

Ecological Impacts of a Trait-mediated Cascade

By

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Abstract

Ecologists have generally recognized that trait-mediated indirect interactions (TMIs) can have important impacts on populations and communities. What is less understood is how and to what degree the interplay of TMIs would affect community dynamics. In this dissertation I take the study of TMIs one step further to examine a specific system in which two interacting TMIs act in concert, significantly regulating consumer-resource dynamics in the system. This system contains two interacting TMIs. The first one consists of a classic ant-hemipteran mutualism. The aggressive arboreal ant, *Azteca sericeasur*, through its aggressive behavior and harassment prevents the coccinellid beetle, *Azya orbiger*, from preying on the hemipteran *Coccus viridis*. The second TMI occurs when the phorid fly, *Pseudacteon lascinosus*, a parasitoid of *A. sericeasur*, induces anti-parasitism behavior of the ant. Since the phorid fly needs movement to attack the ant, the ant adopts a motionless posture when the phorid is present, effectively cancelling the first TMI. This ecological system is found in the coffee agroecosystem of the Neotropics and has important implications for pest management. Through a literature review, field surveys, and laboratory experiments I document the effects of these interacting TMIs on the coccinellid beetle, an important predator of scale insects in coffee. More specifically, my study suggests that the effects of a remote species that never directly interacts with organisms on lateral food chains can significantly affect several demographic parameters of the coccinellid beetle. It influences oviposition, parasitism and sex ratio, each of which is critical to population dynamics. The study also documents the effect of the interacting TMIs on the general arthropod community that uses coffee. My study also suggests that the specific TMIs interactions can have a broad community-wide impact.

Chapter 1 Introduction

Trait-mediated indirect interactions (TMIIIs) occur when the presence of a third species alters the interaction coefficient between two species (Werner & Peacor 2003). These kinds of non-consumptive, non-fatal interactions have been found to be widespread and as, if not more, important, than direct density-mediated interactions for the organisms involved (Abrams 1995; Peacor & Werner 1997; Werner & Peacor 2003; Schmitz *et al.* 2004; Preisser *et al.* 2005; Preisser & Bolnick 2008; Schmitz 2010; Ohgushi *et al.* 2012). Their effects at the population, community and ecosystem levels have been documented and tested with experiments in simple communities (Bolker *et al.* 2003; Werner & Peacor 2003; Schmitz *et al.* 2004; Preisser & Bolnick 2008; Beckerman 2010), and several reviews (Wootton 2002; Abrams 2010; Beckerman 2010) and meta-analyses (Preisser *et al.* 2005; Preisser *et al.* 2007) have synthesized this literature supporting their importance. In addition, and distinct from the density-mediated indirect effects which have been shown to attenuate through out food chains, trait-mediated indirect effects remain strong, a phenomenon recognized in both a meta-analysis (Bolnick & Preisser 2005) and a long-term old field experiment (Schmitz *et al.* 2004; Schmitz 2010).

The occurrence of TMIIIs involves behavioral, morphological and physiological plasticity in organisms. These interactions have been shown in a wide variety of ecosystems including terrestrial and aquatic. For example, in terrestrial ecosystems it is known that, in response to herbivory, plants can release volatile organic compounds that attract natural enemies that suppress herbivores (Vet & Dicke 1992; Dicke & van Loon 2000; Kessler & Baldwin 2001). This chemical ecology of tri-trophic interactions has aroused much attention in the ecological literature and is expanding to include explorations of TMIIIs effects within complex community contexts (Kessler *et al.* 2004; Poelman *et al.* 2008; Dicke & Baldwin 2010; Utsumi *et al.* 2010; Poelman *et al.* 2011; Bukovinszky *et al.* 2012; Poelman *et al.* 2012; de Rijk *et al.* 2013).

In aquatic ecosystems, aquatic preys are also known to respond to chemical cues of predators. For instance, Mowles and colleagues (2011) suggest that the chemical threats from the common shore crab *Carcinus maenas* arouses a strong anti-predation response of *Littorina littorea*, an intertidal gastropod mollusk. *Littorina littorea* is a stronger competitor in the absence of the crab. The competition hierarchy, however, is reversed in the presence of the crab. As in terrestrial ecosystems, chemically-mediated interactions in aquatic systems change the behavior of organisms and, in doing so, strongly affect population structure, community organization and ecosystem function without the direct consumptive effect of the predator (Hay 2009).

Not all behavioral changes are induced chemically. For instance, anuran preys reduce foraging time or shift habitat when predators are within detectable ranges, either chemically or visually (Relyea 2000; Schoener *et al.* 2002; Schmitz *et al.* 2004; Losos *et al.* 2006; Mowles *et al.* 2011). Similarly, it has been reported that, in a long-term old-field experiment, *Melanoplus femurrubrum* grasshoppers differentially shift in space in response to predatory spiders that possess different hunting modes. In particular, sedentary and actively-hunting spiders present different levels of threats - sedentary spiders clearly induce habitat-shifting behavior of the grasshopper. The different predator-prey combinations therefore lead to a different results in species composition of primary producers and plant biomass (Schmitz *et al.* 2004; Schmitz 2010).

Morphological changes in organisms have also been implicated in TMIIIs. For instance, larval anurans can develop wider mouths in a competitive environment. In the presence of predators, however, larval anurans morphologically reduce their mouth widths to pre-competition level. The variation of phenotypic plasticity affects resource consumption and can have an impact on competition outcome, consequently affecting the growth rate of each anuran species *per-se* (Relyea 2000).

Most of the empirical and theoretical research on TMIIIs have focused on simple units of a pairwise interaction with a third modifier species, However, recent literature explores more complex systems with multiple interaction modifications that are likely to coexist in nature

(Arditi *et al.* 2005; Goudard & Loreau 2008; Hutsumi *et al.*, 2010; Golubski & Abrams 2011; Kefi *et al.* 2012; Utsumi *et al.* 2010). These interacting units of TMIs can affect trophic interactions in profound ways. Golubski and Abrams (2011) synthesize the available literature and conclude that interaction modifications could decrease or increase each other's effects, as antagonistic effects become more common. Examining available literature, they highlight the need to explore community structures that include multiple interaction modifications that are located at different trophic levels and do not function in the same way as focal trophic links, as these interactions are lacking investigations. More specifically, they propose explorations into multiple interaction modifications with structures as those described, for example, in Liere and Larson (2010). This system consists of two interacting TMIs. The first one consists of a classic ant-hemipteran mutualism. The aggressive arboreal ant, *Azteca sericeasur*, through its aggressive behavior and harassment prevents the coccinellid beetle, *Azya orbigera*, from preying on the hemipteran *Coccus viridis* (Fig. 1a). The second TMI occurs when the phorid fly, *Pseudacteon lascinosus*, a parasitoid of *A. sericeasur*, induces anti-parasitism behavior of the ant. Since the phorid fly needs movement to attack the ant (Mathis *et al.*, 2011), the ant adopts a motionless posture when the phorid is present, effectively cancelling the first TMI (Fig. 1b; Hsieh and Perfecto, 2012). According to Liere and Larson (2010) the presence of the phorid fly reduces the activity of the ant creating a window of opportunity that allows adults of *A. orbigera* to feed on more *C. viridis*. The indirect effect of the phorid fly therefore increases the interaction coefficient between a consumer and a resource, and hence, a cascading trait-mediated indirect interaction occurs.

In this dissertation, I focus on this system with multiple interaction modifications at different trophic levels (Fig. 2). Four research chapters are included.

- I. Chapter 2: A review paper that explores the community effects of phorid-ant interactions in general.
- II. Chapter 3: The effect of ant chemical ecology on the population dynamics of *A. orbigera*. This is an endeavor driven by the observations of the life history of the study system, and a reflection of the “reliability-and-detectability” of infochemicals as in plant chemical ecology (Vet & Dicke 1992). This paper also examines

- experimentally the effect that the presence of interacting TMIs has on the oviposition of the beetle that preys on the hemipteran (Figure 1-2, question mark 1).
- III. Chapter 4: An extension of the study system to include an organism at the higher trophic level, a parasitoid wasp of the larvae of *A. orbiger*. In this chapter, I explore whether the cascading trait-mediated effects of phorid-ant interactions can affect the parasitism and sex-ratio of *A. orbiger* (see questions marks in Figure 1-2).
 - IV. Chapter 5: Exploration of the potential broader community-wide impacts of phorid-ant interactions. In this chapter, through a field experiment, I explore the effects of the phorid-ant interactions on other arthropods that use coffee plants (Fig. 1-3). Since the ants are very aggressive and tend to attack a broad range of arthropods that use coffee, I expect these arthropods to take advantage of the window of opportunity created by the phorid –ant interaction.
 - V. Chapter 6: a summary of dissertation findings and implications to ecology.

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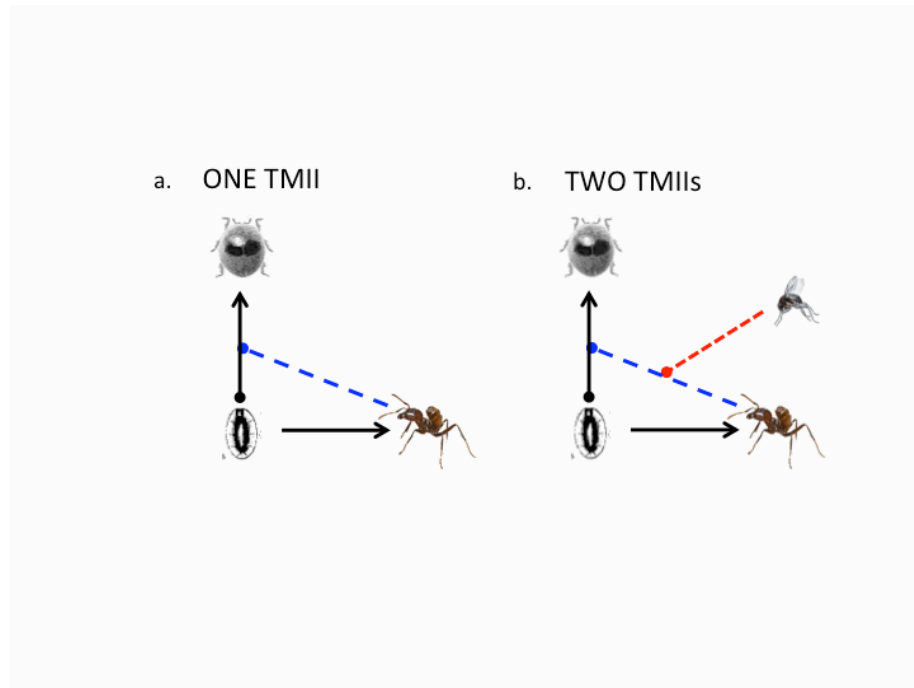


Figure 1-1 Trait-mediated indirect interactions. a) One trait-mediated indirect interaction where an ant disrupt a predator-prey relationship between a coccinellid beetle and an hemipteran; b) Two interacting trait-mediated indirect interaction where a parasitoid cancel the ant's disruption of the predator-prey interaction. Solid black lines represent direct interactions, blue dashed lines represent first level of trait-mediated interactions and red dashed lines represent second level of trait-mediated interactions; arrows indicate positive effects and solid circles indicate negative effects.

Figure 2

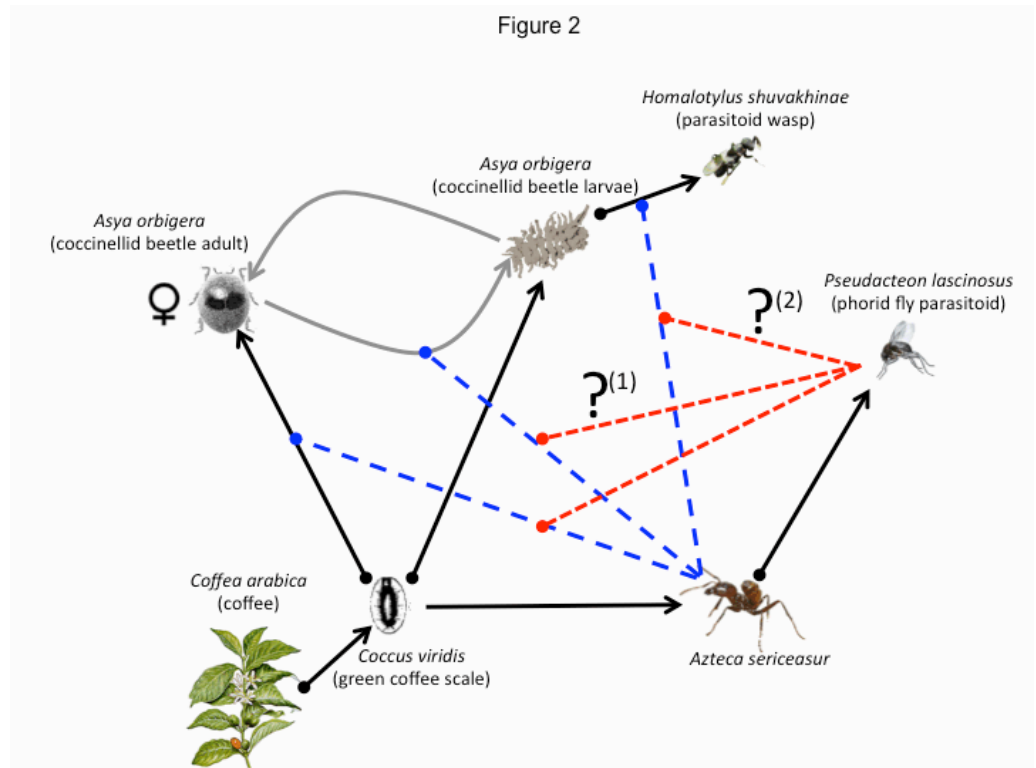


Figure 1-2 A network of multiple TMIs. Solid black lines represent direct interactions, solid grey curves represent reproduction, blue dashed lines represent first level of trait-mediated interactions and red dashed lines represent second level of trait-mediated interactions; arrows indicate positive effects and solid circles indicate negative effects. The two question marks are placed over the interactions that are investigated in this dissertation.

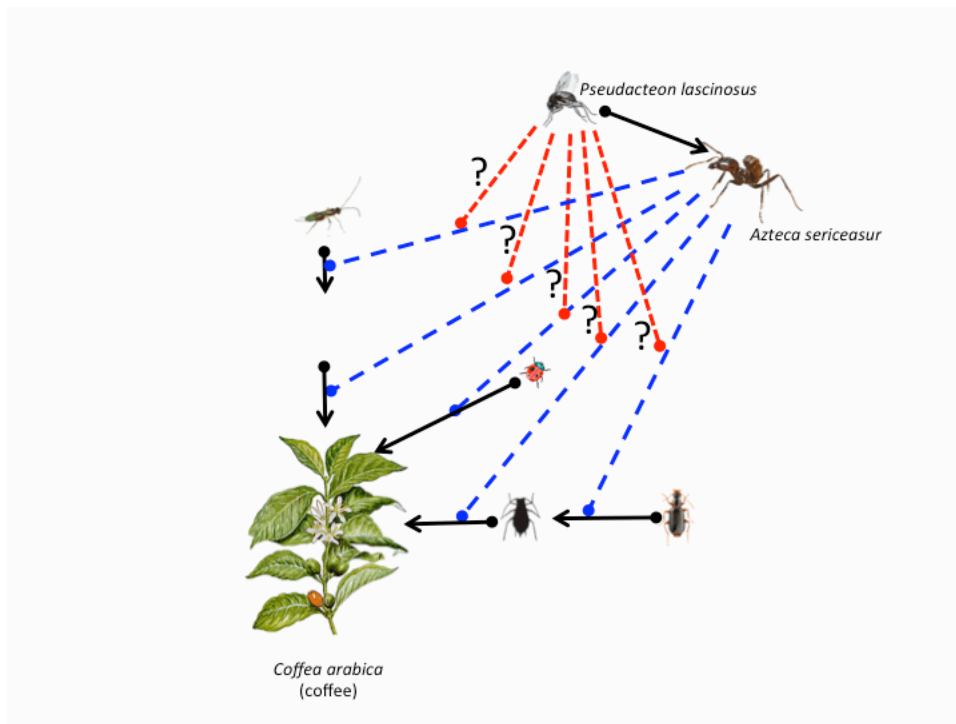


Figure 1-3 Potential community-wide impacts of the phorid-ant interaction through interacting TMIs. Solid black lines represent presumed direct interactions, blue dashed lines represent first level of trait-mediated interactions and red dashed lines represent second level of trait-mediated interactions; arrows indicate positive effects and solid circles indicate negative effects.

Chapter 2 Trait-Mediated Indirect Effects of Phorid Flies on Ants

(This chapter has been published on *Psyche: An Entomology Journal* in 2012).

2.1. Abstract

This paper provides a synthesis of the ecological impact of phorid fly parasitoids on ants. We find the most important impact of phorids on ants to be trait-mediated effects. Phorids diminish the foraging activity of ants, frequently reducing the number and average size of foragers and reducing the amount of food retrieved by a colony. However, ants' coping mechanisms include changing foraging site and time. Phorids can also affect competition, especially through changes in the ability of the host to win in exploitative competition. Factors such as microclimate, resource size, and habitat complexity interact with phorids to change their effect on competition. By being highly specific and attacking ants high in the competitive hierarchy, phorids can alter the linear nature of the competitive transitivity, and by reducing the number of foragers, they can change the discovery-dominance tradeoff that is observed in some ant communities. Trait-mediated effects of phorids also cascade to other trophic levels. As an example, we discuss the trait-mediated cascade of phorids on the *Azteca sericeasur* system in coffee. In this system, by reducing the foraging activity of *A. sericeasur*, phorids reduce the direct and indirect biological control impact of the ant in the coffee agroecosystem.

2.2. Introduction

The best-studied family of ant parasitoids, Phoridae, has been recognized as an important mediator of ant community structure (Feener 2000). Indeed, over the past decades, there have been many studies on the impacts of phorid attacks on ants, from the effects on ant foraging activity, size of foragers, and amount of food retrieval, to the effects at the community level involving several interacting species at different trophic levels. What is clear from this literature is that the main consequences of phorid attacks on ants are not direct density effects

but rather effects mediated by changes in the behavior of ants, the so-called trait-mediated indirect effects (TMIEs) (Werner and Peacor 2003). Although many reviews have been written about TMIE generally (Bolker *et al.* 2003; Werner and Peacor 2003; Schmitz *et al.* 2004), there has not been a review on how phorid flies impact ant communities through trait-mediated indirect interactions (TMIIIs). Since phorid parasitoids attack mainly workers and parasitism rates tend to be very low, their direct impact on the colony is minor (Feener 2000; Morrison and Porter 2005). However, attacking phorid flies elicit ant defensive behaviors that can have repercussions at the community level. These trait-mediated effects have been shown to be important for understanding invasibility of ants (Briano *et al.* 2012) as well as the role of ants in biological control of agricultural pests (Vandermeer *et al.* 2010).

In this paper we provide a synthesis of studies on the ecological impacts of phorids on ant assemblages and ecological networks focusing on TMII. The literature review focuses on studies published over the last ten years, since Feener's review (Feener 2000). However, we also use some of the older literature to support our conclusions. We first examine the effects of phorid parasitoids on ant foraging activity, including number of foragers, forager sizes, and amount of food retrieved. We then examine the evidence for the hypothesis that phorid parasitoids alter the outcome of competition among ants. More specifically, we examine evidence for the impact of phorid flies on exploitative and interference competition and for how parasitoids may alter competitive dominances among ant assemblages (Porter and Savignano 1990; Feener 2000; LeBrun 2005; Feener *et al.* 2008). Finally, we present evidence for trait-mediated effects that transcend ant assemblages and result in changes in the broader community including ant prey, ant mutualists, and the predators of the mutualists. For this last section, we focus on our own work of *Azteca sericeasur* in coffee plantations.

2.3. Direct Density Effects of Phorid Parasitization on Ants

Studies examining direct mortality due to phorid parasitism on ants have reported very low parasitism rates. For example, *Pseudacteon* parasitism on *Solenopsis geminata* has been reported to be only 3% (Morrison *et al.* 1997), while *Apocephalus* parasitization on *Pheidole* has been reported to be 5% (Feener 1981; Feener 1988). Parasitization rates of ants in their introduced ranges can be even lower. Morrison and Porter (2005) reported a 0.058% of

average parasitism rate per colony of *Solenopsis invicta*, a host ant species of *Pseudacteon tricuspis* Borgmeier in a time-span of two years study, in northern Florida. In his review, Feener (2000) also indicated that the effects of density-mediated interaction between phorid parasitoids and host ants are normally very low, with 1-2% as the likely magnitude of the effect of direct phorid parasitism on host ant density reduction. Since most phorid flies that parasitize ants are highly host specific, use ant pheromones to find their host (Mathis and Philpott 2012), and attack workers while foraging, retrieving food resources, or performing other tasks outside their nest, they are bound to elicit specialized defensive behavior that can result in TMIE (Feener 2000). Most of the research on the effects of phorid flies on ants has focused on these trait-mediated interactions between phorids and their ant hosts.

2.4. Trait-mediated Effects on Ant Foraging Activity, Resource Acquisition, and Defense

2.4.1. Number of Foragers

One of the first recognized TMIE of phorids on ants was their effect on foraging activity (Orr 1992). Most ant colonies show behavioral responses to attacks by phorids. The most common behavioral response is a reduction in the number of foragers. Most of the studies show that the number of foragers or ants recruited to a resource is reduced significantly in the presence of phorids (Table 2-1) (Orr 1992; Bragança *et al.* 1998; Folgarait and Gilbert 1999; Morrison 1999; Erthal and Tonhasca 2000; Morrison 2000; Tonhasca *et al.* 2001; LeBrun and Feener 2002; Mehdiabadi and Gilbert, 2002; Philpott *et al.* 2004; Philpott 2005; Vieira-Neto *et al.* 2006; Yackulia and Lewis 2007; Liere and Larsen 2010; Puckett and Harris 2010). In the case of *Azteca sericeasur*, this activity reduction results from two actions on the part of the individual workers. First, some ants go inside their nest, and second, some ants acquire a defensive posture and stop moving (Vandermeer *et al.* 2010). This defensive stationary posture has also been observed in *S. geminata* (Morrison 1999). It has been shown that some species of *Pseudacteon* that parasitize *A. sericeasur* and *Solenopsis* species require movement of the host in order to oviposit (Mathis *et al.* 2011; Mathis and Philpott 2012). The combination of these two actions on the part of *A. sericeasur* and *S. geminata* workers results

in a reduction of 50% of the foraging activity in these two species (Morrison 1999; Philpott 2005; Liere and Larsen 2010). Reductions in ant foraging activity in the presence of phorid flies have also been reported for *Linepithema* (Orr *et al.* 2003; Bragança *et al.* 2008), *Pheidole* (Feener 1981; Feener 1988), and *Atta* (Bragança *et al.* 1998; Tonhasca 1996). Ants can also respond to phorid attacks by increasing foraging activity during periods of time when phorids are not active, for example, at night. This seems to be the case for several species of the leaf cutter ants in the genus *Atta* (Orr 1992; Bragança *et al.* 1998; Kackulic and Lewis 2007), for *Linepithema* (Orr *et al.* 2003), and for *A. sericeasur* (de la Mora, unpublished data).

2.4.2. Size of Foragers

Phorid parasitoids also influence the size of foragers (Table 2-1). The pattern of worker size selection by ovipositing female phorid flies has been described for a few species of *Pseudacteon* on *Solenopsis* (Feener 1987, Morrison 1992; Campiolo *et al.* 1994; Morrison and Gilbert 1998), and *Neodohniphora* on *Atta* (Silva *et al.* 2008; Bragança *et al.* 2009). Mathis and Philpott (2012) discuss ant size as a factor in host acceptance by phorid species. Differences in host size preferences within the fire ants are seen as an effective niche partitioning when several species attack the same host (Orr *et al.* 1997). However, in general, phorid parasitoids tend to prefer larger than average workers. In these cases, the response of the ants to the presence of phorids is to reduce the average size of the foragers (Orr *et al.* 1997; Erthal and Tonhasca 2000; Morrison 2000; Viera-Neto *et al.* 2006; Yackulic and Lewis 2007; Puckett and Harris 2010). Morrison and Gilbert (1998) reported that the size of the emergent phorid was positively related to the size of the host worker with females emerging from a larger host. If ant colonies respond to phorid attacks by changing the size distribution of foragers, this can alter the phorid's sex ratio and can potentially affect the efficiency of phorid parasitoids in biological control of invasive ants (Morrison and Gilbert 1998).

2.4.3. Acquisition of Food Resources

The reduction in the number and size of foragers can have an effect on the ability of ants to obtain and defend food resources (Orr 1992; Bragança *et al.* 1998; Folgarait and Gilbert

1999; Erthal and Tonhasca 2000; Morrison 2000; Mehdiabad and Gilbert 2002; Vieira-Neto *et al.* 2006; Yackulic and Lewis 2007) (Table 2-1). Laboratory and field studies have reported up to 50% reduction in food acquisition by *S. invicta* in the presence of phorid flies (Tonhasca 1996; Morrison 1999; Morrison 2000). In laboratory experiments, Mehdiabadi and Gilbert (2002) showed that the presence of only one phorid fly per 200 workers of *S. invicta* reduced the number of large size workers 50 days later. In the same experiment, they demonstrated that the reduction in foraging and size of foraging workers resulted in a nearly twofold reduction of protein colony consumption. Reduction in the amount of food consumed in the presence of phorid flies has been reported for other ant genera including *Linepithema* (Folgarait and Gilbert 1999) and *Pheidole* (LeBrun and Feener 2002). However, in another laboratory experiment with *S. invicta* and its *Pseudacteon* parasitoids, Morrison (2000) showed that in control trials, where no phorids were present, food retrieval was intermediate to that of the phorid-no-phorid trials, suggesting that ants are foraging more in the no- phorid trials (of the phorid-no-phorid trials) to compensate for the reduction in food retrieval from the phorid-present trials. This kind of compensation can happen in the field if the ants forage more during periods of no-phorid activity, as discussed previously, or shift to forage underground when phorids are present, something that has been shown to happen in *Solenopsis* (Tennant and Porter 1991). Furthermore, in a laboratory experiment, Ramirez *et al.* (Ramirez *et al.* 2006) reported that reduction in food retrieval was not observed when the trials were left running for a period of 72 hours. These experiments suggest that in the long run and under field conditions ants that are attacked by phorid parasitoids compensate for potential losses in the amount of food retrieved by foraging more at times when phorids are not active.

2.4.4. Compensatory Factors

Other factors can help host species compensate for the negative trait-mediated effects of phorid parasitoids. For example, habitat complexity in the form of leaf litter provides refuge from parasitoids for soldier caste of *Pheidole diversipilosa* and *P. bicarinata* resulting in an increased number of foraging soldiers even in the presence of phorid parasitoids (Wilkinson and Feener 2007). Habitat complexity, thus, allows these two species to balance foraging success with the avoidance of parasitism. Likewise, the size and distribution of resources can

have similar effects. In a field experiment, Wilkinson and Feener (2010) demonstrated that the presence of multiple large resources allows colonies of *P. diversipilosa* to redistribute soldier ants from sites that have phorid flies to sites that do not have phorids, therefore maintaining overall numbers of foraging soldiers at the same levels as found in the absence of phorid parasitoids. There is a gap in our knowledge about how many ant hosts mitigate the threat of phorid parasitism by altering regimes, altering posture of exposure, or by foraging on other resources. It is possible that we may be overestimating the population level impact of phorid parasitoids on ants by focusing on daytime interactions or by not measuring other population level parameters such as density, occupancy, and colony migration.

2.5. Phorid Parasitoids and Competition within Ant Assemblages

The kinds of behavioral changes described in the previous section can have important consequences for ant community structure. Since phorid parasitoids tend to be highly specific and attack only one or very few species of ants within a community, they can alter the competitive interactions and change ant community structure (Feener 2000). This effect can be especially important when the host ant is a competitive dominant species, which in the best-studied cases of ant-phorid interactions they frequently are, probably because dominant ant species are evolutionarily more conspicuous (Feener 2000).

When phorid parasitoids are present, host species are faced with a tradeoff between defending themselves against parasitism and maximizing their competitive abilities. The outcome of this tradeoff is not always clear. Based on the evidence of the TMIE of phorid parasitoids on host ants, it is tempting to conclude that phorid parasitoids reduce the competitive ability of host species. However, this is not always the case. Indeed, competitive interactions among ants are complex and influenced by a variety of factors, and therefore, a generalized outcome of the effects of phorid parasitoids on ant competitive interactions is highly unlikely. What we see in the literature is a reflection of that complexity.

2.5.1. Exploitative and Interference Competition

Ants of different species engage in exploitative and interference competition with each other

(Savolainen and Vepsäläinen 1988; Morrison 1999; LeBrun and Feener 2002; Orr *et al.* 2003; Mehdiabadi *et al.* 2004; Perfecto and Vandermeer 2011). Exploitative competition occurs when the removal of a limiting resource by one species makes it unavailable for other species, while interference competition involves direct aggressive interactions between individuals of different species. The presence of phorid parasitoids has been shown to influence both of these types of competition but this is, in no way, a universal phenomenon. For example, in laboratory experiments, phorid parasitoids were found to increase the exploitative competitive ability of *Forelius mccooki*, a competitor of the host species, *S. invicta*. However, phorids did not affect the direct aggressive interactions between the two species (Mehdiabadi *et al.* 2004). Furthermore, although the competitor of the host species increased the number of foragers by a factor of two in the presence of phorid flies, that did not translate into higher colony growth. Similar results have been reported in field experiments. A study of the competitive interactions between *S. geminata* and *S. invicta* in the presence of phorid parasitoids of *S. geminata* found that the host species retrieved 50% less food than the nonhost species in the presence of phorid flies (Morrison 1999). Much the same as in the lab experiment, in the field, phorid parasitoids had no effect on the interspecific aggression between *S. geminata* and *S. invicta* and did not affect the outcome of these interactions at resources. In the case of *A. sericeasur*, phorids also seem to influence exploitative but not interference competition (Philpott 2005; Philpott *et al.* 2009). In field experiments, competitors of *A. sericeasur* were able to access bait resources 12 times more often in the presence of phorids and were able to take over baits only when phorid parasitoids of *A. sericeasur* were present. However, in most cases, *A. sericeasur* did not lose competitive interactions with other species (Philpott *et al.* 2009). The lack of an effect in the interference competition interactions between host and non-host species could be due to the behavioral response of the ants engaged in the fight or a change in behavior of the phorid parasitoids. For example, *S. geminata* has been seen to ignore attacking phorids when engaged in fights with *S. invicta* (Morrison 1999). But, phorid parasitoids have also been observed to lose interest or be distracted by ants that are engaged in active fighting with other ants. Feener (Feener 1981) presented the first evidence for the TMIE of phorids on interference competition between the host species *Pheidole dentata* and its competitor, *Diplorhoptum*

texanum (referred to as *Solenopsis texana*). It is reported that parasitism by phorids was the factor that most strongly influenced the turnover of resources from *P. diversipilosa* to its competitors (LeBrun and Feener 2002). The same study also reports that phorid parasitoids reduce exploitative competitive abilities of *P. diversipilosa* (LeBrun and Feener 2002). On the other hand, Orr et al. (Orr *et al.* 2003) report that phorid parasitoids seldom influence exploitative competition between two *Linepithema* species and their nonhost competitors in Brazil. This field study joins others that have not been able to detect clear effects of phorids on ant competition (Morrison *et al.* 2000).

2.5.2. Factors That Interact with Phorid Parasitoids to Affect Competition

The lack of a clear pattern on the effect of phorid's TMIE on ant competition has to do with the many other factors that are involved in determining the winners and losers of both exploitative and interference competition. Among the potential factors, here we will discuss four, for which there is some evidence in the literature: feedback loops caused by ant chemical pheromones, size and distribution of resources, habitat complexity, and abiotic factors such as temperature and humidity.

Phorid flies are known to use ant kairomones released by their host ant to locate them (Mathis and Philpott 2012). When an ant encounters a competitor, it is more likely to release alarm pheromones that can be used by their phorid parasitoids to find them more easily, causing a positive feedback that may result in a higher turnover rate of resources from host species to their competitors (LeBrun and Feener 2002). There are at least two cases where these kinds of positive feedbacks have been documented. The parasitoid *Apocephalus* sp. discovers faster and arrives in greater numbers at recruitment events where its host species, *P. diversipilosa*, is engaged in competitive conflict than to recruitment events where the host is foraging alone or does not experience conflict (LeBrun and Feener 2002). Likewise, parasitoids of two species of *Linepithema* arrived significantly faster at resources where the host was with another ant species than when it was alone (Orr *et al.* 2003). Furthermore, the rate at which phorid flies arrive at baits depends on the competitor species present and the type of response it elicits from *Linepithema*. Phorid parasitoids arrived faster at baits when the competitor elicits a chemical response versus baits where the competitor elicits primarily

physical aggression (Orr *et al.* 2003). If host ants engaged in direct competition with other species elicit faster and stronger responses from their phorid parasitoids than those that do not encounter competitors, phorids can have an even stronger effect on competition through this positive feedback. Moreover, if the feedback mechanism works for some competitors and not others, as in the case of *Linepithema* and its competitors, the impact of phorids on community structure and colony energetic will depend on these behavioral responses and will be different in different community contexts.

The effects of resource size and distribution and habitat complexity were discussed in the previous section in the context of compensation mechanisms for acquiring resources under the pressure of phorid attacks. These factors can also buffer the impacts of phorids on competitive interactions between host species and nonhost species (LeBrun and Feener 2002; LeBrun 2005; Wilinon and Feener 2007; Wilkinson and Feener 2010). For example, habitat complexity, by allowing continued foraging even when phorids are present, can influence the competitive success of the host species (Wilkinson and Feener 2007). Likewise, widely distributed resources may allow host species to redistribute their foragers to resources not monitored by phorids and continue succeeding in exploitative competition (Wilkinson and Feener 2011). Recruitment to large resources, on the other hand, could increase the number of phorid attacks but the effect of resource size has not been well explored in the literature. In general, ants that recruit to resources tend to recruit more and larger workers to larger resources (Folgarait and Gilbert 1999; LeBrun and Feener 2002). Since phorid parasitoids show a density-dependent response to ants (Philpott *et al.* 2009), higher numbers of ants at a resource will attract higher numbers of phorid parasitoids. Therefore, a higher proportion of large resources at a particular site could represent a liability for those host species that recruit to large resources, which is the case for most species attacked by phorids. However, if a higher proportion of larger resources also results in greater availability of large resources to hosts, ant hosts would be able to switch to resources not monitored by parasitoids (Wilkinson and Feener 2007).

Temperature and humidity affect not only ants but also phorids (Hölldobler and Wilson 1990; Folgarait *et al.* 2003; Chen *et al.* 2005; LeBrun *et al.* 2008). These two variables could

interact to lead to very different competitive outcomes under varying environmental conditions. For example, parasitoid habitat preferences (see Mathis and Philpott 2012) have been shown to cause major differences on parasitism pressure on host ants and their interactions with competitors (Feener *et al.* 2008). In laboratory experiments, Ramirez *et al.* (2006) demonstrated that changes in humidity interact with the presence of phorid parasitoids to alter the competitive outcome of encounters between the invasive *S. invicta* and the native species *S. xyloni*. They attributed the lack of establishment and spread of *S. invicta* in New Mexico to these interactions.

2.5.3. Competitive Dominance Hierarchies and Species Coexistence

Interspecific competition can have profound effects on the abundance, composition, and distribution of species. Communities structured by competition can be organized in a variety of ways that can greatly influence species coexistence and, therefore, the maintenance of diversity within a community. Competitive communities that are organized in a linear transitive dominance hierarchy will tend to have low species diversity because, at equilibrium, the competitive dominant species will exclude all others. On the other hand, intransitive hierarchies, a situation in which the competing species cannot be ranked in a perfect competitive hierarchy, can promote diversity (Buss 1980; Huisman *et al.* 2001; Kerr *et al.* 2003; Laird and Schamp 2008; Rojas-Echenique and Allesina 2011; Vandermeer and Yitbarek 2012). Interspecific competition has been identified as an important factor in structuring ant communities, especially among ground foraging omnivorous ants that forage more or less for the same resources (Davidson 1985; Fellers 1987; Savolainen and Vepsäläinen 1988; Herbers 1989; Anderson and Patel 1994; Perfecto 1994; Human and Gordon 1996; Perfecto and Vandermeer 1996; Sanders and Gordon 2003; Parr *et al.* 2005). However, to date, no competitive intransitivity has been convincingly demonstrated for any ant community. Rather, ant communities have been described to be organized in transitive dominance hierarchies (Fellers 1987; Savolainen and Vepsäläinen 1988; Bestelmeyer 2000; Palmer *et al.* 2000; LeBrun 2005; Feener *et al.* 2008). A question then emerges as to how ant communities are able to maintain species diversity under conditions of transitive dominant hierarchies. TMIE mediated by phorid parasitoids can provide a partial answer to this question (Feener 1981; LeBrun and Feener 2002; LeBrun 2005), although other factors such

as environmental variation [56, 75, 79] and size of resources (Kaspari 1996; Cerdá *et al.* 1998; LeBrun 2005; Wilinon and Feener 2010) have called into question the generality of the transitive dominance hierarchies among ant communities.

By being highly specific and attacking ants that tend to be high in the competitive hierarchy, phorids can alter the linear nature of the competitive transitivity. In a study of the ant community in pine-oak woodlands in Arizona, LeBrun (2005) describes several distinct dominance hierarchies within the ant assemblage. However, the linearity of the dominance hierarchies was determined by the size of the resource and the presence of phorid parasitoids. When competing for fixed resources or for small nonfixed resources in the absence of phorids, the assemblage exhibits significant linear dominance hierarchies. In contrast, in the presence of phorids for both fixed and small resources, this linearity breaks down (LeBrun 2005). For example, on fixed resources, phorids caused the second dominant species to drop to the second most subordinate, and the third species dropped to the fourth position. These changes in the ranking of species dominance generated more indeterminacy in the outcome of individual paired interactions reducing the asymmetries underlying the dominance in the transitive hierarchy. It has been shown, at least theoretically, that when interactions take place locally, which is the case for ants competing for food resources, an increase in symmetry favors diversity (Vandermeer and Yitbarek 2012), providing a potential mechanism for the maintenance of diversity in ant communities.

2.6. Trait-mediated Cascades: The Case of *Azteca sericeasur* in the Coffee Agroecosystem

Ants are an important component of ecosystems in most regions of the world. Since they frequently constitute a great part of the animal biomass in ecosystems, are taxonomically diverse, and act as ecosystem engineers (Folgarait 1998; Vandermeer and Perfecto 2007; Lach *et al.* 2010), they tend to interact with many other organisms. Given the strong TMIE of phorids on ants, and given the wide range of ecological interactions that ants form with other organisms, it should come as no surprise that these TMIEs cascade into other trophic levels of an ecological community. The best-documented case of these sorts of phorid-mediated cascading effects can be found in the *A. sericeasur* system. For more than ten years, we have

been studying the ecological interactions surrounding this ant species in coffee plantations in southern Mexico (for a review see Vandermeer *et al.* 2007). Here we will describe the pivotal role that phorid parasitoids play in shaping these interactions.

Azteca sericeasur is a dominant arboreal ant with a wide distribution in the Americas, from Brazil to Mexico (Kempf 1972). On coffee plantations it is found nesting in shade trees and foraging on both shade trees and coffee plants. This species forms spatial clusters of nests that have a high genetic relatedness (Remfert, unpublished data). The clusters appear to be the result of self-organization emerging from the internal dynamics of the system—short distance dispersal to adjacent trees and density-dependent mortality (Vandermeer *et al.* 2008). One of the main resources for *A. sericeasur* in the coffee plantations is honeydew from *Coccus viridis*, the green coffee scale (Vandermeer and Perfecto 2006). This mutualism plays an essential role in the distribution of the scale insect, which is a potential pest in coffee (Adler *et al.* 2007). *Azteca sericeasur* has been reported to prey on a variety of herbivores in coffee plantations contributing to the control of potential insects pests (Vandermeer *et al.* 2002; Philpott *et al.* 2004; Vandermeer and Perfecto 2006; de la Mora *et al.* 2008; Gonthier *et al.* 2010; Vandermeer *et al.* 2010; Pardee and Philpott 2011). The effect of *A. sericeasur* on deterring herbivores is not only through the direct action of preying or removing herbivores from plants, but also through an indirect effect in which some herbivores avoid plants that have been foraged on by *A. sericeasur*, but were not actually where present when the herbivores arrive (Gonthier *et al.* 2010). Additionally, it has been shown that through a complex network of ecological interactions *A. sericeasur* is a keystone species that contributes to the regulation of insect pests and diseases in coffee (Vandermeer *et al.* 2010; Perfecto and Vandermeer 2008; Jackson *et al.* 2009; Vandermeer *et al.* 2009). *Azteca sericeasur* also competes with other arboreal ant species, especially twig-nesting species (Philpott 2010), and influences the abundance and diversity of ground nesting and arboreal ant species (Perfecto and Vandermeer, 2013; Ennis, unpublished data) and spiders (Marin and Perfecto, 2013). The mutualism between *A. sericeasur* and the scale insects consists of protection of scales from parasitoids and predators, especially the coccinellid beetle, *Azya orbigera* (Perfecto and Vandermeer 2008; Liere and Larson 2010), and removal

of sooty mold (Jha *et al.*, 2012).

Philpott *et al.* (2004) published the first documented case of a phorid parasitoid attacking *A. sericeasur*. At that time it was thought that only one species of *Pseudacteon* was responsible for the attacks. However, recently (Brown & Philpott (2012) three species have been described attacking *A. sericeasur*. *Pseudacteon* spp. have strong TMIE on *A. sericeasur* (Philpott *et al.* 2004; Philpott 2005; Philpott *et al.* 2009), as reported previously. More importantly, these TMIEs cascade to other trophic levels within the community, with important implications for the biological control of insect herbivores and diseases of coffee (Perfecto and Vandermeer 2008; Vandermeer *et al.* 2010).

By reducing *A. sericeasur* foraging activity, phorids disrupt the ability of the ants to remove insect pests from coffee (Philpott *et al.* 2004). In laboratory experiments, it was shown that phorids essentially cancel the ability of *A. sericeasur* to deter coffee berry borer attacks on coffee fruits (Pardee and Philpott 2011). Likewise, we have demonstrated that the presence of phorids reduces the ability of ants to attack, carry away, and force off plants lepidopteran caterpillars that could be potential pests in coffee (Philpott *et al.* 2004).

Higher-order cascading trait-mediated indirect effects have also been documented for this system (Vandermeer *et al.* 2010; Liere and Larson 2010; Perfecto and Vandermeer 2008). The protection that the ants offer to their scale mutualist is the first level trait-mediated indirect effect-the ants disrupt the ability of the predatory beetle to kill and consume scale insects. By causing a reduction in the foraging activity of *A. sericeasur*, phorids disrupt the ability of the ant to protect its mutualist, the green coffee scale (Liere and Larson 2010). This is the second order trait mediated indirect effect (Figure 2-1). When phorids are present, they essentially cancel the protective effect of ants against adults of *A. orbigera*, the coccinellid predator. In laboratory experiments, in the presence of ants and phorids adults of the predatory beetle were able to gain access to the scale and consume the same quantities as when no ants (and no phorids) were present. In other words, through these higher order cascading trait mediated indirect effects, the phorids facilitate the coccinellid beetle (Figure 2-1).

The complexity of this network of interactions increases when the larval stage of the coccinellid beetle is considered. The larva of *A. orbigera* is covered by waxy filaments that protect it from ant predation (Liere and Perfecto 2008). This means that larvae of the main predator of the scales are able to live in patches of high ant activity where the scale is abundant. Furthermore, the ants repel parasitoids in the vicinity of the scale insects, including any parasitoids of the coccinellid beetle, essentially protecting coccinellid larvae (Liere and Perfecto 2008). The presence of phorids could, potentially, eliminate this unintended protective effect of the ants on the coccinellid larvae, by reducing ant patrolling on clusters of scales. However, this interaction has not been yet documented.

Our research also shows that gravid female beetles of *A. orbigera* are able to eavesdrop on the “phorid-alert pheromones” (Hsieh *et al.* 2012; Chapter 4) and oviposit under green coffee scales or other clandestine microsites that workers of *A. sericeasur* and natural enemies of *A. orbigera* would have difficulty finding, removing, and predating. The natural history and interactions between *Pseudacteon* spp., *A. sericeasur*, and *A. orbigera* can well explain why we can find high abundances of *A. orbigera* in the coffee agroecosystem. Since this is the main predator of the green coffee-scale, and it seems to require patches of *A. sericeasur* for the successful development of its larvae, it can be argued that the maintenance of the *Azteca-green* coffee scale mutualism is essential for the successful biological control of the green scale at the level of the entire farm (Vandermeer *et al.* 2008; Vandermeer *et al.* 2010).

Theoretically and empirically, parasitism in spatially distinct patches has been suggested to be an important driver of spatial self-organization of host-parasitoid dynamics (Hassell *et al.* 1991). The *Azteca* system in the coffee agroecosystem adds empirical evidence to the theory of spatial self-organization in host-parasitoid systems. We proposed that *Pseudacteon* spp. contributes to the spatial pattern formation of *A. sericeasur* by acting as a density-dependent control mechanism (Vandermeer *et al.* 2008). Given the fact that the coccinellid beetle is able to capitalize on the trait mediated interaction between *Pseudacteon* spp. and *A. sericeasur*, we suggest that adding trait-mediated cascades to theoretical models would increase our understanding of how complex systems might contribute to spatial self-organization and

system stability. Furthermore, the *A. sericeasur*-*Pseudacteon* spp. - *A. orbiger*a system illustrates how trait-mediated cascades effect biological control in a spatially explicit complex ecosystems.

2.7. Conclusions

Phorid fly parasitoids influence ants mainly through trait-mediated indirect interactions. The presence of phorid flies results in a reduction of foragers, a change in the average size of foragers, mainly toward the smaller sizes, shifts in the time and places of foraging to avoid encounters with phorids, and reduction in the amount of food retrieved. These effects, independently or in combination, have important consequences for the way ants interact with other ant species and with other members of the interacting network within a community.

Through these TMIEs phorids can have important effects on competitive interactions among ants. When phorid parasitoids are present, host species respond behaviorally and can impact their competitive abilities. However, since competitive interactions among ants are complex and influenced by a variety of factors, the outcome of the effects of phorid parasitoids on ant competitive interactions is highly variable. Phorids have been shown to reduce exploitative competitive abilities of some host species but not others. Likewise, they have been shown to affect the interference competition between host and nonhost, but this effect is not widespread among studies. It has been shown that phorid parasitoids can break a competitive hierarchy within ant assemblages by attacking the most competitive dominant species within the hierarchy. Phorids also can influence the dominance- discovery tradeoff that is found in some ant assemblages. The alteration of the competitive structure of ant assemblages could be important in understanding invasibility of ants to ranges where their phorid parasitoids are absent.

Trait-mediated effects of phorids on ants can also transcend the ant assemblage and have cascading effects on other trophic levels and other organisms linked to the host ant species through complex ecological networks. For example, phorid parasitoids can also influence the impact of ants on herbivores. If the host species is an important predator of an herbivore, the presence of phorids can release these herbivores from predation pressure from ants. This

could be important in agroecosystems where ants have been shown to be important predators of insect pests. The study of *A. sericeasur* in coffee plantations presents an excellent case study of these cascading trait-mediated indirect interactions and shows that they could be important in maintaining biological control.

Many areas of research remain open in the study of ant-phorid interactions. In particular there are very few studies that link TMIEs of phorids to population level consequences in ants and other organisms. Making and testing predictions regarding the TMIEs of phorids on population density, occupancy, colonization, and migration patterns across landscapes should be priority of future studies. The *Azteca* system described previously represents a step in the right direction to fill this gap in our knowledge of ant-phorid interactions. However, this system is only one example of the many complex ecological networks that could be influenced by phorid parasitoids. Future studies should focus on these kinds of complex ecological networks and on trait-mediated cascading effects that would be important in understanding the role of ants when they are embedded in complex ecological networks.

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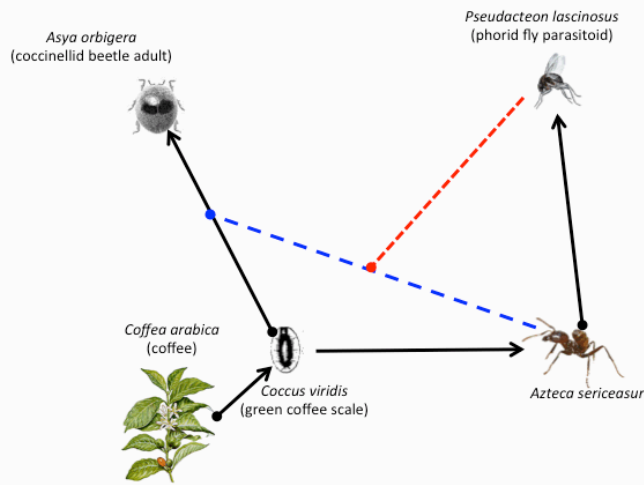


Figure 2-1 Diagrammatic representation of the cascading trait-mediated indirect interactions between *Pseudacteon* spp. and *Azya orbigera*. Arrows represent positive effects and solid circles represent negative effects. Black solid lines represent direct interactions, the blue solid line represent first level trait-mediated indirect interactions, the red solid lines represent the second level trait-mediated indirect interactions, and the dashed purple line represents the resulting cascading trait-mediated indirect interaction between the phorid flies and the coccinellid beetle.

Table 2-1 Ant response to phorid parasitism

<i>Resource retrieval</i>			
<i>Ant spp.</i>	<i>Phorid spp.</i>	Measured ant response to phorid parasitism	Reference
<i>Atta cephalotes</i>	<i>Neodohniphora curvinervis</i>	Reduced resource retrieval	Orr 1992
<i>Atta sexdens</i>	<i>Neodohniphora</i> sp.	Reduced number of loaded ants	Bragança et al. 1998
<i>Atta sexdens</i>	<i>Neodohniphora</i> sp.	Reduced resource retrieval	Tonhasca et al. 2000
<i>Azteca sericeasur</i>	<i>Pseudacteon</i> sp.	Increased time for foragers to carry away resource	Philpott et al. 2004
<i>Pheidole diversipilosa</i>	<i>Apocephalus</i> sp. 8	Increased resource turnover rate by competitor	LeBrun & Feener 2002
<i>Solenopsis geminata</i>	<i>Pseudacteon browni</i> and <i>P. bifidus</i>	Reduced resource retrieval	Morrison 1999
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced colony protein consumption	Mehdiabadi & Gilbert 2002
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced resource retrieval	Morrison 2002
<i>Solenopsis richteri</i>	<i>Pseudacteon</i> sp. (multiple)	Reduced number of workers at resource	Folgarait & Gilbert 1999
<i>Worker size</i>			
<i>Ant spp.</i>	<i>Phorid spp.</i>	Measured ant response to phorid parasitism	Reference
<i>Atta</i>	<i>Neodohniphora erthali</i>	Increased number of hitchhikers	Vieira-Neto et al. 2006
<i>Atta cephalotes</i>	<i>Neodohniphora curvinervis</i>	Sent out workers in smaller size	Bragança et al. 1998
<i>Atta cephalotes</i>	<i>Unreported</i>	Sent out workers in smaller size	Yackulic & Lewis 2007
<i>Atta laevigata</i>	<i>Apocephalus attophilus</i>	Sent out workers in smaller size	Erthal & Tonhasca 2000
<i>Atta sexdens</i>	<i>Neodohniphora</i> sp.	Reduced forager mass	Bragança et al. 1998
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Sent out workers in smaller size	Mehdiabadi & Gilbert 2002
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Sent out workers in smaller size	Morrison 2000

<i>Solenopsis invicta</i>	<i>Pseudacteon</i> sp.	Altered ratio of worker size	Pucket & Harris 2010
<i>Solenopsis invicta</i>	<i>Pseudacteon</i> sp.	Sent out workers in smaller size	Folgarait & Gilbert 1999
<i>Ant activity</i>			
<i>Ant spp.</i>	<i>Phorid spp.</i>	Altered number of foragers	Reference
<i>Atta laevigata</i>	<i>Apocephalus attophilus</i>	Reduced number of loaded ants	Erthal & Tonhasca 2000
<i>Atta sexdens</i>	<i>Myrmosicarius grandicornis</i>	Increased number of unloaded workers returning to nest & decreased number of loaded workers returning to nest	Tonhasca et al. 2001
<i>Atta sexdens</i>	<i>Neodohrniphora</i> sp.	Altered number of foragers	Bragança et al. 1998
<i>Atta sexdens</i>	<i>Neodohrniphora</i> sp.	Altered number of foragers	Bragança et al. 1998
<i>Azteca sericeasur</i>	<i>Pseudacteon</i> sp.	Reduced ant activity	Liere and Larsen 2010
<i>Azteca sericeasur</i>	<i>Pseudacteon</i> sp.	Reduced number of ants	Philpott 2005
<i>Linepithema humile</i>	<i>Pseudacteon</i> sp.	Altered number of foragers	Orr & Seike 1998
<i>Pheidole titanis</i>	<i>Pseudacteon</i> sp.	Reduced ant activity	Feener 1988
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced number of exposed ants	LeBrun and Feener 2002
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Altered recruited ant size	Mehdiabadi & Gilbert 2002
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Altered forager size	Mehdiabadi et al. 2004
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced number of ants at baits	Morrison 2000
<i>Solenopsis invicta</i>	<i>Pseudacteon tricuspis</i>	Reduced number of foragers	Mottern et al. 2004
<i>Solenopsis invicta</i>	<i>Pseudacteo</i> sp.	Reduced number of ants at baits	Puckett & Harris 2010
<i>Solenopsis richteri</i>	<i>Pseudacteon</i> (multiple spp.)	Reduced number of ants at baits	Folgarait & Gilbert 1999
<i>Solenopsis richteri</i>	<i>Pseudacteon</i> (multiple spp.)	Reduced various ant activity	Folgarait & Gilbert 1999
<i>Solenopsis saevissima</i>	<i>Pseudacteon</i> sp.	Reduced ant activity	Orr & Gilbert 1997

<i>Solenopsis sp.</i>	<i>Pseudacteon sp.</i>	Altered number of foragers	Porter et al. 1995
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Chapter 3 Cascading trait-mediated interactions induced by ant pheromones (This chapter has been published in *Ecology and Evolution* in 2012)

3.1. Abstract

Trait-mediated indirect interactions (TMII) can be as important as density-mediated indirect interactions. Here, we provide evidence for a novel trait-mediated cascade (where one TMII affects another TMII) and demonstrate that the mechanism consists of a predator eavesdropping on chemical signaling. Ants protect scale insects from predation by adult coccinellid beetles – the first TMII. However, parasitic phorid flies reduce ant foraging activity by 50% – the second TMII, providing a window of opportunity for female beetles to oviposit in high-quality microsites. Beetle larvae are protected from ant predation and benefit from living in patches with high scale densities. We demonstrate that female beetles can detect pheromones released by the ant when attacked by phorids, and that only female, and especially gravid females, are attracted to the ant pheromone. As ants reduce their movement when under attack by phorids, we conclude that phorids facilitate beetle oviposition, thus producing the TMII cascade.

3.2. Introduction

Long appreciated by traditional naturalists, the idea of a trophic cascade gained popularity in the ecological literature through debates that emerged from Hairston, Smith, and Slobodkin's publication of the famous "green world hypothesis" (Hairston et al. 1960). The enormous literature that emerged from that article cemented in the consciousness of most ecologists the fact that ecological complexity pervaded ecosystems (e.g. Terborgh and Estes 2010). Also long appreciated, at least since Darwin, are the indirect effects on morphologies (and/or physiologies and/or behaviors, etc....) so predominant in nature. In modern literature, these special indirect effects that involve some sort of "trait" of the organism are distinguished from those that affect only the density or biomass of the organism and are thus termed trait-mediated indirect interactions (TMII). It has been shown that, at least in some circumstances, the TMII's can be so strong as to overwhelm any density-mediated effects (Abrams 1995; Werner and Peacor 2003).

Not yet appreciated to any great extent is another formulation that is likely to be familiar to naturalists, although thus far gaining little more than tacit appreciation (e.g. Bolker et al. 2003). For example, a parasitized mouse may have its food-searching ability (a trait) dramatically reduced by an ectoparasite, but the presence of a particular type of vegetation exposes the mice to less parasite attack than in its absence. The vegetation affects the ability (trait) of the parasite to affect the ability (trait) of the mouse to eat – a cascade of trait-mediated effects (species 1 affects the ability of species 2 to affect the ability of species 3 to do something). As with trophic cascades, one’s imagination can create long links from simple natural history storytelling. Although this sort of linkage between TMII units has been discussed in the literature (Schmitz et al. 2004; Ohgushi 2005; Kessler and Halitschke 2007; Poelman et al. 2008; Utsumi et al. 2010), to our knowledge, the specific structure has not been documented experimentally for any terrestrial system other than the one here described (Liere and Larsen 2010). We, here, report on the complex chemical signaling that constitutes the mechanisms driving the TMII cascade. Many TMII are induced by chemical volatiles, such as plant volatiles released in response to herbivory (reviewed by Agrawal 2005; Kessler and Halitschke 2007; Dicke et al. 2009) or predator chemicals that can be detected by the prey and trigger a behavioral change (Rothley et al. 1997; Schmitz et al. 1997, 2004; Relyea 2003; Schmidt-Entling and Siegenthaler 2009; Barbasch and Bernard 2011). Species interactions facilitated by chemical volatiles are common in aquatic and terrestrial insect communities (Bolker et al. 2003; Werner and Peacor 2003; Cardé and Millar 2004; Schmitz et al. 2004), and direct vertical hierarchies have been well documented, whether plant– herbivore, or host–prey interactions (Cardé and Millar 2004). Tri-trophic interactions involving chemicals are also well known in the case of predators and parasitoids that use plant volatiles to find their herbivore host (Vet and Dicke 1992; Ode 2006). However, the exploitation of insect communication systems by other species is less well documented – examples include the use of bark beetle aggregation pheromones by their predators (Wood 1982), the use of a sex pheromone emitted by male stink bugs by their tachinid fly parasitoids (Aldrich et al. 2007), and the use of moth pheromones by egg parasitoids (Fatouros et al. 2008). Beside the well-known cases of chemical camouflage and mimicry (Hölldobler and Wilson 1990; Dettner and Liepert 1994), surprisingly little is known of insects that exploit ant chemical communication systems, even though ant pheromones are especially

reliable sources of information (Hölldobler and Wilson 1990). Reported cases include two ant-eating spiders that use ant alarm pheromones to find their prey (Allan et al. 1996; Clark et al. 2000), phorid parasitoids that use ant alarm or trail pheromones to find their host (Feener et al. 1996; Morrison 1999; Chen and Fadamiro 2007; Mathis et al. 2011; Mathis and Philpott 2012), and two coccinellid beetles, one that uses ant alarm pheromones to find aphids being tended by the ants (Godeau et al. 2003) and the other using ant pheromones to avoid oviposition sites where ants are the most active (Oliver et al. 2008). However, more complex cascading trait-mediated interactions that are facilitated by ant pheromones related to the presence of a third species and have direct consequences for population and community-wide dynamics have not been previously reported.

Ant–hemipteran interactions are among the most ubiquitous mutualisms in terrestrial ecosystems (Buckley 1987). By protecting hemipterans from their predators and parasitoids, ants have access to a reliable and abundant source of energy and nutrients (Buckley 1987), an example of a trait-mediated interaction as ants do not prey directly on the natural enemies, but rather harass them, thus reducing their access to the hemipterans.

Here, we experimentally demonstrate a cascade of trait-mediated indirect interactions involving two TMII units: (1) an ant–hemipteran mutualism unit, where the ants interfere with the ability of predators to attack scale insects, and (2) a phorid-ant-hemipteran unit where the phorids reduce the foraging activity of the ants, thus reducing their ability to interfere with the predator of the hemipteran mutualist. We also demonstrate that the link- age between the TMII units is mediated by semiochemicals, more specifically ant pheromones that are used for alerting nest mates about the presence of parasitoids, thus disrupting the ant’s ability to interfere with the behavior of the predator, especially with the ability of the predator to engage in oviposition behavior.

3.2.1. The study system: the first TMII unit

In a typical ant–hemipteran association, the arboreally nesting ant, *Azteca sericeasur*, protects a significant pest of coffee, the green scale, *Coccus viridis*, against most natural enemies (Vandermeer and Perfecto 2006). Consequently, high densities of green scales can only be found in association with Azteca ants. However, the predatory coccinellid beetle, *Azya orbigena*, has

evolved a capacity to exploit this mutualism (Liere and Perfecto 2008). The larval form is covered with waxy filaments that effectively protect it from ant attacks (Figure 3-1A), allowing it to live in areas with a high density of the green scale, its main prey (Figure 3-1B) (Perfecto and Vandermeer 2008). Furthermore, by scaring away scale parasitoids, the ants inadvertently also scare away parasitoids of the beetle larva, thus providing it with enemy-free space (Liere and Perfecto 2008). On the other hand, adult beetles are harassed and could be killed by the ants (see Appendix D). Both larvae and adults of the beetle are significantly more abundant on coffee plants around Azteca ant nests than in areas without ants, (see Appendix A), suggesting that female beetles are ovipositing on ant-tended plants despite the risk of attacks or egg predation. As ants remove almost all beetle eggs laid bare on ant-tended plants (I. Perfecto, pers. obs.), female beetles must hide their eggs to protect them against ant predation. We have encountered coccinellid beetle eggs on old *A. orbiger*a pupal cases that still have the waxy filaments (Figure 3-1C) and under scale insects (Figure 3-1D), suggesting that adult *A. orbiger*a females are effectively searching out safe microsites for their eggs within ant patrolled plants. The ant-hemipteran mutualism is a well-established trait-mediated indirect interaction unit. However, the presence of coccinellid eggs under scale insects and the high density of coccinellid larvae and adults on plants with *Azteca* ants (Appendix A) suggest that something is interfering with this TMII unit.

3.2.2. The study system: the second TMII unit

A second TMII unit provides clues as to what could be interfering with the ant–hemipteran TMII unit. The phorid fly, *Pseudacteon lacinosus* (Brown and Philpott 2012) is a parasitoid of *A. sericeasur* (Philpott et al. 2004). However, as with many other phorid parasitoids of ants, its main effect on the ant is a trait-mediated effect through a reduction of the ant foraging activity (Philpott et al. 2004, 2009; Philpott 2005; Hsieh and Perfecto 2012). Previous work demonstrated that these phorids are attracted to an alarm-defense pheromone produced by *A. sericeasur*, and also that movement of the ants must be detected by the fly at close range for the latter to oviposit (Mathis et al. 2011). As a consequence of this requirement, when phorids arrive, the ants take on a motionless catatonic state (Appendix E). As this behavior becomes generalized among all ants in the vicinity after a phorid attack, it is evident that a phorid-alert pheromone is released to warn nearby workers that a phorid is in the vicinity. The result of this behavior is that

overall activity of the ants declines by at least 50% in the presence of the phorids (Philpott 2005). This reduction in ant activity is sufficiently large to provide the coccinellid beetles with an effective temporal refuge from the ants (Liere and Larsen 2010). The effect of the phorids can last up to 2 h (Philpott et al. 2004), possibly providing the female beetles with enough time to find high-quality and secure oviposition sites.

The question then is, how do the beetles find these high-quality patches, and can the beetles take advantage of the low ant activity (when the phorids are present) by being able to detect the phorid-alert pheromone?

Here, we test the hypothesis that the coccinellid beetle, *A. orbigera*, is able to detect volatile pheromones from the ants and, more importantly, that the coccinellid beetles have the ability to detect the unique alarm pheromone (or pheromones) released by ants specifically when under attack by phorids, therefore taking advantage of a window of opportunity to search out safe sites for oviposition.

3.3. Materials and Methods

To test the hypothesis that beetles are able to detect volatile pheromones released by ants, we employed a standard olfactometer (Pettersson et al. 1998). We used various chemical attractants of ants on female and male coccinellid beetles, and with mated and unmated females. We also conducted a beetle oviposition experiment, manipulating the presence of phorid flies to determine if the presence of phorids indeed produced a TMII cascade that facilitates beetle oviposition.

Beetles, ants, and phorid flies used to conduct the studies were collected from an organic coffee plantation in the southern part of the state of Chiapas, Mexico (15°10' N, 92°20'W) or reared in the laboratory after field collections from the same site. Five colonies of *A. sericeasur* were collected from the field and maintained in the laboratory for the duration of the studies. Phorid flies were collected from the field minutes before they were used in the experiments. Individuals of *A. orbigera*, the coccinellid beetle, used in the first sets of olfactometer studies were collected directly from the field, kept in the laboratory, and fed with *C. viridis*. The individual beetles used to determine attraction of ant pheromones to mated and unmated female beetles and for the

oviposition experiment were reared in the laboratory from larvae collected in the field.

3.3.1. Olfactometer bioassays

To investigate whether the coccinellid beetle, *A. orbigera*, was able to detect and be attracted to pheromones from *A. sericeasur*, we conducted behavioral assays in a two-arm olfactometer, modified from the four-armed Perplex olfactometer (Pettersson et al. 1998). The apparatus consisted of a central arena 6 x 6 cm with two conical extended arms of 7 cm, to which odors could be introduced from source chambers connected with tubing via 4 mm holes at each at the end of each arm. Airflow in the arena was created by connecting a tube to the center of the arena and attaching it to an air pump, effectively creating two odor environments, a control, and a treatment. The olfactometer was divided into three zones, a neutral zone, consisting of 2 cm in the center of the arena, and the stimulus and control zones, extending to the right and left of the neutral zone and both consisting of 9 cm, separated into six units of 1.5 cm each. At the start of each trial, one beetle was placed in the neutral zone and after a short acclimatization period, the position of the beetle was recorded every 20 sec for 5 min (15 recordings per trial). The position of the treatment was altered every few trials. After each trial, the arena was cleaned with 70% ethanol.

To test beetles' response to the general odor of ants, 20 ants were placed in one of the source chambers and the other left empty. We waited for at least 10 min to let the ants calm down before placing the beetle in the arena and running the assay. To determine beetle attraction to ant pheromones, 20 ants were crushed and placed in one of the source chambers and the other left empty. Crushing the ants liberates all the pheromones produced by ants and is a method frequently used in these types of bioassays (Brown and Feener 1991; Francis et al. 2004; Mathis et al. 2011). Finally, to test for the attraction of *A. orbigera* to *A. sericeasur* while being attacked by phorid parasitoids, we collected five colonies of *A. sericeasur* in carton nests and divided each of them into equal halves. Then we placed the two equal parts of each colony in two transparent 60 x 60 x 60 cm insect-rearing cages (Bug Dorm-2 Insect Tent; Bug Dorm Store, Taichung, Taiwan).

We connected the cages to the two-arm olfactometer and waited for at least 10 min until the ants calmed down. We then released three to five freshly collected phorid flies into one of the tents

and placed one adult beetle in the neutral zone of the olfactometer. When at least one phorid fly started attacking the ants, we started recording the location of the beetle. We conducted these trials with both male and female beetles.

To test the difference between mated and unmated females, we collected larvae and pupae of the beetle from the field and reared them to adults. Some were placed in containers with males and kept there until copulation was observed. The assays to examine differences in the response of mated and unmated females beetles were conducted with unmated females and mated females. We also conducted trials with females that had mated at different times.

To determine the source of the pheromone or pheromones that could attract coccinellid beetles, we conducted additional trials using various body parts of the ants. Both males and female beetles were tested for head, thorax, dorsal part of abdomen and ventral part of abdomen.

Finally, to determine if the adults of *A. orbiger*a used olfactory cues to locate *C. viridis*, we used coffee leaves with *C. viridis* in one chamber and enclosed equal numbers of coffee leaves without *C. viridis* in the other chamber.

3.3.2. Oviposition experiment

To determine if gravid females *A. orbiger*a actually use the chemical cues of the ants when phorid flies are attacking them, we conducted an oviposition experiment. Female *A. orbiger*a were collected from the field, collectively mated, placed in individual containers and daily satiated with scale insects in the lab. When oviposition of a female beetle was observed, the female beetle was used in the experiment. When at least three female beetles were ovipositing, we shuffled individual gravid beetles and randomly assigned them into one of three treatments: (1) no ants/no phorids, (2) ants/no phorids, and (3) ants + phorids. The chambers where the experiment was conducted consisted of containers of 1 L containing a coffee branch with four to six coffee leaves infected with scale insects. The ant/no phorid treatment contained 40 workers of *A. sericeasur* and the ants + phorids treatment contained 40 workers of *A. sericeasur* plus two to three phorid flies. After 24 h, the coffee branch was removed from the experimental chamber and placed under a dissecting microscope, where it was carefully examined for eggs of *A. orbiger*a. The three individual gravid beetles were returned to the beetle pool with other

individuals. For the next trial all three beetles were again randomly selected and assigned to treatments. The experiment was replicated nine times.

3.4. Data analyses

Beetle preferences were determined by directly calculating the probability of finding something other than a 50% response with a binomial distribution using Excel binomial distribution function ($P = 1 - \text{BINOMDIST} [\text{number of beetles that choose treatment, total number of trials, TRUE}]$). For each trial, we added all recordings for the control, the treatment, and the neutral (no-response) zones, and categorized the trial based on the zone that had the higher number of recordings (control or treatment). Trials categorized as “no-response” were eliminated from the analyses. To investigate the relationship between days after copulation and level of attraction to ant pheromones, a simple linear regression analysis was used. In this particular case, instead of using the categories (control or treatment), we calculated a strength index by averaging the number of the position of the beetle at each recording period (from 1 to 6, with 1 being a weak response and 6 being a strong response). To test whether ants deter female beetles from ovipositing and whether phorid parasitoids facilitate oviposition, a Wilcoxon rank-sum test was conducted.

3.5. Results

Both females and males of the coccinellid beetle, *A. orbigera*, are attracted to the green coffee scale (Figure 3-2; see also Appendix B). However, only females showed any response to odors released by ants (Figure 3-2). While females showed no response to live ants that were not being attacked by phorids or that were not alarmed for other reasons, they did show a positive response to crushed ants and to live ants that were being attacked by phorid flies (Figure 3-2B and C).

Through a separate series of two-arm olfactometer assays (testing heads, thoraxes, ventral part of abdomens, and dorsal part of abdomens), we were able to determine that the phorid-alert pheromone is produced in the ventral part of the abdomen, most likely in the Pavan or Dufour's gland (Figure 3-3), distinct from the general alarm pheromone that attracts the phorids and that is produced in the pygidial gland, located on the dorsal side of the abdomen (Mathis *et al.* 2011). These assays also confirmed that only female beetles are attracted to pheromones produced by ants (Figure 3-3D).

Testing mated and unmated females demonstrated that the attraction to ant pheromones is manifested only after mating, and that before mating, female beetles have an aversion to ant pheromones (Figure 3-4A). Female beetles collected from the field showed an intermediate level of attraction to ant pheromones between that of unmated and mated females (Figure 3-4A). Furthermore, the attraction to ant pheromones continues to increase for at least 7 days after copulation (Figure 3-4B).

Results for the oviposition experiment show that the average number of eggs oviposited by *A. orbiger*a on the no ant/no phorid treatment is not significantly different from the average number of eggs oviposited on the ants + phorids treatment (Figure 3-5). It also shows that both of these treatments, on the average, had higher eggs oviposited than the ant/no phorid treatment (Figure 3-5). These results support the hypothesis that ants deter female beetles from ovipositing on plants where they tend scales, and that female beetles used the phorid-alert pheromone (or pheromones) released by *A. sericeasur* to find a window of opportunity to oviposit (Figure 3-5).

3.6. Discussions

This study demonstrates that the coccinellid beetle, *A. orbiger*a, is able to detect pheromones released by the ant *A. sericeasur*. More interestingly, female beetles are attracted specifically to the phorid alarm pheromone released by the ants when under attack by *Pseudacteon* phorid flies. Furthermore, only gravid female beetles showed an attraction to the ant pheromones and this attraction increased with the number of days after copulation. Further evidence that female beetles are attracted to the ants only when they are being attacked by phorids comes from the lack of a significant response to live, undisturbed ants (Figure 3-2). These results also explain the large variance encountered when olfactometer assays are conducted without discriminating among female beetles' gravid status (i.e. beetles collected from the field).

This study also provides strong evidence that the female beetles use the phorid-alert pheromone released by the ants to find windows of opportunity to oviposit in high-quality sites where the larvae will have sufficient food (scale insects) and be protected from parasitoids (Liere and Perfecto 2008). We also showed that both male and female beetles are attracted to chemicals released by scale insects or coffee volatiles induced by herbivory (Appendix B).

Given these results, it is likely that both male and female beetles find patches of high concentrations of scales through chemical compounds found either in the scale insects themselves or from volatiles emitted by coffee plants when being fed on by scales (see Appendix B). As beetle larvae have dramatically restricted movements and are attacked by several parasitoids (Liere and Perfecto 2008), there is clear pressure for female beetles to oviposit in ant-tended areas, where high prey density and low risk of parasite attack are secured. However, the aggressive behavior of ants renders female beetles incapable of ovipositing in these high-quality areas (Figure 3-5). Here, we demonstrate that female beetles can avoid this problem by being able to detect the phorid-alert pheromones released by Azteca ants. This ability allows beetles to take advantage of the low ant-activity periods to search for sites where their eggs can be hidden and protected against ant predation after ants resume their normal activity levels.

More generally, this system is an example of cascading trait-mediated indirect interactions resulting from the linkage between two TMII units: the ant-scale mutualism unit and the ant-phorid-scale unit. Furthermore, in this study, we were able to demonstrate that the linkage between these two TMII units is mediated by the ability of the beetle to eavesdrop on the chemical signaling of the ant. In other words, the ant pheromone initiates the cascading trait-mediated indirect interactions that result in the facilitation of the coccinellid beetle. The first TMII is the ant's interference with the ability of the female beetles to oviposit in sites with high scale abundance, due to the ants' mutualistic interaction with the scale insects. The second TMII is the phorid fly interference with the first TMII (i.e. with the ant's interference of beetle oviposition). These interactions are illustrated in Figure 3-6. Through those cascading trait-mediated indirect interactions (effects on effects on effects), the phorid fly indirectly facilitates the coccinellid beetles.

The facilitation that results from the linkage between these two TMII units can have important consequences at the ecosystem level. The scale, *C. viridis*, is reported to be an important pest of coffee causing considerable damage in some cases (Williams 1982). The coccinellid beetle, *A. orbigera*, is the main regulator of the scale insect at the landscape level (Perfecto and Vandermeer 2008; Vandermeer et al. 2010). Outside the patches of Azteca, the larvae of *A. orbigera* suffer a high percentage of parasitization by at least four different parasitoids and is

unlikely to reach maturity and reproduce (Liere and Perfecto 2008; Vandermeer et al. 2010). Therefore, the Azteca patches represent refuges for the larvae of *A. orbigera*. Once the adults emerge, they fly away from the Azteca patches, thus escaping harassment by the ants. Mark-recapture experiments show that adult beetles move readily between Azteca patches (H. Liere, unpubl. data), suggesting that they control scale insects outside Azteca patches, which in the study area represent only about 3– 5% of the coffee area (Perfecto and Vandermeer 2008; Vandermeer *et al.* 2008).

This is the first report of an insect exploiting the ant chemical communication system through such complex interactions. In other cases, parasitoids or predators were able to use a trail or an alarm pheromone to locate ants to attack them (Allan et al. 1996; Feener et al. 1996; Clark et al. 2000; Chen and Fadamiro 2007; Mathis et al. 2011), locate their hemipteran mutualists (Godeau et al. 2003), or avoid them altogether (Oliver et al. 2008). It is not difficult to imagine how these direct chemically mediated interactions evolved as ant pheromones are known to be reliable sources of information (Hölldobler and Wilson 1990). The evolution of such complex interactions as reported here is more perplexing, especially given that it occurs in an agroecosystem of a non-native species, coffee. *Coffea arabica*, the host plant of *C. viridis*, is not native to the Americas, having been introduced less than 300 years ago from Africa (Vega 2008). *Coccus viridis* is a pan-tropical phytophagous insect thought to have originated either in Ethiopia or Brazil (Zimmerman and Hardy 1948; Gill et al. 1977). The phorids, *Pseudacteon laciniosus*, and possible two other species were recently described as attacking only *A. sericeasur* (Brown and Philpott 2012), therefore it is likely that they are restricted to the geographic range of *A. sericeasur* in the Americas. Although the genus *Pseudacteon* has been collected from the Americas, Europe, Asia, and Australia, its center of diversification is reported to be Brazil (Porter and Pesquero 2001). Finally, *A. sericeasur* is a Neotropical ant whose distribution ranges from Brazil to Mexico (Kempf 1972). Except for coffee, the host plant, the other species involved in this network are likely to have been interacting in natural systems for sufficient time to allow the evolution of such complexity.

Our study demonstrates that *A. orbigera*, the coccinellid beetle, has the ability to capitalize on the “phorid alert” pheromones released by the ant *A. sericeasur* while under attack by phorid

flies. Phorid attacks reduce ant foraging activity, thereby providing female coccinellid beetles with a window of opportunity to oviposit in sites with high scale density. This is an example of linkages between TMII units with potentially important ecosystem-level implications (Schmitz *et al.* 2004; Ohgushi 2005; Kessler and Halitschke 2007; Ohgushi *et al.* 2007; Poelman *et al.* 2008; Utsumi *et al.* 2010).

These chemically mediated indirect complex interactions can have practical implications for the management of coffee, as *C. viridis*, the scale insect, is a pest in coffee, the coccinellid beetle is an important predator of the scale insect and their effect on scales is clearly conditioned by the cascading TMII described here. Given that this system is found in a recently established agroecosystem, it is likely that components of the system (the TMII units) are quite common in natural ecosystems and that these sorts of TMII cascades could be more common than previously thought.

To our knowledge, ours is one of the few studies that document these cascading effects (the others being from the same study system; Liere and Larsen 2010; Pardee and Philpott 2011; Philpott *et al.* 2012), and the only one investigating the mechanism behind the cascading interactions. However, each pairwise interaction involved in this system is common in nature: (1) ant/hemipteran mutualisms are very prevalent and well documented (Buckley 1987), (2) phorid parasitoids frequently elicit trait-mediated effects (Hsieh and Perfecto 2012), (3) ants depend on fairly reliable pheromones for communication among nest mates (Hölldobler and Wilson 1990), and (4) many insect predators and parasitoids have been found to use other insect chemicals to find their prey/host (Wood 1982; Bolker *et al.* 2003; Werner and Peacor 2003; Cardé and Millar 2004; Schmitz *et al.* 2004; Aldrich *et al.* 2007; Chen and Fadamiro 2007). Furthermore, recent studies suggest that TMII do not occur in isolated units, but interact with other TMII units (Utsumi *et al.* 2010). Given the prevalence of these pairwise interactions and the documented linkages between TMII units, it is likely that the complex trait-mediated cascade of interactions documented here are common in nature, but due to the complexity of the interactions have not been documented in other systems.

Ecological research long ago transcended the limits of classical density-dependent interactions, and the literature is now replete with examples of chemical, behavioral, and morphological

changes of organisms in response to challenges from other organisms, such as the threat of predation. Indeed, such trait-mediated indirect interactions significantly affect species coexistence, trophic length, and energy and material flows within ecosystems (Werner and Peacor 2003; van Veen et al. 2005, 2009; Kratina et al. 2010; Loreau 2010; Kefi et al. 2012). Less attention has been accorded to what we report herein, the possibility of cascades of trait-mediated effects. We suggest that such effects may be common in nature and their uncommon occurrence in the literature is a product of investigators failing to search for them in the first place. Current literature of simple trait-mediated interactions is in need of empirical support to bridge gaps generated by theoretical models (Bolker et al. 2003), while theoretical synthesis is expeditiously integrating non-feeding interactions into food webs (e. g. Bolker *et al.* 2003; Golubski and Abrams 2011; Kefi *et al.* 2012). However, we also emphasize that more complicated pathways, such as those described herein, may be paramount in affecting system dynamics and deserve more attention, both theoretical and empirical.

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3.7. Authors' Contributions

H. Hsieh designed and conducted the olfactometer experiments, analyzed the data, and wrote part of the manuscript. H. Liere, helped with project design, gathered the beetle distribution data, and edited the manuscript. E. Jimenez Soto helped with the oviposition and oviposition experiment. I. Perfecto helped design the experiments and wrote part of the manuscript.

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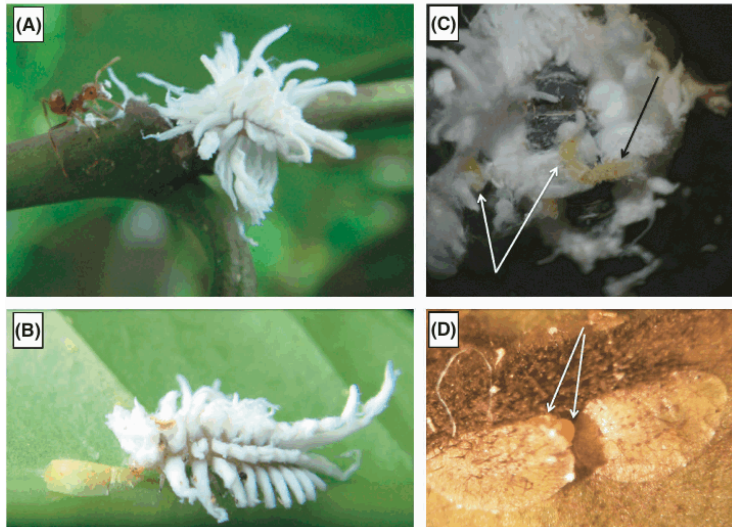


Figure 3-1 Photos of *Azya orbigera*. (A) The ant, *Azteca sericeasur*, attacking *A. orbigera*, but getting its mandibles filled with the sticky waxy filaments that cover the body of the larvae of *A. orbigera*; (B) *A. orbigera* eating a *Coccus viridis*; (C) Eggs (white arrows) and first instar larvae (black arrow) of *A. orbigera* on an old pupal case of the same species; (D) *A. orbigera* eggs (white arrows) hidden under a scale insect.

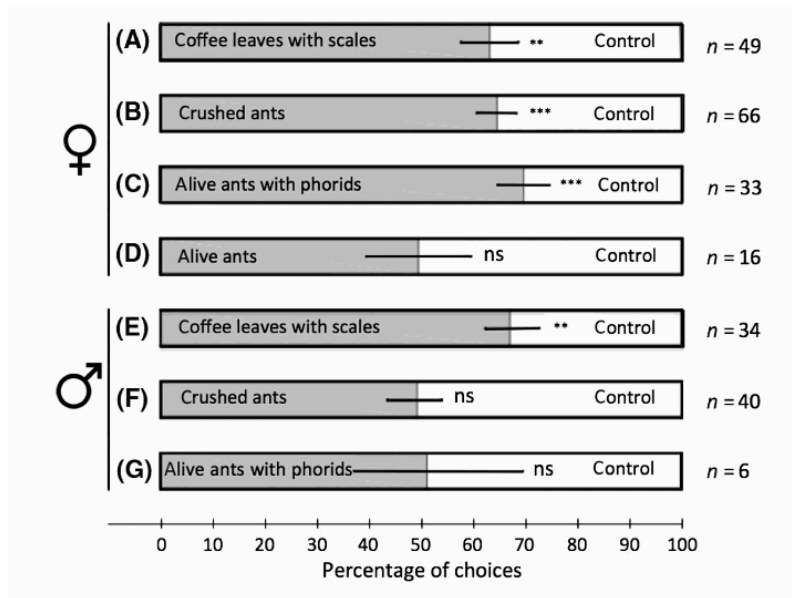


Figure 3-2 Percentage of female and male beetles choosing control versus treatment. Asterisks indicate statistical significance based on binomial distribution; ** $P < 0.01$; *** $P < 0.001$. Error bars based on standard error of the mean.

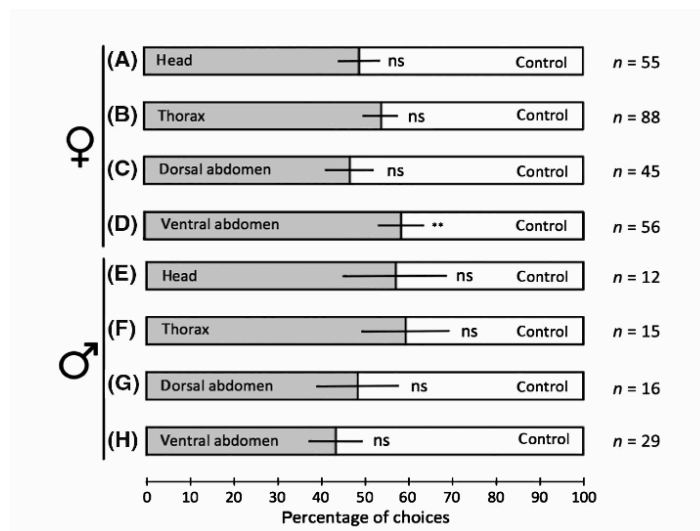


Figure 3-3 Percentage of female and male beetles choosing control versus treatment (various ant body parts). Asterisks indicate statistical significance based on binomial distribution; ** $P < 0.01$; *** $P < 0.001$. Error bars based on standard error of the mean.

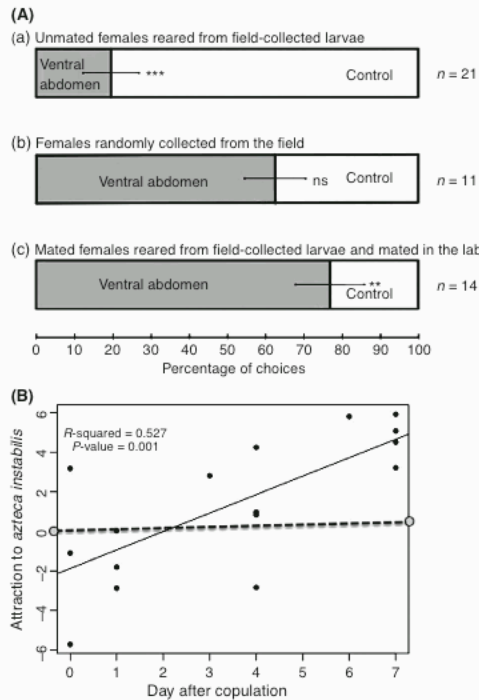


Figure 3-4 Responses of mated and unmated female *Azya orbigera* to ant pheromones. (A) Percentage of unmated, mated, or field-collected female beetles choosing control versus odor from the ventral abdomen of ants. Asterisks indicate statistical significance based on binomial distribution; ** $P = < 0.01$; *** $P = < 0.001$. Error bars based on standard error of the mean. (B) Relationship between days after copulation of female *A. orbigera* and attraction to ants. The dashed horizontal line divides beetles' positive and negative response to ant pheromones, with numbers representing the intensity index (0 = no response; 6 = strong response). P -value based on a simple linear regression between days after copulation and level of attraction to ant odor.

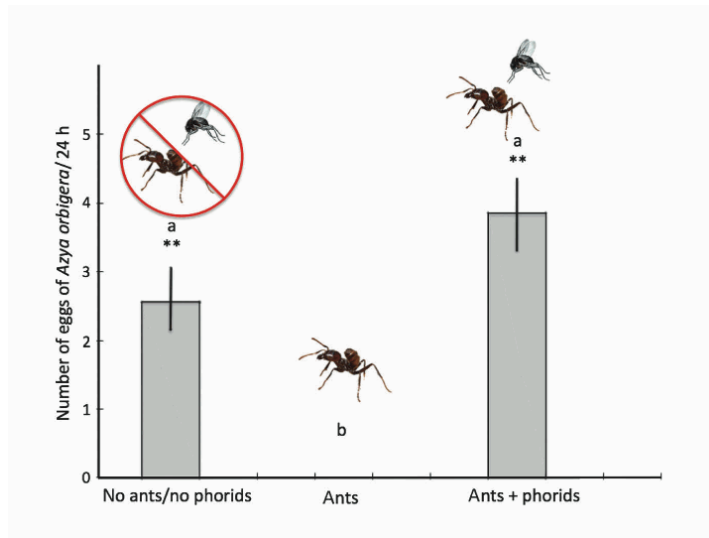


Figure 3-5 Numbers of eggs oviposited by *Azya orbigera* within a course of 24 hours.

Experiment conducted in insect cages with three conditions: including branches of coffee with scale insects, but no ants or phorid flies (no ants/no phorids); with branches of coffee with scale insects and ants, but no phorids (ants); with branches of coffee with scale insects, ants and phorid flies (ants + phorids). Statistical test based on Wilcoxon rank-sum test (treatment comparisons: ants + phorids versus ants, P -value = 0.015; phorids versus no ants/no phorids, P -value = 0.014; no ants/no phorids versus ants + phorids, P -value = 0.87). Mean eggs within 24h + SEM: 2.56 ± 0.40 (no phorids/no ants); 0 ± 0 (ants); 3.86 ± 0.55 (ants + phorids).

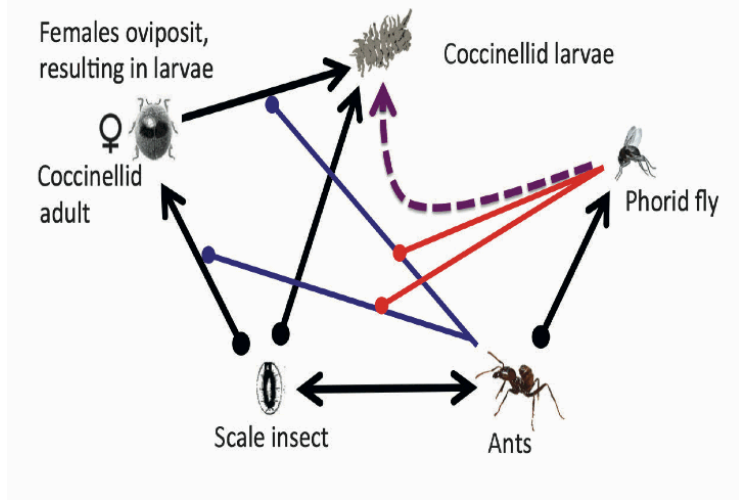


Figure 3-6 Diagrammatic representation of the cascading trait-mediated indirect interactions between *Pseudacteon* spp. and *Azya orbigera*. Arrows represent positive effects and solid circles represent negative effects. Black solid lines represent direct interactions, blue solid lines represent first-level trait-mediated indirect interactions, red solid-lines represent second-level trait-mediated indirect interactions, and dashed purple line represents the resulting cascading trait-mediated indirect interaction between the phorid flies and the coccinellid larvae.

Chapter 4 Effects of trait-mediated indirect interactions on a consumer-resources system

4.1. Abstract

Ecologists have widely accepted that trait-mediated indirect interactions (TMIs) can have important effects on other members of a community. What is less understood is how and to what degree that the interplay of TMIs would affect community dynamics. Recently, a few empirical studies demonstrate that, via the interactions between two TMIs, a remote species can have an effect on a focal trophic link in a lateral food chain. Here, we focus on one, studying how zero, one, and two interacting TMIs affect a focal species. We employ a system that includes two interacting TMIs and broke it down experimentally into a system with no TMII, one TMII and two interacting TMIs. The simplest unit in this system is composed of a parasitoid wasp that consumes a coccinellid beetle (no TMII). The next level of complexity (a single TMII) is the parasitoid wasp and the beetle plus an ant that interferes with the ability of the parasitoid to attack the beetle (i.e. a TMII). The third and final level of complexity includes two interacting TMIs and consists of the parasitoid wasp, the beetle, the ant, and a phorid fly parasitoid of the ant. The latest of the organisms interferes with the ability of the ant to interfere with the attack of the parasitoid wasp to the beetle. Within this experimental system we examine parasitism and sex ratio of the coccinellid, two important demographic characteristics of the beetle. We hypothesize that the second level TMII (the phorid restricting the ant) effectively cancels the effect of only a single TMII (the ant restricting the parasitoid) and, therefore, these two important demographic characteristics should be the same with the two TMII as they were in the system with no TMII (i.e. the direct density-mediated effect of the parasitoid wasp on the beetle). From mesocosm experiments, we find that the ants do exhibit aggressive behavior toward the parasitoid wasp (first level TMII) and that this aggression is reduced in the presence of the phorid fly parasitoid (second level TMII). However, in spite of the aggressive behavior of the ants towards the parasitoid, the presence of this one TMII (ants disrupting the ability of the parasitoid wasp to attack the beetle) did not change the parasitism ratio or sex ratio of the beetle

as compared to the direct density mediated effect (beetle and parasitoid wasp), thus rejecting our initial hypothesis. On the other hand, the presence of the two interacting TMII terms (ants plus phorid fly parasitoids), significantly increases the parasitism probability of the beetle, reduces the survival probability of males and changes the sex ratio of the beetle to a female biased population. Field surveys verify that areas with high rates of phorid fly attacks on ants tend to have a female biased beetle population. The significance of these complex TMII is discussed in the context of the biological control of a scale insect that is the prey of the coccinellid beetle in coffee farms.

4.2. Introduction

Understanding trophic interactions has been one of the pillars of ecological theory (Elton 1927; Lindeman 1942). Historically, ecologists have been concerned with food webs and the direct and indirect effects on the density of organisms through consumption (Hairston *et al.* 1960; Holt 1977; Carpenter *et al.* 1985; Fretwell 1987; Polis & Strong 1996; Polis *et al.* 2000). The lethal effect of a predator on a prey has been regarded as the main mechanism that regulates prey density and other species through density-mediated indirect effects. More recently, however, it has become evident that density-mediated interactions are not the only mechanism that can impact prey and other species connected to the prey through consumption or other ecological interactions. Consumers can induce morphological, physiological, and behavioral and life history traits on their resources and, through these, affect population and community dynamics (Karban & Baldwin 1997; Werner & Peacor 2003; Miner *et al.* 2005; Ohgushi *et al.* 2007). Indeed, it has been found that these trait-mediated indirect interactions (TMIIIs) can be as important or even more important than the density-mediated effects in some cases (Schmitz 1998; Werner & Peacor 2003; Preisser *et al.* 2005; Kishida *et al.* 2010).

For the most part, TMII have been studied as single units of a consumer-resource system plus a third species affecting a trait of the consumer. However, recent literature in trait-mediated indirect interactions (TMIIIs) suggests that the incorporation of multiple units of TMIIIs in ecological communities can have significant impacts on population and community dynamics (Utsumi *et al.* 2010). Golubski and Abrams (2011) encourage considering more complex species units in TMII studies, for they exert different effects from that of single TMIIIs on interaction

coefficients between pairwise trophic links (*i.e.* host-parasitoid interactions and predator-prey interactions) (see for example, Arditi *et al.* 2005; Daugherty *et al.*, 2007; Goudard & Loreau 2008; Kondoh, 2008; Liere & Larsen 2010; de Roode *et al.* 2011; Golubski & Abrams 2011; Hsieh & Perfecto 2012; Hsieh *et al.* 2012; Pages *et al.* 2012; Lin & Sutherland 2014). A few empirical studies emphasize the importance of two connected TMII that cascade the effect of a remote species to consumer-resource dynamics. These studies investigate systems with different trophic levels in both terrestrial and aquatic ecosystems (Figure 4-1). They conclude that, while the first-tier TMII interferes with the consumer-resource link, this interference is reduced or cancelled in the presence of a fourth remote species that has another trait-mediated effect. In other words, this effect can cascade through the food web to influence multiple trophic levels (Liere & Larsen 2010; de Roode *et al.* 2011; Hsieh *et al.* 2012; Pages *et al.* 2012).

These studies, however, as with other TMII studies (but see Losos *et al.* 2006), do not incorporate likely effects of TMII on the directional selection of resource species. Individuals in a population differ in traits. And, while consumption is trait-dependent, individuals in a population with different traits have different degrees of vulnerability (Losos *et al.* 2006). In the presence of a TMII that interferes or facilitates the preferential consumption of individuals based on a particular trait, (for example, males *versus* females), the strength of the selection is altered. The interplay of TMII, depending on its net effect, would further alter the phenotypic distribution of survivors. This effect may have direct ecological impacts on the population and the ecological community where it is embedded, and would probably lead to evolutionary changes in the resource and its consumer. Considering this complexity would enhance our understanding of “species blocks” – units of species that interdependently rely on each other for survival, growth and reproduction – it could also contribute to our understanding of reciprocal impacts between ecology and natural selection – how ecological interactions affect trait distribution, which may consequently influence ecological dynamics (Johnson & Stinchcombe 2007; Schoener 2011).

Here, we employ a naturally occurring interaction network that includes two interacting TMII units to examine the effect of zero, one and two TMII on a focal resource species with a

parasitoid that prefers to parasitize female over male hosts, which leads to a reduction of female ratio in survivors' population. Previous studies suggest that the interplay of the two TMII effectively cancels the negative effects of one TMII on predation and oviposition of organisms (Liere & Larsen 2010; Hsieh *et al.* 2012), we therefore hypothesize that, 1) one TMII that interferes with parasitism will increase the survival of the resource species and inhibit the host selection, and 2) the interplay of the two TMII will result in the same survival probability and sex ratio as if no TMII exist.

Study System

Our study system is part of a larger complex ecological network that has been described in coffee plantations in the State of Chiapas, Mexico (Perfecto & Vandermeer 2008; Vandermeer *et al.* 2010; Hsieh & Perfecto 2012; Perfecto *et al.* 2014). The target consumer-resource system consists of the coccinellid beetle *Azya orbiger* and its parasitoid, the wasp, *Homalotylus shuvakhinae*. The ant *Azteca serieasur* has been found to affect the parasitism of the beetle by the wasp (Liere and Perfecto 2008). This TMII seems to be driven by the mutualistic relationship between the ant and the green coffee scale, *Coccus viridis*. In a typical ant-hemipteran association, the ant consumes the honeydew produced by the hemipteran and protects it from its natural enemies, including the adult coccinellid beetle (Liere and Larson 2010; Uno 2007). However, the ant does not seem to be able to distinguish between different species of parasitoids and attack any parasitic wasp that comes close to the hemipterans. Since the hemipterans are the main prey of the coccinellid beetle, and they tend to aggregate close to their food source, the ant inadvertently scare away the parasitoids of coccinellid larvae. Furthermore, coccinellid beetle larvae are covered by waxy filament that protects them against the aggressive action of the ants (Hsieh *et al.*, 2012). Therefore, the first TMII consists of the ant altering the ability of the parasitic wasp to attack beetle larvae. A second TMII occurs when a phorid fly appears. *Pseudacten lascinosus* is a phorid fly that parasitizes the ant *A. sericeasur*. Since the phorid fly needs ant movement to be able to locate the ant (Mathis *et al.* 2011), the ant enters into a catatonic state when the phorids arrive, dramatically reducing their foraging activity (Philpott *et al.* 2004; Philpott 2005).

This system allows us to examine a consumer-resource system with, 1) no TMIIIs (the beetle larva, *A. orbiger*, and its parasitoid wasp, *H. shuvakhinae*; Figure 4-2A), 2) one TMII (the beetle, the wasp and the ant *A. sericeasur*; Figure 4-2B), and 3) two interacting TMIIIs (the beetle, the wasp, the ant and the phorid fly *P. lascinosus*; Figure 4-2C). Furthermore, in this study system, unpublished data suggest that the parasitoid wasp prefers female beetle larvae hosts over males, allowing us to examine this preference in the context of TMIIIs. In this study, we ask the following questions: (1) do the ants exhibit aggressive behavior toward the parasitic wasp and does the presence of the phorid fly reduce this aggressiveness, leading to the interaction between the two TMIIIs? (2) how does the presence of no TMII, one TMII (presence of the ant and the absence of the fly), and the interplay of two TMIIIs (the presence of both the ant and the fly) influence the probability of beetle survival and the sex ratio of the beetle?; (3) how does the presence of no TMII, one TMII and the interplay of two TMIIIs influence the per-capita fitness of female and male beetles?; (4) is it possible to detect these effects in the field where all four species are present?

4.3. Materials and Methods

4.3.1. Study site

All the laboratory/mesocosm and behavioral experiments were conducted between 2012 and 2013 in *Finca Irlanda*, a 300 hectare shade-grown organic farm located at 950 – 1,100m in southern Chiapas, Mexico [15° 10.428'N, 92° 20.169'W]. The field surveys were conducted between 2009 and 2012 within a 45-hectare plot established in *Finca Irlanda* since 2004.

4.3.2. Behavioral experiments

To verify the existence of the TMIIIs we designed a behavioral experiment to test whether the ant interfere with the wasps and whether the hovering action of the phorid fly reduces the ants' interference. This experiment consisted of recording ant behavior towards the parasitoid wasp in the presence and absence of phorid flies. We placed small coffee branches with two mature leaves containing about 100 scale insects (*C. viridis*) in one-liter transparent cylinder containers. In each container we introduced 20 ants, waited for at least 15 minutes for the ants to calm down and start tending the scale insects, and then inserted one third- or fourth-instar larva of the beetle (*A. orbiger*). For the ant/phorid treatment we inserted 20 ants, waited for at least 15 minutes and

then inserted one third- or fourth-instar larva of the beetle and three or four phorid flies. Once this basic set up was established, we then released one parasitoid wasp (*H. shuvakhinae*) into all containers and made observations for 40 minutes. Each time that a parasitoid wasp encountered an ant worker, we recorded the response of the ant individual to the parasitoid. Ant responses to parasitoids were classified into two categories: 1) ant ignores the wasp; 2) ant attacks the wasp. All insects were used for a single replicate and then discarded. A total of four replicates were completed for both the presence and absence of phorids. Mesocosm experiments were conducted in insect cages of the biostation in *Finca Irlanda*.

4.3.3. *Azya orbigera* parasitism experiments

To test the effect of zero, one and two interacting TMIIIs on parasitism of the beetle by the wasp, we established a mesocosm experiment with three treatments mimicking ecological communities in which different combinations of biotic interactions were included: 1) beetle larvae and its parasitoid wasp (no TMIIIs), 2) beetle larva, its parasitoid and ants (one TMII), and 3) beetle larvae, its parasitoid, ants and phorid flies (two interacting TMIIIs) as in Figure 2A, B and C. All the experiments were conducted in 60cm × 60cm × 60cm insect cages. A coffee branch with 4 to 6 leaves infested with approximately 100 scale insects (*C. viridis*) was placed inside an open plastic container at the center of the cage. For the treatment with only the beetle larvae and the parasitoid wasp (no TMIIIs) we randomly selected 5 third- or fourth-instar larvae (*A. orbigera*) from those being reared in the laboratory and placed them inside the tent on the coffee branch, and then introduced one parasitoid wasp (*H. shuvakhinae*) in the tent. For the treatment with beetle larvae, parasitoid wasps and ants (one TMII) we had the same set up as the previous treatment but before introducing the parasitoid wasp we placed 60 to 80 ants (*A. sericeasur*) in the plastic container and allowed time for them to calm down and start tending the scale insects. Then, we introduced one parasitoid wasp. For the treatment with the beetle larva, parasitoid wasps, ants and phorid flies (two interacting TMIIIs) we had the same set up as the previous treatment but, at the same time that we introduced one parasitoid wasp we also introduced 3 to 5 phorid flies (*P. laciniosus*) into the cage. All insects were used for one replicate and then discarded and a new replicate with new organisms was started. We completed 10 replicates of the no-TMIIIs and two interacting TMIIIs treatments and 11 replicates of the one-TMII treatment.

Since we could sex only *A. orbigera* adults, the assigning process was blind. After letting the experiment run for 24 hours, we collected all the beetle larvae and kept them in the lab until non-parasitized adults emerged. Emerged adult beetles were sexed based on head color (adult females of *A. orbigera* have black heads, adult males have orange heads).

4.3.4. Source of insects used for the experiments

During the summers of 2012 and 2013, we collected adults of *A. orbigera* in a 45-hectare permanent plot in *Finca Irlanda* and mated them in the lab. Individuals from the first generation of these adults were used for the behavioral experiments and the mesocosm parasitism experiments. In the parasitism experiment beetles were sexed upon emergence. A maximum likelihood estimate of the sex ratio in the interaction-free population (insects reared in the laboratory and not exposed to any intra or interspecific interactions), based on the *mle2* function in the *bbmle* R package (Bolker 2008b; Bolker 2008a), indicates that the beetle sex ratio before the experiment was female-biased, with a mean female probability ($\hat{P}(F)$) of 62.82% with 95% confidence intervals of 51.80% and 73.00%. Workers of *A. serieasur* were collected from a single nest in the coffee farm and used within a day of collection. *P. lacinosus* phorid flies were collected from the field while they were attacking foragers of *A. serieasur*, and used within half an hour of collection. *H. shuvakhinae* were obtained from parasitized *A. orbigera* pupae collected from the field and reared in the laboratory. The parasitoids that were used in the experiment were no older than two days post-emergence.

4.3.5. Field surveys

In addition to the lab experiments, we conducted field surveys in a 45-hectare plot within *Finca Irlanda*, to verify the results of the experiments under natural field conditions. We chose 35 sites with shade trees that had active and strong *Azteca sericeasur* nests. To quantify phorid fly activity at each site we used two bowls, each of which contained 50 ants. We smashed a few ants in the bowl, releasing ant pheromones to arouse phorid attacks and we counted phorid attacks on the ants for five minutes in each bowl. To quantify beetle abundance we spent five minutes collecting and sexing each individual of *A. orbigera* adults that we were able to catch on the four

coffee bushes nearest to the shade tree. Finally, we counted the larvae of *A. orbiger*a on each of the four coffee bushes. Surveys were conducted from June (wet season) to December (dry season) in 2009 and again in 2012. The survey was repeated for four times across the rainy season in 2009 and six times in 2012.

4.4. Data Analyses

4.4.1. Ant behavior

To test if ant behavior towards beetle parasitoids changed in the presence of phorid flies, in each trial, we recorded ant responses to the parasitoid whenever an ant worker encountered a parasitoid. For each trial, we calculated the proportion of actions (either none or aggressive) by ants when encountering the parasitoid wasp in the treatments with and without phorid flies. We used R (R Development Core Team, 2011) to conduct a two-sample Mann-Whitney U test on the proportion of ant actions.

4.4.2. Beetle parasitism experiments- effect of TMIIIs on parasitism of *A. orbiger*a

To investigate the effect of zero, one and two interacting TMIIIs on the parasitism of *A. orbiger*a, we performed an analysis of variance (ANOVA). We also developed a nested model to test whether a proposed mechanism of ecological interactions could be responsible for the observed parasitism of the predatory beetle.

We began with the equation

$$\text{logit}(\hat{P}(S)) = a + bA \quad \text{eqn1}$$

where $\hat{P}(S)$ is the probability of an individual being parasitized, A is a binary variable, standing for the absence (0) and presence (1) of ants, a is the baseline probability, and b is the magnitude of parasitism altered by ants in the logistic function. We further hypothesized that phorid attacks modify the strength of the interaction modification that ants exert upon the host-parasitoid interaction. Therefore,

$$b = g + hP \quad \text{eqn2}$$

where P is another binary variable, standing for the presence (1) and absence (0) of phorids. Substituting b , we obtain the following function,

$$\text{logit}(\hat{P}(S)) = a + gA + hAP \quad \text{eqn3}$$

where g represents the effect of ants (one TMII) on the parasitism rate of *A. orbiger* larvae, and h represents the effect of two interacting TMII (the fly's interference with the ant's interference with the wasp) on the parasitism rate of *A. orbiger* larvae. We used binary responses (1: survival; 0: parasitized) of all available beetle individuals across the three treatments. We performed model selection based on Akaike Information Criterion (AIC) and likelihood ratio tests. For the latter, we started model selection by fitting the full model and preceding each step by eliminating the term that had the least significance (the greatest p -value) on the explanation of the dependent variable. The analysis was performed with the application of the *glm* function in R. By doing this, we determined the maximum likelihood estimates of survival probability of the beetle, $\hat{P}(S)$, in the three treatments: (1) $A = 0, AP = 0$ (no TMII); (2) $A = 1, AP = 0$ (one TMII) and (3) $A = 1, AP = 1$ (interacting TMII), and errors associated with these estimates.

4.4.3. Beetle parasitism experiments - effect of TMII on survivors' sex ratio

To test the effect of TMII on the sex ratio of beetle survivors, we developed a model similar to the one above that describes the mechanism affecting the sex ratio of the surviving beetle larvae population in the three treatments (no TMII, one TMII and interacting TMII) of the parasitism experiment. Likewise, we began with the following equation,

$$\text{logit}(\hat{P}(F|S)) = r + mA + nAP \quad \text{eqn 4}$$

where $\hat{P}(F|S)$ is the probability of being female given survival, r is the probability that an individual is female given survival from parasitism while none of the TMII is present (*i.e.* only host-parasitoid interaction exists), m is the effect of ant interference on the probability of being female given survival from parasitism, and n is the effect of interacting TMII on the probability of being female given survival from parasitism. A and P are both binary variables. Respectively, they represent the ant and the phorid fly, and the binary attributes, 0 and 1, denote their absence and presence. As before, model selection and parameter estimates were conducted with AIC and

the application of *glm* function in R. By doing this, we determined $\hat{P}(F|S)$, the estimate of being a female beetle given survival, for the three treatments: (1) $A = 0$, $AP = 0$ (no TMII); (2) $A = 1$, $AP = 0$ (one TMII) and (3) $A = 1$, $AP = 1$ (interacting TMIIIs), and errors associated with these estimates. In addition, we employed the *mle2* function in the *bbmle* package in R to estimate female probability in the absence of TMII (the beetle and the parasitoid alone), in the presence of one TMII (the beetle, the parasitoid and the ant), and in the presence of two interacting TMIIIs (the beetle, the parasitoid, the ant and the phorid fly).

4.4.4. Beetle parasitism experiments – per capita survival probability with regard to sex

A change of sex ratio within the three different community contexts (i.e. with and without TMIIIs) indicates that ecological interactions can have an important effect on the population dynamics of the focal species. To explore likely evolutionary consequences of community structure, however, requires measuring per-capita survival probability with regard to traits. To this aim, we employ Bayes' Theorem to derive the probability of survival given sex. In our study, each probability of survival from parasitism given sex (female or male) was derived based on the three estimated parameters: $\hat{P}(F)$, $\hat{P}(F|S)$ and $\hat{P}(S)$. According to the Central Limit Theorem, the estimates of proportions, $\hat{P}(S|F)$ and $\hat{P}(S|M)$, are approximately normally distributed

$$\hat{P}(S|F) \sim N \left(\hat{P}(S|F), \sqrt{\frac{\hat{P}(S|F) \times (1 - \hat{P}(S|F))}{n^*}} \right)$$

$$\hat{P}(S|M) \sim N \left(\hat{P}(S|M), \sqrt{\frac{\hat{P}(S|M) \times (1 - \hat{P}(S|M))}{n^*}} \right)$$

with means $\hat{P}(S|F)$ and $\hat{P}(S|M)$, and standard deviations $\sqrt{\frac{\hat{P}(S|F) \times (1 - \hat{P}(S|F))}{n^*}}$ and

$\sqrt{\frac{\hat{P}(S|M) \times (1 - \hat{P}(S|M))}{n^*}}$, where $\hat{P}(S|F)$ and $\hat{P}(S|M)$, respectively, are the population proportions of females and males. Here we employ n^* , the smallest sample size among those of the three variables in the Bayesian formulas for males and females. As the three variables have different

sample sizes, n^* guarantees a conservative estimate of standard error, and thus confidence interval, of each derived probability.

4.4.5. Field surveys

For each survey, we computed the mean and standard error of phorid attack intensity, and applied maximum likelihood estimates of female probability with the application of `bbmle` package. 90% and 95% confidence interval of female probability in the field in each survey was obtained by the application of `confint` function in R.

4.5. Results

4.5.1. Ants are aggressive towards parasitoid wasps and the presence of phorid flies reduce that aggressiveness

Corroborating previous work (Liere and Perfecto, 2008; Liere & Larsen 2010; Hsieh *et al.* 2012), our study showed that ants behave aggressively towards parasitoid wasps and this aggressive behavior is disrupted by the presence of the phorid flies. Ants attacked parasitoid wasps 76% of the time in the absence of phorids and this was reduced to 44% when phorids were present. The result is statistically significant (Mann-Whitney U test, p -value = 0.028) (Figure 4-3).

4.5.2. One TMII does not affect beetle parasitism but two interacting TMII do

Contrary to expectation, and in spite of the evidence that ants disrupt parasitoids (Figure 4-3), the presence of the ants (one TMII) did not alter parasitism but the presence of the ants and the phorid flies (two interacting TMII) significantly increased beetle parasitization (Figure 4-4). Likewise, model selections based on AIC suggest that the best model of survival probability contains the effect of the parasitoid (no TMII) and the effect of ants and phorid flies (two interacting TMII), but not the effect of ants alone (one TMII) (Table 4-1A). This is congruent with the result of the likelihood ratio test in the GLM (Table 4-1B), revealing that parasitoids kill 52.94% ($\pm 7.20\%$) of available *A. orbiger* larvae (Table 4-1C). The 95% confidence interval of survival probability in the condition of no TMII falls within the range of 32.94% and 61.18%, suggesting the probability of survival is not different from the probability of parasitism. On the other hand, phorid flies significantly facilitate parasitism by a mean effect of 26.65%, leading to

only 20.41% ($\pm 5.82\%$) survival probability from parasitism in the presence of phorids, with 95% confidence interval of survival probability between 9.01% and 31.81% (Table 4-1C).

4.5.3. One TMII does not affect survivor's sex ratio but two interacting TMIIIs do

By rearing beetles in the laboratory and doing maximum likelihood estimate (*mle2* function in *bbmle* package of R), we were able to determine that in the absence of ecological interactions (intra or interspecific) the population of *A. orbiger* is female biased (68.8 % female). However, when beetle larvae are exposed to the parasitoid wasp (no-TMIIIs) the sex ratio of the survivors change to be sex-balanced (female proportion 43.38% with 95% confidence interval 21.19% and 65.77%). Similar to the results for parasitism probability, we found that the presence of ants alone (one TMII) did not alter the sex ratio of the beetles that survived from parasitism, but the presence of the ants plus the phorid flies (two interacting TMIIIs) revert the sex ratio of the survivors to strongly female biased (80% female with 95% confidence interval 56.36% and 103.64%; Figure 4-5). Model selections based on AIC suggest that the best model of the probability of being female in the survivor population contains the effect of the parasitoid (no TMIIIs) and the effect of the ants and the phorid flies (two interacting TMIIIs; Table 4-2A). This result is also congruent with the result of a likelihood ratio test (Table 4-2B). The GLM result suggests that the mean probability of being female, given survival in the no-TMII condition, is 43.48%, with 95% confidence interval ranges from 21.19% to 65.77%. This suggests that it is not more likely to find a female survivor than a male survivor when the parasitoid wasp acts alone. This probability does not change in the presence of the ant (one TMII). The effect of interacting TMIIIs that cascades from the phorid fly via the ant to the parasitoid wasp significantly increases the female probability in the survivor population by a mean effect of 36.52%, leading to a female-biased population of 80% with 95% confidence intervals of female probability of 56.36% and 103.64% (Table 4-2C).

4.5.4. TMIIIs affect fitness of male vs. female individuals

As we indicated in the methods section, we could not sex *A. orbiger* larvae, and all sexing was completed at the time of beetle emergence. This limits a direct assessment of differential parasitism on sex. Nevertheless, Bayes' Theorem provides a convenient vehicle by which to

assess the unobserved variables, survival from parasitism of females and males, in the three treatments. A maximum likelihood estimate of female probability suggests a female-biased lab-reared population (mean female proportion 62.82%, 95% confidence interval between 51.81% and 73.00%). Since performing the *mle2* functions yields very similar female probabilities in no-TMII, one TMII and interacting TMII communities to those in the GLM, we employ the MLE of $\hat{P}(F)$ as a denominator in Bayes' formula. The derivation of per-capita male survival probability and per-capita female survival probability in the three tested communities are presented in Tables 4-3A and 4-3B. The results suggest that the fitness of males is significantly reduced while interacting TMII are present, as the confidence intervals of survival probability of males in different communities are non-overlapping. This negative phenomenon is not observed in females. This differential response between sexes may contribute to the above-reported differential sex ratio between treatments.

4.5.5. Field surveys: female biased population in areas with high phorid attack intensity

Field surveys show a declining phorid fly activity with time (*i.e.* as the season progress from wet season to dry season). Grouping the data for each of the 4 sampling periods in 2009 shows that the beetle population is female-biased when the number of phorid attacks is high and then the sex ratio of the beetle population shifts to be male-biased or sex –balanced as the phorid fly attack intensity declines (Figure 4-6). A wide range of uncertainty (95% confidence interval) shows this trend. The same pattern in 2012 was observed. Nevertheless, the trend is less clear as only 90% confidence interval reveals this trend. In general, field patterns support the results from the parasitism experiments that show that the presence of two interacting TMII (i.e. the presence of the phorids) changes the sex ratio of the beetle from being sex-balanced to be female biased (Figure 4-5).

4.6. Discussions

Our results show that interacting TMII can have important effects on a consumer/resource system. In particular, we were able to show experimentally that in the presence of the ant, *A. sericeasur*, and the phorid fly (*P. lascinosus*) (two interacting TMII) the coccinellid beetle (*A. orbigera*) suffers higher levels of parasitization from a parasitoid wasp (*H. shuvakhinae*) and

also, that the beetle population changes from being sex-balanced under the influence of the parasitoid wasps alone (no-TMIIs) to be strongly female biased under the influence of the parasitoid wasps, the ants and the phorid flies (two interacting TMIIs). Although our results confirm that the ant interferes behaviorally with the parasitoids of the coccinellid beetles, and that the presence of the phorid parasitoids of the ants largely cancels this interference effect, in the parasitism experiments we did not detect a significant effect of the presence of the ant on the parasitism rate of beetle by the wasp. One previous study with the same species did report that ants marginally reduced the parasitism of the beetle ($p\text{-value} = 0.0615$, Liere and Perfecto (2008)). This discrepancy may arise due to the age difference of the parasitoid wasps in the two studies. While Liere and Perfecto (2008) used wasps of all possible ages (Liere, personal communication), in this experiment, we used adult wasps less than 2 days old (and preferentially one-day-old adult wasps). The use of younger female wasps may have led to lower levels of parasitism in general. This suggests that the wasps do not fully utilize the resource while free from ant interference. On the other hand, the wasp parasitizes more individuals in the ant+phorid fly treatment (two interacting TMIIs) than in the no-ants (no TMII) or the ant-alone (one TMII) treatments. This indicates that egg production was not a limiting factor of parasitism.

The interesting question here is why, even though the ant behaves aggressively towards the parasitoid wasp and interferes with its foraging behavior, it did not have a significant effect on parasitism probability. A potential explanation is that the observed parasitism in the presence of the ant is the net effect of a positive and a negative effect of the ant on the parasitoid wasp. The negative effect is the already established aggressive behavior of the ant towards the parasitoid wasp (Figure 4-3). The positive effect is more speculative. We suggest that female wasps use the ants to search for good patches for oviposition, in other words, they prefer patches with ants for oviposition. Applying a “mother knows best hypothesis” (Martínez *et al.* 2013), we can speculate that the parasitoid wasp might prefer not to lay eggs in locations where ants are completely absent. Since the larvae of endoparasitoids are completely encapsulated within their hosts, potential defenses against predation or environmental disadvantages are limited. Therefore, habitat and host selection by ovipositing female endoparasitoids is critical for offspring fitness (Godfray 1994; Godfray & Shimada 1999). Following this logic we propose

that ant patches might increase the fitness of parasitoids' offspring. We propose that ant patches provide two benefits to parasitoid larvae. First, since ants tend scale insects, the main food resource of *A. orbiger* larvae, ant patches ensure high scale abundance and therefore high *A. orbiger* abundance. Furthermore, as larvae of *A. orbiger* keep growing after being parasitized, patches with high scale abundance will also ensure a healthier and bigger host. Second, ants may also provide enemy-free space, as many studies have indicated (Kaplan & Eubanks 2005; Lescano *et al.* 2012). In our study system, *A. orbiger* larvae experience multiparasitism (Hsieh, personal observation), and we have observed *Prochiloneurus* sp. (Hymenoptera: Encyrtidae), a hyperparasitoid species, attack the parasitoid larvae. Furthermore, as *Azteca* workers forage, they interfere with intruders that can affect the survival of parasitoid offspring.

The significantly higher parasitism probability of the beetle by the wasp in the presence of ants and phorids also supports the hypothesis that the wasp prefers to oviposit on sites with ants. The phorid fly opens up a temporary ant-free space for parasitoid wasps to oviposit in sites with ants. To our knowledge, this is the first study demonstrating a trait-mediated cascading effect (through two interacting TMIs) all the way to a top carnivore. Other studies have shown that the phorid flies facilitate the feeding and oviposition efficiency of *A. orbiger* by inducing a defensive (motionless) behavior by the ant (Liere and Larsen, 2010; Hsieh *et al.*, 2012). In this study, we show that the effect of phorids can be transmitted across the food chain and influence host-parasitoid dynamics between insects at the third and fourth trophic levels (i.e. coccinellid beetle and their parasitoids).

We see that parasitism is context-dependent. Our study shows that the parasitoid wasp prefers to parasitize females to males, as it drives an original female-biased population to be sex-balanced (Fig. 4-5). On the other hand, the presence of ant-phorid interactions reverses the ratio of the survivors' sexes, generating a strongly female-biased population and potentially affecting reproduction of the beetle (Bessa-Gomes *et al.* 2004; Miller & Inouye 2011; Mains *et al.* 2013). These results suggest that community context (absence vs. presence of interacting TMIs) is important in regulating host-parasitoid interactions, as well as likely trait-evolution involved in host selection.

Per-capita survival probability from parasitism, a fitness measure, reveals a different pattern between female and male beetles. Our conservative estimates of confidence intervals suggest that, as the probability of per-capita female surviving from parasitism does not differ across the three treatments, a male individual clearly suffers from a higher probability of mortality due to parasitism in the treatment where both ants and phorids are present (Table 4-3A, Table 4-3B; Table 4-3C). Since the parasitism rate is higher while phorid-ant interactions exist (Table 4-1C; Figure 4-4), the per-capita probability of parasitism of both female and male beetles should be higher when phorids and ants are present, if no other factor is involved. Nevertheless, the effect of interacting TMIs on per-capita survival probability is only present on males. We are aware that a small n^* leads to a large standard error, potentially masking any possible effect of community context on the per-capita survival probability of females. To address this concern, we further used the largest sample size of the three variables ($\hat{P}(S)$, $\hat{P}(F|S)$, $\hat{P}(F)$) in each community, which is 78 (*i.e.* the sample size of $\hat{P}(F)$), to yield the smallest errors (and thus the narrowest ranges of confidence intervals). Interestingly, this additional analysis still does not separate the per-capita female survival probability in one treatment from another. Although the evidence is indirect, this phenomenon merits further investigation. Here we speculate that the differential fitness between sexes could be a consequence of differential resistance to parasitism. As lab-reared populations of the beetle are clearly female-biased, there is an inherent factor that affects the beetle's sex ratio. We hypothesize that this is likely a result of male-killing bacteria, which have been frequently reported to affect sex ratios in other coccinellid populations (Majerus 2006). As male-killing bacteria are endosymbionts, they must confer fitness benefits to hosts, possibly via increasing resistance of female individuals to parasitism, as reported in various studies (Hurst & Hutchence 2010; Feldhaar 2011; Xie *et al.* 2014). Another possible explanation for the differential resistance to parasitism is associated with the possible use of the phorid-ant interaction by the beetle. A previous study suggests that adult female beetles are responsive to phorid-induced ant pheromones (Hsieh *et al.* 2012). It remains unknown whether female larvae are also able to respond to these chemical compounds and have a tendency to associate more, spatially, with ants than the male beetles. By being close to ants, the female larvae may be more protected from parasitism than the male larvae.

Our five-species study system expands the scope of commonly examined pair-wise interactions in research on trait-mediated indirect interactions (TMII) (Werner & Peacor 2003; Schmitz *et al.* 2004; Mowles *et al.* 2011) by including organisms across three trophic levels under the influence of two trait-mediated indirect interactions. The incorporation of this complexity yields interesting results: that interacting TMII have important effects on the host-parasitoid interaction and that by affecting evolutionary traits (i.e. sex ratio), they can have important evolutionary consequences. Our study suggests that host-parasitoid interactions cannot be fully understood without incorporating this complexity. The parasitoid's host and habitat selection preference, organisms that facilitate or interfere with host-parasitoid interactions, and sex ratio of the host itself, can all affect host-parasitoid interactions, as well as the fitness of hosts and parasitoids. Since the genetic structure of a population varies with sexual reproduction, the sex ratio of the parental population can affect the genetic variation of offspring. Preferential parasitism combined with the effect of community context therefore would influence the evolutionary process.

Another broader implication of this study lies in the agro-ecological functions of this complex system (Vandermeer *et al.* 2010). As *A. orbiger* is an important regulator of the green coffee scale (Charanasri & Nishida 1975), its population dynamics has been a central concern in agroecological literature about coffee production (Perfecto & Vandermeer 2008). Our previous study suggests that *A. orbiger* depends on ant-phorid interactions for reproduction (Hsieh *et al.* 2012). Phorid facilitation cancels out ant interference and allows gravid female beetles to gain access to ant-scale patches that can provide sufficient food source and ant protection to offspring (Liere & Larsen 2010; Hsieh *et al.* 2012). Our present study suggests that the phorid-ant interaction also facilitates *A. orbiger* parasitism (Figure 4-7), and this effect is only reflected in male offspring. It is therefore more costly for male individuals to live in strong phorid-ant interaction patches. From a practical point of view, the presence of phorids, and therefore the interaction of TMII, could have positive and negative effects on the coccinellid beetle and therefore affect the predation pressure of scale insects by this predator. The positive effect consists of facilitating beetle access to scale insects and oviposition sites in the presence of ants

(Hsieh *et al.*, 2012) and the negative effect consist of facilitating parasitism of beetle larvae by the parasitoid wasps in the presence of ants (Figure 4-4; Figure 4-7). However, this negative effect may be counteracted by the fact that female larvae appear to resist more parasitism and under high phorid attacks, the population will tend to be female biased (Figure 4-5).

Our field surveys support these results. While both phorid attack intensity and *A. orbiger* density decline over the rainy season, the sex ratio of *A. orbiger* shifts from being female-biased to being sex-balanced or male-biased towards the end of the year when phorid attack is lowest (Figure 4-6). The strength of phorid attacks could thus be associated with the induction of the cascading effects, leading to the temporal variation in sex-ratio of *A. orbiger*. As the beetle experiences strong selection annually, so do the beetles' effects on ecosystem services (e.g. pest control). Finally, since *A. serieasur* is an arboreal ant, the preservation of shade trees in coffee agroecosystems is an important consideration in order to preserve important ecological interactions that strongly affect ecosystem services. In conclusion, interacting TMIs are crucial for the population dynamics of *A. orbiger*, since it affects feeding, oviposition, mortality due to parasitism and sex ratio.

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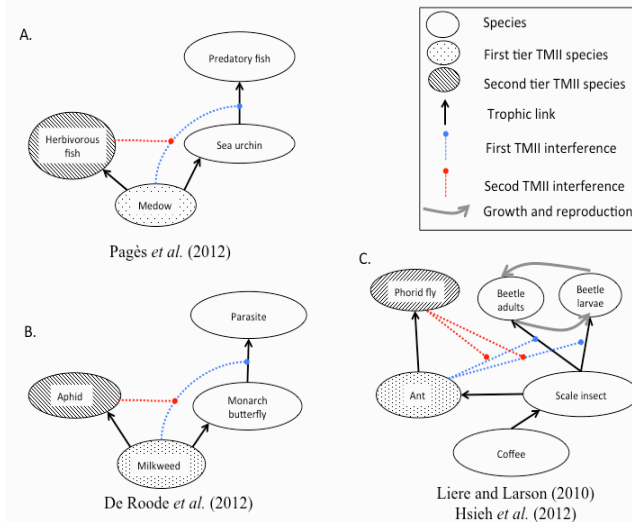


Figure 4-1 Several empirical studies on a specific structure of interacting TMIIIs. A. Meadow provides refuge for sea urchin against the consumption by predatory fish. The presence of herbivory fish shortens the length of the meadow, exposing sea urchin to its predator. Note that the effect of herbivory fish reduces the interference of the meadow with the predator-prey interaction, leading to a cascading trait-mediated effect. **B.** As in A, The secondary metabolite compounds of milkweeds protect the monarch larvae against the parasite. Aphids alter the compositions of the secondary metabolite compounds of the plant, leading a greater vulnerability of monarchs to the parasite. **C.** All insects interact on coffee. The herbivorous scale insect and the ant forms a protection mutualism – the scale insect provides nutrient rewards in exchange of protection against its predatory beetle. The ant interferes with the beetle, reducing its interaction with the scale insect. The hovering action of the phorid fly over the ant, however, cancelling the effect of the ant interference, leading to a predation coefficient as if the ant is absent (Liere and Larsen 2010). The effects of the first tier TMII and its interaction with the second are also reflected on the oviposition of the predatory beetle. Gravid female beetles lay eggs under the scale insect. The ant interferes with this oviposition behavior, and the hovering action of the phorid fly induces the defense behavior of the ant, cancelling the effect of the ant interference with the beetle’s oviposition (Hsieh *et al.* 2012).

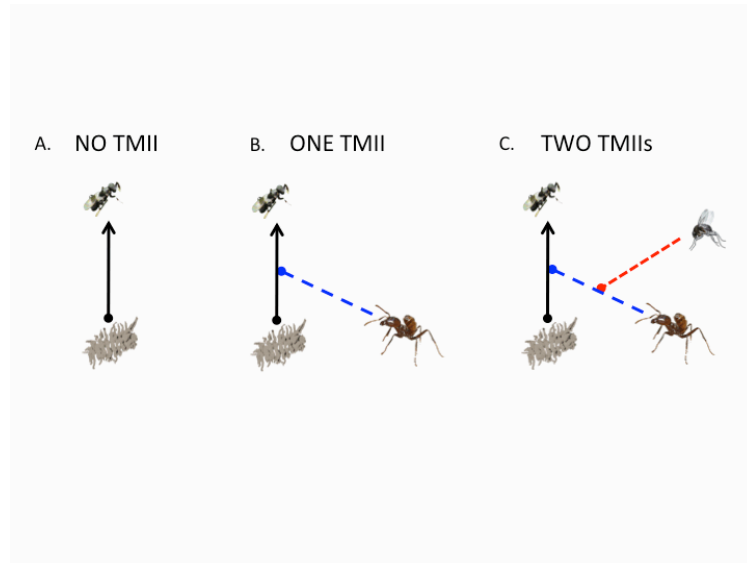


Figure 4-2 The model system is an expansion based on Figure 4.2C, with the addition of the parasitoid wasp of *Azya orbiger*. Coffee is the foundation species on which the green coffee scale (*Coccus viridis*) feeds on. The workers of *Azteca serieasur* forms a protection mutualism with the green coffee scales, harvesting honeydew produced by the scales and interfering with the adults of the predatory beetle (*Azya orbiger*). The larvae of the beetle have naturally-occurring white filaments in protection of ant harassment and take advantage of abundant and quality scale resources at patches with the protection mutualism. On the other hand, the hovering action of the phorid fly, the parasitoid of *Azteca*, induces anti-parasitism behavior of ant workers, creating a space for the beetle adults to feed on and oviposit under the green coffee scale. In the present study, we test whether the effects of trait-mediated indirect interactions in the system can cascade up to a higher (fourth) trophic level, a parasitoid wasp of the beetle larva. We also test if the interplay of TMIIs would affect the sex ratio of beetle survivors and per-capita female vs. per-capita male survival probability. The black solid lines stand for trophic interactions, with arrows standing for positive and dots standing for negative effects. Blue dashed lines present that ant interference with the intruders (the adult beetle and the wasp) and the phorid fly interferes with this interference (cascading trait-mediated effects).

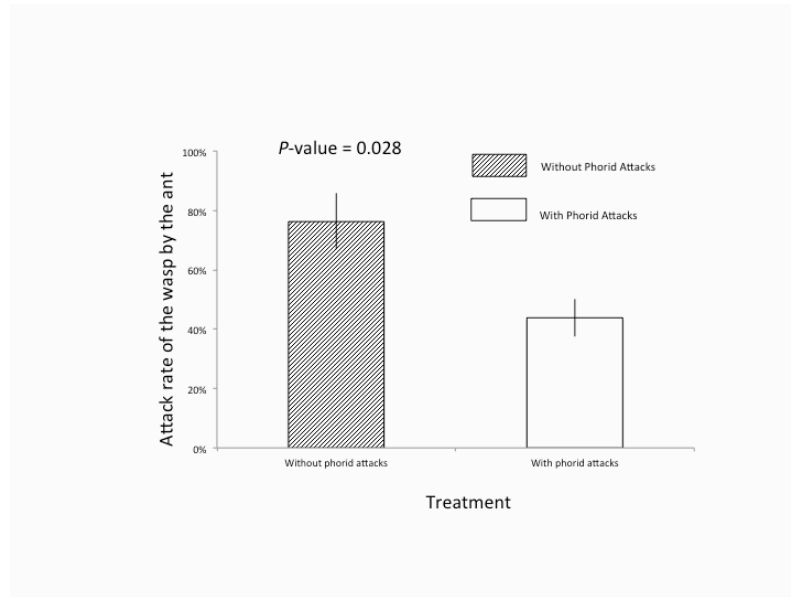


Figure 4-3 The response of the ant (Mean ± S.E.) to the parasitoid wasp in the absence versus the presence of phorid attacks.

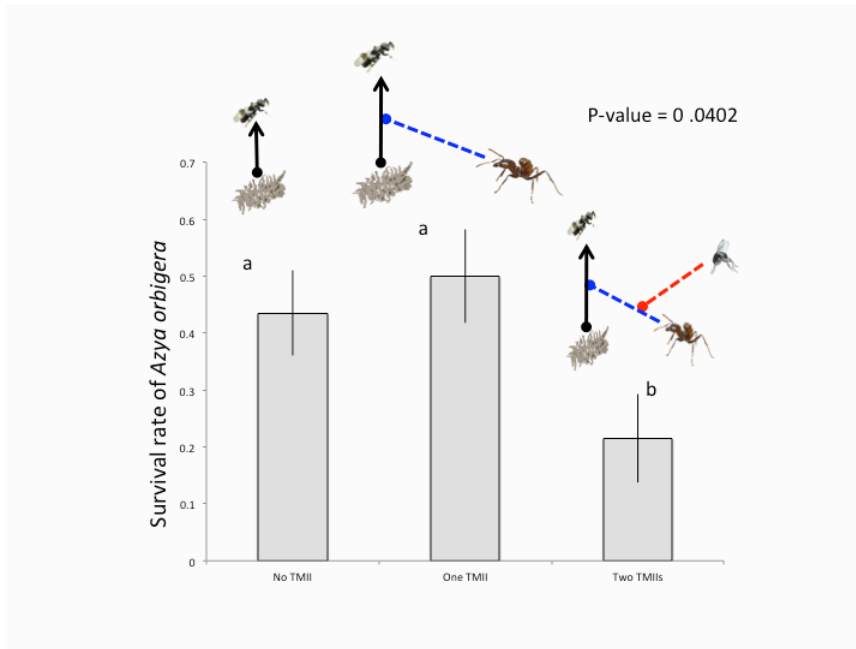


Figure 4-4 The survival rate of *Azya orbigera* in three ecological communities.

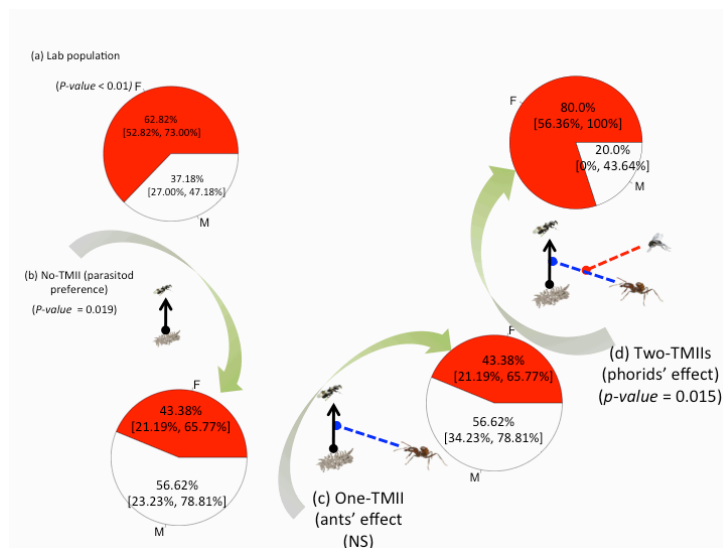


Figure 4-5 Female probability in four different conditions: lab population, no TMII, one TMII and two interacting TMIs. The red portion in a pie chart presents mean female proportion and 95% confidence interval, and the white portion in a pie chart presents mean male proportion and 95% confidence interval.

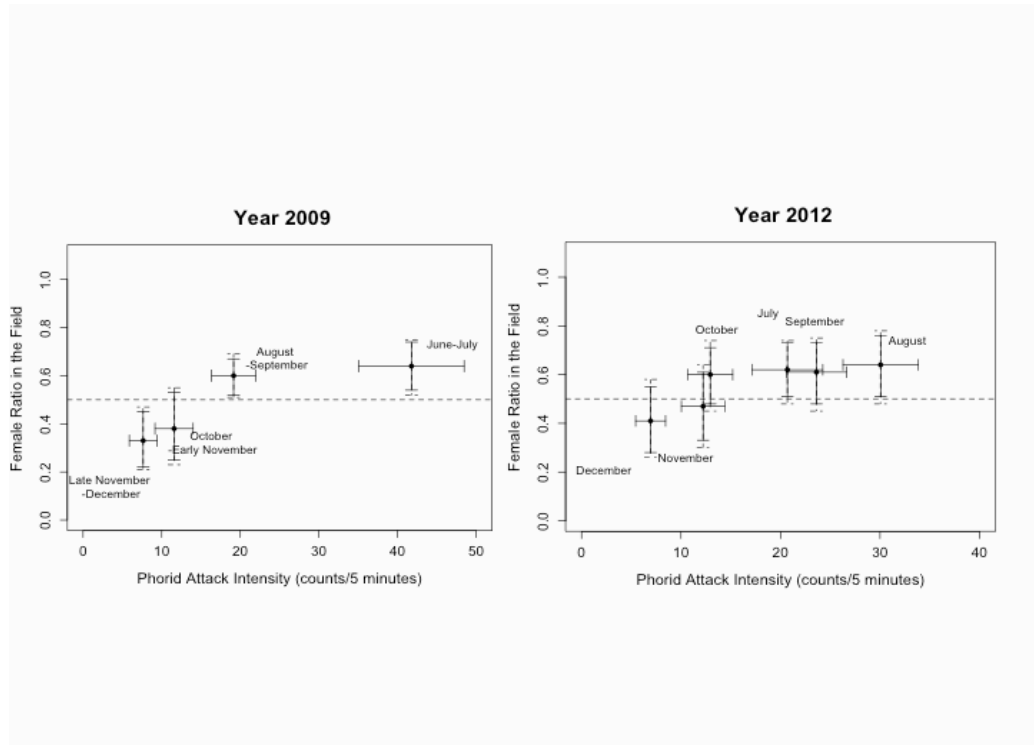


Figure 4-6 Female ratio and phorid attack intensity in multiple surveys in 2009 and 2012. Centers of crosses present means values. Solid lines and dashed lines, respectively, along the *y* – *axes* present the 90% and 95% confidence intervals of female ratio. Wrrors on *x* – *axes* present standard errors of phorid attack intensity.

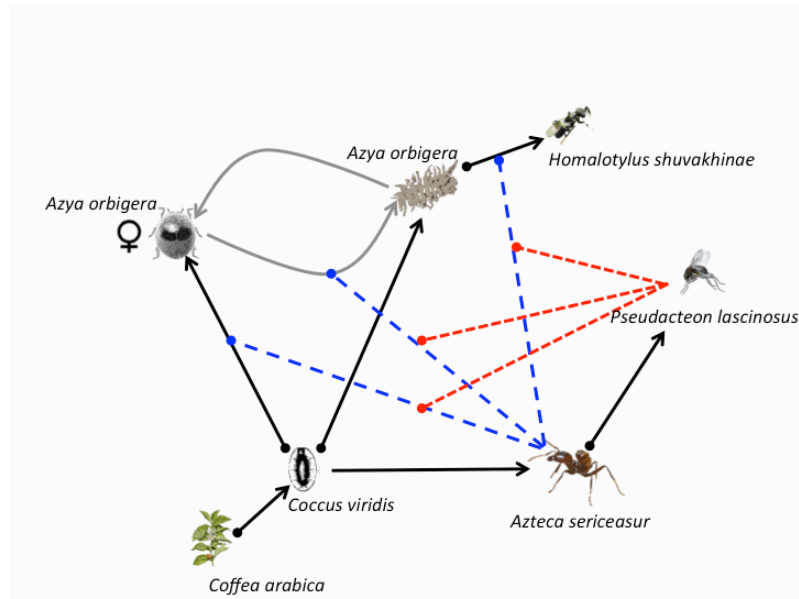


Figure 4-7 The proposed diagram of the dynamics of the study system. Black solid lines present trophic relationships with black dots indicate resources and arrows present consumers. Gray curved arrows present growth and reproduction of the beetle. Blue dashed lines present the first-tier TMI – the indiscriminate interference of the ant with the beetle and the wasp, and red dashed lines presents the disruptions of the first tier-TMI in the presence of the phorid fly, the second-tier TMI organism.

**Table 4-1 The survival probability of *Azya orbiger*a from parasitism
A. Comparisons of generalized linear models**

Model	df	AIC	Residual deviance
1	3	196.24	190.24
2	2	194.64	190.64
3	2	204.30	200.30
4	1	203.14	201.14

Model 1 $\hat{P}(S) = a + b \times A + c \times AP$

Model 2 $\hat{P}(S) = a + c \times AP$

Model 3 $\hat{P}(S) = a + b \times A$

Model 4 $\hat{P}(S) = a$

B. Results of the best generalized linear model for the survival probability of *Azya orbiger*a from parasitism (Model 2), based on AIC and Likelihood Ratio Test

Parameters	Coefficient	S.E.	P-value
<i>a</i>	-0.12	0.20	0.55
<i>c</i>	-1.24	0.41	< 0.01

C. Means, standard errors and 95% confidence intervals of $\hat{P}(S)$ in three communities

Community context	Mean	S.E.	Confidence Interval	
			2.50%	97.50%
Zero or one TMII	47.06%	7.20%	32.94%	61.18%
Two interacting TMIIIs	20.41%	5.82%	9.01%	31.81%

Table 4-2 Probability of being female *Azya* given survival from parasitism

A. Comparisons of generalized linear models

Model	df	AIC	Residual deviance
5	3	78.97	72.97
6	2	76.99	72.99
7	2	80.91	76.91
8	1	79.63	80.91

Model 5 $\hat{P}(F|S) = d + e \times A + f \times AP$

Model 6 $\hat{P}(F|S) = d + f \times AP$

Model 7 $\hat{P}(F|S) = d + e \times A$

Model 8 $\hat{P}(F|S) = d$

B. Results of the best generalized linear model for probability of being female *Azya* given survival from parasitism (Model 6), based on AIC and Likelihood Ratio Test

Parameters	Coefficient	S.E.	P-value
<i>d</i>	-0.26	0.30	0.38
<i>f</i>	1.65	0.84	0.05

C. Means, standard errors and 95% confidence interval of $P(F|S)$ in three communities

Community context	Mean	S.E.	Confidence Interval	
			2.50%	97.50%
Zero or one TMII	43.48%	11.37%	21.19%	65.77%
Two interacting TMII	80.00%	12.06%	56.36%	103.64%

Table 4-3 Per-capita survival probability of female and male in three ecological conditions

A. Statistics of $\hat{P}(S|F)$, per-capita female survival probability in the three ecological communities

	Community context	Mean	<i>n</i>*	S.E.	2.5%	97.5%
Female	No TMII or one TMII	27.05%	19	10.19%	7.08%	47.03%
	Interacting TMIIIs	25.99%	10	13.87%	-1.19%	53.18%

B. Statistics of $\hat{P}(S|M)$, per-capita male survival probability in the three ecological communities

	Community context	Mean	<i>n</i>*	S.E.	2.5%	97.5%
Male	No TMII or one TMII	80.87%	19	9.02%	63.18%	98.55%
	Interacting TMIIIs	10.98%	10	9.89%	-8.40%	30.36%

Chapter 5 Community-wide impacts of interactive trait-mediated interactions: A case study in neotropical coffee agroecosystems

5.1 Abstract

Trait-mediated indirect interactions can have strong effects at the community level. Here we investigate whether a specific combination of two trait-mediated indirect interactions (TMII) has broader impacts on the arthropod community that live on coffee plants. The two TMII are 1) an ant indiscriminately interfering with predators and parasitoids while tending a hemipteran herbivore, and 2) the induced anti-parasitism behavior of the ant while under attack of a phorid fly parasitoid. Previous studies suggest that the complexity of these two interacting TMII affects the population dynamics of a coccinellid beetle that preys on the hemipteran. We explore whether these interacting TMII affect other organisms in the wider-community of arthropods that live and forage on the coffee plants. In 20 sites at each of two coffee farms in Mexico we sampled insects with sticky traps before and after the induction of the interactive TMII. We found that this interaction complexity has an important impact on the activity of coleopteran and hemipteran insects in general, and on a specific rove beetle and a specific leafhopper. Coleopteran insects and the rove beetle increase activity after the induction of the TMII, while hemipterans and the leafhopper decrease after the induction of the interactions. None of the other arthropods sampled showed significant changes after the induction of the interactions. These results suggest that complex interacting TMII can influence the activity of a broader range of arthropods that are living or foraging on plants where the TMII take place, but the effect is not general to all arthropods.

5.2 Introduction

Historically, direct consumptive trophic interactions have been the focus of the study of food webs. However, recent empirical evidence and theoretical explorations support the idea that multiple non-consumptive interactions can have strong population and community level effects

(Arditi *et al.* 2005; Goudard & Loreau 2008; Golubski & Abrams 2011; Kéfi *et al.* 2012; Lin & Sutherland 2014; Kéfi *et al.* 2015). For example, by changing the behavior of other organisms without directly consuming them, a predator can have a strong effect on its prey and this can translate into community-wide effects (Schmitz *et al.* 1997; Schmitz & Suttle, 2001). Furthermore, these trait-mediated indirect interactions (TMIIIs) can be linked to one another forming complex interacting TMIIIs with potentially wider community effects (Utsumi *et al.* 2010).

One such example of interacting TMIIIs has been described for the coffee agroecosystem in Mexico (Perfecto & Vandermeer 2015). This system consists of two connected TMIIIs. The first one consists of the arboreal ant *Azteca sericeasur*, its hemipteran mutualist the green coffee scale, *Coccus viridis*, and the coccinellid beetle, *Azya orbigera*, that preys on the scale insect (Fig. 5-1A). The ant protects its hemipteran mutualist by chasing away and harassing its predators and parasitoids (i.e., a trait-mediated interaction) (Uno 2007). So, even though the ant rarely kills the natural enemies of the scale insects (i.e. a density-mediated interaction), it still benefits the population of the scale through this trait-mediated effect. The second TMIII consists of a phorid fly, *Pseudacteon lascinosus*, that parasitizes the ants and therefore is connected with the first TMIII just described (Fig. 5-1B). Because the phorid fly needs movement to locate the ants (Mathis *et al.*, 2011), as a protective measure, when the phorids arrive, the ants assume a particular stationary posture and reduced their activity by about 50% (Philpott 2005). Therefore the presence of the phorid fly parasitoid effectively cancels the protective effect that the ants have on the scale insects, allowing predators to attack them (Liere & Larsen 2010; Hsieh *et al.* 2012). These interactions have been shown to be sometimes highly fine-tuned. For example, it has been shown that *A. orbigera*, the coccinellid beetle that preys on the scale insects, is able to detect the pheromones that the ants release when the phorids are attacking and use that as a window of opportunity to prey on the scale insects and oviposit on plants with high densities of scales and ants (Liere and Larsen, 2010; Hsieh *et al.*, 2012). More recently, we have discovered that the effect of the phorid fly cascades up all the way to a parasitoid wasp that attacks the coccinellid beetle (Chapter 4).

These results suggest that these interacting TMIIIs could have a broader community level impact. *Azteca sericeasur* is a very aggressive arboreal ant with strong effects on other

arthropods that forage on coffee plants. For example, it has been demonstrated that plants with this ant suffer less damage from one of the main pests of coffee, the coffee berry borer, *Hypothenemus hampei* (Perfecto & Vandermeer 2006). Since the presence of the phorid fly reduces the activity of the ant, providing a window of opportunity for the coccinellid beetle to eat scales and oviposit on coffee plants, it can potentially provide a window of opportunity for other insects that typically forage on coffee plants.

In this study we examine whether these interacting TMIs are important in a wider community context. More specifically, we ask whether the activity of other coffee-associated arthropods is affected by these interacting TMIs. We predict that the presence of the interacting TMIs (Fig. 5-1B) will increase the activity of many other arthropods that use coffee and not only those that are directly involved in the TMIs themselves. Determining the effects of interacting TMIs in the broader community of arthropods would contribute to a better understanding of how these non-consumptive interactions affect ecological networks.

5.3 Methods

5.3.1. Study sites and field experiments

To explore if the interactive TMIs would have community-wide impacts, we established 20 sites at each of two large farms with different management in the Soconusco region of the state of Chiapas, Mexico, *Finca Irlanda* (15°11'N, 92°20'W) and *Finca Hamburgo* (15°10'N, 92°19'W). According to the criteria of Moguel & Toledo (1999), *Finca Irlanda* can be classified as a commercial polyculture, with high shade tree diversity and 57% canopy cover, while *Finca Hamburgo* can be classified as a shaded monoculture, with 15% canopy cover (Iverson, unpublished data). In the two types of farms we selected coffee plants that had a high density of scale insects that were tended by a healthy colony of *A. sericeasur*. To measure how the interacting TMIs (i.e. the presence of the phorid flies) affect the activity of the arthropods that use coffee, we used three 10.16 x 17.78 (cm) double-sided yellow sticky traps (Seabright Laboratories), sampling before and after the phorids arrived. Sticky traps were placed at about 1 – 1.2m in height around a coffee plant and were positioned on twigs at roughly equal distance

from each other, generating three central angles of 120°. We left the sticky traps on the coffee plants for thirty minutes before inducing the interactive TMIs. The induction of the interactive TMIs was demonstrated in a previous field experiment (Appendix F). In this field experiment, we squeezed ant workers, which released alarm pheromones that attracted the phorid fly. The hovering action of the phorid fly reduces the ant activity (Appendix F). In the previous and present field experiments, we also observed no phorid arrival before squeezing ants. We then collected the three sticky traps and placed them on a zip-lock bag. We then induced the interactive TMIs – the interruption of ant interference with arthropods by phorids-induced anti-parasitism behavior of the ant, and placed another three sticky traps on similar locations on the same coffee plants. After thirty minutes we collected this second set of sticky traps and placed them in zip-lock bags. To induce the interactive TMIs, we squeezed a few ant workers to release ant alarm pheromones, which attract *P. lacinosus* (Mathis et al. 2011). The hovering action of *P. lacinosus* stimulates ant workers to assume a motionless status, likely accompanying with specific pheromones, therefore visually and/or chemically signaling other organisms of low ant activity at that time (Hsieh et al. 2012). Collected insects were washed off sticky traps by applying mineral spirits and preserved in 95% ethanol for future identification to order and morphospecies.

Insects were sorted into orders within the orders Coleoptera, Hemiptera, Diptera and Hymenoptera, and then were further sorted into morphospecies (except for the dipterans). At the order level, three response variables were obtained, abundance, species richness, and Simpson's index of diversity.

5.4. Data analyses

Due to the non-normality of the data, we employed a non-parametric Wilcoxon signed-rank test on the effects of the treatment (with vs. without phorid attacks on the ant) on the following variables at order levels:

- (1) Total abundance combined for all Coleoptera, Hemiptera, Hymenoptera and Diptera in both of the farms and in each farm separately.

- (2) Total species richness combined for all Coleoptera, Hemiptera and Hymenoptera in both of the farms and in each farm separately.
- (3) Total species Simpson index of diversity for all Coleoptera, Hemiptera and Hymenoptera in both of the farms and in each farm separately.
- (4) Species abundance of Coleoptera, Hemiptera, Hymenoptera, and Diptera, respectively, in both of the farms and in each farm separately.
- (5) Species richness of Coleoptera, Hemiptera and Hymenoptera, respectively, in both of the farms and in each farm separately.
- (6) Simpson's index of diversity of Coleoptera, Hemiptera and Hymenoptera, respectively, in both of the farms and in each farm separately.

At the species level, we pooled all of the data of the two farms together, and identified those insect morphospecies that accounted for 95% variations in a Principal Component Analysis (PCA). Then we employed a non-parametric Wilcoxon signed-rank test on the effect of treatment on species counts on traps, to examine if treatment has an effect on their activities in both of the farms and in each farm separately.

Finally, we eliminated the one morphospecies that had the highest significant difference (p – values < 0.05) from the dataset and repeated the same analysis so as to determine whether the observed differences at the order level were only a consequence of a specific species. All statistical analyses were conducted in R (R Development Core Team 2011).

5.5. Results

5.5.1. Responses of total arthropods and particular orders

The presence of the phorid parasitoids (interacting TMIIIs) did not change total insect abundance, richness or Simpson Index of diversity of arthropods when we pooled the data for both farms or in *Finca Hamburgo*. Simpson Index of diversity of arthropods significantly increased in *Finca Irlanda*, while species richness and abundance had no change (Table 5-1; Figure 5-2).

Some significant differences appear as we test the effect of the treatment on particular orders and/or in individual farms. In particular, the abundance of insects in the order Coleoptera significantly increased after phorid attacks (Table 5-1). This effect was significant both for the data combined for both farms and as for *Finca Irlanda* (the more diverse farm) alone. Diversity and richness of Coleoptera were marginally significant for the combined data and significant for *Finca Irlanda* (Table 5-1; Figure 5-3). Finally, the abundance of insects in the order Hemiptera decreased significantly after phorid attacks in *Finca Irlanda* but this effect was only marginally significant when data from both farms were combined (Table 5-1; Figure 5-4). Insects in the orders Hymenoptera and Diptera did not respond to the treatment in any direction nor on any of the farms (Table 5-1; Figure 5-5; Figure 5-6).

5.5.2. Responses of Morphospecies

A principal component analysis suggests the first twenty principal components are responsible for 95% of the loadings (Table 5-2A). Nevertheless, only the first few components account for large quantities of variations (Table 5-2A). At the species level, the PCA suggest that *Myrmedonota shimmerale*, a predatory rove beetle symbiotically associated with *A. serieasur* (Mathis & Eldredge, 2014) is responsible for the first principal component in the PCA (Fig. 5-7; Table 5-2B). This rove beetle explains 43.2% of variation of the PCA. Following *M. shimmerale*, a flea beetle (Coleoptera_MS9) is responsible for the second principal component and explains an additional 16.8% of variation (Fig. 5-7; Table 5-2B). The rest of the insects contributing to the leading principle components of the PCA are two Hymenopteran species, two leafhoppers, and *Azya orbiger*, another flea beetle (Coleoptera_MS3), the coccinellid beetle that preys on the scale insects and is known to use the interactive trait-mediated interactions (Liere & Larsen 2010; Hsieh *et al.* 2012). These species all together are responsible for the third component that explains an additional 16% of variation (Table 5-2A; Table 5-2B).

The non-parametric Wilcoxon signed-rank test on *Myrmedonota shimmerale* shows that the rove beetle abundance increases after phorid attacks for the pooled data as well as in *Finca Irlanda* (Table 5-3; Figure 5-8). Similar to the results for the order Hemiptera, the test results indicate that a specific leafhopper (Hemiptera_MS1) decline in abundance after phorid attacks for the pooled data. However, contrary to the results at the order level, this leafhopper showed

significant declines after phorid attacks only in *Finca Hamburgo* but not in *Finca Irlanda* (Table 5-3; Figure 5-8). The rest of the individual species did not change significantly after phorid attacks (Table 5-3).

To see if the effects observed at the order level for Coleoptera and Hemiptera were due to simply to the effect of the rove beetle (*Myrmedonota shimmerale*) and the leafhopper (noted as ‘Hemiptera_MS1 (leafhopper)’ in Table 5-3) respectively, we removed these two species from the data set and performed a Wilcoxon signed-rank test on the effect of Coleoptera abundance, richness, and diversity, and on Hemiptera abundance, richness and diversity on the modified data set. The results show that Coleoptera abundance continues to show a significant increase after phorid attacks in *Finca Irlanda*, but the effect disappeared for the pooled data. The effect also disappears for richness and diversity regardless of whether the data was pooled or not. (Table 5-4). For the order Hemiptera, the results continue to show a significant decline in the abundance for *Finca Irlanda*, but the marginal effect recorded for the pooled data disappeared (Table 5-4).

5.6. Discussions

This study suggests that the activity of some arthropods that use coffee (as measured by their counts on sticky traps) is affected by interacting TMIs (indicated by the appearance and attacks of phorids on ants). This suggests that at least some arthropods do use the window of opportunity provided by the low ant activity that emerges from the TMIs generated by the arrival of phorid flies. However, these broader effects are only observed in the order Coleoptera.

It is clear that the diversity difference between treatments comes from the order Coleoptera exclusively (Table 5-1). We also were able to detect significant changes in the abundance of Coleoptera and Hemiptera but these effects were opposite (Table 5-1; Figure 5-3; Figure 5-4). As we expected, the abundance of Coleoptera in sticky traps increased after phorid attacks. This can be explained by the negative effect that the phorid flies have on the activity of the ants. Given the aggressiveness of these ants, it is not difficult to explain why insects with high mobility, like adult beetles, would increase their activity once there is a decline in ant activity. Ant activity has been reported to decline by 50% when the phorid flies arrive and start

attacking the ants (Philpott, 2005). Frequently ants become catatonic (paralyzed) as a defense against the phorid parasitoids or retrieve to their nest (Perfecto & Vandermeer 2008). The PCA analysis suggests that the main effects of increased abundance after phorid attack is due to Coleoptera, and in particular to the rove beetle, *Myrmedonota shimmerale*, two flea beetles and *Azya orbiger*, the coccinellid beetle that preys on the green coffee scale in coffee. However, when individual analyses were performed on these species, only the rove beetle showed a significant increase with phorid attacks. *Myrmedonota shimmerale* has a strong association with *Azteca sericeasur* and has been shown to be attracted to pheromones that the ants release when under phorid attack (Mathis & Eldredge, 2014). This species has been observed inside *A. sericeasur* nests and has been shown to prey on ants. It is possible that *M. shimmerale* could be using the lower ant activity as a window of opportunity to enter the ant nest. Although the increased abundance of Coleoptera is not only a consequence of the responses of the rove beetle, when this species was eliminated from the data, the effect of the treatment disappeared for the pooled data but remained strong for *Finca Irlanda* (Table 5-4), indicating that other beetle species also increased their activity after phorid attacks on the diverse farm but not on the more intensive farm that has lower abundance and diversity for most orders sampled, including Coleoptera (Table 5-4; Figure 5-9; Figure 5-10; Figure 5-11; Figure 5-12; Figure 5-13). The elimination of the rove beetle also resulted in the disappearance of the effect of the phorids on beetle richness and diversity. This suggests that *M. shimmerale* has a very strong effect on the overall responses observed for the order Coleoptera.

The reduction in the activity of Hemipteran insects after the induced of phorid attacks is harder to explain. We observe significantly fewer individuals of Hemipteran insects after the inductions of phorid attacks in *Finca Irlanda*, and a marginal effect in the same direction for the pooled data (Table 5-1). Although associations between ants and Hemiptera are common in the families Coccoidea, Aphidoidea and Membracidae, associations with Cicadellidae, the leafhoppers, are less common (Moya-Roygoza & Larsen 2008; Stiller 2012)(Hölldobler & Wilson 1990; Larsen *et al.* 2001; Moya-Roygoza & Larsen 2008; Moya-Raygoza & Larsen 2014), with the apparent exception of the genera *Dalbulus* (Nault *et al.* 1983; Moya-Raygoza & Nault 2000) and *Rotundicerus* (Maravalhas and Morai, 2009). Since most of the samples collected in this study were leafhoppers from other genera that have not been described as ant

mutualists, we doubt that the ant-hemipteran association explains the decline. We find no conclusive evidence that hemipteran insects reduce activities while ants are facing the challenge of balancing the trade-off between foraging and parasitism risk. A potential explanation of the observed reduction is a sampling bias intrinsic of the methodology used to assess the effect of the phorid flies. Since we sampled insects before and after the induction of the phorid attacks, those insects that were present at low numbers could have been caught on the sticky traps during the first sampling period (the “before” treatment) leaving very few or no individuals to get trapped during the second sampling period (the “after” treatment). It is possible that the significant reduction in the Hemiptera that was observed in our study is a reflection of this sampling bias given the general low abundance of insects in this order that were captured in the study (Figure 5-11). This bias is likely to be stronger for insects that get attracted to yellow sticky traps, like leafhoppers and aphids (Chu *et al.* 2000).

Removing the most abundant leafhopper species (Hemiptera_MS1) from the dataset does not change the significant decline of the abundance of Hemiptera in the diverse farm but eliminates the marginal significance for the pooled data. This suggests that this effect is not very strong and varies between farms (Table 5-1, Table 5-4). The significant decline in the specific leafhopper (Hemiptera_MS1) was observed for the more intensive farm and for the pooled data but not for the diverse farm, which confirm that the decline in overall Hemiptera observed, was not due to this particular species (Table 5-1, Table 5-4).

The broader arthropod community-wide impact found in this study was limited. However, our sampling efforts were also limited since we sampled for only thirty minutes for each treatment at each site. We decided to limit the time period of the sticky traps because we were interested in documenting potential immediate behavioral effects of the phorid flies on other organisms that were not obvious component of the TMII network. We also wanted to limit the potential depleting effect of the sticky traps (as mentioned above). This short time period could help explain the discrepancy between previous studies with laboratory experiments and our field study. Specifically, laboratory experiments have demonstrated that *A. orbiger* gravid females are attracted to the pheromones that ants release when under attack by the phorid flies (Liere & Larsen 2010; Hsieh *et al.* 2012). Furthermore, Chapter 4 in this dissertation also suggests that the parasitoid wasp of *A. orbiger*, *Homalotylus shuvakhinale*, is facilitated by

phorid attacks. In addition, a study on the interaction between *A. serieasur* and *Myrmedonota shimmerale* and *Myrmedonota xipe* suggest that both species of rove beetles utilize the time period of phorid attacks on *A. serieasur* to mate and predate on ant workers (Mathis & Eldredge 2014). In our dataset, however, only *M. shimmerale* shows a significant increase in activity after phorid attacks. Results from the PCA analysis shows that while *M. shimmerale* explains 43.2% of variation of the PCA, *A. orbiger* accounts for only 5.6% along with several other species (Table 5.2), and *M. xipe* fell into the 15th principal component, which explains less than 1% of variation. We only encountered one individual of *H. shuvakhinae* after phorid attacks and two before phorid attacks in *Finca Hamburgo*. Since the time period for the sticky traps was short, most of these species were represented by just a few individuals (Table 5-5, Table 5-6).

Conducting the study in two farms with different management allowed us to compare abundance, richness and diversity of arthropods between the two types of farms in addition to the treatment effect. Unfortunately, it was not possible to repeat the study in more farms and therefore our conclusions from this analysis are limited. In line with several other studies of biodiversity in coffee farms with different degree of intensification (mostly measured by the number and diversity of shade trees; Perfecto and Vandermeer, 2015), we found the effect of farm management to be strong for most of the variables measured (Table 5-9 to Table 5-14). *Finca Irlanda*, the less intensive farm had significantly greater abundance of total arthropods (Table 5-9), Coleoptera (Table 5-10), Hemiptera (Table 5-11), and Diptera (Table 5-12), but not Hymenoptera (Table 5-13). *Finca Irlanda* also had a significantly greater species richness of Coleoptera (Table 5-10) and Hemiptera (Table 5-11), and a greater diversity of Coleoptera (Table 5-10). In addition, the numbers of *M. shimmerale* and a hymenopteran wasp (Hymenoptera_MS3) were significantly greater in *Finca Irlanda*. *M. shimmerale* accounts for 40% of Coleoptera in *Finca Irlanda*, but it only has a tenth of this magnitude in *Finca Hamburgo*. Rove beetles are often found in moist habitats, especially where there is decaying plant and animal materials (Frank & Thomas 2009) and this may help explain the large difference between the two farms. In addition to the lower density and diversity of trees in the more intensive farm (*Finca Hamburgo*), the application of agrochemicals may contribute to the reduced levels of arthropods in the more intensive farm. Overall, the higher abundance and

diversity of arthropods collected in the less intensive farm in this study may help explain why most of the significant changes observed were observed on this farm.

In sum, we observed significant increases in abundance, richness and diversity as a result of the interactive TMIs (the presence of the phorids reducing the activity of the ants), but this effect was limited to the order Coleoptera. The challenge of balancing trade-offs between foraging and parasitism risk of *A. seriatus* can lead to the differential activities of some insects. As organisms interdependently interact with each other for growth, survival and reproduction, these results suggest that TMIs may have broader impacts than previously thought and should be subject to more in-depth study.

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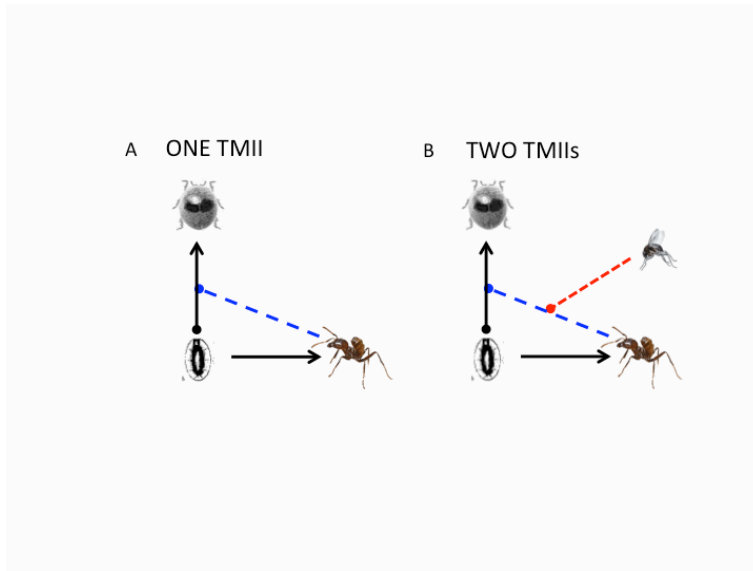


Figure 5-1 The two interactive interactions in this study. **A.** One TMII due to ant interference with organisms in ant-hemipteran patches. **B.** The interruption of ant interference, resulting in cascading effects on organisms in the ecological community

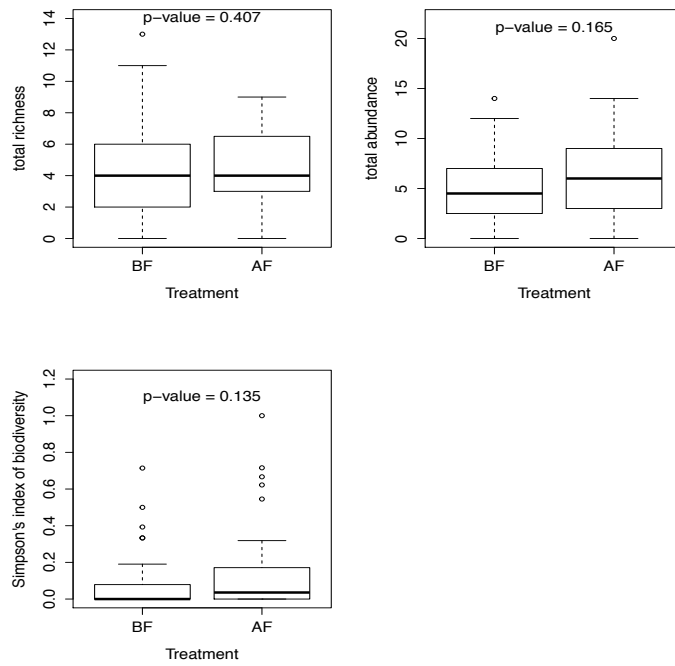


Figure 5-2 Results (*P* - values) of Wilcoxon sign-ranked tests on total species richness, abundance and diversity. BF represents 'before phorid attacks', and AF represents 'after phorid attacks.'

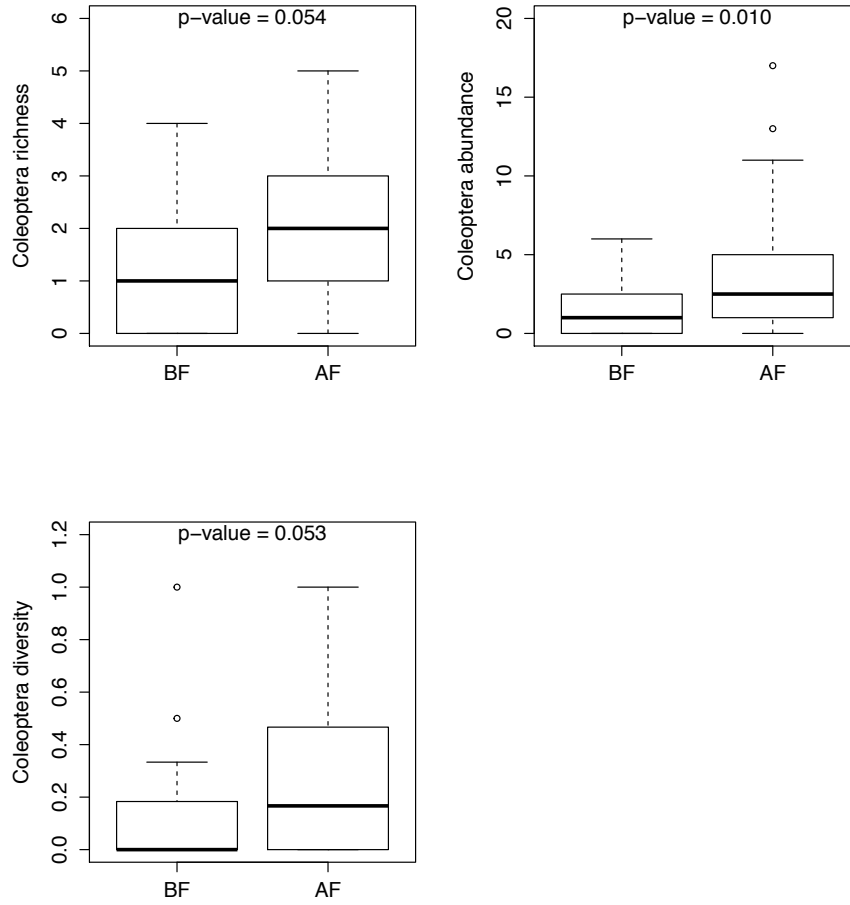


Figure 5-3 Results (*P* - values) of Wilcoxon sign-rank tests on total species richness, abundance and diversity of Coleoptera. BF represents 'before phorid attacks', and AF represents 'after phorid attacks.'

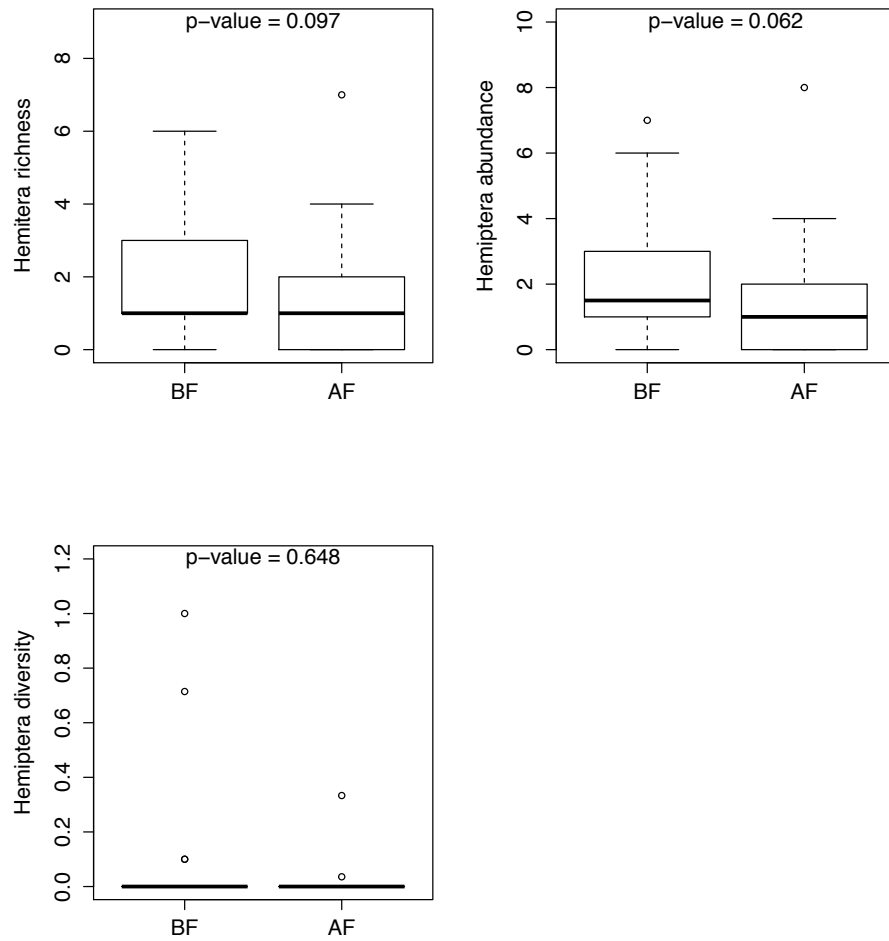


Figure 5-4 Results (*P* - values) of Wilcoxon sign-ranked tests on total species richness, abundance and diversity of Hemiptera. BF represents 'before phorid attacks', and AF represents 'after phorid attacks.'

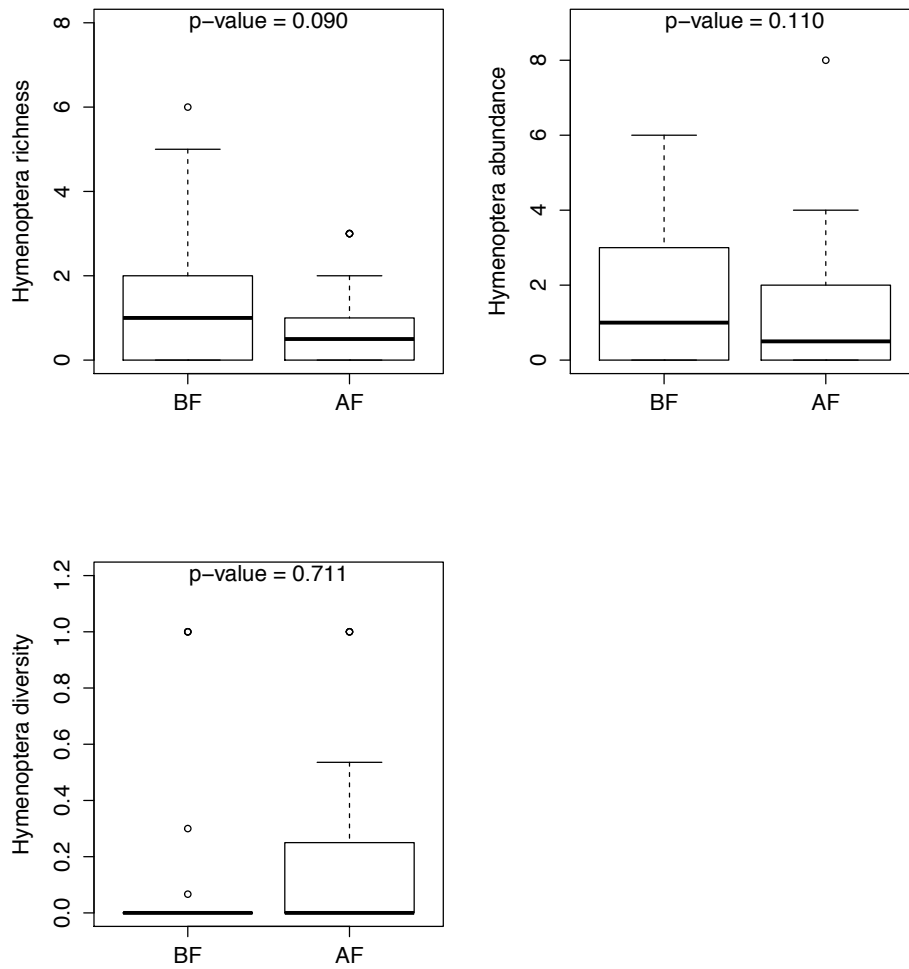


Figure 5-5 Results (*P* - values) of Wilcoxon sign-ranked tests on total species richness, abundance and diversity of Hymenoptera. BF represents 'before phorid attacks', and AF represents 'after phorid attacks.'

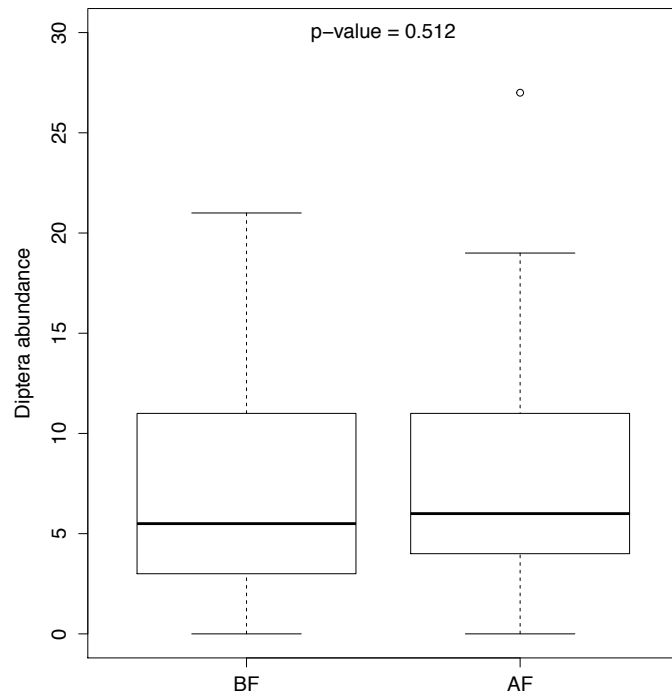


Figure 5-6 Results (*P* - values) of Wilcoxon sign-ranked tests on species abundance of Diptera. BF represents 'before phorid attacks', and AF represents 'after phorid attacks.'

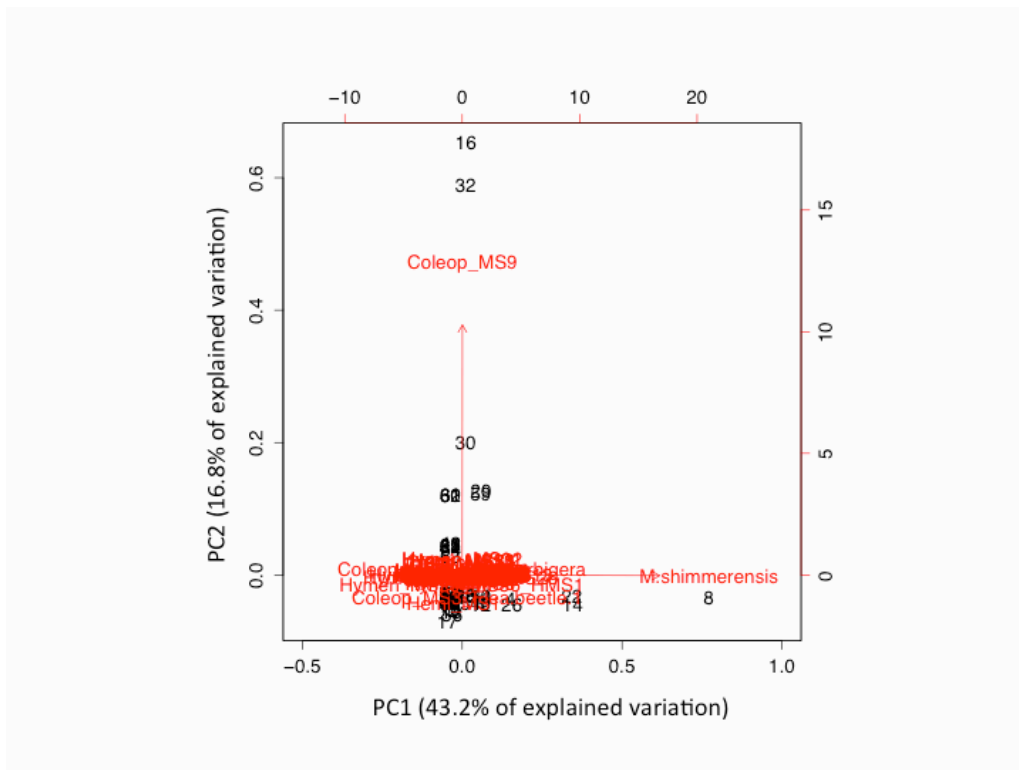


Figure 5-7 Biplot of the PCA. Data are superimposed on the first two principal components. *M. shimmerale* explains the first principal component (43.2% of variation) and Coleoptera_MS9 (flea beetle) explains the second principal component (16.8% of variation).

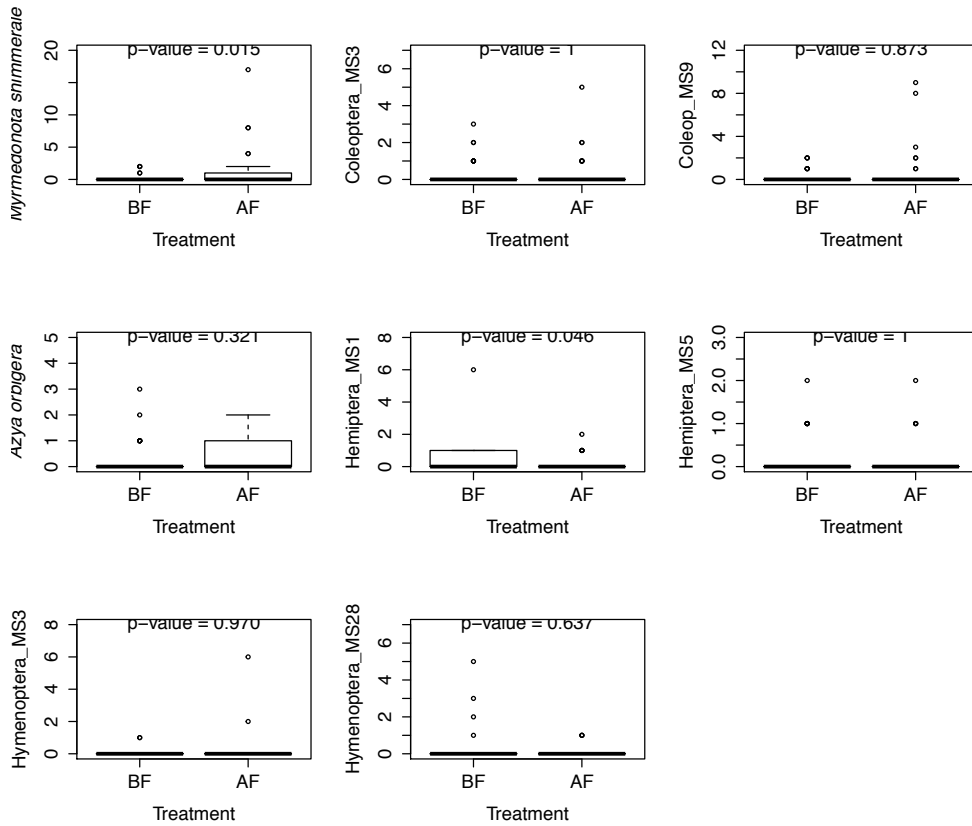


Figure 5-8 Results (*P* - values) of Wilcoxon sign-ranked tests on species counts. BF represents 'before phorid attacks', and AF represents 'after phorid attacks.'

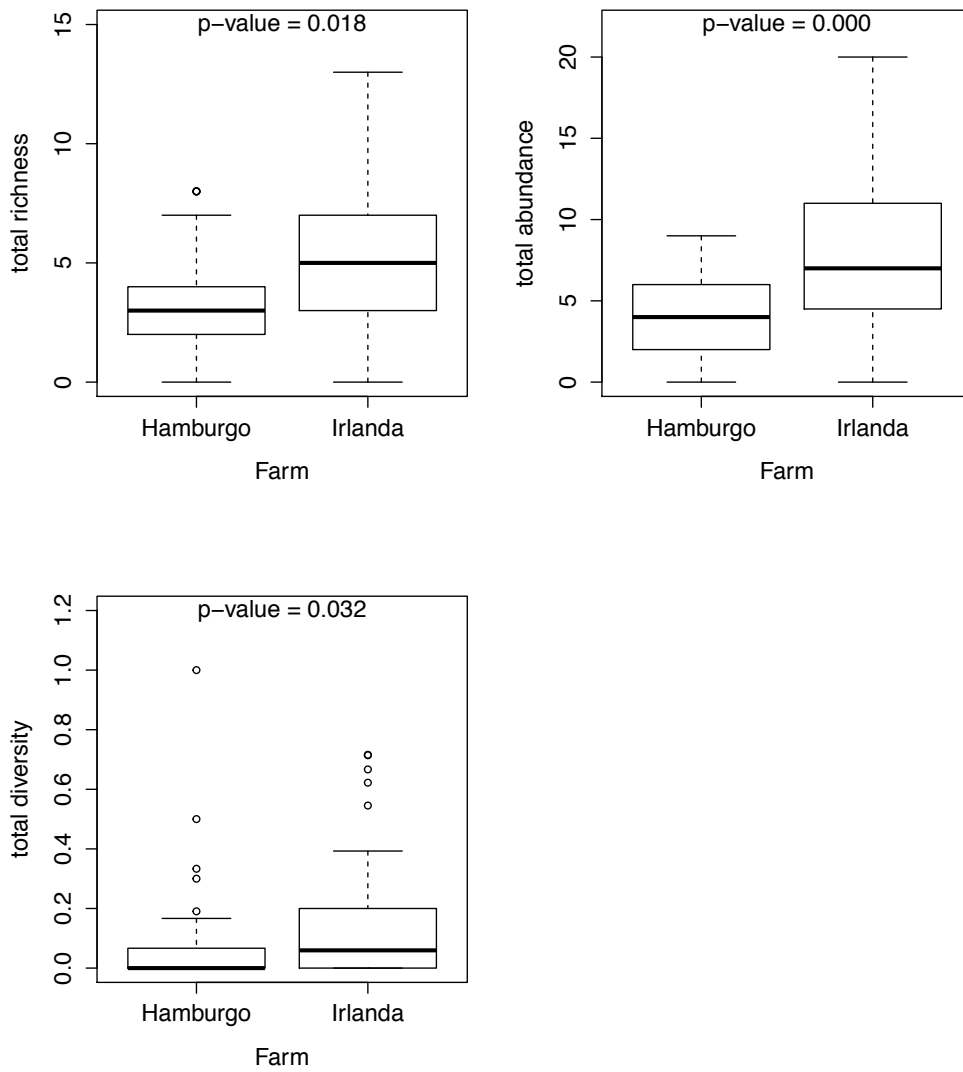


Figure 5-9 Results (*P* - values) of Wilcoxon sign-ranked tests on species richness, abundance and diversity of insects in all orders in the two farms.

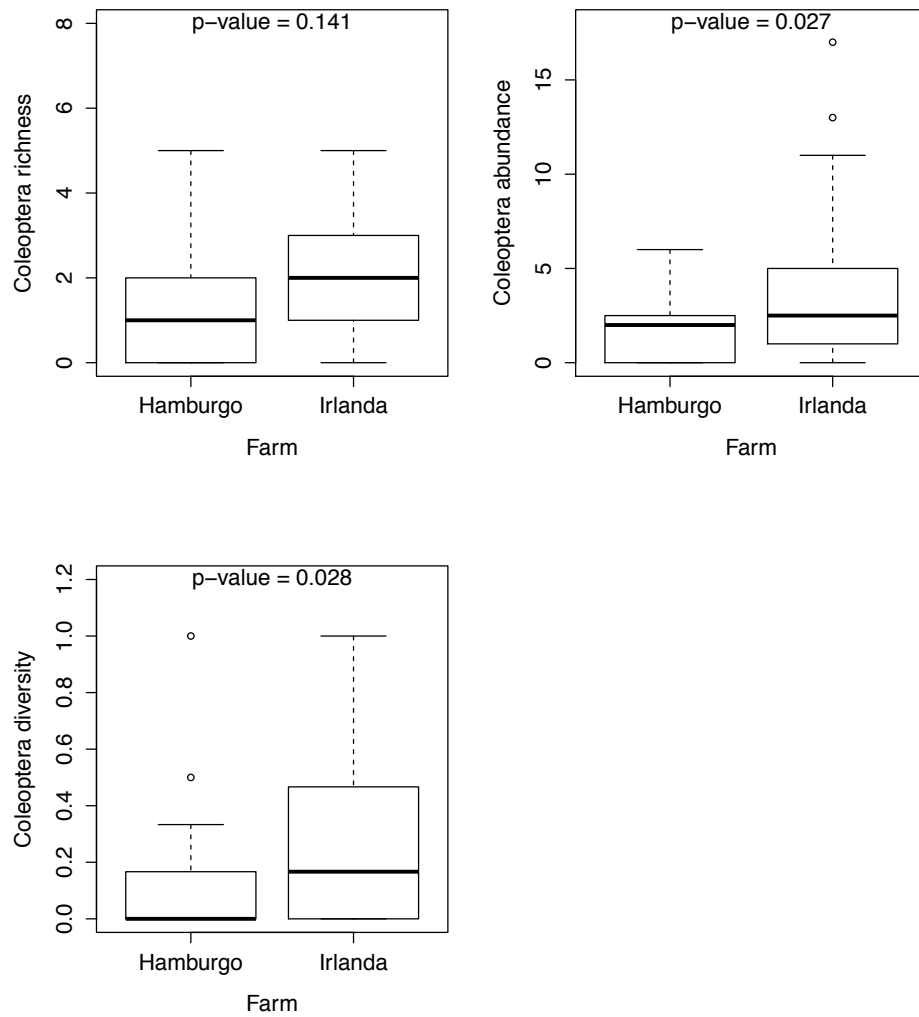


Figure 5-10 Results (*P* - values) of Wilcoxon sign-ranked tests on species richness, abundance and diversity of coleopteran insects in the two farms.

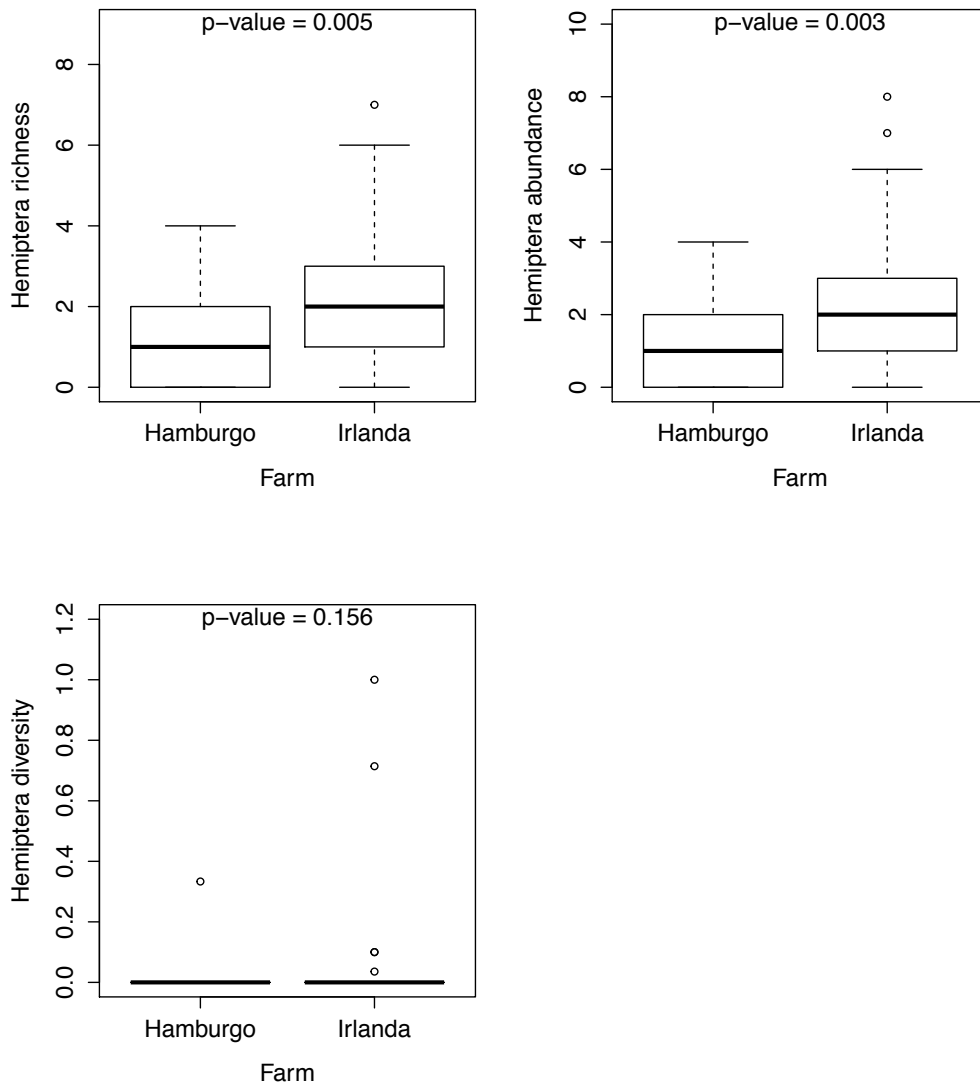


Figure 5-11 Results (P - values) of Wilcoxon sign-ranked tests on species richness, abundance and diversity of hemipteran insects in the two farms.

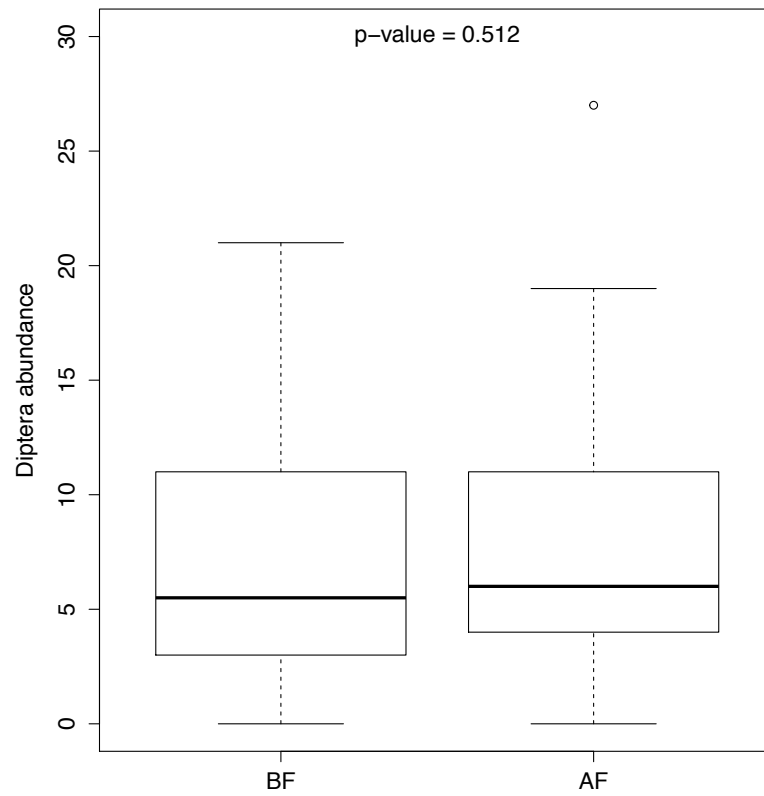


Figure 5-12 Results (*P* - values) of Wilcoxon sign-ranked tests on species abundance of dipteran insects in the two farms.

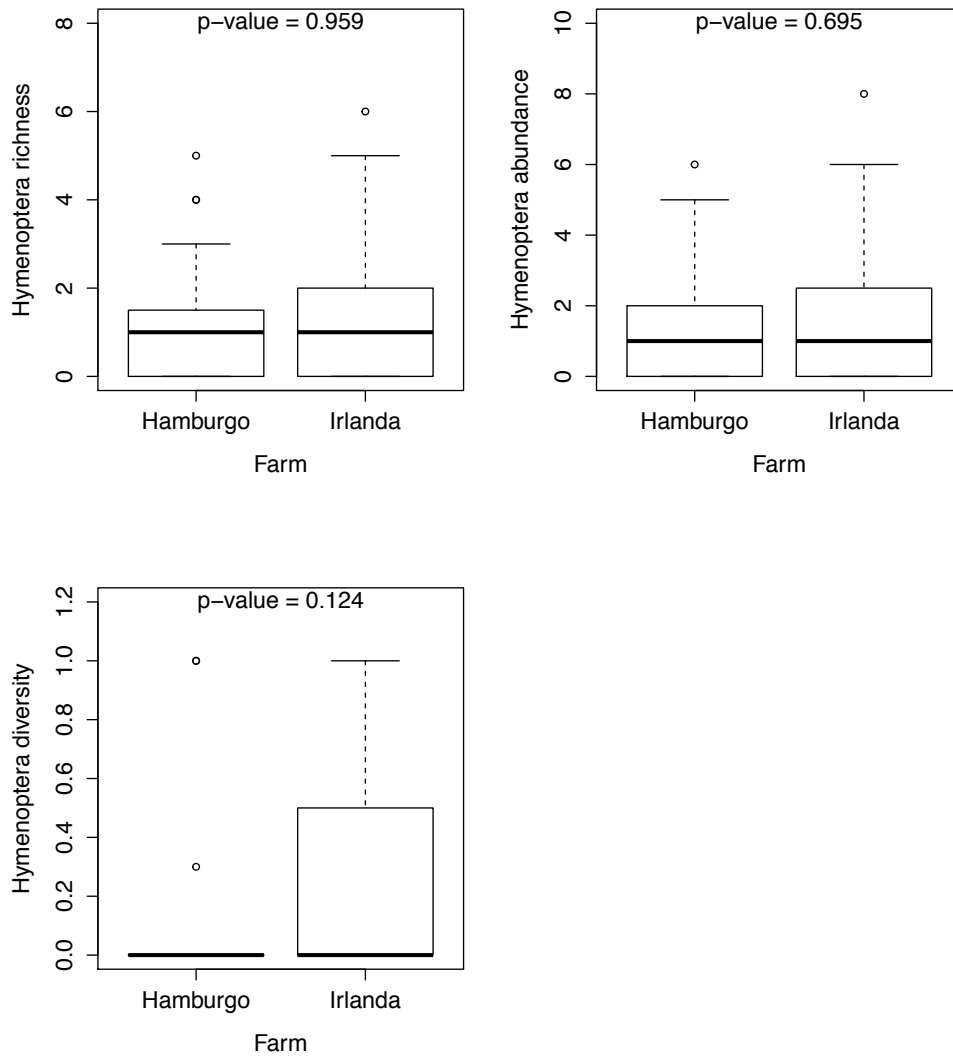


Figure 5-13 Results (*P* - values) of Wilcoxon sign-ranked tests on species richness, abundance and diversity of hymenopteran insects in the two farms.

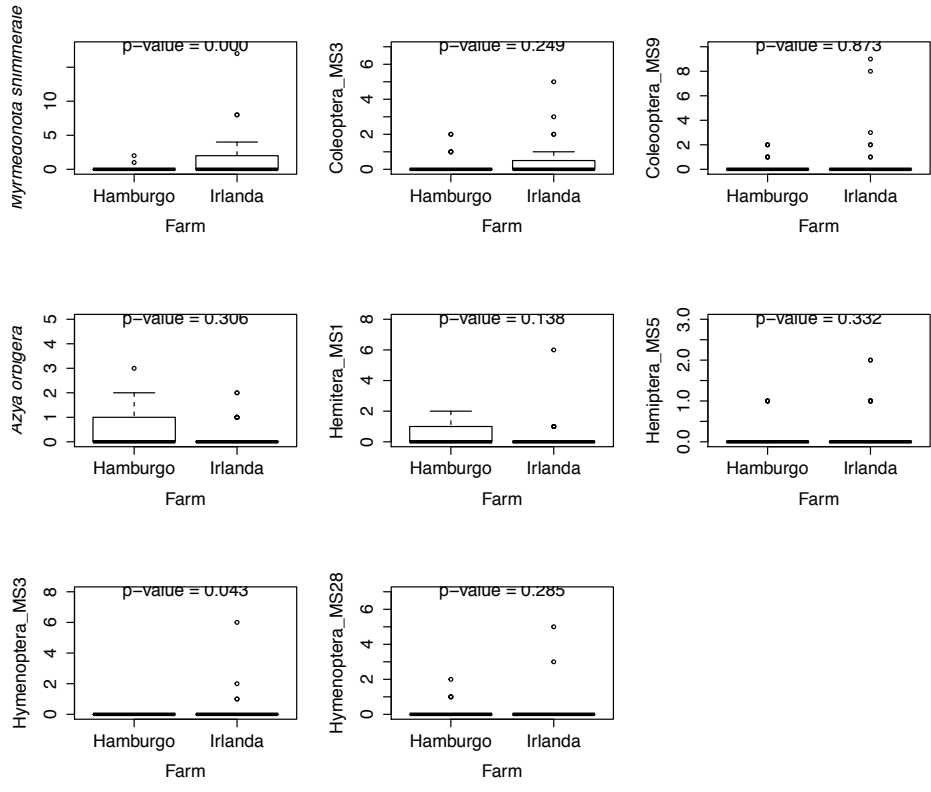


Figure 5-14 Results (*P* - values) of Wilcoxon sign-ranked tests on morphospecies counts in the two farms.

Table 5-1 Results (*p* - values) of Wilcoxon sign-ranked tests on abundance, species richness and diversity in all and individual orders. Data were presented to show significance level of the treatments in both farms together and each of the farms. Arrow é presents increased activities of organisms after phorid attacks, while arrow ê presents the opposite.

	<i>Finca</i>		
	Farms Combined	Irlanda	<i>Finca</i> Hamburgo
Total abundance	NS	NS	NS
Total richness	NS	NS	NS
Total diversity	NS	0.02é	NS
Coleoptera abundance	0.01é	0.02é	NS
Coleoptera richness	(0.054)é	0.00é	NS
Coleoptera diversity	(0.053)é	0.02é	NS
Hemiptera abundance	(0.062)ê	0.02ê	NS
Hemiptera richness	NS	(0.062)ê	NS
Hemiptera diversity	NS	NS	NS
Hymenoptera abundance	NS	NS	(0.057)ê
Hymenoptera richness	NS	NS	NS
Hymenoptera diversity	NS	NS	NS
Diptera abundance	NS	NS	NS

Table 5-2 Results of a Principal Component Analysis (PCA)

A. Standard deviations, proportions of variations and cumulative variations of corresponding principal components						
	Comp1	Comp2	Comp3	Comp4	Comp5	Comp6
Standard deviation	2.34	1.45	0.859	0.793	0.752	0.718
Proportion of variation	0.432	0.165	0.058	0.05	0.045	0.041
Cumulative variation	0.432	0.598	0.656	0.706	0.751	0.791
	Comp7	Comp8	Comp9	Comp10	Comp11	Comp12
Standard deviation	0.633	0.532	0.477	0.405	0.374	0.347
Proportion of variation	0.032	0.022	0.018	0.013	0.011	0.01
Cumulative variation	0.823	0.845	0.863	0.876	0.887	0.897
	Comp13	Comp14	Comp15	Comp16	Comp17	Comp18
Standard deviation	0.335	0.328	0.314	0.302	0.282	0.27
Proportion of variation	0.009	0.009	0.008	0.007	0.006	0.006
Cumulative variation	0.906	0.914	0.922	0.929	0.936	0.942
	Comp19	Comp20				
Standard deviation	0.257	0.253				
Proportion of variation	0.005	0.005				
Cumulative variation	0.947	0.952				

B. Loadings of the Principal Component Analysis. Loadings of first four components and morphospecies that are responsible for the variations are shown

	Comp.1	Comp.2	Comp.3	Comp.4
Morphospecies				
Hymen_MS3			0.401	0.403
Hymen_MS28			-0.184	0.201
Hemi_MS1 (leafhopper)			0.436	0.573
Hemi_MS5 (leafhopper)			0.110	
<i>Myrmedonota shimmerale</i>	0.99962			
Coleop_MS3 (flea beetle)			-0.676	0.619
Coleop_MS9 (flea beetle)		-0.989		0.121
<i>Azya orbiger</i>			0.328	0.108

Table 5-3 Results (p – values) of Wilcoxon sign-ranked tests on species counts of seven species. Data were presented to show significance level of the treatments in both farms together and each of the farms. Arrow é presents increased activities of organisms after phorid attacks, while arrow ê presents the opposite.

	Farm	<i>Finca</i>	<i>Finca</i>
	Combined	Irlanda	Hamburgo
Coleoptera			
<i>Myrmedonota shimmerale</i>	0.01é	0.003é	NS
<i>Azya orbiger</i>	NS	NS	NS
Coleoptera_MS3 (flea beetle 1)	NS	NS	NS
Coleoptera_MS9 (flea beetle 2)	NS	NS	NS
Hemiptera			
Hemiptera_MS1 (leafhopper)	0.04ê	NS	0.03ê
Hemiptera_MS5 (leafhopper)	NS	NS	NS
Hymenoptera			
Hymenoptera_MS3	NS	NS	NA
Hymenoptera_MS28	NS	NS	NS

Table 5-4 The effects of the treatments on Coleoptera and Hemiptera after excluding *Myrmedonota shimmerale* and Hemiptera_MS (leafhopper). Results (*p* -values) of Wilcoxon sign-ranked tests on Coleoptera abundance, richness and diversity and Hemiptera abundance, richness and diversity are presented. Data were presented to show significance level of the treatments in both farms together and each of the farms. Arrow é presents increased activities of organisms after phorid fly attacks, while arrow ê presents the opposite.

<i>Finca</i>			
	Farm Combined	Irlanda	<i>Finca</i> Hamburgo
Coleoptera abundance	NS	0.000é	NS
Coleoptera richness	NS	NS	NS
Coleoptera diversity	NS	NS	NS
<i>Finca</i>			
	Farm Combined	Irlanda	<i>Finca</i> Hamburgo
Hemiptera abundance	NS	0.04ê	NS
Hemiptera richness	NS	NS	NS
Hemiptera diversity	NS	NS	NA

Table 5-5 Insect counts before and after phorid attacks in *Finca Irlanda* and *Finca Hamburgo*. Data are presented in terms of order.

Farm	Treatment	Order			
		Coleoptera	Hemiptera	Hymenoptera	Diptera
Irlanda	Before	33	58	37	183
	After	114	33	28	208
Hamburgo	Before	36	23	33	100
	After	35	19	14	106

Table 5-6 Insect counts before and after phorid attacks in *Finca Irlanda* and *Finca Hamburgo*. Data are presented in terms of morphospecies

Farm	Treatment	Morphospecies				
		<i>M. shimmerale</i>	Coleoptera		Hemiptera	
			Coleop_MS9	Coleop_MS3	<i>A. orbiger</i>	Hemi_MS1
Irlanda	Before	6	2	6	6	9
	After	53	8	11	20	3
Hamburgo	Before	2	9	6	4	10
	After	1	7	2	6	4

Farm	Treatment	Morphospecies		
		Hymenoptera		
		Hemi_MS5	Hymen_MS3	Hymen_MS28
Irlanda	Before	6	2	8
	After	4	8	0
Hamburgo	Before	3	0	0
	After	2	0	3

Chapter 6 Closing Remarks

6.1. Dissertation conclusions

Trait-mediated indirect interactions (TMIIIs) are interactions where a species modify the relationship between two other species by inducing morphological, behavioral, physiological, or life history changes in one of the interacting species (Werner and Peacor 2003). These interactions have been shown as important, or sometimes even more important, than direct density-mediated interactions. However, while most studies of TMIIIs focus on a unit of two directly interacting species and a third species that modifies that interaction, most species are embedded in complex webs of interactions in nature. In this dissertation, I took the study of TMIIIs a step further by examining interconnected units of TMIIIs. Using a network of insect species that are found in the coffee agroecosystems in the Neotropics as a model system, I investigated how connecting TMIIIs affect target species and communities. Through a literature review, laboratory and field experiments and field surveys, I studied a specific combination of trait-mediated indirect interactions (TMIIIs) as the basis for testing several aspects of cascading trait-mediated effects. This complex system is composed of an aggressive arboreal ant species, *Azteca sericeasur*, its phorid fly parasitoid, *Pseudacteon lascinosus*, its herbivorous mutualistic partner, the hemipteran *Coccus viridis*, a predatory beetle of the herbivore, *Azya orbigera*, and the parasitoid wasp of the beetle, *Homalotylus shuvakhinae* (Fig. 6-1). Coffee (*Coffea arabica*) is the foundation species of the agroecosystems where I conducted the research, and it serves as the basis of all interactions of the above insects.

To gain insights on the potential effects of *P. lascinosus*, the phorid parasitoid, on the ant, *A. sericeasur*, I conducted a general literature review on the ecological impacts of phorid fly parasitoids on ants (Chapter 2). I found that the most important effects of the phorid flies on ants are trait-mediated effects rather than direct mortality effects due to parasitism. Most ant species respond to phorid attacks by reducing foraging activity, number of foragers and, in some cases, size of foragers, resulting in a diminished amount of food brought back to the nest to feed the

brood. Ants' coping mechanisms include changes in foraging time and sites. This behavioral change could affect ant competition and the discovery-dominance tradeoff observed in some ant communities. I concluded the review by examining the *Azteca* system, which is the main focus of this dissertation, and describing the cascading effect that the phorid flies have as a consequence of the connection between two TMIs.

As indicated elsewhere in this dissertation, the interference of the ant with adults of *A. orbigera* (Chapter 3) and the parasitoid wasp (Chapter 4) represent the first-level TMII, and the phorid fly induced anti-parasitism behavior of the ant, is the second-level TMII. This second level induces the release of specific pheromones that the ants use to communicate with nest mates and alert them about the presence of the phorid fly parasitoids. The pheromone release triggers a defense behavior on the ants that result in their reduced movement and activity, allowing adult gravid *A. orbigera* to use the reduced ant activity as a window of opportunity to oviposit and hide their eggs to protect them against ant predation once the ants regain their higher activity level (Chapter 3).

In old-field experiments, the grasshopper *Melanoplus femurrubrum* faces trade-offs between foraging and risk of predation (Schmitz *et al.* 2004). Similarly, *A. sericeasur* faces the challenge of trade-off between foraging for food and parasitism risk by the phorid fly. This evolved behavior is chemically mediated by ant pheromones. Through trials with olfactometers, I was able to show that females, but not males, of *A. orbigera*, are able to detect the pheromone that the ants release when they are being attacked by the phorid flies (Chapter 3). I also showed that gravid females are attracted to these pheromones and that there is a positive relationship between the time after mating and the attractiveness of *A. orbigera* females to ant pheromones released during phorid attacks (Chapter 3). Finally, through laboratory experiments, I showed that gravid female beetles lay more eggs in the ants plus phorid treatment, as compared to the ant alone treatment. In the field, a patch with ants and phorids can guarantee an abundant food resource (*Coccus viridis*) and enemy free space for the beetle larvae due to the indiscriminate harassment of the ant towards predators and parasitoids (Liere & Perfect 2008). Furthermore, the presence of the phorid provides a window of opportunity for oviposition without ant harassment.

Given the benefits accrued to the beetle by being able to detect ant pheromones, it is likely that these chemically-mediated interactions affect their fitness (Chapter 3).

One of the parasitoids that the ant harasses is the wasp, *Homalotylus shuvakhinae*, which attacks the larvae of *A. orbigera*. However, it appears that this parasitoid wasp also utilizes the space freed of ant foragers created by the phorid flies to parasitize more host individuals. In the fourth chapter of this dissertation, I examined the impacts of zero, one and two TMIs have on *A. orbigera* through its interaction with *H. shuvakhinae*. This study was conducted in the laboratory with mesocosm containing various combinations of organisms representing zero, one and two TMIs. Zero TMI was represented by the direct density-mediated interaction (the beetle larvae and the parasitoid wasp), one TMI was represented by the parasitoid wasp and the beetle plus the ant that interferes with the ability of the parasitoid to attack the beetle, and two TMIs were represented by the parasitoid wasp, the beetle, the ant, and the phorid fly parasitoid of the ant that interferes with the ability of the ant to interferes with the ability of the parasitoid wasp to attack the beetle. We found that *H. shuvakhinae*, through its direct interaction with the *A. orbigera*, changes the sex ratio of the beetle from female biased to male biased. Furthermore, although ants did show aggressiveness toward *H. shuvakhinae*, the presence of one TMI had no significant effect on either parasitism or sex ratio of *A. orbigera*. Finally, the presence of two interacting TMIs increased the parasitism rate of *A. orbigera* larvae and reverted its sex ratio back to female biased (Chapter 4). Likely, this observed result is a consequence of female resistance to parasitism, but the hypothesized mechanisms remain untested.

Generally speaking, the interplay of the TMIs in this system significantly influences all parameters of the population dynamics of *A. orbigera*. Through increased predation of *C. viridis*, it alters the interaction coefficient between *A. orbigera* and *C. viridis* (Liere & Larsen 2010). Through increased oviposition, it affects the conversion coefficient of *A. orbigera*. Through increased parasitism it affects the mortality coefficient of *A. orbigera*. Finally, having an effect on the sex ratio of the survivors, it influences the reproduction of *A. orbigera*.

Since the ant species at the center of this ecological network is a very aggressive arboreal ants and the presence of the phorid parasitoid reduces ant activity, providing a window of opportunity for *A. orbigera* adults to eat scales and oviposit, it is reasonable to speculate that these interacting TMIs could have effects that extend beyond these particular species. In the

fifth and last research chapter of this dissertation, we explored the potential short-term behavioral effects that these interacting TMIs have on the broader arthropod community. We found that the presence of the phorid fly (triggering the interactions of two TMIs) increased the abundance, richness and diversity of insects in the order Coleoptera. We also detected a significant increase of the rove beetle *Myrmedonota shimmerale*, which is a known predator of *A. sericeasur* and the reduction of a leaf hopper (Chapter 5).

Taken together, these results suggest that the indirect effect of a remote species, the phorid fly, is important in regulating system dynamics of multi-trophic interactions through trait-mediated interactions. Here I provide suggestions of the implications of this study to basic ecology and applied agroecology.

6.2 Implications to Basic Ecology

The trait-mediated cascading system in my study suggests that the interplay of two TMIs can have profound impacts on multi-trophic dynamics of a lateral-food chain composed of an herbivore, its predator and the parasitoid wasp of the predator. The cascading effects of interactive trait-mediated indirect interactions are evident in laboratory experiments, with some supporting evidence from the field. This is the first study that considers the multi-dimensions of trait-mediated cascading effects on the population dynamics of an organism. The intermediate species, *A. sericeasur*, has a different role from other intermediate species in TMII experiments (Relyea 2000; Schmitz *et al.* 2004). In these studies, fear of predation induces shifting of habitat and foraging reduction of intermediate species, leading to trophic cascades, reversed competition outcomes, etc.

Ant interference with other organisms is the key component that distinguishes this study from most other TMII studies. While the intermediate species in other systems are only engaged with competitors or resources in density-mediated interactions, *A. sericeasur* in our system has strong trait-mediated interactions with organisms that attempt to intrude on ant-hemiptern patches. This interaction interacts with the anti-parasitism behavior of the ant when the phorid fly arrives and contributes to the observed dynamics in our experiments. The impacts of the cascading effects of the system are multi-faceted. The evolutionary play (resistance of female *A. orbiger*, the use of phorid-alert pheromones to oviposit by gravid *A. orbiger*, and the preference of host and habitat selections by the parasitoid wasp) folds out with the manifestation

of the interplay of ant interference and phorid-ant interactions. Without the interactive interactions, the system behaves differently as sex ratio of *A. orbiger*a and trophic interactions are all altered.

As the first case study exploring the details of cascading trait-mediated systems, the generality of my study remains to be explored. The question is, how common is the structure of trait-mediated cascades in natural systems? As I pointed out in Chapter 2, ant-phorid interactions are fairly ubiquitous and their ecological effects are primarily trait-mediated effects that result from the ants' behavioral responses to the parasitoid. Ant-hemipteran associations are also fairly common and usually involved TMIIIs since the ants protect their mutualists by harassing their natural enemies. Given the fact that ants interact with many different species and that many ant species are very aggressive toward other organisms, it is very likely that the type of complex trait-mediated indirect interactions described in this dissertation, are fairly common in nature.

Furthermore, as summarized in Chapter 4, a few case studies in the literature do suggest that trait-mediated cascades occur in other systems, and these interactions occur at different trophic levels in both terrestrial and aquatic communities. The shared structure of these systems is the first-tier interference TMII and the second-tier TMII that disrupts the strength of the first-tier interaction. This structure strengthens trophic interactions on lateral food chains and likely provides an alternative pathway for trophic cascades.

6.3 Implications to Agroecology

This study was conducted on coffee farms in the Socunusco Region of Chiapas, Mexico. Previous studies have shown that the activity of *A. sericeasur* affects other herbivores in the system. These include the coffee berry borer (*Hypothenemus hampei*) (Perfecto & Vandermeer 2006) and herbivorous *Margaridisa* sp. flea beetles that feed on *Conostegia xalapensis* (Gonthier 2012). Importantly, the herbivorous damage of coffee by the coffee berry borer forms a negative linear relationship with the strength of the mutualism between *A. sericeasur* and the green coffee scale (*C. viridis*), which is a potential pest in coffee – as the population size of *C. viridis* increases, the boring rate of coffee berries declines. Also, as the foraging activity of the ant increases, the time for the ant to remove the coffee berry borer decreases.

On the other hand, the phorid-ant interaction adds complexity to the potential biocontrol effect of the ant, with a special association with *C. viridis*, the green coffee scale. The multi-

trophic effects of the phorid-ant interaction suggest that it increases the predation rate of *C. viridis* and facilitates the oviposition of *A. orbigera*, but it also increases parasitism probability and female proportion of *A. orbigera*. The net effect of phorid-ant interaction on biocontrol is therefore unclear. In particular, with the time lags due to population growths, the predation risk of *C. viridis* by *A. orbigera* would vary across time. As it requires further studies, my field data collected in 2009 and 2012 suggest the oscillation of biocontrol effects of the complexity (Fig 6-2).

Data that were not included in the previous chapters support the above view. They show that the strength of phorid-ant interactions, in June-July 2009 and July 2012, is negatively correlated with the reversed predation risk of *C. viridis* by *A. orbigera*, a proxy obtained by dividing population size of *C. viridis* with that of *A. orbigera* at site (Fig. 2). As time proceeds, this trend is reversed, suggesting that the reversed predation risk of *C. viridis* by *A. orbigera* is positively correlated with the interaction strength between the phorid fly and the ant in August-September 2009 and August 2012. The predation relaxation of *C. viridis* would prevent the pest from going extinct and thus stabilize the system as well as its biocontrol effect. Whether this is truly so remains to be proven by further studies, however, probably via theoretical modeling and long-term field studies.

A previous study shows that the phorid-ant interaction contributes to the spatial dynamics of the cluster distribution of *A. sericeasur* colonies (Vandermeer & Perfecto 2006). It is suggested that a strong phorid-ant interaction suppresses the spatial expansion of large ant nest clusters, while weak phorid-ant interactions at small ant nest clusters release this stress. The consequence is a power-law distribution of ant nest clusters in space (Vandermeer & Perfecto 2006). As *A. orbigera* feeds on *C. viridis* as the principal resource, and *H. shuvakhinae* is a specialized parasitoid of *A. orbigera*, the added complexity due to these organisms may affect the biocontrol of *C. viridis* in space. We are unclear how this dynamics operates.

The complexity of interacting TMIs limits the potential for using them as agents of practical agroecological planning. Here I was able to use a model system that is well known and experimentally tractable to demonstrate some of the implications of adding such complexity to the study of TMIs.

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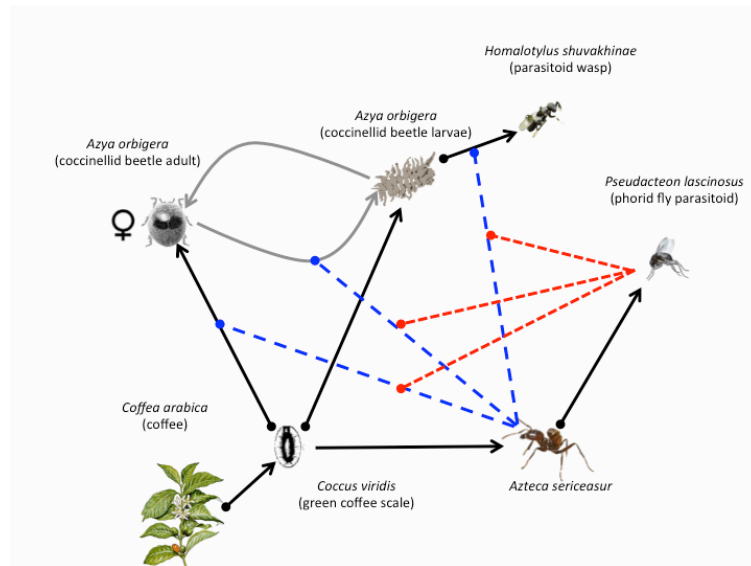


Figure 6-1 A network of multiple TMII. As pointed out in previous chapters, *Azteca sericeasur* serves as the first-tier TMII, interfering with the oviposition of *Azya orbigera* and the parasitoid wasp. The anti-parasitism defense of *A. sericeasur* interrupts the ant interference, leading to trait-mediated cascades.

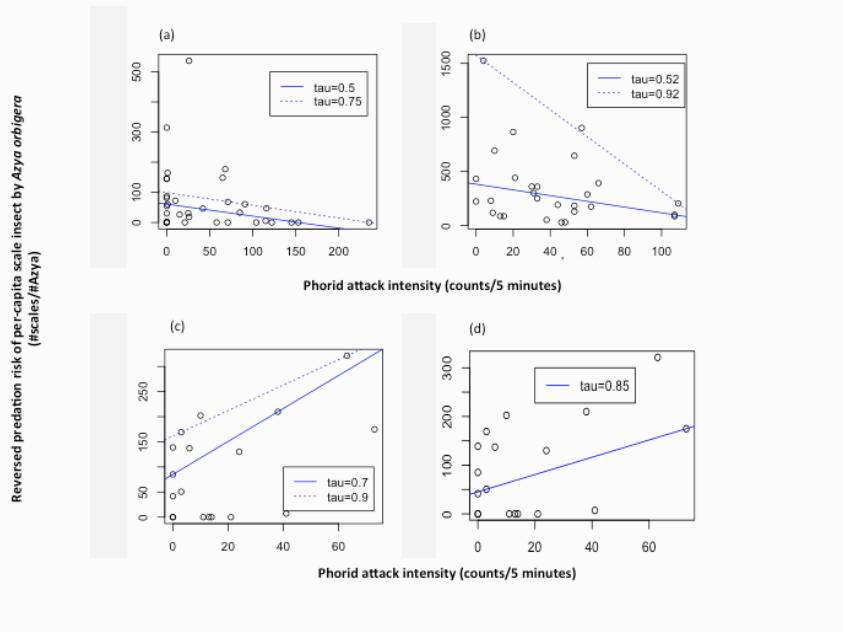


Figure 6-2 Reversed predation risk of *Coccus viridis* by *A. orbigera*. Statistics based on quantile regressions. Tau values in subplots refer to selected percentiles that show significant differences between reverse predation risk and phorid attack intensity.

Appendices

Appendix A. The coccinellid beetle, *Azya orbigera*, is associated with the ant, *Azteca instabilis*

Larvae and adults of *Azya orbigera* are found at significantly higher densities within clusters of nests of *Azteca instabilis* than in areas with no ants. In a 45-hectare plot within an organic coffee plantation we mapped all shade trees and all *A. instabilis* nests (Vandermeer et al., 2008). The plot was divided into 128 subplots of 50X50 m. If no trees with *A. instabilis* nests were found within the plot, then the tree closest to the center of the plot was selected and the 5 closest coffee bushes were inspected for beetles and the number of *A. orbigera* larvae and adults were recorded. If the plot had trees with *A. instabilis* nests, then the tree with *A. instabilis* that was closest to the center of the plot was selected and the coffee bushes that were within a 3 meter radius from the center tree were inspected for beetle presence. The survey was conducted in the summer of 2006. The results of the survey are presented in table A.

Table A. Average number of coccinellid beetles found in coffee bushes in areas with and without *Azteca instabilis*. The numbers in parentheses are standard errors.

	With ants	Without ants	P
Adults	5.76 (0.99)	1.26 (0.21)	<0.000001
Larvae	3.8 (0.82)	1.58 (0.32)	0.005

Appendix B. The coccinellid beetle, *Azya orbiger*, is attracted to volatiles of green coffee scales but not attracted to pierced coffee leaves without scales.

Many plants are known to produce volatiles when damaged by herbivores (Paré and Tomlinson, 1999). We conducted olfactometry experiments using coffee leaves with scale insects and intact leaves and concluded that *A. orbiger* is attracted to leaves with scale insects (Fig. 2a,e). However, in order to determine whether the coccinellid beetles are attracted to volatiles from the scales or induced volatiles from the coffee leaves we conducted additional olfactometer experiments with intact and pierced leaves without scales. Although the production of volatiles can vary from insect damage to mechanical damage, the difference tends to be more in quantity than quality or in the proportion of the volatiles produced (Mithófer et al., 2005). Therefore, if the coccinellids are attracted to leaf volatiles rather than scale volatiles, we expect to find a difference between intact coffee leaves and leaves that have been pierced to mimic scale insect damage.

To conduct these tests, coffee leaves without green coffee scales were collected from the field and wrapped with wet cotton and parafilm within a few seconds of collection. Equal numbers of leaves of similar size were used for the treatment and the control. Twelve female and three male adults of *Azya orbiger*, one at a time, were used in the olfactometry experiment to test if the beetles were attracted to volatiles released from pierced coffee leaves as opposed to intact leaves. A direct binomial distribution test and a Mann-Whitney test showed that *A. orbiger* adults are not attracted to the volatiles released from pierced leaves (Fig. B).

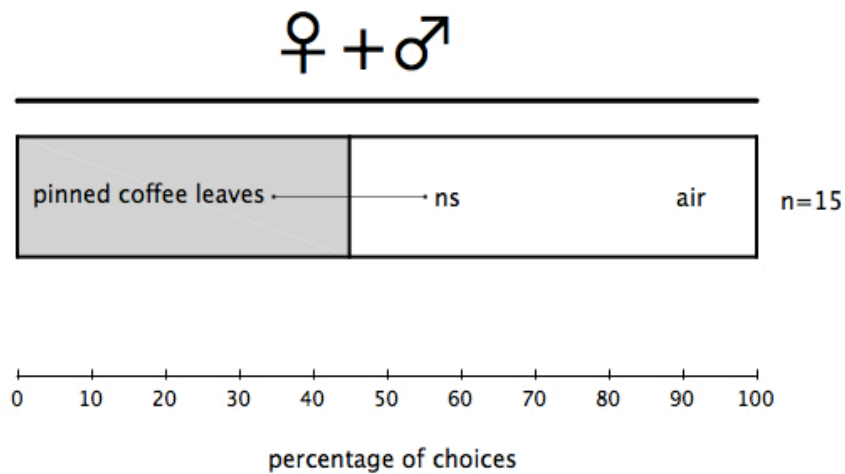


Figure B. Results of trials with a two-arm olfactometer for male and female *Azya orbiger* combined (N=16, p=0.20 direct binomial test).

Appendix C. Number of trial and p -values for binomial distribution tests for olfactometer trials.

		Number of trials	p - value from Binomial Distribution
Females	Scale insect	49	0.004
	Smashed dead ants	66	0.000
	Alive ants	16	0.402
	Phorid attacks	33	0.000
	Ant heads	55	0.658
	Ant thoraxes	88	0.139
	Ventral abdomens	63	0.015
	Dorsal abdomens	45	0.674
Males	Scale insect	34	0.000
	Crushed dead ants	43	0.622
	Alive ants	NA	NA
	Phorid attacks	6	0.344
	Ant heads	12	0.133
	Ant thoraxes	17	0.402
	Ventral abdomens	29	0.907
	Dorsal abdomens	16	0.402

Appendix D. A video of *Azteca* ants harassing adult *Azya oribgera*

<http://onlinelibrary.wiley.com/store/10.1002/ece3.322/asset/supinfo/ece3322-sup-0004-VideoS1.wmv?v=1&s=f3763caa30e8560710b4a451a43262f40dfb3c85>

Appendix E. A video of a phorid fly hovering over a paralyzed *Azteca* worker.

<http://onlinelibrary.wiley.com/store/10.1002/ece3.322/asset/supinfo/ece3322-sup-0005-VideoS2.mov?v=1&s=4226e82a33f9d3fbe96c466da18f334fcb441b5>

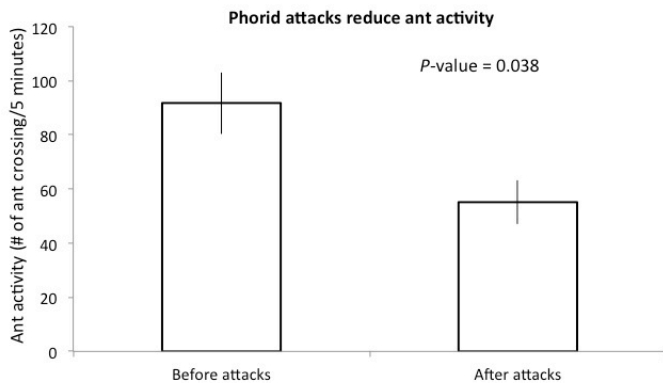
<http://onlinelibrary.wiley.com/store/10.1002/ece3.322/asset/supinfo/ece3322-sup-0005-VideoS2.mov?v=1&s=4226e82a33f9d3fbe96c466da18f334fcb441b5>

Appendix F

Phorid attacks reduce ant activities

In summer 2011, we conducted a field experiment to test the effect of phorid attacks on ant activities in 16 selected sites where *A. sericeasur* actively tended the green coffee scale. In each site, we first counted ant activity, measured as number of ant passing a cross of a twig and a major stem in one minute. We then used two bowls, each of which contained 50 *A. sericeasur* workers collected from a remote nest. We squeezed 4-6 ant workers in each bowl, an action that released alarm pheromones which induce phorid attacks. We allowed phorids to attack the ant for 5 minutes. Afterwards, we returned to the same coffee bushes, measured ant activities at the same fixed locations for one minute.

We summed up ant activities of the four coffee bushes before phorid attacks in one site, and that after phorid attacks in one site. We used the data (n=16) and conducted Wilcoxon signed-rank test on ant activity before vs. after phorid attacks. The results suggest that phorid attacks significantly reduce ant activity (p -value = 0.038)



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