





**A CHINK IN THE ARMOUR: AN INVESTIGATION OF THRIPS SOLDIER  
MORPHOLOGY AND SEX IN RELATION TO FIGHTING ABILITY**

By

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### Abstract

Explaining the persistence of self-sacrificing individuals throughout evolutionary time has been a central area of study for evolutionary biologists since Darwin. In total, seven species of Australian gall inducing thrips have evolved soldiers with enlarged femurs that are used to defend against invertebrate invaders. In the species *Kladothrips intermedius*, the sexes of soldiers differ morphologically – the females have more robust femurs than the male soldiers, which have longer wings, suggesting less commitment to this defensive role. There was no difference in the fighting abilities of the male and female soldiers in *K. intermedius*. Morphology of the soldier, within a sex, was related to the outcome of a battle with an invader, but not in the way expected. Females with slimmer femurs were more likely to battle an invader. Also, female soldiers with shorter wings were better able to dispatch an invader while male soldiers with longer wings were less likely to battle an invader.

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## Table of Contents

Abstract	ii
Acknowledgements	iii
Table of Contents	iv
List of Tables	vi
List of Figures	vii
Chapter 1. Introduction	
1.1. Eusociality	1
1.2. Gall-inducing thrips	10
1.3. Objectives	15
Chapter 2. Methods	
2.1. Collections and Processing	19
2.2. Fighting Assays	20
2.2.1. Morphological Measurements	21
2.3. Infiltrate Gall Assays	22
2.4 Mating Assays	23
2.5 Statistics	25

Chapter 3. Results	
3.1. Gall Data	28
3.2. Fighting Assays and Morphological Measurements	28
3.3. Infiltrate Gall Assays	31
3.4. Mating Assays	31
Chapter 4. Discussion	
4.1. Fighting Assays	39
4.2. Infiltrate Gall Assays	43
4.3. Mating Assays	46
4.4. General Discussion	48
References	57

### List of Tables

- Table 1.1. Relatedness values found in a haplodiploid genetic system where females are produced from fertilized eggs and males are produced from unfertilized eggs. Males are most related to their daughters, and are not related to their sons at all. Females are most related to their sisters and least to their brothers 17
- Table 3.1. Repeatability values of several morphological measurements taken from male and female soldiers of *Kladothrips intermedius*. MSwithin and MSamong were obtained from a one way repeated measures ANOVA in PASW v18; repeatability was calculated using equation 2.1. 33
- Table 3.2. The results of a binary logistic regression performed to determine if morphological variables could accurately predict the outcome of a fight between a soldier of *Kladothrips intermedius* and a *Koptothrips*; only significant results are reported. 36

### List of Figures

- Figure 2.1. The last few abdominal segments of a male and female soldier of *Kladothrips intermedius*. The males have pointed terminal abdominal segments (on the left) while the females are more rounded (on the right). 26
- Figure 2.2. A male soldier of *Kladothrips intermedius* that was marked for a behavioural assay by applying Testor's Enamel paint to the pronotum as to make it distinguishable from the other thrips present in the assay. 26
- Figure 2.3. A half gall set in plasticine within a Petri dish with soldiers of *Kladothrips intermedius* placed inside for the purposes of observing mating. One male soldier was marked by placing brightly colored paint on its pronotum – this individual is seen near the center of the gall and is indicated by the arrow. 27
- Figure 2.4. Soldiers of *Kladothrips intermedius* during copulation. The male thrips mounts the female and wraps his abdomen around the female's abdomen where the aedeagus enters the genital pore of the female. 27
- Figure 3.1. The average body length, wing length, femur length, femur width, pronotum length and pronotum width (mm), along with standard deviations, of male and female soldiers of *Kladothrips intermedius*

- used in the fighting assays. The male and female soldiers were found to differ significantly for all measurements (independent-samples t-test) 34
- Figure 3.2. The proportion of outcomes for the 217 (109 female, 108 male) fighting assays for the female and male soldier. 35
- Figure 3.3. The proportion of individuals (n = 111) that entered a gall for the female *Koptothrips* and male and female soldiers of *Kladothrips intermedius* involved in the “infiltrate gall assays”. 37
- Figure 4.1. A gall of *Kladothrips intermedius* that has experienced desiccation while in the infiltrate gall assay. The ostiole has separated, allowing the brood inside to easily leave the confines of the gall. 55
- Figure 4.2. Cahan and colleagues (2002) schematic representation of the trajectory of social decisions an individual will face throughout its lifetime. The yellow pathway represents the proposed trajectory for female soldiers of *Kladothrips intermedius* as well as the early life trajectory for male soldiers. The blue pathway represents the proposed switch in trajectories that the male soldiers of *K. intermedius* experience. 56

## Chapter One – Introduction

### 1.1 Eusociality

Cooperation has been a feature of several major transitions in the evolution of life: molecules assembling into genes, emergence of multicellularity and the formation of whole-organism societies (Buss 1987; Maynard Smith and Szathmary 1995; Michod 1999). Cooperation at the organism level has emerged across a range of taxa including bacteria (Shapiro 1998; Henke and Bassler 2004; Williams et al. 2007), amoebae (Bonner 1967; Raper 1984; Gadagkar and Bonner 1994; Kessin 2001), flatworms (Hechinger et al. 2010), and most prominently in insects. Social insects have become so successful that they are a dominant feature of our terrestrial landscape, both in terms of biomass and coverage of our planet’s terrestrial surface (Wilson 1990). The paramount presence of these insects and the complexity of cooperation exhibited by some groups have attracted the interest of evolutionary biologists since Darwin. In 1859, Darwin stated that non-reproductive individuals pose a potentially “insuperable” flaw for his theory of evolution by natural selection. These ‘altruistic’ individuals sacrifice their fitness for the purpose of increasing the fitness of another (Aoki 1987; Ito 1989; Stern and Foster 1996). Darwin (1859) struggled with explaining how natural selection can lead to part of a population becoming sterile, and furthermore, how sterility can be maintained in that population. And, this question still remains an important field of study in evolutionary biology (International Union for the Study of Social Insects Conference 2010) and will be the central theme of my thesis.

Insect societies are often referred to as superorganisms in order to draw a parallel between individuals forming specialized castes and cells coming together to form specialized tissues and organs (Wilson 1971). Michener (1969a) proposed categorizing social interactions into presocial behaviours that were considered more complex than the behaviours involved in mating, but were thought of as a precursor to the most complex form of social behaviour, eusociality. 'Eusociality' is traditionally characterized as an overlap of generations (mother with adult offspring), cooperative brood care, and a reproductive division of labour with one or a few main reproductives and the presence of more or less sterile individuals in the form of soldiers or workers (Wheeler 1928; Batra 1966; Michener 1969a; Wilson 1971; Holldobler and Wilson 1990). Michener's (1969a) hierarchy of social classification begins with 'solitary' animals. Animals in this category never exhibit groups with cooperative brood care, nor a reproductive division of labour (no sterile individuals present), and they do not have groups of individuals that consist of overlapping generations. In other words, solitary species exhibit none of the main criteria for eusociality. Next in order of increasing social complexity is 'subsociability', in which adults would care for their own young, be it in the form of eggs, nymphs, or larvae. This parental care mainly involves the provisioning and protection of food for the young and decreasing the parasitization and predation of young (Gullan and Cranston 2010). When the same generation of individuals comes together within a shared nest, but without any cooperative tending of young, we have Michener's 'communal' sociality, and when communal nesters share brood rearing efforts we have a 'quasisocial' society. The next stage in complexity is 'semisociality' and in this category are all the previous

requirements (same generation, same nest, cooperative brood care) plus a reproductive division of labor (with one main reproductive group).

Despite Michener's (1969a) attempt to remove ambiguity by students of social behaviour in the use of social terminology, Wilson (1971) notes that it remained an issue and, therefore, a barrier to a full understanding of social evolution. Some researchers use the three main criteria listed above (Wheeler 1928; Michener 1953b; 1969a; Batra 1966; Wilson 1971) while others have eliminated a generational overlap as a defining point of eusocial societies (Richards and Richards 1951; Emerson 1959). In the early to mid 90s, the debate as to how to define eusocial societies was at its peak. Two main approaches emerged as to how to revamp the eusocial definition and involved either restricting the definition (for example, Tsuji 1992; Crespi and Yanega 1995) or expanding the definition to include all vertebrate and invertebrate societies with helpers (for example, Furey 1992; Sherman et al. 1995). This thinking even went as far as to split eusociality into advanced and primitive such that advanced societies were long-lived, large colonies where workers of a different morphology couldn't mate, while primitive societies were small, annual colonies where workers, similar in appearance to the queen, could mate (for example, Michener 1974; Eickwort 1981; Cowan 1991). It seemed the main issue with defining eusociality was the ambiguity of the term "reproductive division of labour". One response was to use differences in lifetime reproductive success to quantify reproductive division of labour along a continuum, such that a value of zero is where all individuals are breeding, a lack of reproductive skew, and a value of 1 represents a situation where there are main reproductives, a high reproductive skew (Sherman et al. 1995). Societies

then fell into three main categories of lifetime reproductive success, or reproductive skew: low reproductive skew was represented in helper at the nest societies where helpers do not reproduce during the helping period but can successfully do so at a later time; medium reproductive skew was represented in societies with singular breeding and limited direct reproductive opportunities for helpers such that only some helpers will eventually reproduce, either by joining another group lacking a main reproductive, or by inheriting a group; and high reproductive skew was represented in societies where most members do not reproduce, such as those societies that possess physiologically sterile workers (Sherman et al. 1995). Another "continuum" type approach was the eusociality index developed by Keller and Perrin (1995) which quantified the decrease in direct reproduction of helpers that occurs as a consequence of performing altruistic behaviours on other members in the group. Crespi and Yanega (1995) took a different approach to the re-defining of the eusocial concept and stated that these societies must contain permanent castes, which are groups of individuals that become irreversibly behaviourally distinct at some point in their life span prior to reproductive maturity. One of these castes helps the other reproduce, such that one caste has a higher mean lifetime reproduction than the other. This definition does not mention overlap of generations, and does not limit one caste to a relatively low reproductive output. In the wake of the varying criteria used to define eusocial societies, Wcislo (1997a) suggested a "define as you go" approach, where the researcher should define eusociality explicitly in terms of each organism and system of interest, and categorize these organisms in terms of the research questions you are attempting to answer. In light of this, I will, in this thesis, define

eusociality as Crespi and Yanega's (1995) definition: eusocial societies must contain distinct behavioural castes, with one caste having a lower mean lifetime reproduction than the other.

In 1964, Hamilton provided a theoretical solution for the evolution and persistence of altruistic and largely sterile individuals within populations. A gene can persist over time in two main ways. The first involves increasing the survival and/or reproduction of the individual that possesses it, known as direct fitness. The second, known as indirect fitness, works by increasing the survival and/or reproduction of relatives, other than direct offspring, that may have a copy of the gene through common ancestry. The combination of a phenotype's direct and indirect fitness is called inclusive fitness. An altruistic individual may lose direct fitness as a consequence of its helping behaviour, but if that loss is offset or exceeded by positive impacts on a relative, then the genes shaping this helping behaviour will persist or increase in frequency in the population through indirect transmission to the next generation (Haldane, 1932; Fisher, 1930; Maynard Smith, 1964). The idea that inclusive fitness can explain the evolution of eusociality is still debated; some researchers indicate that inclusive fitness theory has little value in explaining occurrences in the natural world (Nowak et al. 2010), while other researchers stand firmly behind the theory as they believe it plays a major role in our understanding of the evolution of eusociality (Abbot et al. 2011). Hamilton (1964) presented a simple mathematical representation of this idea:  $r * b > c$ . The degree of relatedness,  $r$ , is the probability that an individual being helped will possess a copy of the gene for helping. In order for altruistic acts to be favored by natural selection, the cost that the helper incurs,  $c$

(number of offspring not produced by the helper because of the helping behaviour), must be less than the benefit (increased offspring production by the receivers due to the helping behaviour) gained to the helper because of the altruistic acts,  $b$ , times the degree of relatedness,  $r$ , between the two individuals. There is no 'altruistic' gene per se, instead the gene considered above shapes helping phenotypes, and furthermore, the expression of the helping genes must be conditional. The gene is turned on in helpers, but not in those that receive help (Charlesworth 1980; Seger 1981). Differences in the physical, nutritional, and social environment of the individual often determine the expression of these genes (Wheeler 1986).

Based on Hamilton's rule, there are two main hypotheses for the evolution of sociality arising from solitary ancestors (Queller and Strassman 1998). The first hypothesis is relatedness centered such that altruistic individuals are more related to their relatives than their own offspring. The second hypothesis is fitness centered, in which altruistic individuals can provide more benefit to their relatives through helping than the benefit their own offspring would gain. Both relatedness and fitness are required for the evolution of altruistic acts, but these two hypotheses state which is the more prominent factor that has driven altruism and sociality to evolve. One relatedness centered hypothesis that is frequently assumed is Hamilton's haplodiploid hypothesis (1964; 1972). Haplodiploidy is a genetic system in which male offspring are produced from unfertilized eggs such that they are haploid, and female offspring are produced from fertilized eggs such that they are diploid. This system creates relatedness asymmetries as illustrated in Table 1.1. Females are more related to their sisters and least related to their

brothers, while males are most related to their daughters since they contribute their whole genome to create female offspring (fertilized eggs) and are not related to their sons at all, which are produced from unfertilized eggs. Since all Hymenoptera (ants, bees, and wasps) are haplodiploid, it was argued that sociality evolved most often in this taxa because sisters that raise sisters over their own offspring are genetically better represented in the next generation. This supposed preference for raising sisters was supported by the fact that workers in the Hymenoptera are all female. Despite the promise of this hypothesis to explain the evolution of eusociality in hymenopterans, it has been shown to contain some unsupported assumptions. In these haplodiploid societies the relatedness values between females is commonly found to be below 0.75 due to multiple main reproductives and multiple matings (Crozier and Pamilo 1996). Also, even with the high relatedness to their sisters, females are the least genetically related to their brothers, meaning that on average these females are not more related to their siblings than to their own offspring (Trivers and Hare 1976). The low relatedness of females to their brothers proved detrimental for Hamilton's haplodiploid hypothesis, so Trivers and Hare (1976) proposed an alternative explanation for the presence of eusociality in these haplodiploid societies. Female workers, when rearing full sisters, should manipulate the sex ratio so that it is biased towards females at 3:1. This ratio brings into balance the relatedness asymmetries engendered by haplodiploidy from a female worker perspective. Queens were predicted to prefer a 1:1 sex ratio, much different from the worker's preference, bringing them into conflict. Thus, Trivers and Hare (1976) predicted that workers should kill their brothers and rear their sisters so that the resulting ratio was 3

females: 1 male. This biased sex ratio was found in a number of eusocial hymenopterans, yet was not found in numerous other eusocial societies (Alexander and Sherman 1977). Also, as stated before, many societies have multiple main reproductive individuals who are usually multiply mated, reducing relatedness, and therefore the 3:1 sex ratio would not be applicable (Alexander and Sherman 1977). These discrepancies suggest that haplodiploidy alone could sufficiently explain the evolution of eusociality (Crespi, 1994; Roux & Korb, 2004).

In 1998, Queller and Strassman proposed two regimes for the evolution of sociality originating from escaping the effects of predation (life insurance) and escaping predation itself (fortress defense). Life insurance evolved from ancestral parental care and can be seen in ants, bees, and wasps. These societies forage outside the nest where the risk of encountering predators is relatively high. An overlap of generations and helping at the nest ensures that the brood will be raised when adults and parents die as a result of either a short life span in the adult stage or predation while foraging. On the other hand, altruistic acts in the fortress defense situation evolve when the individuals are limited by colony size (the insects live with a finite amount of available space) and resources, and live in a defensible nest site (Andersson 1984; Alexander et al. 1991; Crespi 1994). Even though most social insects live within nest sites, those in the fortress defense situation have very limiting space (such as confined within a gall) which therefore limits the number of individuals produced. Also, these insects have a limited food supply, which can cause competition among the individuals within a nest. This situation is found in termites (Thorne et al. 2003), wood beetles (Kent and Simpson 1992), galling aphids

(Aoki 1987; Stern and Foster 1996), gall-inducing thrips of Australia (Crespi 1992), and even two species of mole rats (Sherman et al. 1991) and sponge-dwelling shrimp (Duffy 1996).

One method of examining the evolution of social systems involves comparisons of common decision points of reproductive and non-reproductive behaviours that individuals face throughout their lifetimes (Emlen and Wrege 1994; Emlen et al. 1995; Cahan et al. 2002). The paths that individuals take in different taxa can then be compared to determine how social decisions change over evolutionary time.

Even with the level of cooperation and self-sacrificing behaviour seen within these social colonies, they are not free of conflict. These social systems in which helping behaviour is prominent are vulnerable to invasion by selfish individuals, or cheaters (Haldane 1932; Maynard Smith 1964; Alexander 1974; Dawkins 1976). Haldane (1932) first recognized that altruistic populations are subject to invasion by selfish alleles which would be likely to spread. Dawkins (1976) even went so far as to assert that altruistic populations are unstable because of the inherent benefit of cheating in which selfish individuals gain all the benefits of the other helping individuals without exerting any costs. Even in completely clonal eusocial societies, such as the galling aphids, where cooperation would be expected to be at an all time high due to complete relatedness ( $r = 1$ ), clonal mixing has been found. The galling aphid species *Pemphigus obesinymphae* possess altruistic soldiers who slow their development and stay in the first instar stage, and sacrifice themselves to defend the gall against invaders, and also perform tasks such

as cleaning and repair of the gall (Benton and Foster 1992; Abbot et al. 2001). Abbot and colleagues (2001) have shown that soldiers of this species of aphid infiltrate galls of unrelated aphids, and furthermore, once inside the non-natal gall, the aphid soldiers forego defense and accelerate their development into the reproductive stage. These aphid soldiers have a condition-dependent altruistic strategy; in their natal gall these soldiers benefit from self-sacrificing defensive behaviours, but in a non-natal gall with unrelated aphids these soldiers should reap the benefits of the defensive brood already present and develop into reproductives as quickly as possible (Foster 2002). Even with this clonal mixing and cheating behaviour, sociality and soldier behaviour is maintained within this species.

Research into the evolution and maintenance of eusociality has been largely centered on the Hymenoptera, but recently has started to shift focus to more disparate taxa such as social aphids (Aoki 1987; Ito 1989) and the gall-inducing thrips of Australia (Crespi 1992a; Mound and Crespi 1995). The gall-inducing thrips of Australia is a recently evolved eusocial lineage, that evolved approximately 6 million years from the present, (McLeish and Chapman 2007). In contrast, the origin of sociality in the ants, bees, and wasps is much more ancient, for example, eusociality in ants evolved, more than 80 million years ago (Hölldobler and Wilson 1990; Bourke and Franks 1995) and that of the termites at approximately 100 million years ago (Andersson 1984). Given its relatively recent evolution, gall-inducing thrips are proving to be very useful in adding to the comparative database for the evolution of sociality since within this clade exists closely related solitary species, allowing for informative comparisons.

## 1.2 Gall-inducing thrips

The order Thysanoptera contains approximately 5,500 described species of thrips (Crespi et al. 2004). Thrips, which have a cosmopolitan distribution, are on average 1 to 3 mm in length, have fringed wings, and possess piercing-sucking mouthparts (Mound and Walker 1982; Kirk 1996). Thrips exhibit a variety of life styles, with the more primitive thrips feeding on fungal matter and the more advanced thrips feeding on leaf cells (using their piercing-sucking mouthparts), with few predatory species (Kirk 1996). Many thrips species, known as thunderbugs, are pests on crops such as wheat and are widely studied due to their ability to transmit pathogenic viruses in plants (Kirk 1996). Thrips possess bladder-like terminal pads at the end of their legs, termed aroliums that helps the thrips adhere to surfaces. All thrips are haplodiploid (in depth explanation in section 1.1), where unfertilized eggs develop into male offspring, and fertilized eggs develop into female offspring. This genetic system creates relatedness asymmetries as described in section 1.1 and shown in Table 1.1.

Several origins of the ability to alter plant growth into a protective domicile exist within this order, with one of the origins of galling represented in Australia on host trees of the genus *Acacia* (Crespi et al. 2004). This genus of plants is the most speciose in Australia, and thrips are found associated with the Juliflorae and Plurinerves divisions (Crespi et al. 2004). A single origin of 10 million years before the present has been inferred regarding the evolution of the Australian gall-inducing habit (Morris et al. 2002) (McLeish and Chapman 2007). These gall-inducing thrips of Australia belong to the

genus *Kladothrips*, a diverse group that contains several life history strategies (Morris et al. 2001). These thrips live in an arid climate in which protection from desiccation and a dependable food source are essential for survival. Because of this, these thrips have an intimate relationship with *Acacia* trees and the ability to create a domicile that provides both protection and nutrition. A single female, known as a foundress, induces a gall on a growing phyllode (modified petiole serving as a leaf) by a mechanism that remains unknown; it is plausible that the thrips delivers a stimulus in which the plant responds by creating a gall, as happens in other galling insects (Ananthakrishnan 1984; Shorthouse and Rohfritsch 1992; Ananthakrishnan and Gopichandran 1993; Williams 1994; Mound and Kranz 1997). A gall, once fully formed, is known as a 'factory fortress', since it provides the necessary food and shelter for the enclosed foundress and her subsequent brood (Crespi 1994; Queller and Strassmann 1998; Chapman et al. 2002). The galls formed by *Kladothrips* are of interest to a kleptoparasitic genus of thrips, *Koptothrips*. *Koptothrips* are specialist invaders of *Acacia*-thrips galls that drive out or kill the inhabitants, producing their own brood within the gall (Crespi 1992a, 1992b; Crespi and Abbot 1999). These kleptoparasites cannot form their own gall, so this infiltration is vital to the life cycle and perpetuation of *Koptothrips*.

Three different life strategies have developed as a response to the invasion pressures presented by *Koptothrips*. One lineage of gall-inducing thrips, known as 'hidlers', produce small, tightly sealed galls in the arid regions of Australia (Chapman et al. 2008). These galls are fairly secure against the invasion pressures of *Koptothrips*. The second lineage of the gall-inducing thrips, known as 'fliers', produce large, diverse galls and

have a short within-gall phase with a high fecundity across species (Chapman et al. 2008). Even with this high throughput life strategy, *Koptothrips* invaders are abundant in this lineage (Crespi and Abbot 1999).

The last lineage, known as 'fighters', consists of the gall-inducing thrips species that have a caste of soldiers and two species that appear to have lost soldiers (Crespi et al. 2004; Chapman et al. 2002). In total, seven species of Australian gall inducing thrips have evolved this soldier morph and have small brood sizes, large eggs, and they create long-lived, small galls, with a high surface area to volume ratio (Crespi 1992a; Crespi and Mound 1997; Crespi et al. 1997a, 1997b; Crespi and Worobey 1998; Kranz et al. 1999, 2001a, 2001b; Willis et al. 2004). In these species, the foundress lays two sets of eggs: the first brood develops into soldiers of both sexes and the second brood develops into the dispersing caste that, like the foundress, will leave their natal gall to initiate their own gall (Crespi 1992a). These soldiers have a pale exoskeleton, reduced antennae, reduced or absent wings, and most importantly, enlarged femurs in comparison to the dispersing caste (Mound, 1971; Crespi, 1992a; Mound and Crespi, 1995; Mound et al., 1996; Crespi and Mound, 1997; Kranz et al., 1999). With the use of these enlarged femurs, the soldiers defend their gall by grasping and repeatedly squeezing the invading *Koptothrips*. These soldiers are not completely sterile but still exhibit varying degrees of reproductive output that is negatively correlated with fighting ability. In addition to dispersers produced by foundresses, there is a second generation within galls produced by the soldiers. These individuals have the same morphology as the dispersers produced by the foundress. Soldiers in some species are highly fecund and contribute significantly to

this second generation of dispersers, yet in two species they show significantly reduced reproduction compared to the foundress and are, incidentally, the most effective fighters (Chapman et al. 2002). The eusocial species vary in a number of life history traits such as the number of soldiers and dispersers produced per gall, the relatedness and inbreeding levels within a gall, and the sex ratio of each caste (Crespi et al. 2004).

This origin of a helping caste in the form of thrips soldiers is seemingly distinct from that of workers in the Hymenoptera. The difference between these trajectories may be due to the difference in the type of helping behaviour: defending a gall versus foraging and rearing a brood. The episodic nature of defending a colony and the difficulty that unarmed individuals may face in encountering aggressive and potentially armed (morphological specializations for fighting) individuals are thought to have led to a weaker trade-off between helping and reproduction for soldiers (Chapman et al. 2002). Foraging helpers, on the other hand, immediately suffer the loss of reproductive opportunities due to the much greater time commitment that foraging for and nursing conspecifics demands. Therefore, soldiers in the termites and aphids were predicted to have undergone a similar evolutionary trajectory to that of thrips (Chapman et al. 2002). Some evidence has been put forward that supports this claim within the termites (Thorne et al. 2003). Early termite soldiers' life time reproductive output may have been similar to queens and kings, as these defenders may have remained in the colony as 'hopeful reproductives'. As well, some aphid soldiers retain the ability to molt into the reproductive form (Foster 1990) and, as such, these soldiers remain totipotent and could be representative of an earlier stage of soldier evolution (Stern and Foster 1997). The

gall-inducing thrips have been an important addition to our comparative database when examining origins of sociality, and further study will increase the importance of this group as a 'model clade'. Investigations centered on closely related solitary species, lineages that have lost sociality (Crespi and Yanega 1997) and those that show flexibility in social form (Schwarz et al. 2007), are important to refining our general understanding of the evolutionary advancement of sociality. In this thesis, I focus on one gall-inducing thrips species that may exhibit the evolutionary advancement of sociality.

Subsequent to the single origin of soldiers, there have been two evolutionary losses (Crespi et al. 2004, McLeish and Chapman 2007). Both losses seem to coincide with a shifting in *Acacia* host lineages (Crespi et al. 2004). A third *Acacia*-thrips lineage that is also thought to have shifted host lineages is *Kladothrips intermedius* Bagnall (Crespi et al. 2004). Soldiers remain in this lineage, but this fighting caste is different from the others: soldiers are partially winged and the caste is completely absent in a small minority of galls. There are also notable sex differences within the soldier caste in this species: male soldiers have a wing length more like the dispersing caste and are generally smaller with slimmer femurs, while the female soldiers possess smaller wings with more robust femurs (Mound 1970, 1971a; Crespi and Vanderkist 1997). Combined, these characteristics have led Crespi and colleagues (2004) to suggest that the soldier caste in *K. intermedius* may be less committed to defense than the castes of other species.

### **1.3 Objectives**

Here I address four main questions using *K. intermedius* as a focal species do: (1) male and female soldiers differ in their willingness to engage invaders in fights and in their effectiveness in killing invaders, (2), morphological characters (wing length, body and femur dimensions), within a sex, correlate with willingness to fight and success in killing invaders, (3) male and female soldiers enter intact galls, and (4) male soldiers display a preference for mating with their sisters versus unrelated females? The first question was addressed through the use of a behavioural assay originally developed by Perry and colleagues (2004). I have slightly modified this assay to directly assess any defensive differences present between the male and female soldiers of *K. intermedius*. Then, I collected several morphological measurements from the soldiers involved in these assays in order to address the second question, relating fighting behaviours to morphological measurements. It has already been known that the wing length is negatively correlated with femur size in female soldiers of this species (Crespi 1992a), but the relationship between morphology and fighting has not been assessed. The third question was addressed by placing male and female soldiers, identifiable by a dot of paint on their pronotum, within a modified Falcon tube with an unopened gall, allowing the soldier the opportunity to infiltrate this gall. Lastly, marked soldiers were placed in assays with either their gall mates or unrelated thrips and observed for the occurrence of mating.

The rest of this thesis will, in detail, address the four main objectives. Chapter two of this document will explain the methodology required for these assays while Chapter three will discuss the results obtained. Lastly, Chapter four will provide a

discussion of the consequences of the outcomes outlined in Chapter three, and place the results in light of the suggested social trajectory by Cahan and colleagues (2002).

Table 1.1. Relatedness values found in a haplodiploid genetic system where females are produced from fertilized eggs and males are produced from unfertilized eggs. Males are most related to their daughters, and are not related to their sons at all. Females are most related to their sisters and least to their brothers.

Sex	Daughter	Son	Sister	Brother
Female	0.5	0.5	0.75	0.25
Male	1.0	0	0.25	0.5

## Chapter Two – Methods

### 2.1 Collections and Processing

The specimens used in all behavioural assays were collected on January 22-25, 2010 from the host tree, *Acacia oswaldii*, near Middleback sheep station (137°23'E 32°57'S). The Middleback homestead, which is almost 1000 km<sup>2</sup>, is located about 400 km NW of Adelaide, South Australia. This area has a semi arid climate, with low amounts of rainfall and high summer temperatures ([www.bom.gov.au/climate](http://www.bom.gov.au/climate)). *Acacia oswaldii* has a sparse distribution on the station, and the distribution of galls on these trees is episodic; not every host tree has galls. Accordingly, GPS coordinates were used to locate host trees that had galls present during a study in April of 2009. Galls were collected from a total of three host trees, with the majority of the collections coming from one large host tree (137° 16' E 32° 51'S). Galls were stored on ice until returned to Flinders University campus (Adelaide) where the behavioural assays were conducted.

Galls were cut longitudinally along the ostiole with a scalpel, and then pried into two separate halves. The ostiole is the area where the plant tissue of the gall joins after invagination through some level of cellular adhesion, but it is still seen as a visible line in the gall's integument. With the use of a fine paintbrush, thrips were transferred to a petri dish to view the specimens under a dissecting microscope. Individuals were identified as either host (*Kladothrips intermedius*) or invader (*Koptothrips* sp.) and placed in 1.5 ml centrifuge tubes. The contents of each gall were recorded, noting the number of soldiers (adults), number of dispersers (adults), number of larvae, and presence of *Koptothrips* species. In preparation for the behavioural assays, adult soldiers and *Koptothrips* were

sexed by examining the sculpturing of the last few abdominal segments, where male thrips have pointed terminal abdominal segments and females are more rounded as seen in Figure 2.1.

## 2.2 Fighting assays

Perry and colleagues (2004) developed a behavioural assay to assess the fighting ability of soldiers in two species of social thrips (*K. intermedius* and *Kladothrips habrus*) against their natural enemies, *Koptothrips*. A gall-defender and a gall-invader were placed in 200  $\mu$ L PCR tubes, approximating the average size of a gall across species. The interactions between these individuals were not instantaneous, so the trials were allowed to continue for up to 40 hours. Consequently, the interactions between the gall-defenders and the gall-invaders typically were not directly observed. Instead, each trial was periodically checked to determine if the thrips remained mobile or if they had become moribund (i.e. unable to stimulate movement using the bristle of a fine paintbrush). The original protocol was modified so that the sex of the soldiers was known before placement in a trial. One female soldier was placed with either a female or a male *Koptothrips*, and the same arrangements were set up for male soldiers. Trials were allowed to continue for 40 hours, and checks were made every 8-12 hours (4 checks in total). Outcomes for these trials were recorded as: soldier and *Koptothrips* mobile (alive) and only soldier or only *Koptothrips* moribund (presumed killed). After the 40-hour trials were completed, 90% ethanol was added to each PCR tube to preserve the specimens for later analysis (see below).

### 2.2.1 Morphological Measurements

The post-battle soldiers (preserved in 90% ethanol) were placed dorsal side up on a microscope slide using a paintbrush. A cover slip, placed on top of the soldier, was gently pressed flat with the end of the paintbrush (exerting approximately equal pressure for each soldier) to achieve maximal focal range. Sufficient care was taken in the process of mounting these soldiers under a cover slip such that the specimens suffered no damage or breakage. An ocular micrometer was calibrated, at 60 X magnification, to millimeters using a stage micrometer, such that, measurements of the soldiers could be taken in metric units. The length of each thrips from the top of the head, between the first antennal segments, to the end of the abdominal tube (body length) was then determined under 60X magnification using this arrangement. Body length was used to set the scale of the measurements in millimeters, so that the other morphological measurements that were taken (see below) could be obtained in metric units. To minimize systematic error in our measurements (Arnqvist and Martensson 1998), three digital pictures were taken of each thrips (dorsal side only) using a *Motic 2.0* digital camera mounted on the dissecting scope. The entire specimen mounting procedure could not be repeated for each image, because removing the cover slip frequently results in breakage of the specimen. For each photo, the mounted thrips was removed from the microscope stage, replaced, and then re-focused. These digital images, combined with the freeware Image J (<http://rsb.info.nih.gov/ij/>, Rasband W., NIH), were used to make the following measurements: femur length, femur width, pronotum length, pronotum width, and wing length (body length, as described above was used to calibrate these measurements). The

femur length and width were taken at their maximums, while pronotum length was measured along the central axis of the pronotum, and width was taken as the widest posterior portion of the pronotum. Wing length was measured from the most proximal portion of the forewing, where it meets the clavus, to the most distal edge of the forewing. The averages of the three sets of measurements for each specimen (from the three photos) were used in the statistical analyses described below. In order to decrease the possibility of an observer bias, measurements were taken several weeks after the behavioural trials were completed, so the specimens were measured blind to outcome of the trial (all trials were identifiable by number only, and were selected randomly for measurements). All images and measurements were taken using the same microscope and digital camera throughout.

Repeatability (R) of each of the six morphological measurements was calculated using equation 2.1:

$$R = \frac{S^2A}{S^2W + S^2A}$$

where  $S^2A$  represents among individual variance (naturally occurring) and  $S^2W$  represents within individual variance (measurement error) (Arnqvist and Martensson 1998).

### **2.3 Infiltrate Gall Assays**

In an attempt to directly test the possibility of movement of these soldiers into non-natal galls, this assay consisted of placing individuals (male soldier, female soldier, *Koptothrips*) into a modified 15 mL Falcon tube with an unopened gall. These modified Falcon tubes were cut off approximately 2 cm above the cap, and this was then sealed with a fine mesh to allow airflow through the tube. The soldiers were marked by placing a small dot of Testor's Enamel paint on the pronotum of the thrips, as seen in Figure 2.2. This mark allowed the identification of the soldier from the natal brood if the soldier did indeed enter the gall in the assay. *Koptothrips* individuals were not marked since they are easily distinguishable from *K. intermedius* and they usually occur at low rates in populations, such that the gall used was unlikely to contain a full brood of *Koptothrips*. The tubes were placed in a plastic container with damp tissue placed along the bottom to prevent desiccation of the galls. Assays were checked twice over the span of 48 hours. During these checks, if the individual placed in the tube could not be located, the gall was then bisected and the contents examined to locate the individual of interest, confirming infiltration. Once the gall was bisected, a general census of the contents was conducted, noting contents of the brood (*K. intermedius* full brood, *Koptothrips* present, etc.) and the state of the individuals inside; were the majority of individuals alive or moribund? An assay was stopped if the individual of interest was declared moribund.

#### **2.4 Mating Assays**

In May of 2009, galls collected in April 2009 from Middleback field station were bisected and individuals were moved into a petri dish using a fine paintbrush. A male

soldier was selected, and was marked with Testor's Enamel paint, using the same method as described above in section 2.3. The frequency of mating of these marked individuals was tested under two conditions: the male soldier among their natal gall-mates and among non-natal individuals. In the natal gall trials, a soldier was selected, marked and then placed back in the petri dish with its brothers and sisters. In the non-natal gall trials, a soldier was selected, then another gall was bisected and the marked soldier was placed with the unrelated brood. The number of male and female soldiers present was recorded for the natal gall and the non-natal gall (if they were placed with an unrelated brood) condition. This set up was viewed using a Leica© DFC420 digital camera mounted on a Leica© MZ95 dissecting microscope, for up to a period of 15 minutes.

This set up was modified in February 2010 (Figure 2.3) such that a gall was bisected, and the half gall was mounted in plasticine, which was then placed within the bottom half of a petri dish. All but the soldiers (including the marked individual) were removed from the half gall; when larvae and dispersers were left within the half gall, it was extremely difficult to keep track of the marked individual and its behaviours. Once the gall was mounted with the individuals inside, the top half of the petri dish was placed on top of the half gall to prevent individuals from escaping. The two conditions (natal and non-natal) were tested using this set up. This set up was viewed under a Dinolite© (AnMo Electronics Corp.) digital microscope, for up to a period of 15 minutes.

Mating of thrips is observed when a male mounts a female, wrapping his abdomen around the female's abdomen (see Figure 2.4), upon which the aedeagus of the

male enters the genital pore of the female. Frequency of mating was observed in each of the conditions.

## 2.5 Statistics

Independent-samples t-tests were carried out to compare the morphological measurements between male and female soldiers. This analysis was also conducted to compare the male and female soldier's mean morphological values between assays in which all individuals were alive or one individual was moribund (without specifying if it was a soldier or invader), and when a soldier or an invader was declared moribund.

Chi-Square tests were used to determine if there were significant differences in a male and female soldier's fighting willingness (either of the two combatants became moribund) and fighting effectiveness (the soldier was alive and the *Koptothrips* was moribund).

Binary logistic regressions were executed separately for each sex to explore the possible role of various morphological dimensions for predicting the willingness of a soldier to engage in a fight, and then their ability to win that fight.

The mean squares values needed to calculate repeatability were obtained from a one-way repeated measures ANOVA. All analyses were done with the program PASW version 18.

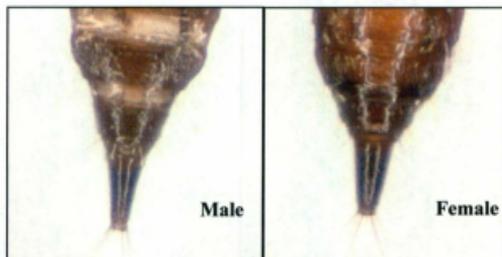


Figure 2.1. The last few abdominal segments of a male and female soldier of *Kladothrips intermedius*. The males have pointed terminal abdominal segments (on the left) while the females are more rounded (on the right).



Figure 2.2. A male soldier of *Kladothrips intermedius* that was marked for a behavioural assay by applying Testor's Enamel paint to the pronotum to make it distinguishable from the other thrips present in the assay.



Figure 2.3. A half gall set in plasticine within a Petri dish where soldiers of *Kladothrips intermedius* were placed inside for the purposes of observing mating. One male soldier was marked by placing brightly colored paint on its pronotum – this individual is seen near the center of the gall and is indicated by the arrow.



Figure 2.4. Soldiers of *Kladothrips intermedius* during copulation. The male thrips mounts the female and wraps his abdomen around the female's abdomen where the aedeagus enters the genital pore of the female.

## Chapter Three – Results

### 3.1 Gall data

Out of the three *Acacia oswaldii* from which galls were collected, only one tree had galls containing full broods, which have all life stages and castes of *Kladothrips intermedius* present. The galls processed from one of the other host trees were inhabited solely by a foundress with eggs (78 out of 78 of the galls processed). The last host tree had 88% (167/190) of the galls containing a foundress with eggs and the other 12% (23/190) of the galls were host to a brood of *Koptothrips*. Accordingly, only galls from the host tree with full broods were used in the assays. The following information is focused on this one host tree that contained full broods, with a general summary of the population structure of this tree. Approximately 56% of the galls had a full brood consisting of adult soldiers, dispersers, and few larvae. These galls are in the late stages of the life history, where the dispersers are about to leave the gall to initiate their own gall. Galls in the earlier stages of the life history, with a live foundress, soldiers and larvae, were present in approximately 15% of the galls processed. *Koptothrips dyskritus* had an invasion rate of about 12% on this tree, while no *Koptothrips flavicornus* were found; therefore all assays utilized the species *Koptothrips dyskritus*.

### 3.2 Fighting Assays and Morphological Measurements

The morphological measurements of the soldiers had repeatability values near 1 (Table 3.1), meaning the variation seen in the measurements was mainly accounted for by naturally occurring among individual variation, not within individual variation, or systematic measurement error.

Female soldiers were found to be significantly larger ( $\bar{x} = 1.468$ ,  $SD = 0.142$ ) than the male soldiers ( $\bar{x} = 1.316$ ,  $SD = 0.130$ ;  $t(215) = -8.211$ ,  $p < 0.001$ , two-tailed). Despite the female soldiers' general larger size, the average wing length of males was significantly greater ( $\bar{x} = 0.619$ ,  $SD = 0.049$ ) than that of the females ( $\bar{x} = 0.534$ ,  $SD = 0.123$ ;  $t(142.076) = 6.657$ ,  $p < 0.001$ , two-tailed; Figure 3.1).

Chapman (2003) created an inclusive fitness model which took into account reproductive opportunities and genetic factors to help explain the evolution of the soldier caste in the gall-inducing thrips. This model suggested that in *K. intermedius* the female soldiers are more inclined to defend their colony than the male soldiers. Therefore, this apparent lack of defensive commitment in the male soldiers should be reflected in their morphology, as seen in their longer wings and less robust femurs as compared to the female soldiers in *K. intermedius*. A total of 217 assays were examined, with 109 *K. intermedius* female soldier trials and 108 male soldier trials. No significant difference was found between female and male soldiers of *K. intermedius* – female soldiers did not fight more often than male soldiers ( $X^2=1.466$ ,  $df=1$ ,  $p = 0.262$ ), nor did they win more battles than the males ( $X^2=2.564$ ,  $df=1$ ,  $p = 0.116$ ; Fig. 3.2).

The femur width of female soldiers involved in assays in which both individuals were alive was significantly larger ( $\bar{x} = 0.138$ ,  $SD = 0.011$ ) than the femur width of female soldiers in assays where the soldier or the invader was found moribund ( $\bar{x} = 0.134$ ,  $SD = 0.011$ ;  $t(107) = 2.044$ ,  $p = 0.043$ , two-tailed). The wing length of the male soldiers in assays in which both individuals were alive was significantly longer ( $\bar{x} =$

0.634, SD = 0.032) than that of males in assays where the soldier or the invader was found moribund ( $\bar{x}$  = 0.612, SD = 0.055;  $t(102.897) = 2.593$ ,  $p = 0.011$ , two-tailed). The mean body length of female soldiers that defeated an invader in an assay was larger ( $\bar{x}$  = 1.528, SD = 0.201) than that of the females who were killed by an invader ( $\bar{x}$  = 1.427, SD = 0.051;  $t(31.009) = -2.644$ ,  $p = 0.013$ , two-tailed). Female soldiers that killed an invader had a shorter average wing length ( $\bar{x}$  = 0.476, SD = 0.142) than those who were killed by an invader ( $\bar{x}$  = 0.560, SD = 0.117;  $t(62) = 2.598$ ,  $p = 0.012$ , two-tailed). Male soldiers who defeated an invader were significantly larger ( $\bar{x}$  = 1.398, SD = 0.207) compared to the male soldiers who were killed ( $\bar{x}$  = 1.278, SD = 0.079;  $t(25.030) = -2.692$ ,  $p = 0.012$ , two-tailed).

Chi-Square analyses revealed that male and female *Koptothrips dyskritus* are just as likely to partake in a fight with *K. intermedius* soldiers ( $X^2=0.409$ ,  $df=1$ ,  $p=0.551$ ), and exhibit no significant differences in their ability to defeat these soldiers ( $X^2=0.048$ ,  $df=1$ ,  $p=0.854$ ). Since there was no detectable difference in male and female *Koptothrips dyskritus* fighting behaviours, the analyses did not take into account sex of the invaders.

Sexes of the soldiers were examined separately using binary logistic regressions to determine if the morphological measurements could predict the outcome of the assays (soldier or *Koptothrips* is discovered as moribund, or the soldier is alive and the *Koptothrips* is moribund). Male soldiers with shorter wings resulted in more moribund individuals (either the male soldier or the invader) than those with longer wings. While no other morphological measure significantly predicted the outcome of the battles for

male soldiers, femur width, body length and wing length were all found to be of significance for female soldiers. Female soldiers with a smaller femur width were found more often in assays with moribund individuals than those with a larger femur width. The more effective (soldier alive, invader moribund) female soldiers had a larger body size and shorter wings (Table 3.2).

### 3.3 Infiltrate Gall Assays

A total of 19 assays were conducted using female *Koptothrips dyskritus*. These female invaders were found to enter a gall 26% (5/19) of the time. After an analysis of the contents of the gall for each of these 5 times, it was discovered that the invader was moribund within the gall, yet three of these assays had a live *K. intermedius* brood present within the gall, and the other two assays the brood was moribund as well. Presumably, the soldiers had defeated the invader after infiltration of the gall in three of these assays. Out of 42 male *K. intermedius* soldier trials, two males (4.8%) were found to enter a gall. In both of these cases, the male soldier was found alive within the gall, along with a live resident *K. intermedius* brood. For female *K. intermedius* soldiers, one female out of 50 entered a gall. In this one case, the female soldier was alive within the gall, but the gall was occupied by a lepidopteran larvae with no resident thrips present (Fig. 3.3).

### 3.4 Mating Assays

In May 2009 a total of 5 trials (3 non-natal and 2 natal trials) were conducted using marked male soldiers to test for any possible differences in mating frequencies of

these male soldiers. The male soldiers were found to mate twice, once in a non-natal trial and once in a natal trial, at 8 minutes, and 10 minutes and 42 seconds after trial initiation, respectively. In both cases, the male soldiers were mating with female dispersers, not female soldiers. During set up of these trials, additional mating was observed with a non-marked male mating with both female dispersers and female soldiers. On one occasion, a male soldier was observed mounting and attempting to mate with a second instar larvae.

In the modified experiment in which a bisected half gall, instead of in a petri dish was used there were 15 trials in total – 14 male soldier trials and 1 female soldier trial. Out of the 14 male soldier trials, five were natal gall trials and nine were non-natal gall trials, while the female soldier was in the female's natal gall. Although the trials had taken place in a more natural set up, no mating was observed. The soldiers would remain very still within the half gall, with little movement or exploratory behaviour.

Table 3.1. Repeatability values of morphological measurements taken from male and female soldiers of *Kladothrips intermedius*. MS<sub>within</sub> and MS<sub>among</sub> were obtained from a one way repeated measures ANOVA in PASW v18; repeatability was calculated using equation 2.1.

Measurement	MS <sub>within</sub>	MS <sub>among</sub>	S <sup>2</sup> W	S <sup>2</sup> A	Repeatability
Body length	0.005	0.156	0.005	0.050	0.909
Wing length	5.209E <sup>-7</sup>	0.047	5.209E <sup>-7</sup>	0.016	0.999
Forelimb length	4.188E <sup>-5</sup>	0.010	4.188E <sup>-5</sup>	3.319E <sup>-3</sup>	0.988
Forelimb width	2.422E <sup>-5</sup>	0.003	2.422E <sup>-5</sup>	9.919E <sup>-4</sup>	0.976
Pronotum length	2.215E <sup>-5</sup>	0.005	2.215E <sup>-5</sup>	0.002	0.987
Pronotum width	2.316E <sup>-5</sup>	0.009	2.316E <sup>-5</sup>	0.003	0.992

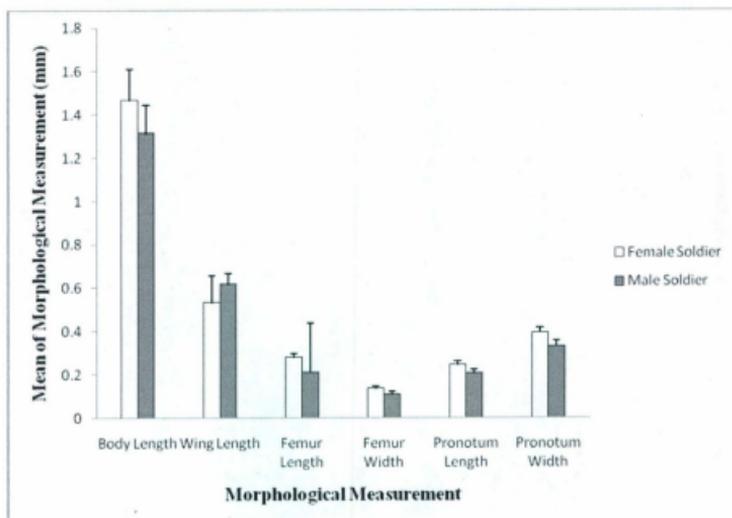


Figure 3.1. The average body length, wing length, femur length, femur width, pronotum length and pronotum width (mm), along with standard deviations, of male and female soldiers of *Kladothrips intermedius* used in the fighting assays. The male and female soldiers were found to differ significantly for all measurements (independent-samples t-test).

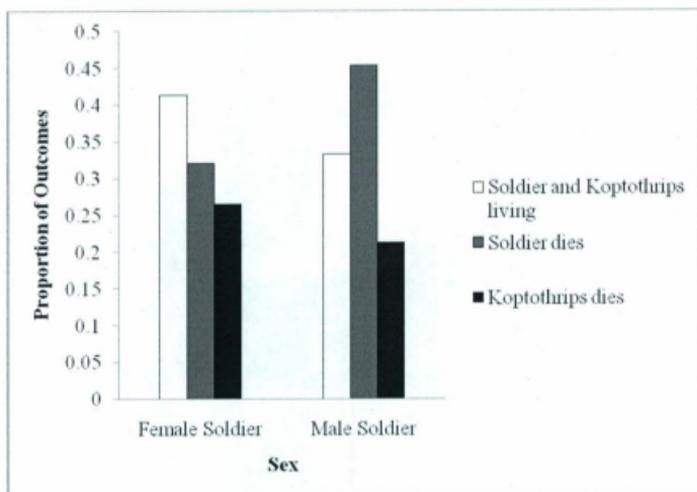


Figure 3.2. The proportion of outcomes for the 217 (109 female, 108 male) fighting assays for the female and male soldiers.

Table 3.2. The results of a binary logistic regression performed to determine if morphological variables could accurately predict the outcome of a fight between a soldier of *Kladothrips intermedius* and a *Koptothrips*; only significant results are reported.

Morphological predictor	Sex of soldier	B	Wald $\chi^2$	p	Odds ratio	95% CI for the odds ratio	Outcome
Wing length	male	-	4.319	0.038	0.000	0.000 – 0.513	Decrease wing length
		11.722					increase odds a fight occurs
Forelimb width	female	-	5.933	0.015	0.000	0.000 – 0.000	Decrease forelimb width
		55.395					increase odds a fight occurs
Body length	female	8.149	4.697	0.030	3460.828	2.787 – 5492301.588	Increase body length
							increase odds of soldier win
Wing length	female	-5.398	5.013	0.025	0.005	0.000 – 0.510	Decrease wing length
							increase odds of soldier win

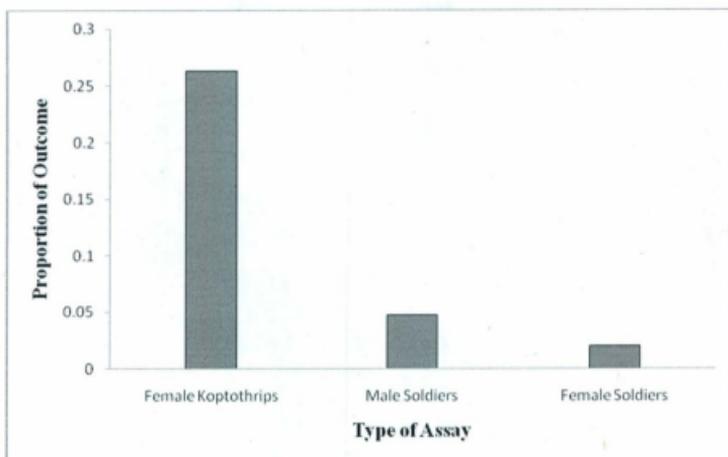


Figure 3.3. The proportion of individuals ( $n = 111$ ) that entered a gall for the female *Koptothrips* and male and female soldiers of *Kladothrips intermedius* involved in the “infiltrate gall assays”.

## Chapter Four – Discussion

### 4.1 Fighting Assays

Direct comparisons of male and female soldiers of *K. intermedius* in this study did not support any differences in their defensive abilities despite their differing morphology. Despite the male soldier's overall smaller size (with the exception of wing length, see Mound 1970, 1971a; Crespi 1992; Crespi and Vanderkist 1997; Figure 3.1), there was no significant difference in the fighting frequency (either soldier or invader moribund) or fighting effectiveness (only invader moribund) of the sexes. This result calls into question the utility of the soldier morphology in defense. If there is an optimal morphology found in these soldiers, natural selection would have had six million years (McLeish and Chapman 2007) to alter the soldier morphology in the direction of the ideal for specialized defense and fighting. Therefore, any variation from this ideal, environmentally or genetically induced, would be selected against. In *K. intermedius*, morphology does vary greatly within a sex, and wide ranges were even noted in the femur, the soldiers' weapon. Even with this wide variation in morphology, male and female soldiers were just as likely to risk a battle with an invader and in a fight were equally effective at dispatching invaders, suggesting that the increase in femur size of the soldiers compared to the dispersing caste is for a purpose other than heightened defensive ability.

What, then, is the purpose of this specialized morphology seen in *K. intermedius* soldiers? Are these morphological changes a consequence of life within a gall? The long occupancy of the gall may contribute to a specialized morphology in the soldiers. Thrips

of the genus *Iotatubothrips* that reside in long lived woody galls (scale of many years) on trees in the genus *Casuarina* produce wing-reduced, permanent gall residents that have short antennae and broad fore femora (Mound et al. 1998). These thrips have been observed to attack kleptoparasitic thrips using these enlarged forelimbs and they also experience variation within this micropterous morph that is related to within-gall density. Since the galls of *Casuarina* are host to many generations of the resident thrips, two strategies arose – a winged, dispersing morph that initiates a gall and a micropterous morph that resides within the gall. It is possible that the longer life span of the galls could, convergently, produce similar strategies in *K. intermedius*, where the soldiers adapt this specialized morphology because they are permanent gall residents. Approximately 15 soldiers are present within each *K. intermedius* gall, but do these soldiers coordinate their defensive efforts? Caravan (2008) conducted a study that involved placing either one or two soldiers against an invader in a 200  $\mu$ L PCR tube (Perry et al. 2004). Since it would be of great benefit to the soldiers and the gall-inhabitants to have coordinated and more effective defense against these invaders, it was expected that the assays with two soldiers would result in more deaths of the invader than those assays with just one soldier. Yet, no evidence was found for cooperation or coordination between pairs of soldiers when battling an invader (Caravan 2008). One possible explanation for a lack of cooperation is that these soldiers are experiencing conflict within the gall. The morphology of soldiers could be a byproduct of this sibling conflict, not a specialization aimed at increased defensive abilities. Being confined to a limited space, enlarged femurs could be used in fighting resident siblings for access to

mates, oviposition sites, or feeding sites. These proposed explanations can be explored further through observations of live soldier interactions in a gall or gall-like environment. Also, a study in 2010 placed 4 soldiers of varying sex ratios (from all male to all female soldiers) against one invader in a tube. This study found no differences in the death rate of the invaders in the different types of assays, even though the assays with all female soldiers were expected to have a higher kill rate than those with all male soldiers (O'Connell 2010). This result again illustrates the lack of cooperation in these soldiers, as well as the lack of specialization of the morphology in the male and female soldiers.

If this morphological variation between sexes doesn't affect fighting ability, is the variation within a sex important? It would seem that natural selection has optimized the soldier morphology differently in male and female soldiers of *K. intermedius*, since their morphologies are drastically different yet they are equally effective in fighting invaders. Male soldiers with shorter wings were engaged more often in interactions with invaders than males with longer wings, suggesting that the longer-winged males are 'actively avoiding' interaction with an invader. Male soldiers of *K. intermedius* may have two options – remain in their natal gall and fight, or disperse and mate elsewhere. These options would mean that the male soldiers with longer wings have dedicated more energy into dispersal and, as such, a battle with an invader becomes riskier than dispersal from the gall. Such a tradeoff between fighting and dispersal is seen in the males of the thrips species *Hoplothrips karnyi* (Crespi 1988). A similar system is also reflected in several species in the aphid genus *Pemphigus*, where the first instar soldiers either stay in their natal gall and defend against invaders, or enter an unrelated colony and accelerate their

development to reproduce while reaping the benefits of defense provided by non-siblings (Abbot et al. 2001; Johnson et al. 2002).

In contrast to male soldiers, assay outcomes of females were significantly associated with wing length, but additionally, femur width and body length were important morphological predictors of assay outcome. Females with shorter wings and longer bodies were more effective than those with longer wings and a smaller body size, killing a higher number of *Koptothrips dyskritus*. Surprisingly, females with a slimmer femur were more likely to have fought a *Koptothrips* (winning or losing), the opposite outcome of what one would predict if the robustness of the limb is directly associated with fighting behaviour. The tradeoff between flight and reproduction in female insects, where wingless females have an increased fecundity, has been found in many taxa: crickets (Tanaka 1976, Roff 1984), aphids (Dixon 1972, Wratten 1977, Walters and Dixon 1983), planthoppers (Kisimoto 1965, Denno et al. 1989, Denno 1994), water striders (Anderson 1973, Zera 1984), corixids (Young 1965), and pea weevils (Utida 1972). The relation of fighting abilities and morphology in the female soldiers of *K. intermedius* could reflect a similar tradeoff in which the female soldiers have dedicated more energy to reproduction resulting in longer bodies and less developed wings and forelimbs. This investment may select for female soldiers to be more dedicated and/or efficient in fighting since they are protecting their own offspring, and, therefore, protecting their their own fitness investment.

If this morphology within the soldier caste doesn't affect fighting effectiveness, then does it affect defensive abilities among castes, in which the morphological differences are even more pronounced? The assays in which this study was based (Perry et al. 2004) concluded that morphology of the caste does affect fighting ability in *K. intermedius*. In the study (Perry et al. 2004), comparison of fighting behaviour between the soldier and the foundress could only successfully be determined in *K. intermedius* and *K. habrus* since it is difficult to find a live foundress once the soldiers are eclosed. Because of this, the foundresses used in the present study were near the end of their life cycle and, therefore, would have a harder time defending against any *Koptothrips* individuals; whereas the soldiers were young, and most likely at the peak of their defensive abilities. The timing of the development of soldiers, just as the foundress is unable to ward off invaders, ensures that the gall is now protected by these young, vigorous soldiers. With an average of 15 soldiers per gall (Crespi et al. 2004) and only a few male soldiers present, who are still equally effective as the females once engaged in a fight, the galls of *K. intermedius* are well-protected once the foundress is no longer living. Even if male soldiers can leave the gall and use their longer wings to disperse and mate elsewhere, or possibly even walk between galls, the loss of the males would not be detrimental to protection of the gall, due to the highly female biased sex ratio (Crespi 1992). Ultimately, this means that the soldier caste in at least this one species of eusocial thrips is a behavioural one in which the timing of development and the decision to remain within the gall defines the caste more than does morphological specialization. This trait may be a byproduct of this species experiencing a host shift. The two other thrips species

that have undergone a host shift have lost soldiers (*K. xiphius* and *K. rodwayi*; Crespi et al. 2004, McLeish and Chapman 2007). It is possible that *K. intermedius* is in the process of losing the soldier caste, explaining the lack of morphological specialization. Similar work, as in this study, needs to be conducted on the other eusocial species that do not have the complication of a host shift. This work would help to determine if the soldier caste of all 7 species is defined by behavioural specialization only, or if morphological specialization is important in all eusocial thrips with the exception of *K. intermedius*.

#### 4.2 Infiltrate Gall Assays

The female *Koptothrips dyskritus* used in this assay served as a control, since it is known that it is the female *Koptothrips*, sometimes accompanied by a male (Gonsalves 2010), that invade the galls of the host thrips (Crespi and Abbot 1999). If movement of these female invaders occurred into the unopened galls within the assays, then the assays were deemed sufficient in detecting and allowing movement into these unopened galls.

It was found that the female invaders did indeed infiltrate the galls in the assays. The invader was always found to be moribund within the gall, but a live *K. intermedius* brood was present in some of the galls (in other assays both the *K. intermedius* brood and the female *Koptothrips dyskritus* were moribund within the gall); presumably the soldiers had successfully detected and defeated the invading individual.

The male soldiers of *K. intermedius* were found to enter a gall 4.8% of the time, or 2 out of 42 assays. It was found that the male soldier was alive, residing within the foreign gall among the resident brood. The fact that the male soldier could infiltrate the

gall and successfully reside within it without conflict may mean that *K. intermedius* lacks the ability to detect non-kin. The resident females within these galls might be more interested in mating with these intruder male soldiers – e.g., the soldier was fit enough to brave the harsh environment and make his way into a gall. On the other hand, it would be beneficial for the resident male soldiers of these galls to find and dispatch this male soldier since it is unlikely that he will participate in any altruistic defensive behaviour. Kin recognition is important in eusocial societies because altruistic acts should be directed towards close relatives to increase inclusive fitness (Hamilton 1964; Holldobler and Wilson 1990; Holmes 2004). Kin recognition, or kin discrimination, has been documented in the many social societies, including: a subsocial desert isopod (Linsenmair 1987); a social huntsman spider (Yip et al. 2009); and many eusocial Hymenoptera (Greenberg 1979; Gadagkar 1985; Michener and Smith 1987; Breed and Bennett 1987; Chapuisat et al. 2005, etc.). Although kin discriminative behaviours are widespread across these social taxa, some groups still lack this ability, making them vulnerable to invasions. One such group is the eusocial aphids, who cannot recognize clone mates (these gall-living species are clonal) or close relatives (Foster and Benton 1992; Miller 1998; Shibao 1999), and clonal mixing is seen in these aphids (Abbot et al. 2001; Johnson et al. 2002). For example, the species *Pemphigus obesinymphae*, produces first instar soldiers but in addition uses what Abbot and Chhatre (2007) coin as an “intruder strategy” in which nymphs invade and exploit unrelated clonal galls without resistance from the non-relative soldiers within the unrelated gall (Abbot et al. 2001). These intruders invade these galls by simply walking along the branches of their host tree

and once inside do not contribute to defense, but instead accelerate their development into the winged life stage of the aphids that then travels to another host plant. It is possible if these thrips also lack kin recognition abilities, the soldiers of *K. intermedius* may be using this intruder strategy in the same way as the aphids. The male soldiers could simply walk to another gall on a branch, or use their longer wings to disperse to another gall, enter, and mate with any females once inside without experiencing any confrontation from the resident soldiers.

Desiccation problems with this assay may have caused the rate of movement into the galls to be underestimated. The galls were found to dry out within the tubes, causing the ostiole to separate, as seen in Figure 4.1. Once the ostiole separates enough, the inner brood is exposed and was found to spill out into the tube. There were several assays in which individuals (*Koptothrips*, and broods of *K. intermedius* including larvae, dispersers, and soldiers) were found outside the gall – both alive and moribund.

When individuals were found outside the gall, it was impossible to decipher if the individual of interest had entered the gall previous to the drying, or if infiltration had not been attempted. This drying out may have also caused the individuals of interest to become moribund faster than if the moisture content was regulated. In the fighting assays, the thrips can survive past the 40 hour period with little to no mortality, unless engaged in a battle. Yet, in the infiltrate gall assays, a large number of soldiers were found to be moribund after just 24 hours. The design of this experiment needs to be revised, perhaps by placing the modified assay tubes within a moisture and temperature controlled container. Once the desiccation issue is addressed, another additional step

would be to obtain morphological measurements (e.g., wing length, body length and femur dimensions) of all the soldiers involved in the assays. By doing so, it could be pinpointed if there is a specific morphological type that is entering the galls; for example, are only longer-winged individuals entering a gall? Even with the desiccation issue, this assay illustrates that the soldiers of *K. intermedius* (both female and male) can and will enter an intact gall, which was previously unknown. It would also be beneficial to examine if the soldiers would voluntarily exit their natal gall, taking note of the sex of the soldier. Also, genetic examination of potential cheating behaviour, such as males entering unrelated galls then producing offspring within, would shed more light on the possibility of inter-gall movement of soldiers.

#### **4.3 Mating Assays**

Mating trials were conducted to quantify any differences in the mating preferences of the soldiers of *K. intermedius*; did the soldiers prefer to outbreed and mate with non-relatives, inbreed and mate with relatives, or not exhibit any preference? Male soldiers of *K. intermedius* were seen to mate twice out of the five trials that took place in May of 2009. These marked males mated with female dispersers, as well, additional non-marked males were seen mating with female dispersers, soldiers, and on one occasion an attempt to mate with a second instar larva was observed. These male soldiers did not show a preference with regard to mating with their sisters or non-relatives since both marked males mated in a non-natal and a natal trial and, additional mating occurred randomly in the trials. In the 'natural' modification of these mating trials (15 trials in

total) no mating was observed. Even in this natural set up (compared to a petri dish), the soldiers were not very active, and usually stayed in the same general area within the gall.

One unexpected observation was that soldiers in the foreign gall assay didn't exhibit any sort of conflict. This lack of conflict points to an absence of kin recognition in *K. intermedius*, as was also suggested when living soldiers were found after entering galls hosting non-relatives in the infiltration assay (addressed in detail in section 4.2). In fact, it seems that the soldiers of *K. intermedius* could have very poor recognition of the life stages of thrips as well – a male soldier would have no advantage trying to mate with a larva. It is possible that the male soldier could not identify the larva and proceeded in an attempted reproductive effort. For example, soldiers did not exhibit conflict with non-relative soldiers, possibly because the soldiers could not detect a foreign individual. Also, soldiers cannot identify a *Koptothrips* invader immediately (e.g., in the fighting assays detection was never immediate), which could be due to reduced sensory structures or because of cryptic adaptations of the invader. Soldiers of *K. intermedius* are known to have a reduced number of sensory neurons in the largest olfactory sensilla in comparison to the dispersing caste (De Facci et al. 2011). This reduction in sensory neurons may affect the soldier's ability to recognize individuals, but more exploration is needed to determine their ability to recognize invaders and kin versus non-kin.

The lack of mating in the February 2010 trials could be attributed to the early life stage of the galls in addition to the time of year. It would stand to reason that male soldiers would benefit more from mating with female dispersers, who will then create

their own gall and directly pass on the male soldier's genes when she lays fertilized eggs. The galls used in the mating trials in May were in the later stages of their life history, in which adult dispersers were present preparing for dispersal. In the February trials, galls were present with adult dispersers, but galls that contained adult soldiers and larvae, with few to no adult dispersers present, were far from rare. Also, whenever dispersers were present within the gall used in the trials, they were removed due to the difficulty of observing the marked soldier. It is possible that the male soldiers experience a switch in their life history strategy in which younger soldiers focus on defense, but as the gall ages and the demographic changes with the presence of adult dispersers, the males focus on direct fitness and mate with these dispersers.

#### **4.4 General Discussion**

A unified approach in studying social animals started with Wilson (1975) and has since been supported by the study of sociality across many taxa, which has advanced our understanding of the evolution of sociality (Emlen 1996; Brockmann 1997; McRae et al. 1997; Mumme 1997; Blumstein and Armitage 1998). However, there are difficulties in this type of approach, such as the emergence of the debate over the definition of eusociality, which was discussed in Section 1.1. There are also difficulties in tying together various social systems since vastly different life histories create a problem when attempting to apply analogous processes across different taxa (Jamieson 1991).

Initially, focus on the evolution of sociality concerned reproductive skew as a driving factor, but these studies did not incorporate any type of non-reproductive

behaviours such as: defense, foraging, and building and maintenance of a nest, which can be of great importance (Cahan et al. 2002). Decision theory is a way of examining the evolution of social systems in which current fitness benefits and prospective fitness benefits are incorporated along with important non-reproductive behaviours. Decision theory works on the basis that an individual makes a series of decisions related to social behaviours throughout its lifetime, and each decision will influence the individual's fitness. For differing sets of decisions, lifetime inclusive fitness can be calculated and compared; this comparison is helpful in determining the best strategy for the individual (Emlen and Wrege 1994).

Cahan and colleagues (2002) expanded the original basis of decision theory (Emlen and Wrege 1994; Emlen et al. 1995) so that comparisons could be made across taxa that had different life histories and could incorporate vertebrates. These researchers break decisions into common decision points that individuals will face both in terms of reproductive and non-reproductive behaviours. There are three main decision points, represented as nodes on a tree, which individuals in social groups face: (1) to disperse or remain in their natal environment; (2) to raise or help relative offspring; and (3) to breed within the group, or to forego reproduction. At these main decision points, the individual evaluates the costs and benefits of each alternative situation. The decision to disperse from their natal environment is based primarily on environmental conditions such as the availability of suitable habitat, population density, predation risks, and any benefits that could be derived from living within a group (Stacey and Ligon 1991). Individuals can either form their own group or join a group of conspecifics. Once in an established

group, the individual can either breed or forego breeding and help. If an individual doesn't breed but displays helping behaviour, the extent of helping can vary; the individuals could display permanent helping, eventual usurpation or territory inheritance, or the helping could be a function of delayed dispersal until suitable habitat is available (Cahan et al. 2002). Members of a group do not necessarily have to evaluate the costs and benefits related to helping in the same manner; many factors can influence the decision to help such as possible future reproductive opportunities of the individual (Cahan et al. 2002; Cockburn 1998).

In the case of *K. intermedius*, it seems possible that the optimal strategy at the various decision points differs for the male and female soldiers. The initial decision to disperse from their natal gall once eclosed would be based primarily on the arid environment and the probability of finding a growing phyllode that would make it possible to initiate a gall. Also, a male would need an accompanying female in order to reproduce within a newly formed gall. It seems that the best decision at this point would be to stay within the natal gall. These thrips are found in a plural breeding system, where two or more of the same sex individuals breed within a group (Brown 1987).

It is known that the soldiers of *K. intermedius* do reproduce, but at a decreased rate equal to approximately 7% of the reproduction displayed by the foundress (Kranz 2005). Fecundity in female soldiers are limited by decreased ovary size, while there is no evidence that the male soldiers are limited physiologically in any such way, meaning their future reproductive opportunities would be higher than the female soldiers. The

male soldiers may be more willing to defend the gall early in the gall life cycle while the dispersing generation is waiting to eclose because of these potential future reproductive opportunities. Because the dispersers of *K. intermedius* eclose within the gall, a trait unknown in other species of thrips, the male soldiers would have a chance to mate with the dispersers in the future. The optimal strategy for the male soldiers could shift over their life span along with any changes in environmental and body condition, age, experience, and group structure (Cahan et al. 2002). When the male soldier first ecloses as an adult, perhaps the best strategy would be to remain within the gall, possibly co-breed with their sister soldiers at a decreased rate, and cooperate by defending the gall if an invasion was to occur. This appears to be the likely strategy for the female soldiers throughout their life span. The trajectory of this strategy along Cahan and colleagues (2002) decision tree is illustrated in the yellow coloured path in Figure 4.2. However, as the gall becomes older and dispersers begin to eclose, the male soldiers' strategy can shift. The male can now take the option of dispersing, (either through flight since their wings are nearly the length of dispersers or simply by walking to a gall on the same branch) and joining an established group by infiltrating an unrelated gall, such as in the gall-inducing aphids. The advantage of joining an unrelated group lies in defense – the male soldiers can allow unrelated soldiers to defend their natal gall, while the infiltrated male soldier can mate freely with dispersers. Therefore, these males could disperse to an established group (gall), breed, and not cooperate as shown as the blue path in Figure 4.2. The present study suggests that male soldiers can indeed infiltrate an unrelated gall without any conflict. Such dispersal by the male soldiers would not leave their natal gall

unprotected; the sex ratio is strongly biased towards females in *K. intermedius* at 3:1, therefore, the female soldiers remaining can still defend the gall. This material presented here is highly speculative, and goes beyond the scope of the data in this thesis, but indeed begs for more investigation into the life strategies for the male and female soldiers of *K. intermedius*.

Gall-inducing thrips may be more flexible in changing their social strategy throughout their lifetime since they are more recently evolved than other social groups, such as the Hymenoptera. In some ant species and nearly all bees and wasps, the individuals within a colony are given specific reproductive roles during development and cannot change their physiology (Wheeler 1986; Holldobler and Wilson 1990). Since the soldiers of *K. intermedius* can still reproduce (in particular the males have no signs of physiological suppression of reproduction) it may be easier for them to switch their optimal social strategy throughout their lifetime. This is especially true for the male soldiers of *K. intermedius*, which possess wings nearly the length of those of the dispersing caste, thus dispersal through flight could be a likely option.

It is possible that the shorter and longer winged male soldiers of *K. intermedius* represent two different strategies. The ant *Cardiocondyla obscurior*, for example, has two male morphs: a wingless fighter and, what is deemed as a peaceful winged male (Cremer et al. 2011). The wingless males remain within the nest and fight using their modified mandibles to gain access to females (Kulger 1984) while the winged males remain within the nest to mate at first, later dispersing to mate with females outside of the

nest (Kinomura and Yamauchi 1987; Cremer et al. 2002; Anderson et al. 2003). These winged males can adjust their behaviour according to mating opportunities available within the nest (presence of receptive females) and any male competition encountered in the nest from the wingless males. Cremer et al. (2011) found that winged males left the nest faster when wingless males were present compared to when only winged males were present. Also, if females within a nest mate more than once, an increase in sperm competition would be observed, local mate competition would increase and the winged males risk death at the mandibles of the wingless males (Cremer et al. 2011). It is known that females of *K. intermedius* average 1.2 mates over their life span (Chapman and Crespi 1998). Maybe the shorter winged, more aggressive (found to fight more often than longer winged males, Section 3.2) male soldiers use their femurs to gain access to females within a gall, since dispersal may not be an option. The shorter winged soldiers may also be aggressive towards the longer winged males. In this case, longer winged males could mate with their sisters within a gall, and then disperse when they experience conflict from their shorter winged brothers. These males could disperse along with the dispersing caste, and have the possibility of encountering another female disperser while she is initiating her gall. Here, the male could mate with the female disperser, and if he encountered any conflict from another male, he could defend himself (long winged male soldiers were just as effective in battle as female and short winged soldiers, see Section 3.2).

Initially, as seen in the termites and possibly the aphids, the form of eusociality in the gall-inducing thrips was thought to have arisen first as helping, leading to

morphological specialization, and then reduced reproduction (Chapman et al. 2002). Yet, it seems that morphology of these soldiers isn't playing a role in defensive behaviours, but may be involved in scenarios such as sibling conflict and mate competition. This outcome changes the proposed evolution of helping behaviour, at least within *K. intermedius*, such that helping leads directly to reduced reproduction, without the presence of morphological specialization for defensive purposes.



Figure 4.1. A gall of *Kladothrips intermedius* that has experienced desiccation while in the infiltrate gall assay. The ostiole has separated, allowing the brood inside to easily leave the confines of the gall.

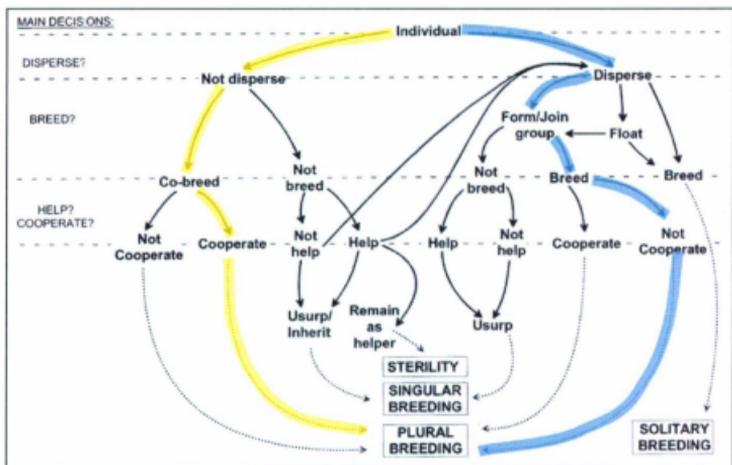


Figure 4.2. Cahan and colleagues (2002) schematic representation of the trajectory of social decisions an individual will face throughout its lifetime. The yellow pathway represents the proposed trajectory for female soldiers of *Kladothrips intermedius* as well as the early life trajectory for male soldiers. The blue pathway represents the proposed switch in trajectories that the male soldiers of *K. intermedius* experience.

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