Complex Interactions and Ecosystem Function:
Auto-regulation of an Insect Community in a Coffee Agroecosystem

by

Heidi Liere

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Doctoral Committee:

        Professor John H. Vandermeer, Chair
        Professor Mercedes Pascual
        Professor Ivette Perfecto
        Associate Professor Robyn J. Burnham
        Associate Professor Beverly J. Rathcke (Deceased)

Dedication

I would like to dedicate this dissertation to my daughter Sybelle, because watching her grow gives me enormous strength and a strong desire to keep doing research that will, hopefully, contribute to make the world a better place.

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Chapter I

Introduction

The importance of conserving biodiversity is widely accepted among scientist and environmentalists. As an implicit justification it is postulated that biodiversity confers benefits to ecosystems; however, how biodiversity promotes ecosystem function and stability remains a highly contentious topic in contemporary ecology (Schwartz et al. 2000; Hooper et al. 2005). An example is pest control in agriculture, thought by many to be enhanced through biodiversity (Andow 1991; Marc and Cannard 1997; Altieri 1999; Wilby and Thomas, 2002; Benton et al. 2003; Cardinale et al. 2003; Gurr et al. 2003; Bianchi et al. 2006). Recent literature suggests that it is the complex nature of interactions embedded in biodiverse agroecosystems that may frequently be the key to the delivery of this and of other important ecosystem services (Vandermeer et al. 2010). This complexity encompasses not only the number of species in a given system but all the different life history strategies of those species, their temporal and spatial distributions, and the various types of interactions among them (linear, non-linear, direct, indirect, density-mediated, trait-mediated). In order to identify the mechanisms that help maintain the health and stability of an ecosystem, it is thus important to take into account the complex nature of ecological communities.

My PhD. dissertation addresses how the interaction complexities of an diverse insect community characteristic of agroecosystems managed with environmentally-friendly techniques, support mechanisms of self-regulation with potential implications

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natural pest control. Specifically, I address how the biocomplexity of a diverse coffee plantation allows the population persistence of important natural enemies, which in turn, maintain the populations of potentially harmful pests below damaging levels.

For decades, coffee has constituted one of the major sources of household income for many Latin American countries. Furthermore, coffee plantations coincide spatially with the most biodiversity-rich areas in the world, such as Soconusco region where my research took place. When grown under traditional practices, i.e. without agrochemical input and high percentage of shade tree cover, coffee agroecosystems can maintain a very high biodiversity, even comparable to nearby natural forests (Perfecto et al. 1996).

My research project is part of a larger project in a diverse, organic, shade-grown coffee plantation in Chiapas, Mexico. Our research team has worked out the details of how four important coffee pests are kept under control in this farm by a complex ecological network (Perfecto and Vandermeer 2008; Vandermeer et al. 2010). The coffee berry borer, the green coffee scale, the coffee rust, and the coffee leaf miner are all potentially devastating coffee pests, all of which are affected either directly or indirectly by the aggressive arboreal ant *Azteca instabilis*. In shaded coffee plantations these ants build their nests in the shade trees and forage on nearby coffee bushes, strongly affecting any organism living or foraging thereon. In a 45-ha permanent plot, we determined that 3 to 5% of shade trees in this farm are occupied by *A. instabilis* nests, and that these nests have a clustered spatial distribution (Vandermeer et al. 2008). Due to the aggressiveness and dominance of this ant species, the spatial distribution of its nests effectively creates a spatial heterogeneity for any organism associated with it. Using computer modeling and field surveys we have presented evidence that the four mentioned potential pests are kept

under control by a combination of stochastic, nonlinear, and spatial effects within the complex web of interactions of which *A. instabilis* appears to be a key component. This web also includes other herbivores, predators, parasitoids, hyperparasites and fungal pathogens (see Vandermeer et al. 2010).

My dissertation focused on a subsystem of this ecological web. I studied the interactions between *A. instabilis*, the green coffee scale *(Coccus viridis),* and the predatory ladybeetle *Azya orbigera*. This voracious predator of green scales is very abundant in the studied plantation and can therefore be considered an important natural controller of green scale populations. I investigated the interactions of this predator with the mutualism between ants and green scales.

As in any typical ant-hemipteran mutualism, *A. instabilis* protects green coffee scales against their natural enemies and the scales in return produce honeydew that the ants use as energy source (Way 1963; Beattie 1985). Although this has not been the case in the plantation where I did my research, population outbreaks of green coffee scales cause devastating harvest loses and are very common in coffee plantations around the world (Purseglove 1987; APHIS 1998; Mau and Kessing 1999). Many authors have accordingly recommended eliminating the mutualistic ants in order to successfully control and prevent population outbreaks of green scales (Purseglove 1987; Hanks and Sadof 1990; Reimer et al. 1993). However, in some instances, ant-hemipteran mutualisms can, directly or indirectly, even benefit the host plants (Beattie 1985; Bach 1991). Given the complex interactions among the various species associated with this particular mutualism, I propose that this recommendation can prove problematic, leading to unexpected consequences. Specifically, I propose that the complex ecological web,

characteristic of this biodiversity-rich coffee plantation, keeps scale populations in check and eliminating one apparently key element of the web may lead to unexpected results.

In the first part of my dissertation I describe the results of lab and field experiments aimed at understanding the details of the local interactions between ants, scales, ladybeetles and associated parasitoids (Chapters II and III). Then I report on a series of large and small scale surveys of a 45-ha plot to determine the spatial distribution, abundance and persistence of the ladybeetle populations (Chapter IV). Finally, I report the results of a computer simulation model that aimed to determine how the ladybeetle influenced and was influenced by the spatial distribution of ant nests and the potential implications for the control of the scale insect populations (Chapter V). In general, I found that the persistence of this important predatory ladybeetle depends on complex local and spatial interactions of which *A. instabilis* is a key element. In what follows, I briefly describe the main objectives and questions of each chapter.

Chapter II: Cheating on a Mutualism: Indirect Benefits of Ant Attendance to a Coccidophagous Coccinellid. In this chapter, I report on the myrmecophily of *A. orbigera*, an important predator of the green coffee scale. Coccinellid beetles, also known as ladybeetles, are generally unable to prey upon ant-tended prey (Way 1963; Sloggett and Majerus 2000). However, particular coccinellid species have morphological, behavioral, or chemical characteristics that render them immune to ant attacks, and some species are even restricted to ant-tending areas (Eisner et al. 1978; Majerus 1989; Hubner 2000; Völkl 1992, 2001). The benefit gained from living in close association with ants can be twofold: 1) gaining access to high-density prey areas and 2) gaining enemy-free space (Völkl 1992, 2001). Here, I investigated three main questions: (1) Are the waxy

filaments of *A. orbigera* larvae effective as defense against attacks of the mutualistic ant partner of *C. viridis*, *Azteca instabilis*?;(2) Does *A. instabilis* reduce the rate at which *A. orbigera* larvae prey upon scales?; and (3), Do *A. orbigera* larvae gain enemy-free space by living in close association with *A. instabilis*? I found that *A. orbigera* larvae 'cheat' on the mutualism between ants and green scales and thus benefit in two ways: they have exclusive access to abundant scale colonies tended by ants and they gain enemy free space.

Chapter III: Cascading Trait-mediation: Disruption of a Trait-mediated Mutualism by Parasite-induced Behavioral Modification. Contrary to *A. orbigera* larvae, adults do not have access to abundant scale colonies tended by ants. However, the presence of parasitoid phorid fly that attacks *A. instabilis* drastically reduces ant activity. In this chapter, I aimed to determine the effect of this parasite-induced change of ant behavior (in other words, this trait mediated indirect interaction or TMII) on the *A. instabilis-C. viridis* mutualism and how that affects the predatory *A. orbigera*. TMIIs are important driving-forces causing trophic cascades in aquatic and terrestrial food webs (Brett and Goldman 1996; Shurin et al. 2002; Schmitz et al. 2004). Furthermore, since most biological communities are not simple food chains but complex networks of interactions (Polis 1991), one TMII within a community might easily be influenced by another TMII (Abrams 1993; 1995; LeBrun and Feener 2002). In other words, TMII themselves can be cascades with potential implications for community dynamics. Here, I report on one of such cascade, where a parasitic fly induces behavioral changes that disrupt a trait-mediated ant-hemipteran mutualism.

Chapter IV: Habitat Heterogeneity and the Abundance, Distribution, and Persistence of a Myrmecophilous Ladybeetle. One of the major questions in population and community ecology has been the role of spatial heterogeneity in predator-prey interactions (Tilman and Kareiva 1997; Murdoch et al. 2003). Myrmecophilous ladybird beetles are examples of organism that can be greatly affected by different habitat types created by interactions among members of the community in which they interact (Majerus 1989; Völkl 1992, 1995; Völkl and Stechmann 1998). For example, the aggressive ant *A. instabilis* creates two habitat types utilized differently by different life stages of the predatory ladybird beetle, *A. orbigera*, which is immune to ant attacks as larva but not as adult. Areas dominated by the ant sustain high prey density and enemy-free space for this ladybeetle larvae. In this chapter, I report on how the presence of a clustered distribution of areas with prey tended by ants embedded in a matrix of areas without ants affected the distribution, abundance, and persistence of this voracious predatory ladybeetle.

Chapter V: Population Regulation from Spatial Heterogeneity: An Example of Autonomous Biological Control Through Ecological Complexity. Spatial heterogeneity is essential for the persistence of many inherently unstable systems such as predator-prey and parasitoid-host interactions (Nicholson 1993; Noy-Meir 1975; Tilman and Kareiva 1997; Dieckmann et al. 2000; Gurney and Veitch 2000; Murdoch et al. 2003). Since biological interactions themselves can create heterogeneity in space, the heterogeneity necessary for the persistence of an unstable system could be the result of local interactions involving elements of the unstable system itself. In this chapter, I report on a predatory ladybird beetle whose natural history suggests that the beetle requires the

patchy distribution of the mutualism between its prey, the green coffee scale, and the arboreal ant, *A. instabilis*. Based on the natural history of the system, I constructed a spatially explicit model and determined that the spatial heterogeneity of ant nests may be key for the persistence of the beetle populations but, at the same time, the effect of the beetles preying upon scales appears to help create the ant spatial distribution.

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Chapter II

Cheating on a Mutualism: Indirect Benefits of Ant Attendance to a Coccidophagous Coccinellid

Abstract

Coccinellids (Coleoptera: Coccinellidae) are generally unable to prey upon ant-tended prey. However, particular coccinellid species that have morphological, behavioral, or chemical characteristics that render them immune to ant attacks, and some species are even restricted to ant-tending areas. The benefit gained from living in close association with ants can be twofold: 1) gaining access to high-density prey areas and 2) gaining enemy-free space. Here, the myrmecophily of *Azya orbigera* Mulsant (Coleoptera: Coccinellidae), an important predator of the green coffee scale, *Coccus viridis* (Green) (Hemiptera: Coccidae), is reported. In this paper, three main questions were investigated: 1) Are the waxy filaments of *A. orbigera* larvae effective as defense against attacks of the mutualistic ant partner of *C. viridis*, *Azteca instabilis* F. Smith (Hymenoptera: Formicidae)? 2) Does *A. instabilis* reduce the rate at which *A. orbigera* larvae prey upon scales? and 3) Do *A. orbigera* larvae gain enemy-free space by living in close association with *A. instabilis*? Laboratory and field experiments were conducted to answer these questions. We found that due to the sticky waxy filaments of *A. orbigera* larvae, *A. instabilis* is incapable of effectively attacking them and, therefore, the predation rate of *A. orbigera* on *C. viridis* does not decrease in the presence of ants. Furthermore, *A. instabilis* showed aggressive behavior towards *A. orbigera's* parasitoids and the presence of ants

reduced the parasitism suffered by *A. orbigera*. This is the first time that this kind of indirect positive effect is reported for an ant and a coccidophagous coccinellid. Furthermore, this indirect positive effect may be key to the persistence of *A. orbigera*'s populations.

Introduction

The coffee green scale, *Coccus viridis* (Green) (Hemiptera: Coccidae) and many ant species have a mutualistic association (Hanks and Sadof 1990), in which the ants protect the scales against their natural enemies and the scales produce a carbohydrate-rich secretion—honeydew—that the ants use as an energy source (Way 1963, Buckley 1987). This mutualism has been regarded as harmful to coffee plantations (Hanks and Sadof 1990, Reimer et al. 1993) since the green scale can sometimes reach pest status (Murphi 1997, APHIS 1998). Even in coffee plantations where the green scale does not normally represent a problem, like the farm in which our study took place, high densities of scales can be found in coffee bushes around nests of its mutualistic ant partner *Azteca instabilis* F. Smith (Hymenoptera: Formicidae) (Vandermeer and Perfecto 2006). Consequently, some authors recommend eliminating the ants from coffee plantations so that the scale insects can be controlled by their natural enemies (Hanks and Sadof 1990, Reimer et al. 1993).

This recommendation does not take into account all the complex direct and indirect interactions that surround the ant-scale mutualism. For example, some natural enemies of honeydew-producing hemipterans have morphological, behavioral or chemical mechanisms that render them immune to ant attacks (Pontin 1959, Eisner et al.

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1978, Sloggett et al. 1998, Völkl 1995, Lohman et al. 2006) and some can mostly or exclusively be found in areas where their prey are tended by ants (Majerus 1989, Slogget and Majerus 2000). In these cases, not only have these myrmecophilous natural enemies access to high-density prey areas, but through indirect interactions, ants can also provide them enemy-free space (Völkl 1992). In this way, the mutualistic association between hemipteran prey and honeydew-collecting ants benefits natural enemies whose population survival and persistence might be key to the natural control of hemipteran populations at the landscape level (Liere et al. in review).

Here we experimentally investigate the myrmecophily of *Azya orbigera* (Mulsant) (Coleoptera: Coccinellidae), an important predator of the coffee green scale in an organic coffee farm in Chiapas, Mexico. Several species of coccinellid scale predators, syrphid flies (Diptera: Syrphidae), and two parasitoid species (Hymenoptera: Encyrtidae) form the community of natural enemies that attack green scales in the studied farm (personal observation). *Azya orbigera* is among the most commonly found near green scale colonies and due its voracity, both as larva and as adult, is likely to be a key enemy maintaining green scale populations below damaging levels in the area (Liere in preparation). The larvae are covered by sticky waxy filaments or secretions that are thought to protect them against ant attacks (Michaud et al. 2002). However, these reports are only anecdotal and no experimental data supporting this affirmation could be found. These waxes do not protect them against parasitism, and four parasitoid species (three species of the family Encyrtidae: *Homalotylus shuvakhinae* Trjapitzin, *Prochiloneurus* sp., and one unknown species; and one unknown species of the family Eulophidae) have been reared from *A. orbigera* larvae (H.L., unpublished data). In general, very little is

known about the biology of this coccinellid (Michaud et al. 2002). In this paper, three main questions are investigated: 1) Are the waxy filaments of *A. orbigera* larvae effective as defense against *A. instabilis* attacks? 2) Does *A. instabilis* reduce the rate at which *A. orbigera* larvae prey upon scales? and 3) Do *A. orbigera* larvae gain enemy-free space by living in close association with *A. instabilis*?

Materials and Methods

The study was conducted during the summers of 2005 and 2006 on Finca Irlanda, an organic shade-grown coffee farm located in the southern part of Chiapas, Mexico, near the border with Guatemala (15° 10' N and 92° 20' W) at approximately 950 m above sea level.

For all laboratory experiments, we collected *A. instabilis* colonies from the field. On its nesting trees, this species of ant builds big carton structures (up to 1 m long), which contain huge amounts of workers, larvae, and pupae; even the queen can be sometimes found inside these structures (personal observation). Pieces of these carton structures collected from the field are thus perfect to keep in the laboratory for behavior experiments, since after a few days the ants regain their 'normal' activities (i.e. collect food, tend scales, defend their nest) and are easily kept alive for long periods of time (personal observation). We collected small pieces of carton (10- 30 cm) and placed them in plastic containers with boarders coated with Fluon ®.

To determine if the waxy filaments protect *A. orbigera* larvae against ant attacks, we took advantage of the fact that these filaments fall off very easily when handled. Thus, we were able to completely remove the filaments from 12 laboratory-reared larvae

with a fine wet brush. This procedure, when performed carefully, does not damage the

larvae (Völkl and Vohland 1996). We did not notice any change in the larval behavior

after the filaments were removed. Another 12 larvae were used as control. Four larvae

from each instar were used for each treatment. All larvae were then placed inside plastic

containers with *A. instabilis* colonies that had been previously collected from the field.

The interactions between the larvae and the ants were observed and the number of attacks

and ant-induced mortality events were noted.

To investigate whether *A. orbigera* larvae were able to prey upon ant-tended

scales, 20 cm branches infested with 35 to 50 adult *C. viridis* were cut from coffee

seedlings. One laboratory-reared larva (from the third or fourth instar) that had been

previously starved for 24 hours was placed on each branch. The branches were randomly

assigned either as 'ant' or 'no ants' treatments. Then the branches were placed, according

to their assigned treatment, inside plastic containers with or without *A. instabilis* colonies

collected from the field. There were a total of eight replicates per treatment. The number

of scales in each branch was counted before the initiation of the experiment. The

coccinellid larvae were then left for 24 hours in the containers and the remaining number

of scales was recorded again after the end of the experiment. The predation percentage

was then calculated for both treatments.

To determine if *A. instabilis* provides enemy-free space for the larvae of *A.

orbigera*, ant removal experiments were established in coffee plants in the laboratory and

in the field. In the field experiment, five scale-infested coffee bushes were randomly

assigned the to the 'ant-exclusion' treatment and five to the 'no exclusion' treatment. All

plants were located around two *A. instabilis* nests, which were about 20 m apart.

Tanglefoot ® (The Tanglefoot Co., Grand Rapids, MI) was applied at the base of the exclusion treatment plants, and all ants were manually removed. Then, five laboratory-reared *A. orbigera* larvae (from the third- or fourth-instar, which according to our observations are the preferred instars for parasitism) were placed in each plant and left there for a week. Additionally, we did 10-minute observations per plant looking for parasitoids and potential predators of the coccinellid larvae (e.g. wasps, spiders, frogs). When retrieved, the larvae were placed in separate plastic containers and kept alive in the laboratory with a constant supply of green scales. We, then, were able to determine if there was a difference in percentage of parasitism between ant and ant-free treatments.

For the laboratory experiments, two coffee seedlings infested with green scales were placed inside transparent 60 x 60 x 60 cm insect-rearing cages. One of the seedlings was placed in a container with a 10-cm carton piece of *A. instabilis* colony and the other was kept without ants. We made sure that the ants were climbing the coffee seedling and tending scales before beginning with the experiment. Five laboratory-reared *A. orbigera* larvae (third- or fourth-instar) were placed on each plant and then five laboratory-reared *H. shuvakhinae* individuals were released inside the cages. The interactions between parasitoids, larvae and ants were observed for one hour. Each treatment had seven replicates, each with different larvae and parasitoids. In the ant-plants, the interactions between ants and parasitoid were recorded following the methodology used by Völkl (1995):

- Ant reactions:
  - Ignore parasitoid
  - Attack parasitoid

- Show non-aggressive behavior towards parasitoid (antennal tapping)

- Parasitoid reactions:

  - Change of walking direction to avoid contact with ants

  - Fly away when encountering an ant

  - Jump away from ants upon encounter

  - Respond to non-aggressive behavior (antennal tapping)

The time the parasitoids spent on each plant was also noted. After two hours, the larvae were collected and kept alive in separate plastic containers to determine and compare parasitism percent between treatments. The larvae were kept alive with a constant supply of green scales during the duration of their larval stage.

Data analysis. For all experiments, to determine if the difference between treatment means was statistically significant, 10,000 trial permutation (resampling without replacement) tests were performed using the Resampling Software Add-in for Excel version 3.2 (Resampling Stats Inc. 2006). First, the difference between treatment means was calculated (Resampling Stats Inc. 2006). Then, the data were randomly shuffled into two groups (without replacement) and the new means and difference between means were calculated; this procedure was repeated 10,000 times (Resampling Stats Inc. 2006). The treatment means are said to be significantly different if differences as large or larger than the observed are rarely seen when the data are randomly shuffled into two groups (Resampling Stats Inc. 2006). The p value is then the proportion of resampling trials (out of 10,000) that yields differences as extreme as the observed difference (Simon 1997), we used a cutoff value of 0.05 to consider a difference statistically significant. Resampling methods require no assumptions about the

distribution of the data or about the sample size (Simon 1997). Due to our small sample sizes and the non-normality of our data, we considered resampling methods more appropriate to detect difference between means that conventional parametric or non-parametric test.

Results

Waxy filaments. Regardless of larval instar, all the experimental larvae (without waxy filaments) were attacked and killed by ants. In contrast, none of the control larvae (with waxy filaments) were damaged or killed. Usually, one or two ants attempted to attack recently placed control larvae, but after getting waxy filaments stuck in their mandibles, they did not attack again nor did any other ant in the colony.

Predation. One larva from the 'without ants' treatment pupated during the experiment, so it was not included in the analysis. The percentage of scales predated by the coccinellid larvae during the 24-hour period of the experiment was not affected by the presence of ants (predation percentage with ants: 33.5%; predation percentage without ants: 27.9%; p=0.15) (Fig. 2.1).

Enemy-free space. *Field experiments*. Many larvae from the field ant-exclusion experiments were not found after the week-long period of the experiment. Out of the total of 25 larvae that were placed on plants with ants, 14 remained after a week, whereas in the plants without ants, only a total of five larvae remained. The number of remaining larvae per plant was too low to allow assess the percentage parasitism per plant. However, it still allowed us to determine the mean number of remaining larvae per plant that were not parasitized, i.e. the ones that were found alive and were not going to die due

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to parasitism. This mean was significantly higher in plants with ants than in plants without ants (p=0.003) (Fig. 2.2). During the 10-min observation periods we did not observe any parasitoids of the coccinellid larvae in the experimental plants. However, we did observe sphecid wasps, vespid wasps, spiders, and small frogs as potential predators. There were more predators in the plants without ants but no significant differences were found (with ants: 2.7 individuals ± 0.5 SE; without ants: 4.5 individuals ±0.6 SE; p= 0.13).

Lab-experiments. Ants repeatedly showed aggressive behaviors towards *H. shuvakhinae* and tried to attack them every time they came within < 1 cm of each other (number of attacks: 35; average number of attacks per trial: 2.5). At greater distances, the ants did not directly follow or pursue the parasitoids but sometimes followed the trail were they had walked. The ants never caught, killed or injured any parasitoid during the observation period (one hour per replicate). The parasitoids avoided close encounters with ants by changing their walking direction. When attacked, the parasitoids avoided being caught by jumping or running away and staying motionless on the underside of a leaf; they would also fly away from the plant (Fig. 2.3 a). On average, the parasitoids spent significantly more time on the plants without ants than in the plants with ants (Fig. 2.3 b). Accordingly, larvae on the plants with ants suffered less parasitism than the larvae in the plants without ants though the difference was marginally non significant (p=0.0615) (Fig. 2.3 c).

Discussion

Coccinellid beetles must have a number of traits in order to successfully prey upon ant-tended prey (Völkl 1995). First, all larval instars and pupal stage have to have morphological, behavioral or chemical mechanisms to avoid ant attacks. Second, adults have to be able to oviposit close enough to the ant nests so that their larvae have access to the ant-tended colonies, and lastly, eggs should be protected against ant predation. The coccinellid beetles *Platynaspis luteorubra* (Goeze) (Völkl 1995), *Coccinella magnifica* Redtenbacher (Sloggett et al. 1998), *Scymnus nigritus* Kugelann and *S. interruptus* (Goeze) (Völkl and Vohland 1996) have these traits, are usually associated with ants, and are thus considered myrmecophilous. Here, the presence of some of these traits in *A. orbigera* were experimentally demonstrated.

We demonstrated that *A. orbigera* larval waxy filaments provide an excellent defense against *A. instabilis*. When ants tried to attack the larvae, the mandibles of the aggressor ended up covered with sticky waxes. After this, the aggressor did not attempt to attack again nor did any other ant in the colony; thus, *A. orbigera* larvae of all instars preyed freely upon ant-tended scales. In contrast, when these filaments were manually removed, *A. instabilis* rapidly attacked and killed larvae of all instars. The same outcome has been found when removing the waxy filaments from *Scymnus* spp. (Völkl and Vohland 1996) and from green lacewing larvae (Eisner et al. 1978). *Azya orbigera* thus gains protection against *A. instabilis* by the presence of sticky filaments and not by chemical traits, as is the case with other myrmecophilous coccinellids (Völkl 1995) or other myrmecophilous insect predators (Lohman et al. 2006). These results contrast with Bach (1991) and Reimer et al. (1993), where *Pheidole megacephala* (F.) (Hymenoptera:

Formicidae) ants removed the waxy filaments and killed *A. orbigera* larvae. The myrmecophily of a coccinellid is thus apparently highly dependent on the ant species involved in the interaction.

We have never observed *A. instabilis* attacking *A. orbigera* pupae, neither in the field nor in the laboratory. Like the myrmecophilous *P. luteorubra* (Völkl 1995), *A. orbigera* pupae appear to be well protected against ant attacks, since the waxy filaments during this stage become very long and dense. The fact that all stages of *A. orbigera*—i.e. all instar larvae, pupae, and adults—are more abundant in *A. instabilis*-tended areas (Liere et al. in preparation), suggests that not only are larvae and pupae protected against ant attacks but also that adults manage to oviposit close enough so that their offspring have access to these prey-rich areas.

Even if adults are able to overcome ant attacks and are able to oviposit within ant-tended areas, ovipositing females still have to cope with the risk of egg predation. Coccinellid eggs are vulnerable to predation, since they have no physical protection if laid bare on the leaf surface (Honek 1996). However, ovipositing females have been observed covering their eggs with exuviae of prey aphids (Kawauchi 1985) or laying the eggs under prey scales (Pantyukhov 1968); behaviors presumably adopted to protect their eggs. Accordingly, we have observed *A. orbigera* eggs and recently eclosed larvae under dead *C. viridis* adults. We have also observed eggs on top of pupal exuviae and parasitized larvae of their own species. Possibly, the waxy filaments provide protection against ant attacks to these vulnerable stages. Coccinellid females ovipositing on pupal exuviae of their own species has been previously reported for *Exochomus flavipes* Thunberg (Geyer 1947). Whether these oviposition behaviors increase offspring survival

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probabilities and whether it is a common behavior among myrmecophilous coccinellids to protect eggs against ant predation remains to be tested.

Being able to prey upon ant-tended scales is, in itself, a great advantage since ant-tended hemipterans tend to be more abundant and persistent than non-tended ones (Bristow 1991). In the coffee plantation where our study was conducted, high densities of green scales can only be found in coffee bushes in the vicinity of shade trees with *A. instabilis* nests (Vandermeer and Perfecto 2006). *Azya orbigera* appears to have all the characteristics to be able to gain access to these high-density prey areas. Furthermore, living in close proximity with ants can potentially give myrmecophilous predators and parasitoids an extra advantage, namely, enemy-free space (Majerus 1989, Völkl 1992, 1995). For example, Völkl (1992) found that the myrmecophilous aphid parasitoid, *Lysiphlebus cardui* (Marshall) (Hymenoptera: Aphidiidae), suffered less hyperparasitism when ants were present. Majerus (1989) found that spider populations were smaller in close proximities of *Formica rufa* L. (Hymenoptera: Formicidae) nests, freeing *C. magnifica* from high predation pressure in these areas, and Völkl (1995) found that the presence of ants decreased the parasitism suffered by the coccinellid *P. luteorubra*. In the same way, *A. instabilis* showed aggressive behavior towards *H. shuvakhinae*, the most common parasitoid of *A. orbigera* larvae in the area.

Insect parasitoids need time to find, handle, and oviposit on their host. If this searching or handling time is interrupted, the parasitism success might be significantly reduced. Even if ants do not often directly attack parasitoids, their presence can significantly reduce the parasitism percentage (Völkl and Mackauer 1993). In our laboratory experiment, *A. instabilis* often attacked *H. shuvakhinae* forcing them to fly off

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the plant before finding their host larva. Thus, ant presence significantly reduced the time

parasitoids spent on coffee plants, resulting in reduced parasitism of *A. orbigera* larvae

on plants with ants relative to those without ants. However, even though *A. instabilis*

interfered with the searching and ovipositing behavior of *H. shuvakhinae*, they never

caught or killed any parasitoid during the experiments. The parasitoids were always able

to avoid being caught by running, jumping, or flying away. Consequently, it appears that

the decreased parasitism of *A. orbigera* larvae in plants with *A. instabilis* is not due to

parasitoid ant-induced mortality but to behavioral changes induced by the aggressive

behavior of ants. This is the first time that this kind of indirect trait-mediated positive

effect is reported for an ant and a coccidophagous coccinellid.

Unfortunately, in the field it was not possible to assess the impact of ants on the

parasitism of larvae, since after one week very few of the experimental larvae remained

in the plants. Nevertheless, the fact that at the end of the experiments more larvae

remained in the plants with ants than in the plants without ants is, in itself, an interesting

result. This result suggests either that the larvae were predated upon more often in ant-

excluded plants, or that the larvae left the plant more often when they did not detect the

presence of ants. The latter is very unlikely since the base of the plants was covered with

Tanglefoot and had a larva crawled down to leave the plant, we would have been able to

detect it stuck in the resin. Additionally, we did observe potential predators of the

coccinellid larvae that might have been responsible for their disappearance. Furthermore,

even though we did not find any statistically significant difference, we observed more

potential predators in plants without ants. This result coincides with Majerus (1989),

where fewer spiders were found near honeydew-collecting ants. Intraguild predation is

known to be an important factor affecting coccinellid survival (Sato et al. 2003), and coupled with parasitism it could drastically reduce coccinellid populations. Consequently, any factor reducing one or both of these sources of mortality would represent a big advantage to the coccinellids, as shown by the great difference in the number of surviving larvae (found alive and not parasitized) between ant and ant-free plants.

Not only do *A. instabilis* ants fail to affect the rate at which *A. orbigera* larvae prey upon *C. viridis*, but they also show aggressive behavior towards *A. orbigera*'s parasitoids. In the same way as other myrmecophilous ladybeetles, like *Coccinella magnifica* and *P. luteorubra* (Majerus 1989, Völkl 1995), *A. orbigera* larvae avoid ant attacks and thus, can gain access to persistent and abundant ant-tended prey colonies and gain potential protection against natural enemies. Due to these advantages, it would be expected that myrmecophilous ladybeetle populations should be more abundant near ant-tended prey colonies. Accordingly, all stages of *C. magnifica* in England (Majerus 1989, Sloggett et al. 2000), *P. luteorubra* in Germany (Völkl 1995) and *A. orbigera* in Mexico (Liere et al. in preparation) were found more often in the vicinities of honeydew-collecting ant nests. The existence and accessibility to these areas of high prey density and enemy-free space could have significant effects on the dynamics and persistence of these myrmecophilous coccinellid populations (Liere et al. in preparation). Due to the complex ecological interactions described here, much more work is needed to determine which would be the practical consequences of eliminating the ants from the plantations.

Acknowledgements

Figures

Fig. 2.1. Percentage of *Coccus viridis* eaten by *Azya orbigera* larvae after 24 hours. The experiment was performed in separate plastic containers with or without *Azteca instabilis* ant colonies. One larva per container was placed in scale-infested coffee branches for a total of eight replicates per treatment. The bars represent one SE from the mean.
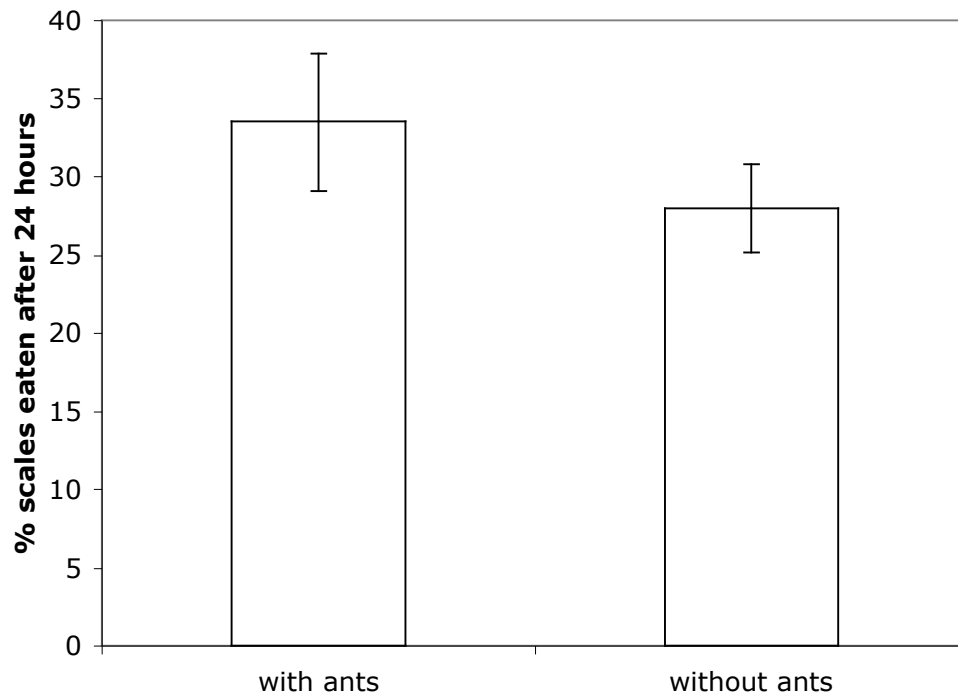
Fig. 2.2. Average *Azya orbigera* larvae per plant found alive and not parasitized in a field ant-exclusion experiment. The experiment started out with five larvae per plant in ant or ant-free coffee bushes in the field; the larvae were left there for a week. There were five replicates (coffee bushes) per treatment. The coffee bushes were infested with *Coccus viridis*. The bars represent one SE from the mean.
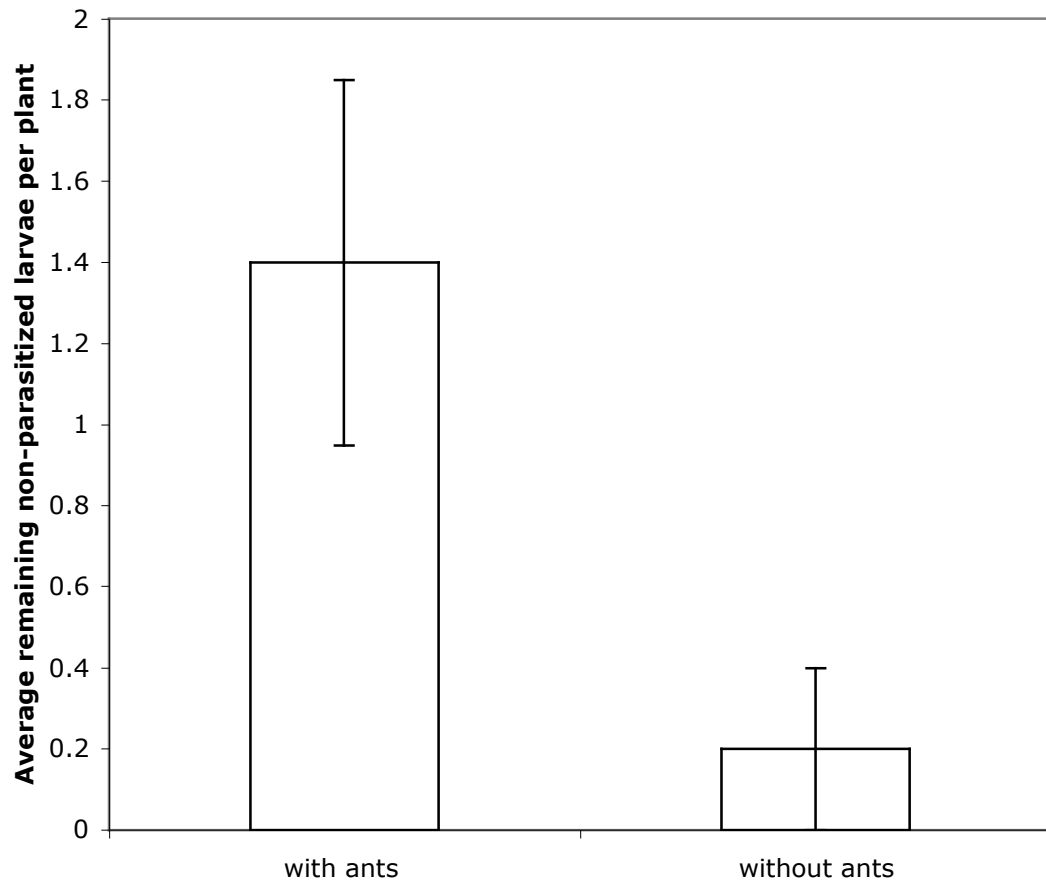
Fig. 2.3. Laboratory ant-exclusion experiment inside insect-rearing cages. Five *Azya orbigera* larvae were placed on a coffee bush infested with *Coccus viridis* tended by *Azteca instabilis;* another five larvae were placed in a coffee seedling without *A. instabilis*. Five *Homalotylus shuvakhinae* were then released inside each cage (n=7). One-hour observations were performed. a) Reactions of *H. shuvakhinae* to avoid encounters and attacks by *A. instabilis*. Numbers indicate the number of times the reaction was observed. b) Mean percentage parasitism of *A. orbigera* by *H. shuvakhinae*. c) Time *H. shuvakhinae* spent in plants with and without ants. The bars represent one SE from the mean.
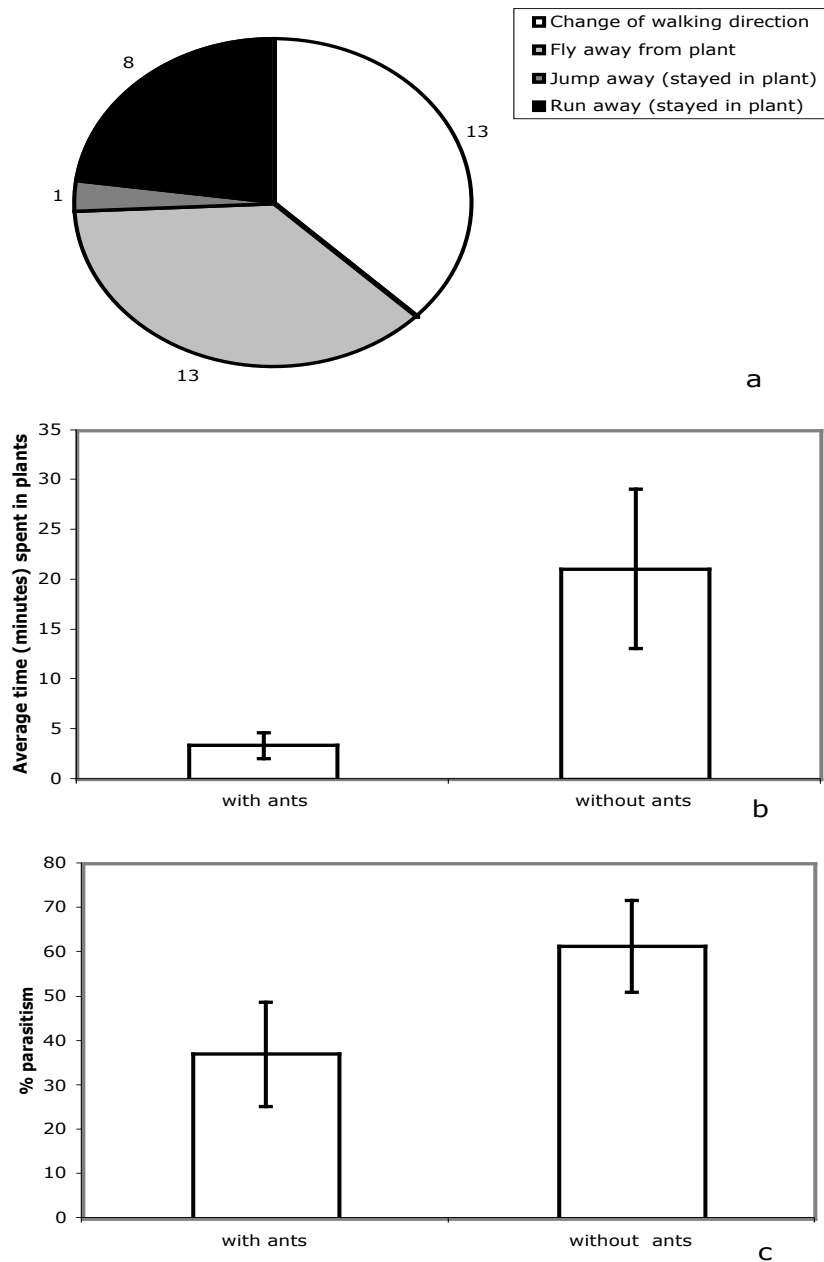
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Chapter III

Cascading Trait-mediation: Disruption of a Trait-mediated Mutualism by Parasite-induced Behavioral Modification

Abstract

Trait-mediated indirect interactions (TMII) are important driving-forces causing trophic cascades in aquatic and terrestrial food webs. Furthermore, since most biological communities are not simple food chains but complex networks of interactions, one TMII within a community might easily be influenced by another TMII. In other words, TMII themselves can be cascades with potential implications for community dynamics. Here we report on one of such cascade, where a parasitic fly induces behavioral changes that disrupt a trait-mediated ant-hemipteran mutualism. We show that during parasite-induced low-activity periods, the ant *Azteca instabilis* fails to protect its mutualistic scale-insect partner against predatory ladybeetles. Thus, in the presence of the parasite, ladybeetles ate as many scales in ant-patrolled plants as they did in ant-free plants. These results demonstrate how, through a cascade of trait-mediated interactions, associations between members of a community can be drastically altered.

Introduction

Cascading effects in food webs are common and well-documented. In tritrophic systems, for example, predators or parasites can have cascading positive effects on a plant population by reducing herbivore pressure (Shurin et al. 2002). These trophic cascades can be triggered either by an actual reduction in herbivore densities, i.e. a density-mediated indirect interaction (DMII) (Abrams 1995, Werner and Peacor 2003) or by changes in herbivore behavioral traits induced by the presence of predators or parasites, i.e. a trait-mediated indirect interaction (TMII) (Abrams 1995, Peacor and Werner 2001, Werner and Peacor 2003, Schmitz et al. 2004). The behavior and consequences of both types of trophic cascades have been broadly studied in terrestrial and aquatic communities (Brett and Goldman 1996, Shurin et al. 2002, Schmitz et al. 2004).

However, most biological communities are not simple tritrophic chains but complex networks of interactions (Polis 1991). Accordingly, multi-trophic communities with multiple and interacting density-mediated effects have been extensively studied (Schoener 1989, Power 1990, Letourneau and Dyer 1998). What is needed next is a more thorough understanding of the consequences of increasing the number of species or links that create new trait-mediated effects or through which trait-mediated effects propagate (Abrams 1993, 1995, LeBrun and Feener 2002). Theoretical studies have modeled four species food webs containing two or more trait-mediated links for some time (e.g. Abrams 1992, Abrams and Matsuda 1993). However, there still remains a dearth of empirical studies tracing how the effects of two or more TMIIs interact and propagate through ecological communities.

Interesting dynamics, like the positive feedbacks found in ant community assemblages (LeBrun and Feener 2002), might arise when multiple non-consumptive effects interact within a community. For example, if through attempting to escape the attack of a predator the herbivore reduces its feeding rate, there would be a positive TMII between the predator and the plant. However, if a top predator is introduced into the system and its presence causes the first predator to reduce its feeding rate, the first TMII would be disrupted, the herbivore could resume its feeding activities, and the plant would again suffer higher levels of herbivory. In such a situation, the top predator has introduced a new trait-mediated effect that interacts with another trait-mediated effect. Such an interaction of two TMIIs that has cascading community-wide effects is what we are calling here cascading trait-mediation.

What would be the effect of adding species that create new TMIIs to competitive and mutualistic associations that are already trait-mediated? For example, behavioral changes induced by predation risk might alter the ability of a superior competitor to acquire resources, allowing the inferior competitor to flourish in the presence of a predator (Feener 1981, Werner 1991, Peacor and Werner 1997, Werner and Peacor 2003). Furthermore, many mutualisms, like ant-hemipteran and ant-plant mutualisms, are also trait-mediated. While ants receive a direct benefit, in the form of honeydew or extra-floral nectar, the benefit received by their mutualistic partners is indirect and trait-mediated. In these associations, plants and hemipterans alike gain enemy-free space not because their natural enemies' density is reduced by the ants, but rather because of the behavioral changes induced by the constant ant patrolling and the resulting difficulty natural enemies have in accessing their food. Adding a top-predator or parasite to such

systems could trigger cascading trait-mediations that could potentially reverse back competitive outcomes and disrupt mutualism associations.

Here we examined the community-wide effects of a cascading trait-mediation triggered by the presence of an ant parasitoid on an insect community in a coffee plantation in Chiapas, Mexico. In our study site, the tree-nesting ant, *Azteca instabilis* F. Smith normally protects the coffee green scale, *Coccus viridis* (Green), by constantly harassing the predatory ladybeetle, *Azya orbigera* Mulsant (Coleoptera, Coccinellidae). However, previous studies have shown that the presence of the parasitoid fly *Pseudacteon* sp. (Diptera, Phoridae) drastically reduces ant activity levels (Philpott et al. 2004). Given that phorids are very active around *A. instabilis* nests during the rainy season, when both scale and beetle populations are at its highest, and ant activity is drastically reduced for up to 90 min even after short periods of phorid attacks (Philpott 2005, Philpott et al 2009), we wanted to determine how this parasite-induced behavioral change altered the interactions between *A. instabilis* and the predatory ladybeetle, *A. orbigera*. With respect to the cascading trait-mediation, we specifically asked three questions: 1) How does phorid presence affect ant activity? 2) How does the reduction of ant activity influence the interactions between ants and ladybeetles? and 3) Does phorid fly presence allow ladybeetles to prey upon ant-tended scales? We hypothesized that in the presence of phorid flies, ants would fail to protect their scale insect partners against predation by the ladybird beetle. The final consequence of this cascading trait-mediated effect would be that the mutualism between *C. viridis* and *A. instabilis* would be disrupted and *A. orbigera* would gain access to otherwise non-accessible prey (Fig. 3.1).

Methods

Study site

Experiments were conducted in a 300ha organic shaded coffee farm located in southwestern Chiapas, Mexico (15°11'N, 92°20'W). The site is 900-1150m in elevation and receives ~4500mm of rainfall annually. Experiments took place during the rainy season between June and August, 2008.

For all laboratory experiments, small pieces of *A. instabilis* colonies were collected from the field and broken into roughly 10 cm in diameter fragments (for details see Liere and Perfecto 2008). Fragments of this size contain a large number of workers, larvae, and pupae, and function as artificial nests that maintain regular activities of food collection and nest defense, and are self-sustainable for several weeks. Fragments were placed in plastic containers with sides treated with Insect-a-Slip Insect Barrier ®(BioQuip Products, Inc., Rancho Dominguez, Ca) and the top edges coated with Tanglefoot®(The Tanglefoot Co., Grand Rapids, MI).

All *A. orbigera* adults used were collected from the field and maintained in the laboratory in insect rearing cages. They were fed *C. viridis* scales on fresh leaves daily, to excess. *Pseudacteon* flies were collected in the field near *A. instabilis* nests between 8-11am and were introduced to the experimental arenas before 1pm on the same day they were collected.

How does phorid presence affect ant activity?

To confirm Philpott *et al*. (2004) results, which show that in the field *A. instabilis* activity is significantly reduced by the presence of phorid flies, we conducted an ant

response experiment to determine how the presence of *Pseudacteon* sp. reduced ant activity levels under controlled laboratory conditions. Coffee branches with one or two *C. virdis* infested leaves were collected from the field and "planted" on a wire base inside a 10cm tall, 6.5cm diameter plastic cup. The "planted" twig was then placed within the larger plastic container holding the artificial *A. instabilis* nest, and together they were placed in the center of transparent 60x60x60cm insect rearing cages. Ant activity was recorded as the number of ants crossing a given point on the "planted" twig in a one-minute period. Ant activity was first evaluated in the absence of phorid flies; two phorid flies were then released inside the cages. Ant activity was reassessed at 0, 5, 10, 15, 20, 30, 60 and 120 minutes after the phorids were released. There were sixteen replicates for this experiment. To minimize potential confounding light or temperature variation, the replicates were conducted one at a time and were spread out throughout six days between June 30th and July 12th, 2008. We completed one to three replicates each day, starting around 11am. Ant activity before and during each time period after phorid introduction was compared using a Repeated Measures Mixed Linear Model, using 'trial' as the subject variable, 'ant activity' as dependent variable, and 'time' (before phorid introduction, 0, 5, 10, 20, 30, 60, and 120 minutes) and 'day' (1–6) as fixed effects.

How does the parasite-induced reduction of activity influence in the interactions between ants and ladybeetles?

We evaluated the interaction between ants and *A. orbigera* adults in 27 ant-tended scale-infested coffee bushes in the field. To understand phorid fly influences on ant activity and indirect influences on *A. orbigera* behavior, we evaluated ant-coccinellid

interactions before and after phorids arrived. First, ant activity in the absence of phorid parasitoids was measured as the number of *A. instabilis* that crossed a single point '*a*' (approximately 30-45cm from the ground) on a coffee branch within a two-minute period. One *A. orbigera* adult was then placed at that point, and the occupation time of the beetle in the coffee plant was noted. Additionally, interactions were qualitatively categorized as either a) ants attacking, capturing and killing the beetle, b) ants attacking the beetle causing it to fall or fly away, or c) beetle flying away on its own. Any beetle that did not leave the bush after 10 minutes was recorded as occupying the bush for 10 minutes and leaving on its own.

In order to evaluate the effect of ant parasitoids on the ant-beetle interactions, we then smashed one ant in the plant to attract a phorid fly (Philpott et al. 2004). We observed that the initial *A. instabilis* response to phorid flies includes running to a hiding place or back towards the nest, so we waited four minutes after the first phorid appeared to measure ant activity (again, number of ants crossing point *a* during a two-minute period). Then, a new *A. orbigera* adult was placed at point *a,* and occupation time and interaction type were recorded.

Since the response of ants to their phorid parasitoids is commonly found to be consistent, irrespective of the number of attacking flies (Feener and Brown 1992, Morrison et al. 2000, LeBrun and Feener 2002, Orr et al. 2003) we did not try to control the number of phorids that arrived or take note if multiple flies were present during a trial. If phorids did not appear within the first five minutes, one additional ant was smashed every five minutes. If no phorids appeared within 15 minutes, or if all phorids left before the observation period, the trial was ended.

Beetle occupation time and ant activity were log-transformed to meet the conditions of normality and then a linear regression was used to evaluate the relationship these two variables with and without phorids. However, we observed that somewhere between twelve and eighteen ants/2 min period there appeared to be a discontinuity or threshold in the data, below which ants did not seem to affect the time beetles stayed in the plants. The exact value of this ant-activity 'threshold' was not relevant for our study and therefore we did not attempt to statistically locate it. What was important to our study was the fact that for low ant activity values the interaction between the two variables was different than for high ant activity values. Therefore, in addition to the regression between the whole data, we performed two separate linear regressions, one above and one below fifteen ants/2 min period. Ant-beetle interactions were then compared with chi-square contingency tables to determine if beetles were as likely to leave a plant on their own as due to ant harassment below and above these ant-activity 'threshold'.

Does phorid fly presence allow ladybeetles to prey upon ant-tended scales?

To establish whether the presence of *Pseudacteon* sp. enabled *A. orbigera* adults to consume ant-tended scales, we performed a laboratory predation experiment. We collected *C. virdis*-infested coffee twigs from the field and counted the number of adult scales; we used only those twigs that had approximately 100 adult scales. The twigs were 'planted' as described above, and then placed inside rearing cages. The twigs were randomly assigned to one of three treatment cages: 1) no ants and no phorids, 2) with ants and no phorids, and 3) with ants and with phorids. There were 14 replicates of each treatment.

Once the ants were actively tending the scales in the rearing cages, one coccinellid adult was randomly assigned and released into each of the three treatment cages. For the treatments with phorids, two flies were released in each cage simultaneously with the coccinellid adult. After 24 hours, we counted the remaining adult scales and calculated the number of scales eaten during this period. To detect difference between treatment means we used an ANOVA test and then a Tukey test for post-hoc comparisons.

Results

How does phorid presence affect ant activity?

Time after phorid introduction had a significant effect on ant activity (F_8= 9.415; $P<0.0001$) and this result did not depend on the day the trial was performed (F_5 = 0.426; $P=0.820$). Ant activity (16.19 ants/min ± 2.21 SE) was halved five minutes after the phorids were released into the cages (8.4 ants/min ± 1.4 SE) and stayed around that level for the remainder of the observation period (2 hours) (Fig 3.2).

How does the parasite-induced reduction of activity influence the interactions between ants and ladybeetles?

Though we never observed any beetle being caught or killed by ants, there was a strong negative relationship between ant activity and beetle occupation time (R^2=0.72, $F_{1,26}$ =69.2, $P<0.00001$). There appears to be a threshold of ant activity below which ants do not seem to affect the time beetles stayed in the plants and this is reflected by the poorer fit to the linear regression at low ant-activity values (Fig 3). Below fifteen ants per

two-minute period, ant activity and beetle occupation time had a very weak and non-significant relationship (R^2=0.0192, $F_{1,11}$=0.47, P=0.5) and beetles were as likely to fly on their own as they were due to ant attacks (26 non attacks vs. 15 attacks; χ^2 P>0.05). Contrastingly, above this value of ant activity there was a strong negative relationship between ant activity and beetle occupation time (R^2 = 0.627, $F_{1,12}$= 20.21, P=0.0007) (Fig. 3.3a) and beetles always flew away or fell off of the plant to escape harassment (0 non attacks vs. 21 attacks; χ^2 P<0.001). After phorids arrived, ant activity was reduced below the threshold in all but two trials; as a result, beetles were no longer affected by ants (R^2 = 0.091, $F_{1,23}$= 2.32, P=0.141) (Fig. 3.3b).

Does phorid fly presence allow ladybeetles to prey upon ant-tended scales?

The number of scales eaten by *A. orbigera* was significantly affected by treatment in the consumption experiment (ANOVA, F_2=6.651, P=0.004) (Fig. 3.4). The presence of tending ants significantly reduced the number of scales eaten (Post-hoc Tukey-test: P=0.005). However, when phorids where present, the protective effect of *A. instabilis* disappeared and *A. orbigera* was able to prey as many scales as in the treatment without ants (Post-Hoc Tukey-test: P=0.836).

Discussion

Trait-mediated interactions are ubiquitous in ecological communities (Preisser et al. 2005), and the potential for behaviorally driven trophic cascades appears similarly widespread (Peacor and Werner 2001, Schmitz et al. 2004). Though TMIIs in tri-trophic interactions have been well studied, few empirical studies have looked how multiple

interacting TMIIs are transmitted throughout a community (but see LeBrun and Feener 2002). Here we show such a system in which through the first trait-mediated interaction, *A. instabilis* ants protect their scale insect partners by non-lethally harassing the predatory ladybeetles forcing them to abandon the scale-infested coffee bushes. These patrolling and foraging activities sometimes attract a parasitic phorid fly which causes *A. instabilis* to seek shelter or alter their behavior in a way that renders them incapable of effectively harassing the predatory ladybeetles, thus the second TMII. Similar to LeBrun and Feener (2002) who showed that two interacting TMIIs could be important in explaining the structure of ant assemblages, here we show how a cascade of trait-mediated interactions can have strong effects on the dynamics of a biological community.

Our results show that, in the absence of ant-attacking parasitoid flies, the *A. instabilis – C. viridis* association works like any other ant-hemipteran mutualism (Way 1963). As expected, in our laboratory experiments, fewer scales were killed in the presence of tending ants, and accordingly in the field, ant harassment reduced the time ladybeetles were able to stay in scale-infested plants, presumably reducing their foraging capability. Thus, we showed that *A. instabilis* tend their mutualistic partners and provide them enemy-free space against predatory ladybeetles. Additionally, despite the fact that we never observed any beetle being caught or killed by ants either in the field or in the lab, ant patrolling did effectively reduce the number of scales eaten, suggesting that the interaction by which the ants provide enemy-free space to their mutualistic partners is indeed indirect and trait-mediated.

The impact of TMIIs on non-trophic interactions, like mutualisms, has received much less attention than their influence on trophic interactions (Bronstein and Barbosa

2002). Traditionally, mutualisms have been studied as pairwise interactions, and the fact that they are embedded within complex ecological communities, and that the effect that the mutualistic partners have on one another might be context-dependent, has only recently been recognized (Bronstein 1994, Holland et al. 2002, Stanton 2003). It is known that the outcome of mutualistic associations is not necessarily stable and that due to biotic or abiotic conditions the net effect of species on each other can vary in space and time. Thus, a mutualistic association can vary from strong mutualism, to weak mutualism, to commensalism, and even to antagonism depending on the circumstance (Bronstein 1998, Stanton 2003). For example, in an ant-hemipteran mutualism, both partners gain a benefit from the association: ants gain a direct benefit in the form of honeydew, and scales gain an indirect and behaviorally driven benefit in the form of enemy-free space (Way 1963, Beattie 1985). However, our results show how the outcome of this trait-mediated association can be altered. In the absence of phorid parasitoids, beetles were usually forced off plants and prevented from preying upon ant-tended scales; conversely, when phorids were present, ants were forced to reduce their activity levels leaving beetles undisturbed to freely prey upon the scales. Therefore, while presumably the scale insects continued producing honeydew, the presence of the parasitoids rendered ants unable to effectively harass the predators of their mutualistic partners. Consequently, our results suggest that through a cascade of trait-mediation, the association between ants and scales might alternate between mutualistic and antagonistic based on phorid presence.

The importance of this cascade of indirect interactions depends on whether or not phorids have persistent effects on the ants so as be consequential for the community

dynamics. In general, phorid flies' ability to significantly affect ant populations has been

extensively studied due to its potential as biological control of imported fire ants

(Jouvenaz 1983, Feener and Brown 1992, Porter et al. 1995, Morrison et al. 2000,

LeBrun and Feener 2002, Mehdiabadi and Gilbert 2002). In our particular system,

phorids also have a great potential to significantly impact *A. instabilis* populations.

Previous studies on the area (Philpott et al. 2004, Philpott 2005, Philpott et al. 2009) as

well as personal observations suggest that wherever phorids are present, they are active

throughout the day and will attack ant colonies when ants become agitated. While

defending their territory, ants release pheromones which act as attractants to nearby

parasitoids (Mathis et al. in review). Consequently, if phorids are in the area, they will

launch attacks, which can diminish *A. instabilis* competitive abilities (Philpott et al 2004,

Philpott 2005). Similarly, phorid presence could cause persistent changes in the ant-

beetle-scale interactions and the results of our laboratory experiments could well

represent what is happening in the field.

It is well known that local community interactions can cause unexpected spatial

patterns (Hassell et al. 1991, Rohani et al. 1994, 1997). Consequently, cascades of trait

mediation could potentially influence the spatial distribution of the organisms in the

community. Our data demonstrate that *A. instabilis* greatly reduces the foraging ability of

A. orbigera and therefore, it would be expected that these beetles would be forced to only

prey upon the smaller, unprotected scale populations present throughout the farm in areas

without colonies of *A. instabilis*. However, we have observed that the ladybeetles, both as

larvae and as adults, are highly concentrated around *A. instabilis* nests (Liere,

unpublished data). Thus their distribution, as well as the ants', is highly clumped

(Vandermeer et al. 2008). We suspect that the presence of phorid flies and their effect on ant activity might explain this counterintuitive spatial pattern. Since adult beetles usually remain within a limited distance of scale-infested plants and repeatedly return to these plants even after being harassed and forced to fly away (H.L. personal observation), they might opportunistically forage in these high prey-density areas when phorids force ants to suspend their tending behavior. These interactions could potentially explain the aggregated distribution of these ladybeetles around ant nests.

Potentially the most persistent effect of the phorid-induced change in ant behavior may be the ability of the ladybeetle to capitalize on the low ant-activity periods to oviposit in ant-tended plants. Due to waxy filaments that cover their bodies and render them immune to ant attacks, *A. orbigera* larvae benefit greatly by living in close association with ants via access to a persistent and abundant food supply and enemy-free space (Liere and Perfecto 2008). Consequently, ovipositing in ant-patrolled plants represents a great fitness advantage for females. However, oviposition might be too difficult and risky under constant ant patrolling since similar to other ladybird beetles (Nais 2008, Kawauchi 1985), *A. orbigera* oviposits under scale exuvia or carcasses to protect their fragile eggs from predation. Thus, they have to be able reach the branches with scales, locate the exuvia or dead scales and lift them to oviposit underneath; a lengthy process that might only be possible during the phorid-induced low ant-activity periods. Even though our experiments tested only for the effects of phorid flies on the foraging efficiency of the beetles, given the nature of the oviposition behavior of the beetles, we suggest that phorid-induced low ant-activity periods also provide excellent opportunities for the adult ladybeetles to oviposit in these high-quality areas. Thus, a

chain of interacting TMII might very well explain the counterintuitive spatial distribution of ladybeetles, and may in fact enable the persistence of the beetle at higher densities than would otherwise be feasible based on the sparse scale populations existing outside of ant-tended plants. Though more research is necessary to confirm these predictions, it seems clear there exists community wide effects stemming from the phorid-ant interaction.

Furthermore, these local interactions might determine the spatial dynamics of ant nests in the area. When beetles attempt to prey upon ant-tended scales, they usually trigger *A. instabilis* attacks. This agitation may attract nearby phorids, which in turn, may force ants to reduce their activity levels long enough for the beetles to oviposit or prey upon scales. Consequently, high phorid abundance around an ant colony could render ants largely incapable of defending their mutualistic partners, whose populations in the area could thus be easily decimated. Without their main carbohydrate source, the ant colony would be forced to move to another tree or face starvation, which corresponds with the high ant nest mortality and turnover in the farm (Vandermeer et al 2008). Moreover, since ant-nest cluster size is positively correlated with phorid densities (Vandermeer 2008) phorid-induced nest mortality and movement might be stronger in areas with high concentrations of ant nests.

Similar interactions have been recorded in an ant-plant mutualism where in the presence of spiders, patrolling ants sought refuge and suspended their tending behavior leaving their mutualistic plants unprotected against herbivory (Gastreich 1999). Since biological communities are complex networks of interactions, cascading trait-mediations most likely extend outside ant-hemipteran or ant-plant mutualisms and might be a feature in most systems. We believe that more attention should be paid to this type of cascade

and that questions related to the extent and strength of these interactions in structuring ecological communities should be addressed. By answering these questions, we might be able to gain a better understanding of context-dependent community interactions and be able to explain counterintuitive and unexpected spatial or temporal pattern.

Acknowledgments

Figures

Fig. 3.1. Hypothesized interactions and cascading effect of the parasitic fly (*Pseudacteon* sp.) on the trait-mediated mutualism between *Azteca instabilis* and *Coccus viridis*. a) The ladybeetle, *Azya orbigera*, avoids ant-tended scale colonies because of the risk of ant attack. In this way, scale insects gain enemy-free space by behavioral changes in the ladybeetle induced by the presence of the ants: TMII mutualism. b) If the presence of the parasite reduces ant activity, ladybeetles would be released from ant influence; the mutualism would be disrupted and the ladybeetles would gain access to scales. Solid arrows represent direct interactions, dashed lines represent indirect interactions. Circles represent negative effects and arrows represent positive effects.
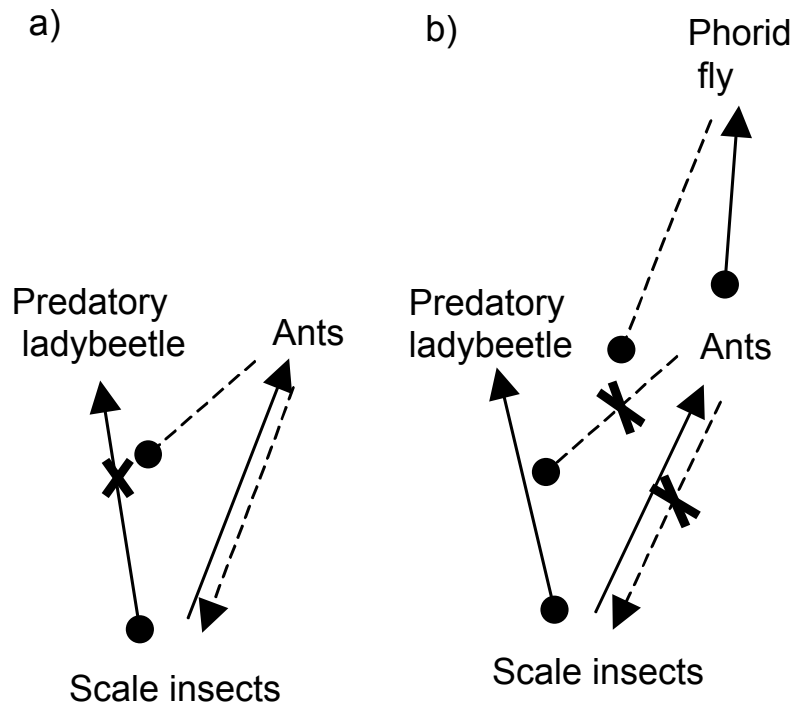
Fig. 3.2. Effect of *Pseudacteon* phorid presence on *Azteca instabilis* activity levels. Data show mean ± SE (n=16). Experiments were conducted in the lab inside rearing cages. Dashed line indicates when the phorids (two per cage) were released. There was a significant effect of time after phorid introduction on ant activity (F_8= 9.415, p<0.0001)
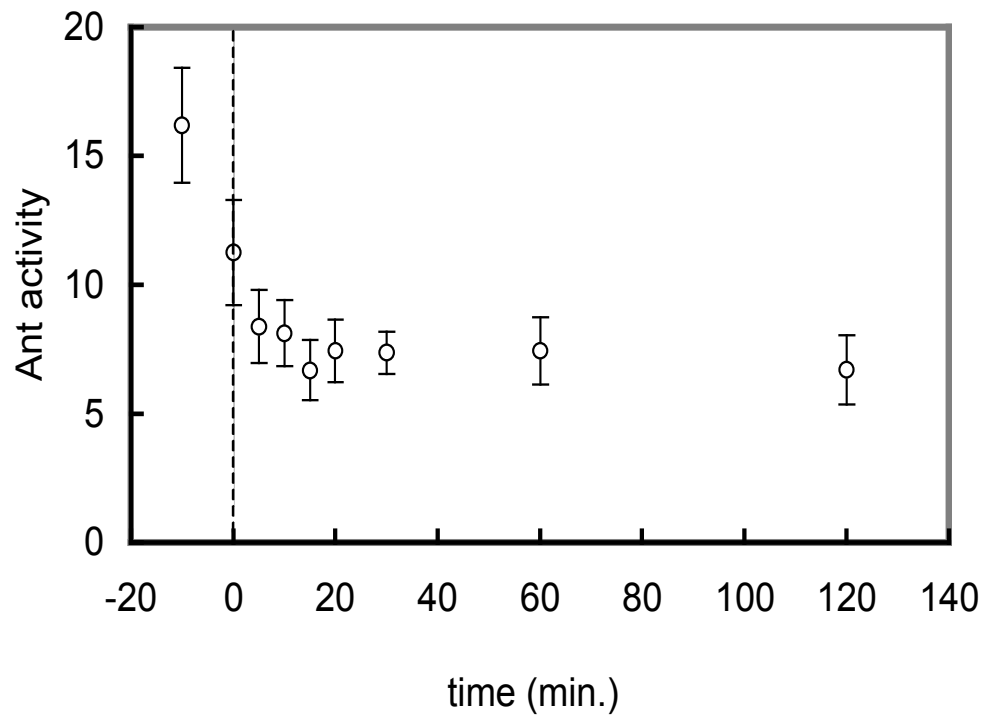
Fig. 3.3. Ant activity effect on beetle occupation time (in seconds) in coffee bushes. Both axes in log (x+1) scale where a value of 2.77 corresponds to the threshold of 15 ants per minute. **a**) Relationship between ant activity and beetle occupation time before phorids arrived. The dashed line indicates the threshold below which beetles appear not to be affected by ant activity (R^2=0.0192, P>0.05, n=13). Above the threshold there is a strong significant negative relationship (R^2=0.627, P<0.005, n=14). **b**) Relationship after phorids arrived to the plant. Ant activity was mostly reduced below the threshold were beetles are not disturbed (R^2=0.09, P>0.05, n=25).
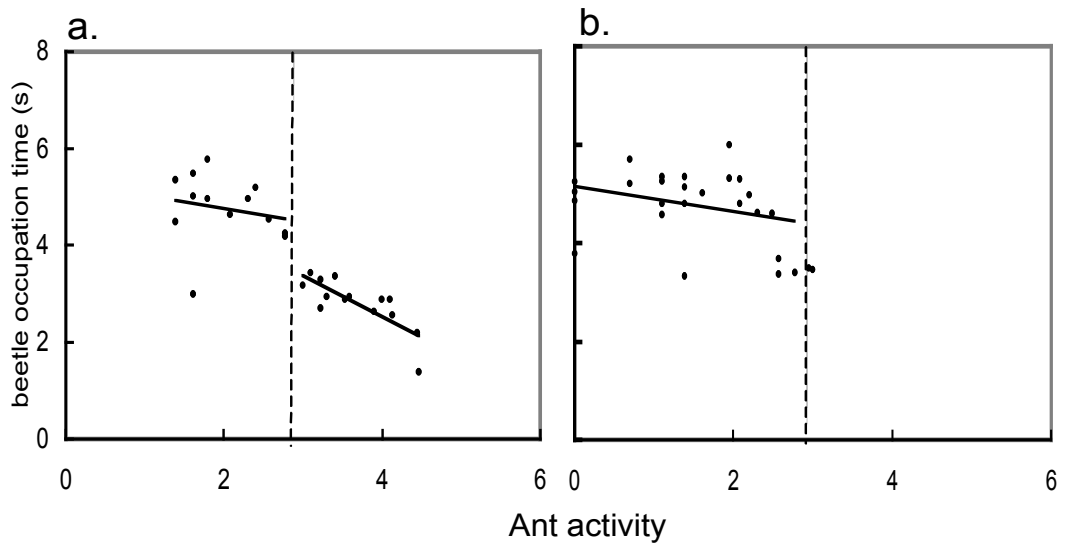
Fig. 3.4. Effect of phorid flies (*Pseudacteon* sp) on the protection given by *Azteca instabilis* to their mutualistic scale partners, *Coccus viridis*, against predation from the ladybeetle *Azya orbigera*. Experiments were conducted in the lab inside rearing cages (n=14). Figure shows mean adult scales eaten in 24 h ± SE. Different letters indicate statistically significant differences between treatments (post-hoc Tukey test p<0.005)
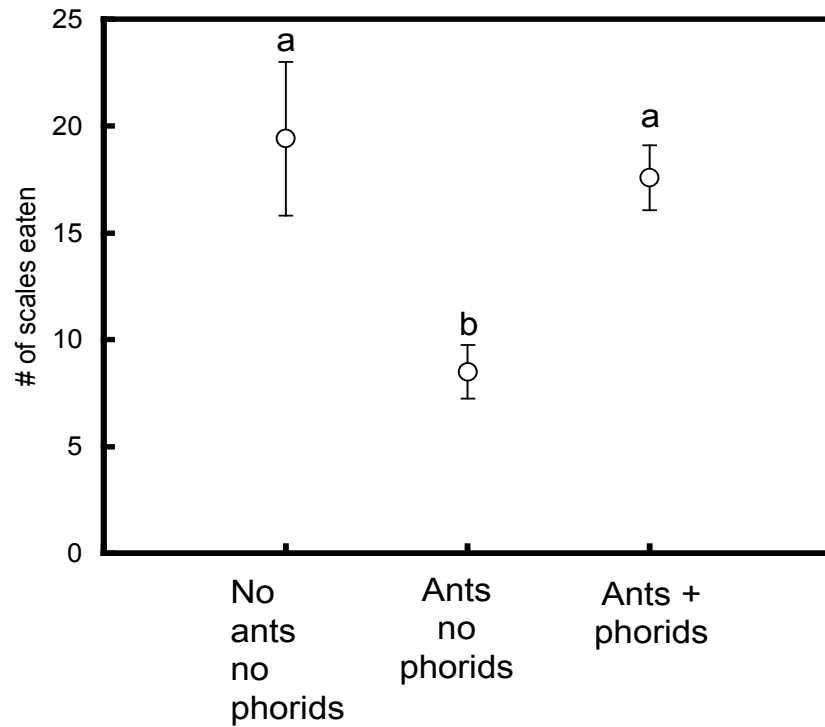
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Chapter IV

Habitat Heterogeneity and the Abundance, Distribution, and Persistence of a Myrmecophilous Ladybeetle

Abstract

Myrmecophilous ladybird beetles can be greatly affected by the different habitat types created from the interactions among members of their community. For example, the aggressive ant, *Azteca instabilis*, creates two habitat types utilized differently by separate life stages of the predatory ladybird beetle, *Azya orbigera*, which is immune to ant attacks as larva but not as adults. Areas dominated by the ant sustain high prey density and enemy-free space for this ladybeetle larvae. Here we report on how the a patchy distribution of areas with prey tended by ants, embedded in a matrix of areas without ants, affected the distribution, abundance, and persistence of this voracious predatory ladybeetle in a coffee farm in Chiapas, Mexico. We surveyed a 45-ha plot where the location of every *A. instabilis* nest is mapped, and also tracked short and long distance movements of *A. orbigera* adults. We found that both adults and larvae are more abundant and persistent in areas with ants but that adults tend to be restricted to the peripheries of the area of ant influence. Our results suggest that ant areas are source habitats for *A. orbigera* larvae but that adults disperse to no-ant areas, probably to feed. Our results suggest that the specific spatial distribution of *A. instabilis* nests plays a key role for the persistence of this important predatory ladybeetle.

Introduction

The vast majority of animal communities are not randomly distributed. Rather they tend to have uniform, clustered, or patchy distributions and the question of how these patterns emerge as well as the consequences of their existence are current and exciting topics in ecological research. Habitat heterogeneity, or the existence of high and low-quality habitat patches, is known to promote coexistence of otherwise unstable systems like parasitoid-host and predator-prey associations (Bailey et al. 1962; Hassell and May 1974; Hasell et al. 1991; Bonsall et al. 2002). Interestingly, the spatial heterogeneity can be created by local interactions among members of the community (Briggs and Hoopes 2004; Comins and Hassell 1996; Hassell 2000). Thus, population persistence can, in many cases, be attributed to the underlying patchy structure of the habitat, which in turn is influenced by the dynamics of the population in question (Liere et al., in review).

The populations of many coccinellid beetles, also called ladybeetles, are often highly vulnerable to local extinctions due to, among other factors, the patchy and ephemeral nature of their hemipteran prey (Dixon 2000). A good oviposition site is particularly important for coccinellids since their larvae have limited dispersal capabilities and their survival thus depends on the mothers' choice of a high-quality oviposition site (Hodek and Honek 1996; Seagraves 2009). Therefore, female ladybeetles need to find oviposition sites where their prey is both abundant and young (Dixon 1959, 2000) and where mortality due to natural enemies is low (Mills 1982; Osawa 1989, 1992; Burgio et al. 2002; Oliver et al 2006). Areas where their hemipteran prey are tended by ants represent potentially high-quality habitats since these frequently have all these

qualities plus the additional advantage of sustaining relatively more persistent prey populations compared to those not tended by ants (Bristow 1991; Mahdi and Whitaker 1993; Sloggett and Majerus 2000a). However, due to the high mortality risk from ant attacks, most ladybeetles, as well as other species of hemipteran natural enemies, stay away from ant-tended areas except in periods of severe prey scarcity (Way 1963; Sloggett and Majerus 2000b). These beetles are thus effectively restricted to areas of low resource abundance and in this way experience a landscape modified by the ants that tend their prey.

Nevertheless, several predators and parasitoids, including ladybeetles (for review see Majerus et al 2007), have evolved morphological, chemical, or behavioral modifications that render them immune or relatively tolerant to ant attacks (Eisner et al 1978; Majerus 1989; Hubner 2000; Völkl 1992, 2001). In this way, besides gaining access to more stable and persistent prey patches, myrmecophilous or 'ant loving' ladybeetles also gain reduced competition and indirect protection against their own natural enemies by the constant ant patrols in these areas (Völkl 1992; Mackauer and Völkl 1993; Liere and Perfecto 2008). The distribution and abundance of myrmecophilous ladybeetles is, accordingly, positively correlated to that of hemipteran-tending ants (e.g. Völkl 1992, 1995; Völkl and Stechmann 1998) and some obligate myrmecophiles, like *Coccinella magnifica* (Sloggett et al. 2002), are exclusively found in the vicinity of the nests of tending ants (Majerus 1989). Thus, contrary to the majority of hemipteran natural enemies, through a combination of accessibility to abundant prey and enemy and competition-free space created by ants, myrmecophilous ladybeetles

experience a habitat heterogeneity with high-quality habitats within areas of ant influence and low-quality habitats outside of them.

The coccidophagous ladybeetle *Azya orbigera* (Coleoptera: Coccinellidae) is a voracious predator whose adults are vulnerable to the aggressive tree-nesting ant *Azteca instabilis* (Hymenoptera: Formicidae) (Liere and Larsen 2010), but whose larvae have waxy filaments that render them immune to ant attacks (Liere and Perfecto 2008). Therefore, larvae are able to prey upon the abundant ant-tended populations of green coffee scales (*Coccus viridis*, Hemiptera: Coccidae) that are common on the coffee bushes in the close vicinity of *A. instabilis* nests. Additionally, when living on ant-patrolled plants these ladybeetle larvae are also relatively free of predation and parasitisization (Liere and Perfecto 2008). Since the risk of larval mortality is significantly lower in ant-tended areas, there is an obvious fitness advantage for females to oviposit in these areas.

Our main objective here was to determine how *A. orbigera* beetles are affected by the spatial habitat heterogeneity created by *A. instabilis*, given that beetles have access to ant-tended areas only during their larval stage. Specifically, we sought to determine how the spatial distribution of the *A. instabilis*-*C. viridis* mutualism affected the spatial distribution, abundance, and persistence of *A. orbigera* in a coffee farm in Chiapas, Mexico. First, in a large-scale survey on a 45-ha permanent plot, we compared the abundance of *A. orbigera* larvae and adults in areas with and without ant nests. To measure short distance effects of ants on beetle distribution and abundance we sampled beetle adults and larvae within a 5-meter radius of isolated ant nests. We hypothesized

that the larvae would be more abundant within areas of ant influence but that due to their susceptibility to ant attacks, adults would show the opposite trend.

Given that *A. instabilis'* nests have a clustered distribution in space and given the strong effect that this ant has on the abundance of their mutualistic partners (Vandermeer and Perfecto 2006), we wanted to determine whether the spatial distribution of these nests would affect *A. orbigera* populations. We hypothesized that the size of the clusters of ant nest affected the abundance and persistence of *A. orbigera* populations. That is, since larger clusters are likely to have more concentrated abundant resources, we predicted that ladybeetle abundance and persistence would be positively correlated with the size of the ant nests cluster.

Finally, to gain a deeper understanding of the population dynamics of this important predator, we investigated the degree to which they disperse among areas with and without ants. We hypothesized that given the inability of adults to prey upon ant-tended scales, they would be forced to move between these different habitat types (for example, by being forced to leave areas with ants after eclosion, or having to move among different areas for oviposition and feeding).

Methods

Study site

The study took place in 'Finca Irlanda' an organic shade-grown coffee farm in Chiapas, Mexico, near the border with Guatemala (15° 10' N and 92° 20' W) at approximately 950 m above sea level. Within this farm, a 45-hectare plot was established in 2004 and all the shade trees larger than 10 cm dbh were identified and located within a coordinate system.

Through biannual surveys, the location of nests of *A. instabilis* was determined and mapped (see Vandermeer et al. 2006 for details of the farm and the 45-ha permanent plot).

Study system

The ecological community under study consists of the green coffee scale, *C. viridis*, its mutualistic tree-nesting ant, *A. instabilis*, and the predatory ladybeetle, *A. orbigera*. *Azteca instabilis* protects the green scales against natural enemies, such that high densities of green scales are only found within close vicinity of ant nests (Vandermeer and Perfecto 2006). These ants build their nests in shade trees and tend the scales living on coffee plants in the surrounding two to three meter radius of the nest. In an established 45-ha permanent plot, only approximately three to five percent of shade trees contain ant nests and these are clustered in space (Vandermeer and Perfecto 2006; Vandermeer et al. 2008). The ladybeetle *A. orbigera*, both as larva and as adult, is a voracious predator of green scales but, as mentioned above, only the larvae, whose body is covered by waxy filaments that protect them against ant attack, have ready access to ant-tended scales (Liere and Perfecto 2008).

Ladybeetle abundance in areas with and without ants

To determine the effect of ant nests on the ladybeetle distribution we sampled coffee bushes around shade trees with and without ant nests. We superimposed a 50 X 50 m grid over the map of the 45-ha permanent plot. Given that within this plot the location of every *A. instabilis* nest is known (Vandermeer and Perfecto 2006; Vandermeer et al.

2008), we could determine whether each of the quadrants was occupied by ants or not. In the cases of quadrants without ant nests, we chose the centermost shade tree and searched the coffee bushes within a 2m radius for *A. orbigera* adults (noting sex), larvae (noting instar and parasitization), and pupae for a period of 30 min. We also counted the number of parasitized larvae. In the cases of quadrants with ant nests, we identified the centermost shade tree with an ant nests and sampled the coffee bushes using the same methodology described above. We excluded all the quadrants at the edge of the plot from the survey. This survey was carried out twice in the rainy season (June/July 2006 and 2007) and twice in the dry season (January/February 2007 and 2008). During the first survey, there was a total of 55 quadrants with ants and 60 quadrants without ants; and 53 with and 63 without during the rest of the surveys.

First, we mapped the distribution of *A. orbigera* populations with respect to *A. instabilis* nests to give a qualitative idea of the effect of the ant nests on the abundance of *A. orbigera*. We then compared the mean number of adults and larvae with and without ants with a 10,000 trial permutation (re-sampling without replacement) tests using the Re-sampling Software Add-in for Excel version 3.2 (Re-sampling Stats 2006) (Liere & Perfecto 2008). For the first data set, rainy season 2006, we also performed separate analyses for males and females; we also compared percent larval parasitism (parasitized larvae/total larvae). Since parasitism is not the only cause of larvae mortality (Liere and Perfecto 2008) we also estimated total larvae mortality, as one minus the proportion of pupae to small larvae (1st, 2nd, and 3rd instar), that is, the proportion of larvae that did not reach the pupal stage.

Effect of ant nest cluster size on ladybeetle abundance and permanence of ladybeetle populations

To determine whether the size of the ant nest clusters had an effect on the abundance and persistence of *A. orbigera* populations, we performed linear regressions between these variables. The size of the ant nest clusters was regarded as the number of nests within a 20m radius of the sampled tree (it thus only included sampled sites with ants) (Vandermeer et al. 2008). The abundance of ladybeetles per cluster size was estimated as the mean number of individuals (adults or larvae) found at each cluster size using the methodology described above; the permanence was estimated as the mean number of times per cluster size (out of the four sampling events) a sampled site was occupied by at least one ladybeetle. Additionally, we did separate regressions for males and females and for larval parasitism and total mortality.

Short-distance effects of ant nests on ladybeetle abundance

To determine the distribution of ladybeetles close to ant nests we sampled adults and larvae in coffee bushes within a 5 m radius of two isolated *A. instabilis* nests (i.e. nests with no neighbor nests within a 20m radius). In both sites, we counted the number of larvae and adults captured in 30 min and annotated the distance from the nest at which each individual was found. Then we grouped individuals based on increasing distance ranges from the nest (0-0.5, 0.6-1, 1.1-1.5, 1.6-2, 2.1-2.5, 2.6-3, 3.5-4, and 4.1-5m), so as to quantify a total number of individuals per distance range. We repeated this procedure three times during the rainy season of 2006. The three sample means were used to

perform linear regressions to determine the relationship between abundance and the distance from the ant nest, for both larvae and adults.

Movement of adult ladybeetles between areas with and without *Azteca* nests

To investigate the movement of *A. orbigera* adults within a relatively small area (50 m^2 plot) that included ant-tended and non-tended plants, we captured, marked, and released adult beetles found in the coffee bushes around every shade tree in the area (total of 80 trees; nine with ants and 71 without ants). In each tree, we searched for 30 minutes and marked, with the point of a toothpick, each captured beetle with Testors enamel paint ® (Medina, Ohio). We used a particular mark to differentiate beetles captured around each shade tree (i.e. all beetles captured in shade tree 'one' had a mark to represent only that tree). We released the beetles immediately after marking them. Five to ten days later we sampled again (with the same methodology) the areas around each tree on the entire plot searching for marked individuals. With these data, we were able to make a qualitative map of A. *orbigera* movement in the 50m^2 plot.

Additionally, since it would have been impossible to track marked ladybeetles over a large area, to determine the large-scale (plot scale) movement of *A. orbigera* adults we did a lab food-deprivation experiment and compared the results to field data to determine adult weights with low and abundant food supplies. In the lab, we used 36 lab-eclosed 1st instar larvae and randomly separated them into two treatment groups. We then reared the larvae to adulthood with either a) an abundant food supply (>30 scales/day) or b) a scarce food supply (<5scales/day). Given that the same treatments occur naturally in nature, with abundant food supplies available to the larvae near *A. instabilis* nests and

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low food supplies everywhere else, we had a total of four treatments: lab low food supply, lab abundant food supply, field low food supply, and field abundant food supply. First, we weighed the recently eclosed adults from the lab to determine how larval food intake affected adult weight. We then captured and weighed seventeen adult beetles in areas with *A. instabilis* and five in areas without the ant. During the experiment, we were unable to find abundant adults in areas without ants and thus had a smaller number of individuals in this treatment. Given that adult weight in ladybeetles greatly depends on the amount and quality of food intake during their larval stage (Hodek and Honek 1996), a difference between adult weights sampled in *A. instabilis* and no *A. instabilis* areas, would mean that beetles stay within the same area where they grew up as larvae. The opposite, that is, no difference between adult weights in sites with *A. instabilis* and without *A. instabilis*, would suggest that the beetles move between these areas. We compared average weights of all treatments with an ANOVA test, with food intake as the independent variable and beetle weight as dependent variable; we then performed a Tukey post-hoc comparison.

Results

In all sampling periods, we found significantly more *A. orbigera* adults and larvae in sites with ants than in sites without ants (Table 4.1), regardless of sex (females: p<0.0001; males p<0.0001). This pattern remained during the dry season when ladybeetle populations were very low (Fig. 4.1). Moreover, during the second dry season sampling we found neither adults nor larvae in sites without ants (Table 4.1). Both parasitism suffered by larvae and total larval mortality were greater in sites without ants, although

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only larval mortality was statistically different (parasitism p=0.15, mortality p=0.03) (Fig. 4.2).

During the first year of survey, abundance of adult ladybeetles had a positive relationship with ant cluster size during the rainy season but not so during the dry season (Fig. 4.3). The second year, we found the same trend but the relationship was weaker and less significant (Fig. 4.3). Thus, we only used the data from the fist year to analyze sexes individually. Only females showed a significantly positive relationship with ant cluster size (R^2= 0.4657, $F_{1,7}$= 6.1, p=0.04; males: R^2= 0.12, $F_{1,7}$=1.01, p=0.34). The abundance of larvae was not significantly related to the size of the clusters of *A. instabilis* nests during either of the two sampling years (Fig. 4.3). The persistence of adult populations, measured as the mean number of times a site was occupied, was positively related to ant cluster size; the same did not hold for larvae (Fig. 4.4). Total larvae mortality significantly increased with ant cluster size while larval parasitism did not (Fig. 4.5).

With regard to short distance effects, the abundance of *A. orbigera* adults had a significantly positive relationship with the distance from the ant nests within a 5m radius, while the larvae did not (Fig 4.6).

In the assessment of movement of *A. orbigera* adults between areas with and without ant nests we found that in the 50x50 m plot, adult beetles regularly moved between nearby areas with and without *A. instabilis* nests (Fig. 4.7).

In the lab, we found that larvae development time was significantly longer in the low-food supply treatment (44 average days from egg eclosion to pupation ± 1.28 SE) compared to the abundant food supply (19 days ± 0.4 SE) (p<0.0001). Furthermore, pupation time was also longer for larvae reared with low-food supply (13.6 days ±0.45)

compared to larvae reared with abundant food (10.2 days ± 0.55) (p<0.001). In the lab low-food supply treatment there was a survival of 61% compared to 100% survival in the abundant food supply treatment.

We found a significant effect of larval diet on adult weight (F_3=20.34, p<0.0001) where the weight of the adults reared in the laboratory with low food supply was significantly lower than the other three groups (post-hoc Tukey p<0.0001), but the other groups where not statistically different from each other (p>0.1) (Fig 4.8).

Discussion

We found that, like other myrmecophilous natural enemies, the abundance, distribution, and persistence of *A. orbigera* is closely related to that of the ants that tend their prey. According to our predictions, *A. orbigera* larvae were significantly more abundant in areas tended by ants and that, at the large scale, the same trend held for adults but these were restricted to the peripheries of strong ant influence. Our results suggest that areas with ants serve as source habitats for the beetle but that areas without ants are also necessary for their survival. Furthermore, our results suggest that the spatial distribution of ant nests might also play a key role for the persistence of this important predatory ladybeetle, as our previous theoretical studies have suggested (Liere et al. in review).

We found that areas with *A. instabilis* sustained larger larval populations than areas without ants. This result is not surprising given that due to their limited dispersal capabilities, ladybeetle larvae face high risk of starvation in areas with low prey densities (Seagraves 2009). Additionally, as our results showed, the deprivation of food increases

their developmental time, likely lengthening the period when they are highly vulnerable to predators and parasitoids. Thus, the relative abundance of accessible prey in areas with *A. instabilis* (Vandermeer and Perfecto 2006) and the release from natural enemies provided by ant patrolling (Liere and Perfecto 2008) makes these places high-quality habitats for *A. orbigera* larvae. Moreover, the lack of weight differences between *A. orbigera* adults collected in the field in sites without *A. instabilis*, with *A. instabilis*, and with abundant food supply in the laboratory, suggests that *A. orbigera* adults in areas without ants actually grew up as larvae in sites with abundant food supply, that is, in high-quality ant-patrolled habitats. Our results suggest that ant-tended areas are source habitats for *A. orbigera* that later disperse as adults throughout the broader areas.

Azya orbigera larvae, however, were not completely restricted to ant-tended areas. Adult ladybeetles have a high predisposition to disperse even from high-quality habitats (Ives 1981; Frazer 1988), partly because they are known to be deterred from ovipositing in areas with abundant conspecific larvae to avoid intraspecific competition and cannibalism (Doumbia et al. 1998; Merlin et al. 1996; Hemptine et al. 2000; Oliver et al. 2006). Furthermore, given their inability to feed in ant-tended scale colonies, they are likely forced to move around the farm, probably looking for the sparsely distributed non-tended scale colonies. Consequently, even though female ladybeetles preferentially oviposit in areas with abundant food supply (Dixon 2000), if unable to find an another area with A. instabilis nest, some *A. orbigera* females might be forced to lay their eggs in low-quality areas. In fact, as females get older or when they have experienced low-quality habitats for a time, they become less selective in their oviposition preferences

(Frechette et al. 2004). This could explain why, despite the high mortality risk, we did

find, albeit in very low densities, *A. orbigera* larvae in areas not tended by ants.

Contrary to larvae, *A. orbigera* adults do not have access to ant-tended prey.

Thus, the risk of ant attacks restricts them to the peripheries of the two to three meter

radius area that ants patrol around their nests. Moreover, due to the low abundance of *C.*

viridis in coffee plants without *A. instabilis* protection, to acquire their necessary nutrition

requirements, *A. orbigera* adults probably have to constantly move to find prey. Adult

ladybeetles are able to survive on alternative food (nectar, pollen, or alternative prey) in

times of food scarcity (Hodek and Honek 1996); however, they do need to feed on

suitable or 'essential' prey in order to reproduce (Hodek 1960; Triltsch 1999; Hodek and

Honek 2009). Indeed, our results show that *A. orbigera* adults move short and long

distances among areas with and without *A. instabilis* nests. Given that ladybeetles can be

very efficient in detecting individual prey (Hattingh and Samways 1992) , *A. orbigera*

adults may easily find the sparsely distributed green scales in the matrix of coffee bushes

not tended by ants. It thus seems to be a situation in which areas under *A. instabilis*

influence are necessary habitats for the larvae of *A. orbigera* and areas without *A.*

instabilis are necessary for the adults. Consequently, population control of green scales at

the plot level probably depends on the constant beetle predation pressure that begins as

larvae in areas with *A. instabilis* and continues as adults in the no-ant matrix.

An inherent contradiction to the above hypothesis, however, arises in that if ant

harassment deters *A. orbigera* adults from preying upon scales, it most likely also deters

them from ovipositing in ant patrolled areas. How do these adults then manage to

oviposit inside ant-tended areas without being attacked or killed? The answer is that they

manage to do so only because of another natural enemy, in this case, a parasitoid fly that

attacks *A. instabilis*. The mere presence of this phorid fly, *Pseudacteon* spp. (Diptera:

Phoridae) drastically reduces ant activity for at least two hours (Philpott et al. 2004;

Philpott 2005; Liere and Larsen 2010) allowing adult beetles to prey upon ant-tended

scales (Liere and Larsen 2010). Phorid presence may thus provide the windows of

opportunity that *A. orbigera* females need to oviposit in high-quality areas, where their

larvae will have abundant food and reduced mortality due to natural enemies. In fact, a

previous study on the same system suggest that *A. orbigera* females are attracted by

alarm pheromones released by *A. instabilis* to indicate that phorids are present (Hsieh et

al. in review). There are many studies that report that adult ladybeetles might respond to

sensory cues and that these cues influence their choice of a particular habitat (for review

see Seagraves 2009). It is thus possible that *A. orbigera* females might be able to locate a

particular ant nests based on chemical cues that indicate high density of phorids in the

area.

The positive relationship between *A. orbigera* females with the size of *A.

instabilis* nest clusters is thus probably also explained by the influence of phorid flies.

Previous studies have shown that phorid density and attack rate is greater in larger

clusters of *A. instabilis* nests (Vandermeer et al 2008). Thus, larger concentrations of ant

nests may provide more frequent periods during which ant activity is sufficiently low to

allow *A. orbigera* females to oviposit and, accordingly, result in a positive density

dependent relationship. As suggested by our short-distance surveys, adults tend to

aggregate in the peripheries of ant influence, probably eating on non-patrolled plants but

waiting for opportunities to oviposit on patrolled ones. Thus, even though *A. orbigera*

adults do not have the morphological traits that larvae have to overcome ant attacks, they relay on behavioral mechanisms to gain enough access so that their larvae are able to exploit high quality ant tended habitats.

Our results show that while *A. orbigera* adults are more abundant and permanent in larger ant clusters, the same did not hold for larvae. These counterintuitive results may be explained again by greater phorid presence in larger ant clusters. Just as *A. orbigera* adults take advantage of phorid-induced low ant activity periods to oviposit, the predators and parasitoids that normally attack *A. orbigera* larvae may also take advantage of greater phorid presence in large ant clusters. *Azya orbigera* larvae in these areas might thus only increase in abundance until phorids break down ant protection leaving larvae vulnerable to their natural enemies for relatively extended periods of time. It is worth noting that our results suggest that the parasitoids of *A. orbigera* larvae do not appear to be able to take advantage of low ant-activity periods. This might be attributed to a long oviposition process or a reluctance to approach plants with ant chemical signals; more detailed studies, however, are needed to confirm this. Nevertheless, given that parasitism was only a small fraction of total larvae mortality, which significantly increased with ant cluster size, higher oviposition rate of female beetles in larger ant clusters was not reflected in higher larvae abundance.

The great advantage of living in close association with tending ants is reflected by the variety of mechanisms that numerous species of natural enemies of ant-tended prey use to deter ant aggression (Majerus et al. 2007). For example, *Platynaspis luteorubra* larvae are chemically and morphologically camouflaged such that they are treated by ants as one of their hemipteran mutualists (Völkl 1995). The ant social parasites *Maculinea*

spp (Schönrogge et al. 2004; Everett et al. 2005), the predatory caterpillar *Feniseca tarquinius*, the lacewing *Chrysopa slossonae*, and the syrphid fly *Syrphus ribesii* chemically mimic the cuticular hydrocarbons of their prey to deter ant attacks (Lohman et al. 2006) while the larvae and pupae of the ladybeetle *Thalassa saginata* mimic the cuticular lipids of the tending ant's brood to be able to live inside the ant nests (Orivel et al. 2004). An interesting example that involves a behavioral mechanisms is the case of the lacewing, *Chrysopa slossonae*, which 'wears' the wooly filaments of their aphid prey and is thus camouflaged from tending ants (Eisner et al. 1978). Thus *A. orbigera*, along with a selected group myrmecophilous predators and parasitoids, have access during one or all their life stages, to high quality ant-tended areas with abundant and persistent food and relative freedom from interspecific competition and natural enemies. Their spatial distribution is, as our results suggest, accordingly affected by the ant-hemipteran mutualism.

Our results suggest that this important predator is not only dependant on the existence of habitats with and without ants, but in the delicate balance between large ant clusters for greater oviposition opportunities, and small clusters, for greater larvae survival. Since the ant nests in the studied plot have a very specific spatial distribution and this distribution is autonomously created by local interactions among the members of the community (Vandermeer et al. 2008; Jackson et al. 2009; Liere et al. in review), the question of whether this specific spatial pattern is essential for beetle population persistence is a very interesting one, from both an empirical and a theoretical point of views.

From an empirical point of view, the persistence of important natural enemies in agricultural systems is key for the natural control of potential pests. Furthermore, studying the details of arthropod food-webs in agricultural systems and consequent spatial distributions is particularly important since in order to keep herbivores below damaging levels, predators have to have the ability to aggregate in high density patches (Murdoch et al 1985; Murdoch and Briggs 1996), as is the case with *A. orbigera*. Furthermore, our results challenge the apparently straightforward implication that ants have a damaging effect to the plantation by protecting potentially harmful hemipteran pests from their natural enemies (Hanks and Sadof 1990; Reimer et al. 1993). In fact, our results suggest that ants and the habitat heterogeneity they help create are necessary for the persistence of the important predatory ladybeetle and thus for the population control of green scales in the farm. From a theoretical point of view, the study of the effects of autonomously created habitat heterogeneity and spatial patterns could shed light on the mechanisms of how biodiversity enhances persistence and stability in biological communities.

Tables

Table 4.1. Ladybeelte (*Azya orbigera*) abundance (mean individuals per site +- SE) in sites with ants (*Azteca instabilis* nests) and without ants in a 45-ha plot in a coffee farm in Chiapas, Mexico.

	Season	With ants	Without ants	P
Adults	Summer 2006	5.76 (0.99)	1.26 (0.21)	<0.000001
	Winter 2007	2.24 (0.9)	0.032 (0.022)	<0.000001
	Summer 2007	5.57 (1.25)	0.26 (0.08)	<0.000001
	Winter 2008	0.77 (0.26)	0	
Larvae	Summer 2006	3.8 (0.82)	1.58 (0.32)	0.005
	Winter 2007	1.47 (1.06)	0.016 (0.02)	0.0006
	Summer 2007	0.46 (0.19)	0.016 (0.015)	0.001
	Winter 2008	0.32 (0.16)	0	

Figures

Fig. 4.1. Map of the spatial distribution of *Azya orbigera* with respect to *Azteca instabilis* nests in a 45-ha permanent plot. The size of the circles is proportional to the abundance of beetles found in each site. Dark circles represent populations sampled in sites with ants and pale circles represent populations sampled in sites without ants. Dark points represent *A. instabilis* nests. The surveys were conducted during a) the rainy season 2006 (May-June) and b) the dry season 207 (January-February)
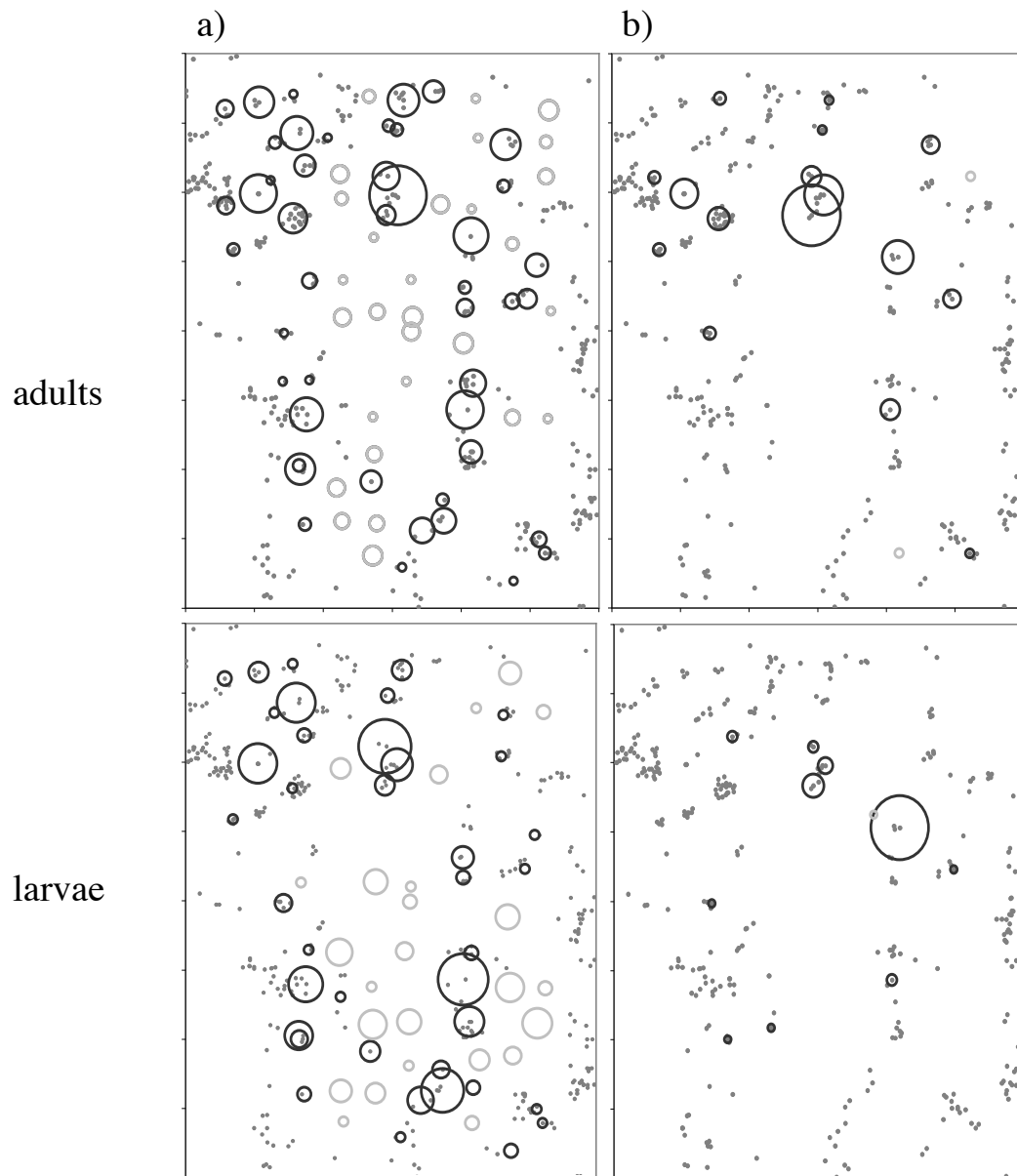
Fig. 4.2. Mortality and parasitism suffered by *Azya orbigera* larvae in sites with and without *Azteca instabilis*. The y-axes is the proportion of larvae that were either parasitized or suffered mortality from unknown causes. * denotes statistically significant differences at the 0.05 level. Error bars represent one SE from the mean.
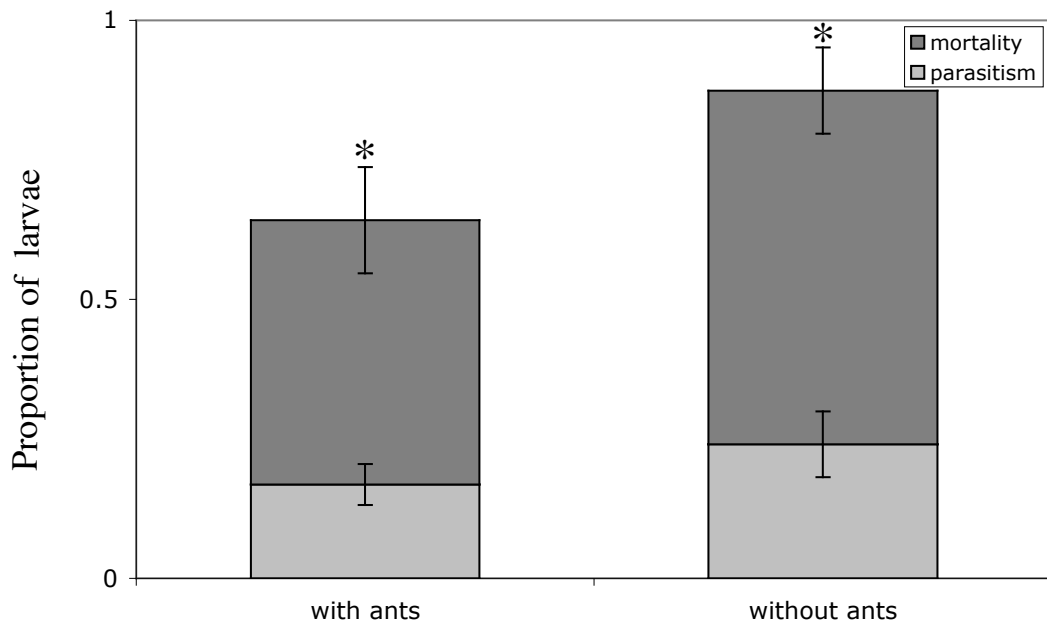
Fig. 4.3. Mean beetle abundance vs. ant nest clump size. Dark circles represent adult beetles, and open circles represent larvae beetles. Clump size was estimated as the number of ant nests within a 20m radius of the sampled tree. Error bars represent one SE. a) Rainy season 2006, adults R^2= 0.66, $F_{1,7}$=13.87 p=0.0074; larvae R^2= 0.093, $F_{1,7}$= 0.71, p= 0.37 b) Rainy season 2007 adult R^2=0.36, $F_{1,8}$= 4.46, p=0.06; larvae R^2=0.12, $F_{1,8}$= 1.06, p=0.33   c) Dry season 2007 adult R^2= 0.05, $F_{1,9}$=0.5, p= 0.49; larvae R^2= 0.089, $F_{1,9}$=0.88, p=0.37 d) Dry season 2008 adult R^2= 0.15, $F_{1,9}$=1.62, p= 0.23; larvae R^2=0.2, $F_{1,9}$=2.25, p=0.17
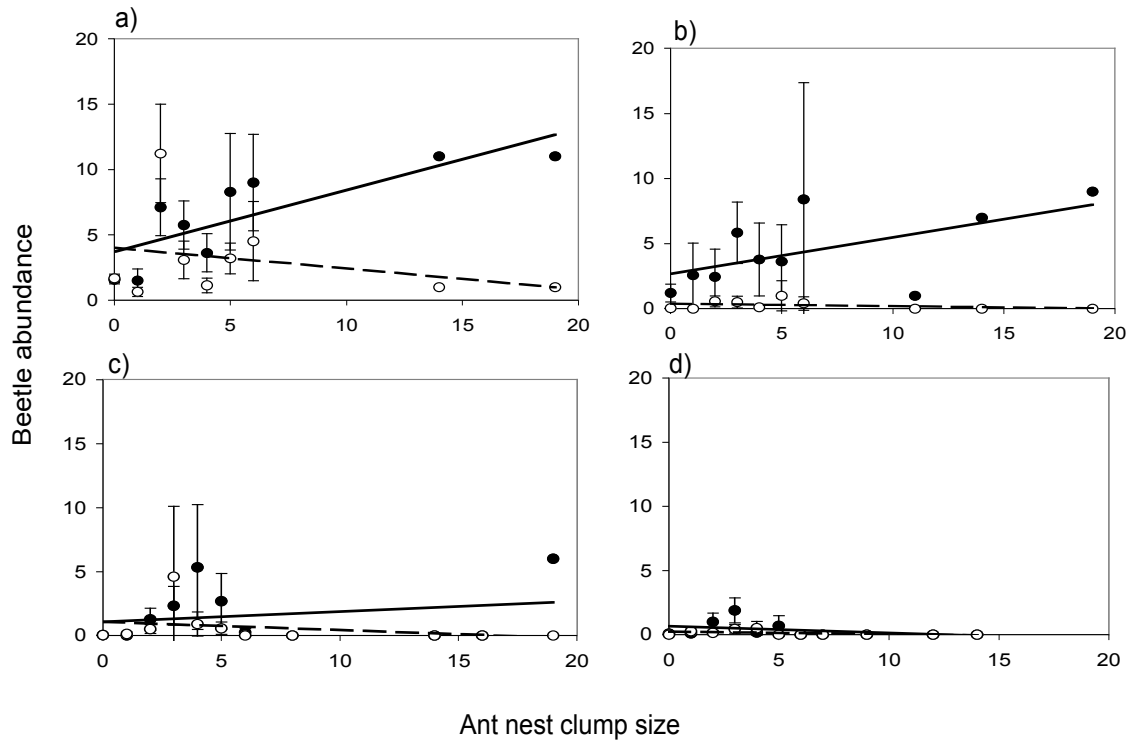
Fig 4.4. *Azya orbigera* mean population persistence vs. size of *Azteca instabilis* nest clump. Dark circles represent adult beetles ($R^2=0.58$, $F_{1,7}=9.76$, p=0.016) and open circles represent larvae beetles ($R^2=0.032$, $F_{1,7}=0.23$, p=0.65). Clump size was estimated as the number of ant nests within a 20m radius of the sampled tree. Persistence was estimated as the proportion of times the beetle population was > 0. Error bars represent one SE.
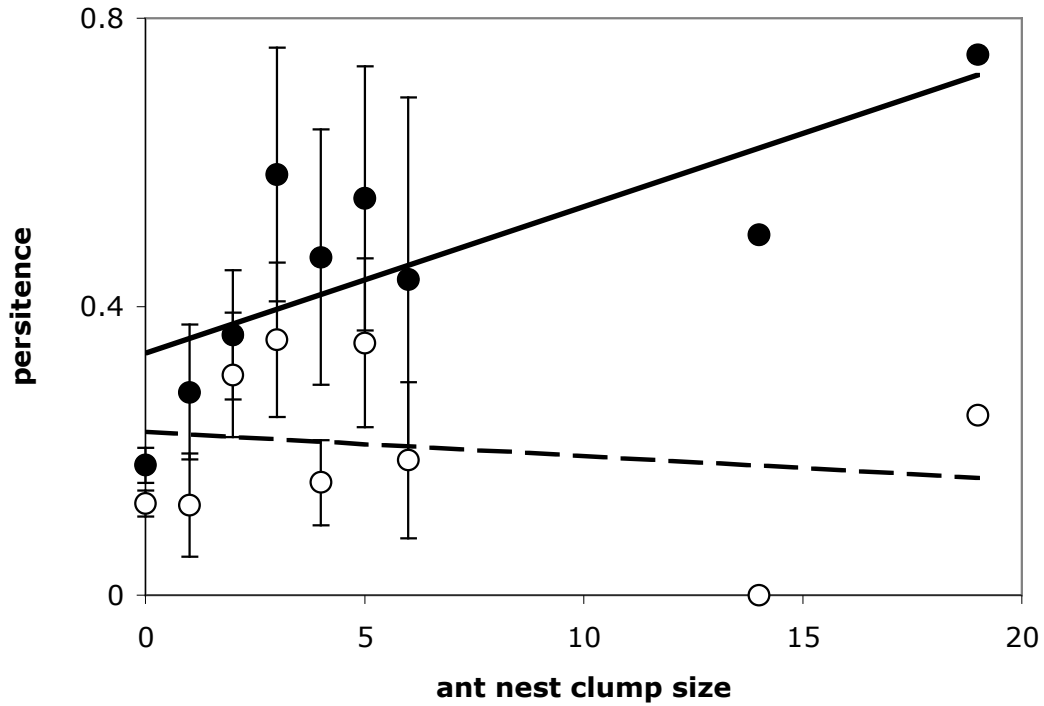
Fig. 4.5. Mean percentage of *Azya orbigera* larvae mortality (dark circles) and parasitism (pale circles) vs. *Azteca instabilis* nest clump size. Clump size was estimated as the number of ant nests within a 20m radius of the sampled tree. Larvae mortality $R^2 = 0.55$, $F_{1.7} = 7.46$, p =0.03; larvae parasitism $R^2 = 0.17$, , $F_{1.7} = 1.25$, p=0.3.
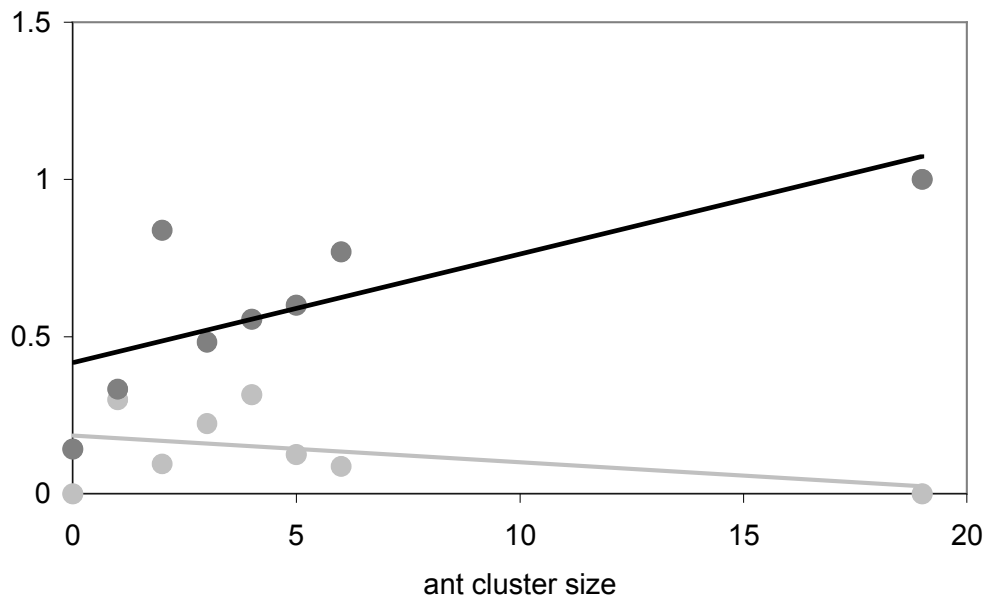
Fig. 4.6. Short distance effects of ant nest on *Azya orbigera* distribution. Dark circles represent adult beetles, and open circles represent larvae beetles. Y axis represents the mean individuals sampled during three different sampling dates. Error bars are one SE from the mean. Larvae $R^2= 1.37$, $F_{1,7}=1.12$, p=0.32; Adult $R^2= 0.56$, $F_{1,7}=9.01$, p=0.019.

mean individuals

distance from nest (m)
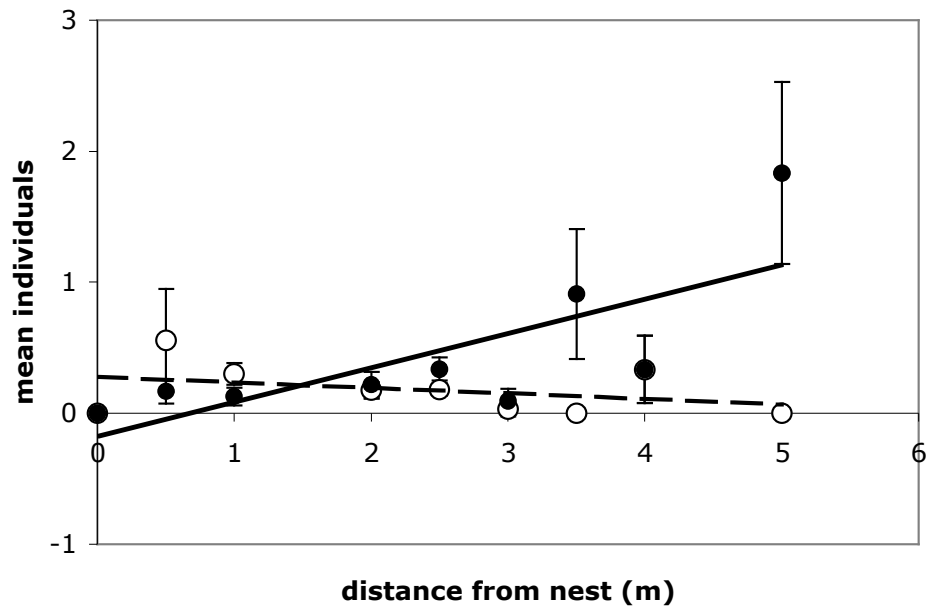
Fig. 4.7. *Azya orbigera* adult movement. Recorded (with mark and recapture events) movements of *Azya orbigera* adults between shade trees with and without *Azteca instabilis* nests in a 50 X 50 m plot. Black points represent shade trees, dark circles represent shade trees with ant nests and pale circles represent shade trees without ant nests. Size of the circles is proportional to the abundance of adults captured in each tree. Arrows represent the direction of the movement.

50 m

50 m
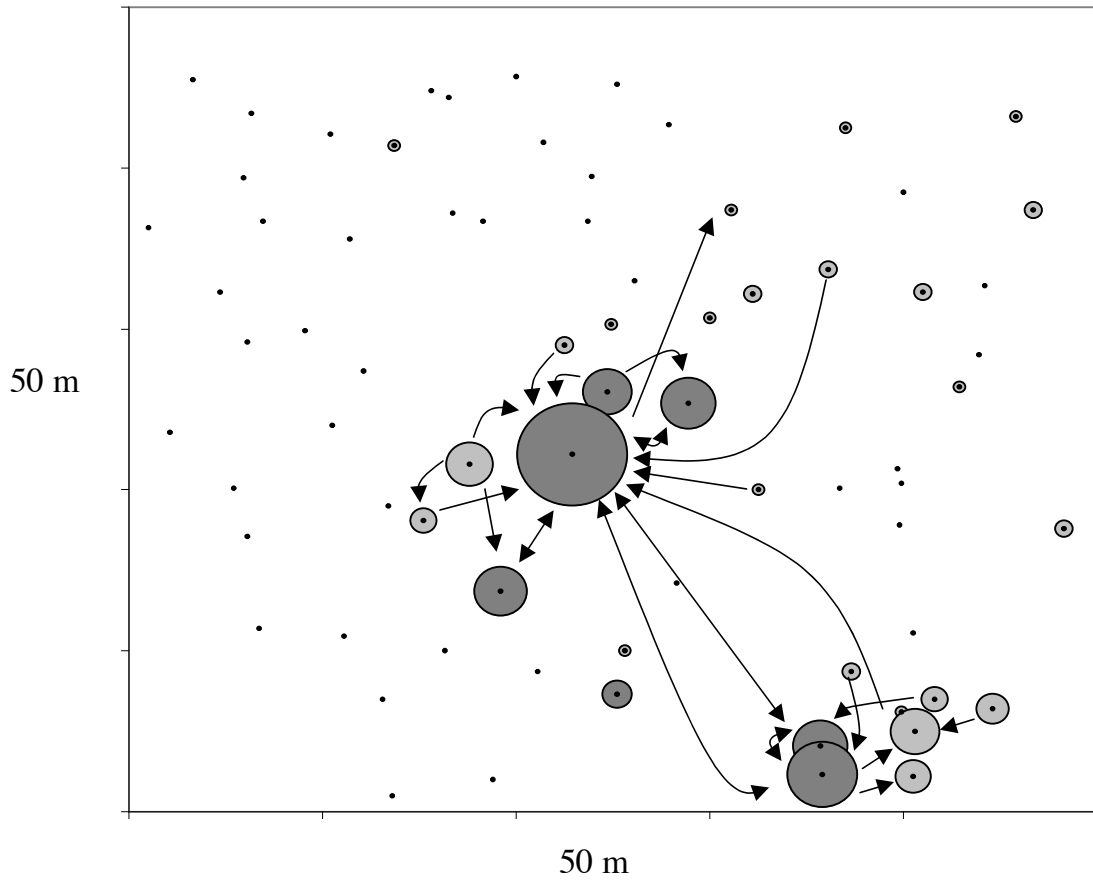
Fig. 4.8. Adult weight experiment. Mean *Azya orbigera* adult weight reared as larvae with low (<5 scales per day) or abundant (>30 scales per day) in the laboratory and captured in low food supply areas (scale colonies not tended by *Azteca instabilis*) and abundant food supply (scale colonies tended by *A. instabilis*). Different letters represent statistically significant differences at the 0.05 level in a post-hoc Tukey multiple comparison test.
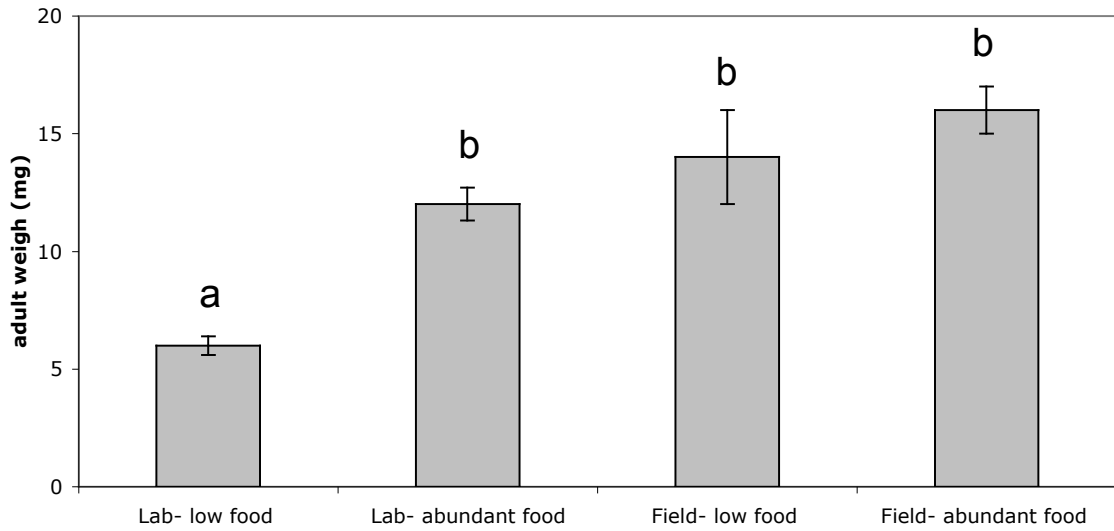
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Chapter V

Population Regulation From Spatial Heterogeneity: An Example of Autonomous Biological Control Through Ecological Complexity

Abstract

Spatial heterogeneity is essential for the persistence of many inherently unstable systems such as predator-prey and parasitoid-host interactions. Since biological interactions themselves can create heterogeneity in space, the heterogeneity necessary for the persistence of an unstable system could be the result of local interactions involving elements of the unstable system itself. Here we report on a predatory ladybird beetle whose natural history suggests that the beetle requires the patchy distribution of the mutualism between its prey, the green coffee scale, and the arboreal ant, *Azteca instabilis*. Based on known ecological interactions and the natural history of the system, we constructed a spatially explicit model and showed that the spatial heterogeneity of ant nests is indeed key for the persistence of the beetle populations. Furthermore, we show that the dynamics of the beetle consuming scale insects can cause the patchy distribution of the mutualistic ants in the first place. It is a unique situation in which a predator indirectly causes a spatial pattern of an organism other than its prey and, simultaneously, the persistence of the predator is dependent on this same spatial pattern.

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Introduction

It is familiar knowledge that local interactions can sometimes create complex and surprising self-organized spatial patterns (Hassell et al. 1991; Rohani et al. 1994; 1997). Much attention has been given to the subject and complex spatial patterns from spots in animal coats to animal distribution patterns which have been shown to arise from simple local dispersion and interactions. For example, the distribution of species in simple predator-prey and parasitoid-host deterministic models in homogeneous environments can form spiral waves, clustered distributions, crystal lattices, and chaotic patterns (Hassell et al. 1991; Comins et al. 1992; Rohani et al. 1994; Rohani and Miramontes 1995). In a separate literature, it has often been noted that spatial heterogeneity can allow coexistence of predator-prey and parasitoid-host associations that are otherwise prone to local extinctions (Nicholson 1933; Noy-Meir 1975; Tilman and Kareiva 1997; Dieckmann et al. 2000; Gurney and Veitch 2000; Murdoch et al. 2003). In the present communication, stimulated by the natural history of a complex insect community in a coffee agroecosystem, we propose that the spatial heterogeneity needed for the persistence of a system might be caused by the dynamics of that very system.

Vandermeer, Perfecto and Philpott (Vandermeer et al. 2008) (hereafter, VPP) recently investigated the spatial pattern of a tropical arboreal ant species in a coffee agroecosystem. This tree-nesting ant occupies about 3% of shade trees in a 45-ha plot and form a clustered distribution in the farm. They defined two nests as belonging to the same cluster if they were less than 20 m apart and attributed the clustered spatial pattern to positive density-dependent spread of nests from tree to tree balanced by a negative

density-dependent control from a natural enemy. Using a stochastic cellular automata,

VPP closely approximated the actual spatial pattern with this hypothesized dynamic, and

attributed the negative density-dependence to the tendency of a phorid parasitoid fly to

concentrate on bigger clusters of ant colonies. The resulting 'self-organized' spatial

pattern creates significant habitat variability and thus a potential influential force for any

organism associated with the species of ant in question, *Azteca instabilis*.

The coccidophagous ladybeetle, *Azya orbigera*, is an example of an organism that

might be strongly influenced by the spatial pattern of *A. instabilis*. This predatory

ladybeetle is a voracious predator of the green scale, *Coccus viridis*, an important coffee

pest. *C. viridis* maintains a mutualistic association with *A. instabilis* and is thus only

highly abundant on coffee plants in close proximity (2-3m) to ant nests (Vandermeer and

Perfecto 2006). For *A. orbigera* this mutualistic association has two contradictory effects.

On the one hand, adult ladybeetles are normally not permitted to eat in ant-tended areas

due to the aggressive action of the ants (Liere and Larsen 2010). On the other hand, given

that larva ladybeetles are covered by waxy secretions that deter ant attacks, they are able

to live and prey in ant-tended plants (Liere and Perfecto 2008, Liere et al. in review).

Furthermore, due to the ants' generalized harassing behavior, natural enemies (predators

and parasitoids) that normally attack the ladybeetle larvae, are prevented from doing so

(see video in the Online Resource 1). Thus, in areas with ants, larvae beetles have

abundant food and enemy-free space, while adults are almost completely prevented from

eating. Contrastingly, in areas without ants patrolling, the poor-dispersing ladybeetle

larvae are under considerable risk of starvation and pressure from their natural enemies,

while adult ladybeetles are able to fly from plant to plant searching for sparsely non-

tended scales. Indeed, field surveys in the area show that both beetle adults and larvae are significantly more abundant in areas with ants but that due to ant harassment adults are forced to disperse to areas without ants to feed (Liere et al. in review). Given this natural history, we suspected that the beetle population must live in a spatially heterogeneous environment with respect to the *A. instabilis* distribution in such a way to provide adequate habitat for both larval and adult stages: the larvae requiring ant habitats to survive and the adults requiring ant-free space.

Furthermore, we suspected that *A. orbigera* itself could contribute to the spatial pattern of the ant nests in the first place. VPP showed that the patchy spatial structure of ants can be attributed to the expansion of ant nest satellites controlled by a negative density-dependence force, hypothesized to be a result of a parasitoid phorid fly acting on the ant. However, the structure of their model included only a generalized density dependent mortality term, which could be due to the phorid, as they suggest, but also could be due to other density-dependent forces. In particular, given that similarly to many honeydew-collecting ants (Way 1963), *A. instabilis* may greatly depend on the energy source provided by its mutualist partners to survive and any organism that reduces the population of scale insects could potentially increase the mortality of ant nests. Additionally, if this control force increased the likelihood of ant nest mortality in a density-dependent fashion (in other words, if this force had a tendency to be stronger in larger clusters of ant nests), it could also play a role in the cluster-forming spatial dynamics of ant nests. A previous study in the area showed that drastic scale mortality events caused by an entomopathogenic fungi caused shifts in the foraging area of *A. instabilis* (Jackson et al. 2009). Additionally, since *A. orbigera* does indeed have a

density-dependent response to ant nest clusters (Liere et al. in review), we propose that this predatory beetle can be responsible for the formation of ant clusters.

Thus, our goal was to determine whether it was possible for a spatial pattern in ant nests to emerge from the natural history of the system and, at the same time, that this pattern is essential for the survival of a species that plays a key role in generating that pattern. Specifically, we used the model to address the following questions: 1) Starting from random distributions, can the beetle/scale insect dynamics generate a clustered spatial pattern of ant nests similar to that seen in nature, and, if so, do the field data support the beetle model more closely than the phorid model proposed by VPP? 2) a. Does the existence of areas with and without ants generated by this spatial pattern play a key role in the persistence of the beetle populations? and b. Are there conditions under which elimination of the spatial pattern will cause the extinction of the beetle population and consequently an outbreak of the scale insect?

Methods

To answer these questions, we constructed a spatially explicit model as a Cellular Automata (CA) to simulate the presence and absence of ant nests. The lattice consisted of 120 cells X 90 cells and represents a 800 m X 600 m (45 ha) study plot from the field. We then coupled the CA model with three Coupled Map Lattices to simulate the scale insects and the ladybeetle populations in the lattice.

We started by randomly scattering ant nests, scale insects, and beetle larvae and adults over the lattice, with some of the parameters delimited by the natural history of the system. We then used a genetic algorithm (GA) to search for values for the remaining

parameters (see below). Then we qualitatively compared the spatial distribution of the ant

nests resulting from our model with that found in the field. Additionally, we compared

the frequency distribution of ant nest clump sizes to that obtained by the VPP model.

To answer the second question, we simulated three different scenarios. In the first

one, we ran the model with the fitted parameters that resulted in clustered ant nest

distributions. For the second and third scenarios, we fixed the ant nest mortality and

expansion parameters (see below) so that a) the ants go extinct and b) the ants cover all

the lattice respectively. We then followed the scale and beetle populations over time to

determine their persistence in the three scenarios.

State variables and scales

The research that led to our understanding of the natural history of the system

took place in a traditional shaded coffee plantation in southern Chiapas, Mexico. Our

model is intended to capture the essence of the biological interactions that create spatial

structure and allow a beetle population to persist in this coffee agroecosystem without

including any unnecessary details (for details on the model code see Appendix 1).

Therefore, our emphasis was on simplicity and not on all of the known aspects of the

natural history of the system.

As with the model in VPP paper, our model uses a 120 cell X 90 cell grid with

periodic boundaries to represent a 800 m X 600 m (45 ha) study plot. Each of the four

populations— ants, scale insects, beetle larvae, and beetle adults — is represented using a

separate 120 cell X 90 cell grid. Each cell in these grids represents a shade tree (where

the ants nest) and its neighboring coffee bushes (where the scale insects live and the beetles forage).

Since we were interested in the effects of ant presence and absence, the ant nest grid in our model contains only zeros and ones representing the presence or absence of ant nests, respectively. By contrast, each cell in the scale insect grid is either empty or with a number representing the population size of the scale insect associated at each time-step; likewise for the beetle larvae and beetle adult.

Process overview and scheduling

The model operates in discrete time and uses synchronous updating. Each time step corresponds to a six-month period, which matches the frequency of field surveys (Vandermeer et al. 2008). In accordance with the natural history of the system this formulation assumes that larvae either eclose or die in one time step, and all adult beetles live only a single time step.

Model parameters: We employed a genetic algorithm implemented using the Java package JGAP (Java Genetic Algorithms Package) to search for parameter values fulfilling certain performance criteria (Goldberg 1989). The performance (fitness) of parameter value combinations was calculated based on the following criteria: number of ant nests, complete extinction of beetles when ants are fixed to occupy 100% of the lattice points, complete extinction of beetles when ants are fixed to occupy 0% of the lattice points, numbers of beetle adults and larvae in locations with ant nests, and numbers of beetle adults and larvae in locations without ant nests (for details, see Appendix 2). The target values for the numbers of ant nests, adults, and larvae were

obtained from field data. Certain parameter values were constrained based on the known biology of the system, e.g., the survivorship of beetle larvae in locations without ants was defined to be less than the survivorship with ants.

Interactions. Interspecific interactions in this model include: 1) the predator-prey relationship between beetle larvae and scale insects; 2) the predator-prey relationship between beetle adults and scale insects; 3) the mutualism between ants and scale insects, which is incorporated, on the one hand, in the increased ant nest mortality with decreased scale insect density and, on the other, in the increased scale insect intrinsic growth rate and the reduced predation pressure suffered by scale insects in the presence of ants; 4) the inadvertent protection of beetle larvae by ants, which is incorporated in the ant nest-dependent survivorship rates of the larval beetles; and 5) the inhibitory effect of ants on beetle adults, which is incorporated in the ant nest-dependent consumption and survivorship rates of the adult beetles. There are also conspecific interactions implicit in 1) the logistic growth rate of the scale insects, 2) the density-dependent expansion of ant nests, and 3) the density-dependent migration rates of scales and beetle adults.

Model description

The model starts with randomly scattering ant nests (ones and zeros), scales and beetles over the lattice (for details see Appendix 1).

1) *Beetle population growth*. For each iteration of the model, we first allow the beetles to reproduce and eat scales according to the cell type (with or without ants) inspired by the first term of a typical Rosenzeig-MacArthur model:

$$A_{t+1} = e\left(\frac{c_L N_t L_t}{1 + g N_t}\right)$$

$$L_{t+1} = b\left(\frac{c_{Ai}N_t A_t}{1 + gN_t}\right)$$

Where each time step or generation time coincides with the six month sampling of ant nests we have from the field. A_t and A_{t+1} represent the beetle adult population at time t and t+1 respectively; L_t and L_{t+1} represent the beetle larvae population at time t and t+1 respectively; and N_t is the population of scale insects at time t. Where e represents the eclosion rate (from larvae to adults); c_L is the consumption rate of larvae; g is the functional response term for both larvae and adults; b is the beetle birth rate; c_A is the consumption rate of adults and according to the natural history of the system, adult consumption rate of scales is smaller in patches with ants ($c_{A(A)}$) than in patches without ants ($c_{A(noA)}$) (Liere and Larsen 2010). Contrastingly, larval consumption rate of scales (c_L) is not affected by the presence of ants (Liere and Perfecto 2008). Thus in the first step of our model, the population of beetle adults in time t+1 depends on the larvae to adult eclosion rate (*e*) and on the consumption rate of the larvae at time t. The larvae population at time t+1 depends on the birth rate (*b*) and on the consumption rate of adult beetles at time t.

2) *Ant nest mortality, nest satellite expansion (colonization of cells in the Moore neighborhood) and long distance migration (propagule rain).* These are all modeled probabilistically following the VPP model with the exception of the mortality effect. In particular, satellite expansion probability increases linearly with the local (Moore neighborhood) number of nests. Here we formulated the probability of mortality of an ant nest as a decreasing linear function of the local population density of the scale insect.

Thus if a cell population of scale insects in a given cell is low, the probability of mortality is higher than for a cell where the populations of scale insects are high.

3) *Beetle and scale insect local and long distance migration*. We allow a random proportion of the local scale and adult beetle population in each cell to stochastically migrate both locally to the Moore neighborhood and globally as a propagule rain. For local migration, a proportion of the adult beetle population in each cell was allowed to migrate to the neighboring cells that had stochastically been chosen to receive migrants (so that zero to eight cells could receive migrants). For global migration, the likelihood that each cell received migrants was also stochastically determined. Then a proportion of individuals from all occupied cells sent migrants to the chosen cells.

4) *Scale local dynamics*. Scale population size is determined by equations inspired by a Rosenzweig-MacArthur model (with K=1):

$$N_{t+1} = r_i N_{t+1}\left(1 - N_{t+1}\right) - \left(\frac{c_{Ai} A_{t+1} N_{t+1}}{1 + g N_{t+1}}\right) - \left(\frac{c_L L_{t+1} N_{t+1}}{1 + g N_{t+1}}\right)$$

Where $r_{(i)}$ is the intrinsic rate of increase of scale insects, and which was slightly lower in cells without ants as suggested by field data (Jha et al. unpublished ms). Thus, scale insects in our model have a logistic growth and experience death rates that depend on the predation terms already described for the beetle adult and larvae.

5) *Beetle differential survival:*

Adult mortality:

$A_{t+1} = A_{t+1} * s_{A(i)}$

Larvae mortality:

$$L_{t+1} = L_{t+1} \; {}^*s_{L(i)}$$

Given the natural history of the system, the proportion of surviving adults in cells

with ants $(s_{a(A)})$ is smaller than the proportion of surviving adults in cells without ants

$(s_{a(noA)})$; while the opposite is true for the proportion of surviving larvae $(s_{L(i)})$.

After this, the number of ant nests and the population size of scales and beetles was

calculated and the spatial distribution was plotted.

Comparing the model and field data

To compare the model output to the field data, we examined two indicators of spatial

pattern: first, the approximation to a power law distribution of cluster sizes (as in VPP)

and second, clustering of ant nests as measured by Ripley's K (Haase 1995). To

calculate Ripley's K, the number of other nests in the neighborhood of each nest is

compared to the number expected for a random (Poisson) distribution. The neighborhood

is defined by a sampling circle with a specific radius. To determine the degree of spatial

clustering at different spatial scales, Ripley's K is calculated for a range of sampling

circles. Deviations from the random expectation indicate that the spatial pattern is either

more clustered or more uniform than random, depending on the direction of the deviation

(Haase 1995).

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Results

</div>

Starting from a random distribution of ants, scale insects, and beetles, and using the

parameters found with the GA (see Appendix 2, SM Table 1), the clustering of nests that

emerges from our model is similar to that observed in the field (Fig. 5.1). Thus, casting the beetle population as an indirect density-dependent negative force, indeed produces qualitative patterns that are similar to those observed in the field and reported by VPP. Like the VPP model, our model also produces a distribution similar to the field data, with a close fit to a power function (Fig. 5.2).

The profile of Ripley's K-function, transformed such that the expectation for all spatial scales is zero for a random spatial pattern and greater than zero for a clustered pattern (Goreaud and Pélissier 1999) (Fig. 5.3) suggests that the field data might be better explained by the VPP model than by the beetle model at short spatial scales, and the other way around for large spatial scales. Thus, when the sampling circle is between 0 and approximately 25 m, the data closely approximate the pattern from the phorid model while between 25 and 75 m the beetle model gives a better fit. Above 75 m, the overlap of the two models does not allow to statistically distinguish them. There is, however, a general tendency to follow the mean of the beetle model more than that of the phorid model.

Regarding the persistence of the beetle population, the dynamics of the four relevant populations are shown as time-series in Fig. 5.4. As expected from the natural history of the beetle, and with the parameter set displayed in the Appendix Supplementary Material 4 (SM Table 2), simulations on a 90 x 120 lattice reveal that 1) if the ants are dynamically maintained within the system, forming a spatial pattern effectively caused by the beetles, the beetles persist indefinitely, 2) if the ant mortality parameters are set such that the ants occupy 100% of the lattice points, the beetle population goes extinct, 3) if the ant mortality parameters are set such that the ants

completely disappear from the system, the beetle population goes extinct (Fig. 5.4). Note that in both cases when the beetles go extinct, the scale insects almost double their densities after being released from their predators' control.

Discussion

Our model provides a conceptual representation of a subset of the key interactions in the system, and a possible explanation for both the emergence of spatial clustering and the persistence of the interacting species in the field. Just as VPP showed that with very simple local interactions a clustered pattern of ant nests similar to the one in the field could be generated, we showed that with an implicit density dependent control force (i.e. the beetle eating scales) a qualitatively similar pattern of ant nests can form for a feasible range of parameter values. Specifically, our model simulations show that starting with all the populations randomly distributed, ant satellite expansion coupled with predation pressure by the beetle on scale insects can form a clustered distribution of ant nests. At the same time, the existence of areas with and without ants created by this emergent spatial pattern was key to the persistence of the predatory beetle itself. Thus, we propose that through a complex network of interactions the beetles might be helping to create ant clusters that, in turn, provide the habitat heterogeneity necessary for their own survival.

Since our model was intended to produce a qualitative results, the details of the parameters are not that important and therefore, a full sensitivity analysis was not performed. However, the speed at which the multiple GAs found parameter values that converged to an acceptable performance of our model, suggests that the model output is relatively robust to changes in parameter values (for details see Appendix 2, SM Fig. 2).

In our model, the beetle acting as an indirect cause of ant nest mortality is the control force that counteracts the expansion of ant nests by satellite nest formation, and contributes to the formation of the clustered spatial distribution of ant nests. Thus, our model can qualitatively generate the cluster formation of ant nests in nature (Fig. 5.5b): The scale populations in areas with ant nests can increase to relatively high values compared to areas with no ants, probably because of the combined action of *A. orbigera* (explicit in our model in the predation term) and other natural enemies. In areas with ant nests, where the protection by ants excludes most other natural enemies, except *A. orbigera* (and mostly larvae of this species), scale populations thrive and grow in size. This is followed by an increase of the beetle population, which eventually impose a sufficient predation pressure to cause scale populations to decrease. These dynamics would leave ants without their main carbohydrate source, and in doing so, increase their probability of mortality. Because of the build up of beetle populations in areas with ants, these areas act as sources for beetle adults that then disperse to the rest of the farm and contribute (most likely, in conjunction with other predators, pathogens and parasitoids) to the maintenance of low scale populations. Due to the tendency of beetles to concentrate in areas with large clusters of ant nests (Liere et al. in review) and the diffusive nature of beetle migration, there is a stronger effect of beetle predation in larger clusters of ant nests, resulting in a density dependent effect.

A central assumption of the model is that beetles are able to decrease scale populations, indirectly increasing ant nest mortality. Even though we do not have direct evidence to support this, some indirect evidence does suggest that this is possible in the field. First, we know that while *A. orbigera* is not the only natural enemy of *C. viridis* in

the area, it is a voracious predator both during its larvae and its adult stages. Being able to consume an average of 25 scales per day (Liere unpublished data), an individual beetle could consume as much as 600 scales during its time as a larva, and 1200 as an adult (Liere unpublished data). Given that in the field, on average, there are 3.8 larvae per coffee bush in areas with ants, and 5.8 adults in the peripheries of these areas, beetles could indeed be a strong population regulation control for the scales (Liere et al. in review). Second, while it is possible that *A. instabilis* could survive on alternative carbohydrates and extra floral nectaries thus not depending solely on *C. viridis'* honeydew, only some shade trees in the farm have extrafloral nectaries and populations of other species of scale insects are not as abundant as *C. viridis* (personal observations). Furthermore, a previous study in the area suggests that *A. instabilis* colonies do migrate in response to high levels of *C. viridis* mortality caused in turn by local concentrations of a fungal pathogen, *Lecanicelium lecanii* (Jackson et al. 2009). Thus, although we do not propose that the beetle is the sole driving control force in the field (other candidates being the phorid fly, *Pseudacteon* spp., and the entomopathogenic fungi), we suggest that given its voracity and natural history, the beetle could easily be able to decrease scale populations so as to increase the probability of *A. instabilis* mortality. Furthermore, we suggest that given the tendency of beetles to aggregate in larger clusters of ant nests (Liere et al. in review), just as VPP show for the phorid flies, the beetle could also be a density dependent force driving the formation of the clusters of ant nests.

The result that *A. orbigera* requieres areas with and without *A. instabilis* to survive is based on the assumption that *A. orbigera* is a specialist consumer of *C. viridis*. First, while it is true that *A. orbigera* adults, like the majority of ladybeetle species, are

able to survive feeding on alternative prey (Hodek and Honek 1996), most other species of scale insects are generally scarce throughout the farm and even if tended by ants (*A. instabilis* or other ant species) they are never as abundant as *C. viridis* (personal observation). Second, while ladybeetle adults could survive for a while feeding on extrafloral nectaries, nectar or pollen, in absence of their preferred or 'essential' prey, coccinellids are not able to properly reproduce and larvae mortality is very high (Hodek and Honek 1996, Hodek and Honek 2009). Thus, we believe that even if not strictly a specialist on *C. viridis*, this resource is important for the beetle proper growth, development and reproduction. This suggests that the existence of habitats with and without ants, as well as adult dispersal between habitat types, is essential for the beetle's population persistence (Fig. 5.5a).

Our model is a necessary simplification of a complex web of interactions within this community in the field, and it is likely that a combination of different forces is acting directly and indirectly on ant nest mortality. For example, when comparing the frequency distribution of ant nest cluster sizes resulting from our model with the one resulted with the VPP model, we see that the field data fall somewhere between the pattern predicted by the two models. This result suggest that both density-dependent forces, i.e. the beetle predation of scales and phorid fly parasitism, may be involved in the creation of the pattern. Evidence gathered from the field strongly suggests that the phorid and the beetle are behaviorally interactive with one another (Liere and Larsen 2010). In fact, by a reduction of ant activity, the presence of the phorid fly indirectly facilitates the consumption of scales by the coccinellid beetle and is also thought to increase the oviposition chances for female beetles in ant tended areas (Liere et al. in review),

supporting a synergistic role of these two forces in pattern formation and species persistence.

Furthermore, the Ripley's K-function suggests that the field data correspond more to the phorid model than to the beetle model at short spatial scales and more to the beetle model at larger scales. The different ways in which the density dependence appears to operate both in the field and in the two models probably explains why the models seem to correspond to the field data at different scales. While in the phorid model density dependent mortality is very local (operating strictly in the Moore neighborhood of a nest), the beetle affects ant nest mortality by reducing scale populations not only in the immediate neighborhood but throughout the lattice. Because of the buildup of the beetle population and subsequent dispersal away from ant nests, the effect is stronger in larger ant clusters. We believe this difference reflects what happens in nature, with the phorid flies directly affecting ants locally by forcing them to reduce their tending and foraging activities (Philpott et al. 2004, Philpott 2005), and the beetles preying on scales all over the farm, thus indirectly affecting the landscape of the ants at a larger scale. Thus, in nature both the beetles and the phorids are likely to affect the distribution of the ants. Both models appear to explain the formation of the spatial patterns but at different spatial scales.

In our simulations, beetle population were only able to survive in the scenario where the ants form a clustered pattern similar to the one in the field, which the beetles themselves helped create. When the beetles went extinct, the scale insects almost doubled their densities after being released from their predators' control. The modeled scenarios represent the distribution of ant nests in the field and two extreme cases (no ants and ants

everywhere). While the clustered distribution of ant nests generated by our model follows a power-function distribution similar to the one in the VPP model and the one found in the field, this does not necessary imply that this particular spatial distribution is vital to the beetle survival. Field evidence suggests that due to indirect effects of the phorid fly, the existence of both large clusters and small clusters of ant nests could indeed be important for the ladybeetle survival (Liere et al. in review). Our model simply suggests, however, that through community interactions this particular clustered distribution is the pattern that the beetles generate and that this pattern provides the habitat variability that the beetles require to survive.

There have been several theoretical studies reporting that predators (or parasitoids) can cause the formation of high and low prey-density patches in a homogeneous environment. In turn, prey spatial heterogeneity has been shown to be essential for the regional persistence of the predator/parasitoid population (Briggs and Hoopes 2004; Comins and Hassell 1996; Hassell 2000). What is unique about our system is not that a predator-prey system is able to persist by a self-organized spatial pattern, but that the predator indirectly can generate a spatial heterogeneity of an organism other than its prey, and that this heterogeneity, in turn, is required by the predator for reasons other than variability in prey densities. The beetle persists because the ant is spatially structured and the ant is spatially structured because the beetle persists. We speculate that empirical examples of such interactions may be common but have been widely overlooked. Finally, we note that since the scale insect is a persistent pest of coffee in many coffee producing areas in the world, its maintenance below damaging levels in this particular farm may be

an example of an important ecosystem service provided by complex local and spatial dynamics.

Acknowledgments

Figures

Fig. 5.1. Snapshot of the distribution of ant nests. a) in the 120 x 90 lattice from the theoretical model. b) From a 45 ha plot field survey conducted in the summer of 2006, evidently similar, qualitatively, to the model results (for methods see VPP). We use this size lattice as in VPP, since the study system in nature is approximately that size and contains about 11,000 potential ant nesting sites (shade trees).
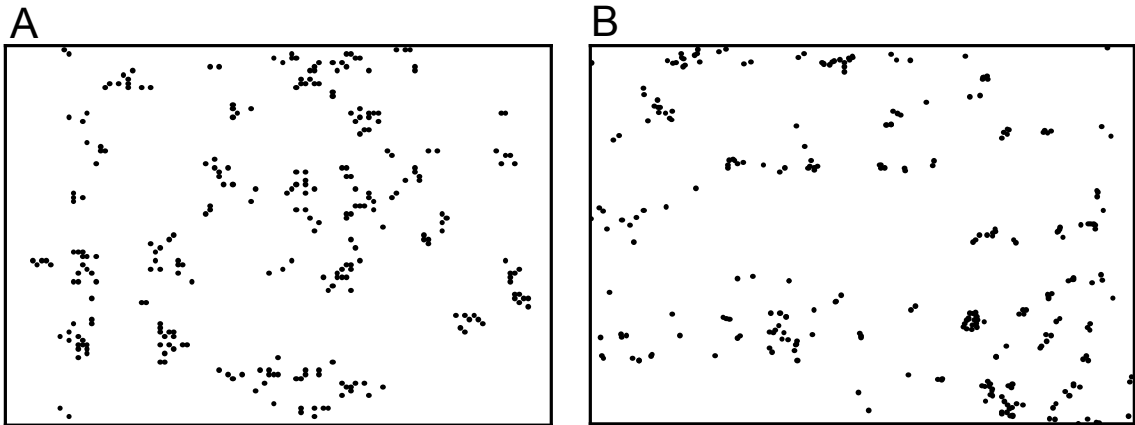
Fig. 5.2. Log of cumulative frequency of log cluster sizes of ant nests. A. From field-parameterized cellular automata stochastic model (VPP) where a parasitic phorid fly is the cause of density dependent ant nest mortality. B. From field samples (rainy and dry season 2004), based on a minimum distance of 20m between nests that are judged to the in the same cluster (see VPP). C. From cellular automata coupled map latticed stochastic model where beetle predation on scale insects is the indirect cause of ant nest mortality.

A

$y = -1.4289x + 5.385$
$R^2 = 0.9639$

B

$y = -1.7374x + 5.2853$
$R^2 = 0.9652$

C

$y = -1.9999x + 6.57$
$R^2 = 0.9597$

Ln (frequency)

Ln (cluster size)
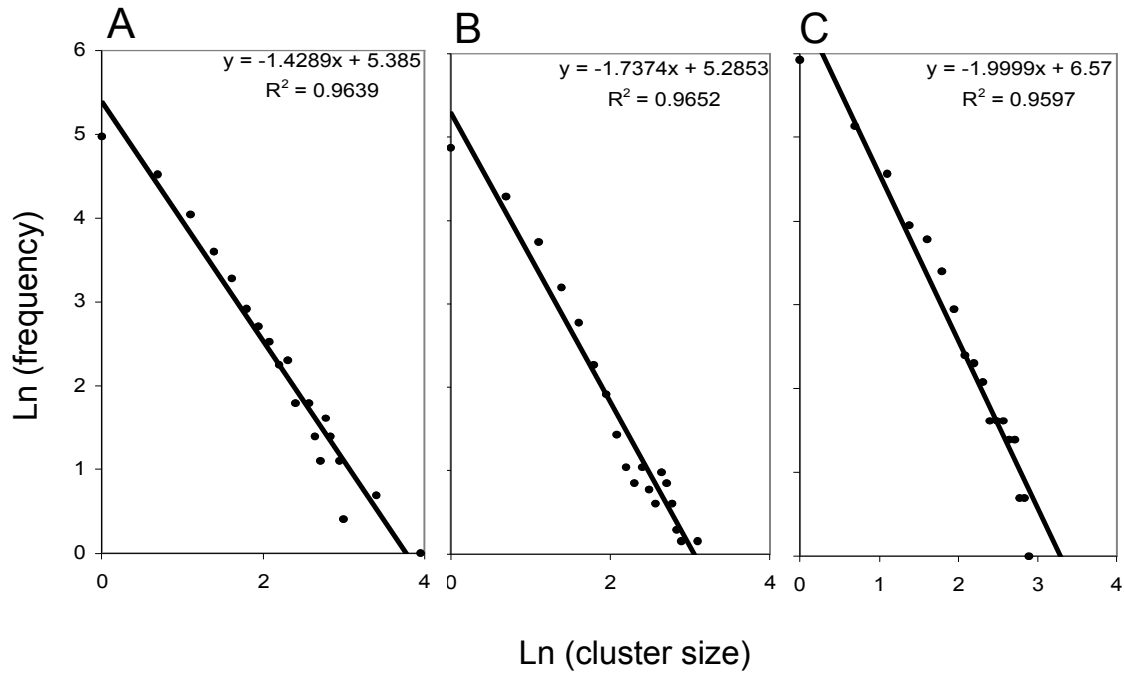
Fig. 5.3. Ripley's K versus size of sampling circle around each tree. The purple color represents the results from the phorid model (VPP) (the dashed line is the mean and the shaded area are the 95% confidence limits); the grey color represents the results from the beetle model; the solid black lines are the results from two field surveys in 2004 of the plot. Note how the field data seem to correspond to the phorid model at very low circle sizes whereas they track more closely the beetle model at higher circle sizes.
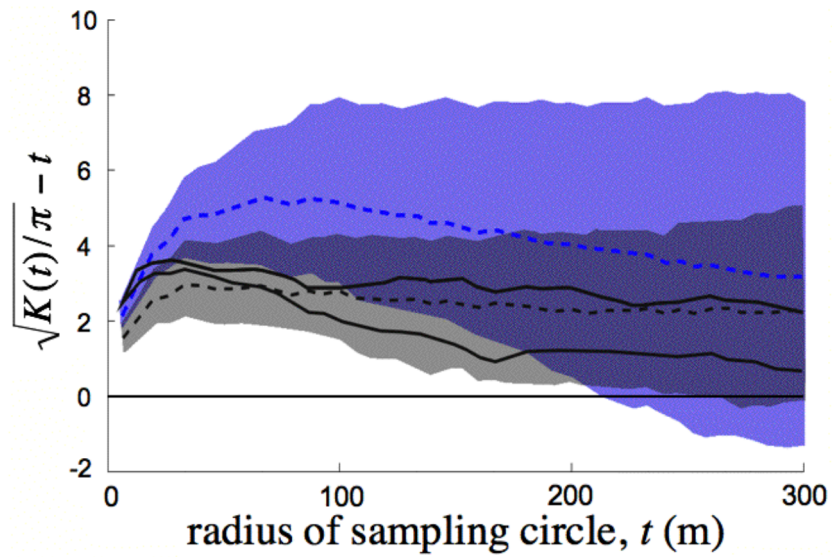
Fig. 5.4. Simulation time series of the four populations (ants, scales, larvae beetles and adult beetles). The oscillatory behavior of the basic predator/prey system and the phase coordination of the mutualistic subsystem is illustrated. Population sizes on the y-axis are on a log+1 scale. A) Simulation in which the ant population goes extinct (ant mortality parameters: $d_o = 0.85$; $d_1 = 0$. B) Simulation in which the ant population occupies the whole lattice (ant mortality parameters: $d_o = 0.2$; $d_1 = 0.3$). C) Simulation in which the ant population is similar to the one observed in the field.

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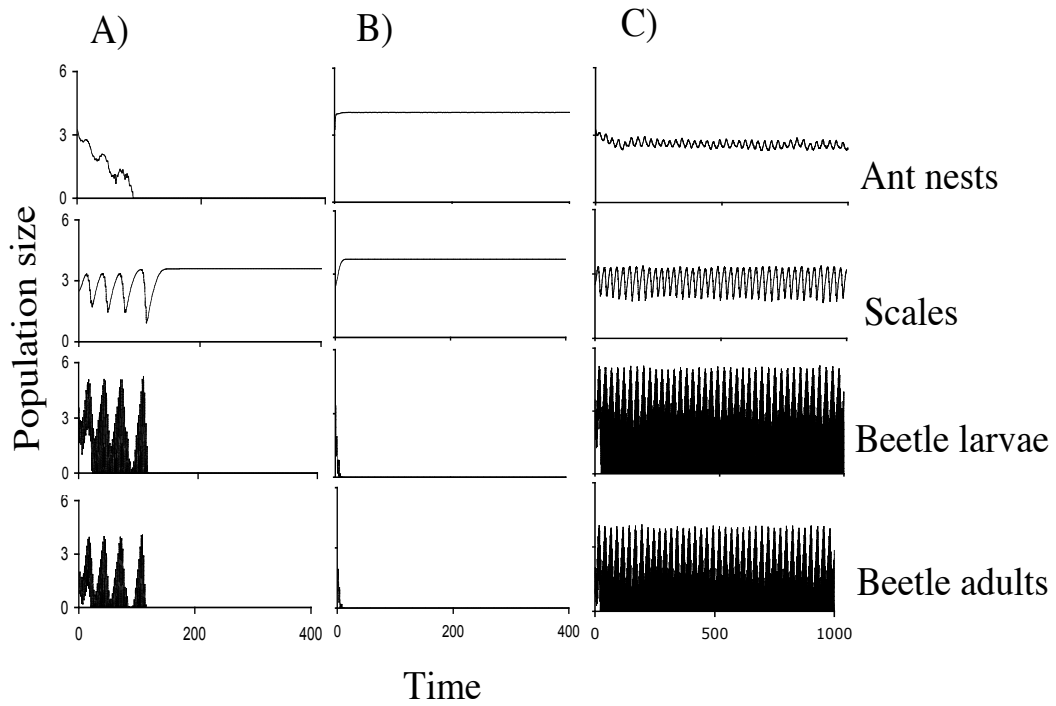
Fig. 5.5. Diagrammatic representation of the proposed mechanisms allowing beetle population persistence (A.) and the formation of clusters of ant nests (B.). Arrowheads indicate positive effects, closed circles indicate negative effects, x indicates a blockage of the potential effect (see text).

A. PROPOSED MECHANISM ALLOWING BEETLE POPULATION PERSISTENCE

B. PROPOSED MECHANISM FORMING CLUSTERS OF ANT NESTS

AREAS WITH ANT NESTS

AREAS WITHOUT ANT NESTS

Ants

mutualism

ants prevent adult beetles from feeding

Ants provide enemy-free space to larvae

Abundant scale insect populations

Predation

Sparce scale insect populations

Predation

Beetle larvae

High larvae mortality due to natural enemies

Beetle larvae

Reproduction

Eclosion

Reproduction

Beetle adults

Beetle adults

Adult beetle dispersal

1.
Ant colony establishes in a nearby tree with scarce populations of scales

6.
Some ant colonies die from lack of food

Ant nest

2.
Ant colony grows and occupies nearby trees forming a cluster

5.
Beetle predation reduces scale insects

3.
Under protection from ants, scale insects increase population

4.
Beetles find clusters and thrive due to abundance of scale insects and enemy-free space

Appendix 1. Model pseudo-code

The model starts with randomly scattering ant nests (ones and zeros), scales and

beetles all over the lattice. Average initial number of organisms used:

Ant nests: 241 (of a total of 10800 cells)

Scales: 1680 cells populated with an average of 0.16 scales per cell

Beetle adults: 241 cells populated with an average of 0.0228 adults per cell

Beetle larvae: 241 cells populated with an average of 0.0228 larvae per cell

Submodels

Beetle adult local dynamics:

FOR all cells in beetle adult grid

IF with ants THEN

$$A_{t+1}' = (e*c_{L(A)}*L_t*N_t)/(1+g*N_t)$$

ELSE

$$A_{t+1}' = (e*c_{L(nA)}*L_t*N_t)/(1+g*N_t)$$

END IF

END FOR

Beetle larvae local dynamics:

FOR all cells in beetle larvae grid

IF with ants THEN

$$L_{t+1}' = (b*c_{A(A)}*A_t*N_t)/(1+g*N_t)$$

ELSE

$$L_{t+1}' = (b * c_{A(nA)} * A_t * N_t)/(1 + g * N_t)$$

END IF

END FOR

Ant nest satellite expansion

$D_{t, Moore}$ = number of ant nests in the Moore neighborhood at time t

FOR all cells in ant nest grid

rand = random number between 0 and 1 drawn from a uniform distribution

IF cell is not occupied by an ant nest THEN

IF *rand* $< x_o + x_1 * D_{t, Moore}$

cell becomes occupied by an ant nest

END IF

END IF

END FOR

Ant nest mortality

FOR all cells in ant nest grid

rand = random number between 0 and 1 drawn from a uniform distribution

IF cell is occupied by an ant nest THEN

IF *rand* $< d_o - d_1 * N_t$

cell becomes unoccupied

END IF

END IF

END FOR

Scale long distance migration:

$propagule\_rain\_count = 0$

FOR all cells in scale grid

$N_{t+1}{'} = N_t$

$rand$ = random number between 0 and 1 drawn from a uniform distribution

IF $rand < w$ THEN

mark cell to receive scale propagule rain

$propagule\_rain\_count = propagule\_rain\_count + 1$

END IF

END FOR

$propagule\_rain = p_x*$(total # of scales in grid)$/propagule\_rain\_count$

FOR all cells marked to receive propagule rain

$N_{t+1}{'} = N_t + propagule\_rain$

END FOR

Scale local migration:

$N_{t,\,Moore}$ = number of scales in the Moore neighborhood at time t

FOR all cells in scale grid

$rand$ = random number between 0 and 1 drawn from a uniform distribution

IF $rand < v_0 + v_1*N_{t,\,Moore}$ THEN

$$local\_migration = 0$$

FOR all cells in Moore neighborhood of cell

$$N_{t+1}' = N_{t+1}' - m_l * N_t$$

$$local\_migration = local\_migration + m_l * N_t$$

END FOR

$$N_{t+1}' = N_{t+1}' + local\_migration$$

END IF

END FOR

FOR all cells in scale grid

$$N_{t+1}' = N_{t+1}' * (1 - p_x)$$

END FOR

Beetle adult long distance migration:

$$propagule\_rain\_count = 0$$

FOR all cells in beetle adult grid

$$A_{t+1}'' = A_{t+1}'$$

rand = random number between 0 and 1 drawn from a uniform distribution

IF with ants THEN

IF *rand* < u_A THEN

mark cell to receive beetle adult propagule rain

$$propagule\_rain\_count = propagule\_rain\_count + 1$$

END IF

ELSE

IF *rand* < u_{nA} THEN

    mark cell to receive beetle adult propagule rain

    propagule_rain_count = *propagule_rain_count* + 1

END IF

END FOR

propagule_rain = p_A*(total # of beetle adults in grid)/*propagule_rain_count*

FOR all cells marked to receive propagule rain

    A_{t+1}'' = A_{t+1}' + *propagule_rain*

END FOR

Beetle adult local migration:

    A_{t+1}'$_{,Moore}$ = number of beetle adults in the Moore neighborhood, using A_{t+1}'

    FOR all cells in beetle adult grid

        rand = random number between 0 and 1 drawn from a uniform distribution

        IF *rand* < a_0 + a_1*A_{t+1}'$_{,Moore}$ THEN

            mark cell as a sink

        END IF

    END FOR

    FOR all cells in beetle adult grid

        IF cell marked as a sink THEN

            local_migration = 0

            FOR all cells in Moore neighborhood of cell

                migration_fraction =

min(m_2, 1/number of sinks in source cell's Moore

neighborhood)

$A_{t+1}'' = A_{t+1}'' - migration\_fraction*A_{t+1}'$

$local\_migration = local\_migration + migration\_fraction*A_{t+1}'$

END FOR

$A_{t+1}'' = A_{t+1}'' + local\_migration$

END IF

END FOR

FOR all cells in beetle adult grid

$A_{t+1}'' = A_{t+1}''*(1-p_A)$

END FOR

Scale insect local dynamics:

FOR all cells in scale grid

IF with ants THEN

$N_{t+1} =$

$r_A*N_{t+1}'*(1-N_{t+1}')$

$- (c_{A(A)})*(A_{t+1}'')*(N_{t+1}')/(1+g*N_{t+1}')$

$- (c_{L(A)})*(L_{t+1}')*(N_{t+1}')/(1+g*N_{t+1}')$

ELSE

$N_{t+1} =$

$r_{nA}*N_{t+1}'*(1-N_{t+1}')$

$- (c_{A(nA)})*(A_{t+1}'')*(N_{t+1}')/(1+g*N_{t+1}')$

$$- (c_{L(nA)})*(L_{t+1}')*(N_{t+1}')/(1+g*N_{t+1}')$$

END IF

END FOR

Beetle adult mortality:

FOR all cells in beetle adult grid

IF with ants THEN

$$A_{t+1} = A_{t+1}''*s_{A(A)}$$

ELSE

$$A_{t+1} = A_{t+1}''*s_{A(nA)}$$

END IF

END FOR

Beetle larvae mortality:

FOR all cells in beetle larvae grid

IF with ants THEN

$$L_{t+1} = L_{t+1}'*s_{L(A)}$$

ELSE

$$L_{t+1} = L_{t+1}'*s_{L(nA)}$$

END IF

END FOR

Fig. SM. 1. Flow chart of model execution
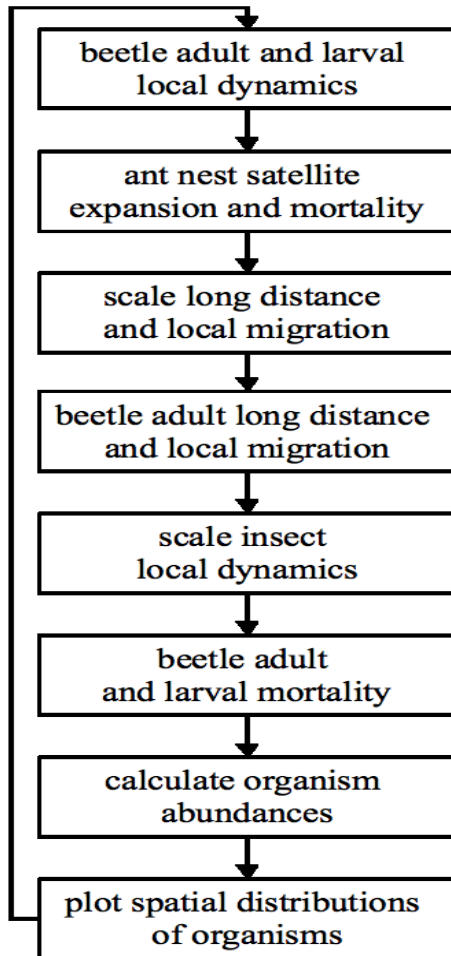
Appendix 2. Genetic algorithm (GA) description

We employed a genetic algorithm (GA) implemented using the Java package JGAP

(Java Genetic Algorithms Package) to search for parameter values fulfilling certain

performance criteria. The performance (fitness) of parameter value combinations was

calculated based on the following criteria: number of ant nests, complete extinction of

beetles when ants are fixed to occupy 100% of the lattice points, complete extinction of

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beetles when ants are fixed to occupy 0% of the lattice points, numbers of beetle adults

and larvae in locations with ant nests, and numbers of beetle adults and larvae in

locations without ant nests, as follows,

$$f = \sum_{i=1}^{n} \left[w_i \left(\left\| \left\| \begin{cases} F_{min} & : x_i < min\_x_i \\ F_{min} + \left(\frac{x_i - min\_x_i}{target\_x_i - min\_x_i} \right) (F_{max} - F_{min}) & : min\_x_i < x_i < target\_x_i \\ F_{max} - \left(\frac{x_i - target\_x_i}{max\_x_i - target\_x_i} \right) (F_{max} - F_{min}) & : target\_x_i < x_i < max\_x_i \\ F_{max} & : x_i > max\_x_i \end{cases} \right\| \right\| \right) \right]$$

where f is the fitness; n is the total number of performance criteria; and x_i is the value

of criterion i at the end of the simulation run. For each set of parameters, the model was

run three times: once with the ant nests appearing and dying dynamically, as defined by

the rules of the cellular automata and coupled map lattice models (dynamic ants), a

second time with ants occupying every cell in the lattice (all ants), and a third time with

no ant nests (no ants). The values and descriptions of the coefficients are found in SM

Table 1.

Table SM.1 GA fitness function coefficients

| Coefficient	Description	Value
F_{min}	minimum fitness contribution before weighting	0
F_{max}	maximum fitness contribution before weighting	100000
n	total number of performance criteria	15
$target\_x_1$	number of ant nests (dynamic ants)	300
$target\_x_2$	number of adult beetles per cell in patches with ants (dynamic ants)	5.76
$target\_x_3$	number of adult beetles per cell in patches without ants (dynamic ants)	1.27
$target\_x_4$	number of beetle larvae per cell in patches with ants (dynamic ants)	3.8
$target\_x_5$	number of beetle larvae per cell in patches without ants (dynamic ants)	1.58
$target\_x_6$	number of ant nests (all ants)	300
$target\_x_7$	number of adult beetles per cell in patches with ants (all ants)	0
$target\_x_8$	number of adult beetles per cell in patches without ants (all ants)	0
$target\_x_9$	number of beetle larvae per cell in patches with ants (all ants)	0
$target\_x_{10}$	number of beetle larvae per cell in patches without ants (all ants)	0
$target\_x_{11}$	number of ant nests (no ants)	300
$target\_x_{12}$	number of adult beetles per cell in patches with ants (no ants)	0
$target\_x_{13}$	number of adult beetles per cell in patches without ants (no ants)	0
$target\_x_{14}$	number of beetle larvae per cell in patches with ants (no ants)	0
$target\_x_{15}$	number of beetle larvae per cell in patches without ants (no ants)	0
min_x_1	minimum number of ant nests (dynamic ants)	
min_x_2	minimum number of adult beetles per cell in patches with ants (dynamic ants)	0.001
min_x_3	minimum number of adult beetles per cell in patches without ants (dynamic ants)	0.001
min_x_4	minimum number of beetle larvae per cell in patches with ants (dynamic ants)	0.001
min_x_5	minimum number of beetle larvae per cell in patches without ants (dynamic ants)	0.001
min_x_6	minimum number of ant nests (all ants)	1
min_x_7	minimum number of adult beetles per cell in patches with ants (all ants)	-1
min_x_8	minimum number of adult beetles per cell in patches without ants (all ants)	-1
min_x_9	minimum number of beetle larvae per cell in patches with ants (all ants)	-1
min_x_{10}	minimum number of beetle larvae per cell in patches without ants (all ants)	-1
min_x_{11}	minimum number of ant nests (no ants)	1
min_x_{12}	minimum number of adult beetles per cell in patches with ants (no ants)	-1
min_x_{13}	minimum number of adult beetles per cell in patches without ants (no ants)	-1
min_x_{14}	minimum number of beetle larvae per cell in patches with ants (no ants)	-1
min_x_{15}	minimum number of beetle larvae per cell in patches without ants (no ants)	-1
max_x_1	maximum number of ant nests (dynamic ants)	11000
max_x_2	maximum number of adult beetles per cell in patches with ants (dynamic ants)	10000000
max_x_3	maximum number of adult beetles per cell in patches without ants (dynamic ants)	10000000
max_x_4	maximum number of beetle larvae per cell in patches with ants (dynamic ants)	10000000
max_x_5	maximum number of beetle larvae per cell in patches without ants (dynamic ants)	10000000
max_x_6	maximum number of ant nests (all ants)	11000
max_x_7	maximum number of adult beetles per cell in patches with ants (all ants)	2
max_x_8	maximum number of adult beetles per cell in patches without ants (all ants)	2
max_x_9	maximum number of beetle larvae per cell in patches with ants (all ants)	2
max_x_{10}	maximum number of beetle larvae per cell in patches without ants (all ants)	2
max_x_{11}	maximum number of ant nests (no ants)	11000
max_x_{12}	maximum number of adult beetles per cell in patches with ants (no ants)	2
max_x_{13}	maximum number of adult beetles per cell in patches without ants (no ants)	2
max_x_{14}	maximum number of beetle larvae per cell in patches with ants (no ants)	2
max_x_{15}	maximum number of beetle larvae per cell in patches without ants (no ants)	2

Coefficient	Description	Value
w_1	weight for number of ant nests (dynamic ants)	0.11
w_2	weight for number of adult beetles per cell in patches with ants (dynamic ants)	0.11
w_3	weight for number of adult beetles per cell in patches without ants (dynamic ants)	0.11
w_4	weight for number of beetle larvae per cell in patches with ants (dynamic ants)	0.11
w_5	weight for number of beetle larvae per cell in patches without ants (dynamic ants)	0.11
w_6	weight for number of ant nests (all ants)	0
w_7	weight for number of adult beetles per cell in patches with ants (all ants)	0.11
w_8	weight for number of adult beetles per cell in patches without ants (all ants)	0
w_9	weight for number of beetle larvae per cell in patches with ants (all ants)	0.11
w_{10}	weight for number of beetle larvae per cell in patches without ants (all ants)	0
w_{11}	weight for number of ant nests (no ants)	0
w_{12}	weight for number of adult beetles per cell in patches with ants (no ants)	0
w_{13}	weight for number of adult beetles per cell in patches without ants (no ants)	0.11
w_{14}	weight for number of beetle larvae per cell in patches with ants (no ants)	0
w_{15}	weight for number of beetle larvae per cell in patches without ants (no ants)	0.11

In order to find acceptable parameter values more rapidly, the GA was run on 12

computers simultaneously. Each instantiation of the GA had a population of 100 possible

solutions, with the parameter values in the first generation chosen at random. The

maximum fitness per generation for each of the 12 instantiations is shown in Figure

SM.2. There was significant variability in the speed of the computers used, so there was

substantial variation in the number of generations the machines completed before the

search was terminated. All of the instantiations converged rapidly to marginally

acceptable or acceptable performance, suggesting that the range of acceptable parameter

values is relatively large, i.e., the model output is relatively robust to changes in

parameter values.

Figure SM.2 Fitness versus generation for 12 instantiations of the GA
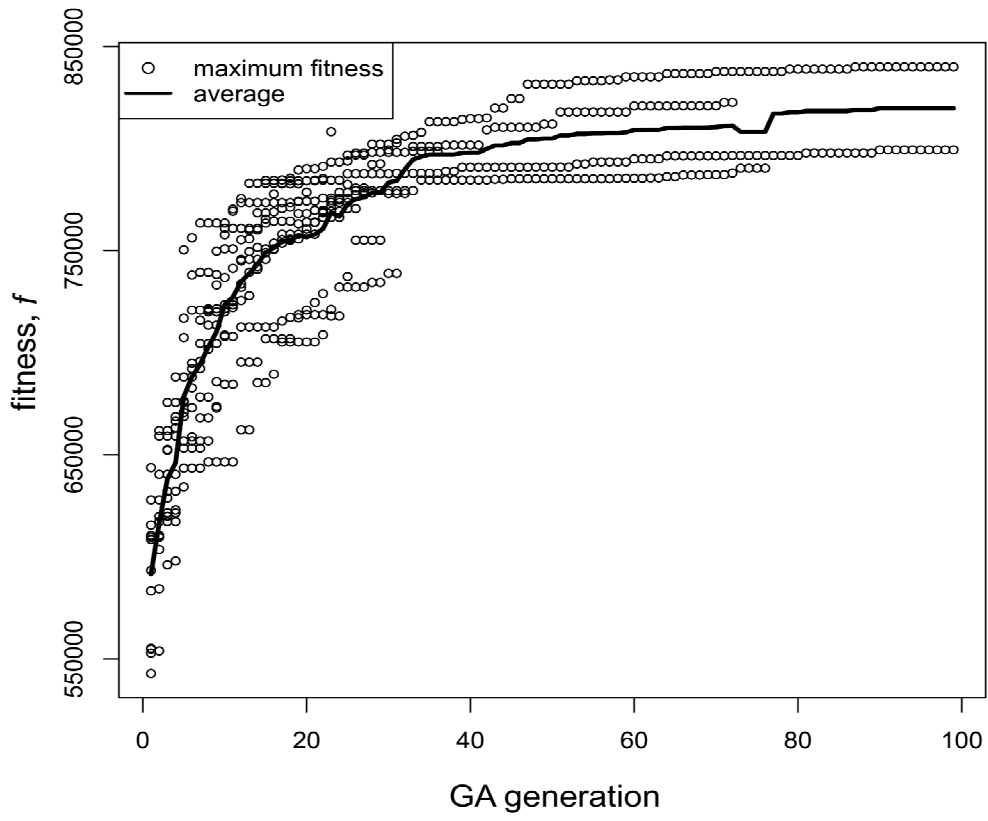
Appendix 3. The parameters

Table SM. 2. Parameter values used for the model

Parameter	Description	Value
x_0	intercept of ant nest density-dependent local expansion function	2.0×10^{-7}
x_1	slope of ant nest density-dependent local expansion function	0.7365
d_0	intercept of ant nest scale-dependent mortality function	0.82
d_1	slope of ant nest scale-dependent mortality function	0.3
v_0	intercept of scale density-dependent local migration function	0
v_1	slope of scale density-dependent local migration function	0.7129
w	probability of a cell receiving propagule rain of scales	1.0×10^{-3}
p_X	coefficient of propagule rain of scales	2.0×10^{-4}
m_1	coefficient of migration of scales	0.02
r_A	intrinsic growth rate of scales with ants	1.9968
r_{nA}	intrinsic growth rate of scales without ants	1.5938
c_L	predation rate of larval beetles with or without ants	0.7568
$c_{A(A)}$	predation rate of adult beetles with ants	0.0107
$c_{A(nA)}$	predation rate of adult beetles without ants	0.3231
g	functional response of larval and adult beetles	2.0989
e	eclosion rate of beetles	1
b	birth rate of beetles	962.7603
$s_{A(A)}$	survivorship rate of adult beetles with ants	0.4690
$s_{A(nA)}$	survivorship rate of adult beetles without ants	0.8841
$s_{L(A)}$	survivorship rate of larval beetles with ants	0.9345
$s_{L(nA)}$	survivorship rate of larval beetles without ants	0.6341
a_o	intercept of adult beetle density-dependent local migration function	0
a_1	slope of adult beetle density-dependent local migration function	0.5
m_2	coefficient of migration of adult beetles	0.8914
p_A	coefficient of propagule rain of adult beetles	0.1129
u_A	probability of a cell with ants receiving propagule rain of beetle adults	0.2332
u_{nA}	probability of a cell without ants receiving propagule rain of beetle adults	0.1994

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Chapter VI

Conclusions

The green coffee scale (*Coccus viridis),* can reach very high densities in coffee plantations around the world causing devastating harvest loses (Purseglove 1987; APHIS 1998). Conversely, in some coffee agroecosystems, like in the one where my research took place, the presence of green scales does not present a problem to the plantation. Therefore, this insect and the ecological community with which it interacts is an ideal system to address the question that has puzzled agroecologists and pest managers for decades: why do potential pests never acquire pest status in some farms? The answer, as my research suggests, could lie in the rich complexity of interactions maintained in biodiverse agroecosystems. My results, along with accumulating evidence from our research team, are leading us to the conclusion that the maintenance below damaging levels of this and other important pests in this farm are examples of "intrinsic" biological control. The complex nature of the ecosystem enables the ecological communities to regulate themselves, hindering pest population explosions. The result is a healthy agroecosystem with little necessity for external inputs for pest management (as summarized in Vandermeer et al. 2010).

Throughout my dissertation I have shown the complexity and context dependence of the nature, strength, and direction of the interactions between the ant *Azteca instabilis*, the green coffee scale *C. viridis,* and the predatory ladybeetle *Azya orbigera* as well as how their rich web of direct and indirect interactions enables the community to persist

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and regulate itself in the system. In Chapter II, I showed that the larvae of the predatory *A. orbigera* are able to "cheat" on the mutualism between *A. instabilis* and the green coffee scale. While *A. instabilis* usually protects the green scale from predators, it is not able to do so against *A. orbigera* larvae due to the sticky waxy filaments that cover the body of these predatory larvae. Furthermore, since *A. instabilis* showed aggressive behavior towards *A. orbigera*'s parasitoids, the presence of ants reduced the parasitism suffered by *A. orbigera*. As a result, and contrary to the majority of *C. viridis* natural enemies, these ladybeetle larvae have access to the abundant scale colonies patrolled by ants and, by living in these areas, they gain indirect protection against their own natural enemies.

In Chapter III, I demonstrated that the interactions between ants, green scales and ladybeetles are drastically altered in the presence of a parasitic fly. I presented evidence that during parasite-induced low-activity periods, the ant *A. instabilis* fails to protect its mutualistic scale-insect partner against predatory ladybeetles. Thus, in the presence of the parasite, ladybeetle adults, which are otherwise almost completely prevented from preying upon ant-tended scales, ate as many scales in ant-patrolled plants as they did in ant-free plants. These results demonstrate how, through a cascade of trait-mediated interactions, associations between the members of this community were drastically altered. My results suggest that whether an area under ant influence is beneficial or detrimental to ladybeetle larvae and adults depends on the presence and abundance of this parasitoid fly.

In Chapter IV, I present evidence that, at large spatial scales, both adults and larvae ladybeetle are more abundant and persistent in areas with ants; adults, however,

tend to be restricted to the peripheries of areas of ant influence. The results from Chapter IV suggest that even if areas with ants are high quality habitats for the ladybeetle larvae and are functioning as source habitats for them, as adults they are forced to disperse to areas without ants, probably to feed. The results from this chapter also suggest that the specific spatial distribution of *A. instabilis* nests might also play a key role for the persistence of this important predatory ladybeetle.

Lastly, in Chapter V, I showed, using a computer simulation based on the natural history of this ecological web, that the spatial heterogeneity of ant nests might be key for the persistence of the ladybeetle populations. Moreover, I argued that the dynamics of the ladybeetle consuming green scales can, in turn, cause the clustered distribution of the mutualistic ants. This is a unique situation in which a predator indirectly causes the spatial pattern of an organism other than its prey and, simultaneously, its persistence is depends on this spatial pattern. As such, the ladybeetle persists because the ant exhibits a spatial pattern and the ant maintains this spatial pattern because the ladybeetle persists.

As the brief description of each chapter's findings show, throughout my dissertation I have presented evidence of how both local interactions and spatial distributions in this ecological web promote mechanisms of community self-regulation. Specifically, I have shown the importance of complex interactions associated with the ant-scale insect mutualism for the persistence, spatial distribution, and dispersal of the voracious *A. orbigera*, which in turn, is prevents the green scale populations from increasing above damaging levels. My research thus suggests that, at least for this diverse agroecosystem, the disappearance of ants would not enhance the natural control of green scales as has been previously suggested (Reimer et al. 1993). On the contrary, the

absence of ants in this system would probably cause the complex ecological web to collapse rendering populations of important predators highly prone to local extinctions. Given that green scales are a persistent and damaging coffee pest around the world, the fact that their populations are maintained below damaging levels in this particular farm seems to be an example of an important ecosystem service provided by complex local and spatial dynamics characteristic of diverse agroecosystems.

Broader implication of my research

On a superficial level, massive losses of biological diversity due to disappearance of natural habitats and economical losses in agriculture due to pest damage might seem like two completely unrelated issues. Yet, these two serious problems are in fact closely connected and, therefore, solutions for both might only come to light if the link between the two is thoroughly understood. Most biodiversity hotspots today are, for better or worse, landscapes composed of fragmented forests embedded in a matrix of agricultural land (Janzen 1983, Vandermeer and Perfecto 2007, Perfecto et al. 2009). Given that even large and well-preserved forest fragments face high extinction rates (Newmark 1995; Ferraz et al. 2003), recolonization is crucial for the preservation of biological diversity in these habitats (Hanski and Beverton 1994, Hanski and Ovaskainen 2000). Whether an agricultural matrix acts as a barrier against migration or, on the contrary, allows migration of organisms between forest patches (Ricketts 2001) and can therefore be inhabited temporarily, or perhaps even permanently, by forest species, depends mostly on the type of agricultural management practiced therein (Daily et al. 2001, Greenberg et al. 1997, Perfecto et al. 1996). Forest fragments can be 'source' habitats or serve as refuges

for myriad organisms that provide invaluable ecosystem services like decomposition (Altieri 1999), pollination (Klein 2009, Kremen et al. 2002, Ricketts 2004, Ricketts et al. 2004) and pest control (Altieri 1999, Andow 1983, Thies and Tscharntke 1999). Thus, if a forest fragment and its associated biodiversity disappears, nearby farmers may experience significant economic losses (Ricketts et al. 2004). Consequently, farmers ought to be concerned with the preservation of nearby forest fragments and conservationists should allocate resources to ensure that the agricultural matrix surrounding fragmented forests is managed with environmentally friendly techniques. Understanding ecological processes occurring in agroecosystems is crucial to break the current trend of complete reliance on expensive and hazardous pest control agrochemicals and to develop agricultural managing techniques that may contribute both to agricultural sustainability and to the conservation of biological diversity.

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