life span and ultimately fitness. The provisioning of offspring is one example of the optimal resource allocation challenge, as it often requires individuals to make a temporary sacrifice – an investment – for improved long-term gains. Many social animals make these decisions as part of a joint venture. For example, in cooperatively breeding species, parental investment is extended to whole groups of individuals making joint sacrifices for a collective reward. Kin selection theory explains how groups of related individuals can benefit from undertaking such collective ventures (Hamilton, 1963, 1964; Griffin and West, 2003), but the dynamics of cooperation amongst non-kin are less well understood (Clutton-Brock, 2009). The lower termites present a unique opportunity to investigate collective investment amongst non-kin because of their capacity to live in either isolated or merged groups of cooperating, unrelated individuals.

In the lower termites, pseudergates are totipotent workers that can differentiate into either dispersing reproductives (alates) or sterile soldiers. While pseudergates perform most provisioning labour, soldiers defend the nest from intruders such as predators, thereby producing a public good – colony defence. Unlike pseudergates, soldiers are morphologically specialised for their role, and this specialisation means that soldiers are no longer capable of becoming reproductive; they instead rely on indirect fitness, and altruistically assist their sibling alates. Because of this, differentiation of a soldier removes a potential alate from the maximum number that a colony can produce, representing a cost to the colony in lost reproductive opportunity. Soldiers also rely on trophallaxis for nutrition, representing an additional cost because while a pseudergate is feeding a soldier, it cannot be provisioning the rest of the brood – i.e. potential future alates (McMahan, 1963; Song et al., 2006). However, soldiers appear to play an important role in the rearing of future reproductives and experimental evidence suggests that colonies with no soldiers produce fewer alates (Roux and Korb, 2004). This cost/benefit trade-off is reflected in their seasonal fluctuations, with peak soldier numbers coinciding with alate emergence, when their benefit is likely highest (Waller and La Fage, 1988).

Soldiers could be particularly important during interactions with neighbouring colonies, when they typically show aggression towards non-nestmates (Johns et al., 2009). A range of outcomes has been observed following these interactions from annihilation to colony fusion. Colony fusion occurs when two colonies merge to act as one multi-colony group, typically following an initial period of fighting (Thorne et al., 2003; Johns et al., 2009; Howard et al., 2013). Colony fusion is important in single-piece nesting termites because their confinement to a single piece of wood means that interactions can be frequent, and competition over nutrient-rich nesting space is strong (Shellman-Reeve, 1994). However, it is not only single-piece nesting termites that form fused groups – *Reticulitermes* species are nomadic and commonly found in multi-colony groups. Following a fusion event, some pseudergates differentiate into neotenic reproductives to replace the original reproductives, which are often killed during the initial period of aggression (Johns et al., 2009). In this way, pseudergates can gain direct fitness after fusion, avoiding the reduction in indirect fitness that comes with a sudden decline in average relatedness to the group (Hamilton, 1963, 1964; Korb and Roux, 2012). A less intuitive outcome of group fusion is the continued differentiation of soldiers from both colonies in the group (Howard et al., 2013).

In a fused group, each colony benefits from the public good produced by each soldier, regardless of the colony of origin of that soldier. We can model this as a ‘collective investment’ game (Madgwick et al., 2018) in which colonies jointly invest in the common good that is the soldier caste. Individual colonies pay a cost to invest in soldiers, which ultimately benefits their success. By jointly investing, colonies can share this cost. However, there are risks associated with games of cooperation, primarily that the interaction partner ‘defects’ (makes little or no investment), gaining the benefit from the public good without suffering the costs of investment. This could result in additional costs to both parties due to insufficient investment (in the case of termite soldiers, this cost could be vulnerability to predation, for example). However, if both colonies invest maximally in the public good, this could result in wasted investment, representing a cost in lost reproductive opportunity. Colonies in a fused group therefore face a dilemma about how to invest in soldiers.

Here we re-derive the collective investment game (Madgwick et al., 2018), and extend it to investigate how investment in soldiers might be expected change as a result of colony fusion in termites. To reflect the dependence of post-fusion investment on pre-fusion conditions, we first modelled soldier investment in a colony before it meets another. We then used a simple tug-of-war contest success function (Hirshleifer, 1989) to allow a second, post-fusion investment model to depend on these pre-fusion conditions. We modelled a typical violent fusion event between two unrelated groups, during which some individuals are expected to die, therefore changing relative proportions of each colony in the fused group. We assumed colonies of equal size to draw our main conclusions, but demonstrate how a size differential might change the outcome of colony fusion. We additionally explored the effects of fusion on a measure of colony-level fitness (successfully dispersed alates) to draw conclusions about when fusion might be beneficial at the colony level. We discuss the limitations of a colony-level model for lower termites, whose workers retain the capacity to become reproductive throughout development.

The model

We present a two-part model, which extends the collective investment model by Madgwick et al. (2018). Madgwick et al. (2018) derive the collective investment game to describe investment in sterile stalk cells in uni- and multi-clonal *Dictyostelium discoideum* aggregations. Multi-clonal *D. discoideum* aggregations and multi-family termite groups face similar investment decisions because groups contain a mixture of closely related and unrelated individuals; uni-clonal *D. discoideum* aggregations are analogous to un-fused termite colonies, living in kin groups. Termite group fusion occurs in stages, and the initial meeting of colonies is often violent. By modelling the dynamics of this conflict, we extend the collective investment game to link pre- and post-fusion soldier investment to investigate how optimal investment might change depending on the outcome of conflict during the initial meeting, and its consequences on the composition of the fused group.

Part I: Before fusion

The first part of the model describes a single colony before it meets a second colony. We therefore consider the cost and benefit of investing in the public good (soldiers) in a colony of equally- and closely-related individuals. We define cost as the loss in fecundity per unit investment in soldiers, and the benefit as the increased dispersal success of each reproductive (reproductive efficiency) due to the presence of a soldier. We assume a linear relationship between both cost and benefit and the number of soldiers.

As in other models of intergroup conflict (e.g. Fearon, 1995; Miller and Engemann, 2004; reviewed in Sherratt and Mesterton-Gibbons, 2013; Rusch and Gavrilets, 2017), we treat the colony as a unitary actor. We therefore assume that individuals in a group share the same preferred outcome of an interaction, and we assume a mechanism by which individuals in the group reach a consensus decision on soldier investment. We ignore within-group differences between individuals that could result in individual-level conflicts of interest over interaction outcome and soldier caste ratio. Investment in soldiers is therefore given as the proportion of the total 'budget' that a colony can allocate towards either soldiers or reproductives. We evaluate the limitations that this simplifying assumption introduces – particularly in the lower termites – in the Discussion.

In a termite colony, fecundity is related to the number of dispersing alates as these individuals leave the nest to breed and found a new colony. For every soldier that a colony produces, there is one less potential reproductive; additionally, soldiers are nutritionally dependent on nestmates, which might cause further reductions in fecundity. Fecundity (F) for a given colony, i, is therefore given by:

$$F_i = 1 - c_i I_i \tag{4.1}$$

where I_i is the level of investment in soldiers as a proportion of the total possible investment and c_i represents the cost per unit investment in soldiers. Fecundity is defined between 0 and 1, such that the maximum fecundity, 1, occurs when there is no investment in soldiers.

Soldiers benefit the colony by providing defence, which allows the alates to develop and disperse (Roux and Korb, 2004). The success of alates for colony i (S_i) is given by:

$$S_i = 1 + b_i I_i \tag{4.2}$$

where b_i represents the survival enhancement to alates conferred per unit investment in soldiers.

Following from optimal brood size models (Smith and Fretwell, 1974), we can multiply fecundity and survival to give a measure of colony-level (parental) fitness. We define the fitness of a colony, i (W_i) as:

$$W_i = (1 - c_i I_i)(1 + b_i I_i), \tag{4.3}$$

such that the minimum 'fitness', 0, occurs when I_i is 1 (i.e. there is total investment in soldiers).

The proportion of resources allocated towards soldiers that maximises this function for a given cost and benefit we term optimal investment. Optimal investment for colony i (O_i) is given by:

$$O_i = \left\langle \frac{1}{2}\left(\frac{1}{c_i} - \frac{1}{b_i}\right)\right\rangle \tag{4.4}$$

(see Appendix B.1 for derivation; Madgwick et al., 2018), where $\langle x \rangle = \max(x, 0)$, such that when O_i is negative, it is defined as 0. This is because there can be no negative investment (the transition from pseudergate to soldier is irreversible). As the benefit to alate dispersal (b_i) increases, optimal investment increases up to an asymptote at 0.5 (Figure 4.1a); as the cost of soldier production increases, optimal investment decreases exponentially (Figure 4.1b).

Conflict phase

Part I of the model presented here describes a lone colony's investment in soldiers, which produce a public good that aids success of dispersing reproductives. We now move on to model the initial aggression that typically characterises the early stages of group fusion. The outcome of this aggression determines the composition of the subsequent fused group, and is therefore an important part of group fusion (Johns

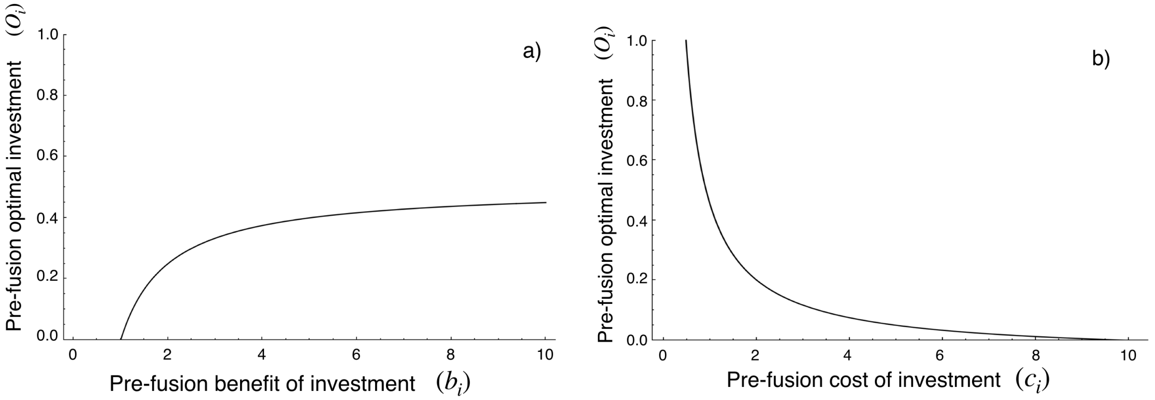


Figure 4.1: **A higher benefit and a lower cost of soldiers increases the optimal level of investment.** Optimal investment reaches an asymptote at 0.5 as benefit increases, so there is always lower investment in soldiers than alates. Optimal investment reaches zero when the benefit is equal to the cost. Parameters: a) cost (c_i) = 1; b) benefit (b_i) = 10.

et al., 2009). Since soldiers are the primary aggressors, investment in soldiers is clearly important to the outcome of this initial meeting. Here, we used a tug-of-war contest success function to determine how differential investment in soldiers might affect composition of a fused group (Hirshleifer, 1989). We can say that the representation of colony i (R_i) in the fused group is given by its soldier investment relative to that of colony j, scaled by the difference in size (s) between the two groups (Hirshleifer, 1989). Size here represents the relative number of pseudergates, which could play a role in this conflict as they are known to also show aggression (Thorne, 1982). Assuming that both colonies invest optimally given the environmental conditions that each experiences before they meet, the proportion of the fused group that colony i comprises is given by:

$$R_i = \frac{O_i}{O_i + sO_j} \tag{4.5}$$

where O_i and O_j are the (pre-interaction) optimal investment levels of two colonies i and j, respectively, as determined by Equation 4.4, and s is the relative size of colony j, where a value of 1 would mean that groups are equally sized; $s < 1$ denotes that colony j is smaller than colony i and $s > 1$ denotes that colony j is larger than colony i. Because we assume that there are two interacting groups, the representation of the second colony, j, is simply $1 - R_i$. Representation in the fused groups is important because, assuming that pair-wise relatedness is roughly equal within a group, but zero between individuals of different groups, representation of colony i can be used as a proxy for the average relatedness of an individual from colony i to the whole group. Figure 4.2 shows the effects of relative investment and size on representation in the final group.

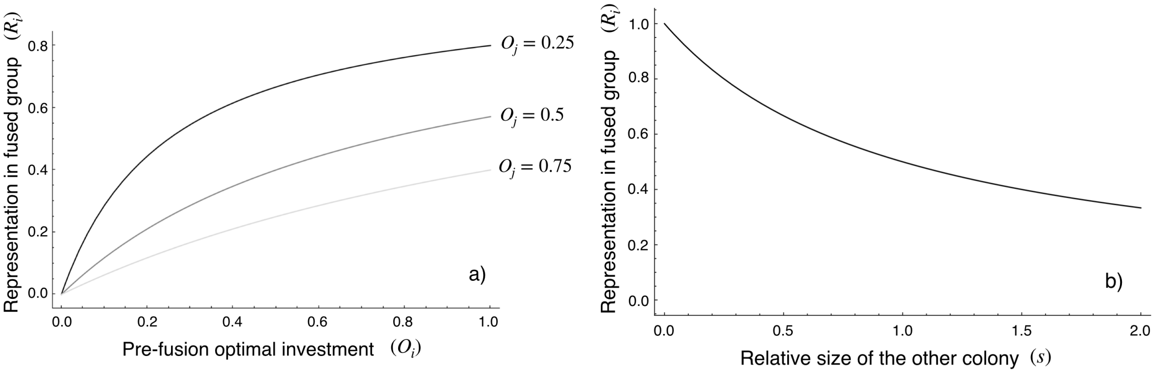


Figure 4.2: **Greater investment in soldiers and smaller opponent size result in higher representation in the fused group.** a) Colonies that invest more in soldiers initially are better represented in the fused group, but higher investment by the other decreases the rate at which investment 'pays off' and results in more equal overall representation; each line represents a different level of investment by the other colony: light grey shows high investment by the other colony, dark grey, an intermediate investment and black a low investment by the other colony. b) The larger the other colony's (colony j) relative size (s), the lower the representation of the focal colony (colony i), in the fused group.

Part II: After fusion

Following fusion, the two colonies behave as a single, larger colony so that the public good (colony defence) produced by each soldier helps members of both original colonies. This assumption is broadly appropriate because of the nature of the public good provided – a general reduction in predator presence benefits both colonies, for example. Each colony therefore faces a dilemma about how much to invest given that some of this investment could be 'wasted' if it benefits unrelated individuals from the other colony. Additionally, if both colonies invest in soldiers, the group might have redundant soldiers that do not provide sufficient additional benefit to outweigh the cost in lost fecundity. However, if a colony i invests in no soldiers, it risks leaving alates undefended if the other colony produces insufficient soldiers to defend all the alates in the colony. The former option might therefore result in wasted investment, while the latter risks the overall group investment being too low.

Because the production of soldiers is assumed to benefit members of both colonies and kin recognition is assumed to be absent, the probability that a soldier from one colony will help an alate from the same colony is proportional to the representation of that colony in the group. According to Hamilton's rule, we would expect that the greater the likelihood of conferring an altruistic benefit on an individual with shared genes (i.e. that is related), the greater the level of altruism shown (Hamilton, 1964). Since soldiers are altruists, the level of soldier investment by a colony should therefore increase with its representation in the fused group. We can extend the previous, single-colony optimal investment function to include these parameters and a new post-fusion cost (c') and benefit (b') to give the post-fusion optimal investment for colonies i and j (O'_i, O'_j):

$$O'_i = \left\langle \frac{1}{2}\left(\frac{1}{c'} - \frac{1}{b'R_i} - \frac{O'_j(1-R_i)}{R_i}\right)\right\rangle \tag{4.6}$$

$$O'_j = \left\langle \frac{1}{2}\left(\frac{1}{c'} - \frac{1}{b'(1-R_i)} - \frac{O'_iR_i}{(1-R_i)}\right)\right\rangle, \tag{4.7}$$

where the first term, $\frac{1}{c'}$, represents the cost of soldiers in lost reproductive opportunity; this term is the same for both colonies because they now share resources and so the cost of soldiers is assumed to be identical. The second term represents the benefit per unit investment, multiplied by the probability that that benefit will go towards the colony that produced that soldier. The final term represents an additional opportunity cost in lost reproductives caused by soldier investment by the other colony. This is a form of Hamilton's rule (see Appendix B.2; B.3 for the fully expanded model, and its relation to Hamilton's rule, respectively).

In a fused group, the success of alates from each colony depends on the combined investment of both colonies in the group. Because each colony invests proportionally to their representation in the group, the joint investment of the group (O_G) is defined as the weighted average level of investment by each colony ($O_G = \sum O_i' R_i$). This means that post-fusion fitness of colony i (W_i') depends on the group rather than colony investment:

$$W_i' = (1 - c' O_i')(1 + b' O_G). \tag{4.8}$$

Model interpretation

With this model, we investigate the collective investment decision of a colony of termites in a fused group. We choose parameter values in figures to exemplify their effects, but these results hold qualitatively across a wide range of values. Firstly, we explore how optimal investment changes following fusion, and then how post-fusion optimal investment is influenced by representation in the group and the other colony's investment, and how this is constrained by the cost and benefit of soldier production in a fused group. Using the measure of fitness defined above (Equation 4.8), we then examine how different outcomes of fusion, in terms of group composition, can affect colony-level fitness.

Results

Optimal investment in soldiers before and after fusion

Optimal investment is typically lower following fusion than before. However, when the cost of soldiers (lost reproductive opportunity) is sufficiently lower after fusion, then a colony's optimal investment in soldiers can be higher following fusion (Figure 4.3). Once pre-fusion cost is high enough that it constrains a colony's representation in the fused group (because fewer soldiers are present during the conflict phase), then the consequent low level of representation can cause post-fusion investment to be lower compared to pre-fusion investment (Figure 4.3). This is also dependent on the pre-fusion investment by the other colony – high pre-fusion investment by colony j will lead to lower representation and therefore lower post-fusion investment by colony i.

Changes in cost and benefit (increased reproductive efficiency) of soldiers produce similar patterns of optimal investment before and after fusion, with lower cost and higher benefit resulting in increased optimal investment. Following fusion, the greater proportion of the group that a colony makes up (the higher its representation), the higher its optimal investment because the efficiency of the investment is greater – it has a higher probability of benefiting the colony that produced the soldier. However, a colony's optimal investment decreases with increasing post-fusion investment by the other colony because the opportunity cost increases with the presence of soldiers from the other colony (Equation 4.6; Figure 4.4).

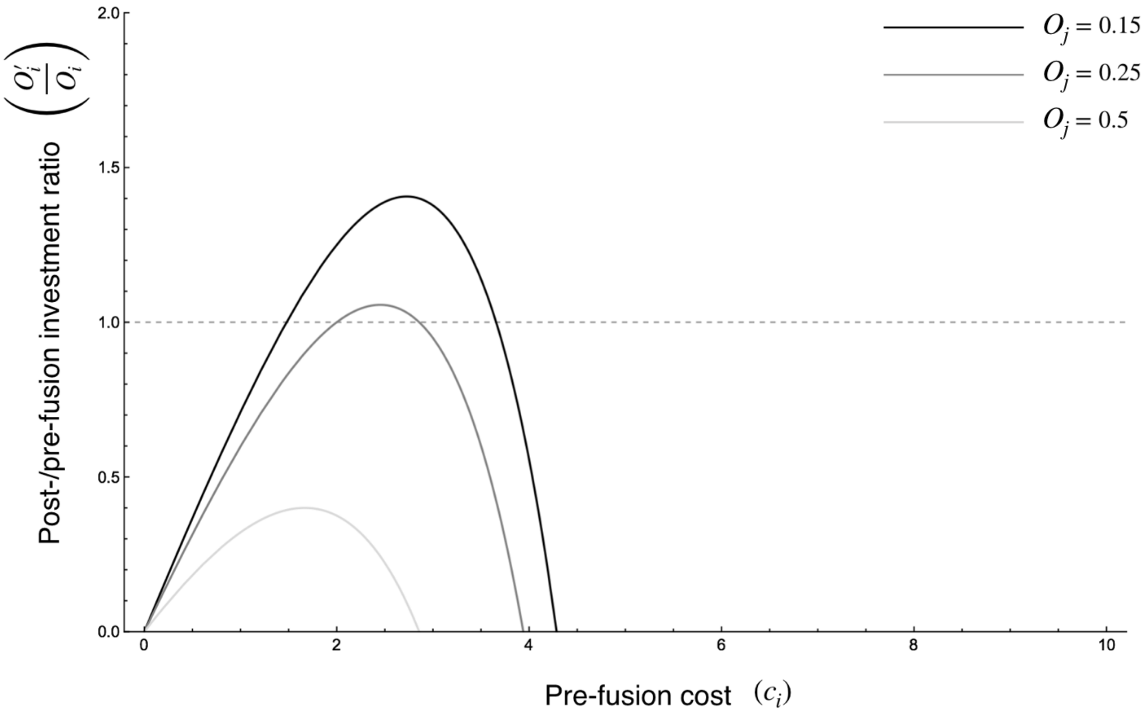


Figure 4.3: **Post-fusion investment is typically lower than pre-fusion investment, but can be higher if the cost decreases after fusion, and depending on representation.** A higher pre-fusion cost causes pre-fusion – but not post-fusion – investment to decrease; when pre-fusion cost begins to constrain representation in the fused group, post-fusion investment falls faster than pre-fusion investment. Cost and benefit parameters: $b_i = 10$; $c' = 1$; $b' = 10$.

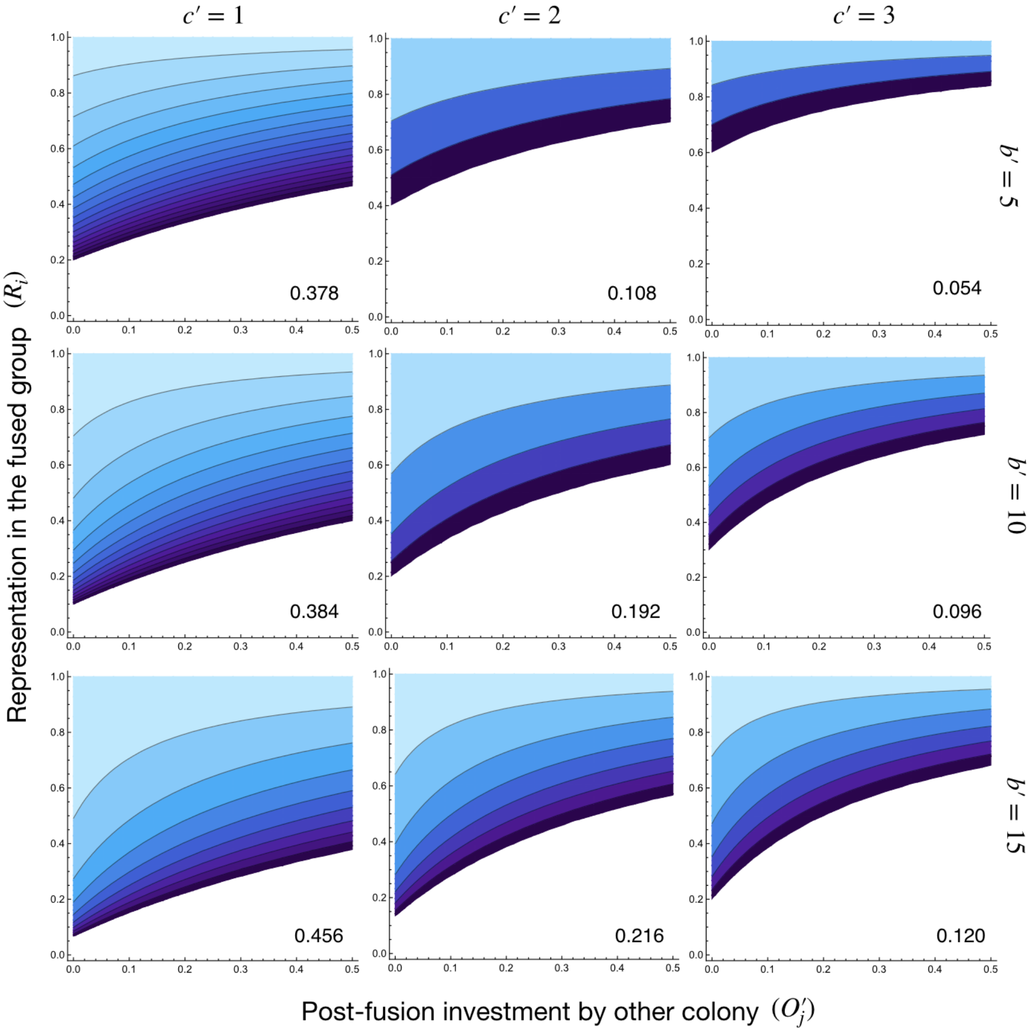


Figure 4.4: **Higher representation in the fused group and lower investment in soldiers by the other colony results in higher optimal investment.** Optimal investment by colony i (O'_i) is shown in blue, lighter blue represents a higher level of investment – the value of the highest contour is shown in the figure. White space represents the region where optimal investment by colony i is zero.

Fitness outcomes of colony fusion

The fitness impact of colony fusion depends on a colony's representation in the fused group and their consequent soldier investment (Equation 4.8a,b). When colony j makes a limited investment, colony i is only predicted to benefit from fusion if it is in the extreme minority, and so makes little or no investment. However, when

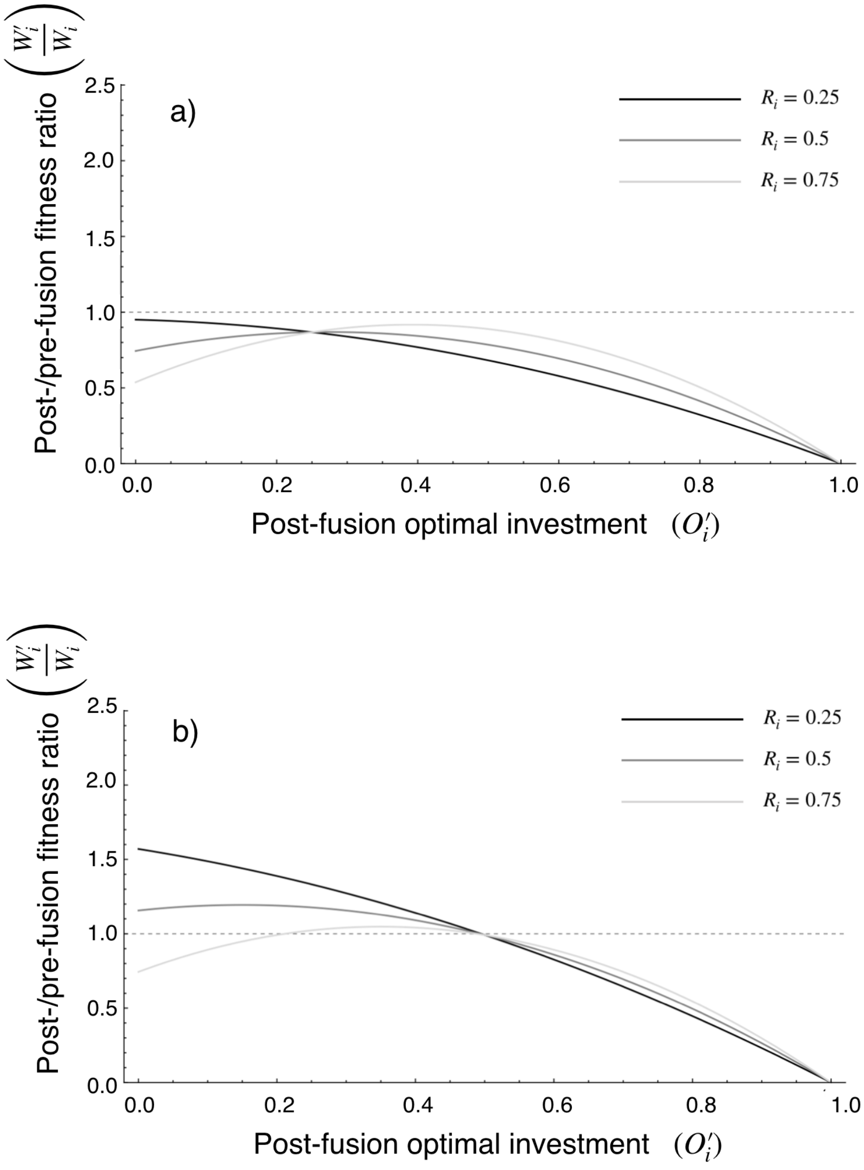


Figure 4.5: **A colony can gain a fitness advantage after fusion if there is sufficient post-fusion soldier investment.** Following fusion, if the other colony does not invest much in soldiers ($O_j' = 0.25$; figure a), then a colony is predicted to have lower fitness after fusion no matter how much it invests itself. However, if the other colony makes a high investment in soldiers ($O_j' = 0.5$; figure b), then a colony can gain a fitness advantage from continued investment after fusion. The way in which a colony's own investment affects its fitness also depends on its representation in the fused group – a colony in the minority ($R_i = 0.25$ is shown in the figure but this pattern holds when $R_i < 0.4$) does not benefit from investing, while a colony in the majority often benefits from some investment. Parameters: $c' = 1$ and $b' = 10$.

the other colony makes a high investment, then fusion can be beneficial even for a majority colony (Figure 4.5b). The fitness of the colony in the minority is predicted to be higher than that of a majority colony, because a minority colony is predicted to make a lower investment (see Appendix B.4 for an alternative formulation of the model illustrating this). Figure 4.5 shows these patterns of fitness for two example levels of investment by the other colony ($O'_j = 0.25$ and $O'_j = 0.5$) and three levels of representation ($R_i = 0.25$, $R_i = 0.5$, and $R_i = 0.75$), but these patterns also hold for a broader parameter space.

Discussion

Here we developed a game theoretical model of collective investment in soldiers by a colony of termites following fusion with another colony, after which the benefits (but not costs) of investment are shared between the two colonies. This model predicts that optimal investment in soldiers typically decreases following fusion, and that fitness also typically falls. However, if colony fusion causes the cost/benefit ratio of soldier production to decrease, then a colony's optimal investment in soldiers and fitness can increase following fusion. Fitness can also increase following fusion if a colony is able to take advantage of the other colony's soldier investment, making limited investment itself.

Optimal investment in soldiers before and after fusion

The prediction that soldier investment will decrease following fusion is contrary to some empirical evidence that suggests that colonies in fused groups continue to produce soldiers (Howard et al., 2013). However, the model predicts that a colony's investment can be higher following fusion under two scenarios: when the other colony makes little or no investment, and when the cost/benefit ratio of soldier production decreases after fusion. A minority colony is predicted to invest very little, so a colony that finds itself in the majority in a fused group might optimally invest more in soldiers than it did before fusion to compensate. This is tentatively supported empirically by Johns et al. (2009) who find that most of the soldiers in a fused group originate from the majority colony. However, the skew in soldier origins is slight in this study, and there is only limited evidence that these soldiers differentiated after the fusion event.

Following fusion, the model predicts that a colony can invest more than it did before fusion if the cost/benefit ratio of soldiers decreases. The benefit that soldiers confer could increase following fusion if larger groups are more vulnerable to predation, for example. However, the benefit of soldiers could also decrease because colony fusion removes a potential competitor from the environment, making another violent encounter less likely. Costs of soldier production could fall after fusion if there are more resources available per capita than before fusion, as resource availability can limit soldier number (Song et al., 2006). This might be relevant for colonies of single-piece nesting termites, which could gain access to important, patchily distributed resources by fusing (Shellman-Reeve, 1994). It is less clear what cost reduction nomadic termites like *Reticulitermes* species might experience, since they are less constrained by resource availability.

Fitness outcomes of colony fusion

In most post-fusion scenarios, a colony's best outcome in terms of producing successful alates is when it avoids investing in soldiers but can exploit the investment of the other colony. This prediction is supported by evidence that *Reticulitermes speratus* colonies will preferentially fuse with another colony that has a low ratio of nymphs to workers, and are aggressive towards colonies with a higher ratio of nymphs (Matsuura and Nishida, 2001). The nymph is the developmental stage before the alate, and it is suggested that a colony could benefit from fusing with another that has a lower proportion of nymphs because the colony can gain additional workers to provision their own nymphs (Matsuura, 2001).

Alternatively, a colony could benefit from fusion if the costs of soldier production decrease or the benefits increase as a result of colony fusion. Lower costs enable higher investment in soldiers, providing more benefit to the colony. Higher benefit means that less soldier investment is needed to gain their maximum benefit, allowing greater resource allocation towards alates. As we discuss above, the cost and benefit of soldier production is likely to change for a colony following fusion, but it is unclear in which direction and to what extent these changes might occur.

Taking these points together, the model predicts that there are two ways in which group fusion could be beneficial to a colony: 1) if the cost/benefit ratio of soldiers decreases following fusion, meaning that higher investment is beneficial; or 2) if the other colony's investment can be exploited. These two options could be relevant to termite species of different nesting types. Single-piece nesting termites might be more likely to benefit by gaining access to patchily distributed resources, meaning that they can afford to invest more in defensive soldiers following fusion (Shellman-Reeve, 1994). Conversely, foraging termites are less likely to benefit from gaining resources post-fusion, but they could benefit from another colony's worker caste (Matsuura and Nishida, 2001).

Future directions

This model predicts that optimal soldier investment should decrease following fusion. However, this assumes no within-group conflict, and does not reflect what is reported from experimentally staged colony fusions. This discrepancy could be a result of the difference in potential fitness of termite castes – soldiers have no future direct fitness opportunities, but pseudergates can and do gain direct fitness (Johns et al., 2009; Korb and Roux, 2012). The differences in potential fitness outcomes introduce the potential for within-colony conflicts of interest, which could affect the consequences of fusion. For example, there is evidence that the presence of hormones from reproductives can trigger soldier differentiation (Lüscher, 1960; Maekawa et al., 2012). Pseudergate reproductive disinhibition following colony fusion might therefore result in additional soldier differentiation. This represents a potential mechanism by which within-colony reproductive conflict could result in behaviour that benefits individuals from both colonies – i.e. cooperation between groups.

To investigate this potential effect, modelling frameworks that deal with individual-level fitness decisions – for example, n-player extensions of the optional prisoner's dilemma, or the volunteer's dilemma, in which individuals choose whether or not to invest in a public good – could offer insight into the adaptive causes of differentiation into soldiers following fusion at the individual level (Diekmann, 1985; Hauert et al., 2002; Archetti, 2009). Experimental manipulation of hormones that regulate caste differentiation during fusion could simultaneously help to reveal the proximate mechanisms that underpin caste differentiation during colony fusion.

Colony fusion is thought to be a selective force in the evolution of pseudergate totipotency, constraining the evolution of a true worker caste. Understanding the causes and consequences of colony fusion could help our understanding of the role of ecology in the evolution not only of cooperation between groups, but of reproductive division of labour and social organisation.

Chapter 5

General discussion

Colony fusion in the lower termites raises questions about the relative roles of ecological and social drivers in the evolution of eusociality. Experimental work has laid the foundations for exploring these questions by establishing mechanisms by which individuals might help their kin or target enemies, and identifying physiological pathways of caste and colony development. But colony fusion in the lower termites also presents broader questions that are applicable across taxonomic boundaries: why should unrelated individuals from different groups work together? How can apparently altruistic behaviour evolve with low kinship? In this thesis I began by trialling a potential individual marking method that could facilitate individual-based behavioural experiments in termites. I then experimentally investigated nestmate recognition to explore the mechanistic causes of colony fusion, before using a theoretical model to examine its functional and adaptive consequences.

Nestmate recognition and the causes of colony fusion

In Chapter 3, I found that pseudergates of a single-piece nesting lower termite, *Zootermopsis angusticollis*, appear to identify non-nestmates as they show increased identity-checking behaviour (antennation) towards an introduced non-nestmate, and a recoiling response that is associated with a negative stimulus (Thompson et al., 2020; Howse, 1965). The behavioural response to non-nestmates does not appear to be dependent on broad-scale dietary differences between individuals. This indicates that nestmate recognition is present in lower termite pseudergates, and could have a strong non-environmental component in single-piece nesting termites, or could be environmentally mediated at a finer scale than substrate type, such as that of microbial heterogeneity within a log (Shellman-Reeve, 1994).

The apparent plasticity in response to non-nestmates implied by the variation in results from similar studies across the literature could indicate that termites use a range of nestmate recognition cues. Experimental design also appears to play a role in the level of aggression shown in non-nestmate introduction trials (Cornelius and Osbrink, 2009), suggesting that short-term changes in the environment could affect perception of threat. Explicitly testing the role of nest proximity in intercolony aggression would facilitate the design of experiments that can test behavioural responses to non-nestmates at a finer scale and potentially remove confounding effects of experimental design from future studies.

Contrary to prediction, we found that pseudergates displayed cooperative behaviour (allogrooming) towards non-nestmates and nestmates equally. This observation supports the hypothesis that pseudergates gain direct fitness benefits from cooperating with non-nestmates when this leads to colony fusion, when pseudergates gain the opportunity to become reproductive (Thorne et al., 2003; Johns et al., 2009; Korb and Roux, 2012; Howard et al., 2013). The ability of pseudergates to become reproductive introduces the potential for within-group conflict as soldiers relying on indirect fitness might benefit from maintaining group boundaries so that they direct help towards kin, while pseudergates might gain direct fitness benefits from colony fusion (Korb and Roux, 2012).

A link between within- and between-group cooperation and conflict has been documented across a variety of taxa, with several studies suggesting that between-group conflict promotes within-group cooperation (e.g. in birds, Radford, 2008, 2011; in mammals, Thompson et al., 2017b; in fish, Bruintjes et al., 2016). Theory also shows support for these observations, suggesting that individuals can benefit more from investing in between-group than within-group conflict if between-group competition and within-group kinship are high (Reeve and Hölldobler, 2007), or if within-group cooperation promotes success in intergroup conflict (Choi and Bowles, 2007).

Studies in which lower termite pseudergates are found to be cooperative towards non-nestmates in order to gain direct fitness benefits suggest that this relationship could also go the opposite way and that within-group conflict (over reproduction, for example) could make individuals more willing to cooperate with other groups (Korb and Roux, 2012). This is supported by theory, which shows that when competition is high amongst kin, this can outweigh kin-selected benefits of helping (Murray and Gerrard, 1984; Wilson et al., 1992). The presence of individuals in a group that have selfish interests that are better met by out-group affiliation or cooperation than by maintaining group cohesion could therefore promote intergroup cooperation or even group fusion, as appears to be the case in termites (Johns et al., 2009; Howard et al., 2013).

Experimentally manipulating within- and between-group conflict could help to uncover the interaction between conflict and cooperation at different levels. For example, reproductive conflict can be created within groups of the lower termites by removal of the primary reproductives from the colony, triggering the development of pseudergates into replacement reproductives (Thorne et al., 2003; Johns et al., 2009; Penick et al., 2013). When this happens, pseudergates perform behavioural displays, such as butting against the substrate and increasing rates of social contacts, to establish reproductive dominance (Penick et al., 2013). Manipulating within-group conflict by removing primary reproductives, and simultaneously altering perceived levels of intergroup conflict (as in Thompson et al. (2020), for example), could allow analysis of the behavioural responses to conflict at different levels. Direct competition assays in other systems, such as slime molds and bacterial biofilms, in which within-group relatedness can be directly manipulated (e.g. Madgwick et al., 2018; Griffin et al., 2004) could also be important to establish the generality of these trends.

Soldier investment and the consequences of colony fusion

In Chapter 4, I used a game theoretical collective investment model to investigate the consequences of group fusion on colony-level soldier production and alate dispersal, a colony-level measure of fitness. I found that soldier investment and alate dispersal were both typically predicted to fall following fusion. If the cost of soldiers fell sufficiently then soldier investment could be higher after fusion. Asymmetry in representation of colonies in a fused group resulted in potentially higher post-fusion alate production for the minority colony, which was predicted to produce few, if any, soldiers. The model predicted that colonies could benefit (in terms of alate dispersal) from jointly investing in soldiers only when the costs of soldier production are low.

Considering colony-level fitness as we did in Chapter 4, only a small region of the parameter space – when costs of soldier production were low and the benefits high – was predicted to result in an increase in alate dispersal for both colonies following fusion. This does not necessarily match with empirical findings that around 30% of lower termite groups comprise fused colonies since this frequency suggests that encounter rates would provide sufficient selection pressure for resistance to unwanted fusions if all fusion was exploitative (Deheer and Vargo, 2004; Korb and Schneider, 2007; Howard et al., 2013).

This mismatch suggests that individual-level factors like the direct fitness that pseudergates can gain might be more important in the evolution of termite colony fusion than group-level fitness effects (Korb and Roux, 2012). While classical game theory and the extension of dyadic games to groups has provided valuable insights into intergroup conflict, development of theoretical frameworks that incorporate heterogeneity in individual- or (in eusocial societies) caste-level fitness effects will likely improve our understanding the causes of intergroup cooperation and the role that intergroup cooperation plays in the evolution of social groups and the sometimes-blurred boundaries between them.

Conclusions

The spectrum of social and ecological niches that termites occupy makes them a valuable system for studying the evolution of complex social behaviour. The social organisation of the lower termites in particular, with their hybrid of cooperative breeding and strict eusociality, uniquely places them as a model to address the link between within- and between-group cooperation and conflict. Further experimental work to investigate the mechanisms, both physiological and behavioural, that underpin the maintenance of these social groups will help to address questions about the relative roles of punishment and kinship, for example, and help to answer questions such as: why should unrelated groups cooperate or fuse? How is reproductive altruism maintained in groups of non-relatives? And what selects for the evolution of irreversible altruism, as in the helpers of ants and the higher but not lower termites? These questions link to broader ideas about conflict resolution and cooperation in animal societies in general and could aid our understanding of the formation and transformation of social groups.

The development of theoretical models that take into account individual-level fitness during intergroup interactions will further expand the range of questions that we can address about social organisms. Combining theoretical and empirical work has already proved a powerful tool in the development of our understanding of the evolutionary forces that govern the origin and maintenance of social groups from genomes to populations. Developing these ideas with novel experimental methods and extensions of current theoretical frameworks will facilitate further expansion of our understanding of the interplay between the conflict and cooperation that shape the living world.

Appendix A

Appendix A: VIE trial

A.1 Code for survival simulations

```
proportion_censored <- length(f_data$Day_survived[which(
f_data$Day_survived==63)])/length(f_data$Day_survived)

num_deaths <- length(f_data$Day_survived[which(
f_data$Treatment==0 & f_data$Day_survived < 63)])
deaths_per_day <- (num_deaths/63)
deaths_pd_pi <- deaths_per_day/65
hazard_function <- function(t){deaths_pd_pi}

all_simdata <- list()
all_sim_time <- c()
all_sim_status <- c()
all_sim_treatment <- c()
all_sim_surv_object <- c()
```

```
all_sim_fit1 <- c()
all_pvals <- c()
all_sim_fit <- list()

for (i in 1:1000){
  simdata <- sim.survdata(N=130, T=63, num.data.frames=1, X=cov,
        beta=c(0.253), censor = proportion_censored,
        hazard.fun = hazard_function)
  sim_time <- simdata$data$y
  sim_status <- 1-simdata$data$failed
  sim_treatment <- simdata$data$Treatment
  sim_surv_object <- Surv(sim_time, sim_status)
  sim_fit1 <- survfit(Surv(sim_time, sim_status) ~   Treatment,
        data = simdata$data)
  all_simdata[[i]] <- simdata
  all_sim_time <- c(all_sim_time,sim_time)
  all_sim_status <- c(all_sim_status,sim_status)
  all_sim_treatment <- c(all_sim_treatment,sim_treatment)
  all_sim_surv_object <- c(all_sim_surv_object,sim_surv_object)
  all_sim_fit[[i]] <- sim_fit1
  all_pvals <- c(all_pvals, surv_pvalue(sim_fit1)[[2]])
}
```

A.2 Origin of individuals that died

Colony ID	Treatment	Number died	Mean group weight on day 0 ± SD
HC2	VIE	4	0.02618 ± 0.00117
HC2	Control	0	0.02880 ± 0.00521
RW6	VIE	4	0.03776 ± 0.00636
RW6	Control	0	0.04220 ± 0.00791
RW4	VIE	1	0.06342 ± 0.0119
RW4	Control	0	0.06220 ± 0.0139
BB1	VIE	0	0.06540 ± 0.0141
BB1	Control	5	0.07528 ± 0.0173
BB2	VIE	3	0.08720 ± 0.00423
BB2	Control	5	0.09298 ± 0.00957
HC3	VIE	1	0.07178 ± 0.0187
HC3	Control	1	0.07216 ± 0.0272
RW3	VIE	0	0.03804 ± 0.00683
RW3	Control	1	0.03166 ± 0.00839
BUT1	VIE	2	0.04712 ± 0.00339
BUT1	Control	5	0.04958 ± 0.00200
HC1	VIE	1	0.02580 ± 0.00117
HC1	Control	0	0.02900 ± 0.00521
FON1	VIE	0	0.05434 ± 0.0192
FON1	Control	0	0.05044 ± 0.00607
RW7	VIE	0	0.04716 ± 0.0112
RW7	Control	0	0.05324 ± 0.00877
RW8	VIE	0	0.04618 ± 0.00518
RW8	Control	0	0.05356 ± 0.00694
SLR1	VIE	0	0.06972 ± 0.0120
SLR1	Control	0	0.06002 ± 0.00989

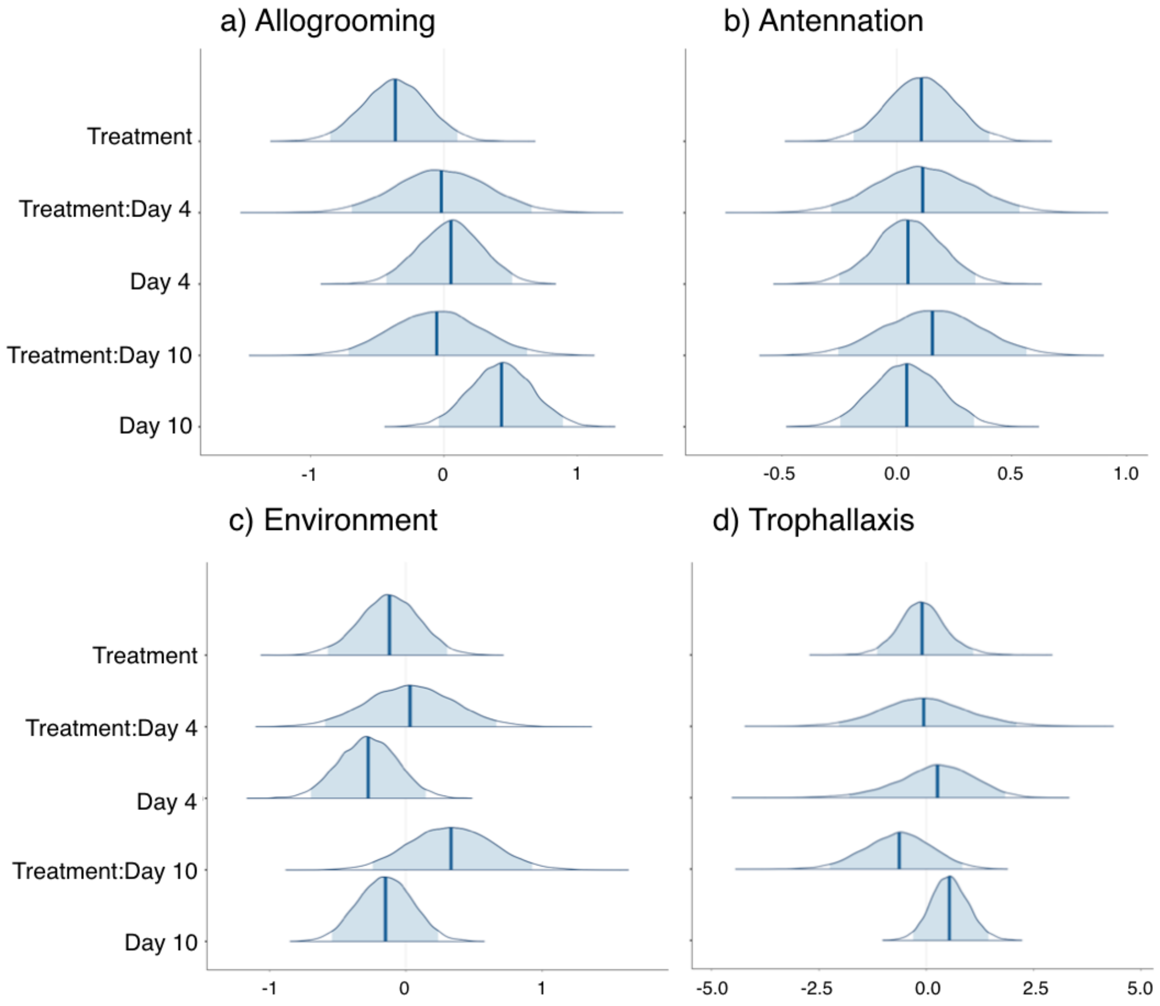
Table A.1: **Treatment, number (of out the group of five) that died and mean group weight for each arena.**

A.3 Pearson correlation coefficients for behaviours

	Allogrooming	Antennation	Butting	Environment	Self-grooming	Trophallaxis
Allogrooming	1.00	0.13	0.01	-0.10	-0.02	**0.18**
Antennation	0.13	1.00	-0.04	-0.07	-0.06	0.06
Butting	0.01	-0.04	1.00	-0.03	0.04	0.00
Environment	-0.10	-0.07	-0.03	1.00	-0.03	-0.03
Self-grooming	-0.02	-0.06	0.04	-0.03	1.00	-0.04
Trophallaxis	**0.18**	0.06	0.00	-0.03	-0.04	1.00

Table A.2: **Pearson correlation coefficients showing that there are no strong correlations between behaviours.** Bold values show the significant correlation (after Bonferroni correction) between allogrooming and trophallaxis.

A.4 Distributions of coefficient estimate



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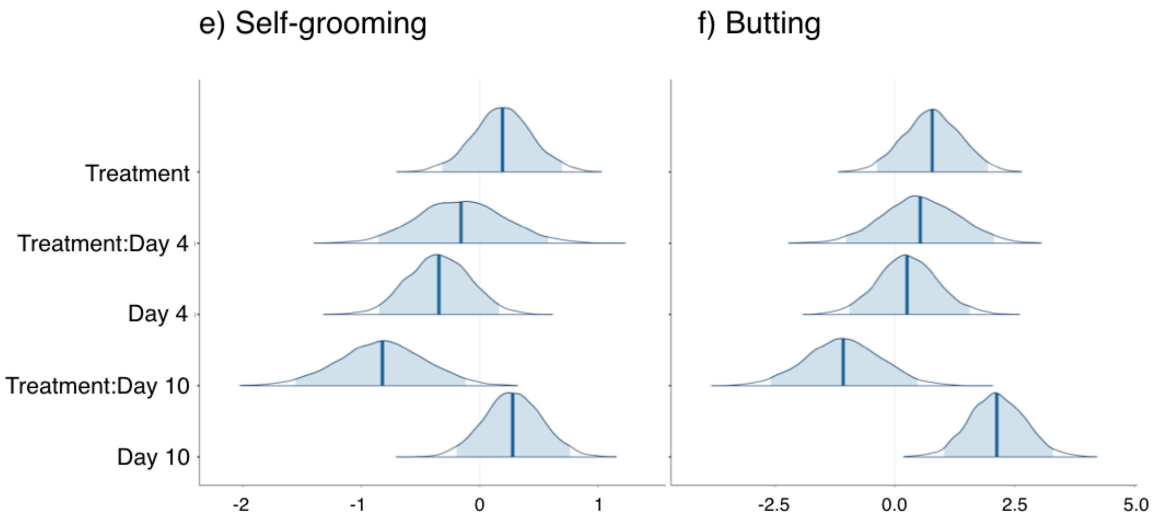

Figure A.1: **Posterior distributions of coefficient estimates from GLMMs for behaviours observed in the Visible Implant Elastomer trial.**

Appendix B

Appendix B: Soldier investment model

B.1 Deriving optimal investment from the fitness curve

Fitness in the model is given by:

$$W = (1 - cI)(1 + bI),$$

producing the fitness curve shown in Figure B.1. To find the optimal investment, the fitness curve can be differentiated to find its maximum:

$$\frac{dW}{dI} = -2bcI + (b - c).$$

When $\frac{dW}{dI} = 0$, we can rearrange this to:

$$I = \frac{c - b}{2bc}$$

which is equivalent to:

$$O_i = \frac{1}{2}(\frac{1}{c} - \frac{1}{b}).$$

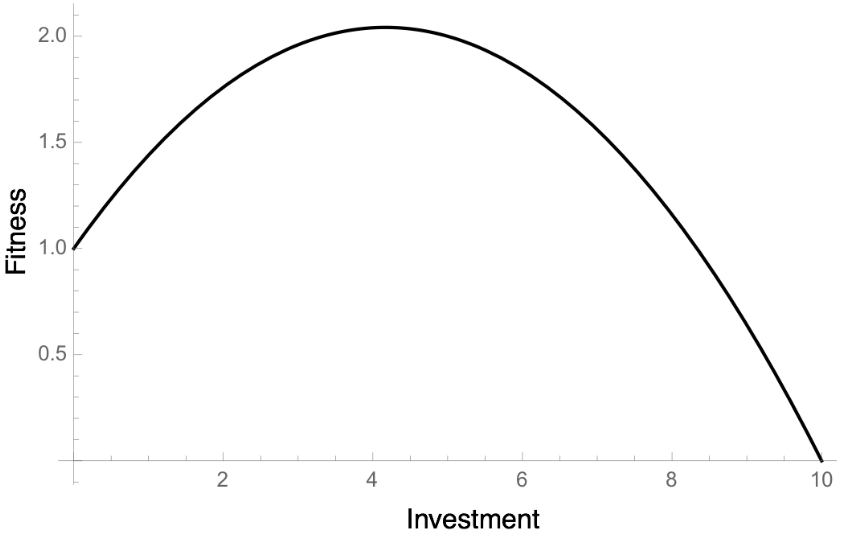

Figure B.1: **Example fitness curve**. Peak fitness occurs at an intermediate level of investment.

B.2 Fully expanded optimal investment model

Post-fusion optimal investment is given by:

$$O'_i = \left\langle \frac{1}{2} \left(\frac{1}{c'} - \frac{1}{b' R_i} - \frac{O'_j(1 - R_i)}{R_i} \right) \right\rangle . \tag{B.1}$$

Which is equivalent to:

$$O'_i = \left\langle \frac{1}{2} \left(\frac{1}{c'} - \frac{1}{b' \frac{O_i}{O_i + sO_j}} - \frac{O'_j(1 - \frac{O_i}{O_i + sO_j})}{\frac{O_i}{O_i + sO_j}} \right) \right\rangle , \tag{B.2}$$

and:

$$O'_i = \left\langle \frac{1}{2} \left(\frac{1}{c'} - \frac{1}{b' \frac{\left\langle \frac{1}{2}\left(\frac{1}{c_i}-\frac{1}{b_i}\right)\right\rangle}{\left\langle \frac{1}{2}\left(\frac{1}{c_i}-\frac{1}{b_i}\right)\right\rangle + s(\left\langle \frac{1}{2}\left(\frac{1}{c_j}-\frac{1}{b_j}\right)\right\rangle)}} - \frac{O'_j(1 - \frac{\left\langle \frac{1}{2}\left(\frac{1}{c_i}-\frac{1}{b_i}\right)\right\rangle}{\left\langle \frac{1}{2}\left(\frac{1}{c_i}-\frac{1}{b_i}\right)\right\rangle + s(\left\langle \frac{1}{2}\left(\frac{1}{c_j}-\frac{1}{b_j}\right)\right\rangle)})}{\frac{\left\langle \frac{1}{2}\left(\frac{1}{c_i}-\frac{1}{b_i}\right)\right\rangle}{\left\langle \frac{1}{2}\left(\frac{1}{c_i}-\frac{1}{b_i}\right)\right\rangle + s(\left\langle \frac{1}{2}\left(\frac{1}{c_j}-\frac{1}{b_j}\right)\right\rangle)}} \right) \right\rangle, \tag{B.3}$$

where: O'_i is the optimal investment post-fusion for colony i; c' is the post-fusion cost of investment; b' is the post-fusion benefit of investment; c_i is the pre-fusion cost of investment for colony i; b_i is the pre-fusion benefit of investment for colony i; s is the relative size of colony j; O'_j is the post-fusion optimal investment of colony j; c_j is the pre-fusion cost of investment for colony j; b_j is the pre-fusion cost of investment for colony j.

B.3 Post-fusion optimal investment is a form of Hamilton's rule

$$O_i' = \left\langle \frac{1}{2}\left(\frac{1}{c'} - \frac{1}{b'R_i} - \frac{O_j'(1-R_i)}{R_i}\right)\right\rangle$$

$$0 < \frac{1}{2}\left(\frac{1}{c'} - \frac{1}{b'R_i} - \frac{O_j'(1-R_i)}{R_i}\right)$$

$$0 < \left(\frac{1}{c'} - \frac{1}{b'R_i} - \frac{O_j'(1-R_i)}{R_i}\right)$$

$$0 < \frac{1}{c'} - \frac{1}{b'R_i}$$

$$\frac{1}{b'R_i} < \frac{1}{c'}$$

$$\frac{1}{b'} < \frac{R_i}{c'}$$

$$c' < R_i b' \equiv C < rB$$

B.4 Relating the continuous model to bimatrix games

Although level of soldier investment is continuous, it can be more intuitive to interpret payoffs in the context of a binary decision – to invest or not? This changes the assumptions of the model so that colonies can now invest in soldiers either at a high level or not at all, and this is decided in a one-shot decision immediately after fusion. A high level of investment is considered to be 0.5 because this is the maximum level of investment that the continuous model ever predicts. We calculated the payoffs using the post-fusion fitness function, $W'_i = (1 - c'O'_i)(1 + b'O_G)$, given a low, intermediate and high cost to soldier production. Benefit of soldiers was held constant at 10.

We find that different costs and levels of asymmetry in representation can result in very different payoff structures (Table A3). Mutual investment is only an evolution-

Table A3: **Payoff tables for different costs and representation asymmetry in the collective investment game.** Each bordered cell shows the payoff table for the corresponding representation (R_i) and cost of investment (c'). The values show the payoffs for colony i (left) and j (right) given their investment decision. The bold values show the best payoff for each colony. The greyed cells show the evolutionarily stable set.

			Colony j							
			$R_i = 0.25$			$R_i = 0.5$			$R_i = 0.75$	
Colony i	$c' = 0.5$		**Invest**	**Defect**		**Invest**	**Defect**		**Invest**	**Defect**
		Invest	4.5, **4.5**	1.69, 2.25	**Invest**	**4.5, 4.5**	2.63, 3.5	**Invest**	**4.5**, 4.5	3.56, **4.75**
		Defect	**4.75**, 3.56	1, 1	**Defect**	3.5, 2.63	1, 1	**Defect**	2.25, 1.69	1, 1
	$c' = 1$		**Invest**	**Defect**		**Invest**	**Defect**		**Invest**	**Defect**
		Invest	3, **3**	1.13, 2.25	**Invest**	3, 3	1.75, **3.5**	**Invest**	**3**, 3	2.38, **4.75**
		Defect	**4.75**, 2.38	1, 1	**Defect**	**3.5**, 1.75	1, 1	**Defect**	2.25, 1.13	1, 1
	$c' = 5$		**Invest**	**Defect**		**Invest**	**Defect**		**Invest**	**Defect**
		Invest	-9, -9	-3.38, **2.25**	**Invest**	-9, -9	-5.25, **3.5**	**Invest**	-9, -9	-7.13, **4.75**
		Defect	**4.75**, -7.13	1, 1	**Defect**	**3.5**, -5.25	1, 1	**Defect**	**2.25**, -3.38	1, 1

arily stable set (ESS; Smith and Price, 1973) when colonies are equally sized and the costs of investment are low. Otherwise, the best strategy for a colony depends on the size asymmetry. Low (post-fusion) costs of soldier production can result in investment being the best strategy for a majority colony, even if the minority colony makes no investment, as is their best strategy. This scenario is therefore an example of the snowdrift game in which the ESS is to do the opposite of what the other player does. This, we interpret as showing that some division of labour, whereby one colony invests in soldiers and the other invests exclusively in alates, can be stable at intermediate costs when there is no size asymmetry. This is not complete division of labour because the soldier-producing colony must also produce some alates to gain (colony-level) fitness assuming as we do that the colonies are unrelated. However, this shows that cooperative partial division of labour can emerge from interactions between unrelated actors.

In contrast, a high cost means that a colony can always gain by defecting (i.e. making no investment in soldiers). Assuming that a colony knows its representation in the group, a minority colony should never invest, while a majority colony should always invest unless the cost of investment is high. If colonies have no information about their representation in the fused group, then a pure strategy ESS can only emerge if the outcome of fusion is always that colonies are equally represented, otherwise, a mixed strategy should emerge.

B.4.1 Calculating payoff tables

Payoff tables were calculated from the post-fusion fitness function ($W_i' = (1-c'O_i)(1+b'O_G)$) using the following R code with the cost and representation changed accordingly.

```
cost = 5

benefit = 10

prop1 = 0.75

prop2 = 1-prop1


# Both invest

p1a = 0.5 # player 1 investment

p2a = 0.5 # player 2 investment

Ga = (p1a*prop1+p2a*prop2) # group investment

a1 = (1-cost*p1a)*(1+benefit*Ga) # player 1 fitness

a2 = (1-cost*p2a)*(1+benefit*Ga) # player 2 fitness


# P1 defects

p1b = 0

p2b = 0.5

Gb = (p1b*prop1+p2b*prop2)

b1 = (1-cost*p1b)*(1+benefit*Gb)

b2 = (1-cost*p2b)*(1+benefit*Gb)


# P2 defects

p1c = 0.5

p2c = 0

Gc = (p1c*prop1+p2c*prop2)

c1 = (1-cost*p1c)*(1+benefit*Gc)

c2 = (1-cost*p2c)*(1+benefit*Gc)
```

```
# Both defect

p1d = 0

p2d = 0

Gd = (p1d*prop1+p2d*prop2)

d1 = (1-cost*p1d)*(1+benefit*Gd)

d2 = (1-cost*p2d)*(1+benefit*Gd)

print(c(a1,a2,b1,b2,c1,c2,d1,d2))
```

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