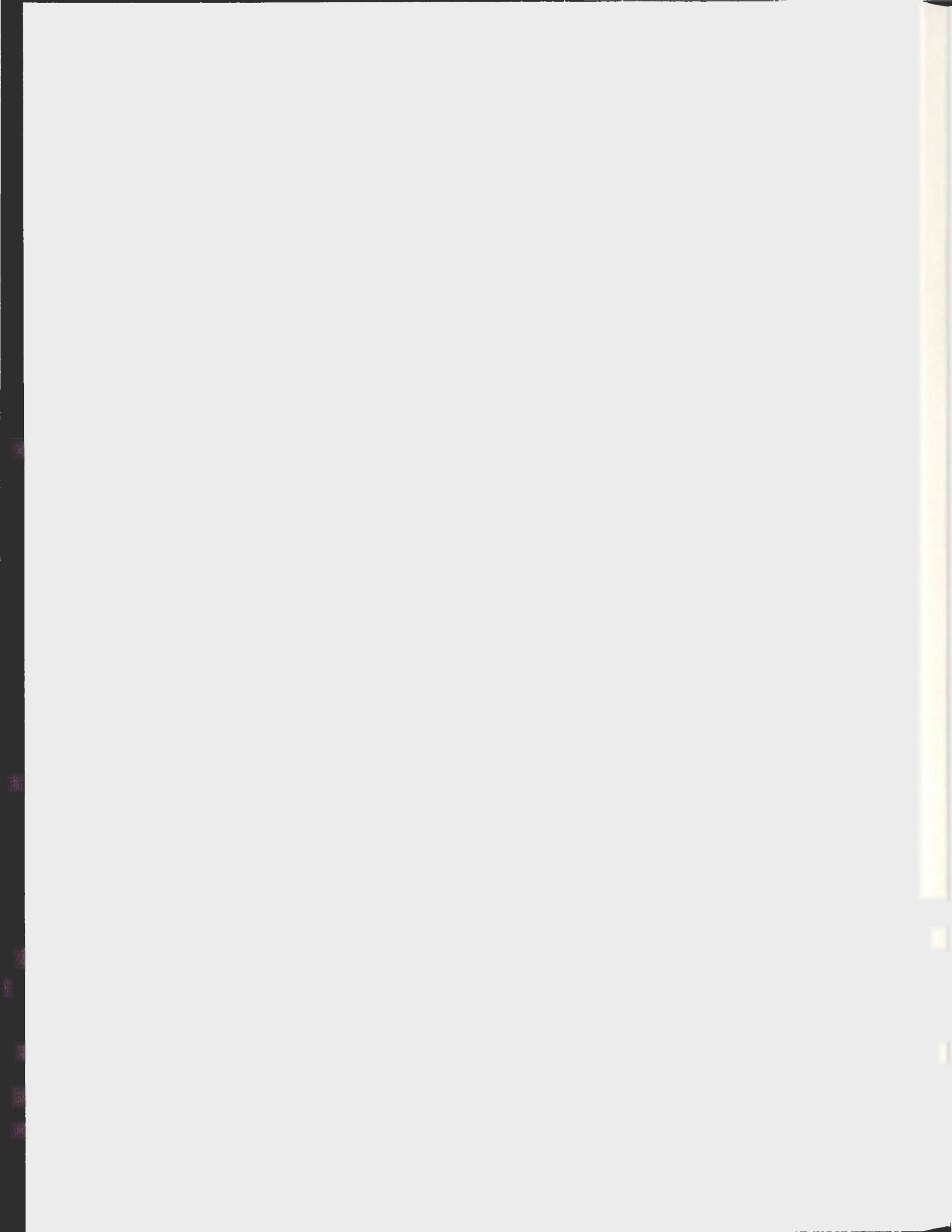


FIGHT AND FLEE:
CASTE DECISIONS DURING AN INVASION

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FIGHT AND FLEE: CASTE DECISIONS DURING AN INVASION

By

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Abstract

Sociality is a trade-off between personal reproduction and investment in helping others to survive and reproduce. Seven species of Australian gall-inducing thrips have a helping caste (soldiers). A kleptoparasite in the genus *Koptothrips* that invades and destroys thrips colonies is hypothesized to have evolutionarily shaped this caste. The two investigations presented here both center on one potential evolutionary reaction to the threat of invasion; the production of an alarm pheromone. In the first study, reproductives and soldiers were assessed in the presence or absence of a putative alarm pheromone. Reproductives moved more directly and quickly than soldiers in the trials, but no impact of the pheromone was detected. In the second study, soldier willingness to fight was assessed in relation to this chemical (or blend). Again, no change in behaviour was detected in the trials. Taken together, these two investigations suggest that chemical communication may not have arisen within the gall inducing thrips.

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physogastry. In addition to this, there was a reduction in gall volume and, as result, a smaller brood size, and longer gall duration. At point 'C' there is a loss of male founders. At point 'D' there is a decrease in soldier reproductive output, which is associated with an increase in soldier efficiency.

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Chapter 1. The evolution of eusociality in Australian gall-inducing thrips: a general introduction

Eusociality is characterized by overlapping generations, cooperative brood care, and a reproductive division of labor with some individuals being more or less nonreproductive (Michener, 1969; Wilson, 1971, p. 548). The existence of nonreproductive individuals has long been recognized as problematic for evolutionary theory. Darwin, for example, stated that sterile workers in eusocial insect colonies pose “one special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory” (Darwin, 1859, p.236). The problem can be posed as follows: if natural selection favours individuals of higher fecundity, how could it lead to the production of sterile individuals who have a fitness of zero? A theoretical solution to this problem was provided by Hamilton (1964). His theory of kin selection shows that the direct fitness loss of helping or being sterile could be offset if the individual being helped is a relative, thereby permitting the nonreproductive to gain indirect fitness (Haldane, 1923; Fisher, 1930; Maynard Smith, 1964). In the case of haplodiploid insect colonies, such as Hymenoptera (ants, bees, and wasps) and Thysanoptera (such as the Australian gall-inducing thrips), females are more closely related ($r = 0.75$) to their sisters than to their daughters ($r = 0.5$) and, thus, would better propagate their genes by helping raise reproductive sisters than by breeding. This pattern is found within eusocial Hymenoptera, in which females help to raise their sisters instead of producing offspring of their own.

Despite this apparent ability of the haplodiploid hypothesis to explain the origin of eusociality in haplodiploid insects, a fatal flaw soon became evident. In haplodiploid insects, females are more related to their sisters than their own offspring but they are less related to their brothers ($r= 0.25$). Thus on average hymenopteran females are not more related to siblings than to offspring of their own (Trivers and Hare, 1976). Trivers and Hare (1976) suggested that female workers of haplodiploid insect colonies should manipulate the sex ratio until there is a female biased ratio of 3:1. Although Trivers and Hare found a female biased sex ratio of 3:1 in a number of eusocial Hymenoptera, it was later determined that many eusocial species did not have this sex ratio (Alexander and Sherman, 1977). In addition, many eusocial insect colonies contain more than one queen or have a single queen that is multiply mated, both of which reduces relatedness (Alexander and Sherman, 1977). Both of these observations, as well as the fact that there are eusocial diploid species, refuted the idea that a haplodiploid genetic system alone could explain the origin of eusociality (Crespi, 1994; Roux and Korb, 2004). Despite this, there was an emphasis on genetic factors for quite some time. Genetic factors alone, however, are not sufficient to explain the origin of eusociality (Crespi, 1994; Roux and Korb, 2004). In addition to genetic factors, phenotypic characteristics, such as degree of parental care and a defensible nest site (Andersson, 1984; Alexander, Noonan, and Crespi, 1991) were identified as factors also playing a role in the evolution of eusociality. Moreover, extrinsic environmental factors are hypothesized to be selective agents that act upon intrinsic genetic and phenotypic traits. An example of an

environmental trait that could function as a selective agent is an ecological constraint on individual reproduction (Emlen, 1992), such as a lack of space within a nest site.

Although a large proportion of the research investigating the evolution and maintenance of eusociality has used insects from the orders Hymenoptera and Isoptera, the use of Australian gall-inducing thrips has provided important insights into the evolution of eusociality that may otherwise have not been observed. The utility of this group is that this is a recently evolved eusocial lineage (approximately 6 million years from the present; McLeish and Chapman 2007), relative to sociality in the ants (more than 80 million years ago; Hölldobler and Wilson, 1990; Bourke and Franks, 1995) and the termites (approximately 100 million years ago; Andersson, 1984). An additional advantage of studying thrips eusociality is that there are closely related solitary species, which have not evolved a helper caste, and can, therefore, be used in comparative studies to better understand the evolution of eusocial behaviour. In contrast, the termites and ants have no extant taxa that are entirely lacking castes. All ant species without castes have secondarily lost normal colony formation and instead have become obligate social parasites (Bourke and Franks, 1995).

The thysanopterans comprise 4500 described species (Mound and Walker, 1982), and have a worldwide distribution. In general, thrips are minute (less than 2 mm in length), darkly coloured, and possess two sets of fringed wings. However, the main morphological characteristics that distinguish thrips from other insect orders are unique piercing and sucking mouthparts and the bladder-like terminal pads (arolium) on their

feet that they use to adhere to various surfaces. Most thrips feed on fungus, with many feeding on fungal spores and pollen. Approximately 300 species have formed a close relationship with plants, the growth of which they can manipulate to form galls. One of the independent origins of gall-forming ability and subsequent species radiation is the Australian gall-inducing thrips that parasitize species of *Acacia* trees and shrubs. These thrips create a domicile in developing phyllodes (a petiole modified to serve as a leaf), thereby, gaining both shelter and nutrients. Galls are induced by a single foundress, or, in some species, a founding female-male pair, by feeding on the developing phyllode which causes it to curl or evaginate so that opposite sides of the phyllode meet to form an enclosed chamber (Crespi and Mound, 1997). Once the female is enclosed within this chamber she deposits eggs on its inner surface. In eusocial species, the first of her brood develop into soldiers, which are morphologically distinct with enlarged forelimbs, reduced antennae, reduced wings, and often a pale exoskeleton (Mound, 1971; Crespi, 1992a; Mound and Crespi, 1995; Mound, Crespi, and Kranz, 1996; Crespi and Mound, 1997; Kranz, Schwarz, Mound, and Crespi, 1999). The remainder of the brood develops into a dispersing morph (possessing fully developed wings) and, in most species, the dispersers leave the gall as second-instar larvae to pupate in the soil, with a few exceptions (such as *Kladothrips intermedius*) that develop into adults in the gall (Morris, Schwarz, Crespi, and Cooper, 2001). The foundress produces a large proportion of the second brood while soldier contribution varies with relatively high soldier reproduction in basal lineages and low soldier reproduction in derived lineages (Chapman, Kranz, Bejah, Morris, Schwarz, and Crespi, 2002; Kranz, 2005), such as *K. intermedius*. The

reproductive capacity of female soldiers is limited by the space available for their offspring within the gall (Crespi, 1992a). Thus there is a reproductive division of labor present in the galls of eusocial thrips (Chapman and Crespi, 1998).

The evolution of eusocial behaviour in gall-inducing thrips is thought to have arisen due to the unique nature of the gall as a resource and the harsh environment of the Australian interior (Crespi, 1994; Chapman, Crespi, and Perry, 2008). Thrips can only induce galls when the *Acacia* is producing new phyllodes, which is dependent on rainfall. Since rainfall is infrequent in the Australian interior, new phyllodes are produced annually in regions with a predictable wet season and less frequently in areas of unpredictable rainfall (Chapman et al., 2008). The gall provides both food and shelter for the thrips creating a habitat that is ideal for the rearing and protection of offspring. The harsh environment outside the gall will, presumably, result in death for the thrips. Since the gall is such a valuable resource and cannot readily be produced, they are a coveted resource for natural enemies, such as thrips from the genus *Koptothrips*. For this reason, soldier morphs have arisen in many species of gall-inducing thrips for the defense of the gall and its inhabitants.

Thrips in the genus *Koptothrips* are kleptoparasitic and specialize in invading galls of gall-inducing thrips, driving out or killing the inhabitants, and breeding within the gall (Crespi, 1992a, 1992b; Crespi and Abbot, 1999). *Koptothrips* can be common in natural populations and are thought to be a major selective force underlying the evolution and maintenance of soldier morphology and behaviour (Crespi, 1992a; Crespi and Mound,

1997; Crespi and Abbot, 1999; Kranz, Chapman, Crespi, and Schwarz, 2001a; Kranz, Schwarz, Wills, Chapman, Morris, and Crespi, 2001b).

Despite the recognized importance of *Koptothrips* in the evolution of soldiers, little research has investigated the response of gall-inducing thrips during an invasion. In other eusocial insects, such as Hymenoptera and Isoptera, a response to an invasion typically involves some means of communication between nest mates in order to coordinate caste behaviour. For eusocial insects, the primary means of communication is via pheromones (Blum, 1974). Pheromones are chemicals that are emitted by one individual causing a behavioural response in others (Shorey, 1973). An example of using pheromones to coordinate caste behaviour during an invasion is found in honeybees. Guard honeybees, stationed at the entrance of the hive, will mark an enemy with an alarm pheromone to facilitate the orientation of other bees to the enemy (Blum, 1969; Shorey, 1973). To date, there have been no published studies investigating communication with Australian gall-inducing thrips. However, many species of thrips, including Australian gall-inducing thrips, have been observed to raise their abdomen up and over their head (a scorpion-like curl) and produce an abdominal tube droplet (Hodson, 1935; Lewis, 1973; personal observation, June 2008). In some species of thrips, these secretions have been shown to function as an alarm pheromone (Teerling, Pierce, Borden, and Gillespie, 2003), a predator repellent (Howard, Blum, and Fales, 1983; Howard, Blum, Jones, and Tomalski, 1987; Blum, 1991; Blum, Footitt, and Fales, 1992), and a trail pheromone (Kiestler and Strates, 1984). The purpose or utility of this action for the gall-inducers is unknown, but touching the thrips using a single bristle from a small paintbrush can

induce this behaviour. And, anecdotally, the droplet has been seen to be produced when a soldier encounters a *Koptothrips* (personal communication between Chapman and Bejah, 1997). Although there could be many functions of this droplet production, I will be exploring the possibility that it may contain volatile substances that act as a chemical signal to others in the gall that an invasion is underway. Understanding communication within Australian gall-inducing thrips would not only provide insight into their response to an invasion, but would also provide insight into the understanding of the evolution of social behaviour, since Australian gall-inducing thrips are a recently evolved eusocial lineage. The study of communication within this group provides an opportunity to examine the early stages in the evolution of chemical communication. Comparative studies of the origin of chemical communication are also possible within this group since there is a closely related solitary species. A multispecies study is desirable, but the first step is to establish the function of the droplet in one species.

In the following chapters, the movement of reproductives, termed dispersers, and the defensive behaviour of helpers, termed soldiers, was quantified. In addition to this, the effect of the abdominal tube droplet on such behaviours was analyzed. *Kladothrips intermedius* was used since it is the most evolutionary derived species and was abundant in the study area.

Chapter 2 quantifies movement of soldiers, dispersers, and larvae upon the breach of their gall. Since soldiers are gall bound, meaning they remain in their natal gall and protect its inhabitants from invaders, they should remain relatively still compared to

dispersers and larvae. Once fully developed, dispersers will leave their natal gall to create their own gall and reproduce. For this reason, dispersers and larvae should not risk death by an invader and should flee the gall when breached. Gall-inducing thrips produced droplets from their abdomens when cues associated with danger are present, such as, bisection of the gall or probing with a paintbrush (personal observation, June 2008). Since the function of this droplet is currently unknown and it is produced upon the breach of the gall, the effect of this droplet on fleeing behaviour was analyzed. If this droplet contains an alarm pheromone, as found within other species of thrips, soldiers would be expected to aggregate at the source, while dispersers and larvae should flee from the source.

In Chapter 3, the defensive behaviour of thrips was analyzed and, again, the effect of the abdominal tube droplet on this behaviour was determined. If the abdominal tube droplet contains an alarm pheromone, then in the presence of this droplet soldiers should seek out and attack an invader. In addition to this, the effect of morphological (wing length and forelimb size) and sex on soldiers willingness to help was analyzed. Soldiers of gall-inducing thrips use their enlarged forelimbs to grasp the invader, raise them into the air, and squeeze the victim in slow pulses until it is moribund. Enlarged forelimbs may lead to a greater ability to grasp and hold a *Koptothrips* and subject it to greater squeezing force, relative to the slimmer forelimbs of dispersers (Perry, Chapman, Crespi, and Schwarz, 2004). Some soldiers have forelimb and wing size closer to that of a disperser. If the variation in soldier morphology is a tradeoff between dispersal and helping, then soldiers with enlarged forelimbs and reduced wings would be more willing

to defend than those soldiers with wing and forelimb morphology closer to that of a disperser. In addition to this, males are less related to individuals within their natal gall than females, due to the nature of haplodiploidy, and thus should be less willing to defend than females (Chapman, 2003).

Chapter 2. Novel approach to measuring and analyzing insect movement

reveals strong caste differences in a social insect colony

2.1 Introduction

A major question in the study of social behaviour is: what are the necessary and sufficient conditions for the origin and maintenance of eusociality (Crespi, 1994)? Traditionally, theories have focused on genetic conditions that should favor eusocial behaviour. Hamilton's theory of kin selection (1964) is a genetic theory that suggests that the direct fitness loss of helping can be offset if the individual being helped is a relative, thus allowing the helper to gain some indirect fitness. Such a situation is found within haplodiploid insect colonies, where females are more closely related to their sisters ($r=0.75$) than they are their own offspring ($r=0.50$) and thus would better propagate their genes by helping to raise sisters than their own offspring. This type of reproductive system is found within Hymenoptera (ants, bees, and wasps) and Thysanoptera (Australian gall-inducing thrips). Although it first appeared that the haplodiploidy hypothesis could explain eusociality in haplodiploid insects, it was soon found to have shortcomings. Although females in a haplodiploid system are more related to their sisters ($r=0.75$), they are distantly related to their brothers ($r=0.25$) and thus, on average, females are not more related to their siblings than to offspring of their own (Trivers and Hare, 1976). It appears, therefore, in order to determine the necessary and sufficient conditions for the origin of eusociality, one must consider a combination of genetic, phenotypic, and ecological factors.

Australian gall-inducing thrips have both solitary and eusocial species. Eusocial species in this group have a defensive caste, termed soldiers, which have reduced fecundity relative to their 'foundress' mother (Chapman and Crespi, 1998; Kranz, 2005). Soldiers are morphologically distinct from the reproductives, termed dispersers, with enlarged forelimbs, reduced antennae, reduced or absent wings, and often with a pale exoskeleton (Mound, 1971; Crespi, 1992a; Mound and Crespi, 1995; Mound et al., 1996; Crespi and Mound, 1997; Kranz et al., 1999; see Figure 2.1) Soldiers are a non-dispersing morph that remains gall-bound, protecting the gall and its inhabitants rather than leaving the gall to produce offspring of their own. The morphological and behavioural specialization of soldiers is thought to have evolved in response to a kleptoparasite, the genus *Koptothrips*, which invade the gall of gall-inducing thrips, drive out or kill the inhabitants, and breed within the gall.

The gall is a vital resource for gall-inducers, providing both food and shelter. Since new phyllodes and gall formation on *Acacia* can only be induced after rainfall, which is infrequent in the Australian interior, if a gall is lost by its inhabitants it cannot be replaced. Due to the harsh environment of the Australian interior, the loss of the gall would likely mean death for all inhabitants. Since galls are such a valuable resource, they are an attractive resource for natural enemies, such as *Koptothrips*. Both solitary and social species are parasitized by *Koptothrips* and, as a result, have evolved two life history strategies: (1) production of a large gall and either fast development of dispersers or dispersers that leave prior to maturity, or (2) production of a small gall, and consequently a smaller brood size, with the presence of soldiers that increases survival of

these eusocial thrips (Willis, Chapman, Kranz, and Schwarz, 2001; Willis, Chapman, Mound, Kranz, and Schwarz, 2004; Crespi, 2004). Species of *Kladothrips* are primarily invaded by two species of *Koptothrips*, *Koptothrips flavicornis* and *Koptothrips dyskritus*. *Koptothrips flavicornis* can kill a soldier within minutes of piercing them with their fore-tarsal teeth, which are thought to be venomous (Crespi and Mound, 1997), and thus are much more lethal than *Koptothrips dyskritus*, which are hypothesized to be much more primitive in their gall invasion strategy. *Koptothrips dyskritus* primarily invades a complex of solitary sibling species while *Koptothrips flavicornis* invades most of the social species and two species that were inferred to have evolved and secondarily lost soldiers [*Kladothrips xiphius* and *Kladothrips rodwayi* (Crespi, Morris, and Mound, 2004)].

Although, gall invasion by *Koptothrips* is important for understanding the evolution and maintenance of social behaviour within Australian gall-inducing thrips few studies (Crespi, 1992b; Perry et al., 2004; Chapman, Francis-Geyer, and Schwarz, 2006) have looked at the behaviour of thrips during an invasion, which is most likely due to the difficulties of observing behaviours that are confined within the gall. It is known that upon the bisection of a gall, dispersers and larvae swiftly leave the gall while soldiers remain within the gall (personal observation, June 2008). Whether individuals respond similarly to invading *Koptothrips* is not known. In other eusocial insect orders, a response to an invasion typically involves coordinating caste behaviour by use of some means of communication (Hölldobler and Carlin, 1987). Many species of thysanopterans have been observed to raise their abdomen up and over their head (scorpion-like curl) and

produce a large clear droplet (Hodson, 1935; Lewis, 1973; personal observation, June 2008). In flower thrips, *Frankliniella occidentalis*, this droplet appears to function as an alarm pheromone (Teerling et al., 1993), whereas in other species of thrips it appears to function as a predator repellent (Howard et al., 1983, 1987; Blum, 1991, 1992) and a trail pheromone (Kiestler and Strates, 1984). The function of this droplet within Australian gall-inducing thrips, however, is currently unknown.

In this study the movement of the two castes, soldiers and dispersers, and larvae was examined. In addition, the effect of the abdominal tube droplet on movement was analyzed. Two parameters, curvilinear velocity (VCL) and linearity (LIN), were used to analyze the movement of thrips. We predict that because soldiers are gall bound (i.e., remaining in their natal gall), that when a gall is invaded, soldiers should remain in the breached gall, protecting the gall and its inhabitants. Reproductives, however, should move away from a breached gall since dispersers and larvae (upon eclosing within the soil) can produce a gall of their own and, thus, should not risk death by an invader. If the droplet functions as an alarm pheromone then soldiers should aggregate at the source, while dispersers and larvae should move away from the source swiftly.

2.2 Methods

2.2.1 Life History

Seven species of Australian gall inducing thrips have evolved a soldier morph (Crespi 1992a; Crespi and Mound 1997; Crespi, Carmean, and Chapman, 1997a; Crespi, Carmean, Mound, Worobey, and Morris, 1997b; Kranz et al. 1999, 2001a, 2001b; Willis

et al. 2004). These species have remained associated with a group of closely related *Acacia* with the exception of *Kladothrips intermedius*, which appears to have undergone a host-plant shift to *Acacia oswaldii* (Chapman et al. 2008). *Kladothrips intermedius* is one of the most evolutionary derived species and differs from other eusocial species in many ways: (1) dispersers of *Kladothrips intermedius* fully develop within the gall, while dispersers of other social species leave as second-instar larvae and eclose within the soil (presumably), (2) soldiers have partial wings instead of having no or near-absent wings (Crespi 1992a, 1992b; Chapman et al. 2008), and (3) galls without soldiers are not uncommon, which is not the case in other social species (Chapman et al. 2008). These differences are likely due to the host-plant shift, which are typically associated with phenotypic and life history change in Australian gall-inducing thrips (Crespi et al. 2004; Chapman et al. 2008).

2.2.2 Collections

Galls of *Kladothrips intermedius* were collected on the Middleback station property, located 400 km Northwest of Adelaide, Australia. The station is almost 1000 km² and is heavily impacted by human activities, with mining on one edge and a major town on the other edge. In addition to this, sheep have been present on the property for 100 years. The host tree, *Acacia oswaldii*, is found throughout the station but is very patchy in its distribution. The distribution of galls on these trees is also patchy; not every tree has galls and it was rare that a single tree had more than 50 galls. For example, over a 22km transect, only 6 trees out of 100 had galls but of those trees with galls, only three

had more than 50 galls. The two collection sites for *Kladothrips intermedius* [(1) 137° 10'E 33° 0'S and (2) 137° 24'E 32° 55'] that were chosen were 22km and < 1km, respectively from the Research Station managed by the University of Adelaide and a total of three host trees were used. Approximately 70% of the galls were obtained from a relatively young 1.7 m shrub-like tree at site 2. Galls were collected at both sites from June 18 to June 23, 2008. Sampled galls were placed in sealed bags and then transported to the Middleback field station where they were stored in a refrigerator until used for behavioral assays. To obtain sufficient thrips for this experiment, approximately 75 galls were processed (bisected at the ostiole and observed under a dissecting microscope). Voucher specimens were prepared, using the protocol described by Watson and Chandler (1999), and are lodged at Memorial University.

2.2.3 Analysis of movement

Trials were conducted at the Middleback field station 1-3 days following gall collection. Previous research indicates that gall inhabitants are not negatively affected by having their galls removed from the host tree for periods under one week (Bejah, 1997). Trials were conducted at a temperature of 17°C (ambient temperature) under artificial light. A thrips was placed in the centre of a round piece of filter paper (5.5cm diameter) and the time (seconds) it took the thrips to reach the edge of the filter paper was recorded. In addition to this, the path the thrips took to the edge of the filter paper was traced by following closely (approximately 2-3 body lengths) behind the thrips with a pencil. See Figure 2.2 for an example of the path taken by an adult disperser (a) and a soldier (b).

Path length was measured using Image-J software (see below for process) and, with time data, two parameters (VCL and LIN, described below) were calculated. In the experimental condition, there was a teardrop-shaped piece of filter paper covered in abdominal tube droplets (see below for process), placed at the centre of the round piece of filter paper. In the control condition, there was nothing in the centre. There were a total of 131 trials, 56 in the control and 75 in the droplet condition, and a new thrips was used for each trial.

2.2.4 Induction of abdominal tube droplets

A 0.74 cm in length teardrop-shaped piece of filter paper was cut using a round base point punch and used in the collection of the anal droplet for the assays. Galls were bisected at the ostiole, pried open, and observed under a dissecting microscope. The tip of the filter paper was used to touch the thrips on the pronotum causing the thrips to curl (raising the abdomen over the head; a scorpion-like curl) and produce a large clear droplet, approximately 0.15 mm diameter, at the end of the abdominal tube. Thrips frequently touched the droplet to the filter paper, and if not, the filter paper was pressed against the end of the abdominal tube to collect the droplet. Approximately 5 droplets were collected on the same piece of filter paper within 60 seconds. Droplets were collected from thrips at different life stages and castes (dispersers, soldiers, and second-instar larvae) in order to replicate a natural invasion. Upon the bisection of a gall, all individuals have been observed to produce a droplet and, when stimulated with a

paintbrush, individuals produce a droplet more than 50% of the time (personal observation, June 2008). A new filter paper with droplets was used for each trial.

2.2.5 Calculation of motility parameters using Image- J software

Two parameters, curvilinear velocity (VCL) and linearity (LIN), were used to analyze the movement of thrips. These parameters are primarily applied to describe sperm motility, but they can also be used to describe insect movement since both animal sperm and insects move in a similar curvilinear path. The use of these parameters is a novel approach for the measurement and analysis of insect movement, as previous research has used the basic protocol described by Turchin, Odendaal, and Rauscher (1991). This protocol involves dividing a continuous pathway into a series of linear segments and using this information to calculate path length. VCL, also known as total velocity, is calculated by dividing the total curvilinear distance by the time it took the thrips to travel this distance. LIN is calculated by dividing the rate of net space gain (distance from start to end point divided by time to travel this distance) by VCL and multiplying this number by 100%. To calculate each of these parameters, the distance of the path travelled by the thrips is required. The distance of this path was approximated using a series of connected straight lines, as described by Turchin et al. (1991), using the program, Image J. Image J is a Java-based image processing program developed by the National Institution of Health (<http://rsb.info.nih.gov/ij/>, Rasband W., NIH). First, each filter paper was scanned to obtain a jpeg image that could be uploaded into the program. Using Image J, the picture was scaled to true size and then the 'segmented lines selection'

tool was used. This tool allows the user to click a series of points on the picture and then connect these points with straight lines. The distance of each straight line is then combined to get a total distance for the path. This information is used to calculate the parameters of interest, VCL and LIN.

2.2.6 Statistical Analysis

Four generalized linear models were used to analyze the data. The first model was used to analyze the effect of caste/life stage on relative speed (body lengths/sec) using the parameter VCL. The second model was used to determine if castes/life stages differ in path directness to the edge of the filter paper (using the parameter LIN). The last two models were used to determine if each caste/life stage differed in relative speed (body lengths/sec) or path directness in the control versus droplet condition again using the parameters VCL and LIN, respectively.

2.3 Results

2.3.1 Analysis of VCL

There was no significant difference in the VCL between droplet versus control, $\Delta G = 0.0263$, $df = 1$, $p = 0.871$, but there was a difference in VCL between the life stages/castes ($\Delta G = 7.19$, $df = 2$, $p = 0.027$) as VCL of soldiers were much slower ($M = 0.104$, $SD = 0.144$) than that of second-instar larvae ($M = 0.500$, $SD = 0.210$) and dispersers ($M = 0.698$, $SD = 0.348$; Figure 2.3 and 2.4).

2.3.2 Analysis of LIN

A second model was analyzed using linearity (LIN), how directly the thrips moved away from the centre of the filter paper, as the response variable with the same explanatory variables (droplet versus control). Again, the analysis revealed that there was no significant difference in linearity of thrips movement between droplet and control treatments, $\Delta G = 0.119$, $df = 1$, $p = 0.730$, but there was a difference in LIN between life stages/ castes ($\Delta G = 11.9$, $df = 2$, $p = 0.0025$) as LIN of soldiers was much lower ($M = 25.6$, $SD = 0.144$) than that of second-instar larvae ($M = 44.6$, $SD = 0.210$) and dispersers ($M = 56.9$, $SD = 0.348$; Figure 2.5 and 2.6). Typically, dispersers and second-instar larvae moved from the center of the filter paper to the edge in a direct movement (higher LIN), while soldiers were largely stationary (low LIN), rotating and shifting position slightly (See Figure 2.2).

2.4 Discussion

As predicted, soldiers have a lower VCL and LIN than dispersers and larvae. Although this has been previously observed, to date no published studies have described movement of Australian gall-inducing thrips. Soldiers were predicted to move slower than the reproductives (larvae and dispersers) because soldiers remain in their natal gall protecting its occupants from a *Koptothrips* invasion whereas reproductives leave the natal gall upon maturity to produce a gall of their own (Morris et al., 2001). Thus if a gall is invaded, dispersers and larvae are predicted to avoid risking death from *Koptothrips* by

fleeing the gall whereas soldiers should remain to defend the natal gall as life outside the gall would result in death.

In addition to analyzing caste differences in movement, the function of the abdominal tube droplet was also investigated and we predicted that, in the presence of the droplet, dispersers and larvae should have a VCL and LIN higher than that of those in the control condition since they would be informed, assuming the droplet contained an alarm pheromone, of the presence of an invader. Soldiers were predicted to become hyper vigilant in the presence of the droplet and thus have a VCL and LIN lower than that of soldiers in the control condition. The results, however, did not support the hypotheses. The presence or absence of the abdominal tube droplet had no apparent effect on the movement of the castes/ life stages. This result was unanticipated because in some species of thrips, the abdominal tube droplet has been shown to function as an alarm pheromone (Teerling et al., 1993).

The absence of a behavioural change in the presence of the abdominal tube droplet could be interpreted in two ways; first, that there is no alarm pheromone present in the droplet and second (more probable), that more research is necessary to draw any conclusions. A behavioural response may not have been elicited because the droplet dissipates too quickly on the exposed filter paper. The filter paper with the droplets was placed in the PCR tube within 10 seconds of collecting the last droplet. Pheromones that send messages to conspecifics often dissipate quickly (Schoonhoven, Sparnaay, Van Wissen, and Meerman, 1981) and alarm pheromones, in particular, must dissipate

especially rapid after the perceived threat has passed. For example, an alarm pheromone emitted by the pea aphid, *Acyrtosiphon pisum*, dissipates within 10-20 seconds (Sharp and Andrade, 1994). Since gall-inducing thrips live in an enclosed space, and the droplet likely evaporates much more quickly on the exposed filter paper, the lack of response of the thrips could be due to the inability of the thrips to sense any volatile chemicals prior to dissipation. Future research using the droplet should either determine dissipation time prior to the experiment or perform the experiment within an enclosed space, mimicking a gall.

The bisection of the gall prior to experimental trials may have led to unseen droplet production and, thus, influenced behavior such that the addition of more droplets in the treatment condition would not alter behaviour. Subsequent studies should place thrips removed from a gall in holding chambers until normal behaviour is presumed to have returned and then initiating trials using filter paper with droplets.

Because the pressure of *Koptothrips* invasions is hypothesized to be the selective agent underlying the evolution of soldier behaviour and morphology (Crespi, 1992a; Crespi and Mound, 1997; Crespi and Abbot, 1999; Kranz et al., 2001a, 2001b) it is vital to understand castes/life stage response to a *Koptothrips* invasion. Since a response to an invasion typically involves coordinating caste behaviour by use of pheromones, as with other groups of social insects, future research should further investigate the function of the abdominal tube droplet in Australian gall-inducing thrips. The present study found no effect of the droplet on fleeing behaviour and, since the droplet is produced in the

presence of dangerous stimuli, future research should determine the effect of this droplet on fighting behaviour of soldiers.



Figure 2.1. The reproductive (left) and helper (right) caste of a eusocial species of Australian gall-inducing thrips, *Kladothrips intermedius*.

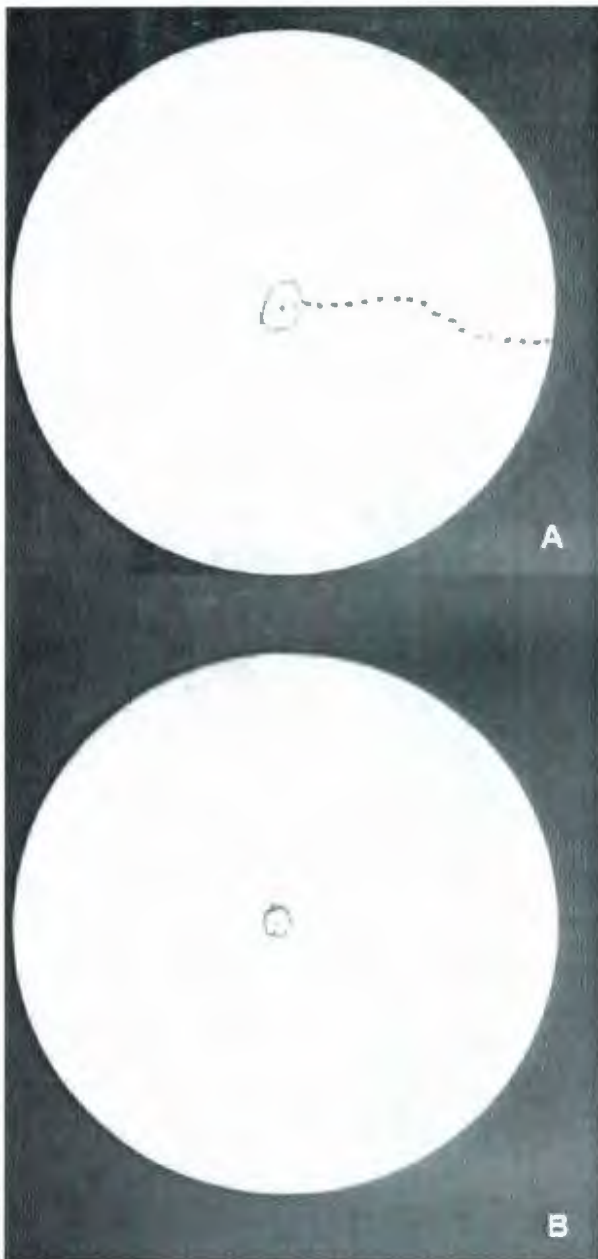


Figure 2.2. Two 5.5 cm diameter circles of filter paper showing examples of pencil tracings of thrips movements. The typical direct movement of an adult disperser, moving from the center of the filter paper to the edge, is shown in (A) and the largely stationary behavior of soldier (soldiers were observed to rotate and shift position slightly) is illustrated in panel (B).

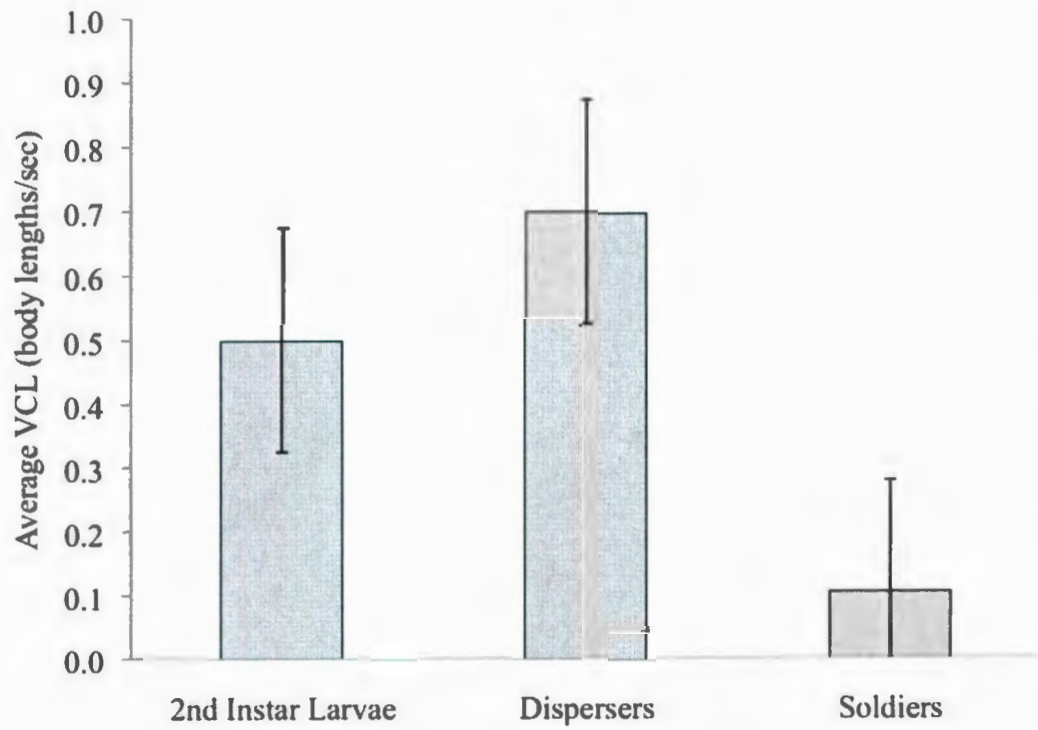


Figure 2.3. The curvilinear velocity (VCL; body length/sec, with standard error) is on the y axis, with thrips of different life stages/ castes on the x axis. VCL was calculated by dividing the total curvilinear distance traveled by the thrips by the total time taken to travel that distance.

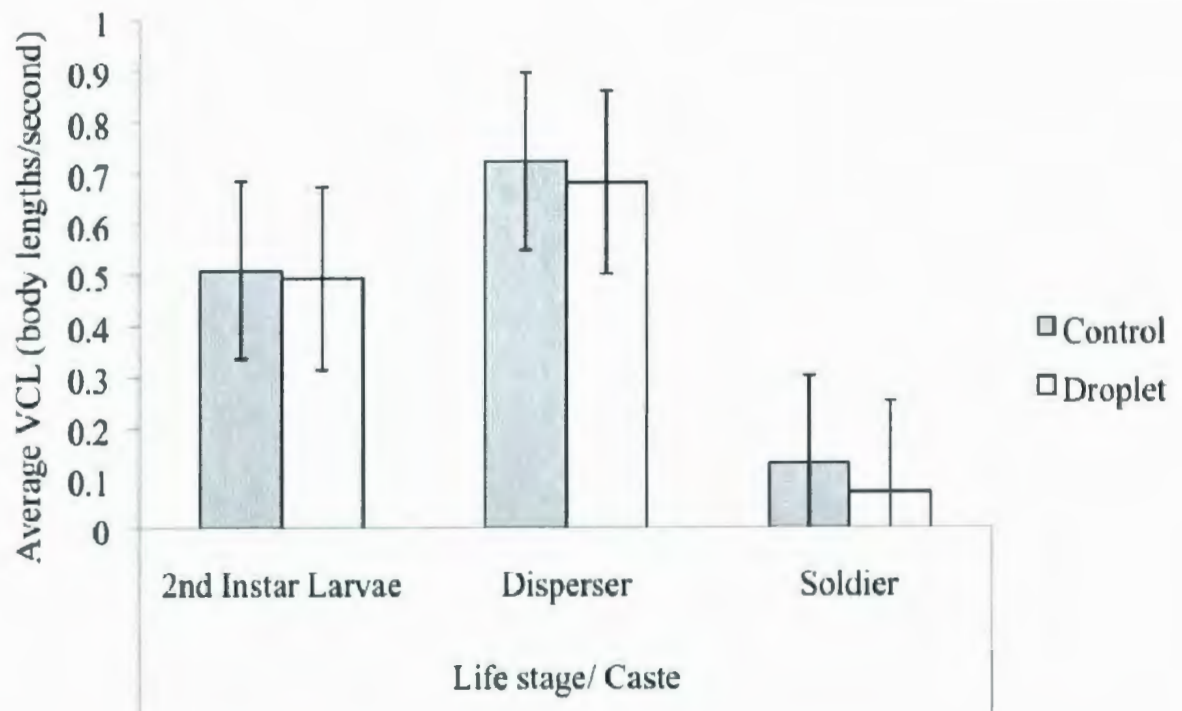


Figure 2.4. The curvilinear velocity (VCL; body length/sec, with standard error) is on the y axis, with thrips of different life stages/ castes on the x axis. VCL was calculated by dividing the total curvilinear distance traveled by the thrips by the total time taken to travel that distance. Trials were conducted where no abdominal tube droplets were added (Control, darkly filled bars), or droplets were added to the trial (Droplet, unfilled bars). Droplets were collected from all castes and life stages of the thrips on a small tear drop shaped piece of filter paper. The thrips in the droplet trials were placed in contact with this small piece of filter paper.

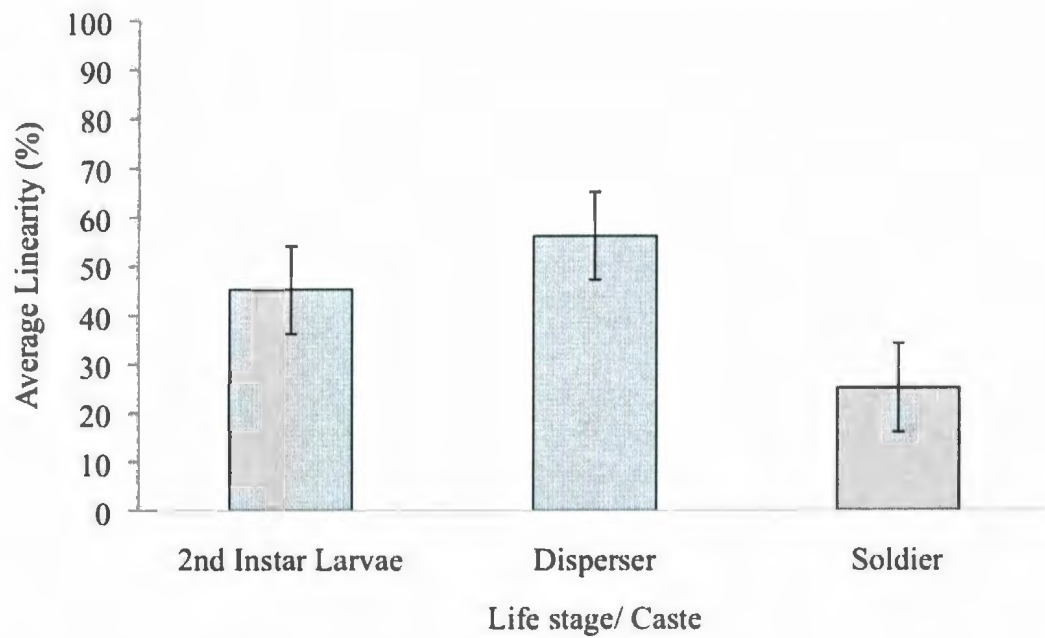


Figure 2.5. The average linearity (LIN; with standard error) is on the y axis, with thrips of different life stages/ castes on the x axis. The linearity (LIN) of the thrips movement was calculated by dividing the rate of net space gain of the thrips (the distance from the start to the end of the movement pattern divided by time) by the VCL calculation, and then multiplied by 100.

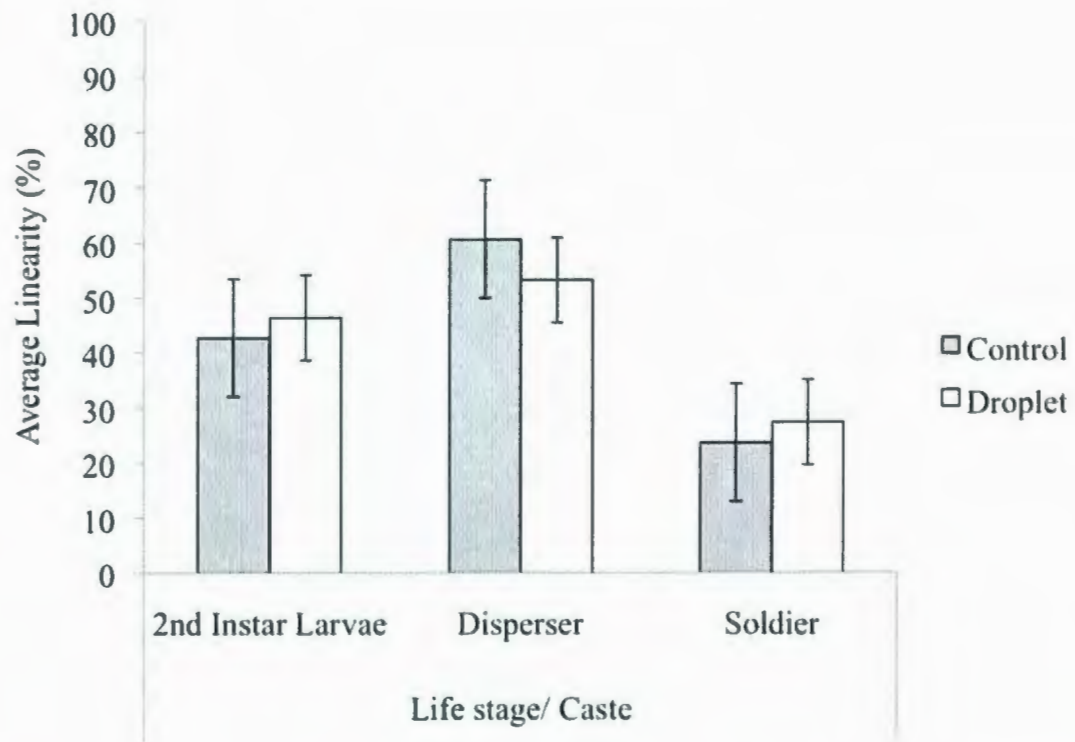


Figure 2.6. The average linearity (LIN; with standard error) is on the y axis, with thrips of different life stages/ castes on the x axis. The linearity (LIN) of the thrips movement was calculated by dividing the rate of net space gain of the thrips (the distance from the start to the end of the movement pattern divided by time) by the VCL calculation, and then multiplied by 100. Trials were conducted where no abdominal tube droplets were added (Control, darkly filled bars), or droplets were added to the trial (Droplet, unfilled bars). Droplets were collected from all castes and life stages of the thrips on a small tear drop shaped piece of filter paper. The thrips in the droplet trials were placed in contact with this small piece of filter paper.

Chapter 3. The effect of the abdominal tube droplet on defense by soldiers of gall inducing thrips (*Kladothrips intermedius*)

3.1 Introduction

Large social units consisting of different castes performing characteristic functions typically evolve highly sophisticated communication systems (Shorey, 1973). Communication can be defined as a transfer of information between two or more individuals that results in a behavioural change (Shorey, 1973). Pheromones are chemicals used for communication and are widely documented to be the primary means of communication within eusocial insects (Blum, 1974). In a pheromone-based communication system, pheromones are emitted by certain individuals causing the appropriate behavioural response in others. Pheromones have been observed to release a wide range of behaviours, including aggregation, dispersion, sexual behaviour, oviposition, foraging, and alarm behaviour. The use of pheromones by eusocial insects has been widely documented within social Hymenoptera and Isoptera (termites). An example of the use of pheromones to coordinate caste behaviour during an invasion is found in honeybees. Guard honeybees, stationed at the entrance of the hive, will mark an enemy with an alarm pheromone to facilitate the orientation of other bees to the enemy (Blum, 1969; Shorey, 1973).

Although chemical communication has been documented in Hymenoptera, Isoptera, and some species of Thysanoptera, to date, there have been no published studies investigating communication within social species of Australian gall-inducing thrips.

Individuals of many thrips species, including Australian gall-inducing thrips, have been observed to raise the abdomen up and over the head (a scorpion-like curl) while producing an abdominal tube droplet (Hodson, 1935; Lewis, 1973; personal observation, May 2008) and, in some species of non-social thrips, secretions have been shown to function as an alarm pheromone (Teerling et al., 1993), a predator repellent (Howard et al., 1983, 1987; Blum, 1991, 1992), and a trail pheromone (Kiestler and Strates, 1984). The importance of pheromones to the release of social behaviour in Australian gall-inducing thrips would provide an important insight into the understanding of the evolution of social behaviour because they are a recently evolved eusocial lineage, relative to ants, termites, and advanced bee and wasp groups. In addition, there are solitary species of gall-inducing thrips that have not evolved soldiers and thus can be used in a comparison study of the origin of chemical communication.

Seven described species of eusocial Australian gall-inducing thrips have evolved a non-dispersing 'soldier' morph characterized by enlarged forelimbs, reduced antennae, reduced or absent wings and often, a pale exoskeleton (Mound, 1971; Crespi, 1992a, 1992b; Mound and Crespi, 1995; Mound et al., 1996; Crespi and Mound, 1997; Crespi et al., 1997a, 1997b; Kranz et al., 1999, 2001a, 2001b). While reduced antennae and melanization could be assumed to be an evolutionary response to exclusively living in a gall, it is the modification of the forelimbs that has been hypothesized to have evolved in response to a kleptoparasite, *Koptothrips*. *Koptothrips* invade a gall, killing or driving out the inhabitants, and thereafter breed and raise their offspring within the gall (Crespi, 1992a, 1992b; Crespi and Abbot, 1999). Soldiers of gall-inducing thrips use their

enlarged forelimbs to grasp *Koptothrips* around the thorax then raise them into the air and squeeze the victim in slow pulses until it is moribund, after which it is released (Caravan, personal communication, February 2010). These enlarged forelimbs may lead to a greater ability of soldiers to grasp and hold a *Koptothrips* and subject it to greater squeezing force, relative to the slimmer forelimbs of dispersers (Perry et al., 2004).

Soldiers and dispersers, the reproductive caste of gall-inducing thrips, differ in their residency within galls. In most species, the dispersers leave the gall as second-instar larvae to pupate in the soil. The one exception is *Kladothrips intermedius* in which dispersers fully develop within the gall (Morris et al., 2001). In contrast, soldiers with reduced or absent wings do not leave their natal gall and are referred to as gall-bound. Soldiers of *K. intermedius*, however, vary in wing length and forelimb size, with some soldiers having fully developed wings resembling dispersers (Crespi and Vanderkist, 1997; Chapman, personal communication, May 2008). Soldiers having more developed wings and slimmer forelimbs may represent a tradeoff between dispersal and defense. These soldiers could potentially leave their natal gall and initiate their own gall and, as a result, may choose to flee instead of fight during a *Koptothrips* invasion.

In addition to wing length and forelimb size, the sex of soldiers of *K. intermedius* may influence whether they choose to flee or fight during a *Koptothrips* invasion (Chapman, 2003). The alteration of inbreeding levels between the mixed-mating of foundresses and the complete inbred mating by soldiers caused oscillations in genetic relatedness within the simulated population that selectively favored a female to take a

helping role in the colony (Chapman, 2003; McLeish, Chapman, and Crespi, 2006). These oscillations produced a situation where female soldiers were more related to individuals within their natal gall than their own offspring than if they left to produce their own gall. Meanwhile, the same oscillations produced a situation where males are less related to individuals within their natal gall, besides their own daughters, due to the nature of haplodiploidy. Support for this model can be found in natural populations of *K. intermedius* where a female soldier bias sex ratio (79%) has been reported (Crespi et al., 2004). In addition, Crespi and Vanderkist (1997) found high levels of fluctuating asymmetries between male and female soldiers of *K. intermedius*. The wing length for male soldiers was near the values found in dispersers. Male soldiers were also reported to have a decreased femur length, which could mean they have less effective weaponry compared to female soldiers. As a result, males may choose to flee instead of fight during a *Koptothrips* invasion.

In this study we first examined whether the abdominal tube droplets of Australian gall-inducing thrips, *K. intermedius*, contain an alarm pheromone that functions to warn other defenders of an intruder by determining if the presence of a droplet in a behavioural assay increased agonistic interactions between *Koptothrips* and soldiers. Second, we quantified wing length, forelimb size, and sex of each soldier used in the assays to determine whether they were correlated with the outcomes of the behavioural assays. If the variation in soldier morphology is a tradeoff between dispersal and defense, then those soldiers with morphology similar to that of a disperser should be less willing to fight an invader. If this is true, then trials with soldiers with slim forelimbs and developed

wings should have a lower death rate than those trials with soldiers with enlarged forelimbs and reduced or absent wings. In addition to this, trials with male soldiers should have a lower death rate than those trials with female soldiers.

3.2 Methods

3.2.1 Life History

Refer to section 2.2.1

3.2.2 Collections

Refer to section 2.2.2

3.2.3 Behavioural assay

Behavioural assays were conducted at the Middleback field station, located 400 km Northwest of Adelaide, Australia, 1-3 days following gall collection. Previous research indicates that gall inhabitants are not negatively affected by having their galls removed from the host tree for periods under one week (Bejah, 1997). The experimental setup of this study was based on a previous study conducted by Perry and colleagues (2004). Galls were bisected at the ostiole, where opposite sides of the phyllode meet, and the contents were removed with a paintbrush onto a plastic Petri dish. The contents of the gall were then examined using a dissecting microscope or a hand lens. Approximately 1 in 30 galls contained the species *Koptothrips dyskritus* and it was, therefore, necessary to open many galls before sufficient numbers of *Koptothrips* were found. Both *Koptothrips* and soldiers of *K. intermedius* were collected and placed into 200- μ l Polymerase Chain

Reaction (PCR) tubes until needed. Soldiers from the same gall were placed in the same tube since gall mates reside naturally within a gall apparently without conflict (Crespi, 1992a; Bejah, 1997).

For each trial, a piece of filter paper with *K. intermedius* droplets (see next section) or a piece of filter paper with nothing on it (control) was placed into a marked PCR tube. A *K. intermedius* soldier followed by a *Koptothrips dyskritus* was then placed into the tube and the trial began. Assays were set up over a two-day period for a total of 140 trials, each containing a soldier and an invader. Trials were conducted over a period of 3-4 days, until most individuals of both species of thrips were dead, and were stored at room temperature in a dark drawer. The assays were checked every 4-13 hours and the outcome of a trial was recorded as death or no death. Deaths were used as an indicator of an interaction between a *Koptothrips dyskritus* and a soldier since a soldier in the presence of a droplet should seek out and attack an invader, but do not always emerge victorious. An outcome was coded as a 'death' if either the *K. intermedius* soldier or the *Koptothrips dyskritus* was dead at the end of the trial and an outcome was coded as 'no death' if both specimens were alive at the end of the trial.

3.2.4 Induction of abdominal tube droplets

Refer to section 2.2.4

3.2.5 Measurement of wings and forelimbs

The body length of each soldier was determined using an ocular micrometer mounted on a dissecting microscope. A stage micrometer was used to determine the number of ocular units in one millimeter and this number was used to convert the body length measurement to millimeters. Using a camera mounted on a light microscope, digital images of the wings and forelimbs were taken. Image J software was used to obtain multiple forelimb and wing measurements (Figure 3.1). All measurements obtained are given in millimeters.

3.2.6 Sex determination

The sex of adult soldiers can be determined by viewing the sculpturing of the last few abdominal segments: females appear more rounded than males. However, verification of sex was also performed by clearing the cuticles (Watson and Chandler 1999) of soldiers where the sex specific structure of males, the phylloblast, can be viewed using a light microscope (Figure 3.2). The determination of the sex of a soldier was repeated by an individual blind to both the putative sex of the individual and the outcome of the battle assay.

3.2.7 Statistical Analysis

A logistical regression was used to determine if the odds of death for the interaction between a soldier of *K. intermedius* and a *Koptothrips dyskritus* were significantly higher in the droplet versus control condition. Because there are several aspects of *K. intermedius* life history that may influence the outcome of the behavioural assays, i.e., the influence of sex on helping behaviour and the presence of wing and

forelimb polymorphism, a second regression model using the morphological characters (wing length and forelimb size), sex, and condition (control and droplet) on the outcome (death or no death) was analyzed.

3.3 Results

There was no significant difference in the odds of death for a *K. intermedius* soldier and a *Koptothrips*, in the droplet versus control condition, $\Delta G = 2.252$, $df = 1$, $p = 0.066$ (Figure 3.3).

In the second model, controlling for sex, wing length, and forelimb size, there was also no significant difference in the odds of death for each condition ($\Delta G = 5.80$, $df = 5$, $p = 0.327$; Figure 3.4, 3.5, and 3.6, respectively).

3.4 Discussion

The difference in the outcomes, the number of interactions of *Koptothrips* and soldiers resulting in death, in the droplet versus the control condition was expected to be small. A small difference between conditions was expected due to the possibility of self-stimulation or stimulation by conspecifics while the gall was being bisected. In addition to this, there is no current data on the volatility of the substances within the droplet and thus we were unable to determine how the manipulations could have impacted this. The results do show a small difference in the right direction for the outcomes between the droplet and control condition, but this difference was not significant at the standard alpha level ($\alpha = .05$).

We predicted that female soldiers should fight more aggressively than male soldiers because in *K. intermedius* female soldiers are more related to individuals within their natal gall than offspring of their own, whereas males are less related to individuals within their natal gall. In this situation, female soldiers should be more willing to help than males and thus should have fought an invader more aggressively than males. If the abdominal tube droplet contains an alarm pheromone, then females should be more likely to seek out and attack the invader than males when the droplet is present. This relationship would result in a higher death rate, which was observed but again was found not to be significant. A possible explanation for the lack of significance of sex on death rate could be the small proportion of males (N=10) versus females (N=88) used in the experiment. As previously mentioned, *K. intermedius* have a female soldier biased sex ratio which led to the paucity of males in this experiment (Crespi et al., 2004).

We predicted that those soldiers with morphology similar to that of a disperser would be less willing to fight an invader because these soldiers may represent a tradeoff between dispersal and defense. Soldiers with disperser-like morphology could potentially leave their natal gall and initiate their own gall and, as a result, may choose to flee instead of fight during a *Koptothrips* invasion. When morphological characteristics were statistically controlled for, there was no significant difference in death rate between the droplet and control condition. These results indicate that there is no difference in willingness to fight an invader between those soldiers with slim forelimbs and developed wings (similar to morphology of dispersers) and soldiers with enlarged forelimbs and reduced or absent wings.

The age of the soldiers could have influenced their ability to detect the droplet and/or perform defensive behavior. Perry and colleagues (2004) used specimens collected in February and included foundresses in their study, which can only be identified if it is the only disperser within a gall of soldiers and/or larvae. Specimens for this study were collected in June and the majority of galls bisected for this study contained fully developed dispersers, indicating that the gall and the soldiers within were relatively old. The age of the soldiers could have influenced their ability to detect the droplet and/or perform defensive behaviour.

Species of Kladothrips are primarily invaded by two species of Koptothrips, *Koptothrips flavicornis* and *Koptothrips dyskritus*. *Koptothrips flavicornis* are thought to have venomous fore-tarsal teeth, since soldiers will die within minutes of being pierced (Crespi and Mound, 1997), and thus are much more lethal than *Koptothrips dyskritus*, which are hypothesized to be much more primitive in their gall invasion strategy. *Koptothrips dyskritus* are opportunistic, in that females will sometimes breed within a damaged gall, instead of invading an inhabited gall, enclosing themselves using cellophane-like partitions (Crespi and Abbot, 1999). Perry and colleagues (2004) used *Koptothrips flavicornis* while the behavioural assays in the current study used *Koptothrips dyskritus*. As previously mentioned, *Koptothrips flavicornis* was reported by Crespi and colleagues (2004) to be the primary invader for most of the social species and two species that were hypothesized to have lost soldiers, while *Koptothrips dyskritus* primarily invades a complex of solitary species, *Kladothrips rugosus*. Despite this, *Koptothrips dyskritus* was the primary invader discovered in the galls of *K. intermedius*

for the current study. Since *Koptothrips flavicornis* appears to be much more lethal than *Koptothrips dyskritus*, assays for the current study, using *Koptothrips dyskritus*, would likely have a lower overall kill rate than if *Koptothrips flavicornis* were used.

Large social units typically evolve highly sophisticated communication systems (Shorey, 1973). Eusocial insects primarily use pheromones, chemicals used for communication. To date, there have been no published studies investigating communication within eusocial Australian gall inducing thrips, although thrips have been observed to produce a large clear droplet when cues associated with danger are present (personal observation, June 2008). The results from this study do not confirm the presence of an alarm pheromone in the abdominal tube droplet but further investigation into the function of the droplet is required. Understanding communication within Australian gall-inducing thrips would not only provide insight into their response to an invasion, but would also provide insight into the understanding of the evolution of social behaviour since Australian gall-inducing thrips are a recently evolved eusocial lineage, relative to sociality in ants, termites, and advanced bee and wasp groups, which is inferred to be much more ancient.

Future research into the function of this droplet should use a eusocial species with a less complicated life history, such as *Kladothrips habrus*. *Kladothrips intermedius* has a life history that differs in many ways from other eusocial species of gall inducing thrips. Larvae of gall-inducing thrips will eclose within the gall instead of leaving the gall as second instar larvae and eclosing in the soil. In addition to this, *Kladothrips intermedius*

has a strong female bias for soldiers and dispersers, not observed in other species (Crespi et al., 2004). Soldiers of *Kladothrips intermedius* have variation in wing length, while other eusocial species have near absent wings. These characteristics of *Kladothrips intermedius* life history likely cause behavioural difference in soldiers compared to other eusocial species. For this reason, it would be better to use a eusocial species that has fewer confounding variables within the life history that have to be considered.

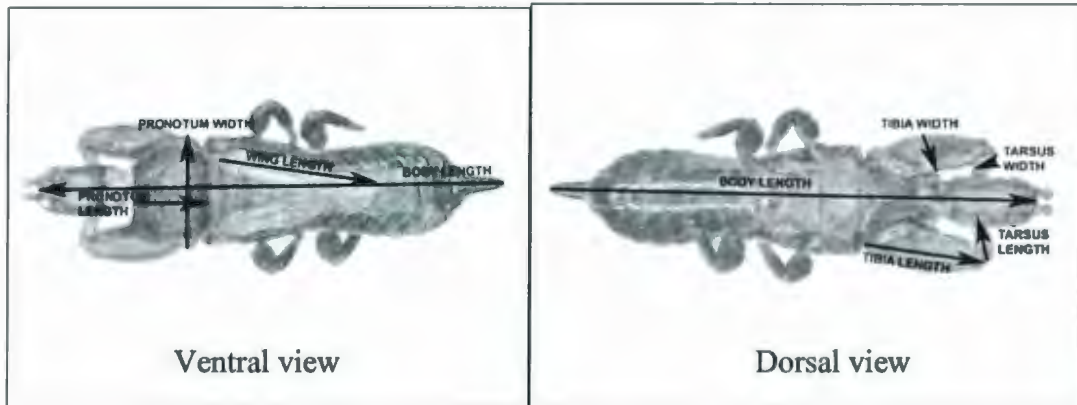


Figure 3.1. The ventral and dorsal view of a soldier (*Kladothrips intermedius*) with the measurements made on each specimen, using Image J, to determine forelimb size and wing length.

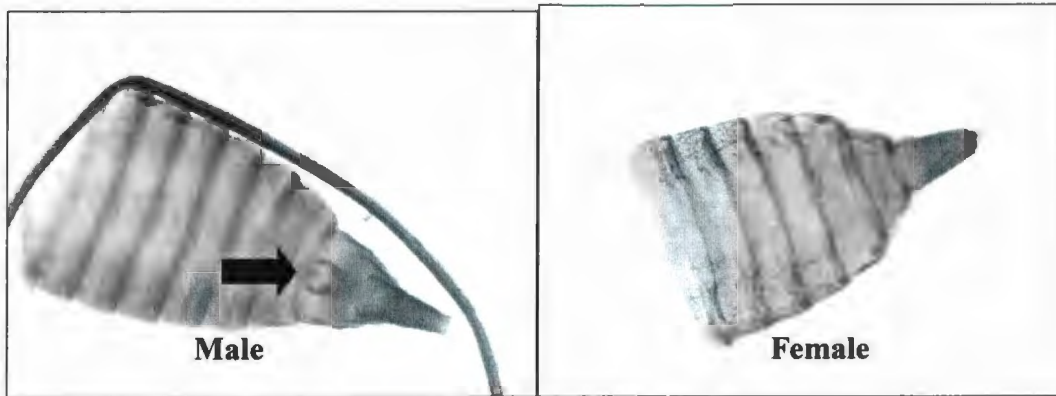


Figure 3.2. Abdomens of two soldiers of *Kladothrips intermedius*. Cuticles were cleared using the protocol described by Watson and Chandler (1999) in order to determine the sex by identification of the phylloblast in males (indicated by the arrow).

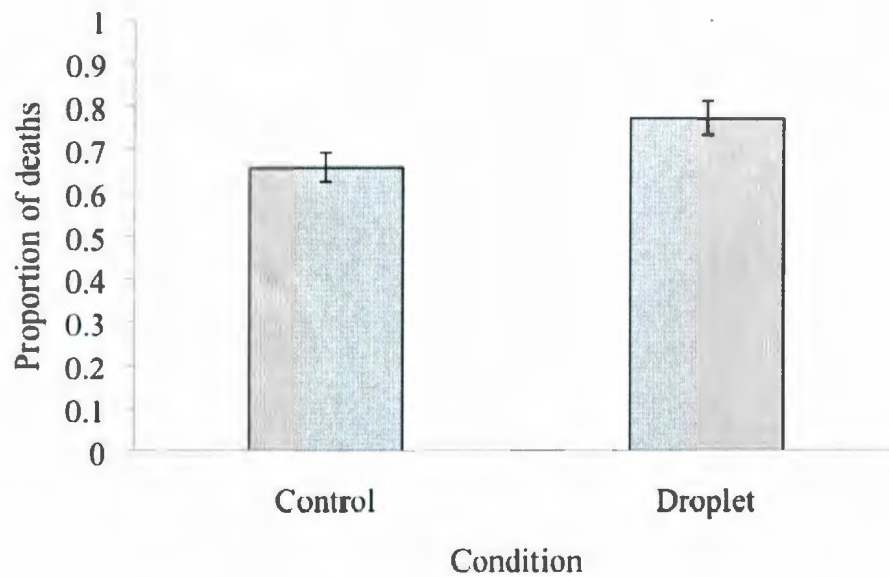


Figure 3.3. The proportion of deaths is on the y-axis and condition is on the x-axis. Trials were conducted where no additional abdominal tube droplets were added (Control) or droplets were added to the trial (Droplet). Droplets were collected from all castes and life stages of the thrips on a small tear drop shaped piece of filter paper. The filter paper was then placed into a PCR tube with a *K. intermedius* soldier and a *Koptothrips dyskritus*.

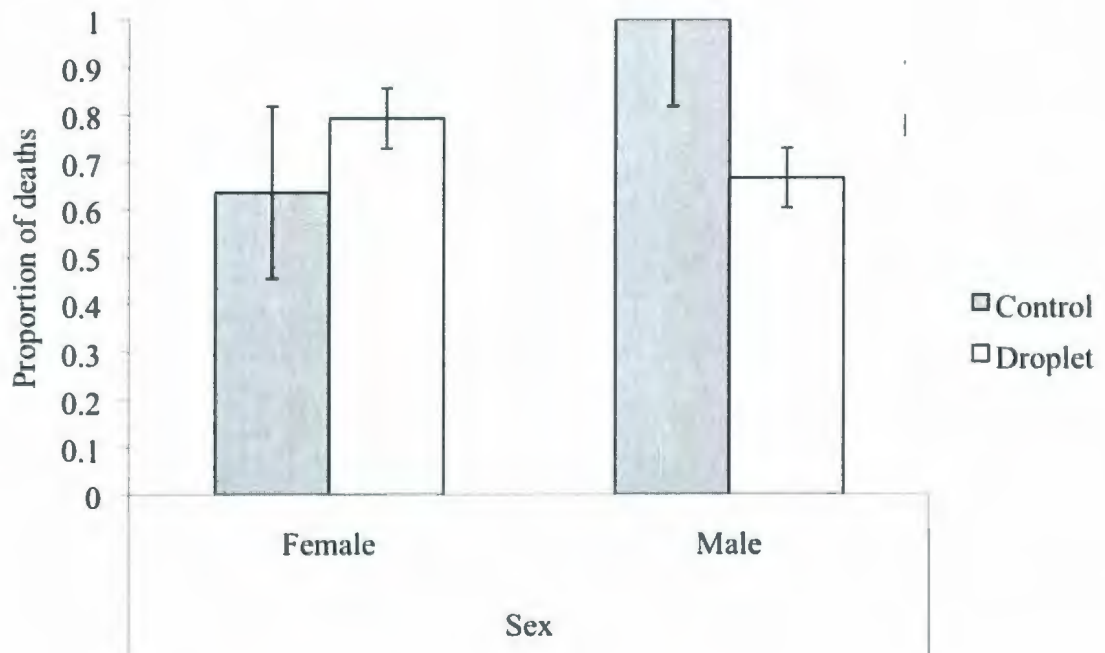


Figure 3.4. The proportion of deaths is on the y-axis and sex is on the x-axis. Trials were conducted where no additional abdominal tube droplets were added (Control, darkly filled bars), or droplets were added to the trial (Droplet, unfilled bars). After the trials were complete, sex was determined by clearing the cuticle using the protocol described by Watson and Chandler (1999) and identifying the phylloblast in males.

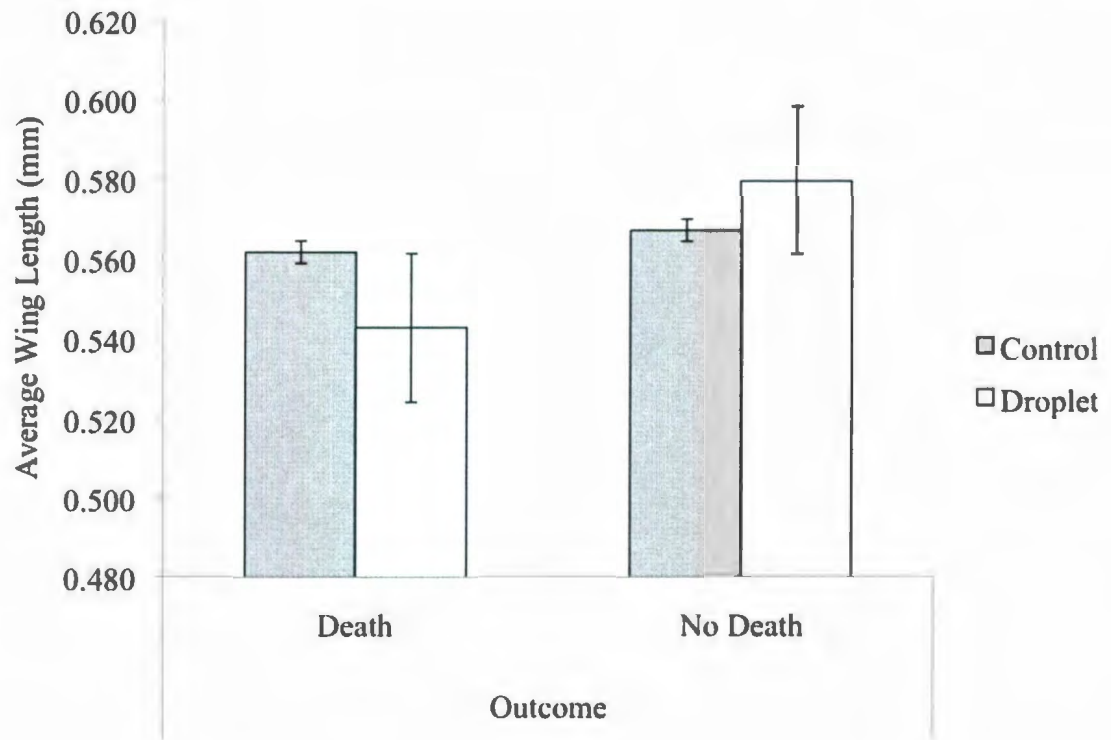


Figure 3.5. Average wing length (mm) of soldiers is on the y-axis and the outcome of the trial on the x-axis. An outcome was coded as a 'death' if either the *K. intermedius* soldier or the *Koptothrips dyskritus* was dead at the end of the trial and an outcome was coded as 'no death' if both specimens were alive at the end of the trial. Trials were conducted where no additional abdominal tube droplets were added (Control, darkly filled bars), or droplets were added to the trial (Droplet, unfilled bars). Digital images of the wings were taken and Image J software was used to obtain wing measurements (mm).



Figure 3.6. Average tibia length (mm) of soldiers is on the y-axis and the outcome of the trial on the x-axis. An outcome was coded as a 'death' if either the *K. intermedius* soldier or the *Koptothrips dyskritus* was dead at the end of the trial and an outcome was coded as 'no death' if both specimens were alive at the end of the trial. Trials were conducted where no additional abdominal tube droplets were added (Control, darkly filled bars), or droplets were added to the trial (Droplet, unfilled bars). Digital images of the wings were taken and Image J software was used to obtain wing measurements (mm).

Chapter 4. General Discussion

The studies described in Chapters 2 and 3, taken together, do not convincingly provide evidence for a communication function for the abdominal tube droplet of *Kladothrips intermedius*. Neither fleeing (Chapter 2) nor fighting (Chapter 3) was altered in the presence of the droplet. There are four possible explanations for the observed results: (1) chemical communication is absent in Australian gall-inducing thrips, (2) chemical communication was lost in *Kladothrips intermedius*, (3) communication does occur within this species but the experimental design used in the current studies were flawed, and (4) chemical communication does occur within this species but the droplet does not function as an alarm pheromone.

If chemical communication between individuals is absent in the gall-inducers, this would suggest that communication was not present at the origin and thus could not facilitate the evolution of eusociality. Such knowledge would provide a significant contribution to the study of social behaviour since one of the major questions of this field is whether communication is necessary for the evolution of social behaviour or if it is a byproduct, since all social groups have some means of communication (Shorey, 1973). For most eusocial insects, such as those found within Hymenoptera and Isoptera, the origins of eusociality are so ancient that such studies would be difficult or even impossible (Hölldobler and Wilson, 1990; Chapman et al., 2008).

Perhaps chemical communication was lost in *Kladothrips intermedius*. This lineage (Figure 4.1) contains all species having soldiers and two species (*Kladothrips*

xiphius and *Kladothrips rodwayi*) without soldiers. Both species of thrips without soldiers and *K. intermedius* have undergone a host-plant shift. Possible scenarios for this pattern and number of origins and losses of soldiers include: (1) one origin of soldiers at the base of the lineage, indicated by 'B', and two losses, in *Kladothrips xiphius* and *Kladothrips rodwayi* or (2) two origins, one in *Kladothrips hamiltoni* + *Kladothrips harpophyllae* and one in *Kladothrips morrissi* + *Kladothrips waterhousei* + *Kladothrips habrus* + *Kladothrips intermedius* + *Kladothrips rodwayi*, followed by a loss of soldiers in *Kladothrips rodwayi* (Morris et al., 2001; Chapman et al., 2008). Chapman and colleagues (2008) suggest that the former scenario is more likely since it is evolutionarily difficult to gain eusociality but it is easy to lose (Wcislo and Danforth, 1997). Host-plant shifts appear to be associated with life history changes in Australian gall inducing thrips, which in turn may also be associated with the presence of chemical communication (Crespi et al., 2004; Chapman et al., 2008). The life history of *Kladothrips intermedius* differs from that of other eusocial species in several ways: (1) dispersers fully develop within the gall instead of leaving as second instar larvae and eclosing in the soil, (2) soldiers have partial wings instead of near absence of wings (Crespi, 1992a, 1992b; Chapman et al., 2008), (3) galls without any soldiers are not uncommon, which is not the case in other species (Chapman et al., 2008), and (4) there is a strong female bias, not observed in other species (Crespi et al., 2004). These differences are likely due to the host-plant shift, which is apparently always associated with life history changes in Australian gall inducing thrips (Crespi et al., 2004; Chapman et al., 2008). It is evident that after the origin of soldiers (Figure 4.1 indicated by 'B') a host-plant shift was

associated with a loss of soldiers, as in *Kladothrips xiphius* and *Kladothrips rodwayi*, but the same is not true for *Kladothrips intermedius*. It is possible that *Kladothrips intermedius* lost soldiers after the host-plant shift and regained them after sufficient environmental pressures from natural enemies. In this scenario, in addition to a loss of soldiers, *Kladothrips intermedius* could have suffered a loss of the communication function of the droplet. If chemical communication was lost in *Kladothrips intermedius*, it is possible that it is still present in other eusocial species that have not undergone a host-plant shift and thus further investigations using these species is necessary.

Perhaps chemical communication does occur within *Kladothrips intermedius*, but the experimental design used in the current studies was problematic. One potential problem that could affect the results obtained for both experiments is that the bisection of the gall may have resulted in unseen droplet production. If droplets were produced prior to the experiment, it may have influenced all individuals prior to the experiment in both the control and droplet conditions. A possible solution to this problem would be to remove the thrips from the gall and place them under a small lid prior to the experiment until normal behaviour is observed to have returned. A possible flaw in the experimental design outlined in Chapter 2 is unknown dissipation time of the droplet. Pheromones that send messages to conspecifics typically have a fast dissipation time (Schoonhoven et al. 1981). Gall-inducing thrips live within an enclosed space so the droplet likely dissipates much more quickly on the exposed filter paper than it would within the gall. For this reason, the lack of response observed could be due to the thrips inability to detect the droplet prior to evaporation.

A fourth explanation for the observed results is that chemical communication does occur within this species but the droplet it is not where it is found or dissipated. This droplet has been shown to function as an alarm pheromone in flower thrips, *Frankliniella occidentalis*, (Teerling et al., 1993), but this droplet has been shown to function as a predator repellent (Howard et al., 1983, 1987; Blum, 1991, 1992) and a trail pheromone (Kiestler and Strates, 1984) in other species of thrips. However, the function of the droplet in the social thrips remains an enigma and future studies should remain open to alternative explanations for this behaviour including non-adaptive explanations.

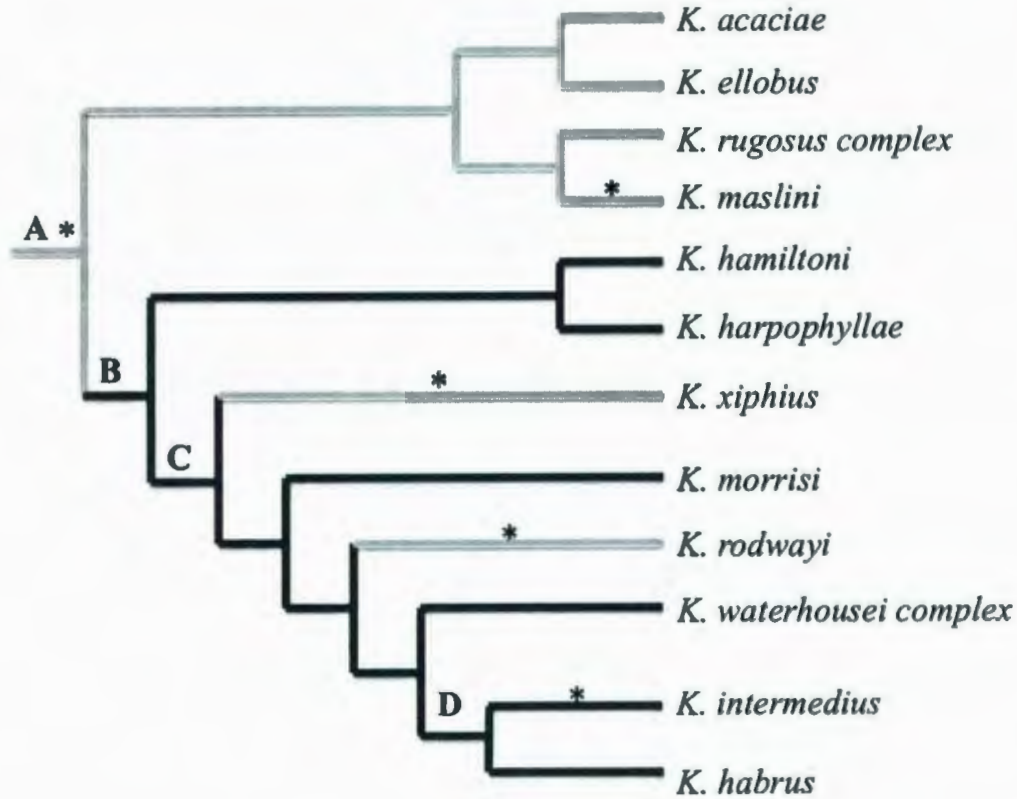


Figure 4.1. Phylogeny for the evolution of social behaviour in gall-inducing thrips on Acacia, from Chapman et al. (2008). Lineages shown in black have soldiers, while grey lineages indicate an absence of soldiers. Host shifting events (between species of *Acacia*) are indicated by '*' above the lineage where the shift is hypothesized to have occurred. At the point marked 'A' there has been a host plant shift. In addition to this, at this point, male founders are hypothesized to accompany females, parasitism by *Koptothrips* arose, and larvae leave the gall to pupate in the soil. The point indicated by 'B' is hypothesized to be the origin of soldiers, accompanied with a loss of physogastry. In addition, there was a reduction in gall volume and, as result, a smaller brood size, and longer gall

duration. At point 'C' there is a loss of male founders. At point 'D' there is a decrease in soldier reproductive output, which is associated with an increase in soldier efficiency.

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