HOST EXPLOITATION AND FIDELITY IN ACACIA GALL-INVADING PARASITES

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HOST EXPLOITATION AND FIDELITY IN *ACACIA* GALL-INVADING PARASITES

By

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ABSTRACT

The form of social organization seen in the *Acacia* gall-inducing thrips genus, *Kladothrips*, is credited to the invasion pressures exerted by species of the parasitic thrips genus, *Koptothrips*. Critical to this conclusion is the assertion that parasite evolution is more or less in lockstep with the divergence of the hosts. *Koptothrips* populations are thought to specialize in invading specific *Kladothrips* taxa. I assessed host exploitation patterns of *Koptothrips flavicornis* and *Koptothrips dyskritus* within a single host, *Kladothrips intermedius*. I also investigated, using DNA sequence data, the connectivity of various *Koptothrips flavicornis* and *Koptothrips dyskritus* populations. Results from host exploitation investigations suggest that *Koptothrips flavicornis* and *Koptothrips dyskritus* exhibit different patterns when invading a common host, while genetic investigations indicate the absence of host fidelity in regions of overlapping host distribution. My study, while narrow in scope, casts some doubt on the existing model for host/parasite coevolution in this system.

DEDICATION

Dedicated to my parents,

Who inspired in me the curiosity and desire

To learn more about the world around us

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Chapter 1

General Introduction

1.1. Rationale

Parasites are acknowledged to influence community structures and host population dynamics (Price et al., 1986; Minchella and Scott, 1991; Poulin, 1999). They play an important role in generating biodiversity (Mouritsen and Poulin, 2005; Hudson et al., 2006). Mates displaying resistance to parasites are preferred partners for reproduction (Hamilton and Zuk, 1982). Groups of individuals in numerous insect species engage in communal nesting and establish colonies as a defence response aimed at lowering the risk against parasitism (Anderrson, 1984; Schmid-Hempel, 1998). The origin of a defence response to counteract the threat of nest parasites has been a vital step in the evolution of insect societies (Lin, 1964; Lin and Michener, 1972; Alexander, 1974; Anderrson, 1984; Schmid-Hempel, 1998).

The gall-inducing thrips genus, *Kladothrips* Froggatt, is a valuable addition to the comparative database available for studying the factors contributing to the rise of social evolution (Crespi, 1992a; Crespi and Mound, 1997; Chapman and Crespi, 1998; Chapman et al., 2000; Kranz et al., 2002 and Chapman et al., 2008). The galls induced by them are also exploited by species of the kleptoparasitic genus, *Koptothrips* Bagnall. *Kladothrips* induced galls are essential for the *Koptothrips* to complete their life cycle. These galls provide food and shelter to both the original inhabitants and the invaders (Chapman et al., 2008). The life history of the *Kladothrips* has evolved to counter the invasion threat of the *Koptothrips*. Some *Kladothrips* species avoid the invaders by inducing tightly closed galls (Hiders), while others exit the gall during the early stages of

development (Fliers) (Crespi et al., 2004). Species belonging to the third suite have a specialized caste of individuals that display a fighting behaviour when presented to a *Koptothrips* (Fighters) (Crespi, 1992a; Crespi, 1992b; Crespi et al., 2004). Rates of *Koptothrips* invasions vary across the three *Kladothrips* suites; they are almost absent in the 'Hiders', low in the 'Fliers' and high in the 'Fighters' (Crespi et al., 2004). Sociality in the *Kladothrips* is attributed to the *Koptothrips* invasion pressures (Crespi and Mound, 1997). *Koptothrips* are thought to speciate in parallel with their *Kladothrips* hosts (Crespi and Abbot, 1999). But a gap exists in our understanding of how these coevolving invaders influence the evolution of sociality in their hosts. My dissertation investigates the patterns of *Koptothrips* specialization resulting potentially from coevolution and the effect that it may have on the form of sociality seen in the *Kladothrips*.

1.2. A Note on nomenclature

Throughout the dissertation, the names of species belonging to the two genera, *Kladothrips* and *Koptothrips* have been abbreviated. The names of *Kladothrips* species, for example, are written as *K. intermedius*, *K. waterhousei*, *K. rugosus* etc. Names of *Koptothrips* species have been written as *Ko. xenus*, *Ko. zelus*, *Ko. dyskritus* and *Ko. flavicornis*. An exception to this stylistic convention is when the species names are written at the start of a sentence.

A reference is also made to two types of hosts in this system - plant host and thrips host. When referring to plant hosts, I refer to the *Acacia* plant species on which the

Kladothrips induce their galls. Thrips hosts refer to the *Kladothrips* and their galls, which are invaded by the *Koptothrips*.

1.3. Classification of Thrips

Thrips belong to the insect order Thysanoptera. Although Thysanoptera means fringed wings, many thrips species have wingless adults (Mound, 2005). Thrips can be recognized by the presence of a sac like vesicle known as the arolium, which is present at the apical end of the tarsus on each leg (Mound, 1980; Heming, 1971). When compared to other insects, the thysanopteran arolium is larger and Heming (1971), attributes this feature to thrips walking on the tips of their legs with the aid of their expanded arolia. Only the left mandible is fully developed in thrips; the right is reabsorbed during its embryonic development (Mound and Morris, 2000). They undergo hemimetabolous development and the number of larval instars and pupal stages vary according to the suborder (Morse and Hoddle, 2006). Thrips are known to use wind currents to disperse aerially (Mound, 2005; Lewis, 1964; Lewis, 1965). Movement of plants and animals by people has also led to thrips being inadvertently throughout the world (Mound, 2004). Worldwide, 5500 species have been described (Mound 2002a; Mound 2002b; Morse and Hoddle 2006; Mound 2007) but their diversity may be closer to10, 000 species (Moritz et al., 2001). Thysanoptera consists of two sub orders, Terebrantia having eight families and Tubulifera having one family, the Phlaeothripidae (Crespi et al., 2004). Within Phlaeothripidae, 700 species are placed in the sub family

Idolothripinae and 2500 species are placed in the sub family Phlaeothripinae (Crespi et al., 2004).

1.4. *Acacia* plant hosts and associated thrips

Host phenology is crucial for the survival and success of phytophagous insects (Mopper, 2005). Gall inducing insects exhibit a strict association with their host plant species and to specific organs of the associated plant (Raman et al., 2004). While Raman and colleagues (2004) suggest that the distribution patterns of thrips is related to climatic and geographic factors, they also note that the density diversity of galls induced by them are specific to certain regions.

Across Australia, 250 thrips species belonging to the sub family Phlaeothripinae are associated with 100 species belonging to the plant genus *Acacia* (Morris et al., 2002; Crespi et al., 2004; Mound 2004; Mound and Morris, 2004). The association of thrips with *Acacia* is almost exclusively restricted to two Sections, *Plurinerves* and *Juliflorae*, with a few of them belonging to *Phyllodineae* (Morris et al., 2002; Crespi et al., 2004). These three Australian *Acacia* sections do not have bipinnate leaves, which are characteristic of the floral family *Leguminosae*. The leaves are replaced by a leaf like extension of the petiole called the phyllode (Morris et al., 2002). The life history and galling traits of the thrips hosts (*Kladothrips*) and the invaders (*Koptothrips*) are intricately linked to the phenology of the *Acacia* hosts. Three *Acacia* species are the focus in this thesis: 1) *Acacia Oswaldii* is a dense shrub about 2-6m tall. It has linear

phyllodes (2-10mm wide and 3-8cm long) while its seedpods appear coiled (Kutsche and Lay, 2003). Although not commonly found, it has widespread distribution throughout Australia's semi-arid and arid zones (Maslin, 2001). 2) *Acacia papyrocarpa* appears spread out and is usually about 3-7m tall. The spreading of the canopy is more pronounced with age. It can be easily identified during a flush of new leaf growth as the phyllodes appear silvery green. Its growth rate is slow and reproduction from seeds occurs only after an exceptional rainfall (Kutsche and Lay, 2003). Its distribution extends from Western Australia to South Australia (Maslin, 2001). 3) *Acacia calcicola* is shrub measuring 3-5 m tall. It has a spreading bushy canopy with its branchlets appearing silvery coloured. It is widespread across the arid region in Central Australia (Maslin, 2001).

Morris and colleagues (2002), classify *Acacia* thrips into four different suites: (1) Gall-inducers - Thrips that induce galls on *Acacia* phyllodes, (2) Domicile- builders - Thrips that glue phyllodes together, (3) Parasites (but described as 'Exploiters' by Crespi et al., (2004)) - Thrips that usurp galls or glued phyllodes and thrips that cohabit with the gall-inducers or domicile builders and (4) Opportunists - Thrips that live in abandoned domiciles or in empty niches not produced by other thrips. Both Parasites and Opportunists are able to utilize galls and domiciles that are not their own to produce their offspring and complete their life cycle. Species within the four suites have evolved a variety of morphological and physiological traits aiding exploitation of their physical environments. The variation observed among them aptly fits Schluter's (2000) definition of adaptive radiation. All of these suites are remarkably diverse (within themselves) with

regard to their sheltering, defence and reproductive strategies (Crespi, 1992a; Crespi et al., 1997; Crespi and Worobey, 1998; Chapman et al., 2002; Crespi et al., 2004; Perry et al., 2004; Kranz, 2005).

1.5 Defining eusociality

Wilson (1971) defines attributes of eusociality as: (i) overlap in generations between parents and their offspring; (ii) cooperative brood care and (iii) reproductive division of labour. However, a number of complex social systems do not fit Wilson's (1971) criteria precisely (Costa and Fitzgerald, 2005). Crespi and Yanega (1995) define eusocial societies as those containing castes that become irreversibly distinct in their behaviour prior to reproductive maturity. They also eliminate Wilson's (1971) criteria for the presence of a generational overlap between parents and their offspring. Throughout my thesis, I refer to the gall-inducing thrips as eusocial based on the criteria defined by Crespi and Yanega (1995).

1.6. *Acacia* gall-inducing thrips

The gall-inducing thrips clade, *Kladothrips* is associated with approximately 50 *Acacia* species (Crespi et al., 2004). *Kladothrips* are a monophyletic group that has diversified over time (Morris et al., 2002). Their diversification is closely linked to the range expansion of their *Acacia* plant hosts (McLeish et al., 2007a; McLeish et al., 2007b). *Kladothrips* evolved approximately 10-15 million years ago coinciding with an

increasing aridification in Australia (McLeish et al., 2007a; McLeish et al., 2007b). Subsequent radiation within this clade has resulted in the formation of species complexes in at least four described species (Crespi et al., 2004) The gall-inducers are classified into three generic groups ('Hiders', 'Fliers' and 'Fighters') based on their ecological, morphological and behaviour adaptations (Crespi et al., 2004).

1.6.1. Hiders

Species belonging to this group are found in highly arid regions and they induce galls on plants belonging to the *Acacia* Section *Juliflorae*. Galls induced by them are generally spherical. The foundress is highly physogastric (increased egg producing capacity). The larvae eclose within the gall and this behaviour is be linked to the highly arid environment and unpredictable rainfall, since the brood would remain within the gall until favourable conditions arrive (Crespi et al., 2004).

1.6.2. Fliers

Fliers are found mainly in the semi arid zone. Like the hiders, they too induce nearly spherical galls, exhibit high foundress fecundity and produce large broods. They induce galls on plants of the *Acacia* Section *Plurinerves*. Males are present with the female foundress during gall induction and beyond in at least 50 % of galls and the larvae eclose in the soil (Crespi 1992b; Crespi et al., 2004). One species, *Kladothrips rugosus* Froggatt is thought to be a species complex that is found on numerous *Acacia* species (Crespi and Mound, 1997; Crespi and Worobey, 1998). A gall may be thought of as a

phenotypic extension of its inducer (Stone and Schönrogge, 2003) and a variation in the morphology of galls induced by the same taxa across different host plants may be indicative of the underlying differences between cryptic populations (Crespi and Worobey, 1998). Genetic investigations within the *K. rugosus* species complex have revealed the presence of cryptic species that induce several highly different gall variants (McLeish et al., 2006; McLeish et al., 2007a; McLeish et al., 2007b). One of these putative taxa was found to have sufficiently high levels of evolutionary divergence to be re-described as a new species, *Kladothrips nicolsoni*, McLeish, Chapman and Mound (McLeish et al., 2006).

1.6.3. Fighters

Species belonging to this suite have a distinct caste of individuals that have reduced antennae and wings as well as enlarged forelimbs. This morphological specialization suggested that they were adapted to fight and defend the gall from enemies. Consequently, they were termed 'soldiers' (Crespi, 1992b). Female soldiers show reduced ovarian development (Chapman et al., 2002) when compared to females from the dispersing caste (named so because individuals from this caste disperse from the gall on maturity). Seven species are described as eusocial based on Crespi and Yanega's (1995) definition for eusociality (Crespi, 1992b; Mound and Crespi, 1995; Crespi and Mound, 1997; Kranz et al., 2001; Willis et al., 2004). The group is generically referred to as 'fighters' (Crespi et al., 2004). Overall, the 'fighter' suite is monophyletic and strong bootstrap values support most of the internal nodes in this group (Chapman et al., 2008).

All 'fighter' species induce galls on *Acacias* within the *Plurinerves* Section (Crespi et al., 2004). Species within this group induce elongate galls which (with the exception of *K. morrisi*) support smaller brood sizes (Crespi and Worobey, 1998; Crespi et al., 2004). The soldier caste has originated once, approximately 6.3 million years ago (McLeish and Chapman, 2007). Crespi and colleagues (2004) also place two species that have lost soldiers (*Kladothrips rodwayi* Hardy and *Kladothrips xiphius* Mound, Crespi and Kranz) and are closely related to the 'fighters' within this group. Barring *Kladothrips intermedius* Bagnall and *K. rodwayi*, whose larvae eclose within the gall, the larvae of all other 'fighter' taxa eclose in the soil (Crespi et al., 2004).

1.7. Influence of *Koptothrips* invasions on the life histories of the gall-inducers

Parasites foster diversification both within their exploited hosts as well as among themselves (Price 1977; Drès and Mallet, 2002; Summers et al., 2003). Natural enemies may influence the form and type of diversification within their hosts (Vamosi, 2005). Shifting of hosts to escape their natural enemies can also promote speciation within galling insects (Brown et al., 1995). *Koptothrips* invasions have led to the evolution of diverse morphological, behavioural and reproductive adaptations within the *Acacia* galling clade (Crespi et al., 1997; Crespi and Mound, 1997; Crespi and Abbot, 1999; Crespi et al., 2004; Chapman et al., 2008). Life histories of the solitary (those

species that lack the 'soldier' caste – viz., those belonging to the 'Hider' and 'Flier' suites) and soldier caste possessing gall-inducers are directly related to the rates of *Koptothrips* invasions. 'Hiders' inhabit highly arid areas and they suffer from little or no *Koptothrips* invasions. Most produce large galls which support large broods that persist within the gall for long periods of time (Crespi et al., 2004). However, taxa from both the 'flier' and 'fighter' lineages are often attacked by *Koptothrips*. Their life histories have evolved as a counter response to the *Koptothrips* invasions. The 'fliers' have evolved an early exit strategy and galls of this suite are relatively short lived (Crespi et al., 2004). Flier foundresses are highly physogastric and produce large broods to compensate for an early exit (Crespi et al., 2004). In contrast, the 'fighter' (social gallers) suite consists of social species that induce relatively longer lived galls produce smaller broods and invest in the production of soldiers to defend the gall from *Koptothrips* (Crespi and Mound, 1997; Crespi et al., 2004).

1.8. The evolutionary history of *Koptothrips* spp.

The kleptoparasitic thrips genus, *Koptothrips* Bagnall consists of four described species: *Koptothrips xenus*, *Koptothrips zelus*, *Koptothrips flavicornis* and *Koptothrips dyskritus* (Mound, 1971). The *Koptothrips* evolved independently as a monophyletic group with a single origin of kleptoparasitism that is associated with a host plant shift. No evidence is present to indicate a reversal to a non-parasitic life style (Morris et al., 1999; Morris et al., 2002). Engaging in a parasitic behaviour would offset

the costs associated with having to adapt and live on a novel host plant (Crespi and Abbot, 1999). The evolution of kleptoparasitism in *Koptothrips* has involved a facultative behaviour that resembles an ancient transitory phase. This behaviour is evident in three species, *Ko. xenus*, *Ko. zelus* and *Ko. dyskritus* where females use anal secretions to seal off open or damaged galls that are devoid of the gall-inducers (Crespi and Mound, 1997; Crespi and Abbot, 1999; Crespi et al., 2004). Additionally, multiple female invaders of *Ko. xenus* and *Ko. zelus* can each form a substructure with a single gall, which may be used to raise their respective broods (Crespi and Mound, 1997). The transition in usurpation strategy, from using damaged and abandoned galls to taking over a gall by killing the inhabitants has been a key feature in the successful radiation of *Koptothrips* across a large number of *Acacia* species in the *Plurinerves* section and a few in the *Juliflorae* section (Crespi et al., 2004). Within the group, the lineage of the present day *Ko. flavicornis* has evolved in parallel to the lineage that subsequently evolved into three distinct species (*Ko. xenus*, *Ko. zelus* and *Ko. dyskritus*) (Crespi and Abbot, 1999). Of these three species, *Ko. zelus* evolved first, followed by *Ko. xenus* and *Ko. dyskritus* respectively.

1.9. Behavioural ecology of *Koptothrips* spp.

Upon entering a *Kladothrips* gall, *Koptothrips* are attacked by the foundress and soldiers (Crespi, 1992b). *Kladothrips* foundress and soldiers attempt to grasp *Koptothrips* with their enlarged fore legs that contain pointed fore tarsal teeth at the apex

(Crespi 1992a, b; Crespi and Mound 1997; Perry et al., 2004; Crespi et al., 2004). The *Koptothrips* respond by stabbing their fore tarsal teeth into the *Kladothrips. Koptothrips* are highly efficient in killing *K. hamiltoni* and *K. morrisi* whereas they are least successful in fighting off *K. intermedius*, *K. habrus* and *K. waterhousei* (Perry et al., 2004). *Koptothrips xenus*, *Ko. zelus* and *Ko. dyskritus* use their enlarged fore legs to fight off the gall-inducers and perhaps, their conspecifics as well (Crespi et al., 2004). By comparison, *Ko. flavicornis* is smaller in size and has less developed fore legs. Crespi and Mound (1997), suggest that *Ko. flavicornis* may be using venom, which is delivered through the fore femoral gland to kill the host thrips (Crespi et al., 2004). The 'fighters' are engaged in defending the gall from the kleptoparasites and being driven off is likely to result in death (Crespi et al., 2004). For the *Koptothrips*, not being able to take over a gall also means certain death (Crespi et al., 2004). Therefore, the gall is vital for the survival of both *Kladothrips* and *Koptothrips*.

Koptothrips invasion rates are higher in the social ('fighter') species than in the solitary ('fliers') ones (Crespi and Abbot, 1999). This may seem paradoxical since the presence of soldiers should mean that the invaders are less successful in taking over a gall (Crespi et al., 2004). But this observation is also consistent with the longer life spans of 'fighter' galls. 'Fighters' produce longer-lived galls and the production of soldiers underlies the importance of protecting such a resource if it is to persist (Crespi et al., 2004). By entering a gall that persists long enough, *Koptothrips* are able to maximize their reproductive success at least for a brief period of time when the gall can still be used

even after the gall-inducers have been killed. However, it also suggests that soldiers may not be effective at defending galls or that the *Koptothrips* invade before soldiers eclose (Perry et al., 2004; Crespi et al., 2004; Chapman et al., 2006, Chapman et al., 2008)

1.10. Model for *Koptothrips* evolution

Diversification in parasites may occur as a result of cospeciation where parasites evolve along the lineages of their hosts (Price, 1977; Thompson, 1994; Thompson, 1999). Crespi and Abbot (1999) suggest a model for *Koptothrips* evolution along the lineages of their hosts (Fig. 1.1). They hypothesize that *Koptothrips* have evolved and diversified along the lineages of their *Kladothrips* hosts on a macro scale with *Ko. dyskritus* invading galls of multiple solitary species ('flier' suite) and *Ko. flavicornis* attacking galls of multiple social species ('fighter' suite). Lineages of *Ko. zelus* and *Ko. xenus* specialize in invading solitary species, *K. ellobus* and *K. acaciae* respectively (Crespi and Abbot, 1999). When present on the same *Acacia* tree, it is possible that *Ko. flavicornis* could enter galls of solitary *Kladothrips* taxa (Crespi and Abbot, 1999).

1.11. Thesis overview

Previous studies on *Koptothrips* have mainly focused on understanding their impact on the ecological and behavioural diversity that is seen in their galling hosts. The

origins, timing and reasons behind the evolution of kleptoparasitism within *Koptothrips* are themes that remain largely unaddressed. *Koptothrips* are a strong selective pressure that have facilitated morphological and behavioural diversity within the *Kladothrips* (Crespi et al., 2004). Life history variation within the *Kladothrips* has emerged as a consequence of either avoiding or defending galls from the *Koptothrips* (Crespi et al., 2004). Little is known about their life cycle, the nature of their invasions or about inter-species competition for commonly exploited habitats. Their radiation onto numerous *Kladothrips* hosts within the 'Flier' and 'Fighter' suites across various *Acacia* species has promoted remarkable variation within them. Consequently, localized host-invader dynamics, which may contribute to inter-population variation within the *Koptothrips*, have not yet been explored. Populations of both *Ko. flavicornis* and *Ko. dyskritus* that attack different *Kladothrips* are believed to be either host races or suite of closely related sibling species (Crespi and Abbot, 1999). Determining the level of intra-species divergence would be the first step towards clarifying the taxonomic status of populations within these two invaders.

The overarching aim of my dissertation is to begin a study that focuses exclusively on investigating the pattern of ecological and evolutionary diversification within the *Koptothrips*. In Chapter 2, I look at *Koptothrips* invasion patterns in a single host, *K. intermedius*. The aim of this chapter is to investigate the invasion patterns of two invaders in competition for one highly valuable domicile resource. Chapter 3 focuses on investigating the extent of genetic divergence between *Koptothrips* populations collected

from various *Kladothrips* hosts. These *Kladothrips* hosts include those that overlap in geographical distribution as well as those from distinct locations. My objective here is to determine the degree of host specific invader specialization between different *Ko. flavicornis* and *Ko. dyskritus* populations. I also present the overall conclusions taken from both chapters and use this summary to suggest future research directions.

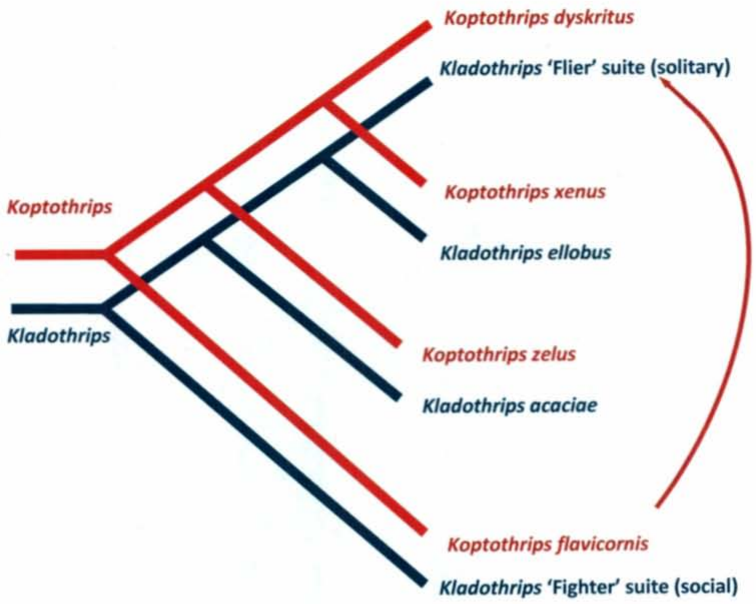

Figure1.1. Model of *Koptothrips* evolution (adapted from Crespi and Abbot, 1999). *Koptothrips zelus* and *Koptothrips xenus* specialize in invading specific 'Flier' species, *Kladothrips acaciae* and *Kladothrips ellobus* respectively. *Koptothrips dyskritus* and *Koptothrips flavicornis* broadly invade galls of numerous species within the 'Flier' (solitary) and 'Fighter' (social) suites respectively. *Koptothrips flavicornis* can also attack galls of 'Flier' species when present on the same *Acacia* tree.

Chapter 2

Koptothrips flavicornis and *Koptothrips dyskritus* gall-exploitation patterns in a population of *Kladothrips intermedius*

2.1. Introduction

Localized adaptation of parasites to their hosts is a dynamic process that can often change in a particular environment over time (Kaltz and Shykoff, 1998). Relative to individuals that may originate from other habitats, native parasitic populations would have higher fitness in their local environment (Kawecki and Ebert, 2004). Consequently, the effects that parasites may have on their hosts are highly variable in environments that provide an opportunity for movement between hosts both on a temporal and spatial scale (Thompson, 1994; Thompson, 1999; Gandon and Michalakis, 2004).

Parasitic pressures can influence the origin and subsequent maintenance of sociality within their hosts (Crespi and Abbot, 1999). The genus *Kladothrips* Froggatt shows distinct life history adaptations as a response to the parasitic pressures exerted by invader genus, *Koptothrips* Bagnall (Crespi and Mound, 1997; Crespi et al., 2004). Galls produced by 'hiders' are rounded and tightly enclosed making it difficult for the *Koptothrips* to enter the gall. 'Fliers' produce large broods that mature outside the gall and attempt to exit before a potential invasion. 'Fighters' have a morphologically and behaviourally distinct soldier caste, which defends the gall against *Koptothrips* invasions. With the exception of *Kladothrips morrisi*, which produces larger brood in comparison to other social gallers, the trade-off for the investment in the production of soldiers has resulted in smaller brood sizes (Kranz, 2001; Kranz 2005). Seven soldier bearing *Kladothrips* species have been described as eusocial (Crespi 1992a; Crespi 1992b; Crespi, 1994; Mound and Crespi, 1995; Wills et al., 2004). Galls of social species are thought to

be long lived since they have soldiers to fight off *Koptothrips* (Crespi et al., 2004). A longer life span makes galls of social *Kladothrips* an ideal target for *Koptothrips* invasions since gall longevity is thought to be crucial for the successful survival and brood development of the invaders (Crespi et al., 2004). A preference for longer-lived galls, however, comes with a potential risk of death as *Koptothrips* have to fight soldiers.

Dependence on *Kladothrips* induced galls meant that the invaders have had to develop an effective strategy that took into account potential face-offs with the hosts. The *Koptothrips* are highly specialized invaders and their radiation has broadly tracked the evolution of the *Kladothrips* clade (Crespi and Mound 1997; Crespi and Abbot, 1999). *Koptothrips flavicornis* Bagnall and *Koptothrips dyskritus* Mound specialize in invading a broader suite of *Kladothrips* species with *Ko. dyskritus* specializing in invading solitary *Kladothrips* hosts belonging to the 'flier' group and Ko. *flavicornis* attacking social *Kladothrips* hosts that belong to the 'fighter' group. These two *Koptothrips* species are likely to represent suites of 'sibling' species. There is some DNA sequence data available that supports the evolutionary independence of some populations within these two species complexes (Crespi and Abbot, 1999). Evolutionary patterns of both *Ko. dyskritus* and *Ko. flavicornis* suggest that these two invaders have diversified by co-speciation and host shifting (Crespi and Abbot, 1999; Crespi et al., 2004). Abrahamson and Blair (2008) suggest that while host shifting may have contributed to *Koptothrips* speciation, they are subjected to additional selection pressures such as adapting to a novel host *Acacia* plant, competing for galls and fighting the gall-inducers.

The social species *Kladothrips intermedius* Bagnall induces galls on *Acacia oswaldii*. *Acacia oswaldii* has a diffused but wide distribution throughout Australia's semi-arid and arid climatic zones (Maslin, 2001). Within the social gall-inducers suite, *K. intermedius* is considered unique as it may have shifted its plant host in the past, which could imply that it was parasite free for a certain period where soldiers may not have been present for a period of time (Crespi et al., 2004; Chapman et al., 2008). However, previous studies have indicated that there is a soldier caste in the *K. intermedius* population at Middleback, South Australia and that its galls are invaded by *Ko. flavicornis* (Chapman et al., 2006). Investigating *Koptothrips* exploitation patterns may give us insights into how these kleptoparasites have shaped sociality in their gall-inducing hosts. The main objectives of my study were: (i) What is a *Koptothrips* invasion composed of? (ii) Is *K. intermedius* exclusively invaded by *K. flavicornis* as suggested by Crespi and Abbot's model (1999) for *Koptothrips* evolution along social and solitary host lineages? (iii) If *K. intermedius* is not exclusively invaded by one species, how do the two invaders exploit a common resource?

2.2. Materials and Methods

2.2.1. Field site and gall collections

In June 2008, galls were collected from a single *Acacia oswaldii* tree. This tree was located in close proximity to the Middleback Field Station building (S 32° 56.765' E 137° 23.696') which is situated approximately 20 km North West of the port

city of Whyalla, South Australia. *Acacia oswaldii* is found throughout the station property (approximately 1000 km^2). In 2008, a survey of trees along a 22km stretch of dirt track on the property resulted in 6 trees (of 50-60 that were investigated) identified as having a population of galls, but only the one tree mentioned above had sufficient numbers to be the focus of this investigation. All available galls (approximately 170) were collected from this tree. In May 2009, we surveyed trees within a 10 km radius from the field station building as well as a location about 30 km north of the field station (S 32° 57.927' E 137° 14.607'). From both these locations, approximately 630 galls were collected from a total of ten trees. Galls were stored in zip-loc bags and transported back to the field station where they were maintained at 4-8 °C. After less than a week at the field station, these galls were preserved in 100% ethanol in anticipation of transport to Canada (Memorial University, St. John's, Newfoundland and Labrador).

2.2.2 Gall measurements and dissections

A gall starts as a furrow oriented along the length of the developing phyllode. The foundress sits in this furrow as the two sides of the furrow continue to swell. Eventually these sides will come together above the female such that she is interred in the hollow that is created. Where the sides of the phyllode meet is called the ostiole, the lip of the gall. The length of the gall is the exterior measurement taken along this ostiole. The width is the widest measurement perpendicular to the ostiole. Gall measurements were taken using a pair of digital vernier callipers.

Galls were opened by creating a small incision along the ostiole with a scalpel and then pried open along the length of the ostiole to minimize damage to the gall

inhabitants. The inhabitants of a gall were then emptied into a petri dish using a fine paintbrush. A compound microscope (up to 50X magnification) was used to observe the thrips.

2.2.3. Species identifications

An identification key and species descriptions from Crespi and colleagues (2004) aided thrips identification in this study. Distinguishing the gall inducer (*K. intermedius*) from the invaders (*Ko. flavicornis* and *Ko. dyskritus*) was straightforward when viewing adult thrips, and the invaders are very different in size (*Ko. dyskritus* is much larger than *Ko. flavicornis*). Larvae are not covered in these keys and descriptions, but observation of gall broods where only one of the species is present at the adult stage (presumably, the only individuals that could have produced the larvae in the galls) shows that the larvae are also easily distinguishable (see description below).

2.2.3.1. Gall-inducers – *Kladothrips intermedius*

Kladothrips intermedius dispersers have a distinctly pale brown yellow 3rd antennal segment, while the rest of the antennae is dark brown (Crespi et al., 2004). The dispersers also have longer wings and more slender forelegs than the other adult form of this species, the soldiers. Soldiers have reduced antennae, truncated wings and enlarged forelegs. Larvae of *K. intermedius* in this population were always observed to have two brownish yellow spots behind their heads (see Figure 2.1).

2.2.3.2. Gall-invaders – *Koptothrips flavicornis* and *Koptothrips dyskritus*

Koptothrips flavicornis has a slender head, with lateral margins of the eye converging to the anterior (Mound, 1971), very different from that of the gall-inducer and

the second invader, *Koptothrips dyskritus*. Also, the antennal segments III-VIII of *Ko. flavicornis* are brownish yellow (Crespi et al., 2004). The large size of *Ko. dyskritus* was the most obvious distinguishing characteristic which did not require a microscope to observe. But this species also has light brown markings apically on antennal segments III-VIII, their eyes are rounded, and the mesopraesternum is reduced to two lateral triangles (Crespi et al., 2004). However, variation in colour has been observed for several collections of these two species taken from different populations (Mound, 1971). The larvae of the gall-invaders have 'distinct' eyes and there are no brownish yellow spots (see Figure 2.1) that are characteristic of the larvae of the gall inducer *K. intermedius*, noted above.

2.2.3.3. Sex identification

The adults of *Ko. flavicornis* and *Ko. dyskritus* can be separated into the two sexes by noting the sculpturing of the terminal segments of the abdomen. The cuticular pigmentation of all specimens was removed so that the hard internal structures associated with the adeagus could be observed to confirm males or the genital pore (fustis) to confirm that an individual was a female (although the fustis was not always observed). The cuticular pigmentation clearing (removing) technique for thrips described by Kirk (1996) was used to clear the individuals and prepare voucher specimens. Voucher specimens of both *Ko. flavicornis* and *Ko. dyskritus* males and females have been deposited in the Department of Biology, Memorial University (room SN4113) and with the Entomology Laboratory, Plant Health and Production, Ottawa in the Canadian National Collection.

2.2.4. Gall census and analysis

The content of each gall was censused. The juveniles, soldiers and dispersers of *K. intermedius* were recorded as present or absent. Juveniles and adults of *Ko. flavicornis* and *Ko. dyskritus* were counted. Note that pupae were rarely present, so larvae and pupae were counted together as 'juveniles'. Adult *Koptothrips* were then stored in 100% ethanol at 4°C before they were cleared (see section 2.2.3.3) to identify sex. The data was analyzed using Minitab version 16. An exploratory data analysis was carried out to obtain descriptive data from adults and juveniles of both *Koptothrips* species as well as to detect any outliers present within the dataset. Observed outcomes for differences in females, juveniles and gall size preferences were calculated and recomputed by randomizing the data (approximately, 500 times). The observed outcome was then compared to the distribution of the randomized outcomes so that a two-tailed p-value could be computed. A correlation analysis was also done between juveniles and females for both invader species.

2.3. Results

2.3.1. Hosts

In general, galls containing *K. intermedius* only had soldiers, dispersers and juveniles. This observation indicates that this population was nearing the end of the gall's life-history when dispersers have all eclosed.

2.3.2. Invaders

In 2008, galls invaded by *Koptothrips* composed approximately 12 % (37/170) of the sample. Of these invaded galls, 56 % (21/37) were invaded by *Ko. flavicornis* and 44% (16/37) were invaded by *Ko. dyskritus.* In 2009, approximately 5% (36/630) of the galls were invaded by *Koptothrips.* Of these galls, 98% (35/36) were invaded by *Ko. dyskritus* and 2% (1/36) were invaded by *Ko. flavicornis.* The presence of *Ko. dyskritus* invading galls in large numbers was not expected as a previous study reported predominantly *Ko. flavicornis* (Chapman et al., 2006). Invasion rates for the *Koptothrips* are summarized in Table 2.1. In both collections, *Ko. flavicornis* and *Ko. dyskritus* were never found in the same gall. Since only a single *Ko. flavicornis*-invaded gall was found in 2009, subsequent comparison and analyses of the two invader species are from the 2008 collection only, except where noted.

Galls invaded by *Ko. flavicornis* were found with *K. intermedius* soldiers 33% of the time. Galls invaded by *Ko. dyskritus* were never found in the presence of soldiers (2008 or 2009). In three cases, a single *Ko. flavicornis* adult female was present in a gall and in one of these cases an adult male was present. Five *Ko. dyskritus* invaded galls had a single female and only one of these galls had a male present as well. These observations suggest that in both *Ko. flavicornis* and *Ko. dyskritus* an invasion consists of a minimum of one female who is sometimes accompanied by a male, presumably a mate. Descriptive data for *Ko. flavicornis* and *Ko. dyskritus* is summarized in Table 2.2. Differences in the number of females present in an invaded gall was non-significant (n=

36, two tailed, P>0.05) (Table 2.2). However, differences in the number of juveniles for the two invaders was significant (n=36, two tailed, P<0.05) (Table 2.2).

The galls being a valuable resource could be exploited by numerous *Koptothrips* females to begin brood production. Consequently, a test of correlation between the offspring and adult females might help to elucidate how the two (offspring and adult females) are associated to each other. A significant correlation between the number of females and juveniles in a gall would support the hypothesis that female *Koptothrips* adults are not part of a single brood that have matured within the gall, but rather that they are contributing to the larval population in that gall by laying eggs. In contrast, a non-significant correlation between females and juveniles might suggest that the females present in the gall are likely to be the daughters of a single foundress that invaded a gall. Results of the exploratory data analysis for the number of *Ko. dyskritus* females per invaded gall showed three outliers present beyond the largest value of the distribution range. A test of correlation between *Ko. dyskritus* females (three outlying values were included in the analysis) and juveniles was non-significant ($r = +0.462$, two-tailed, $P > 0.05$, $n = 16$). When the three outlying values were excluded from the analysis, the strength of the correlation coefficient (r) decreased to 0.12; however, it still remained non-significant ($r = +0.12$, two-tailed, $P > 0.05$, $n = 13$). The relation between juveniles and females did not show a trend (Figure 2.2). For *Ko. flavicornis* females, no outliers were detected and the correlation between *Ko. flavicornis* females and juveniles was significant ($r = 0.497$, $P < 0.05$, two-tailed, $n = 20$). A trend between *Ko. flavicornis* females and juveniles was observed (Figure 2.3).

Do the invaders prefer different gall sizes? The differences in body size might suggest that *Ko. dyskritus* would prefer larger galls. However, comparisons for differences in lengths (n=33, two tailed, P>0.05) and widths (n=33, two tailed, P>0.05) of invaded galls indicate no difference in gall preference (Table 2.2).

2.4. Discussion

Kladothrips intermedius is invaded by two kleptoparasitic species, *Ko. flavicornis* and *Ko. dyskritus* (Table 2.1). This observation differs with a previous study conducted in the same region (Chapman et al., 2006), where *Ko. flavicornis* was the only invader found. Perry and colleagues (2004) also collected *Koptothrips* from Middleback but they do not specify the invader species used for their study. However, Chapman (personal communication), who is a coauthor on the Perry et al. (2004) study, confirmed that it was *Ko. flavicornis* that was collected from *K. intermedius* galls.

Crespi and Abbot's (1999) model for *Koptothrips* evolution is, in part, based on the notion that populations of *Ko. flavicornis* have specialized on attacking social hosts (Chapman et al., 2006 study is consistent with this view), while populations of *Ko. dyskritus* have specialized on solitary hosts. And, any observation of the 'wrong' invader in a gall would be considered incidental and not a challenge to this view. Since the 2008 collection came predominantly from one tree, perhaps the prevalence of *Ko. flavicornis* was due to sampling error. However, the 2009 collection was geographically broader and yet *Ko. flavicornis* invaded fewer galls than did *Ko. dyskritus*. The two collections show

that *Ko. dyskritus* (the 'novel' invader) is found in similar frequencies (2008) or they are substantially more common (2009) in this population than is *Ko. flavicornis*. My observation challenges the idea that lineages of invaders have specialized on social or solitary species and the notion that invasion frequencies can be used as host-species specific traits. Although not specifically noting the invader species, an average of 31% of *K. intermedius* galls were reported, previously, to be invaded by *Koptothrips* (Crespi and Abbot, 1999). In contrast, my study showed a much lower rate of *Koptothrips* invasions (12% in 2008 and 5% in 2009). This rate of invasion falls below the range of invasion rates for social species in general (23% - 32%, Crespi et al. 2004), but well within that of solitary species (2% - 40%, Crespi et al. 2004). Variation in invasion frequency within a population does demand caution when using these estimates in comparative studies, but *K. intermedius* may also be a special case.

The descriptive data obtained from both *Koptothrips* species are the first observations related to the natural history of the two invaders (Table 2.2). These observations provide an insight into the number of individuals that may be present in a *Koptothrips* invaded gall (Table 2.2). A statistical analysis was done to determine whether there are differences in the natural history of the two invaders. Although the difference in the number of females is non-significant, a statistically significant difference in the number of juveniles provides some support for the assertion that there may be a difference in the natural history of the two invaders (Table 2.2). Additionally, differences in female vs. juvenile correlations may also indicate a difference in the natural history (Table 2.2). A significant correlation between *Ko. flavicornis* females and larvae

suggests that more than one female may be reproducing simultaneously and the larvae present in the gall are the offspring of this group of females. The adult *Ko. flavicornis* females present within the gall are more likely to be non-brood members engaging in brood production. It is not clear whether these females are tolerant of each other, perhaps they are cooperating sisters, or they may have difficulty detecting one another. A non-significant correlation between *Ko.* dyskritus females and juveniles suggests that both are part of the same brood. The females were most likely to have emerged as adults within the gall rather than being a group of conspecifics invading a gall. The adult *Ko. dyskritus* females are likely be brood members that have matured rapidly within the gall itself.

Competition for a common resource leads to parasites engaging in diverse host exploitation patterns to avoid potential competition and conflicts with their competitors (Summers et al., 2003). While *Ko. flavicornis* and *Ko. dyskritus* do not show a preference in gall size (Table 2.2), their partitioning of *K. intermedius* galls could be linked to the timing of their invasions. Gall growth and development is an opportunity for resource exploitation by natural enemies (Stone and Schönrogge, 2003). Soldiers are the first to eclose within a *K. intermedius* gall, which is at the early stages of its life cycle. They were found in 33% of *Ko. flavicornis* invaded galls. This observation is consistent with the assertion that *Ko. flavicornis* invades host occupied galls, perhaps earlier in the life cycle of *K. intermedius*.

The presence of living host soldiers in an invaded gall suggests that *Ko. flavicornis* is behaving more like an inquiline which (as noted by Crespi et al., 2004) rears its offspring even when the original inhabitants are present. There have been instances in

non-galling *Acacia* thrips as well as in non-*Acacia* thrips where hosts and invaders have found to be cohabiting domiciles. Bono (2007), reports the presence of the parasitic thrips genus *Xaniothrips* in the domiciles of the phyllode gluer *Dunatothrips*. *Gynaikothrips ficorum*, which induces galls on *Ficus*, is sometimes seen to share its domicile with the kleptoparasite *Mesothrips jordani* (Tree and Walter, 2009). Crespi (1992a) suggests that multiple adults of both *K. intermedius* and *Ko. flavicornis* could sometimes coexist in a gall. It may be interesting to note here that the transition of the *Koptothrips* to a parasitic lifestyle has involved an inquiline stage in the past. *Koptothrips* would share a gall with the hosts rather than taking over (Crespi and Abbot, 1999; Crespi et al., 2004). Retaining some characteristics of an inquiline, *Ko. flavicornis* could enter a gall undetected and, probably, unchallenged. It could begin brood production even when the hosts are present. Cohabitation would enable *Ko. flavicornis* to exploit a gall while the hosts continue to maintain it. The effects that *Ko. flavicornis* might have on its host have not been investigated in this study. However in gall-inducing aphids, the offspring production of *Tamalia coweni* is negatively impacted when its galls are co-occupied by the inquiline, *Tamalia inquilina* (Miller, 2004).

Galls that were invaded by *Ko. dyskritus* were never found in my sample to also contain living host. This observation is consistent with *Ko. dyskritus* killing the hosts or entering galls that have already been abandoned. Populations of *Ko. dyskritus* that invade gall-inducers in the *Kladothrips rugosus* Froggatt species complex are known to be able to repair damaged and abandoned galls using anal secretions (Crespi and Mound, 1997; Crespi and Abbot, 1999; Crespi et al., 2004). An example of domicile utilization

that maybe similar to *Ko. dyskritus*' behaviour is seen in *Tamalia inquilinus*, where the inquilines are capable of occupying and reproducing in old abandoned galls long after the gall-inducers have emerged (Miller, 2005).

Neither *Koptothrips* species was ever observed to share a gall. This observation might indicate that when they do invade the same gall one or the other species is immediately driven out. Behavioural observations of *Ko. flavicornis* females in the presence of *Ko. dyskritus* females would be an important step to establishing if these two species are competitors. Unfortunately, my investigation did not note the condition of the gall before it was destroyed to census its contents. A subsequent study that notes breaches and repairs to the gall could more strongly establish whether *Ko. flavicornis* and *Ko. dyskritus* are exploiting the gall population differently and are thus avoiding competition. Similarly, by examining gall contents for debris from host bodies, we could infer whether the hosts were killed when they were invaded by *Ko. dyskritus*.

However, if these two invaders are directly competing with one another and periodically extirpating each other, they could persist in this population if there are refugia from which they can re-emerge. In the Middleback area there are other populations of gall-inducers that could act as reservoirs. In the next chapter, I utilize DNA sequence data to test connectivity between this population of *K. intermedius* invaders with the invaders of other gall-inducing species that are also present in the Middleback sheep station property.

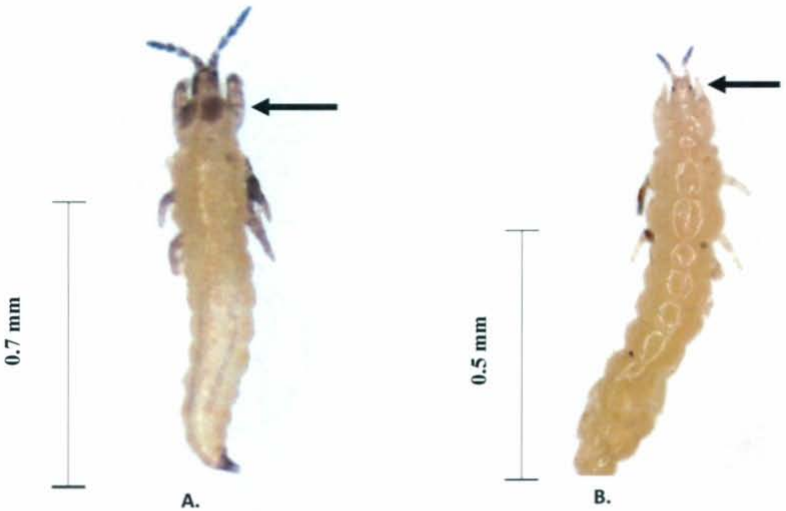

Figure 2.1. Images of *Kladothrips intermedius* and *Koptothrips flavicornis* larvae highlighting their distinguishing characteristics.

A. Arrow indicating the two brownish yellow spots on the dorsal side of the head of *Kladothrips intermedius* larva.

B. Arrow indicating the distinct eyes of the larva of *Koptothrips flavicornis*.

Note: A lack of identification keys for *Koptothrips* larvae makes it difficult to morphologically distinguish between *Ko. flavicornis* and *Ko. dyskritus* larvae. In this study, the presence of adult invaders within a *K. intermedius* invaded gall was used as a proxy to infer the taxonomic identification of the larvae. In no *Koptothrips* invaded gall were adults of both *Ko. flavicornis* and *Ko. dyskritus* found to be present. The characteristic distinct eyes described above for the larvae of *Ko. flavicornis* are also seen in *Ko. dyskritus* larvae.

Table 2.1. *Koptothrips* invasion rates in *Kladothrips intermedius* gall collections from Middleback, South Australia

Year	Galls collected	Galls invaded by *Koptothrips*	Invaded Galls		Galls containing live soldiers	
			Koptothrips flavicornis	***Koptothrips dyskritus***	***Koptothrips flavicornis***	***Koptothrips dyskritus***
2009	630	36	1	35	0	0
2008	170	37	21	16	7	0
2006[+]	48		*			
2004[++]	294	24	*			
1999[a]	423	131				

Note: A study by Chapman et al., 2006 [+] noted that *Ko. flavicornis** was found in these collections. Perry and colleagues (2004[++]) also collected *Koptothrips* for their study but they do not specify the species. However, Chapman (personal communication), who is a coauthor on the study, confirmed that *Ko. flavicornis** was collected from *K. intermedius* galls.

[a] Crespi and Abbot (1999) report *Koptothrips* invasion rates in *K. intermedius* galls that were not exclusively collected from Middleback, SA but from different locations in Australia. They are presented for comparative purposes.

Table 2.2. Summary of the descriptive data and test statistics obtained from *K. intermedius* galls invaded by *Ko. flavicornis* and *Ko. dyskritus*

	Koptothrips flavicornis	***Koptothrips dyskritus***
Average no. of females in a gall	6±1.08 (1-17)	7±2.66 (1-39)
Average no. of juveniles in a gall	51±5.51	19 ±3.89
Differences in no. of females in an invaded gall	n= 36, two tailed, P>0.05	
Differences in no. of juveniles in an invaded gall	**n= 36, two tailed, P<0.05**	
Differences in gall length preference	n= 33, two tailed, P>0.05	
Differences in gall width preference	n= 33, two tailed, P>0.05	
Correlation between *Ko. dyskritus* females and juveniles when the three outliers were included in the analysis	r = +0.462, P > 0.05, two-tailed, n = 16	
Correlation between *Ko. dyskritus* females and juveniles when the three outliers were excluded from the analysis	r = +0.12, P > 0.05, two-tailed, n = 13	
Correlation between *Ko. flavicornis* females and juveniles	**r = +0.497, P < 0.05, two-tailed, n = 20**	

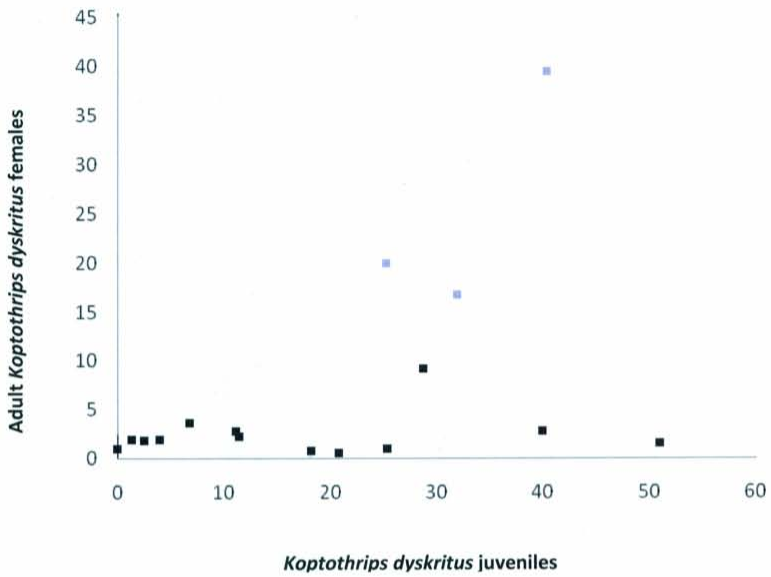

Figure 2.2. A scatter plot showing the relation between the number of *Ko. dyskritus* females and the number of *Ko. dyskritus* juveniles present within a gall. Data points that do not fit the observed trend are highlighted in grey.

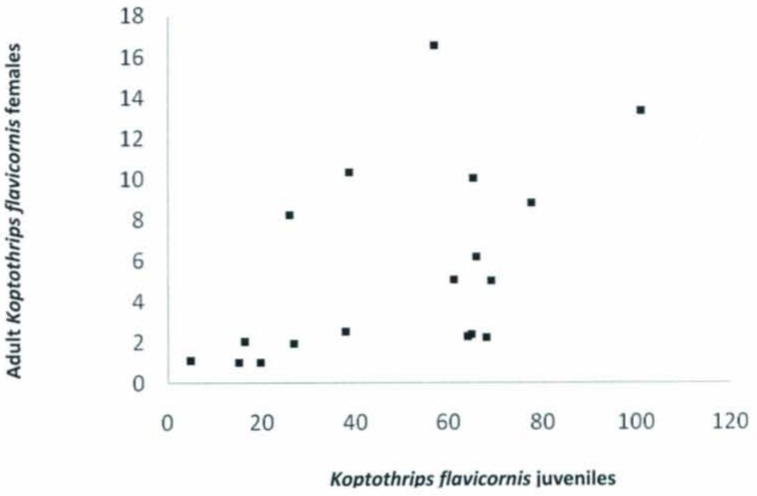

Figure 2.3. A scatter plot showing the relation between the number of *Ko. flavicornis* females and the number of *Ko. flavicornis* juveniles present within a gall.

Chapter 3

Host fidelity in *Koptothrips flavicornis* and *Koptothrips dyskritus* populations

3.1 Introduction

A major contribution to the generation of biodiversity is the coevolutionary relationship between insects and their plant hosts (Ehrlich and Raven, 1964; Farrell et al., 1992; Thompson, 1994). Insect-plant interactions are vital to our understanding of host specialization and speciation mechanisms among phytophagous insects (Mitter et al., 1988; Jaenike, 1990; Barraclough et al., 1998). In turn, insect-plant relations also affect the natural enemies of the herbivores (Price et al., 1980; Bernays and Graham, 1988; Fox et al., 1990).

Kladothrips display highly conserved host plant associations and a subsequent radiation in the host plants has led to diversification among the gall-inducing inhabitants (McLeish et al., 2007a; McLeish et al., 2007b). The strict *Acacia – Kladothrips* association is also thought to be mirrored in the host specific gall invasions of *Koptothrips* spp. Specialization on solitary *Kladothrips* hosts is observed among *Ko. xenus* and *Ko. zelus*, while invasions of multiple social and solitary *Kladothrips* taxa are observed in *Ko. flavicornis* and *Ko. dyskritus* respectively. My gall exploitation study (refer to Chapter 2) showed that both *Ko. flavicornis* and *Ko. dyskritus* are found in galls of the social host, *K. intermedius*. Having different invasion patterns to utilize the resources of a common host may have enabled *Ko. flavicornis* and *Ko. dyskritus* to successfully persist together on *K. intermedius* (refer to Chapter 2). The difference in invasions could have emerged much earlier in the life history of *Ko. flavicornis* and *Ko. dyskritus* when their niches were strictly partitioned along social and solitary hosts

respectively. Parasite divergence could occur through cospeciation with radiating hosts or through host shifts where they move between closely related hosts (Jaenike, 1990; Norton and Carpenter 1998; Roy 2001; Clayton et al., 2003; McCoy 2003; Clayton et al., 2004). The emergence of a geographical overlap between *Acacia papyrocarpa* and *Acacia oswaldii* around Middleback could have provided an opportunity for *Ko. flavicornis* and *Ko. dyskritus* to invade galls of the potentially parasite free *K. intermedius* lineage on *Acacia oswaldii*; and their independently evolved exploitation patterns may enable them to coexist in the long term without competitive exclusion. Crespi and Abbot's (1999) model for *Koptothrips* evolution suggests that *Ko. flavicornis* could move from social to solitary *Kladothrips* when present on the same *Acacia* tree. This assertion is vital to the hypothesis that if the invaders can shift *Kladothrips* hosts on *Acacia papyrocarpa*, they could also move to *K. intermedius* that occurs exclusively on *Acacia oswaldii* in instances of distributional overlap of the *Acacia* hosts.

At Middleback, *K. rugosus* (solitary), *K. waterhousei* (social) and *K. nicolsoni* (solitary) share a common host plant, *Acacia papyrocarpa*. Together with *K. intermedius*, (social) these four gallers are susceptible to *Koptothrips* invasions. Given the presence of overlapping galling hosts, two possible scenarios emerge as result of the invasion patterns of both invaders. The first scenario would be indicative of host fidelity, where populations of *Ko. flavicornis* and *Ko. dyskritus* are host-specific in their invasions. Galls of different *Kladothrips* species are partitioned among the *Koptothrips* populations that invade them. Such a resource partition is an opportunity for the generation of new species

(Nyman, 2009). Divergent niches contribute to the development of phenotypic and reproductive isolation (Ferguson, 2002; Nosil and Sandoval, 2008). The alternate scenario to host fidelity is the possibility of unrestricted invasions. Populations of both *Koptothrips* can freely move between galls of different *Kladothrips* on *Acacia papyrocarpa* and *Acacia oswaldii*. Shifting between *Kladothrips* species would imply that the *Koptothrips* populations do not specialize in invading specific hosts.

Determining if there is any form of gene flow between overlapping *Koptothrips* populations will enable us to interpret which scenario is likely. Molecular genetic studies are ideal for exploring levels of evolutionary divergence. Genetic divergences between *Koptothrips* populations that invade different hosts can be used as a proxy to infer the extent of host specialization. In this study, I investigated *Koptothrips* populations from two locations in South Australia, Middleback and Oodnadatta. Middleback is an ideal location to test host fidelity among *Koptothrips* as they have a range of host choices. Collections from Oodnadatta were compared with collections from Middleback to estimate the level of genetic differences between populations from the two locations. The level of nucleotide sequence divergence at the Cytochrome Oxidase I (COI) gene region was used to characterize the populations. COI is an effective bar-coding gene region (Hebert and Gregory, 2005) and nucleotide polymorphisms observed in this region are considered to be representative of evolutionary divergence between populations (Hebert et al., 2003). Sequence data obtained from CO I regions of different

individuals can also be used to estimate the level of divergence between populations within a phylogenetic approach (Hajibabaei et al., 2007).

3.2. Materials and Methods

3.2.1. Field sites and gall collections

Host *Kladothrips* galls used for this study were collected from two locations in South Australia – Middleback and Oodnadatta.

3.2.1.1. Middleback

Details of the field site at Middleback, *K. intermedius* gall collections and gall handling techniques are described in Chapter 2. *Kladothrips intermedius* galls were collected over two field seasons (2008 and 2009) for this study. In 2009, galls of *K. nicolsoni* were collected from *Acacia papyrocarpa*, which is commonly found in the region.

3.2.1.2. Oodnadatta

Oodnadatta is located approximately 800 km north of Middleback in South Australia's arid climatic region. *Acacia calcicola*, the host plant of *K. morrisi*, is commonly found along dry creek beds in this region. During the 2009 field season, galls of *K. morrisi* were collected along the Painted Desert–Evelyn Downs–Copper hills section of the Oodnadatta Track, an unpaved track that runs between Marla and Marree in South Australia.

3.2.2. Species identifications

The only gall inducer known from *Acacia oswaldii* is *K. intermedius*. Verification of *K. intermedius* and its invaders was made using the species descriptions and species keys developed by Crespi and colleagues (2004), (more detail provided in Chapter 2). Unlike *Ac. oswaldii* that is sparsely distributed in the Middleback property, *Acacia papyrocarpa* is more commonly found throughout the region. The gall-inducers on this tree [*K. rugosus*, *K. nicolsoni*, and *K. waterhousei* (*K. waterhousei* was not found in large enough numbers to collect the invading taxa as part of this study)] can be distinguished from one another by the shape of their galls. Identification keys and descriptions from Crespi and colleagues (2004) were used to verify *K. rugosus* and its invaders. Based on gall morphology, DNA sequence and microsatellite data, McLeish and colleagues (2006) newly described *K. nicolsoni*, although this species will still key out to *K. rugosus* using the key in Crespi et al. (2004).

Galls of *K. morrisi* are found on *Acacia calcicola* in the Oodnadatta region. *Acacia calcicola* can be identified by its silvery phyllodes (Maslin, 2001). *Kladothrips morrisi* galls are elongate and tubular with thick walls (Crespi et al., 2004). Again, identification keys from Crespi and colleagues (2004) were used to verify *K. morrisi* and its invader. Descriptions of characters that aided species identification of *K. intermedius*, *Ko. flavicornis* and *Ko. dyskritus*, are covered in Chapter 2 while those for *K. rugosus*, *K. nicolsoni* and *K. morrisi* are described in the following subsections.

3.2.2.1. *Kladothrips rugosus* (as described by Crespi et al., 2004)

Kladothrips rugosus individuals display a variably yellow colouration along their abdominal segments I-IV. Their heads are slender and their cheeks, while being convex, converge basally. Segments II-VIII of the antennae are invariably yellow; however, a yellowish brown coloration is seen along the fore tarsi and fore tibiae.

3.2.2.2. *Kladothrips nicolsoni* (as described by McLeish et al., 2006)

Until it was described as a separate species, *Kladothrips nicolsoni* was considered to be one of the sibling species within the *K. rugosus* complex. Identification keys for *K. rugosus* as described by Crespi and colleagues (2004) will still aid in identifying *K. nicolsoni*. However, the following description provided by McLeish and colleagues (2006) will help distinguish *K. nicolsoni* from *K. rugosus*. *Kladothrips nicolsoni* adults show a unique form of abdominal sculpturing that distinguishes them from *K. rugosus*. The sculpturing on the third abdominal tergite consists of reticulations that are uniformly sized in comparison to *K. rugosus*.

3.2.2.3. *Kladothrips morrisi* (as described by Crespi et al., 2004)

The III antennal segment of the adult dispersers is yellow while segments IV-VI are brown. Antennal segment IV has two sense cones. The pronotum is wider than long. Cheeks are convex. The forelimbs of the soldiers are enlarged while their wings and antennae are reduced. For the soldiers, the cheeks on the head are almost parallel.

3.2.2.4. Voucher specimens

Voucher specimens for *Ko. flavicornis* populations collected from the galls of *K. intermedius* and *K. nicolsoni* and for *Ko. dyskritus* populations collected from galls of

K. intermedius, *K. nicolsoni* and *K. morrisi* have been lodged at the Department of Biology, Memorial University (room SN4113) and with the Entomology Laboratory, Plant Health and Production, Ottawa in the Canadian National Collection.

3.2.3. DNA extractions, Polymerase Chain Reaction (PCR) amplification, and Cytochrome Oxidase subunit 1 (CO I) sequencing

DNA was extracted from adults and juveniles of *Ko. flavicornis* and *Ko. dyskritus* using a Qiagen DNEasy Extraction Kit. The entire body of the animal was used for DNA extraction. The extracted DNA was amplified in 15 µl reaction volumes. Each reaction tube consisted of 7.5 µl of ProMega GoTaq Colourless Master Mix, 4.7 µl of Nuclease free water, 0.6 µl of 10 µM concentration of each primer, 0.4 µl of 25 mM concentration of $MgCl_2$ and 1.2 µl of template DNA (average concentration, 12 ng/µl). The primers C 1-J-2183 (CAACATTTATTTTGATTTTTGG) and A-2735 (AAAAATGTTGAGGGAAAAATGTTA) (Morris et al., 2002) were used to amplify bi-directional partial sequences of the CO I region. DNA extractions were amplified using the following PCR protocol: 92 °C, 2 min Hot Start; 92 °C, 30 sec denaturation; a 10 cycle touchdown of 67 °C annealing (lowered by 3 °C every subsequent cycle) and a 72 °C 45 sec extension, which was followed by 41 cycles of 92 °C, 30 sec denaturation; 47 °C 45 sec annealing; and 72 °C 45 sec (increased by 1 second at every cycle) extension; and a final elongation at 72.0 °C for 8 min. PCR products of approximately 480-500 base pairs were obtained and then sequenced on an automated Applied Bio-Systems (ABI) 3730 DNA Analyzer through the Genomics and Proteomics (GaP) Laboratory at

Memorial University. Each reaction tube consisted of 0.5 µl of Big Dye Terminator (ABI v3.1 Cycle Sequencing Kit), 0.5 µl of 5X ABI Sequencing Mix, 15.8 µl of Nuclease free water and 2 µl of PCR product (average range of concentration (10-50 ng/ µl). Two sets of reactions tubes were prepared, and each tube within a set contained 0.32 µl (10mM concentration) of one primer (either C 1-J-2183 – forward primer or A-2735 – reverse primer) only to obtain a bidirectional sequence. The Sequencing Mix was added only after a hot start at 98 °C for 5 mins. Following this, the PCR protocol consisted of 25 cycles of 96 °C 10 sec denaturation, 47 °C 30 sec annealing and 60 °C 4 min elongation. The PCR products were maintained at 4°C. A clean-up step was followed before loading the PCR products onto the DNA Analyzer. Five µl of 125 mM EDTA and 6 µl of 95% ethanol were added to each sample and they were incubated for 30 min at 4°C. Post incubation, samples were centrifuged at 3000 rpm for 30 mins and the supernatant was discarded. A second wash of 150 µl of 70% ethanol was added to each tube and centrifuged at 3000 rpm for 15 mins. The supernatant was discarded and the samples were air-dried for 10-15 mins. Each sample was then re-suspended in 15 µl of ABI Hi-Di Formamide before being loaded onto the Analyzer.

3.2.4. Sequence accuracy

The *Koptothrips* bidirectional partial CO 1 sequences were edited using Sequencher (version 4.9) and a consensus sequence for each unique haplotype was obtained. Consensus sequences were then queried using the 'dicontiguous megablast' and 'blastn' search programs in GenBank's nucleotide database at NCBI to find similar

sequences. Quality parameters [such as organism name, its bit score and expect value (E-value)] of the search results were used as a criteria to assess the accuracy of the newly obtained *Koptothrips* sequences.

3.2.5. Genetic distance analysis

Estimating evolutionary distances are useful to interpret divergence times between populations (Tamura et al., 2007). Divergence between populations can be obtained by calculating genetic distances between taxa of interest. Genetic distance measures accumulated nucleotide differences between haplotypes (Nei, 1972). It is obtained by dividing the total number of nucleotide differences seen to the total number of nucleotides compared (Tamura et al., 2007).

Using ClustalW (Thompson et al., 1994), sequences were aligned with CO 1 sequences of both gall-inducers and gall-invaders that were deposited in GenBank prior to the start of this study (refer to Table 3.1 for Accession nos. and collection details). All sequences were then imported into MEGA (version 4.1), (Tamura et al., 2007) to estimate genetic distances between them. Distances were estimated between various gall-inducing taxa as well as between host- specific *Koptothrips* populations. This was done to determine whether the divergence observed among described *Kladothrips* species is congruent to the divergences observed in the *Koptothrips* populations that invade them. Pairwise distances were estimated using the Maximum Composite Likelihood Model for nucleotide variation analysis (Tamura et al., 2007). Analysis preferences for estimating evolutionary distances included pair wise deletion, both transition and transversion

nucleotide substitutions and a heterogeneous substitution pattern with a gamma parameter of 1 among lineages (Tamura & Nei 1993; Tamura et al., 2007).

3.3 Results

3.3.1. Gall collections

Koptothrips flavicornis was collected from galls of *K. intermedius* and *K. nicolsoni*. *Koptothrips dyskritus* was collected from *K. nicolsoni*, *K. rugosus*, *K. intermedius* and *K. morrisi*.

3.3.2. Haplotype diversity

3.3.2.1. *Koptothrips flavicornis*

A total of 22 specimens were sequenced. Sixteen specimens were sequenced from *K. intermedius* gall collections while six were sequenced from *K. nicolsoni* collections. From both collections, three distinct haplotypes were obtained. Each host-specific *Ko. flavicornis* population had one distinct haplotype as well as one that was common to both of them (Table 3.2). Two variable sites were observed within a 493-bp-length fragment (refer to Appendix for sequences). The sequences have been deposited in GenBank (Accession numbers HM 856187, HM 856188 and GU 979211).

3.3.2.2. *Koptothrips dyskritus*

A total of 17 specimens were sequenced. Twelve specimens (six each) were sequenced from *K. intermedius* and *K. nicolsoni* gall collections, three from *K. morrisi* and two from *K. rugosus* galls. Five distinct haplotypes were obtained from the *Ko.*

dyskritus populations collected from *K. intermedius* galls. Six distinct haplotypes were obtained from the remaining collections – two each for populations collected from the galls of *K. rugosus*, *K. nicolsoni* and *K. morrisi* (Table 3.2). A total of 67 variable sites were observed within a 488-bp-length fragment (refer to Appendix for sequences). The sequences have been deposited in GenBank (Accession numbers, HQ530529-HQ530539).

3.3.3. Sequence Accuracy

The queried *Koptothrips* sequences were a close match to the *Koptothrips* sequences that were already deposited in GenBank. E-values for the top three hits were always zero. This result ensured that the hits (GenBank deposited sequences) obtained were statistically significant (NCBI Handbook, 2002). For *Ko. flavicornis* haplotype, the closest match was the *Ko. flavicornis* CO I region (Accession no. AF448296) sequence followed by the CO I sequences of *K. intermedius* (Accession no. AY902988) and *Koptothrips* (species name not mentioned) (Accession no. AF448295). This was the first confirmed *Ko. dyskritus* haplotype sequence to be deposited in GenBank. The closest match for the *Ko. dyskritus* haplotypes sequenced in this study was also *Ko. flavicornis* CO I region (Accession no. AF448296) followed by CO I sequences of *Koptothrips xenus* (Accession no. AF448285) and *Koptothrips* (species name not mentioned) (Accession no. AF448295). Details of the sequence accuracy results are summarized in Table 3.3.

3.3.4. Genetic distances

Genetic distances have been expressed as percentages in the Results and Discussion sections while in the Tables (3.4a, 3.4b, 3.5a, 3.5b), they are written in decimal format.

3.3.4.1. *Kladothrips*

For the *Kladothrips* taxa that were host to *Ko. flavicornis*, genetic distances between them ranged from 18%-26% (Table 3.4a). The *Kladothrips* hosts of *Ko. dyskritus* differed by 7% when present on the same *Acacia* while differences between *Kladothrips* found on different *Acacias* in a common geographical region ranged between 14%-18% (Table 3.4b). Differences in *Kladothrips* collected from *Acacias* in different regions (Middleback and Oodnadatta) ranged between 12%-18% (Table 3.4b).

3.3.4.2. *Koptothrips flavicornis*

Within a common geographical region (Middleback, South Australia), genetic distances were estimated to be less than 1% between haplotypes of samples collected from galls of two different *Kladothrips* on different *Acacia* trees. When haplotypes from Middleback compared to haplotypes from another geographical region (Mildura, New South Wales, refer to Table 3.1 for sampling details), the collections from were about 5% divergent (Table 3.5a).

3.3.4.3. *Koptothrips dyskritus*

Differences between *Ko. dyskritus* haplotypes collected from *Kladothrips* galls induced on the same *Acacia* tree were less than 1%. For haplotypes collected from *Kladothrips* galls induced on different *Acacias* within a common geographical region, the differences ranged between 4%-5%. When comparing haplotypes from two different regions (Middleback and Oodnadatta), the differences ranged between 13%-15% (Table 3.5b).

One of the haplotypes (Hap. 1-5) collected from the galls of *K. intermedius* differed by as much as 9.8% from the other *Ko. dyskritus* individuals collected from this host. However, when compared to *Ko. dyskritus* collected from *K. morrisi*, this haplotype differed by just 3.9% (Table 3.6).

3.3.5. Evolutionary divergence and nucleotide variation (π)

3.3.5.1. *Koptothrips flavicornis*

Mean evolutionary divergence within subpopulations (collections from different *Kladothrips* hosts) and nucleotide diversity (π) the entire population was found to be low (less than 1%) (Table 3.7).

3.3.5.2. *Koptothrips dyskritus*

Mean evolutionary divergence within subpopulations collected from galls of *K. intermedius*, *K. nicolsoni* and *K. morrisi* was less than 1%. For collections from galls of *K. rugosus* the mean evolutionary divergence was 1%. Nucleotide diversity (π) for the entire population (collections from Middleback and Oodnadatta taken together) was found to be high (6.7%), (Table 3.8).

3.4 Discussion

Koptothrips dyskritus, which is thought to be specializing in invading galls of solitary *Kladothrips* species, was present in galls of social *Kladothrips* (*K. intermedius* and *K. morrisi*). Similarly, *Koptothrips flavicornis*, which be specializes in invading social *Kladothrips* species, was present in galls of *K. nicolsoni*, a solitary species. Genetic

distances between populations that overlapped spatially were less than 1% for *K. flavicornis* while for *K. dyskritus*, the highest difference was 5%. Taken together, the invader species found in various gall inducing hosts and the genetic distances between populations suggest that *Koptothrips* specialization may not be strictly partitioned along host lineages as suggested by Crespi and Abbot (1999).

The sequence accuracy results confirmed that the samples sequenced in this study were indeed the Cytochrome Oxidase sub unit I gene region of various *Koptothrips* individuals (Table 3.3). The results also ensured that subsequent analyses were conducted on the right organisms, i.e. between the *Koptothrips* populations that are the focus of this study.

Crespi and Abbot (1999) suggested that in instances where social and solitary *Kladothrips* share a common plant host, *Ko. flavicornis* could potentially move between its normal social host and the solitary species. This movement between hosts could have happened once with subsequent specialization on this new host (solitary species). The resulting two populations could then grow evolutionarily distinct, leading to increasing genetic distances. Alternatively, this population of *Koptothrips* could remain opportunistic on both hosts and, therefore, remain one interbreeding population and show little to no genetic divergence. In the case of *K. nicolsoni* and *K. rugosus*, *Ko. dyskritus* was expected to be present in this population and *Ko. flavicornis* could have invaded from its normal social host, *K. waterhousei* on *Acacia papyrocarpa*. Unfortunately, I did not find sufficient numbers of *K. waterhousei* galls to have been able to recover any of the invaders in that population. I was able to collect *Ko. dyskritus* from *K. nicolsoni* and *K.*

rugosus, which induce galls on the same host tree, *Acacia papyrocarpa*, as *K. waterhousei*. Based on these observations, the model for *Koptothrips* evolution (Crespi and Abbot, 1999) could usefully be extended to include movements of *Ko. dyskritus* between solitary-living species that co-occur on the same host tree and that estimates of genetic distance between these populations can provide insights into the likelihood of movements of invaders between gall-inducing hosts occupying the same tree in general.

Genetic distances between host specific *Koptothrips* populations must be sufficiently high to consider them as independently evolving taxa. Therefore, it is imperative to determine what levels of genetic divergence must be expected from the *Koptothrips* before they are described as host-specific invaders. An effective step in this direction would be to use the observed divergences between various host *Kladothrips* as a standard level of differentiation that would be expected from an evolutionarily distinct *Koptothrips* population. Tables 3.4a and 3.4b highlight genetic distances between various *Kladothrips* host and therefore, set the levels of divergences expected between *Koptothrips* populations, which maybe independently evolving as host-specific invaders.

Genetic distances for *Ko. flavicornis* populations collected from the galls of *K. intermedius* and *K. nicolsoni* were less than 1% (Table 3.5a). The hosts, *K. intermedius* and *K. nicolsoni* showed 18% divergence (Table 3.4a). Differences between *Ko. flavicornis* from Middleback and *Ko. flavicornis* collected from Mildura, New South Wales (located approximately 630 km from Middleback - refer to Morris et al., 2001 for collection details) were 5%-6%. In contrast, the following *Kladothrips* hosts, *K. habrus*, (collected from *Acacia melvelli* - refer to Morris et al., 2001 and Morris et al., 2002), *K.*

nicolsoni and *K. intermedius* differed by 18%-26% (Table 3.4a). The results presented here are consistent with Crespi and Abbot's (1999) observations of divergences between *Ko. flavicornis* collected from different host thrips taxa and *Acacia* species ranging 2.5% to about 7% (averaging about 3%). *Koptothrips flavicornis* that were collected by Crespi and Abbot, (1999) from host thrips (*K. rugosus and K. waterhousei*) on the same plant (either *Acacia loderi* or *Acacia ammophila*) differed by as little as 0.2%. While Crespi and Abbot (1999) collected *Ko. flavicornis* from galls of *K. rugosus*, there is a possibility that they may have also collected them from *K. nicolsoni*, which was part of the *K. rugosus* species complex at the time of their study. In this study, *Ko. flavicornis* was collected from a solitary host, *K. nicolsoni*. Both Crespi and Abbot's (1999) and my study indicate that *Ko. flavicornis* is shifting hosts, presumably from a social host (*K. waterhousei*) to solitary hosts (*K. rugosus* and *K. nicolsoni*). My results suggest that the invaders from all three gallers (*K. intermedius*, *K. rugosus* and possibly, *K. waterhousei*) are conspecifics that could be moving freely between hosts.

For *Ko. dyskritus*, populations collected from two hosts (*K. rugosus* and *K. nicolsoni*) on the same tree (*Acacia papyrocarpa*) were less than 1% different (Table 3.5b). However, differences between their *Kladothrips* hosts, *K. rugosus* and *K. nicolsoni* (found on *Acacia papyrocarpa*) were about 7.6% different (Table 3.4b). *Koptothrips dyskritus* collected from *Kladothrips* found different *Acacias* in the same region differed by 4.5%-5% (Table 3.5b). Differences between the hosts (*K. intermedius*, *K. nicolsoni* and *K. rugosus*) ranged from about 14% to 18% (Table 3.4b). Crespi and Abbot (1999), found divergences between *Ko. dyskritus* collected from different *Kladothrips* on

different *Acacias* ranging from 0.5%-6.9% (Crespi and Abbot, 1999). When *Ko. dyskritus* from Middleback was compared to *Ko. dyskritus* from Oodnadatta (from host thrips *K. morrisi* on *Acacia calcicola*) the differences ranged between 13% to about 16%, while differences between the *Kladothrips* ranged between 12%-16%. These differences among the invaders are high but not surprising since the large geographic distance between Middleback and Oodnadatta (approximately, 800 km) may have contributed to the genetic isolation between the *Ko. dyskritus* populations.

One exception to the above observations on *Ko. dyskritus* genetic divergences was the presence of a unique haplotype (Hap. 1-5) among *Ko. dyskritus* individuals from *K. intermedius* (Table 3.6). The level of divergence between this haplotype and the other *Ko. dyskritus* haplotypes was 9.8% . But when compared to *Ko. dyskritus* haplotypes obtained from *K. morrisi*, the differences were only 3.9%. Thrips are known to utilize wind currents to disperse aerially across larger distances (Lewis, 1964; Lewis, 1965; Mound, 1983; Lewis, 1991). It is not unexpected to find a *Ko. dyskritus* haplotype that is more closely related to a population found approximately 800 km away (Oodnadatta) since the individuals bearing this haplotype may have been carried away in circulating air streams to from Oodnadatta to Middleback.

While there is a higher rate of CO I divergence within the *Kladothrips* hosts, intra-species differences in *Ko. flavicornis* and *Ko. dyskritus* vary (Tables 3.4a, 3.4b, 3.5a and 3.5b). For populations collected from different *Kladothrips* on different *Acacias* that a share a geographical region, differences in *Ko. flavicornis* were less than 1% (Table 3.5a), while in *Ko. dyskritus*, they were as high as 5.1% (Table 3.5b). Nucleotide diversity

within the entire population was less than 1% in *Ko. flavicornis* and 6.7% in *Ko. dyskritus* (Tables 3.7 and 3.8). The high levels of nucleotide diversity (π) within the entire *Ko. dyskritus* collection (Table 3.8) is most likely to be influenced by the exceptional haplotype (Hap. 1-5) that was found in the galls of *K. intermedius* from Middleback (Table 3.6). For both species however, intra-population (host-gall specific) divergence is low (Tables 3.7 and 3.8). Variation in intra-species divergence for *Ko. dyskritus* individuals obtained from different *Kladothrips* and *Acacias* in a common geographical region merit further investigation.

Johnson and colleagues (2002) examined CO I sequences of populations belonging to the parasitic genera *Columbicola* and *Physconelloides* (Insecta: Phthiraptera), commonly known as dove lice. For both genera, they estimated divergences between species and between individuals belonging to the same species. Sequence divergence between species belonging to *Columbicola* ranged from19.5% to 25.6%, while for *Physconelloides* it was 8.9%-17.7%. Between individuals of a species, they report a range of 0%-21.4% for *Columbicola* and 0%-17.2% for *Physconelloides*. The estimates for intra-species variation in the above mentioned study are higher than what was obtained for either *Ko. flavicornis* (0.004%-5.8%) or *Ko. dyskritus* (0.04%-15.8%) (refer to Tables 3.5a and 3.5b). If the *Ko. flavicornis* haplotype from *K. habrus* (not sequenced in this study but obtained from GenBank) and the *Ko. dyskritus* haplotype from *K. morrisi* (collected at Oodnadatta) are excluded, the highest divergence estimate for *Koptothrips* populations from Middleback is 5.1% (refer to Tables 3.5a and 3.5b).

Specialization along *Kladothrips* host lineages that overlap in spatial distribution is almost nonexistent in *Ko. flavicornis*. *Koptothrips dyskritus* collections from different *Kladothrips* taxa on *Acacia* hosts in a common geographical region maybe as high as 4%-5%, but this data alone is insufficient to conclude that these populations are diverging from each other (Whalberg et al., 2003; Whinnett et al., 2005). The differences within *Ko. dyskritus* are non-congruent with the differences seen in their *Kladothrips* host. Host specialization would be manifested in the form of genetic isolations between the *Koptothrips* populations. However, the results presented here suggest that the *Koptothrips* shift between their hosts. While they can move freely between hosts on the same tree, it is not unexpected that they can move between host *Kladothrips* on different *Acacias* that overlap in distribution. Shifting between hosts provides an opportunity for the *Koptothrips* to maximize their opportunities for survival. *Koptothrips flavicornis* would find it easier to invade solitary hosts since they specialize in invading social *Kladothrips*. The distributional overlap of *Acacia oswaldii* and *Acacia papyrocarpa* in Middleback may have aided a shift from host thrips on *Acacia papyrocarpa* onto *K. intermedius* on *Acacia oswaldii*. *Koptothrips dyskritus* is able to enter *K. intermedius* galls as soldier production in this social host could be intermittent due to its relatively parasite free evolutionary history (Crespi et al., 2004; Chapman et al., 2008).

The presence of *Ko. dyskritus* in galls of *K. morrisi*, a social species is noteworthy. Perry and colleagues (2004) also collected *Ko. dyskritus* from galls of *K. morrisi*. *Kladothrips morrisi* soldiers have a lower propensity to fight *Koptothrips* (Perry et al., 2003). Unlike soldiers from other social species, they fight the *Koptothrips* by

grasping the abdomen and not the thorax of the invaders (Perry et al., 2003). The lower attack rates could be attributed to a higher fecundity in soldiers (Kranz et al., 2001; Perry et al., 2003; Kranz 2005). Soldiers of *K. morrisi* have a higher fecundity compared to other gall-inducers (Kranz et al., 2001; Kranz, 2005), which could result in a decrease in fighting ability. A reduced propensity to fight would make it easier for *Ko. dyskritus* to invade galls of *K. morrisi*. Further work around Oodnadatta could be directed towards determining the source population for *Ko. dyskritus* since there is more than one host gall inducer and host *Acacia* within the area. At Middleback, it would be interesting to note if *Ko. dyskritus* could invade galls of the social species, *K. waterhousei* since it is present on the same tree, *Acacia papyrocarpa* that plays host to *K. nicolsoni* and *K. waterhousei*.

3.5 Research highlights and suggestions for future research

Results of Chapter 2 indicate that in Middleback, *Ko. dyskritus* is present in the galls of a social host, *K. intermedius*. In the galls of *K. intermedius*, *Ko. flavicornis* was found cohabiting with soldiers while *Ko. dyskritus* was never found with living hosts. Multiple *Ko. flavicornis* females cohabited a gall and produced offspring. In contrast, *Koptothrips dyskritus* adult females are likely to be part of the same brood as the juveniles. Additionally, both *Ko. flavicornis* and *Ko. dyskritus* were never found to cohabit the same gall. Results of Chapter 3 indicate host shifting and an absence of host specialization among *Ko. flavicornis* and *Ko. dyskritus*. In Middleback, both *Koptothrips flavicornis* and *Koptothrips dyskritus* are opportunistic invaders of *K. intermedius* and *K.*

nicolsoni. Genetic divergence between *Koptothrips* populations in Middleback is low. The low levels of divergence imply that *Koptothrips* could be moving between hosts on *Acacia papyrocarpa* and *Acacia oswaldii*.

Shifting onto *K. intermedius* may force *Ko. dyskritus* and *Ko. flavicornis* to compete since there is now only one available host instead of the potentially three hosts on *Acacia papyrocarpa*. While Crespi and Abbot's (1999) model for *Koptothrips* evolution predicts invader specialization via host/parasite coevolution, my study indicates that there is movement between hosts and an absence of host specialization. Given *K. intermedius*' parasite free evolutionary history resulting in a facultative production of soldiers (Crespi et al., 2004; Chapman et al., 2008), the prospects of not encountering soldiers at all could also explain why *Ko. dyskritus* invasions are high in *K. intermedius* galls. *Acacia oswaldii* (host of *K. intermedius* at Middleback) is not closely related to other *Acacia* species that host *Kladothrips* (McLeish et al., 2007b). Therefore, *K. intermedius*' shift onto *Acacia oswaldii* is unusual, given that radiation among the gall-inducers has involved diversification along lineages of closely related *Acacias* (Crespi et al., 2004; McLeish et al., 2007b). *Acacia oswaldii* has a wide geographic distribution (Maslin, 2001; Crespi et al., 2004) and consequently, in areas where it overlaps with other *Acacia* hosts, *Koptothrips* could shift onto *K. intermedius* from other gall-inducers. While soldiers in eusocial hosts have evolved as a response to counteract the invasion pressures of the *Koptothrips* (primarily, *Ko. flavicornis* according to the model for *Koptothrips* evolution), their utility against *Ko. dyskritus* invasions remains to be tested. Investigating

whether other eusocial *Kladothrips*, especially those that share their *Acacia* hosts with solitary *Kladothrips*, suffer from *Ko. dyskritus* invasions would be invaluable to our understanding social evolution in this system.

Acacias that are host to both social and non-social gallers are ideal for testing hypotheses related to the evolutionary history of the *Koptothrips*. Any form of host specialization can be effectively tested in regions where there are both overlapping host *Acacias* as well as host *Kladothrips*. With overlapping distribution of gall-inducers, it is possible to test whether the *Koptothrips* have co-evolved and subsequently, co-radiated with their hosts. Noting the *Koptothrips* species invading a particular host can be used a direct inference for specialization. The presence of more than one gall inducer species offers the *Koptothrips* a choice of hosts. Therefore, specialization in invading a particular host can be confirmed when one invader is present in them while the other is absent. Alternatively, the presence or absence of both invaders in a particular host would be indicative of non-preferential invasions. Interpreting the absence of one or both invaders however, does demand caution as the seasonality and timing of invasion by either *Ko. dyskritus* or *K. flavicornis* could influence their presence in the galls of their *Kladothrips* hosts.

Building on the existing nucleotide database would be an effective start towards developing a well supported phylogeny for the *Koptothrips*. Sampling several *Koptothrips* populations from a wider geographical distribution would enable us to determine whether an invasion pattern similar to that observed in Middleback is present

elsewhere. A wider geographical coverage provides a greater opportunity to gather data related to invasion preferences and for testing the robustness of the *Koptothrips* evolutionary model. The use of differences in the CO I gene region as a sole indicator to interpret divergences between *Koptothrips* populations is controversial (Will and Rubinoff, 2004; DeSalle et al., 2005; Will et al., 2005; Galtier et al., 2009). Although microsatellites are highly effective in detecting genetic variability in social insects (Hughes and Queller, 1993; Queller et al., 1993), a combination of nuclear and mitochondrial markers would be ideal to investigate phylogeographic patterns (Sunnucks, 2000) between the morphologically-cryptic *Koptothrips* populations.

Complementing the molecular work would be behavioural experiments such as battle assays (similar to trails conducted by Perry et al., 2004) between *Ko. dyskritus* and *Ko. flavicornis*. Such a study would be useful to determine whether they can successfully fight off each other should they be found invading a common host. Moving *Ko. flavicornis* and *Ko. dyskritus* between their gall-inducing hosts from various *Acacias*, as suggested by Crespi and Abbot (1999), would provide us an insight into whether these kleptoparasites face any restrictions in movements between *Kladothrips* species. Invasion rates of *Koptothrips* collected from galls different hosts would give us a better insight on the extent of specialization or generalization between various populations as well as between the two invaders. Collecting descriptive data on the nature of gall breach during *Koptothrips* invasions and detecting evidence for gall repairing capabilities would enhance our knowledge on the natural history of these animals. Together, using these

three approaches (molecular, behavioural and natural history) in an integrated manner would aid our efforts to interpret the evolutionary history of the *Koptothrips*.

Table 3.1. Collection details and GenBank Accession Nos. of *Kladothrips* and *Koptothrips* sequences used for estimating genetic distances this study. These taxa were referenced from the following studies done on the *Acacia* thrips - Morris et al., 2001; Morris et al., 2002 and McLeish et al., 2006.

Taxon name	GenBank Accession No.	Host plant	Location and collection date
Kladothrips nicolsoni	AY827475	*Acacia papyrocarpa*	Middleback, SA, February 2002
Oncothrips tepperi (renamed as *Kladothrips intermedius*)	AF386687	*Acacia oswaldii*	Whyalla, SA, April 1999
Oncothrips habrus (renamed as *Kladothrips habrus*)	AF386692	*Acacia melvillei*	Mildura, NSW, June 1999
Oncothrips morrisi (renamed as *Kladothrips morrisi*)	AF386684	*Acacia calcicola*	Coober Pedy, SA, January 1999 (Coober Pedy is located near Oodnadatta)
Koptothrips flavicornis	AF448296	*Acacia melvillei*	Mildura, NSW, June 1999

Table 3.2. Distinct haplotypes observed in the various *Koptothrips* collections from different hosts *Kladothrips* and *Acacias*. These haplotypes have been submitted to GenBank.

Distinct haplotype*	Accession Nos.	Invader	Source gall	Host plant
Hap. 1-1	HQ530529	*Ko. dyskritus*	*K. intermedius*	*Acacia oswaldii*
Hap. 1-2	HQ530530	*Ko. dyskritus*	*K. intermedius*	*Acacia oswaldii*
Hap. 1-3	HQ530531	*Ko. dyskritus*	*K. intermedius*	*Acacia oswaldii*
Hap. 1-4	HQ530532	*Ko. dyskritus*	*K. intermedius*	*Acacia oswaldii*
Hap. 1-5	HQ530533	*Ko. dyskritus*	*K. intermedius*	*Acacia oswaldii*
Hap. 2-1	HQ530535	*Ko. dyskritus*	*K. nicolsoni*	*Acacia papyrocarpa*
Hap. 2-2	HQ530536	*Ko. dyskritus*	*K. nicolsoni*	*Acacia papyrocarpa*
Hap. 3-1	HQ530534	*Ko. dyskritus*	*K. rugosus*	*Acacia papyrocarpa*
Hap. 3-2	HQ530537	*Ko. dyskritus*	*K. rugosus*	*Acacia papyrocarpa*
Hap. 4-1	HQ530538	*Ko. dyskritus*	*K. morrisi*	*Acacia calcicola*
Hap. 4-2	HQ530539	*Ko. dyskritus*	*K. morrisi*	*Acacia calcicola*
Hap. 5-1	HM856187	*Ko. flavicornis*	*K. intermedius*	*Acacia oswaldii*
Hap. 5-2	HM856188	*Ko. flavicornis*	*K. nicolsoni*	*Acacia papyrocarpa*
Hap. 5-3	GU979211	*Ko. flavicornis*	*K. intermedius* and *K. nicolsoni*	*Acacia oswaldii* and *Acacia papyrocarpa*

Note:

* Distinct haplotypes refers to the different haplotypes found in *Ko. flavicornis* and *Ko. dyskritus* collections. Accession Nos. HQ530529- HQ530539 have not yet been made public on GenBank's nucleotide database. Please see Appendix for CO 1 sequences obtained in this study.

Table 3.3. An example of the search results obtained when *Koptothrips* sequences were queried in GenBank's nucleotide database. The name of hit obtained, its E (Expect)-value and Bit (Maximum) score results ensured positive identification. An E-value that is close to zero indicates that the hit is significantly relevant to the query while a high Bit score indicates that the hit obtained is well-aligned to the queried sequence (NCBI Handbook, 2002).

Koptothrips flavicornis			
Quality Parameters	**Search results - Top 3 hits**		
	First	**Second**	**Third**
Organism Name (gene region)	*Koptothrips flavicornis* (CO I gene region)	*Kladothrips intermedius* (CO I gene region)	*Koptothrips* sp. DM 467 (CO I gene region)
Accession No.	AF448296	AY902988	AF448295
Expect value (E-value)	<<0.001	<<0.001	<<0.001
Bit Score	737	659	652
Koptothrips dyskritus			
Quality Parameters	**Search results - Top 3 hits**		
	First	**Second**	**Third**
Organism Name (gene region)	*Koptothrips flavicornis* (CO I gene region)	*Koptothrips xenus* (CO I gene region)	*Koptothrips* sp. DM 467 (CO I gene region)
Accession Nos.	AF448296	AF448285	AF448295
Expect value (E-value)	<<0.001	<<0.001	<<0.001
Bit Score	684	666	663

Table 3.4a. Genetic distances between *Kladothrips* species from which *Ko. flavicornis* populations were collected. These sequences were deposited in GenBank from previous studies (please refer to Table 3.1 for collection details and Accession Nos.)

Genetic distances between different *Kladothrips* taxa			
	K. intermedius	*K. nicolsoni*	*K. habrus*
K. intermedius			
K. nicolsoni	0.18*		
K. habrus	0.26**	0.192**	

Note:

* Genetic distances between *Kladothrips* taxa on two different *Acacia* hosts (*Acacia oswaldii* and *Acacia papyrocarpa*) in Middleback, South Australia.

** Genetic distances between *Kladothrips* taxa on two different *Acacia* hosts (*Acacia papyrocarpa* and *Acacia melvelli*) in two different regions – Middleback, South Australia and Mildura, New South Wales.

Table 3.4b. Genetic distances between *Kladothrips* species from which *Ko. dyskritus* populations were collected. These sequences were deposited in GenBank from previous studies (please refer to Table 3.1 for collection details and Accession Nos.).

Genetic distances between different *Kladothrips* taxa				
	K. intermedius	*K. nicolsoni*	*K. rugosus*	*K. morrisi*
K. intermedius				
K. nicolsoni	0.18**			
K. rugosus	0.149**	0.076*		
K. morrisi	0.121***	0.188***	0.167***	

Note:

* Distances between *Kladothrips* taxa sharing the same *Acacia* tree (*Acacia papyrocarpa*) in Middleback, South Australia.

** Distances between *Kladothrips* taxa on two different *Acacia* hosts (*Acacia oswaldii* and *Acacia papyrocarpa*) in Middleback, South Australia.

*** Distances between *Kladothrips* taxa on three different *Acacia* hosts (*Acacia oswaldii*, *Acacia papyrocarpa Acacia calcicola*) in two different regions (Middleback and Oodnadatta) in South Australia.

Table 3.5a. Genetic distances between *Koptothrips flavicornis* populations

Genetic distances between *Ko. flavicornis* populations collected from different hosts			
	Ko. flavicornis (from *K. intermedius*)	*Ko. flavicornis* (from *K. nicolsoni*)	*Ko. flavicornis* (from *K. habrus*) *
Ko. flavicornis (from *K. intermedius*)			
Ko. flavicornis (from *K. nicolsoni*)	**0.005****		
Ko. flavicornis (from *K. habrus*)*	**0.058*****	**0.055*****	

Note:

* For collection details of *Ko. flavicornis* from galls of *K. habrus*, please refer to Table 3.1.

** Distances between *Ko. flavicornis* populations collected from galls of *Kladothrips* taxa on two different *Acacia* hosts (*Acacia oswaldii* and *Acacia papyrocarpa*) in Middleback, South Australia.

*** Distances between *Ko. flavicornis* populations collected from galls of *Kladothrips* taxa on two different *Acacia* hosts (*Acacia papyrocarpa* and *Acacia melvelli*) in two different regions – Middleback, South Australia and Mildura, New South Wales.

Table 3.5b. Genetic distances between *Koptothrips dyskritus* populations

Genetic distances between *Ko. dyskritus* populations collected from different hosts				
	Ko. dyskritus (from *K. intermedius*)	*Ko. dyskritus* (from *K. nicolsoni*)	*Ko. dyskritus* (from *K. rugosus*)	*Ko. dyskritus* (from *K. morrisi*)
Ko. dyskritus (from *K. intermedius*)				
Ko. dyskritus (from *K. nicolsoni*)	**0.045****			
Ko. dyskritus (from *K. rugosus*)	**0.051****	**0.005***		
Ko. dyskritus (from *K. morrisi*)	**0.158*****	**0.138*****	**0.147*****	

Note:

* Distances between *Ko. dyskritus* populations collected from galls of *Kladothrips* taxa sharing the same *Acacia* tree (*Acacia papyrocarpa*) in Middleback, South Australia.

** Distances between *Ko. dyskritus* populations collected from galls of *Kladothrips* taxa on two different *Acacia* hosts (*Acacia oswaldii* and *Acacia papyrocarpa*) in Middleback, South Australia.

*** Distances between *Ko. dyskritus* populations collected from galls of *Kladothrips* taxa on three different *Acacia* hosts (*Acacia oswaldii*, *Acacia papyrocarpa* and *Acacia calcicola*) in two different regions (Middleback and Oodnadatta) in South Australia.

Table 3.6. Genetic distance between the Exceptional *Koptothrips dyskritus* haplotype (Hap. 1-5) collected from *Kladothrips intermedius* and *Koptothrips dyskritus* haplotypes collected from *Kladothrips morrisi*

	Exceptional *Ko. dyskritus haplotype* (Hap. 1-5) (from *K .intermedius)* *
Ko. dyskritus (from *K. intermedius*)	**0.098**
Ko. dyskritus (from *K .morrisi*)	**0.039**

Note:

Exceptional *Ko. dyskritus* haplotype (from *K. intermedius*)* - The *Koptothrips dyskritus* haplotype that showed a high level of divergence when compared to other haplotypes from galls of *K. intermedius* and low level of divergence when compared to haplotypes from galls of *K. morrisi*.

Table 3.7. Evolutionary divergence and nucleotide diversity (π) within *Koptothrips flavicornis*

	Koptothrips flavicornis
Mean evolutionary divergence within collections from *K. intermedius* galls	0.001
Mean evolutionary divergence within collections from *K. nicolsoni* galls	0.001
Mean nucleotide diversity for entire population* (π)	0.002

Note:

Entire population*- The term 'entire population' refers to entire collection of *Ko. flavicornis* used in this study.

Table 3.8. Evolutionary divergence and nucleotide diversity (π) within *Koptothrips dyskritus*

	Koptothrips dyskritus
Mean evolutionary divergence within collections from *K. intermedius* galls*	0.007
Mean evolutionary divergence within collections from *K. nicolsoni* galls	0.001
Mean evolutionary divergence within collections from *K. rugosus* galls	0.01
Mean evolutionary divergence within collections from *K. morrisi* galls	0.003
Mean nucleotide diversity for entire population** (π)	0.067

Note:

1) * The exceptional haplotype (Hap. 1-5) (refer to Table 3.5) was not included when estimating the mean evolutionary divergence within collections from *K. intermedius*.

2) Entire population**- The term 'entire population' refers to entire collection of *Ko. dyskritus* (from both Oodnadatta and Middleback) used in this study. The 'exceptional haplotype' (refer to Table 3.5) was included when estimating the nucleotide diversity for the entire *Ko. dyskritus* population.

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Appendix

Cytochrome Oxidase sub unit I sequences of *Koptothrips dyskritus* individuals

Distinct haplotypes obtained from different *Koptothrips dyskritus* populations. Please refer to Table 3.2 for collection details and Accession Nos. for each sequence.

Dots indicate identical nucleotides throughout the sequence while polymorphic sites are indicated by the using the appropriate IUPAC code.

```
Hap._1-1 TTG GAA TTA TTT CCC AAG TAA TTT CTC ATG AAG TAG GAA AAA AAA GAT GTT TTG GGA ACT TGG GAA TAA TTT
Hap._1-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Hap._1-3 ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... .A. .T. ... ... ... ...
Hap._1-4 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Hap._1-5 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Hap._2-1 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Hap._2-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Hap._3-1 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Hap._3-2 ..T .G. ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ...
Hap._4-1 .C. ... ... ... .T. ... ... ... ... ... ... ... .T. ... ... ... ... ... .A. .T. .A. ... ... ...
Hap._4-2 .C. ... ... ... .T. ... ... ... ... ... ... ... .T. ... ... ... ... ... .A. .T. .A. ... ... ...
```

```
Hap._1-1 ACG CAA TAT TAT CTA TTG GCT TTT TAG GCT TTA TTG TTT GAG CTC ATC ATA TAT TTA CTA TTG GTA TAG ATA
Hap._1-2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Hap._1-3 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Hap._1-4 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .C. ... ... ... ... ... ...
Hap._1-5 ... ... ... ... ... ... .A. ... .G. .A. ... ... ... ... ... ... ... .C. ... ... ... .C. .A. ... ...
Hap._2-1 ... ... ... ... ... ... ... ... ... .T. ... ... ... ... .C. ... ... ... .C. ... ... ... ... ...
Hap._2-2 ... ... ... ... ... ... ... ... ... .T. ... ... ... ... .C. ... ... ... .C. ... ... ... ... ...
Hap._3-1 ... ... ... ... ... ... ... ... ... .T. ... ... ... ... .C. ... ... ... .C. ... ... ... ... ...
Hap._3-2 ... ... ... ... ... ... ... ... ... .T. ... ... ... ... .C. ... ... ... .C. ... ... ... ... ...
Hap._4-1 .T. ... ... ... ... ... .A. ... .G. .A. ... ... ... ... ... .C. .C. ... ... ... .C. .A. ... ...
Hap._4-2 .T. ... ... ... ... ... .A. ... .G. .A. ... ... ... ... ... CC. .C. ... ... ... .C. .A. ... ...
```

Hap._1-1 TTG ATA CTC GGG CAT ATT TCA CTG CAG CCA CTA TAG TAA TTG CTG TAC CTA CTG GAG TTA AAG TTT TTA GTT

Hap._1-2 ... ...

Hap._1-3 ... ...

Hap._1-4 ... ...

Hap._1-5 ... ... .A. ... ... .T. ... ... .A. ... ... .A. ... .A. ... .A. ... ...

Hap._2-1 ... ... .A. ... ...

Hap._2-2 ... ... .A. ... ...

Hap._3-1 ... ... .A. ... ...

Hap._3-2 ... ... .A. ... ...

Hap._4-1 ... ... .A. ... ... .T. ... ... .T. ... ... .A. ... ... .A. ... .A. ... .A. ... ...

Hap._4-2 ... ... .A. ... ... .T. ... ... .T. ... ... .A. ... ... .A. ... .A. ... .A. ... ...

Hap._1-1 GGT TAT CTA CCA TTA GAG GAA CTC TAA AAA AAA AAC TAT TCA AAT CCA CTA ATT TGT GGA GAT TAG GAT TTA

Hap._1-2 ... ...

Hap._1-3 ... ...

Hap._1-4 .A. ... ...

Hap._1-5 .A. ... ... .G. .GT ... ...A ... .T. ... ... .C. A. .A. ... ...

Hap._2-1 .A. ... ...T ..T ... ... .T. ... ... .A. ... ...

Hap._2-2 .A. ... ...T ..T ... ... .T. ... ... .A. ... ...

Hap._3-1 .A. ... ...T ..T ... ... .T. ... ... .A. ... ...

Hap._3-2 .A. ... ... .G. .GT. AT ... ...A ... .T. ... .T. ... ... .A. ... ...

Hap._4-1 .A. ... ...T ..T ... ... .T. ... ...C .A. ... ...

Hap._4-2 .A. ... ...T ..T ... ... .T. ... ...C .A. ... ...

Hap._1-1 TTT TTT TAT TCA CTT TAG GAG GAT TAA CAG GGG TTA TTC TTT CAA ACT CCT GTT TGG ATA TTA TAT TAC ATG

Hap._1-2 ... ...

Hap._1-3 ... ... .C. ... ...

Hap._1-4 ... ...

Hap._1-5 ... ... .C. ... ... .A. .A. ... ... .T. ... ..T .C. .A. ... ..C... .T. ...

Hap._2-1 ... ... .A. ..G ... ... .T. .T. ... ...

Hap._2-2 ... ... .A. ..G ... ... .T. .T. ... ...

Hap._3-1 ... ... .A. ..G ... ... .T. .T. ... ...

Hap._3-2 ... ... .A. ..G ... ... .T. .T. ... ...

Hap._4-1 ... ... .C. ... .T. .C. ... ... .A. .A. ... ... .T. ... .T..C. .A. ... ..C... .T. ...

Hap._4-2 ... ... .C. ... .T. .C. ... ... .A. .A. ... ... .T. ... .T..C. .A. ... ..C... .T. ...

Hap._1-1 ATA GTT ATT ATG TTG TTG CAC ATT TTC ATT ATG TAT TAT CAA TAG GTG CCG CTT TCG CAA TTT TTT CAG GAT

Hap._1-2 ... ...C. ... ...

Hap._1-3 ... ...C. ... ...

Hap._1-4 ... ...C. ... ... .T. ... ...

Hap._1-5 ... ... .T. ... ...

Hap._2-1 ... ... .C. ... ... .C. .C. ... ... .C. ... ...

Hap._2-2 ... ... .C. ... ... .C. .C. ... ... .C. ... ...

Hap._3-1 ... ... .C. ... ... .C. .C. ... ... .C. ... ...

Hap._3-2 ... ... .C. ... ... .C. .C. ... ... .C. ... ...

Hap._4-1 ... ... .C. .. .. .T. ... .T. ... ... .G C

Hap._4-2 ... ... .C. .. .. .T. ... .T. ... ... .G C

Hap._1-1 TTA TTT TTT GGT ACC CTC TGA TTA TAA ATT TAA CTT TAA ATG AAT TTT TAT TAA TAA AA

Hap._1-2 ... ...

Hap._1-3 ... ...

Hap._1-4 ... ...T .A. ... ...

Hap._1-5 ... .C. ... ... .T. ..T .A. ... ... .C. ... ...

Hap._2-1 ... ...T .A. ... ...

Hap._2-2 ... ... .T. ..T .A. ... ...

Hap._3-1 ... ... .T. ..T .A. ... ...

Hap._3-2 ... ... .T. ..T .A. ... ...

Hap._4-1 ... .C.... ... .T. ..T .AG... ... ... .C. ... ...

Hap._4-2 ... .C.... ... .T. ..T .AG... ... ... .CT ... ...

Cytochrome Oxidase sub unit I sequences of *Koptothrips flavicornis* individuals

Distinct haplotypes obtained from different *Koptothrips flavicornis* populations. Please refer to Table 3.2 for collection details and Accession Nos. for each sequence.

Dots indicate identical nucleotides throughout the sequence while polymorphic sites are indicated by the using the appropriate IUPAC code.

```
Hap._5-1  ATT TGG AAT TAT TTC TCA AGT AAT TTC CCA TGA AGT AGG AAA  GAA AAG ATT GTT TTG GAA ATC TAG GGA TAA
Hap._5-2  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...
Hap._5-3  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...

Hap._5-1  TTT ATG CTA TAT TAT CTA TTG GGT TTT TAG GAT TTA TTG TTT  GAG CCC ATC  ATA TAT TTA CTA TTG GAA TAG
Hap._5-2  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...
Hap._5-3  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...

Hap._5-1  ATA TTG ATA CCC CAG CAT ATT TTA CTG CAG CTA CTA TAG TAA  TTG CCG TTC  CTA CTG GAG TAA AAG TTT TTA
Hap._5-2  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... .T. ... ... ... ... ... ... ...
Hap._5-3  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... .T. ... ... ... ... ... ... ...

Hap._5-1  GCT GAT TAT CTA CAA TTA GAG GAT CTT TAA AAA GTA AAT TAT  TTA AAT CTA  CTA ATT TAT GAA GAC TAG GAT
Hap._5-2  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...
Hap._5-3  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...

Hap._5-1  TTA TTT TTT TAT TTA CTT TAG GGG GTT TAA CAG GAG TAA TTC  TTT CTA ATT  CCT GTT TAG ATA TTA TAC TTC
Hap._5-2  ... ... ... ... ... ... .G. ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...
Hap._5-3  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...

Hap._5-1  ATG ATA GTT ATT ATG TAG TTG CAC ATT TTC ACT ATG TAT TAT  CAA TAG GTG  CTG CCT TTG CAA TTT TTT CAG
Hap._5-2  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...
Hap._5-3  ... ... ... ... ... ... ... ... ... ... ... ... ... ...  ... ... ... ... ... ... ... ... ...
```

Hap._5-1 GTC TTA TTT TTT GAT ATC CTT TAG TTA TAA ATC TAG TTT TAA ATG AAT TTT TAT TAA AAA T

Hap._5-2 ... ...

Hap._5-3 ... ...