Aggression and its function as a hub mediating host defense against the slavemaking ant *Protomognathus americanus*

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This thesis is based on the following five research articles, which are presented as chapters 1 to 5 and referred to in the introduction and discussion by their respective numbers:

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Scharf, I., Pamminger, T., Foitzik, S. 2011. Differential response of ant colonies to intruders: attack strategies correlate with potential threat. **Ethology**, 117: 731-739.

Pamminger, T, Modlmeier, A.P., Suette S., Foitzik S. Raiders from the sky: slavemaker founding queens select for aggressive host colonies. **Biology Letters**, in press.

Pamminger, T., Leingärtner A., Achenbach A., Kleeberg I., Pennings P.S., Foitzik S. Geographic distribution of the anti-parasite trait "slave rebellion. **Evolutionary Ecology**, in press.

Pamminger, T, Metzler, D., Foitzik, S., Pennings, P.S. Oh sisters, where are you? Indirect fitness benefit could maintain a host defense trait. (to be submitted)

Abstract

In my doctoral thesis I investigated host defense strategies against the social parasite *Protomognathus americanus* ("slavemaker"). I focused on the effectiveness and evolution of defense behaviors in the ant host species *Temnothorax longispinosus* ("slave"). We discovered a novel inducible defense strategy, enabling host colonies to react flexible to the unpredictable threat of a parasite attack. In addition, we studied how host colonies modulate their collective defense against a variety of opponents. We documented strong qualitative and quantitative variation in host responses, fine-tuned to the potential threat of the opponent. These findings indicate that selection can shape defense traits of host colonies against different threats independently. In a third study we demonstrate that parasites can directly alter the evolutionary trajectory of host colony aggression (defense), documenting the strong impact of the parasite on colony defense in its host. In the last two publications we examine an unusual defense trait "slave rebellion" and try to answer the question how a trait can evolve, if the individual carrying it does not reproduce. In a meta-analysis we document the wide distribution and strong variation in expression in different populations, and provide empirical evidence for kin selection being the driving force in the evolution and maintenance of this defense trait.

Zusammenfassung

In meiner Dissertation beschäftigte ich mich mit unterschiedlichen Verteidungsstrategien, deren Effektivität und Evolution, der Ameisenart *Temnothorax longispinosus* ("Sklaven"), gegenüber einem sozialen Parasiten - der nahverwandten, sklavenhaltenden Art *Protomognathus americanus* ("Sklavenhalter"). Wir entdeckten eine neue Kategorie der Verteidigungsstrategie, welche es dem Wirten ermöglicht, flexibel auf die nicht vorhersagbaren Angriffe des Parasiten zu reagieren. Darüber hinaus erforschten wir, wie die Wirte ihre kollektive Verteidigung an eine Vielzahl unterschiedlicher Angreifer anpassen können. Wir konnten feststellen, dass Wirte in der Lage sind ihre kollektive Verteidigung dem Grad der Bedrohung anzupassen. Dies weist darauf hin, dass Selektion die Verteidigung gegen unterschiedliche Typen von Angreifern voneinander unabhängig beeinflussen könnte. In einer dritten Studie belegten wir experimentell, dass die Parasiten die Evolution der Kolonieaggressivität der Wirtsart direkt beeinflussen. Die letzten beiden Publikationen beschäftigten sich mit Sklavenrebellion, einer rätselhaften Verteidigungsstrategie, da noch unklar ist, wie eine Eigenschaft von nicht reproduzierenden Individuen vererbt werden kann. In einer Metaanalyse konnten wir die weite Verbreitung und hohe Variabilität dieser Eigenschaft dokumentieren, und fanden Hinweise, dass Verwandtenselektion eine mögliche Erklärung für die Evolution dieses Merkmals darstellen könnte.

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"The great thing about them (ants) is that they can be modeled as a 2D bipolar fluid which can reach a local density of zero (yes they can indeed die)."

S. in Vienna early 2010

General Introduction

Organisms moving through their habitat will meet a variety of other species during their lifetime. Many of these encounters are swift, interchangeable interactions or even go unnoticed by both participants. But some of these species - species encounters severely affect both participants, with a variety of possible outcomes for the interacting organisms. Encounters influencing both participants include, for example, pollinators finding a flowering plant, a predator attacking its prey and a parasite infecting its host. During such interactions the fitness of both organisms is - suddenly - not just defined by their own genetic makeup and the environmental conditions, but by another organism and their genetic makeup as well. Such species-species-environment interaction, the process of reciprocal evolutionary change via natural selection (coevolution), is probably the most common evolutionary processes in nature (Thompson 2005).

By introducing the mosaic theory of coevolution (MTC) over a decade ago, Thompson (1999, 2005) formalized the scientific methology of coevolutionary processes by providing a theoretical framework to deal with a variety of coevolving systems. Coevolution is an inherent hierarchical process with species-species interactions on the population level being the most fundamental operational unit. These coevolving populations are subject to variation (e.g. ecological conditions, starting frequencies of the interacting species or genetic drift) and consequently differ in the outcomes of the interaction on a local level (Thompson 1997). Local populations are not isolated in space, but connected by migration of individuals (geneflow). Such individuals carry their coevolutionary history with them (e.g. novel resistance traits) and, if able to interbreed within the new population, might influence the outcome of the coevolutionary interaction in the new population. Consequently, the MTC is operating on a meta-population level, resting on three evolutionary hypotheses (Thompson 1999, Thompson 2005, Gomulkiewicz et al. 2007):

1. **Geographic selection mosaics**. The outcome of coevolutionary dynamics varies between populations, since coevolutionary processes are rather species-species-environment interactions than species-species interactions - and environmental conditions may vary in between populations.
2. **Evolutionary hotspots**. Coevolutionary interactions have reciprocal effects in some populations, but not in all. Populations are embedded in a network of evolutionary coldspots, where no reciprocal selection occurs (e.g. species' geographic distributions do not completely overlap and coevolution can exclusively happen in areas where both occur; or one coevolving species is replaced by another species in some habitats). In order to understand local coevolutionary dynamics, one has to consider coevolutionary dynamics on a meta-population level, envisioned as a network of intermingled hot- and coldspots connected by migration.
3. **Trait remixing**. The local genetic structure of coevolving species varies in time and space. Processes such as mutation, gene-flow, local extinction and genetic drift alter the allelic composition of the local populations and, consequently, the coevolutionary outcome of local interaction.

In the past decade empirical evidence has accumulated for all three basic processes postulated by the MTC (Forde et al. 2004, Brodie et al. 2002, Rudgers et al. 2004, Zangerl et al 2008). These three processes generate testable ecological predictions. For example: a. local populations will differ in coevolved traits (e.g. Benkman 1999). b. Trait matching (between coevolved traits) occurs in local populations only, while traits mismatch in other populations (Hanfin 2009) and c. Coevolved traits rarely go to fixation as it is unlikely that they are favorable in all populations (e.g. Burdon 1999). The MTC argues that a wide spectrum of coevolutionary dynamics follows these fundamental principles, ranging from mutualistic symbiotic or mutualistic in between free-living species to grazing, predation and, finally, parasitism. Though we have to take care not to mistake all variation observed in nature as coevolutionary process (Thompson 1999, 2005).

Parasitism (defined as organisms exploiting other organisms as a resource and potentially harming them in the process) can be considered an evolutionary success story. As such, parasitism is one of the most common lifestyles on earth (Price 1980), shaping food webs, influencing biodiversity and driving the development of major evolutionary innovations such as sex (Hamilton 1990, Wommack 2000, Daszak 2000, Morran 2011). By using another organism as a resource, parasitism is usually associated with high, sometimes lethal, costs for the species exploited (Price 1980, Schmid-Hempel 1989). These costs exert severe selection pressures on the host to limit the negative effects of the parasite. Indeed, there is strong evidence that exploited organisms can respond to this selection pressure evolving defense traits and lowering the effects of parasite exploitation (e.g. Gross 1993, Lyon 2003). Since the evolution of host defense mechanisms inevitably causes decreased parasite fitness, parasites respond by developing counter-adaptations, starting a cycle of reciprocal adaptations and counter adaptations. Such a coevolutionary dynamic may escalate, a process well described by the analogy of an evolutionary arms race (Dawkins and Krebs 1979). Once caught in an evolutionary arms race, host and parasite have a slim chance of escaping this escalating process. If one participant fails to adapt to a new innovation of the other party, it could eventually lead to its local extinction (Dawkins and Krebs 1979, Hanifin et al. 2008).

In the late 80's and the early 90's of the 20th century, brood parasites emerged as ideal systems to investigate the escalation dynamics of host-parasite interactions (Davies et al. 1989, Rothstein et al. 1990). Brood parasitism is a form of parasitism in which parasites avoid the costs of rearing their own offspring (time and energy) by "outsourcing" these investments to another species (interspecific brood parasitism). It is a common phenomenon and has evolved multiple times independently where parental care is a considerable investment but can be provisioned by others than the biological parents (Payne 1977, Sato 1986, Buschinger 2009).

Brood parasitism in birds has been intensively investigated for a long time - the fact that cuckoo chicks are raised by a different bird species has already been mentioned in the old Vedic literature in India (approximately 1500–1000 BC) and, later, by Aristotle (384-322 BC) (Friedmann 1965). The costs of raising your own offspring appear to be so substantial that laying eggs in the nests of another species, and thereby transferring the costs to a foster parent, has independently evolved multiple times in birds (Krüger 2007). Out of the ~10 000 bird species worldwide, 100 appear to be obligate brood parasites, with an uneven distribution among the six major bird families (Winfree 1999). Interspecific brood parasitism is a well-documented phenomenon among social insects too, especially hymenopterans such as wasps, bumblebees and ants, and is often referred to as social parasitism (Cervo 2006, Antonovics et al. 2011, Buschinger 2009, Brandt et al. 2005). Within the order Hymenoptera, social parasitism appears to be most common among ants (Hymentoptera: Formicidae) and approximately ~230 out of the

~12 500 known species are social parasites (Bolton 2003). Their distribution among the 22 subfamilies is unequal (Buschinger 2009), similar to brood parasitism in birds. Social parasitism in ants can be classified in four broad categories (Buschinger 2009):

1. Xenobiosis (guest ants)
2. Temporary parasitism
3. Permanent parasitism without slavery (inquilinism)
4. Permanent parasitism with slavery (dulosis)

Xenobiosis

Guest ants share a nest with another species (host) but maintain their own nest, usually within the nest material of the host colony. They keep their brood strictly separate from the host brood but depend on them in terms of food and shelter. In contrast to the other forms of social parasitism in ants, Xenobiosis ants raise their own young, thus the costs for the host are restricted to the loss of food, which is acquired directly from the workers or stolen during trophallaxis of two host workers (see Buschinger 2009, Hölldobler and Wilson 1990, Lenoir et al. 1997).

Temporal parasites

- depend on their host species during colony foundation only. After the mating flight the temporal parasites' queen invades a host colony and replaces the resident queen. They are accepted by the host workers, which start caring for the eggs laid by the parasite queen. These eggs develop into workers fully capable to take care of the colony. In the beginning parasite- and host workers tend the colony together, and the host worker force is gradually replaced due to natural host worker aging and death. This process eventually leads to a self-sustaining colony entirely composed of parasitic workers and their queen (see Buschinger 1986, Buschinger 2009, Hölldobler and Wilson 1990).

Permanent parasitism without slavery (inquilinism)

Inquilines are ant parasites that have lost their worker caste. A parasitic queen invades a host nest and produces sexuals, which are raised by host worker force. Usually, inquilines coexist with the resident queens, but in some cases they kill the queen, which in return limits colony lifetime because the workforce is not replenished (see Buschinger 1986, Buschinger 2009, Hölldobler and Wilson 1990).

Permanent parasitism with slavery (dulosis)

- is the fourth category of social parasitism found in ants and the focus of this thesis. *Temnothorax longispinosus*, a small *Myrmicine* ant belonging to the Formicoxinini tribe, is the preferred host species of *Protomognathus americanus,* an obligatory dulotic species (for details on the ecology of both species see publications 1-5). About a forth of the 230 social parasites in ants are dulotic (Buschinger 1986, Buschinger 2009), though the Formicoxinini appear to be a hotspot for the evolution of social parasitism with six independent origins of slavemakers in this relatively small tribe (Bleibl 2005).

Similar to temporal parasites, freshly mated queens penetrate the host colony directly after the nupal flight and engage in intense, aggressive interactions, trying to expel or kill the resident queen (Wesson 1939, Buschinger 1974). In contrast to temporal parasites, dulotic queens do not blend in with the workers but expel the host workers (Brandt 2005, Fig. 1). After successful usurpation of the host colony the parasite queen takes over the remaining brood, which will hatch and become the first generation of slaves. This first generation accepts the parasite queen within the first days after hatching and starts maintaining the colony, raising parasitic workers laid by the queen (Alloway 1982, Achenbach 2010). These parasite workers are usually not able to carry out nest maintenance, foraging or brood care and even need to be fed by the host workers (Buschinger 2009). As the slaves work force will inevitable diminish, due to aging and regular worker loss, parasites need to acquire additional slaves to compensate for the losses. Parasite workers regularly attack surrounding host colonies, killing or driving away the resident ants and stealing their brood (Cool-Kwait and Topoff 1984, Herbers and Foitzik 2002, Fig.1). These so-called "slavemakers" are well equipped for the raiding events, utilizing chemical and morphological weaponry (Brandt 2005). These features include a thick cuticle, a broad postpetiole and antennal scrobes, interpreted as defensive structures. In addition, parasites utilize chemical substances interfering with colony communication ("propaganda substances"), which are being used during raiding events to confuse the host workers (Brandt 2005, Allies et al. 1986). The high frequency and destructiveness of the parasite raiding events (Foitzik et al. 2001b) result in strong selection pressure on the host, which is in dire need to evolve strategies to defend itself. There is good indication that hosts have indeed responded to the selection pressure exerted by the parasite through developing developed parasite specific recognition (Alloway 1990) and elaborate defensive strategies (Foitzik et al. 2001c). These parasite specific traits are potential candidates for having evolved as a response to the parasite threat and coevolutionary processes. Indeed, there is good indication that *T. longispinosus* and

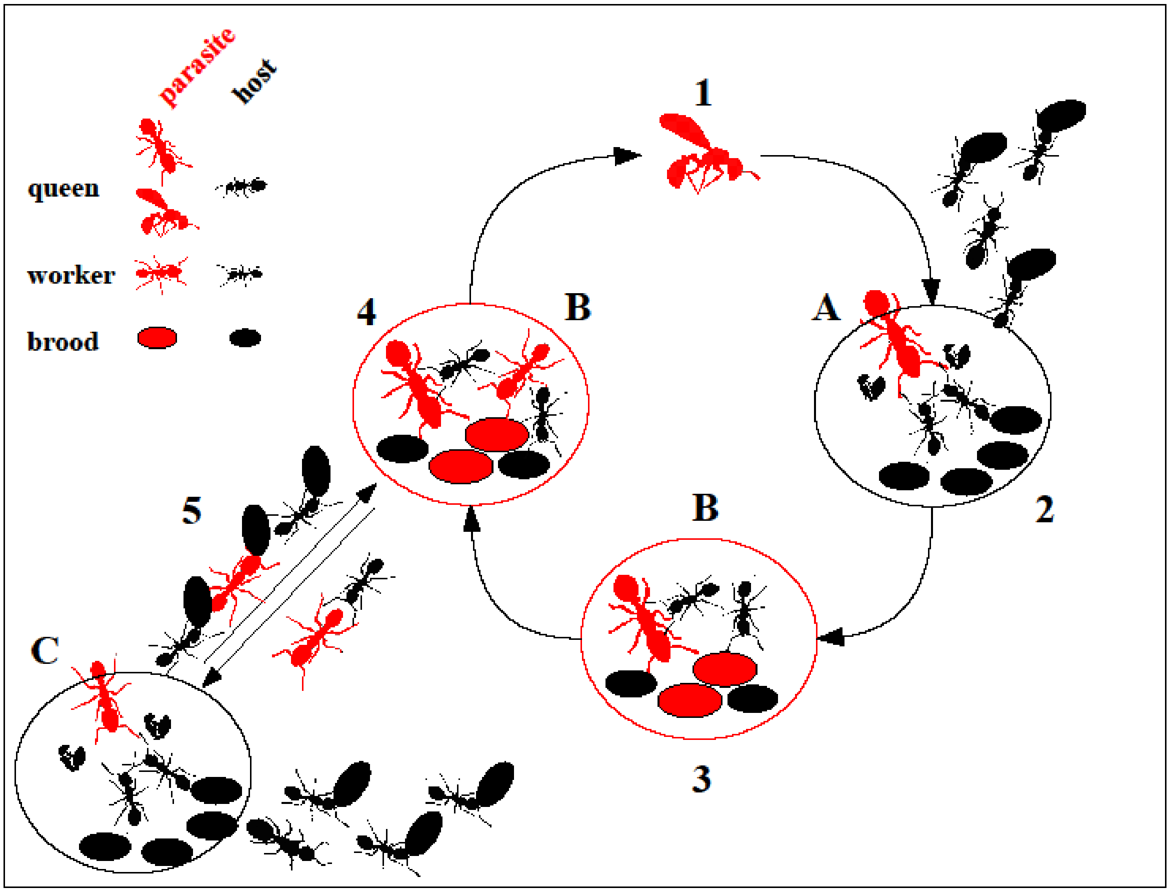

Figure 1: Illustrating the **parasite** life cycle (1-5) and main interaction points between parasite and **host** (A, B and C). **1.** A freshly mated parasite queen searches for a host nest. **2.** Once a suitable nest is located she will invade the nest, kill or drive away the resident adults and take over the remaining host brood. **3.** The parasite queen starts to produce parasite brood and, once the first host workers eclose, they will become the first generation of slaves. **4.** As soon as the first parasite worker emerge a fully functional parasite nest is established, with parasite and slave workers coexisting in the same nest. **5.** The parasite workers will start to attack surrounding host colonies during so-called raiding events and will steal brood with the help of the enslaved host workers, ensuring a steady income of new slave workers. When a parasite nest is mature it will start to produce sexuals and the cycle restarts. In summary, hosts have three regular encounters

with their parasites. Two of these (**A** and **C**) are characterized by open aggression and the host trying to defend their brood. While enslaved (**B**) host workers apparently coexist peacefully.

P.americanus are entangled in a coevolutionary arms race, which makes this system not only interesting from an ecological and behavioral point of view, but also from the evolutionary perspective (Foitzik and Herbers 2001a, Foitzik et al 2003, Brandt et al. 2005, Pennings et al. 2010). As such, we would expect additional host and parasite traits coevolving and ready to be discovered.

During the life cycle of the parasite *T. longispinosus*, hosts may frequently encounter *P. americanus* during three different events (Fig.1), two of which involve open aggressive interactions between the host colony and the intruding parasite(s). Both, during raiding events and parasite queen nest foundation, one or more parasites try to enter the nest and evict the colony in order to gain access to the brood (Alloway 1979). In both cases an individual *T. longispinosus* worker would not stand a chance against the parasite invasion, and successful host defense involves a coordinated and collective defense by the entire colony (Foitzik et al. 2001c). Thus, selection does not act directly on the individual level but on the entire colony (Korb and Heinze 2004). Individual level aggression appears to have some degree of heritability in many animals (Benus et al. 1991) and is accompanied by a complex genetic architecture in insects (Zwarts et al. 2011). Colony level aggression in *T. longispinosus* colony level, both across context and worker generations, is consistent. Such a consistency indicates a genetic basis of this collective trait and, consequently, selection may act on it (Modlmeier et al. 2012). At first sight, the third potential encounter a host worker can have with a parasite involves no open aggression.

Once host workers eclose in a parasite nest and start working as slaves they apparently coexist peacefully. However, this peaceful coexistence is misleading as individual slaves apply modified aggression in order to subvert the parasite suppression (Achenbach and Foitzik 2009).

In my thesis I studied host defense behavior, evolved in this tightly linked coevolutionary dynamic, in both the laboratory and the field. All of these adaptations are centered host aggression (on the colony or individual level) at all three interaction points between host and parasite during of the parasites' life cycle (Fig.1). In addition we were interested if we could find indication that parasites shape these defensive traits as expected in a tightly linked coevolutinary dynamic.

Publication 1

- was inspired by the ability of *T. longispinosus* to recognize their parasite (Alloway 1990). Under natural conditions, the most obvious encounters of hosts and parasites take place during raiding events. Raids of *P. americanus* are a sequence of events, which can be divided into two main stages: 1. the scouting stage and 2. the actual raiding event (Alloway 1979). During the scouting stage individual slavemaker leave their nest in search for suitable host nests (Pohl 2011a, b). Once they detect a host colony they often enter the nest and investigate it (Alloway 1979). After they reach the 'decision' (Pohl 2011a) to raid this particular colony they return home to recruit a raiding party consisting of additional slavemakers as well as slave workers (Alloway 1979), or try to take over the colony directly. Consequently, the presence of a *P. americanus* worker is always 'bad news'. It means that the colony is detected and in immediate danger - either to be taken over directly by the parasite worker or to be raided soon. We investigated if *T. longispinosus* colonies can defend themselves against intruding parasites and use this information (presence of a slavemaker within the nest), in order to prepare for the upcoming raiding event. As a first step we developed a bioassay to screen the aggression of a large number of host colonies in a standardized way and within reasonable time. After that we dissected the raiding event into an encounter sequence, simulating a raiding event as perceived by the host colony. By measuring the aggressive response of the colonies at every step of the encounter sequence, we wanted to find out if the information of the encounter sequence can be used by the host colony to alter their aggression in preparation of an upcoming raiding event.

Publication 2

- takes a closer look at colony level aggression of *T. longispinosus* colonies against different kinds of intruders and investigates if colonies are able to adjust their aggression level and defense strategies to match the threat of a specific intruder. We confronted the colonies with four different types of potential intruders, probably posing different levels of threat to the colony. We used the bioassays developed in publication 1 to measure colony level aggression. In addition, we used the qualitative information collected during the assays to investigate the specific defense strategy implemented by the colony.

Publication 3

In this study we had the rare chance to investigate the founding event of slavemaker queens under natural conditions. In 2010 we set up a field manipulation to investigate how density and parasite presence influence host colony aggression under field conditions. We collected data on 200 *T. longispinosus* colonies (including colony demographics and aggression) and conducted a full factorial field manipulation under semi-controlled natural conditions (in outdoor enclosures).

Due to an exceptionally warm spring the mating flight of the parasite occurred earlier than expected and the founding queens heavily targeted our enclosures, taking over more than 25% of our host colonies. This unexpected event gave us the unique opportunity to investigate a critical event in the life cycle of *P. americanus,* normally inaccessible to experimental investigation. Since we had collected many key parameters of the host colonies we could investigate parasite queen takeover success, local parasite pressure during the event as well as host colony defense.

Publications 4 and 5

- Center on a post-enslavement defense mechanism termed "slave rebellion" and described by Achenbach and Foitzik in 2009. It has been argued that post-enslavement behavioral defense mechanisms cannot evolve in dulotic systems, because slaves lack the behavioral options to increase their fitness directly and, as a result, post-enslavement behavioral defenses could no be selected for (Gladstone 1980). Instead of rearing the brood of their slavemakers successfully, as their own offspring, slaves kill or neglect a high proportion of the parasitic brood.

Publication 4

We investigated this defense trait on a large geographic scale including three well-studied parasitized and one unparasitized population, combining data from 6 years in an meta-analysis. Using this large data set we were interested in the expression of the slave rebellion trait on a large geographic scale and on how local differences in the ecological conditions shape the outcome of this aspect of host-parasite coevolution.

Publication 5

We examined the phenomenon from an evolutionary point of view that regards the slave rebellion phenomenon as somewhat puzzling. As mentioned above, slaves should not be able to actively increase their fitness via behavioral modifications. Additionally, at least as far as we know, slaves do not reproduce. So, the question remained how a trait can evolve if the individuals caring it do not reproduce, thus cannot increase their fitness directly. Publication 5 tackles this problem empirically. We hypothesized that slaves can increase their fitness not directly but indirectly via kin selection. Hence, we argue that indirect (kin) selection could explain the evolution and maintenance of this defense trait. Earlier studies showed that smaller slavemaker nests perform fewer successful raids each season (Foitzik et al. 2001b). By reducing slavemaker colony size through killing a proportion of slavemaker workers (Achenbach and Foitzik 2009, Publication 4), rebellious slaves should lower the parasite pressure on surrounding host colonies. If hosts within the potential raiding range of slavemaker colonies are on average higher related to the rebellious slaves, their behavior could increase the survival probability of closely related host colonies. A modeling approach indicates that a hypothetical slave rebellion allele could spread indeed, if the host population is adequately structured (Metzler unpublished). In this publication we investigated if the host population of *T. longispinosus* is adequately structured to create a potential indirect fitness benefit for the slaves and offer a potential escape from the evolutionary dead end (Gladstone 1981) slaves were thought to be trapped in.

Chapter 1: Increased host aggression as an induced defense against slavemaking ants

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Running title: Induced anti-parasite defense in ants

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Summary

Slavemaking ants reduce the fitness of surrounding host colonies through regular raids, causing the loss of brood and frequently queen and worker death. Consequently, hosts developed defenses against slave raids such as specific recognition and aggression towards social parasites and indeed we show that host ants react more aggressively towards slavemakers than towards non-parasitic competitors. Permanent behavioral defenses can be costly and if social parasite impact varies in time and space, inducible defenses, which are only expressed after slavemaker detection, can be adaptive. We demonstrate for the first time an induced defense against slavemaking ants: cues from the slavemaker *Protomognathus americanus* caused an unspecific, but long-lasting behavioral response in *Temnothorax* host ants. A five minute within-nest encounter with a dead slavemaker raised the aggression level in *T. longispinosus* host colonies. Contrarily, encounters with non-parasitic competitors did not elicit aggressive responses towards non-nestmates. Increased aggression can be adaptive if a slavemaker encounter reliably indicates a forth-coming attack and if aggression increases post-raid survival. Host aggression was elevated over three days, showing the ability of host ants to remember parasite encounters. The response disappeared after two weeks, possibly because by then the benefits of increased aggression counter-balance potential costs associated with it.

Keywords: aggression, behavior, phenotypic plasticity, parasites, social insects.

Introduction

Parasitism is a common lifestyle, and parasites reduce the fitness of most organisms by exploiting host resources for their own benefit. Consequently, host species have evolved anti-parasite defences to prevent parasitic exploitation as well as subsequent proliferation of parasites. Anti-parasite adaptations range from general responses, such as behavioural avoidance of areas with high infection risk (Christe et al., 1994) and morphological changes in plants (Schmid-Hempel and Ebert, 2003) up to complex and highly specific defence systems, such as the vertebrate adaptive immune system. All types of anti-parasite defences aim to improve host survival and reproduction, but the development and maintenance of these defence mechanisms are costly for the hosts (Sheldon and Verhulst, 1996). This has been clearly shown for the vertebrate immune system and experimental evidence is accumulating for less-specific defences in invertebrates as well (Kraaijeveld and Godfray, 1997, Moret and Schmid-Hempel, 2000). The evolution of host defence mechanisms frequently cause a decreased parasite fitness and consequent counter-adaptations of the parasite may result in an escalation of the host-parasite interaction (i.e., coevolutionary arms-races (Dawkins and Krebs, 1979)).

As defense strategies against parasites are costly, temporal and spatial variation in parasite presence may favor the evolution of inducible defenses, only expressed after parasite contact (Harvell, 1990). Inducible defenses are a general strategy against enemies with a patchy distribution in time and space, resulting in a varying impact on their victims. Inducible defenses were modeled either by applying game theory or by using environmental threshold approaches (Hazel et al., 2004). These models point to the following conditions favoring a flexible induced defense strategy over a fixed strategy: an unpredictably changing environment (biotic or abiotic), the availability of reliable cues associated with an attack, competitive interactions within patches and the cost of maintaining the defense (Harvell, 1990; Hazel et al., 2004). In addition, they reveal that the switch point or threshold at which it is beneficial for an individual to induce a defense should be when the expected fitness of an undefended individual equals that of a defended one (Hammill et al., 2008). There are numerous examples for such flexible anti-enemy defenses, mainly from predator–prey systems, such as water fleas (Tollrian, 1995) or frog tadpoles (Teplitsky and Laurila, 2007), which respond to cues of aquatic predators. These induced changes can be very complex and include shifts in victim morphology, behavior and life history strategies (Lass and Spaak, 2003).

Social parasitism, a wide-spread phenomenon in social insects is the association between two closely related species of social insects, where one species – the parasite – utilizes the brood care behavior of another species (the host) and / or uses its socially managed resources (e.g., food) (Buschinger, 2009). The behavior of insect social parasites resembles that of the well-studied avian brood parasites such as cuckoos or cowbirds, which also exploit the brood care behavior of another species (Kilner, 2006). The obligate social parasite and slavemaking ant *Protomognathus americanus* can use three different *Temnothorax* species as hosts. This parasite exerts especially strong selection pressures on its main host species *T. longispinosus*. Its high prevalence and frequent slave raids (Foitzik and Herbers, 2001), often lead to the destruction of attacked colonies, thus greatly reducing host fitness (Foitzik et al., 2009). *P. americanus* colonies are patchily distributed within host populations, resulting in an unequal parasitism risk for host colonies (Herbers and Foitzik, 2002). In addition, both host and parasite colonies are long-lived but frequently relocate their nests so that the local risk of a parasite attack also varies over time. Considering the theoretical predictions favoring inducible defenses, we expected that flexible defense mechanisms could have evolved in the *T. longispinosus – P. americanus* system in addition to the known fixed defenses.

In the slavemaking *P. americanus – Temnothorax* system fixed host defenses include enemy recognition and elevated aggression directed towards slavemakers (Alloway, 1990) and a recently shown behavioral defense of enslaved workers, which destroy parasite brood (Achenbach and Foitzik, 2009). Slavemakers are larger than their hosts and well-equipped with chemical and morphological weaponry (Brandt et al., 2005b), therefore host defenses against slavemaking ants occur predominantly on a cooperative level. Intruding slavemakers are simultaneously attacked by many host workers, because a single host worker would be overpowered by a parasite.

The slave raids of *P. americanus* colonies can be divided into two stages (Alloway, 1979): the scouting phase and the raiding event. When a *P. americanus* scout discovers a suitable target, i.e., a host colony, it enters the nest site (Pohl and Foitzik, in press) and after inspection, returns to its colony to recruit additional slavemaking workers as well as enslaved *Temnothorax* workers to participate at the following raid (Alloway, 1979). Therefore, raiding parties include workers of both ant species, i.e., slavemaker and host workers (Fig.1). Enslaved host workers that participate in raids often harm attack host colonies more than slavemakers, because these *Temnothorax* slaves frequently attack and sting defending host workers (Foitzik and Herbers, 2001). In contrast, *Protomognathus* workers only try to drive away defenders and use glandular secretions to cause confusion among host workers, but never sting (Foitzik and Herbers, 2001). Scouting events invariably precede slave raids, so the presence of a slavemaker worker within or close to the host nest provides a reliable indicator of a slavemaker attack on the host colony in the near future.

In the current study, we investigate whether an encounter with a slavemaker scout induces behavioral changes in free-living host colonies. More specifically, we test whether host colonies of the species *T. longispinosus* (the preferred host species) show an inducible defense against the *P. americanus* slavemaker when encountering an intruder within their nest site. Since slave raids are preceded by a visit of a slavemaker scout within or close to the nest, hosts are expected to attack such a scout more vigorously than conspecific workers from a different nest or a worker of a related, non-parasitic species. If scouts are reliably detected and killed, a raiding attack could be averted. However, if the slavemaker scout manages to escape, a slave raid can be anticipated and the colony should use the time to prepare for an attack. As raiding parties regularly consist of both slavemaker and slave workers, host colonies should not only become more aggressive towards slavemakers, but also to non-nestmate workers of their own species. In line with these expectations, we show that *T. longispinosus* host workers do not only react more aggressively towards slavemaking ants than to conspecifics during a first encounter, but in addition that a contact with a slavemaker worker induces an aggressive response towards non-nestmate conspecifics. This is the first example of an induced anti-social parasite defense in social insects.

Methods

Study system

The slavemaking ant *P. americanus* parasitizes three host species of the genus *Temnothorax*. Its preferred host, *T. longispinosus,* inhabits mixed deciduous forests across the northeastern USA, nesting in hollow acorns, hickory nuts and twigs in the leaf litter. Colonies were collected at the

Huyck Preserve, Albany County, NY (N 42° 31'35.3" W 74° 9'30.1") in March-April 2009. *T. curvispinosus* colonies, which belong to a congeneric, but non-parasitic species, were collected in August 2009 in the Watoga State Park, Pocahontas County, WV (N 38°06′13″ W 80°08′59″). Nests of both species were kept in artificial nests (7.5cm×2.5cm×0.5 cm) in plastic boxes (10cm×10cm×1.5cm) with a plastered floor in a climate chamber in Munich (day:night temperatures 20°C:15°C) under identical conditions at least half a year before the experiment started. Ants were fed weekly with honey, water and crickets. The experiments were conducted at room temperature.

Experimental set-up

We tested whether host colonies demonstrate an inducible response by confronting host nests with a sequence (four cycles of encounters) of dead ants, simulating the encounter sequence of an actual raiding event in nature (Fig.1). We used dead ants to eliminate behavioral variation among the stimuli and focus on the host ants' response to the chemical parasite stimulus. Our experiments resemble earlier studies on hosts of avian brood parasites, in which stuffed cuckoos (an artificial stimulus) were used to simulate a parasite threat (Moksnes et al., 1991). Experiments were conducted in October 2009 – March 2010. Ninety host colonies were split randomly into four experimental groups (three treatment groups and a control; Fig. 1). Host colonies included different social organizations representing natural variation including queenless, monogynous and polygynous colonies (see electronic supplement). The experiment consisted of four cycles (i.e., encounters with opponents), as explained below. Treatments differ in which dead opponent was introduced into the host nest in cycles 2 and 3 (Fig. 1).

Cycle 1: To estimate the base aggression of host colonies, each nest was confronted with a dead conspecific worker (hereafter, non-nestmate conspecific). The opponents belonged to *T. longispinosus* colonies from the same location (NY), not used in the experiment, and the colony reaction towards this dead worker was recorded.

Cycle 2: Three days later, treatment 1 and 2 were exposed to a dead slavemaking worker simulating the presence of a slavemaker scout within the host nest while the control group was again confronted with a dead non-nestmate conspecific worker. Colonies in treatment 3 were exposed to a worker of a related congeneric species, *T. curvispinosus* (see electronic supplement for sample sizes). The experiments with treatment group 3 were conducted three months after the original experiment. We included the 3rd treatment group to determine whether the observed behavioral reactions were caused specifically by the slavemaker or by differences in chemical recognition cues in general. Previous chemical analysis has already shown that even though *T. curvispinosus* is phylogenetically closer to *T. longispinosus*, it is chemically more distant to it than the slavemaker *P. americanus*, which mimics the odor of its host (Brandt et al., 2005b). Assuming that chemical distance triggers the observed response, colonies should react to *T. curvispinosus* workers equally or more aggressively than to a *P. americanus* worker.

Cycle 3: Three days after cycle 2, treatment 1 was confronted with a dead non-nestmate conspecific worker representing an enslaved non-nestmate conspecific participating at a raiding event (following the encounter with a slavemaker scout), while treatment 2 was again confronted with a dead slavemaking worker representing a slavemaker present at a raiding event. Treatment 3 and the control were again confronted with a dead non-nestmate conspecific.

Cycle 4: Fourteen days later, all treatments except for treatment 3 faced a dead non-nestmate conspecific worker (cycle 4), to determine the persistency of the induced response (Fig.

1). The purpose here was to estimate how long a possible induced aggression triggered by an encounter with a slavemaker should last.

All opponents (i.e., non-nestmate conspecifics, related species workers or slavemaking workers) were frozen one day before the experiment and stored at -20°C. Due to the small number of slavemaking workers present in a nest (Foitzik and Herbers, 2001), slavemaking workers belonging to 25 nests were pooled and randomly chosen for the experiment. Each opponent was used three times. To exclude possible effects of reusing opponents, each colony always received the opponent in the same order (e.g., if the colony encountered a non-nestmate conspecific, which was used twice before, it then encountered a slavemaking scout, which was also used twice before). We refer to this order of encounter as the 'replication number'. Opponents were defrosted before the experiment and left outside the colony for five minutes before re-usage.

At the beginning of each encounter the opponent was placed ~1 cm away from the colony center inside the artificial nest. After the placement of the opponent the interactions of all ants in direct contact with the opponent (antennal contact) were recorded every 20 sec during the first minute and every 30 seconds for the following four minutes (11 observations in total). We scored antennation events (**a**) as non-aggressive interactions and six other behavior types as aggressive interactions: Mandible spreading (**m**), biting (**b**), holding (**h**), dragging (**d**), and stinging (**s**). All behavioral reactions were easily distinguished. Behavioral responses were summed for the 11 observations, and are usually composed of more than one ant responding simultaneously. An aggression index was calculated for each encounter using the formula $\frac{\frac{m}{2}+b+h+d+s}{a+m+b+h+d+s}$ expressing the colony aggression as percentage of aggressive interactions of all interactions with the opponent. Mandible spreading was scored as 0.5 times an aggressive interaction, because there was no physical contact between ants in this case and it involved only threatening (in contrast to actual attacks). We used another index to test whether our results were robust to changes in the way aggression was measured. We used the total number of aggressive interactions observed during the five minute encounter (the numerator of the above formula). The results did not differ qualitatively (see electronic supplement). We thereafter only refer to the analysis based on the ratio between aggressive interactions / all interactions, because it controlled for colony size and activity of the colony.

Statistical analysis

Colony structure for all treatments is summarized in Table 1 (electronic supplemental material). All aggression measurements were log-transformed, because they were not normally distributed. First, we tested for homogeneity of the experimental groups regarding replication number, social structure (0, 1 or more than 1 queen) and worker number using one-way ANOVA for worker number as the dependent variable, and a Pearson χ^2 tests for social structure. Groups indeed were found to be homogenous in respect to worker number ($F_{3,86} = 1.94$, $P = 0.13$) and social structure ($\chi^2 = 5.47$, df = 6, $P = 0.49$). Then, we tested for the effects of social structure, worker number and replication number on base aggression (i.e., first cycle experiencing the same stimulus: non-nestmate conspecific), using an ANCOVA. We did not find any effect of worker number ($F_{1,84} = 0.47$, $P = 0.50$) or social structure ($F_{2,84} = 2.03$, $P = 0.14$) on base aggression, but aggressive response decreased with replication number ($F_{1,84} = 7.14$, $P = 0.009$). Therefore, only replication number, treatments and cycles were included in the later analysis.

The main analysis of the data involved three repeated-measures ANOVA tests, with experimental groups and replication number, as the between subjects factors, and cycle, as the within subjects factor. The dependent factor was always the aggression index. The first test compared the 1st and 2nd cycle, re-examining Alloway's (1990) demonstration that slavemakers are treated more aggressively than either non-nestmate conspecifics or ants of a related species. For this analysis we combined treatment 1 and treatment 2 since both groups received the same treatment in cycle 2. We used a Fisher LSD post hoc analysis to test for differences in aggression directed towards different opponents (i.e., non-nestmate conspecific (control), slavemaker and related species). The second repeated-measures ANOVA tested for induced aggression following an encounter with a slavemaker. We compared among the four treatments (control and treatments 1-3) between the 1st and 3rd cycle. No difference in this test between treatments 1 and 2, in spite of the different opponent (non-nestmate conspecific in treatment 1 and slavemaker in treatment 2), would indicate an induced response after an encounter with a slavemaker. We investigated potential differences between treatment 1 and 2 using a post-hoc testing for the difference among the treatment groups. Finally, the third repeated-measures ANOVA included only 3 groups (control, treatment 1 and 2) and aimed at testing whether the elevated aggression persists for 14 days. We tested cycle 1 and 4 as the within subjects variable. No differences in aggression levels would indicate that the induced aggressive response collapsed. Finally, we were interested to see whether there was a consistency in aggression of specific colonies in successive encounters. We correlated aggression levels in cycle 1 with those in cycle 4 using a Pearson's correlation test on log-transformed aggression values. We chose these cycles, because all colonies received the same treatments (encounter with a non-nestmate conspecific).

Results

The repeated-measures ANOVA between cycle 1 and 2 showed a higher aggression level towards the slavemaker than the non-nestmate conspecific (control) and the related species (the interaction term cycle × treatment was significant: $F_{2,81} = 11.40$, $P < 0.0001$). The Fischer LSD post hoc indicated a significant difference between colonies facing a slavemaker and a non-nestmate conspecific ($P = 0.010$) and a related species, i. e. *T. curvispinosus* worker ($P < 0.0001$), but no difference between non-nestmate conspecific and related species ($P = 0.17$). Replication number (i.e., whether the opponent, either slavemaker, conspecific or related species, was used on the first, second or third time) was taken into account and was marginally significant ($F_{2,81} = 3.00$, $P = 0.056$), and none of its interactions with other factors were significant ($P > 0.1$ for all interactions).

The repeated-measures ANOVA between cycle 1 and 3 showed that the previous encounter affects aggression in cycle 3, i.e., aggression towards non-nestmate conspecifics was elevated only if colonies had encountered a slavemaker before (the interaction term cycle × treatment was significant: $F_{3,78} = 17.56$, $P < 0.0001$; post-hoc indicated a significant difference between the control and the two treatments ($P = 0.005$, $P = 0.004$), but not between treatment 1 and 2 ($P = 0.94$). In addition, encounters with *T. curvispinosus* did not differ from the control (non-nestmate conspecific; post-hoc: $P = 0.10$) but was different from both other treatments ($P < 0.0001$, $P < 0.0001$). Replication number was taken into account and had a significant effect ($F_{2,78} = 6.72$, $P = 0.002$), but its interactions with other factors were not ($P > 0.3$ for all interactions).

The last repeated-measures ANOVA between cycle 1 and 4 demonstrated that the elevated aggression levels collapsed after 14 days: There was no difference between cycles ($F_{2,57} = 1.34$, P = 0.27). Replication number was taken into account and was significant ($F_{1,57}$ = 3.82, P = 0.028), but its interactions with other factors were not (P > 0.45 for all interactions).

In order to show whether colonies showed consistent aggression levels, we correlated between the aggression levels when all colonies faced non-nestmate conspecifics (cycle 1 and 4). The correlation was significant (Bartlett χ^2: 12.25, df = 1, P < 0.001, r = 0.42), indicating behavioral consistency of colonies.

Discussion

By simulating a scouting event (i.e., the presence of a slavemaker ant within a host colony), that invariably precedes destructive slave raids, we were able to induce a long lasting and strong aggressive reaction of host colonies, directed towards non-nestmate conspecifics. After the encounter with a slavemaking ant, host colonies reacted towards non-nestmate conspecific similarly aggressive as towards slavemakers. In addition, we confirmed the findings of Alloway (1990) showing that host colonies treat slavemaking ants more aggressive than either non-nestmate conspecifics or a congeneric species.

As Alloway (1990) has demonstrated, host colonies are able to discriminate slavemaker ants from other congeneric ant species and react with elevated aggression directed towards them. The response towards slavemaker ants is fixed and always aggressive independent of context. This fixed aggressive response makes intuitive sense, since an encounter with a slavemaker worker within the nest could either present a reliable cue indicating a forthcoming or an ongoing slave raid. In both cases elevated aggression should increase the host colony fitness either by eliminating the scout, preventing the slavemaking raid or by improving the chance of surviving the raiding event. Behavioral experiments have demonstrated that higher aggression directed towards slavemakers (e.g., number of injured and killed slavemakers) result in a higher proportion of brood being rescued by attacked host colonies (Foitzik et al., 2001).

The response towards non-nestmate conspecifics, on the other hand, is context-dependent and therefore flexible, as it is adjusted according to a previous encounter with the slavemaker (more aggressive after such an encounter). An induced elevated aggressive response towards conspecifics could be adaptive under natural conditions if contact to a single slavemaking ant in the nest is a reliable cue that a slave raid is forthcoming, and if the consequent lasting aggressive response towards conspecifics results in higher survival probability of the attacked colony. In the raiding context, non-nestmate conspecifics (enslaved host workers) are tricked to cooperate with slavemakers (Alloway, 1979) and behavioral observations show that their impact on attacked host colonies is even stronger than that of slavemakers (Foitzik et al., 2001). Therefore, conspecifics encountered during a raid pose a much greater threat to host colonies than conspecifics in other contexts. *Temnothorax* host colonies are susceptible to raids of slavemaking ants. In the studied environment, host colonies have a chance as high as 50% each year to be attacked (Foitzik et al., 2001). Such a high attack probability can explain the evolution of an induced defense.

Conspecific colonies, albeit competing for the same resources, normally do not represent a similar high and immediate threat to host colonies. Behavioral experiments have revealed that aggression directed towards competitors is variable and environment-dependent. Colonies originating from high density areas were found to be more aggressive towards non-nestmate conspecifics than colonies from low density areas (Modlmeier & Foitzik, in review). Moreover, host colonies were shown to react more aggressively towards enslaved host workers during the raiding season in summer than in spring, when *Protomognathus* colonies never go on raids

(Brandt et al., 2005a). In addition to this seasonal adjustment in the aggression level, we provide evidence here that *T. longispinosus* colonies up-regulate their aggressive response towards non-nestmate conspecifics in reaction to parasite cues.

We initially expected that host colonies would increase their aggression towards both non-nestmates and slavemakers, because it is known from other ant species that the repeated encounters between non-nestmates can cause elevated aggression levels (Van Wilgenburg et al., 2010). In contrast, our results show that aggression following confrontation with a slavemaker increased only towards non-nestmates, while aggression against slavemakers was always on a high level. One explanation could be that hosts are always as aggressive as possible against slavemakers, but are able to adjust their aggressiveness against non-nestmate conspecifics. The variable aggression of host colonies directed towards non-nestmate conspecifics supports the assumption that host colonies can adjust their aggression against non-nestmate conspecifics (Modlmeier and Foitzik, in review).

This observed induced aggressive response must be mainly stimulated by chemical cues, because we used dead opponents in the experiment, which eliminate behavioral components in the detection process. Ants are known to use specific cuticular compounds, mainly hydrocarbons, to differentiate between nestmates and non-nestmates (Hefetz, 2007). Slavemaker chemical profile was sufficient to trigger the observed host reaction, while a congeneric species, which is chemically more distant, *T. curvispinosus* (Brandt et al., 2005b) did not result in a strong aggressive response of the host colonies nor did it trigger a similar induced behavior as observed after a slavemaker stimulus (Fig. 2). This indicates that the signal generating the observed behavior is slavemaker-specific. Dead ants elicited strong host responses and we expect that living parasites could induce even stronger reactions. Therefore, our results may even underestimate the level of aggression under natural conditions.

The observed elevated aggression levels persist for at least three days. The stimulation followed by a down-regulation of aggression levels suggests that potential fitness costs are associated with the observed behavior. Substantial costs might be associated with high levels of aggression, in accordance with the recent descriptions of behavioral syndromes (Sih et al., 2004). Possible costs could be lowered colony efficiency if increased aggression interferes with colony routines or even aggression towards nestmates. Detecting possible costs of induced defense are important for explaining why such behavior or morphology is not always maintained, regardless of the predator/parasite presence. Possible major costs are reduced longevity, fecundity and body size and increased development time. Such costs were recently found to be less severe than expected. They are expressed mostly in stressful habitats, and are therefore context-dependent (Van Buskirk and Steiner, 2009). The types of induced response of prey against potential predators can also be context-dependent. Predators can induce morphological changes in their potential prey (e.g., neckspines in water fleas; Tollrian, 1995) or behavioral changes (e.g., hiding, decreasing activity level and spatial avoidance of predators (Lima, 1998). It is expected that behavioral changes induced by predators should be the strongest when competition is not that strong and resources are not scarce. Otherwise, potential prey would keep foraging and remain active in order to avoid starvation (Teplitsky and Laurila, 2007). In such cases, morphological defense can be induced. Therefore, the expression of different anti-predatory induced responses is environment-dependent. In our system, we speculate that competition with conspecifics colonies is less important than avoiding raids by slavemaking ants. Otherwise, host colonies would react always more aggressively to conspecifics.

Finally, data of different host-slavemaker systems indicate that the aggression level of free-living colonies increases during the raiding season of the slavemakers (Brandt et al., 2005a, D'Ettorre et al., 2004). Our findings offer a potential proximate explanation: the increased encounter rate of host workers with slavemaking workers during the season, followed by the triggered and lasting defense reaction could contribute to the elevated aggression observed. An interesting finding in our experiment showed consistency in aggressive responses of host colonies, unrelated to treatments, indicating individual innate aggression (i.e., strong correlation among the aggression level of colonies in successive cycles). Some colonies were generally more aggressive than others, while a proportion of colonies showed a consistent weak response towards intruders. Understanding what keeps such behavioral variation in the population is an interesting future direction, because both natural selection and drift operate to remove variation from populations (Brockmann, 2001). Variation is kept due to various mechanisms such as favoring distinct phenotypes under different environmental situations or under different developmental stages, and negative frequency dependent selection, operating when the success of a phenotype depends on its frequency in the population (Brockmann, 2001). An additional possible future direction includes quantifying potential costs of the induced aggressive behavior as well as measuring the actual fitness benefit host colonies may gain by this induced behavioral defense.

References

ACHENBACH, A. & FOITZIK, S. 2009. First evidence for slave rebellion: enslaved ant workers systematically kill the brood of their social parasite protomognathus americanus. *Evolution,* 63**,** 1068-1075.

ALLOWAY, T. M. 1979. Raiding Behavior of 2 Species of Slave-Making Ants, Harpagoxenus-Americanus (Emery) and Leptothorax-Duloticus Wesson (Hymenoptera, Formicidae). *Animal Behaviour,* 27**,** 202-210.

ALLOWAY, T. M. 1990. Slave-Species Ant Colonies Recognize Slavemakers as Enemies. *Animal Behaviour,* 39**,** 1218-1220.

BRANDT, M., FOITZIK, S., FISCHER-BLASS, B. & HEINZE, J. 2005a. The coevolutionary dynamics of obligate ant social parasite system-between prudence and antagonism. *Biological Reviews,* 80**,** 251-267.

BRANDT, M., HEINZE, J., SCHMITT, T. & FOITZIK, S. 2005b. A chemical level in the coevolutionary arms race between an ant social parasite and its hosts. *Journal of Evolutionary Biology,* 18**,** 576-586.

BROCKMANN, H. J. 2001. The evolution of alternative strategies and tactics. *Advances in the Study of Behavior, Vol 30,* 30**,** 1-51.

BUSCHINGER, A. 2009. Social parasitism among ants: a review (Hymenoptera: Formicidae). *Myrmecological News,* 12**,** 219-235.

CHRISTE, P., OPPLIGER, A. & RICHNER, H. 1994. Ectoparasite Affects Choice and Use of Roost Sites in the Great Tit, Paras Major. *Animal Behaviour,* 47**,** 895-898.

D'ETTORRE, P., BRUNNER, E., WENSELEERS, T. & HEINZE, J. 2004. Knowing your enemies: seasonal dynamics of host-social parasite recognition. *Naturwissenschaften,* 91**,** 594-597.

DAWKINS, R. & KREBS, J. R. 1979. Arms Races between and within Species. *Proceedings of the Royal Society of London Series B-Biological Sciences,* 205**,** 489-511.

FOITZIK, S., ACHENBACH, A. & BRANDT, M. 2009. Locally adapted social parasite affects density, social structure, and life history of its ant hosts. *Ecology,* 90**,** 1195-1206.

FOITZIK, S., DEHEER, C. J., HUNJAN, D. N. & HERBERS, J. M. 2001. Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. *Proceedings of the Royal Society of London Series B-Biological Sciences,* 268**,** 1139-1146.

FOITZIK, S. & HERBERS, J. M. 2001. Colony structure of a slavemaking ant. II. Frequency of slave raids and impact on the host population. *Evolution,* 55**,** 316-323.

HAMMILL, E., ROGERS, A. & BECKERMAN, A. P. 2008. Costs, benefits and the evolution of inducible defences: a case study with Daphnia pulex. *Journal of Evolutionary Biology,* 21**,** 705-715.

HARVELL, C. D. 1990. The Evolution of Inducible Defense. *Parasitology,* 100**,** S53-S61.

HAZEL, W., SMOCK, R. & LIVELY, C. M. 2004. The ecological genetics of conditional strategies. *American Naturalist,* 163**,** 888-900.

HEFETZ, A. 2007. The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae) – interplay of colony odor uniformity and odor idiosyncrasy. A review. *Myrmecological News,* 10**,** 59-68.

HERBERS, J. M. & FOITZIK, S. 2002. The ecology of slavemaking ants and their hosts in north temperate forests. *Ecology,* 83**,** 148-163.

KILNER, R. M. 2006. The evolution of egg colour and patterning in birds. *Biological Reviews,* 81**,** 383-406.

KRAAIJEVELD, A. R. & GODFRAY, H. C. J. 1997. Trade-off between parasitoid resistance and larval competitive ability in Drosophila melanogaster. *Nature,* 389**,** 278-280.

LASS, S. & SPAAK, P. 2003. Chemically induced anti-predator defences in plankton: a review. *Hydrobiologia,* 491**,** 221-239.

LIMA, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions - What are the ecological effects of anti-predator decision-making? *Bioscience,* 48**,** 25-34.

MOKSNES, A., ROSKAFT, E. & BRAA, A. T. 1991. Rejection Behavior by Common Cuckoo Hosts Towards Artificial Brood Parasite Eggs. *Auk,* 108**,** 348-354.

MORET, Y. & SCHMID-HEMPEL, P. 2000. Survival for immunity: The price of immune system activation for bumblebee workers. *Science,* 290**,** 1166-1168.

POHL, S.& FOITZIK, S. Slave-making ants prefer larger, better defended host colonies. *Animal Behaviour*, in press.

SCHMID-HEMPEL, P. & EBERT, D. 2003. On the evolutionary ecology of specific immune defence. *Trends in Ecology & Evolution,* 18**,** 27-32.

SHELDON, B. C. & VERHULST, S. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution,* 11**,** 317-321.

SIH, A., BELL, A. & JOHNSON, J. C. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution,* 19**,** 372-378.

TEPLITSKY, C. & LAURILA, A. 2007. Flexible defense strategies: Competition modifies investment in behavioral vs. morphological defenses. *Ecology,* 88**,** 1641-1646.

TOLLRIAN, R. 1995. Predator-Induced Morphological Defenses - Costs, Life-History Shifts, and Maternal Effects in Daphnia-Pulex. *Ecology,* 76**,** 1691-1705.

VAN BUSKIRK, J. & STEINER, U. K. 2009. The fitness costs of developmental canalization and plasticity. *Journal of Evolutionary Biology,* 22**,** 852-860.

VAN WILGENBURG, E., CLEMENCET, J. & TSUTSUI, N. D. 2010. Experience influences aggressive behaviour in the Argentine ant. *Biology Letters,* 6**,** 152-155.

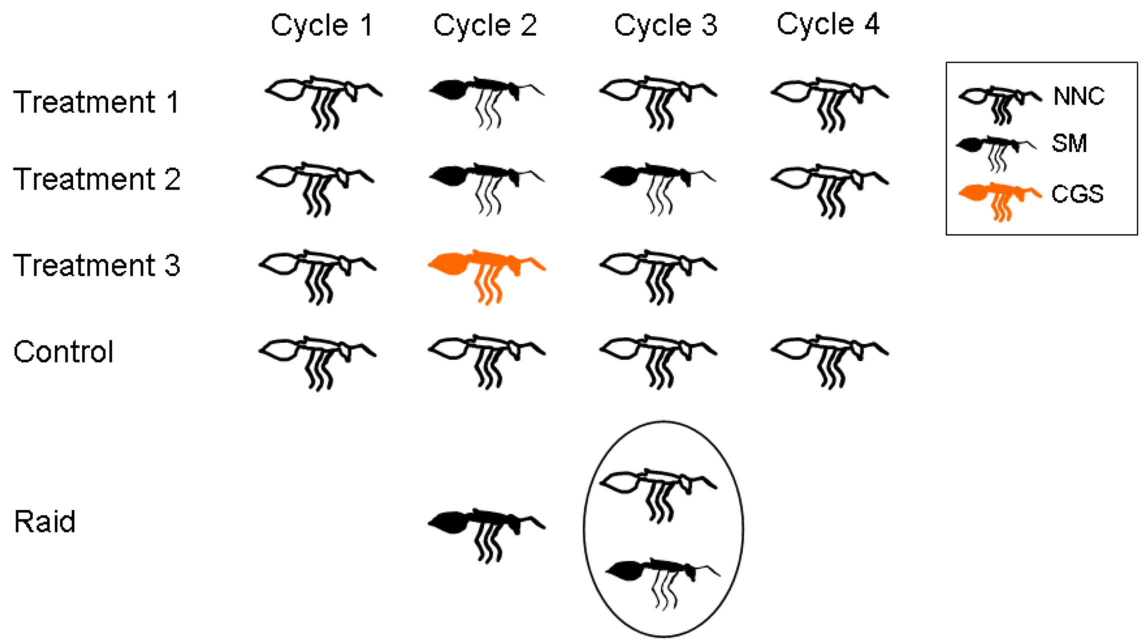

Fig. 1: The experimental setup, demonstrating the encounter sequence of the three different treatments and the control during the experimental cycles. "NNC" stands for a non-nestmate *T. longispinosus* conspecific; "SM" stands for a slavemaking worker of the species *P. americanus* and "CGS" stands for congeneric non-parasitic species (*T. curvispinosus*). First, all treatments were exposed to non-nestmate conspecifics, to evaluate the base aggression of each colony. Second, treatments 1 and 2 were exposed to a slavemaking worker, representing a slavemaker scout. Treatment 3 was exposed to a congeneric species and the control was again confronted with a non-nestmate conspecific. In order to test whether an encounter with a slavemaking worker induces elevated aggression, treatments 1 and 2 were exposed to a non-nestmate conspecific and again to a slavemaking worker. Treatment 3 and the control investigate whether high aggression is induced by other stimuli (non-nestmate conspecific and a congeneric species).

The last cycle aims at estimating the duration of the induced aggression. In addition, the encounter sequence of a raiding event, consisting of the scouting phase (cycle 2) and the raiding event (cycle 3), from the perspective of a host colony, is presented.

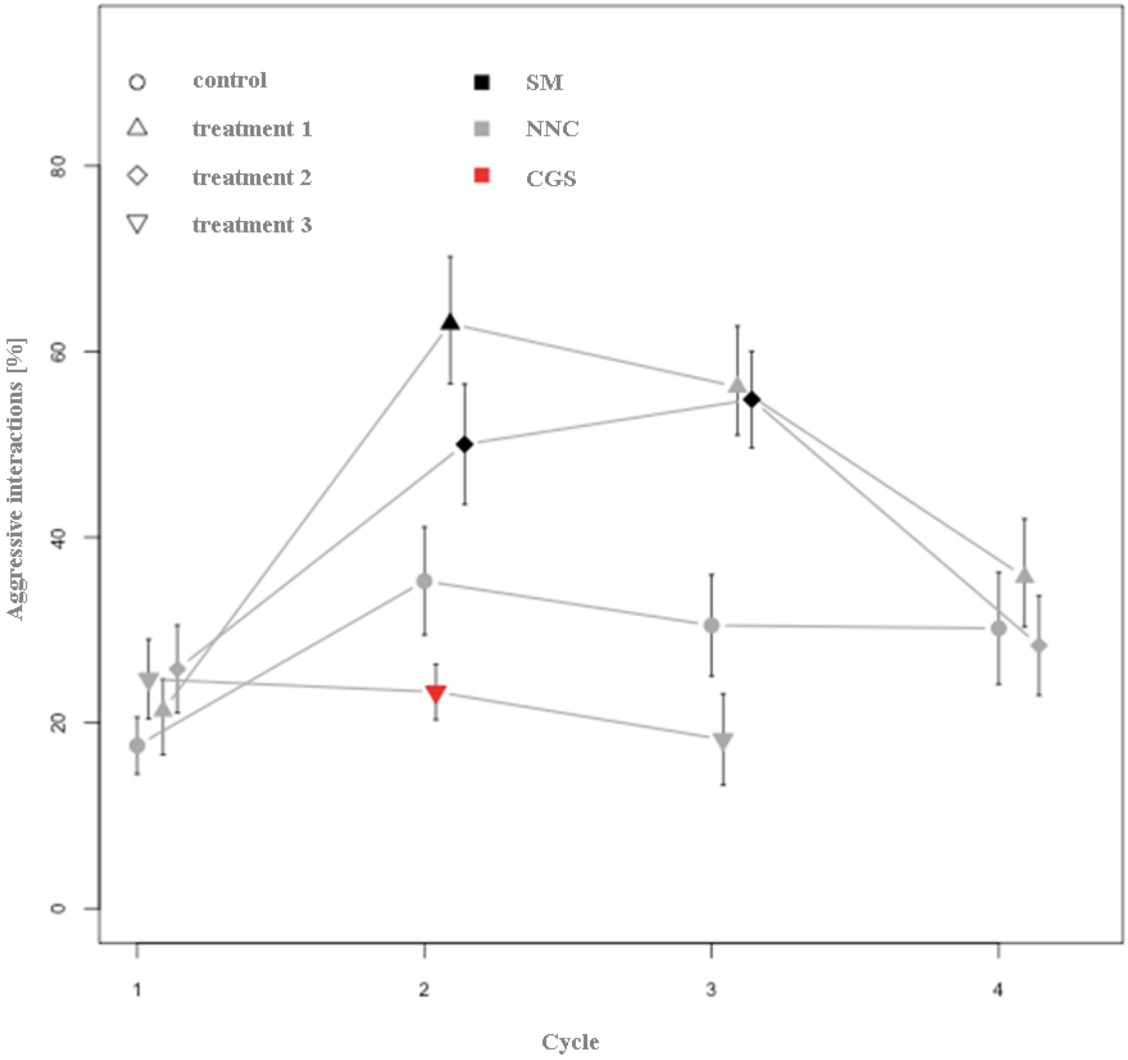

Fig. 2: Mean aggression per treatment and cycle. Control: circles; treatment 1: triangles (upwards); treatment 2: diamonds and treatment 3: triangles (down wards). Non-nestmate *T. longispinosus* opponent (non-nestmate conspecific, NNC) (grey), slave-making worker of the species *P. americanus* (slavemaker, SM) (black) and *T. curvispinosus* (congeneric species, CGS)

(red). Cycle 2, 3 and 4 took place 3, 6 and 20 days after cycle 1, respectively. Means ± 1 SE are presented.

Electronic supplemental

Table 1: Sample size and average number of queens and workers per treatment

	N	Queens (mean ± 1 S.D.)	Workers (mean ± 1 S.D.)
Control	22	1.6 ± 1.2	33.8 ± 11.7
Treatment 1	20	1.5 ± 0.5	38.7 ± 18.7
Treatment 2	24	1.2 ± 0.7	33.2 ± 14.6
Treatment 3	24	1.3 ± 0.8	27.2 ± 18.0

Alternative data analysis based on total aggression

The repeated-measures ANOVA between cycle 1 and 2 showed a higher aggression level towards the slavemaker than the non-nestmate conspecific (control) and the related species (the interaction term cycle × treatment was significant: $F_{2,81} = 10.32$, $P < 0.0001$). The Fischer LSD post hoc indicates a significant difference between colonies facing a slavemaker and the non-nestmate conspecific ($P = 0.008$) and a related species ($P < 0.0001$), but no difference between non-nestmate conspecific and related species ($P = 0.09$). Replication number (i.e., whether the opponent, either slavemaker, conspecific or related species, was used on the first, second or third time) was taken into account and was marginally significant ($F_{2,81} = 3.00$, $P = 0.055$), and none of its interactions with other factors were significant ($P > 0.14$ for all interactions).

The repeated-measures ANOVA between cycle 1 and 3 showed that the previous encounter affects aggression in cycle 3, i.e., aggression was elevated by non-nestmate conspecifics only if colonies had encountered a slavemaker before (the interaction term cycle × treatment was significant: $F_{3,78} = 18.75$, $P < 0.0001$; post hoc indicates on a significant difference between the control and the two treatments ($P = 0.01$, $P = 0.006$) but not between treatments ($P = 0.94$). The related species did differ from the control (post-hoc: $P = 0.01$) being treated less aggressive then the control and different from both other treatments ($P < 0.0001$, $P < 0.0001$). Replication number was taken into account and was significant ($F_{2,78} = 5.4$, $P = 0.006$), but its interactions with other factors were not ($P > 0.69$ for all interactions).

The last repeated-measures ANOVA between cycle 1 and 4 demonstrated that the elevated aggression levels collapsed after 14 days: There was no difference between cycles ($F_{2,57} = 0.5$, $P = 0.56$). Replication number was taken into account and was significant ($F_{1,57} = 3.87$, $P = 0.026$), but its interactions with other factors were not ($P > 0.3$ for all interactions).

Chapter 2: Differential response of ant colonies to intruders: attack strategies correlate with potential threat

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Abstract

Animals are often threatened by predators, parasites or competitors and attacks against these enemies are a common response, which can help to remove the danger. The costs of defense are complex, and involve the risk of injury, the loss of energy/time and the erroneous identification of a friend as a foe. Our goal was to study the specificity of defense strategies. We analyzed the aggressive responses of ant colonies by confronting them with workers of an unfamiliar congeneric species, a non-nestmate conspecific, a co-occurring congeneric competitor species and a social parasite – a slavemaking ant. As expected, the latter species, which can inflict dramatic fitness losses to the colony, was treated with most aggression. A co-occurring competitor was also attacked, but the ants used different behaviors in their responses to both enemies. While the slavemaker was attacked by biting, stinging and was approached with spread mandibles, the competitor was dragged, a behavioral strategy only possible if the defending ant is similar in size and strength to the opponent. Non-nestmate conspecifics were treated aggressively as well, but less than the slavemaker and the co-occurring competitor, presumably because they are less easily recognized as enemies. An unfamiliar congeneric species was rarely attacked. This first detailed study comparing the aggressive responses of ant colonies towards slavemaking ants to other species posing different threats indicates that the responses of ant colonies are adjusted to the risk each opponent poses to the colony.

Introduction

Animals often fight against predators, parasites and competitors in different ways, such as by direct physical attack (e.g., territorial defense; Hölldobler & Wilson 1990, ch. 10) or indirectly (e.g., developing special morphology as spines in water fleas against fish predation; Tollrian 1995). The benefits of defense are apparent – the danger can be removed and the potential decrease in fitness is circumvented. The costs of defense involve three components: risk of injury/failure, loss of energy/time including other physiological trade-offs, and risk of an erroneous identification (e.g., Zuk & Stoehr 2002; Rivera-Marchand et al. 2008). Animals should therefore trade-off the benefits and costs, and if the latter are too high, they should use an alternative strategy, such as flight (Rivera-Marchand et al. 2008; Arnott & Elwood 2009).

Social insects face two major problems in succession: First, how to identify potential threats and distinguish between friends and foes (e.g., Guerrieri & D'Ettorre 2008); second, in which way to respond to these dangers (e.g., Hughes & Goulson 2001). In social insects, recognition is predominantly based on a comparison between the cuticular hydrocarbon profiles of opponents and defenders and to a lesser extent by chemicals produced by the mandibular gland, i.e., the alarm pheromone (e.g., Jaffe & Sanchez 1984; Hefetz 2007). Correct identification of enemies becomes challenging if profiles are similar (e.g., if the intruder is related to the colony). Interestingly, although the cuticular hydrocarbon profile is generally heritable, it can be also modified by the environment or by the addition of external substances. This process can lead to rejection of nestmates that have been kept for long time out of the nest or in a different nest site type (e.g., Heinze et al. 1996; Katzav-Gozanski et al. 2004). Second, potential enemies are treated differently, based on the threat they inflict on the colony (e.g., Alloway 1990; Whitehouse & Jaffe 1996; Von Beeren et al. 2011). The reason for this gradual response is the costs associated with elevated aggression and the context (see references in Tanner & Adler 2009), e.g., familiarity with the risk (the 'dear-enemy phenomenon'; Heinze et al. 1996; Dimarco et al. 2010). Social insects fight against competitors for territory, nest sites and resources (Hölldobler & Wilson 1990, ch. 10; Dornhaus & Powell 2010) and the tendency to fight against competitors varies among species according to their behavioral dominance (e.g., Pearce-Duvet & Feener 2010).

The level of aggression exhibited by colonies towards intruders often differs between non-nestmate conspecifics and heterospecifics (e.g., Stuart 1991, 1993; Guerrieri & D'Ettorre 2008). Intra-specific competitors fill the same ecological niche, and due to more intense competition should elicit stronger responses. On the other hand, non-nestmate conspecifics are more difficult to recognize as they are chemically closer to the colony than different species. Empirical results are mixed: heterospecifics are attacked more frequently in some but not all species (see references in Stuart 1991). Other traits, such as colony and body size, should also correlate with aggression (e.g., Stuart 1991; Nowbahari et al. 1999; Pearce-Duvet & Feener 2010; but see, Stuart & Herbers 2000; Satoh & Hirota 2005).

Social parasitism occurs between two social insect species, one of which takes advantage of the brood care behavior of the other species and/or uses its socially managed resources (e.g., food) (Hölldobler & Wilson 1990, ch. 12; Buschinger 2009). Slavemaking ants, a specific group of social parasites, depend on other ants to complete their lifecycle. A mated slavemaking ant queen invades a host ant nest, takes over, and expels or kills host workers and their queen. The slavemaking workers do not perform routine tasks of workers in ant colonies (such as foraging, cleaning the nest and taking care of the brood). Instead, they specialize in host colony detection and attack, from which they replenish their work force through organized slave raids (Hölldobler & Wilson 1990, ch. 12; Buschinger 2009). The brood is stolen and many workers and occasionally the queen(s) are killed during the fights (Alloway 1979; Foitzik & Herbers 2001). Due to the high costs of slave raids, host colonies show a strong aggressive response towards invading slavemakers (Alloway 1990; Pamminger et al. 2011). Surprisingly, the behavioral response of host colonies towards intruding slavemaking ants has been rarely studied (but see Alloway 1990; Pamminger et al. 2011) and these few studies lack ethological details, such as a detailed differentiation of ant aggression and the changes over time.

In this study we analyze in detail the aggressive response of the ant *Temnothorax longispinosus* facing four different invaders into their nests: (1) *T. curvispinosus*, a related species, not co-occurring with *T. longispinosus* in the habitat of origin; (2) non-nestmate conspecifics; (3) *T. ambiguus*, a competitor co-occurring in the same habitat; and (4) a slavemaking worker of *Protomognathus americanus*, a social parasite of *T. longispinosus*. We use fine-scale observations (short time intervals) and document several aggressive behaviors, in order to define the defensive strategies towards different opponents. This study has two main goals: (1) Presenting a detailed ethological analysis of the aggressive responses of potential host colonies to slavemaking ants. (2) Analyzing the aggressive response of *T. longispinosus* to several opponents varying in familiarity and the threat they can pose to the colony.

We predict that individual and colony aggression towards opponents would increase with the risk they pose to the colony (unfamiliar species < non-nestmate conspecifics < competitor species < slavemaking ants). Aggression towards the slavemaking ant should include biting and stinging, the most aggressive behavioral actions. Moreover, biting/stinging can be a collective response inflicting cumulative damage. Dragging, in contrast, is a less aggressive behavior, aimed at removing the opponent from the nest, and should be triggered against less risky intruders. Such behaviors (e.g., dragging and biting) should be quantified separately, as we expect differences not only in total aggressive behaviors, but also in the components of aggression. The aggressive response towards non-nestmate conspecifics may be either stronger or weaker than the response to heterospecifics (e.g., Stuart 1993; Whitehouse & Jaffe 1996). We expect aggression to be positively correlated with colony size (Holway & Case 2001; Palmer 2004).

Materials and methods

Study system and experimental design

The ant *T. longispinosus* inhabits mixed deciduous forests across the north-eastern USA, nesting in hollow acorns, hickory nuts and twigs in the leaf litter. Colonies were collected at the Huyck Preserve, Albany County, New York (N 42°31'35.3" W 74°9'30.1") in March-April 2009. The social parasite of *T. longispinosus*, the slavemaking ant *P. americanus*, co-occurs with its host at the NY study site. Colonies of the co-occurring competitor *T. ambiguus* were collected in the same locations in July 2009. Colonies of the congeneric species *T. curvispinosus* were collected in August 2009 in the Watoga State Park, Pocahontas County, WV (N 38°06′13″ W 80°08′59″). This species does not occur in the NY study site. Colonies of all species were kept in artificial nests (7.5×2.5×0.5cm) in plastic boxes (10×10×1.5cm) with a plastered floor in a climate chamber at the University of Munich (day:night temperatures 20°C:15°C) under identical conditions at least half a year before the experiment started. Ants were fed weekly with honey, water and crickets. Experiments were conducted at room temperature (~22°C).

Trials with all opponents except of *T. ambiguus* were conducted between October 2009 and March 2010; trials with *T. ambiguus* were conducted in October 2010. In each experiment, a dead ant belonging to one of the four species was entered into the focal *T. longispinosus* nest, and all interactions with the dead ant were recorded for 5 minutes, every 20-30 seconds (total of 11 observations). Ants were killed by freezing in -20˚C at the morning of each experiment. They were taken out of the freezer 5 minutes prior to each trial. Dead ants were placed at the artificial nest's entrance with floppy forceps and carefully pushed ~2 cm inside using a needle, in a similar way in all colonies and using all opponents. Dead ants were used to eliminate behavioral variation among the stimuli and focus on the focal colony's response to the chemical stimulus (similarly to Pamminger et al. 2011). Of course, using live individuals could lead to quantitatively different results, depending on the behavior of each opponent, affecting consequently the costs and benefits of host aggressive behavior. In addition, keeping colonies in the lab for a long period can moderate their aggression level, as they are kept in standardized conditions. Note also that the period of time colonies were kept prior to each experiment is not always identical. Some of the trials were part of a different study, in which we analyze only total aggression without a reference to specific behaviors (Pamminger et al. 2011).

The entrance to the colony was blocked during the experiment (following Stuart 1991). We documented events of antennating, mandible spreading, biting, dragging/holding and stinging. Our sample size included 105 *T. longispinosus* colonies, which were split randomly into four experimental groups, facing different opponents [*T. curvispinosus* (24), non-nestmate conspecifics (22), *T. ambiguus* (25) and *P. americanus* (34)]. This is the sample size in all statistical analyses performed and we focus on the colony level. Host colonies comprised different social organizations representing natural variation including queenless, monogynous and polygynous colonies, but social organization did not differ between treatment groups. See Fig. 1 for a *T. longispinosus* colony interacting with a *T. ambiguus* opponent.

Statistical analysis

To test for differences among treatments in aggressive and antennating behaviors we used two principal component analyses (PCA). The first analysis included all behaviors and the second one focused on the aggressive behaviors (i.e., excluding antennating). We used two PCAs, because antennating was clearly different from the aggressive behaviors (see Results), and since we mainly aimed at understanding how aggressive behaviors correlate with each other. Prior to analysis we used the Z-score transformation, which controls for the variance of the variables and fits well to variables in different units (Gotelli & Ellison 2004, ch. 12). It was partially the case here (i.e., many more mandible spreading than stinging events). A multivariate analysis can detect correlations between the occurrences of different behaviors, which cannot be identified by separately analyzing each behavior. We presented the PC loadings, eigenvalues and percentage of the variance explained for all axes (Table 1). We referred only to PCs demonstrating eigenvalues larger than one (Gotelli & Ellison 2004, ch. 12), and did not use the varimax rotation, sometimes helpful in clarifying the dominant factors in each PC, because it did not change the results much. We compared the PC loadings among different opponents using three ANCOVAs (taking into account colony size and its interaction with opponent). The interaction term was removed when not significant and the test was redone, using Bonferroni post-hoc tests to pinpoint the effects of opponent on aggression.

We were also interested in the rate of behavioral events and whether it differed among treatments. We referred separately to antennating, which differed from all other behaviors, to dragging/holding, which was especially frequent in the response towards the familiar competitor, and to the sum of other aggressive behaviors (i.e., mandible spreading, biting and stinging). We did not pool across the experiment duration as in the PCA. Instead, we regressed each dependent variable (antennating, aggression or dragging/holding) over time (seconds from the experiment's beginning). We got 3×105 slopes, three for each colony. To verify the linearity of the data, we fitted an additional squared term to all regressions, using non-linear regression and the formula: $rate = a \times time^2 + b \times time + c$. If the confidence intervals of the squared term 'a' included zero, it could be removed, and a linear model could be used. Data was usually linear, as the squared term was not significant in ~89% of the cases. We therefore used linear regressions on all data. We were interested to understand whether the slopes were usually negative, positive or did not significantly differ from zero, indicating a decrease, an increase or no clear change in antennating or aggression rate with time. For that purpose we used the percentile bootstrap (Manly 1997, ch. 3), to estimate the 95% confidence limit intervals for the slopes. For instance, if the confidence intervals are both negative, the rate of behavioral interactions decreases with time. Next, we compared using three ANCOVAs the slopes among opponents, taking colony size and the two-way interaction into accounts. Antennating and aggression rates did not deviate from normal distribution (one-sample Kolmogorov-Smirnov tests; P = 0.91 and P = 0.10 for antennating and aggression rates, respectively). Dragging/holding rate was not normally distributed and was log-transformed. Statistical analyses were performed using MATLAB v.7.8 (Mathworks, Natick, USA) and SYSTAT v.11 (SYSTAT Software, San Jose, USA).

Results

The PCA was meant to explore the components of all behaviors directed towards each opponent in general, and aggressive behavior in particular. The PCA on all behaviors clearly showed that antennating is a distinct behavior, as it had a positive loading on the 1st PC while all aggressive behaviors scored negatively (Table 1), and we referred to this PC as 'antennating vs. aggressive behaviors'. The 1st PC differed among opponents ($F_{3,100} = 3.71$, P = 0.014). The main difference was between the response towards the unfamiliar congeneric species and the slavemaking ant (a Bonferroni post-hoc test; P = 0.0037), and as values decreased colonies became more aggressive (Fig. 2a, 1st PC). Neither colony size nor the interaction term opponent × colony size were significant ($F_{1,100} = 1.26$, P = 0.26 and $F_{3,97} = 1.05$, P = 0.37, respectively). While the 1st PC showed a trade-off between antennating and all other behaviors, the 2nd PC represented a trade-off between dragging/holding scoring positively, and all other behaviors scoring negatively (Table 1). Mandible spreading scored most negatively, so we referred to this PC as 'dragging/holding vs. mandible spreading'. The 2nd PC was positively correlated with colony size only when encountering the familiar competitor (the interaction term opponent × colony size was significant; $F_{3,97} = 3.65$, $P = 0.015$; Fig. 2, 2nd PC). When including the interaction term, opponent and colony size as main effects were not significant, since the variance was better explained by the interaction ($F_{3,97} = 0.59$, $P = 0.62$ and $F_{1,97} = 0.86$, $P = 0.36$, respectively). A Bonferroni post-hoc test suggested that the major difference was between the slavemaking ant and the familiar competitor ($P < 0.0001$), but the familiar competitor differed from the two other opponents as well ($P < 0.004$ for all comparisons). In addition, encounters with the unfamiliar congeneric species differed from the slavemaking ants ($P = 0.021$; Fig. 2). The 3rd PC had an eigenvalue of 0.90, and it explained less of the variance (18%). It represented a trade-off between biting and stinging: colonies stinging their opponents less often bit, and vice versa. This PC did not differ among opponents ($F_{3,97} = 0.97$, $P = 0.41$; Fig. 2a, 3rd PC), nor was it influenced by colony size or the interaction term ($F_{1,100} = 0.01$, $P = 0.91$ and $F_{3,97} = 0.41$, $P = 0.75$, respectively).

To pinpoint correlations among aggressive behaviors, we redid the PCA excluding antennating. The 1st PC scores, representing mandible spreading, biting and stinging events, differed among opponents ($F_{3,100} = 11.04$, $P < 0.0001$; Fig 3), and were especially high when colonies encountered the slavemaking ant (the difference according to a post-hoc Bonferroni test was mainly between the response to the slavemaking ant and all the other opponents; $P < 0.007$ for all pairwise comparisons). Colony size and the two-way interaction were not significant

($F_{1,100}$ = 1.88, P = 0.17 and $F_{3,97}$ = 0.45, P = 0.72, respectively). The 2nd PC (representing dragging/holding events) showed a similar pattern to that shown by the 2nd PC when PCA is done on all behaviors: a positive correlation of the 2nd PC scores with colony size, but only for the familiar competitor (a significant interaction term opponent × colony size; $F_{3,97}$ = 3.52, P = 0.018). When including the interaction term, opponent by itself was not significant (differences among opponents were explained by the significant interaction; $F_{3,97}$ = 0.13, P = 0.94), but colony size was significant ($F_{1,97}$ = 5.06, P = 0.027). According to the post-hoc Bonferroni test, the aggressive behavior towards the familiar competitor was characterized by more frequent dragging/holding events than all other opponents (P < 0.0001 for all pairwise comparisons). The interaction term suggested, similar to the 2nd PC of the first PCA, that dragging/holding behavior was correlated with colony size, but only for the familiar competitor. The 3rd PC represented the same above-described trade-off between stinging and biting, but had a low eigenvalue (Table 1). It did not differ among opponents ($F_{3,100}$ = 0.76, P = 0.52) and was not influenced by colony size or the opponent × colony size interaction ($F_{1,100}$ = 0.08, P = 0.78 and $F_{3,97}$ = 0.27, P = 0.85, respectively). We present in the Appendix an additional figure, demonstrating specific behaviors, i.e., antennating and different aggressive behaviors (means of total interactions per colony).

We obtained 105 slopes each for antennating, aggression and dragging/holding events through time. The confidence intervals for antennating, aggression and dragging/holding were [-0.0014, -0.0001], [-0.0022, -0.0010] and [+0.0005, +0.0018], respectively. These results point to a qualitative difference among rates of behaviors: rates of antennating and aggression behavior decreased with the experiment's duration, while dragging/holding increased. None of the rates of behavioral events differed among opponents or was influenced by colony size (*antennating:* opponent: $F_{3,100}$ = 0.45, P = 0.72; colony size: $F_{1,100}$ = 0.20, P = 0.66; opponent × colony size:

$F_{3,97} = 1.19$, $P = 0.32$; *aggression:* opponent: $F_{3,100} = 1.11$, $P = 0.35$; colony size: $F_{1,100} = 0.63$, $P = 0.43$; opponent × colony size: $F_{3,97} = 1.02$, $P = 0.39$; *dragging/holding:* opponent: $F_{3,100} = 2.06$, $P = 0.11$; colony size: $F_{1,100} = 1.80$, $P = 0.18$; opponent × colony size: $F_{3,97} = 1.02$, $P = 0.39$).

Discussion

Temnothorax longispinosus colonies did not show an "all-or-nothing" reaction towards intruders of different species, but a differentiated and gradual response. The ants responded with low aggression to the unfamiliar *T. curvispinosus* but showed a very aggressive response towards the slavemaking ant *P. americanus*. The responses towards non-nestmate conspecifics and the co-occurring competitor *T. ambiguus* were somewhere in-between (Fig. 2a, 1st PC). Although the aggression exhibited against the competitor was quite high, it differed qualitatively from the response to the slavemaking ant: The competitor was more often dragged, probably to remove it from the nest, while the slavemaking opponent faced all other aggressive behaviors (Fig. 2a, 2nd PC; Fig. 3, 2nd PC). Recruitment is often threat-specific and does not occur when ants are exposed to ant species not perceived as a threat or to disturbance (Lamon & Topoff 1981; Powell & Clark 2004). “Enemy specification” or specific defense/attack responses are common in ant systems (e.g., Hölldobler & Willson, p. 429; Cerda & Retana 1998; Powell & Clark 2004). Not only that specific ant species are attacked while others are ignored, attack and defense are often species-specific, as we also show here. The mechanism leading to differences in aggression levels is unclear, but probably involves specific profiles of cuticular hydrocarbons, which are identified as risky or belonging to different potential enemies.

An encounter with a single slavemaker indicates a slave raid in the near future, because slave raids usually occur after a slavemaking scout detected a potential host nest (Alloway 1979; Foitzik & Herbers 2001). Therefore, host colonies should prevent the scout, which detected the colony location, from returning to its nest. Biting and stinging (and also mandible spreading as a step prior to biting) probably aims at killing the slavemaker, while dragging a slavemaker out of the nest without inflicting major damage is dangerous for the host colony. A slavemaking worker, equipped with large head and strong mandibles, will not be deterred by being removed from a host nest. The purpose of dragging/holding, directed towards the competitor, may be not to inflict damage but to remove it from the nest, as also dead nestmates are carried out of the nest. The joint biting behavior is probably used due to the size difference: Host workers are smaller than slavemakers and a single host worker would be unable to damage a slavemaking opponent. The difference between these two aggressive responses emphasizes the need for a better characterization of aggressive behavior and a careful selection of the behavioral indices. Total aggression could suggest similar responses towards the slavemaking and the competitor species, but a more detailed ethological analysis revealed a clear difference.

Attacking an enemy involves considerable costs. Therefore, animal fights are usually gradual and escalate only when the strength asymmetry between opponents is small (e.g., Arnott & Elwood 2009). Ant colonies often modulate the expressed aggression according to the opponent. For example, as nestmates are isolated for a longer time, they face stronger aggression when reintegrated into the colony (Heinze et al. 1996; Katzav-Gozansky et al. 2004). Several *Camponotus* and *Formica* species respond with increasing aggression to other ant species as the phylogenetic distance increases (e.g., Jaffe & Sanchez 1984; Geurrieri & D'Ettorre 2008). Defending against parasites is also gradual and corresponds to the damage each parasite potentially inflicts (e.g., Von Beeren et al. 2011). As expected, we see in our case a gradual/hierarchical response. Responding with the highest aggression possible to each intruder involves a waste of energy and time and may also induce some intra-nest fighting (ants erroneously attacking other nestmates). Yet, colony responses are not always gradual. For example, *Lasius niger* shows an "all-or-nothing" response to discovered resources: The intensity of trail-laying behavior was not correlated with the quantity of food provided (Mailleux et al. 2000).

The response of host colonies to the unfamiliar *T. curvispinosus* was the least aggressive (Fig. 2a, 1st PC). We interpret this response as *T. longispinosus* workers not perceiving *T. curvispinosus* intruders as a threat to the colony. Both congeneric ant species present a different chemical profile (Brandt et al. 2005) to the focal one, but only the co-occurring species elicited an aggressive response. It indicates that a deviant chemical profile alone does not invariably elicit aggressive reactions, but that the focal species developed recognition and defense behavior specifically as a reaction to its most important enemies. Heterospecific competitors (*T. ambiguus*) elicited slightly more aggression than non-nestmate conspecifics. Stronger reactions towards conspecifics could be adaptive, because they compete for the same resources. However, conspecifics show a similar chemical profile and the risk of erroneously attacking a nestmate is higher. This is especially the case for polygynous ant species (e.g., Starks et al. 1998), in which due to their diverse genetic composition of the colony, the chemical profile of nestmates is more variable. The costs and benefits of defense against enemies may also differ based on social structure and relatedness within the colony (e.g., Stuart 1991).

Body size differences in favor of the attacker should encourage attack and determine the fighting results (see references in Arnott & Elwood 2009). Colony size in social insects, as analogous to body size, often determines dominance of certain species (Holway & Case 2001; Palmer 2004). We therefore expected that larger colonies would initiate a stronger attack when a threat is detected. However, colony size was usually not correlated with the aggression intensity.

An exception was the 2nd PC in both analyses representing mandible spreading vs. dragging/holding or dragging/holding alone. In both cases, colony size was correlated with the response only towards the competitor *T. ambiguus*. We suggest that as long as the colony does not react aggressively, colony size does not matter much, but when the colony initiates an attack, its magnitude can depend on the available number of workers. For some reason, colony size was mainly important when the prominent behavior was dragging/holding and weaker in other cases. Previous works correlating colony size and aggression were inconsistent. Stuart (1991) found a positive relationship in *T. ambiguus* colonies, but Stuart and Herbers (2000) could not find such a correlation in *T. longispinosus*, the focal species in this study (for another negative evidence see Satoh & Hirota 2005).

Mandible spreading was a frequent and dominant behavior (e.g., Fig. 2a; 2nd PC), which by itself causes no harm. Some studies explained it as threatening the opponent, prior to attack (e.g., Carlin & Hölldobler 1986; Gurrieri & D'Ettorre 2008). However, we suggest that since vision is not a primary sensory channel used by ants, the addressee would be unable to receive the signal. Instead, mandible spreading could be interpreted as an 'alert status'. Ants spreading their mandibles are prepared and in a better position for an upcoming attack (e.g., biting). Whatever the reason for spreading the mandibles is, it should be considered as a less aggressive behavior than biting. A less significant trade-off, only evident in the 3rd PC in both analyses, is biting vs. stinging, which did not differ among opponents. It can represent a behavioral trade-off of individual ants involved in the attack (individuals that sting do not bite). Alternatively, this association may result from our observation methodology. Stinging is often accompanied by biting or holding, since the ant tries to hold the opponent in a certain position while stinging. If an ant bit or held the opponent, we recorded this as biting or dragging/holding respectively, but if it was stung we recorded this as stinging, leading to documentation of fewer biting events.

The interest of the colonies in the opponents decreased with exposure time, when considering antennating and aggressive behavior s (i.e., negative slopes). Workers possibly lose their interest owing to the lack of counter-response from the opponent (which was dead). Neutralizing the behavior of the opponent may provide more accurate results by decreasing the variance distinct behaviors of opponent would induce, but it can also affect the relative costs and benefits of aggression. In contrast to the two other behaviors, dragging/holding occurred in an increasing rate. None of the behavioral rates differed among opponents, but since dragging/holding was more common against the familiar competitor, we suggest that the competitor still differed to some extent in the rate of behavior it induced. Note also that Dragging/holding was a deviant aggressive behavior according to both PCAs (e.g., 2nd PC of the 1st PCA) as well as according to the rate analysis.

Although two previous studies have pointed to the elevated aggression towards the slavemaker *P. americanus* by its host species *T. longispinosus* (Alloway 1990; Pamminger et al. 2011), this is the first detailed ethological analysis of the aggressive behavior towards a slavemaking ant in particular and a social parasite in general. We also compare the aggressive response to two other related species and show specific behavioral responses. We emphasize the importance of ethological studies in order to better understand the different behavioral components of aggressive interactions. We suggest two important future directions. First, a model of the aggression level expected in response to different threats (possibly by using a game-theory approach) would help formulating the trade-offs underlying colony defenses. Second, it will be interesting to compare the response of the focal species to *T. curvispinosus* in habitats from which it is absent (like in the current study) to populations in which it is present (e.g., *T. longispinosus* populations in West Virginia), expecting a much higher aggression, if both species co-occur.

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References

Alloway, T. M. 1979: Raiding behaviour of two species of slave-making ants, *Harpagoxenus americanus* (Emery) and *Leptothorax duloticus* Wesson (Hymenoptera: Formicidae). Anim. Behav. **27**, 202 –210.

Alloway, T. M. 1990: Slave-species ant colonies recognize slavemakers as enemies. Anim. Behav. **39**, 1218–1220.

Arnott, G. & Elwood, R. W. 2009: Assessment of fighting ability in animal contests. Anim. Behav. **77**, 991–1004

Brandt, M., Heinze, J., Schmitt, T. & Foitzik, S. 2005: A chemical level in the coevolutionary arms race between an ant social parasite and its hosts. J. Evol. Biol. **18**, 576–586.

Buschinger, A. 2009: Social parasitism among ants: a review (Hymenoptera: Formicidae). Myrmecol. News **12**, 219–235.

Carlin, N. F. & Hölldobler, B. 1986: The kin recognition system of carpenter ants (Camponotus spp.): I. Hierarchical cues in small colonies. Behav. Ecol. Sociobiol. **19**, 123–134.

Cerda, X. & Retana, J. 1998: Interference interactions and nest usurpation between two subordinate ant species. Oecologia **113**, 577–583.

Dimarco, R. D., Farji-Brener, A. G. & Premoli, A. C. 2010: Dear enemy phenomenon in the leaf-cutting ant *Acromyrmex lobicornis*: behavioral and genetic evidence. Behav. Ecol. **21**, 304–310.

Dornhaus, A. & Powell, S. 2010: Foraging and Defence Strategies. In: Ant Ecology (Lach, L., Parr, C. L. & Abbott, K. L., eds). Oxford University Press, Oxford, pp. 210–230.

Foitzik, S. & Herbers, J. M. 2001: Colony structure of a slavemaking ant. II. Frequency of slave raids and impact on the host population. Evolution **55**, 316 –323.

Gotelli, N. J. & Ellison, A. M. 2004: A primer of ecological statistics. Sinauer, Sunderland.

Guerrieri, F. J. & D'Ettorre, P. 2008: The mandible opening response: quantifying aggression elicited by chemical cues in ants. J. Exp. Biol. **211**, 1109–1113.

Hefetz, A. 2007: The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae) – interplay of colony odor uniformity and odor idiosyncrasy. A review. Myremecol. News **10**, 59–68.

Heinze, J., Foitzik, S., Hippert, A. & Hölldobler, B. 1996: Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. Ethology **102**, 510–522.

Hölldboler, B. & Wilson, E. O. 1990: The Ants. Springer, Berlin.

Holway, D. A. & Case, T. J. 2001: Effects of colony-level variation on competitive ability in the invasive Argentine ant. Anim. Behav. **61**, 1181–1192.

Hughes, W. O. H. & Goulson, D. 2001: Polyethism and the importance of context in the alarm reaction of the grass-cutting ant, *Atta capiguara*. Behav. Ecol. Sociobiol. **49**, 503–508.

Jaffe, K. & Sanchez, C. 1984: On the nestmate-recognition system and territorial marking behaviour in the ant *Camponotus rufipes*. Insect. Soc. **31**, 302–315.

Katzav-Gozansky, T., Boulay, R. Vander Meer, R. & Hefetz, A. 2004: In-nest environment modulates nestmate recognition in the ant *Camponotus fellah*. Naturwissenschaften **91**, 186–190.

Lamon, B. & Topoff, H. 1981: Avoiding predation by army ants: defensive behaviors of three ant species of the genus Camponotus. Anim. Behav. **29**, 1070–1081.

Mailleux, A.C., Deneubourg, J.L. & Detrain, C. 2000: How do ants assess food volume? Anim. Behav. **59**, 1061–1069.

Manly, B. F. J. 1997: Randomization, bootstrap and Monte Carlo methods in biology, 2nd edn. Chapman & Hall, London.

Nowbahari, E., Feneron, R. & Malherbe, M. C. 1999: Effect of body size on aggression in the ant, *Cataglyphis niger* (Hymenoptera; Formicidae). Aggressive Behav. **25**, 369–379.

Palmer, T. M. 2004: Wars of attrition: colony size determines competitive outcomes in a guild of African acacia ants. Anim. Behav. **68**, 993–1004.

Pamminger, T., Scharf, I., Pennings, P. S. & Foitzik, S. 2011: Increased host aggression as an induced defense against slavemaking ants. Behav. Ecol. **22**, 255–260.

Pearce-Duvet, J. M. C. & Feener, D. H. Jr. 2010: Resource discovery in ant communities: do food type and quantity matter? Ecol. Entomol. **35**, 549–556.

Powell, S. & Clark, E. 2004: Combat between large derived societies: A subterranean army ant established as a predator of mature leaf-cutting ant colonies. Insect. Soc. **51**, 342–351.

Rivera-Marchand, B., Giray, T. & Guzman-Novoa, E. 2008: The cost of defense in social insects: insights from the honey bee. Entomol. Exp. Appl. **129**, 1–10.

Satoh, T. & Hirota, T. 2005: Factors affecting internest variation in the aggressiveness of a polygynous ant, *Camponotus yamaokai*. Entomol. Sci. **8**, 277–281.

Starks, P. T., Watson, R. E., Dipaola, M. J. & Dipaola, C. P. 1998: The effect of queen number on nestmate discrimination in the facultatively polygynous ant *Pseudomyrmex pallidus* (Hymenoptera: Formicidae). Ethology **104**, 573–584.

Stuart, R. J. 1991: Nestmate recognition in leptothoracine ants: testing for effects of queen number, colony size and species of intruder. Anim. Behav. **42**, 277–284.

Stuart, R. J. 1993: Differences in aggression among sympatric, facultatively polygynous *Leptothorax* ant species. Anim. Behav. **46**, 809–812.

Stuart, R. J. & Herbers, M. 2000: Nest mate recognition in ants with complex colonies: within- and between-population variation. Behav. Ecol. **11**, 676–685.

Tanner, C. J. & Adler, F. R. 2009: To fight or not to fight: context-dependent interspecific aggression in competing ants. Anim. Behav. **77**, 297–305.

Tollrian, R. 1995: Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. Ecology **76**, 1691–1705.

Von Beeren, C., Maruyama, M., Hashim, R. & Witte, V. 2011: Differential host defense against multiple parasites in ants. Evol. Ecol. **25**, 259–276.

Whitehouse, M. E. A. & Jaffe, K. 1996: Ant wars: combat strategies, territory and nest defence in the leaf-cutting ant *Atta laevigata*. Anim. Behav. **51**, 1207–1217.

Zuk, M. & Stoehr, A. M. 2002. Immune defense and host life history. Am. Nat. **160**, S9–S22.

Table 1: Results of two principle component analyses of behavioral responses of ant colonies to intruders (see also Methods)

First PCA, all behaviors

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	1.88	1.21	0.90	0.60	0.40
% var. explained	37.7%	24.3%	18.0%	12.0%	8.0%
Antennating	+0.535	-0.408	+0.047	-0.433	+0.598
Mandible spreading	-0.415	-0.592	-0.001	-0.544	-0.427
Biting	-0.465	-0.173	-0.705	+0.170	+0.477
Dragging/holding	-0.376	+0.611	+0.152	-0.612	+0.298
Stinging	-0.429	-0.284	+0.691	+0.337	+0.380

Second PCA, only aggressive behaviors

	PC1	PC2	PC3	PC4
Eigenvalue	1.58	1.00	0.89	0.52
% var. explained	39.6%	25.0%	22.4%	13.0%
Mandible spreading	+0.644	-0.278	-0.019	+0.712
Biting	+0.517	-0.033	+0.721	-0.460
Dragging/holding	+0.192	+0.960	+0.036	+0.203
Stinging	+0.531	+0.024	-0.692	-0.489

Fig. 1: *T. longispinosus* (black) colony workers interacting with a dead *T. ambiguus* opponent (brown). In the upper photo, four workers are antennating the opponent while an additional worker is stinging it. In the lower photo, two workers are antennating the opponent and an additional worker is opening its mandible, possibly prior to biting.

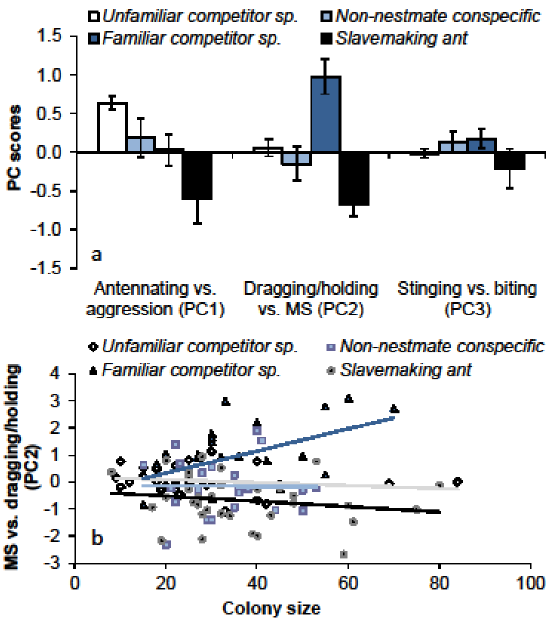

Fig. 2: (a) First, second and third PC axes of the PCA performed on all behaviors including antennating, separated according to opponents. The 1st PC, antennating (positive) vs. aggression (negative), differed between the unfamiliar congeneric species (*T. curvispinosus*) and the slavemaking ant (*P. americanus*). The 2nd PC, dragging/holding (positive) vs. mainly mandible spreading (negative), differed especially between the familiar competitor (*T. ambiguus*) and all other opponents. The 3rd PC, stinging (positive) vs. biting (negative), did not differ among opponents. (b) The relationship between colony size and the 2nd PC, representing dragging/holding vs. mandible spreading. A positive correlation was evident only for the familiar competitor.

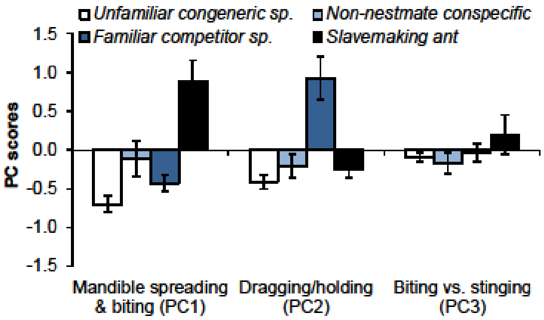

Fig. 3: First, second and third PC axes of the PCA performed on aggressive behaviors excluding antennating, separated according to opponents. The 1st PC represented mostly mandible spreading, biting and stinging events, and differed between the slavemaking ant (*P. americanus*) and other opponents. The 2nd PC represented mostly dragging/holding events, and differed between the familiar competitor (*T. ambiguus*) and other opponents. The 3rd PC, representing a trade-off between biting and stinging, did not differ among opponents.

Chapter 3: Raiders from the sky: slavemaker founding queens select for aggressive host colonies

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Keywords: parasite, personality, dispersal, aggression, fitness

Summary

Reciprocal selection pressures in host-parasite systems drive coevolutionary arms races that lead to advanced adaptations in both opponents. In the interactions between social parasites and their hosts, aggression is one of the major behavioural traits under selection. In a field manipulation, we aimed to disentangle the impact of slavemaking ants and nest density on aggression of *Temnothorax longispinosus* ants. An early slavemaker mating flight provided us with the unique opportunity to study the influence of host aggression and demography on founding decisions and success. We discovered that parasite queens avoided colony foundation in parasitized areas and were able to capture more brood from less aggressive host colonies. Host colony aggression remained consistent over the two months experiment, but did not respond to our manipulation. However, as a fifth of all host colonies were successfully invaded by parasite queens, slavemaker nest foundation acts as a strong selection event selecting for high aggression in host colonies.

Introduction

Personalities, i.e. consistent inter-individual differences in behaviour, affect the fitness of animals in many species, have been shown to be heritable to some degree [1] and thus result in potential selection on behavioural traits. In particular, aggressiveness and boldness can increase the reproductive success in competitive environments [2]. The fitness consequences of aggression have been demonstrated in multiple species: aggressive females were able to produce more offspring in Ural owls and in red squirrels [3, 4]. Aggressive interactions also play an important role in the life of the ant *Temnothorax longispinosus*. In addition to severe intraspecific competition for nest sites in dense populations [5], this species is the preferred host of the slavemaking ant *Protomognathus americanus* [6]. These obligatory slavemakers are unable to found colonies on their own and invade host colonies where they acquire their first slaves as pupae. In addition, established parasite colonies conduct destructive raids to replenish their slave workforce [7]. Contrary to micro-parasites-host associations, slavemakers and their hosts are similar in size and their interactions are mainly behavioural. During slave raids and parasitic queen nest take-overs hosts and slavemakers behave highly aggressively [7, 8]. In both cases host workers react to the intruding parasite with counter attacks and immediate nest evacuation. Thereby, they can usually rescue some of their brood and the queen.

We previously found that *T. longispinosus* colonies in dense populations are more aggressive [9]. This could be either due to natural selection for aggression in crowded habitats or behavioural plasticity as a response to environmental or social conditions. Indeed, a genetic basis of aggression was indicated by behavioural consistency over different worker generations and aggressive colonies could have an advantage under severe intraspecific competition [10]. On the other hand, host aggression can be to some extent plastic, as aggressiveness towards conspecifics was shown to increase for several days after slavemaker contact [11].. We aimed to identify how natural selection and/or behavioural plasticity shape *T. longispinosus* aggressiveness under natural conditions. We conducted a cross-fostering field experiment in which we manipulated nest density and slavemaker presence. We started the two-month field manipulation in late spring and planned to finish it before the slavemakers' mating flight. However, due to an exceptionally warm spring, the nuptial flight of the parasite occurred early that year and about a fifth of the host colonies were usurped by *P. americanus* queens. This selection event allowed us to study the founding behaviour and success of slavemaking queens. In particular, we were able to analyse host nest preference, dispersal and brood take-over success in relation to host aggression and demography under semi-controlled natural conditions. To our knowledge this is the first time that this critical stage of the parasitic life cycle has been accessible to an experimental investigation.

Material and methods

Study system, field collection and aggression experiments

In May 2010 we collected and censused 16 mature *P. americanus* colonies (containing a parasite queen and at least two slavemaking workers) and 160 *T. longispinosus* host colonies at the Huyck Preserve, Albany County, NY (N 42° 31'35.3" W 74° 9'30.1"). Aggression against intruders was determined for all *T. longispinosus* colonies in a standardized set-up by the two first authors ([9]; see electronic supplementary material). Colonies of the different treatments did not differ in the nest density of the source area or in aggression (Kruskal-Wallis-test: $p > 0.05$). Each colony was allowed to move into an individual artificial nest sites (dowel [5]) and placed in field enclosures within two days of collection.

Field manipulation

During May - June 2010 we constructed eight enclosures in a homogenous forest area of about 50m x 50m. Each of the enclosures was composed of four compartments, two larger ones (9 m^2; 3 x 3 m) and two smaller ones (2.25 m^2; 1.5 x 1.5 m) that were separated by 40 cm high aluminium flashing, anchored 10 cm in the ground. The leaf litter and thereby all suitably nest sites were removed from the enclosures to prohibit nest relocation. Thereafter, five *T. longispinosus* colonies in artificial wooden nest sites were placed in each compartment, resulting in nest densities of 2.2 colonies per m^2 in the dense compartments and 0.6 colonies per m^2 in the less dense ones. We placed a slavemaker colony in the centre in one of the high and low density compartments creating four different treatments: (a) high density with slavemaker, (b) high density without slavemaker, (c) low density with slavemaker and (d) low density without slavemaker. All colonies remained in the field for about two months and enclosures were carefully searched for ants in late July. Unless destroyed by raiding or by a parasitic founding queen, we assume that colonies remained in the same artificial nests. We recollected 92 *T. longispinosus* colonies that were censused and subjected to a second standardized aggression test [11].

Statistical analyses

To test for consistency in aggressive behaviour, we performed an ANCOVA analysis with the aggression after treatment as dependent variable, observer and treatment as categorical predictors and aggression before treatment as a continuous predictor.

We analysed how host colony survival was influenced by treatment and host demography using c^2-tests and t-tests. Further, we investigated whether founding events were related to treatment, host colony size and aggression (see electronic supplementary material).

Take-over success was calculated as the number of brood captured by the slavemaker queen divided by the brood originally present in the colony. We used an ANCOVA analysis with arcsine transformed % brood captured by the slavemaker queens as the dependent and colony aggression as a continuous and observer as a categorical predictor to investigate if more aggressive colonies can evacuate more brood. To control for potential cofounding effects (e.g. host colony growth rates) we conducted additional tests (please see supplementary).

Results

The mating flight of *P. americanus* took place during the manipulation and 21 % of our experimental colonies were taken-over by founding *P. americanus* queens. Moreover, a larger fraction of colonies (34 %) disappeared from slavemaker treatments than from parasite-free plots (10 %; c^2_1 =13.2, $p < 0.0005$), suggesting that about a quarter of all colonies in the slavemaker treatments were destroyed during raids. Host colonies that disappeared in slavemaker treatments were smaller than those that survived (t-test: $t = 2.0$, $p < 0.05$; $N_{1,2}$= 53, 27), whereas colony size did not affect host nest survivorship in parasite-free experimental plots (t-test: $t = 1.1$, $p = 0.28$; $N_{1,2}$= 72, 8).

Treatment, observer and the interactions had no effect on host aggression measured after the experiment (all $p > 0.05$). We found a significant positive correlation between aggression measured before and after the manipulation (ANCOVA: aggression before treatment: $F_{1,86}$ = 5.87, $p < 0.02$) demonstrating further that colony identity was largely the same at the end of the experiment.

Moreover the frequency of successful parasitic colony founding depended on treatment (c^2_3 =10.2, $p < 0.02$): while there was no effect of host density (c^2_1 = 0.3, $p = 0.56$), fewer parasitic founding events occurred in plots in which we released a slavemaker colony (c^2_1 = 5.8, $p < 0.02$). These analyses were based on the number of host colonies present in the enclosures after the manipulation and thus cannot be explained by the raiding activity of the slavemakers directly. Whether a host colony was taken-over by a parasite queen was unaffected by host colony size (t-test: $t = -1.12$, $p = 0.27$, $N_{1,2}$ = 33, 91) or aggression (MWU-test: $z = 0.39$, p = 0.70; $N_{1,2}$ = 33, 91). However, *P. americanus* queens that invaded more aggressive host colonies obtained a lower percentage of their brood (ANCOVA: $F_{1,29} = 4.19$, $p < 0.05$). Observer and the interaction did not co-vary with the percentage of brood captured ($p > 0.05$).

Discussion

The external conditions for *T. longispinosus* colonies at our study site are favourable leading to high nest densities and colony productivity. However, these ant colonies suffer from severe competition for nest sites and food [5, 12, 13] and are regularly attacked by social parasites trying to steal their brood [14]. In such an environment dominated by antagonistic interactions, aggression should be favoured. Indeed, we found that more aggressive host colonies were able to rescue a higher fraction of their brood from invading slavemaker queens. Aggression did not play a role for parasite aversion, attacked host colonies only escaped with more brood, which should translate into a fitness benefit. The selection pressure through parasitic nest foundations was found to be high, as 20% of the host colonies were successfully usurped by slavemaking queens within one season. In addition, we can demonstrate that about a quarter of the host colonies were destroyed during raids and smaller host nests were destroyed more often. Hence, the combination of parasitic founding and raiding events selects for larger and more aggressive host colonies. From the parasite perspective, host aggression strongly influences the future of the slavemaking nest, as parasite queens, which obtained only a low fraction of the host brood will start their colony with only few slaves.

Our density and parasite treatments did not influence host colony aggression, but the later was correlated over two months in the field. This is in accordance with earlier work showing consistency in colony aggression over different worker generations [10]. Albeit behavioural experiments showed that host colonies respond to slavemaker contact with an induced short-term increase in aggression against conspecifics [11], the presence of a slavemaking colony within a plot did not result in higher aggression of host colonies. Possibly the encounter frequency of host colonies with slavemakers in parasitized plots is too low to lead to a consistent increase in aggression.

Nevertheless, slavemaker queens invaded host colonies less often in experimentally parasitized plots. This can be explained by host nests in parasitized plots exhibiting a better nest defence either because parasite encounters induced aggressive responses specific to slavemakers or because slave raids selected for better defended host nests. Alternatively slavemaking queens might have been able to detect slavemaking colonies by their odour and actively avoid competition with already established slavemaking nests. Indeed, slavemaking queens of the related species *Harpagoxenus sublaevis* do not return to their home locale, but actively search for new host colonies [15].

Our experiment allowed us to analyse how a personality trait and demography influence the ability of host colonies to defend themselves against parasitic invasions. Parasite queens obtained less brood from aggressive host colonies, and thereby selected for host aggression. Furthermore, slavemaking queens actively avoided invading host nests in parasitized areas and / or the conditions prohibited successful invasion, which should influence parasite distribution within the host population. This study adds another dimension to the growing knowledge of the influence of parasites on animal personalities [16].

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References

1. Réale D., Reader S.M., Sol D., McDougall P.T., Dingemanse N.J. 2007 Integrating animal temperament within ecology and evolution. *Biological Reviews of the Cambridge Philosophical Society* **82**, 291-318. (doi:10.1111/j.1469-185X.2007.00010.x).
2. Smith B.R., Blumstein D.T. 2008 Fitness consequences of personality: a meta-analysis. *Behaviorial Ecology* **19**(2), 448-455. (doi:10.1093/beheco/arm144).
3. Kontiainen P., Pietäinen H., Huttunen K., Karell P., Kolunen H., Brommer J.E. 2009 Aggressive Ural owl mothers recruit more offspring. Behavioral Ecology **20**,789–796.
4. Boon A.K., Réale D., Boutin S. 2007 The interaction between personality, offspring fitness and food abundance in North American red squirrels. Ecology Letters, **10**, 1094–1104.
5. Herbers J.M. 1986 Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. *Behavioral Ecology and Sociobiology* **19**, 115-122.
6. Herbers J.M., Foitzik S. 2002 The ecology of slavemaking ants and their hosts in north temperate forests. *Ecology* **83**, 148-163.
7. Brandt M., Foitzik S., Fischer-Blass B., Heinze J. 2005 The coevolutionary dynamics of obligate ant social parasite systems--between prudence and antagonism doi:10.1017/S1464793104006669 *Biological Reviews of the Cambridge Philosophical Society* **80**, 251-267.
8. Foitzik S., Deheer C.J., Hunjan D.N., Herbers J.M. 2001 Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. *Proceedings of the Royal Society of London Series B Biological Sciences* **268**, 1139-1146.

9. Modlmeier A.P., Foitzik S. 2011 Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behaviorial Ecology* **22**(5), 1026-1032. (doi:10.1093/beheco/arr086).

10. Modlmeier A.P., Liebmann J.E., Foitzik S. 2012 Diverse societies are more productive: a lesson from ants. *Proceedings of the Royal Society of London Series B: Biological Sciences*. (doi:10.1098/rspb.2011.2376).

11. Pamminger T., Scharf I., Pennings P.S., Foitzik S. 2011 Increased host aggression as an induced defense against slave-making ants. *Behav Ecol* **22**(2), 255-260. (doi:10.1093/beheco/arq191).

12. Foitzik S., Backus V.L., Trindl A., Herbers J.M. 2004 Ecology of *Leptothorax* ants: impact of food, nest sites and social parasites. *Behavioral Ecology and Sociobiology* **55**, 484-493. (doi:10.1007/s00265-003-0718-9).

13. Foitzik S., Achenbach A., Brandt M. 2009 Locally adapted social parasite affects density, social structure, and life history of its ant hosts. *Ecology* **90**(5), 1195-1206.

14. Foitzik S., Herbers J.M. 2001 Colony structure of a slavemaking ant. II. Frequency of slave raids and impact on the host population. *Evolution* **55**, 316-323.

15. Buschinger A. 1974 Experimente und Beobachtungen zur Gründung und Entwicklung neuer Sozietäten der sklavenhaltenden Ameise *Harpagoxenus sublaevis* (Nyl.). *Insectes Sociaux* **21**, 381-406.

16. Barber I., Dingemanse N.J. 2010 Parasitism and the evolutionary ecology of animal personality. *Philos Trans R Soc Lond B Biol Sci* **365**(1560), 4077-4088. (doi:365/1560/4077 [pii]10.1098/rstb.2010.0182).

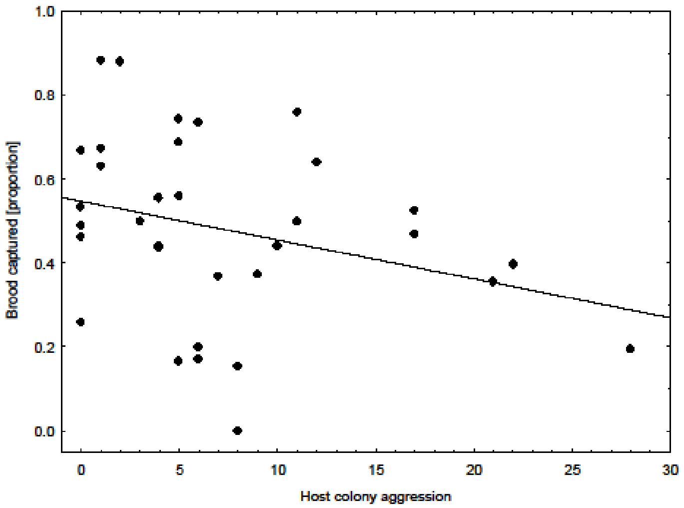

Figure 1. Relationship between brood capture rate of founding *P. americanus* ant queens and host colony aggression of *T. longispinosus* ant colonies. Presented are 33 founding events from a field experiment.

Chapter 4: Geographic distribution of the anti-parasite trait “slave rebellion”

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Running title: Geographic variation in slave rebellion

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Abstract

Social parasites exploit the brood care behavior of other species and can exert strong selection pressures on their hosts. As a consequence, hosts have developed defenses to circumvent or to lower the costs of parasitism. Recently, a novel, indirect defense trait, termed slave rebellion, has been described for hosts of a slave-making ant: Enslaved *Temnothorax longispinosus* workers reduce local parasite pressure by regularly killing pupae of their obligatory slavemaking parasite *Protomognathus americanus*. Subsequently, growth of social parasite nests is reduced, which leads to fewer raids and likely increases fitness of neighboring related host colonies. In this study, we investigate the presence and expression the slave rebellion trait in four communities. We report its presence in all parasitized communities, document strong variation in its expression between different geographic sites and discuss potential explanations for this observed variation.

Keywords: coevolution, selection mosaic, parasitism, social parasites, slavemaking ants, host defense

Introduction

Parasitism is the most common life style on earth and virtually all organisms are affected by it (Price 1980). Parasites are organisms which are closely associated with at least one other species (the host), exploiting its resources and harming it in the process. Parasites reduce the fitness of hosts in a variety of ways ranging from direct extraction of nutrients (Price 1980) and castration (Clay 1991; Yu and Pierce 1998; Lafferty and Kuris 2009) to specialized manipulation of the hosts' behavior to increase transmission and with an often lethal outcome for the host (Berdoy 2000; Thomas et al. 2005).

Parasite pressure has led to the development of defensive strategies in hosts, which, in turn, resulted in the evolution of counter-adaptations in parasites, a process which may lock both species in a coevolutionary dynamic, potentially escalating in an evolutionary arms race (Dawkins and Krebs 1979). The interactions between brood parasites (or social parasites) and their hosts are model systems to study these dynamics (Thompson 2005).

Brood parasitism describes the interaction between two species, one of which exploits the brood care behavior of the other, thereby avoiding the costs of rearing their own offspring. This form of parasitism has evolved in various taxonomic groups including birds, fish and social insects, in which brood care represents a substantial investment and can be transferred to individuals other than the parents (Sato 1986; Rothstein 1990; Davies 1999; Beibl et al. 2005; Kruger 2007; Buschinger 2009). In the hosts of cuckoos and cowbirds (e.g. Lorenzana and Sealy 2001) and in those of social parasites (Hare and Alloway 2001; Fischer-Blass et al. 2006; Foitzik et al. 2009) severe fitness costs have been documented, resulting in strong selection on the hosts to develop defenses to avoid parasitic exploitation (Alloway 1990; Moksnes et al. 1991; Roskaft et al. 2002) or to reduce the costs once parasitized (Langmore et al. 2003; Kruger 2007; Achenbach and Foitzik 2009).

Hosts of avian brood parasites exhibit well-developed behavioral defenses to prevent parasites from laying eggs in their nests, which include mobbing of parasites as soon as they are spotted (Moksnes et al. 1991; Roskaft et al. 2002). In addition, various host populations have developed the ability to recognize parasite eggs by their size or coloration. In brood parasites, on the other hand, egg mimicry has evolved, which counteracts host egg detection (Kruger 2007).

Slave-making ants are social parasites that depend on ant workers of other species – the hosts - either during colony founding or during their entire life cycle (Buschinger 2009). Workers of slavemaking ants often exhibit a limited behavioral repertoire: they are unable to perform routine tasks such as foraging, brood care and nest maintenance and depend on enslaved host ants – the slaves - to perform these essential chores. Slavemaker workers are specialized on searching and attacking nearby host colonies during slave raids, during which they kill or expel all adult host ants. They then rob the host brood, which will develop into a new generation of slaves in the slavemaker nest.

Workers of the slavemaking ant *Protomognathus americanus* are well-equipped for these dangerous raids with chemical and morphological adaptations (Brandt et al. 2005; Brandt, Heinze et al. 2006). Due to the high frequency and destructiveness of these raiding events (Foitzik and Herbers 2001; Foitzik et al. 2009) this evolutionary old social parasite (Beibl et al. 2005) exerts high selection pressure on its three host ant species of the genus *Temnothorax*.

This host-parasite interaction has been studied extensively over the past two decades, revealing several host defense mechanisms. Most studies have focused on adaptations prior to enslavement including enemy recognition, adjustment of the recognition threshold, fighting abilities, inducible aggression, fast evacuation and escape from the attacked host colonies (Alloway 1990; Foitzik et al. 2001; Brandt et al. 2005, Pamminger et al. 2011; Scharf et al. 2011). Based on theoretical considerations it was long thought that defense behaviors of enslaved workers are unlikely to evolve, because slaves cannot escape and reproduce, hence no behavior could increase their direct fitness (Gladstone, 1981). Indeed, we have never found evidence that slaves reproduce in the field (Foitzik et al. 2001, Pamminger et al. unpublished). However, we recently discovered a post-enslavement host defense, which has been termed “slave rebellion” (Achenbach and Foitzik 2009; Achenbach et al. 2010). Instead of raising the brood of their social parasite *P. americanus* to adulthood, enslaved *Temnothorax* were observed to kill a large proportion of the slavemaker pupae either by direct attack or by neglect (Achenbach and Foitzik 2009). Under the same conditions, non-enslaved host workers cared well for their own brood. By selectively killing or neglecting slave-making pupae, enslaved workers decrease the growth of slavemaker colonies and reduce parasite pressure on nearby host colonies. If those nearby colonies are related to the slaves, and recent genetic analyses suggest that this is the case, the killing behavior could spread through kin selection (Metzler et al. unpublished ms).

There are two main differences between egg or brood rejection in hosts of avian brood parasites and of the social parasites that we study. First, a bird that correctly identifies a parasite egg and rejects it will directly benefit from this behavior. It will not waste energy on raising a brood parasite chick, nor will this parasite kill its own young, as young cuckoos do. Ant slaves, on the other hand, that kill parasite pupae cannot directly profit from this behavior and their only fitness benefit derives from increased survival of related colonies. Secondly, there is a difference in the recognition mechanism of the parasite brood. In contrast to avian systems, in which parasites and their eggs are detected visually, ants mainly use chemical cues for recognition and enemy detection (Hefetz 2007). Chemical analyses revealed that parasite pupae show a cuticular hydrocarbon profile distinct from that of their hosts (Achenbach et. al. 2010) and these chemical differences could be used by the slaves to identify parasite brood.

In this study, we investigate the brood care behavior of the main host species, *T. longispinosus,* both in its own nests and in slavemaker nests. We study hosts from three communities in New York (NY), West Virginia (WV) and Ohio (OH), which differ in their community composition, but are similar in parasite prevalence (Herbers and Foitzik 2002; Brandt and Foitzik 2004). Moreover we analyze the brood care behavior of a *T. longispinosus* population in Michigan (MI), where the slavemaker *P. americanus* is absent. Another, undescribed slave-making species (*Temnothorax* sp.; Beibl et al. 2005) occurs in very low densities. Previous studies found differences between the three parasitized communities, which may be important for our study. First of all, slavemaker nests at the NY site have higher raiding frequencies than those in WV (Foitzik and Herbers 2001). Secondly, raiding experiments showed that the slavemakers from NY are more effective and destructive during raids, compared to the two slavemaker populations in WV and OH. Moreover, the hosts from NY are generally better defended compared to the other two parasitized sites. For example, hosts from NY are able to rescue more brood during a raiding attack than hosts from either WV or OH (Foitzik et. al. 2001; Brandt and Foitzik 2004). Thirdly, the species composition of the communities varies. The NY and WV community are dominated by the host species *T. longispinosus* that constitutes between 84% - 98% of the host ant community. Secondary hosts, *T. ambiguus* in NY and *T. curvispinosus* in WV, occur at low densities and are less important for the host-parasite interaction at both sites. In contrast, at our OH study site, *T. curvispinosus* is more common, comprising 60% of the host community, while *T. longispinosus* occurs at a rate of 40% (Table 1), and the slavemaker is exploiting both species. In the Michigan community, *T. longispinosus* is more common (65%) than *T. ambiguus* and *P. americanus* appears to be absent. In this study, we focus on the interaction between *P. americanus* and its main host *T. longispinosus* and we test the following five hypotheses.

We hypothesize that if the slave rebellion trait is present in all parasitized host populations, the survival rate of *P. americanus* brood will be lower than that of *T. longispinosus* brood in unparasitized nests from the same community.

We expect to find geographic variation in *P. americanus* brood survival rates. The key requirement for spatial variation is that the population dynamics are not synchronized by gene flow between different sites. Indeed, genetic analyses detected strong structuring in host and parasite populations (Brandt et al. 2007; Pennings et al. 2011) and geographic differences in the expression of other defense behaviors have been documented (Brandt and Foitzik 2004).

Various studies in avian host-brood parasite systems have found that local parasite pressure results in stronger host defense mechanisms. If parasite pressure is important in our ant system as well, we anticipate that in communities with stronger parasite pressure on the host, i.e., in NY, enslaved workers should show higher brood killing rates.

Previously, higher killing rates of parasite queen pupae and to a lesser extend also of worker pupae were reported (Achenbach and Foitzik 2009). We investigate whether caste specific killing rates are also found in different host and parasite populations.

Most *P. americanus* nests are small with on average less than five slavemakers, but we occasionally find very large slavemaking colonies containing up to 50 slavemaking workers (Pamminger, unpublished). We hypothesize that slavemaker pupae suffer less from killing in larger parasite colonies because these nests contain less rebellious slaves If this is true, we expect the survival of parasite pupae to increase with nest size.

Material and Methods

Study system, collection and housing

Protomognathus americanus, an obligate social parasite of three host species of the genus *Temnothorax*, inhabits the leaf litter of mixed deciduous forests along the East coast of the United States and the southern part of Canada. Over five years (2005 – 2008, 2011) we collected *P. americanus* nests as well as nests of its preferred host species *Temnothorax longispinosus* in four different communities. Ants were collected in early summer at the Huyck Preserve in Albany County, New York (42°31′ 35″ N 74◦9′ 30.1″ W; 2005-2008) in Harpersfield, Ashtabula County, Ohio (41°5′ 34.2″ N 80°57” 55.7″ W; 2005, 2006, and 2008), Watoga State Park, Pocahontas County, WV (N 38°06′ 13″ W 80°08′ 59″; in 2007) and at Sleeping Bear Dunes National Lake Shore, Empire, MI (44° 54′ 47″ N, 86° 1′ 13″ W; in 2011). To investigate pupal survival rates in the four communities, the ant colonies were transported to the laboratory in their natural nests (e.g. an acorn or small rotten sticks), counted, and transferred into artificial nests (7.5cm×2.5cm×0.5 cm). The ants were housed in three-chambered plastic boxes (10cm×10cm×1.5cm) with a plastered floor and kept in a climate chamber (day:night temperatures 20°C:15°C) and fed on a diet of water, honey and crickets.

All colonies were collected between May and June before the onset of the *P. americanus* raiding season, which takes place between July and September. As a consequence of this timing, slavemaking colonies only contained *P. americanus* brood and adults and adult enslaved *T. longispinosus* workers originating from raiding events of previous years. All *P. americanus* colonies containing slaves of species other than *T. longispinosus* were excluded from the analysis. Non-parasitized *T. longispinosus* colonies only contained their own conspecific brood.

Survival rate of pupae

We contrasted the brood rearing success of enslaved *T. longispinosus* workers to that of free-living *T. longispinosus* workers. The number, caste (worker, male, queen) and condition of all brood items (prepupae and pupae) were recorded on a daily basis until seven days after eclosion of the pupae to adult ants. As both species belong to the subfamily Myrmicinae, the pupae lack cocoons and their caste can be identified by shape and size. All pupae that were decapitated or cut in pieces were counted as dead and all missing pupae and callows (freshly eclosed workers) were assumed to have died as well. For details on the behavior of pupae killing and the observation protocol we refer to Achenbach and Foitzik (2009). We collected data from 158 *P. americanus* and 102 *T. longispinosus* colonies from the four communities. This dataset was extended with the data from Achenbach and Foitzik (2009) including 51 *P. americanus* colonies and 41 *T. longispinosus* colonies from New York and Ohio. In total, we monitored the brood development in 352 colonies (WV: 16 host and 15 slavemaker colonies, NY: 51 host and 166 slavemaker colonies, OH: 48 host and 28 slavemaker colonies, MI: 28 host colonies). Most of these colonies had brood of different castes and we recorded the total number of worker, male and queen pupae, and how many of them reached adulthood (N = 896 measurements).

Statistical analysis

We used logistic regressions to analyze the pupal survival data. To fit the logistic regressions, we use the “glmmPQL” command (implemented in the nmle package (Pinheiro et al. 2011)) in the R statistical computing software with a logit link function fit overdispersed models. For more details on the statistical analysis, we refer to the supplementary material. The proportion of surviving brood is modeled as a function of species of the brood (*P. americanus* vs. *T. longispinosus*), community of origin, nest size (total number of *T. longispinosus* workers) and caste of the brood. We included colony as random factor in the model to control for colony identity. We did not include sampling year as random variable as it explained less than four % of the total variation when included in the model. As we performed the main analysis on the survival of brood of both species, we could neither include the number of slavemaker workers per nest (only present in slavemaker nests), nor the unparasitized MI population (slavemaker colonies absent) into the main analysis. We conducted an additional analysis based on only the slavemaker colonies to include the number of slavemaker workers per nest and also an additional analysis with only the host colonies, in order to include the MI population. For model selection, we start with a parameter-rich model, including all factors, covariables and the interactions of the cofactor "species" with the other factors and the covariables and then we followed a stepwise elimination procedure. We report the minimal adequate model, in which all factors and covariables or their interactions have a significant effect.

To study the effect of the number of slavemaker workers on parasite brood survival we analyzed the slavemaker dataset using the same modeling approach as in the main analysis, with the only difference that we now exclude species as a factor but include the number of slavemaker workers. In order to contrast pupae survival in the unparasitized MI population to all other host populations, we applied the same modeling procedure as in the main model but without the slavemaker colonies and therefore without the factor species.

Results

Using the full data set with nests of both species from NY, WV and OH, we find that pupae in *P. americanus* nests show a much lower survival than pupae in free-living *T. longispinosus* nests ($W_{1, 326} = 32$, $p < 0.001$, see Table S1, Fig.1). On average, pupae in free-living *T. longispinosus* nests have a survival of about 85% while *P. americanus* pupae only have a 45% survival probability. Moreover, pupae survival varied between communities ($W_{2, 326} = 9$, $p < 0.0002$) with a higher survival in Ohio (77%) compared to both West Virginia (54%) and New York (63%) (NY vs. OH: t = 3.52 p = 0.0005, WV vs. OH: t = 3.78, p = 0.0002), but no difference between NY and WV (NY vs. WV t = -1.57, p = 0.12, Table S1, Fig.1). Nest size (N of *T. longispinosus* workers) had a positive effect on pupae survival ($W_{1,219} = 7.7$ p = 0.006). In addition, the castes differed in survival ($W_{2,219} = 5.3$, p = 0.0055) with queens surviving less well than workers and males (males vs. queens: t = -2.87, p = 0.0045, workers vs. queens: t = -3.12, p = 0.002), but no difference between males and workers (t = 0.27, p = 0.78).

When analyzing the survival rate of slavemaker brood separately, we find an effect of community ($W_{2,211} = 4.5$ p = 0.012) with lower survival rates in WV (27%) compared to the two other communities (NY vs. WV: t = -2.54, p = 0.012, OH vs. WV: t = -2.96, p = 0.0035) but no difference between New York (49%) and Ohio (58%) (t = 1.32, p = 0.19, Fig.1). We find an effect of caste ($W_{2, 157} = 6.0$ p = 0.003) with slavemaker queen pupae survive less well than worker or male pupae (males vs. queens t = -2.95 p = 0.0037, worker vs. queens t = -3.34, p = 0.001) and no difference between the latter two ($t_{157} = 0.55$, p = 0.57). We find a positive effect of the number of *T. longispinosus* workers on brood survival ($W_{1,211} = 6$, p = 0.015), but no effect of the number of the slavemaker workers (p = 0.89). The complete results of the minimal adequate model are given in table S2 in the supplementary material.

In the *T. longispinosus* data set, we find a significant difference between communities ($W_{3.140} = 9.9$ $p < 0.0001$) and castes ($W_{2,93} = 8.1$ $p = 0.0006$), but no effect of the number of *T. longispinosus* workers ($p = 0.19$). Host pupae survived significantly better in Ohio (96%) compared to New York (77%) and West Virginia (WV 80%) all comparisons $t > 2.7$ $p < 0.007$) and the latter two populations did not differ in pupae survival ($t_{140} = 0.38$, $p = 0.71$). Host pupae survived better in the unparasitized Michigan population (89%) than in New York ($t_{140} = -1.96$, $p = 0.05$), and less well than in Ohio ($t = 2.70$, $p = 0.008$). Host pupae survival rates did not significantly differ between the Michigan and West Virginian population ($t = -1.11$, $p = 0.27$). When looking at the survival rate of the different castes, we find that queens survive worse compared to the two other castes (males vs. queens $t = -2.7$ $p = 0.0081$, workers vs. queens $t = -3.94$, $p = 0.0002$), but we found no difference between workers and males. All results are summarized in Table S3 and Figure 1.

Discussion

In this study, we investigated geographic variation in offspring survival in the obligate social parasite *P. americanus* and its main host *T. longispinosus.* We find severely reduced brood survival rates in *P. americanus* nests compared to *T. longispinosus* nests in all three parasitized communities, even though in both types of nests *T. longispinosus* workers care for the brood. This phenomenon is intriguing, because the observed difference in brood survival could be explained by the host defense trait “slave rebellion” (Achenbach and Foitzik 2009). If slavemaker brood rarely survives, slavemaking colonies will remain small and nearby host colonies, which are potentially related to the slaves in the slavemaker nest, will experience a reduced raiding risk. The low parasite brood survival can explain why *P. americanus* slavemaker nests are so small compared to nests of related species (Foitzik and Herbers 2002).

Before we discuss our findings in the context of an co-evolutionary dynamic we want to explore two alternative explanations for the observed difference in brood survival. One potential explanation of the observed low survival of slavemaker pupae could be that the laboratory environment is unfavorable for the parasite. Secondly, the observed low survival of slavemaker pupae could be the result of enslaved workers generally caring less well for allospecific brood compared to conspecific brood. We can reject both of these explanations, because under laboratory conditions slavemaker larvae develop normally under the care of enslaved host workers with a survival rate until pupation of over 95% (Achenbach and Foitzik 2009). Larvae have to be fed as well as cleaned and thus need more care than pupae. The high survival rate during the larval stage indicates adequate care by slave workers at this developmental stage. We believe that the data are best explained as a defense trait. In addition to the previous argument, we would like to note two other observations which support our view. First we have direct observational evidence that enslaved host workers attack seemingly healthy parasite pupae and tear them apart (Achenbach and Foitzik 2009). In the study by Achenbach and Foitzik (2009) 25% of all slavemaker pupae were observed to be actively killed. Finally, the strong geographic variation in survival rates reported here indicates that the low survival is not due to a general inadequacy of *T. longispinosus* workers to care for *P. americanus* pupae.

The following part of the discussion is organized along the five expectations we stated in the introduction. (1.) We find evidence for a post-enslavement defense to be present in all three parasitized populations, reflected in a severely reduced survival of *P. americanus* pupae compared to host pupae (Tab.S1, Fig.1). In these communities about half of the parasite pupae do not survive to adulthood. Our results, based on a substantially larger data set and an additional community, confirm the findings of Achenbach and Foitzik (2009) (2.) Because the populations we study are genetically differentiated and differ in other defense traits, we expected quantitative differences in the expression of the rebellion trait in different sites and, indeed, our data indicate pronounced differences between communities (Fig.1). While we find that slavemaker pupae survival is lower than host pupae survival in all habitats, the survival rates for parasite pupae were lower in the West Virginia study site than in the two other communities. This finding could provide an explanation for the observation that slavemaker nests in the field are smaller in WV than in other populations (Herbers & Foitzik 2002).

We expected (3.) to find higher slavemaker killing rates in host populations under strong parasite pressure. It is not easy to quantify parasite pressure, because it depends on several parameters such as parasite prevalence, raiding frequency and destructiveness as well as community composition. Previous studies have shown that the parasitation rate is very similar in the three communities (Table 1), but NY slavemaker colonies have a higher frequency of successful raids compared to WV and OH (Foitzik et al. 2001, Brandt & Foitzik 2004). Parasite pressure is therefore likely to be higher on the *T. longispinosus* population in NY than at the two other sites, yet we found the lowest slavemaker survival in WV and not in NY (Fig.1). The fact that we find strong behavioral defenses against raids, but lower pupae killing in NY and the reverse pattern in WV resembles the situation in avian brood parasites in which hosts often either possess strong egg- or chick recognition abilities, but not both. Based on theoretical considerations, Britton et al. (2007) suggested that if multiple defense strategies are utilized in a host-parasite system the presence of one effective defense strategy could relax selection for alternative defenses. If, indeed, defenses have additive effects, a well-developed nest defense could reduce selection on pupae killing behavior in NY, and vice versa in WV. While we found relatively high pupal survival in host colonies from the unparasitized Michigan population, the survival rate was slightly lower than in Ohio, indicating again that additional factors (other than parasite pressure) influence pupal survival rates.

(4.) In our previous study (Achenbach and Foitzik 2009), we found lowest survival rates for *P. americanus* queen pupae, low survival for worker pupae, but very high survival rates of parasite male pupae. In this study we find that parasite queens have the lowest survival probability, but male and worker survival did not differ in this larger data set. In contrast to males and workers, queens are much larger than the two other castes and the lower survival rate might be due to a less stable development. As we find lower survival rates in *T. longispinosus* queens as well, we conclude that the observed effect appears to be a general phenomenon and not specific to parasite brood survival.

We hypothesised (5.) that part of the variation in nest size of *P. americanus* could be explained by variation in killing behavior between nests. We predicted that only colonies with higher brood survival are able to reach large colony size. We do not find an association between *P. americanus* pupae survival and *P. americanus* worker number, but our analysis indicates that brood survival is better in *P. americanus* nests with many slaves. In contrast, we do not find such an association in free living *T. longispinosus* nests. This indicates that groups of *Temnothorax* workers are not per-se better in raising brood, but that this association between slave worker number and pupae survival is specific to parasite nests.

In summary, we demonstrate the presence of the slave rebellion trait in three different communities supporting the results of Achenbach and Foitzik (2009). Moreover, the pronounced variation in trait expression between communities compatible with the presence of a geographic mosaic of coevolution (Thompson 2005). Local parasite pressure did not explain parasite survival, possibly because alternative defense mechanisms change the selective regime on additional traits (Britton et al. 2007). In studies on avian brood parasites, such relationship between local parasite pressure and the expression of host defense is traitwas often found (e.g. Soler and Moller 1990, Stokke et.al. 2008). However, a recent study found no such relationship (Vikan et. al 2010). Vikan et al. document a lack of variation in the defense trait between populations experiencing different levels of parasite pressure. The authors suggest that strong geneflow between the populations might synchronize the different evolutionary dynamics in the studied populations. Such explanation is probably not true for our system, as we find pronounced differences in the expression of the defence trait between populations, indicating that geneflow is limited. Larger studies on more populations are needed to find out what factors can explain the differences between populations in the slavemaker system. In addition potential future research should focus on the experimental investigation of this defense trait. For example it would be interesting to confront colonies originating from unparasitized populations with parasite brood and compare their rearing success to colonies from parasitized population in order to investigate if these colonies also express the defense trait.

The outcome of local coevolutionary dynamics depends on multiple factors ranging from geneflow between sites, local parasite pressure, predation, potential interference of multiple defense traits, up to from ecological conditions resulting in a complex picture of host defense traits (Britton et al. 2007, Thompson 2005, Lorenzi and Thompson 2011). Our results support these findings and add to the growing knowledge of the spatial distribution of host defenses.

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References

Achenbach A, Foitzik S (2009) First Evidence for Slave Rebellion: Enslaved Ant Workers Systematically Kill the Brood of Their Social Parasite *Protomognathus Americanus*. Evolution 63 (4): 1068-1075.

Achenbach A, Witte V, et al. (2010) Brood exchange experiments and chemical analyses shed light on slave rebellion in ants. Behavioral Ecology 21 (5): 948-956.

Alloway TM (1990) Slave-Species Ant Colonies Recognize Slavemakers as Enemies. Animal Behaviour 39: 1218-1220.

Beibl J, Stuart RJ, et al. (2005) Six origins of slavery in *Formicoxenine* ants. Insectes_Sociaux 52 (3): 291-297.

Berdoy M, Webster JP, et al. (2000) Fatal attraction in rats infected with *Toxoplasma gondii* Proceedings of the Royal Society of London Series B-Biological Sciences 267 (1452): 1591-1594.

Brandt M, Fischer-Blass B, et al. (2007) Population structure and the co-evolution between social parasites and their hosts. Molecular Ecology 16 (10): 2063-2078.

Brandt M and Foitzik S (2004) Community context and specialization influence coevolution between a slavemaking ant and its hosts. Ecology 85 (11): 2997-3009.

Brandt M, Heinze, J, et al. (2005) A chemical level in the coevolutionary arms race between an ant social parasite and its hosts. Journal of Evolutionary Biology 18 (3): 576-586.

Brandt M, Heinze J, et al. (2006) Convergent evolution of the Dufour's gland secretion as a propaganda substance in the slave-making ant genera *Protomognathus* and *Harpagoxenus*. Insectes Sociaux 53 (3): 291-299.

Britton NF, Planque R, Franks NR (2007) Evolution of defence portfolios in exploiter-victim systems. Bulletin of Mathematical Biology 69 (3): 957-988 DOI: 10.1007/s11538-006-9178-5

Buschinger A (2009) Social parasitism among ants: a review (Hymenoptera: Formicidae). Myrmecological News 12: 219-235.

Clay K (1991) Parasitic castration of plants by fungi. Trends in Ecology & Evolution 6 (5): 162-166.

Davies NB (1999) Cuckoos and cowbirds versus hosts: Co-evolutionary lag and equilibrium. Ostrich 70 (1): 71-79.

Dawkins R, Krebs JR (1979) Arms Races between and within Species. Proceedings of the Royal Society of London Series B-Biological Sciences 205 (1161): 489-511.

Fischer-Blass B, Heinze J, et al. (2006) Microsatellite analysis reveals strong but differential impact of a social parasite on its two host species. Molecular Ecology 15 (3): 863-872.

Fischer B and Foitzik S (2004) Local co-adaptation leading to a geographical mosaic of coevolution in a social parasite system. Journal of Evolutionary Biology 17 (5): 1026-1034.

Foitzik S, Achenbach A, et. al. (2009) Locally adapted social parasite aff-+ects density, social structure, and life history of its ant hosts. Ecology 90 (5): 1195-1206.

Foitzik S, DeHeer CJ, et al. (2001) Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. Proceedings of the Royal Society of London Series B-Biological Sciences 268 (1472): 1139-1146.

Foitzik S, Herbers JM (2001a) Colony structure of a slavemaking ant. I. Intracolony relatedness, worker reproduction, and polydomy. Evolution 55 (2): 307-315.

Foitzik S, Herbers JM (2001b) Colony structure of a slavemaking ant. II. Frequency of slave raids and impact on the host population. Evolution 55 (2): 316-323.

Frank SA (1996) Models of parasite virulence. Quarterly Review of Biology 71 (1): 37-78.

Gandon S, Buckling A, et al. (2008) Host-parasite coevolution and patterns of adaptation across time and space. Journal of Evolutionary Biology 21 (6): 1861-1866.

Hare J F, Alloway TM (2001) Prudent *Protomognathus* and despotic *Leptothorax duloticus*: Differential costs of ant slavery. Proceedings of the National Academy of Sciences of the United States of America 98 (21): 12093-12096.

Herbers JM, Foitzik S (2002) The ecology of slavemaking ants and their hosts in north temperate forests. Ecology 83 (1): 148-163.

Hefetz A (2007) The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae) - interplay of colony odor uniformity and odor idiosyncrasy. A review. Myrmecological News 10: 59-68

Kaltz O, Gandon S, et al. (1999) Local maladaptation in the anther-smut fungus *Microbotryum violaceum* to its host plant *Silene latifolia*: Evidence from a cross-inoculation experiment. Evolution 53 (2): 395-407.

Kaltz O, Shykoff JA (1998) Local adaptation in host-parasite systems. Heredity 81: 361-370.

Kaspari M, Vargo EL (1995) Colony Size as a Buffer against Seasonality - Bergmanns Rule in Social Insects. American Naturalist 145 (4): 610-632.

Kruger O (2007) Cuckoos, cowbirds and hosts: adaptations, trade-offs and constraints. Philosophical Transactions of the Royal Society B-Biological Sciences 362 (1486): 1873-1886.

Lesnoff M, Lancelot, R (2010) aod: Analysis of Overdispersed Data. R package version 1.2, URL http://cran.r-project.org/package=aod

Lafferty KD, Kuris AM (2009) Parasitic castration: the evolution and ecology of body snatchers. Trends in Parasitology 25(12): 564-572.

Langmore NE, Hunt S, et. al. (2003) Escalation of a coevolutionary arms race through host rejection of brood parasitic young. Nature 422 (6928): 157-160.

Lively CM (1989) Adaptation by a parasitic trematode to local-populations of its snail host. Evolution 43 (8): 1663-1671.

Lorenzana JC, Sealy SG (2001) Fitness costs and benefits of cowbird egg ejection by gray catbirds. Behavioral Ecology (12) 3: 325-329, DOI: 10.1093/beheco/12.3.325

Moksnes A, Roskaft E, et al. (1991) Behavioral-Responses of Potential Hosts Towards Artificial Cuckoo Eggs and Dummies. Behaviour 116: 64-89.

Pamminger T, Scharf I, Pennings PS, Foitzik S (2011) Increased host aggression as a induced defence against slave-making ants. Behavioral Ecology *22 (2): 255-260.*

Pinheiro J, Bates D, DebRoy S, Sarkar D, the R Development Core Team (2011). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-101.

Price PW (1980) Evolutionary biology of parasites. – Princeton University Press, Princeton

Pennings P.S, Achenbach A, et al. (2011) Similar evolutionary potentials in an obligate ant parasite and its two host species. Journal of Evolutionary Biology 24 (4): 871-886.

Poulin R (1995) "Adaptive" changes in the behaviour of parasitized animals: A critical review. International Journal for Parasitology 25 (12): 1371-1383.

Roskaft E, Moksnes A, et al. (2002) Aggression to dummy cuckoos by potential European cuckoo hosts. Behaviour 139: 613-628.

Rothstein SI (1990) A model system for coevolution – avian brood parasitism. Annual Review of Ecology and Systematics 21: 481-508.

Sato T (1986) A Brood Parasitic Catfish of Mouthbrooding Cichlid Fishes in Lake Tanganyika. Nature 323 (6083): 58-59.

Scharf I, Pamminger T, Foitzik S (2011) Differential Response of Ant Colonies to Intruders: Attack Strategies Correlate With Potential Threat. Ethology 117 (8): 731-739

Soler M, Moller AP (1990) Duration of Sympatry and Coevolution between the Great Spotted Cuckoo and Its Magpie Host. Nature 343 (6260): 748-750.

Stokke BG, Hafstad I, et al. (2008) Predictors of resistance to brood parasitism within and amoung reed warbler populations. Behavioural ecology 19: 612-620

Thomas F, Adamo S, et al. (2005) Parasitic manipulation: where are we and where should we go? Behavioural Processes 68 (3): 185-199.

Thompson JN, (2005) The Geographic Mosaic of Coevolution. University of Chicago Press: Chicago, IL, USA

Vikan JR, Stokke BG, et. al. (2010) Evolution of defences against cuckoo (*Cuculus canorus*) parasitism in bramblings (*Fringilla montifringilla*): a comparison of four populations in Fennoscandia. Evolutionary Ecology 24, 1141—1157.

Yu DW and Pierce NE (1998) A castration parasite of an ant-plant mutualism. Proceedings of the Royal Society of London Series B-Biological Sciences 265 (1394): 375-382.

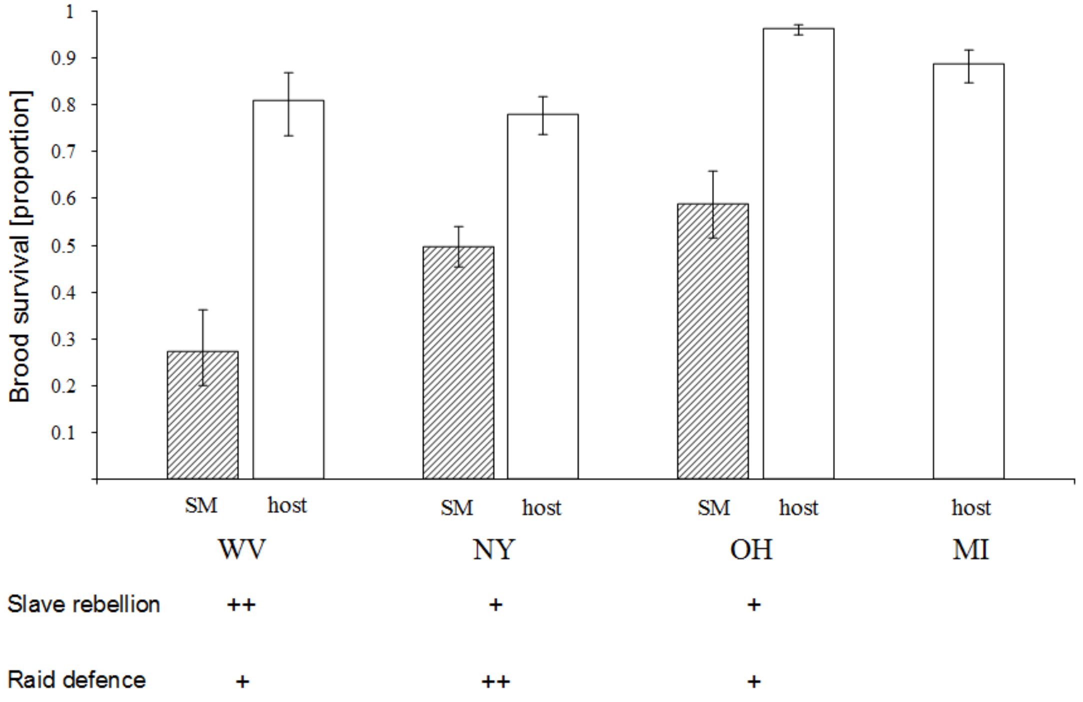

Figure 1.: Survival probabilities of pupae of the slavemaker *P. americanus* and the host *T. longispinosus* in the four communities West Virginia (WV), New York (NY), Ohio (OH) and Michigan (MI). Presented are means and error bars indicating standard error predicted by the glmmPQL model. Below the graph we present a summary of known host defence traits. Presented are performance during raiding events and killing rates of parasite pupae (++ well defended + defended).

Table 1: In this table we present the composition of the ant community in the four study sites. Parasitiation rate is given as the number of host nests (*Temnothorax*) per parasite nest (*P. americanus*). Species contribution to the ant community is given in percent (Herbers & Foitzik 2002; Brandt & Foitzik 2004).

Community	**Parasitation rate**	***T. longispinosus***	***T. curvispinosus***	***T. ambiguus***
New York	10	97.7	0	2.3
West Virginia	8.4	83.6	16.4	0
Ohio	10.2	29.0	71.0	0
Michigan	-	64.6	0	35.4

Supplementary material

1. Software used

To fit generalized linear mixed models to our data we used the glmmPQL (general linear mixed model using Penalized Quasi-Likelihood) with the setting "familiy = quasibinomial" command of the R statistical programming environment implemented in the lmne package (Pinheiro et al. 2011). Dispersion parameters were estimated by the glmmPQL routine. During model selection procedures we checked for a significance of regression coefficients using a Wald test based on an F-distribution as implemented in the aod package (Lesnoff and Lancelot, 2010). We used a backward elimination procedure ending up with the minimum adequate model where all factors are significant. We used the command wald.test to perform the analysis.

2. Factors influencing pupae survival in the complete dataset

Using the glmmPQL command, we applied an overdispersed binomial logistic regression to the main dataset (NY, WV and OH) to test whether the three cofactors species (*P. americanus* or *T. longispinosus*), community (NY, WV, OH), caste (male, worker, queen), and the covariables nest size (number of *T. longispinosus* workers) have an effect on the proportion of pupae that survived to become adults. To control for nest identity we included colony as a random factor in the analysis. The following table is the result of the model that we obtained after our variable selection procedure (backward elimination). The minimal adequate model and the cofactors, covariables and interactions which were removed are shown in table S1.

Table S1

Minimal adequate model	**W**	**df**	**p**
Species	32	1, 326	<0.0001
Nest size	7.7	1, 219	0.0061
Community	9	2, 326	0.0002
Caste	5.3	2, 219	0.0055
Excluded terms			
Species x Nest size	0.063	1, 323	0.8
Species x Caste	2.4	2, 217	0.092
Species x Community	2.4	2, 324	0.089

Post hoc comparisons	**df**	**t-value**	**p**
Community			
NY x OH	326	3.521	0.0005
NY x WV	326	-1.566	0.1182
OH x WV	326	-3.784	0.0002
Caste			
Males x Queens	219	-2.867	0.0045
Males x Worker	219	0.271	0.7876
Worker x Queens	219	-3.122	0.0020

3. Factors influencing survival of slavemaker pupae

Using the glmmPQL command, we applied an overdispersed binomial logistic regression to the dataset containing only the slavemaker nests, to test whether the two cofactors community, caste and whether the two covariables number of slavemaker workers and number of host workers have an effect on the proportion of pupae that survived to become adults. To control for nest identity we included colony as a random factor. The following table is the result of the model that obtained after our variable selection procedure (backward elimination). The minimal adequate model and excluded terms are shown in table S2.

Table S2

Minimal adequate model	**W**	**df**	**p**
Community	4.5	2, 211	0.012
Caste	6.0	2, 157	0.003
Nest size	6.0	1, 211	0.015
Excluded terms			
Number of SM worker	0.031	1, 159	0.86
Post hoc comparisons	**df**	**t-value**	**p**
Community			
NY x OH	211	1.323	0.1872
NY x WV	211	-2.536	0.0119
OH x WV	211	-2.956	0.0035
Caste			
Males x Queens	157	-2.950	0.0037
Males x Worker	157	0.555	0.5790
Worker x Queens	157	-3.342	0.0010

4. Factors influencing pupae survival in the host data set

Using the the glmmPQL command, we applied an overdispersed binomial logistic regression to the dataset containing only the host nests, to test whether the two cofactors community, caste and the two covariables and number of host workers have an effect on the proportion of pupae that survived to become adults. The following table is the result of the model that obtained after our variable selection procedure (backward elimination). The minimal adequate model and terms dropped from the model are shown in table S3.

Table S3:

Minimal adequate model	**W**	**df**	**p**
Community	9.9	3, 140	>0.0001
Caste	8.1	2, 93	0.0006
Excluded terms			
Nest size	1.7	1, 93	0.19
Post hoc comparisons	**df**	**t-value**	**p**
Community			
MI x NY	140	-1.964	0.0515
MI x OH	140	2.703	0.0077

MI x WV	140	-1.106	0.2704
NY x OH	140	5.227	>0.0001
NY x WV	140	0.377	0.7066
OH x WV	140	-3.385	0.0009
Caste			
Males x Queens	94	-2.705	0.0081
Males x Workers	94	1.292	0.1995
Workers x Queens	94	-3.943	0.0002

Chapter 5: Oh sisters, where are you? Indirect fitness benefit could maintain a host defence trait.

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Running title: Fitness benefits of Slave rebellion

Word count: 5747

Introduction

Organisms exploiting other organisms as a resource can be considered an evolutionary success story. Indeed, parasitism is one of the most common lifestyles on earth (Price 1980), shaping food webs, influencing biodiversity and driving the evolution of major innovations such as sex and sociality (Hamilton 1990, Wommack 2000, Daszak 2000, Morran 2011). The high costs often associated with being parasitized exert strong selection pressure on the host to defend themselves (e.g Booth et al. 1993). Hosts can respond by developing various forms of defence mechanisms limiting the harmful effects of parasites (Minchella 1985, Brunham 1993, Gross 1993, Bennett 1994, Sheldon et al. 1996, Morran 2011). Studying such counter adaptations in detail is crucial in order to better understand the factors shaping the evolution of parasite virulence and host resistance.

The outcome of such host-parasite dynamics have been the focus numerous theoretical studies (e.g. Bremermann 1983, Boots 1999). However, most of these consider well-mixed populations and largely ignore local population structure (Boots et al. 2004). In recent years it has become apparent that population viscosity can have a strong effect on parasite virulence (Boots et al. 2004) as well as on host resistance (Best 2011). Most natural populations exhibit spatial structure at least on some scale this structure can affect the outcome of host-parasite interactions. In the current study we investigate the impact of local population structure on a host-social parasite interaction in ants.

In contrast to micro-parasites, which directly extract resources from their hosts (Price 1980), brood parasites exploit their hosts' brood care behavior (Petrie 1991). A large proportion of the adult life of many organisms is devoted to the production of offspring, which is costly concerning time and energy investments. Brood parasitism is a strategy to reduce those costs. Indeed, brood parasitism has evolved in various systems independently (e.g. Kilner 2011) in which rearing the offspring can be performed by individuals other than the parents (e.g., in birds and fish Sato 1986; Rothstein 1990). Slavery (dulocis) is a variant of brood parasitism encountered only in ants (Buschinger 2009). Obligatory slavemakers are unable to perform basic tasks such as colony maintenance, foraging, nest relocation and brood rearing. All these tasks are outsourced to workers of another species (e.g. Stuard and Alloway 1985). These so-called slave workers are acquired during destructive raiding events where slavemakers steal brood of surrounding host colonies (Alloway 1979, Foitzik and Herbers 2001). The future worker generation is transported back to the parasite nest where they hatch and start working.

In our study system, host-parasite interactions take place on the behavioural level, because host and parasite are of approximately equal size and engage in fierce encounters during raiding events (Alloway 1990, Foitzik 2001). Consequently the host's struggle to defend itself can be observed with the naked eye and usually involve some kind of aggressive interaction (Alloway 1990, Foitzik et al. 2001, Scharf 2011, Pamminger et al. 2011). The ant *Protomognathus americanus* is an obligatory slavemaker species and various defence mechanisms have been described in its main host species *Temnothorax longispinosus* prior to enslavement. They range from parasite detection and nest evacuation and elaborate collective defence strategies, up to induced colony aggression after the encounter with a parasite (Alloway 1990, Foitzik et al. 2001, Pamminger et al. 2011), all of which potentially increase the host's fitness directly.

It has been argued that post enslavement defense mechanisms can not evolve in slavemaker systems because slaves are probably unable to reproduce and lack the behavioural options to increase their direct fitness directly, therefore there should be no selection on their behavior (Gladstone 1981). However, in his argument, Gladstone neglected that slaves may also have opportunities to increase the number offspring of their free-living relatives by reducing the raiding frequency of the slavemakers, if they could reduce the number of slavemakers in the nest. Such a behavior was found by Achenbach and Foitzik (2009), who described a post-enslavement behavioral defense mechanism in *T. longispinosus* which they termed “slave rebellion”. Instead of rearing the brood of their slavemakers, the slaves were found be extremely ineffective in rearing slavemaker brood. This phenomenon appears to be wide spread, occurring to various extend in all populations we investigated (Pamminger et.al. 2012).

We know from earlier studies that smaller slavemaker nests perform fewer successful raids per season (Foitzik 2001). Therefore, it is plausible that reducing the slavemaker colony size by killing a proportion of slavemaker workers (Achenbach 2009, Pamminger et al. 2012) rebellious slaves could lower the parasite pressure on surrounding host colonies. This behavior could be selected for if hosts within the potential raiding range of slavemaker colonies are more related to the rebellious slaves than colonies further away are. A modeling approach indicates that indeed a hypothetical slave rebellion allele could spread if the host population is adequately structured (Metzler et al. unpublished manuscript). In order to investigate the potential benefits of slave rebellion we mapped the small scale population structure of two well-studied sampling sites on the east coast of the United States (New York and West Virginia). In this paper we will:

1. Investigate whether we find any indication of slave reproduction and therefore direct fitness benefits of slave rebellion.

2. Estimate the potential raiding range of slavemaker colonies in order to find the adequate scale to study host population structure.
3. Test if we find increased relatedness levels between slaves and surrounding host colonies within the raiding range of the slavemaker nest.
4. Try to uncover the origin of the observed structure by analysing the small scale population genetics of the host population in detail.

Material and methods

Ant collection and sampling location

Ant nests of the host species *T. longispinosus* and slavemaker *P. americanus* were collected in July 2009 at Huyck Preserve in Albany County, New York (N 42◦31’ 35.3” W 74◦9’ 30.1”) and at the Watoga State Park, Pocahontas County, West Virginia (N 38◦06’13” W 80◦8’59”). To investigate small scale genetic structure, we mapped two transects per habitat consisting of six plots (6 x 3m) each, spread over a distance of approximately 100 m.

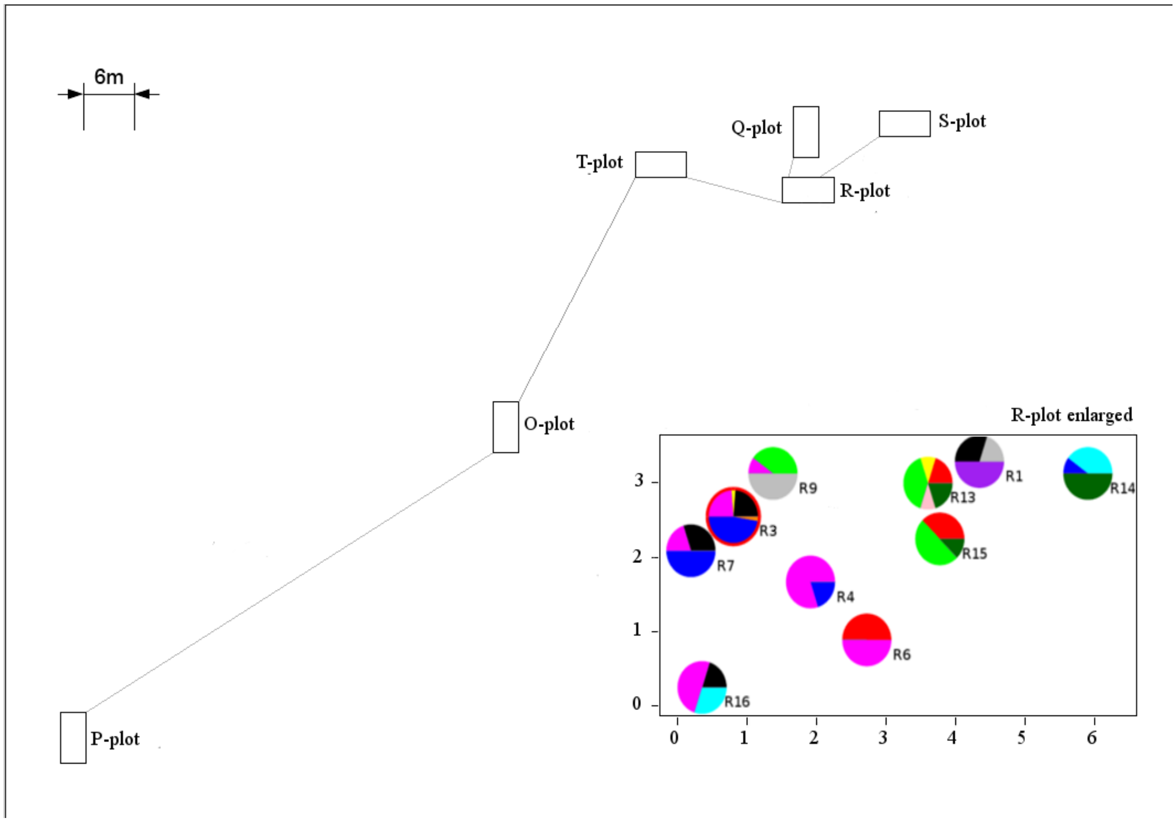

Figure 1: Example of the distribution of plots within one transect in WV. In the lower right corner plot R is enlarged to demonstrate the distribution of colonies within the plot and show the distribution of GT1 alleles among colonies. R3 is a slavemaker nest and shares many alleles with R7. While R13 and R15 are free living host colonies in close proximity and appear to be related. The missing R colonies were of a different species (*Temnothorax curvispinosus*) not included in this analysis.

In WV we mapped two additional plots separated from the two transects. We recorded nest location within each plot and distances between plots, which enabled us to calculate distances between all nests in a given transect. Ant nests were censused directly after collection and transferred in 100% ETOH for genetic analysis. We amplified six highly variable microsatellite loci for five workers per non-parasitized *T. longispinosus* nest and up to 20 enslaved *T. longispinosus* workers of the *P. americanus* nests. In total we collected data from 1181 non-parasitized *T. longispinosus* workers (241 *T. longispinosus* nests) and 683 enslaved *T. longispinosus* workers (41 *P. americanus* nests). From now onwards, we will refer to *T. longispinosus* workers from non-parasitized *T. longispinosus* nests as "free-living" and to the enslaved *T. longispinosus* workers sampled from *P. americanus* nests as "enslaved".

1. Potential direct benefits of slave rebellion (Slave reproduction)

It has been assumed that slave behavioural defence mechanisms can not evolve in slavemaker systems, because enslaved ant workers are unable to reproduce or locate and be accepted in their mothers' nest from which they were taken as pupae (Gladstone 1981). Indeed, in free-living nests, reproduction is monopolised by the *T. longispinosus* queen if she is present in the nest (Heinze et al. 1997). In accordance with these findings, *T. longispinosus* workers were never observed to reproduce in the presence of a *P.americanus* worker or queen (Foitzik & Herbers 2001 Evol a,b). Enslaved *T. longispinosus* workers appear to accept *P. americanus* ants as dominant reproductive individuals and consequently do not reproduce in their presence. However, once the queen or the slavemaker is removed, it is likely thatb*T. longispinosus* workers will start to develop their ovaries and produce males (Konradt et al. 2012, Heinze and Oberstadt 1999). Therefore, it may be possible for enslaved *T. longispinosus* workers to reproduce if they outlive their slavemakers. Hence, slaves might have a direct fitness benefit from killing slavemaker brood, increasing the probability of outliving them and produce male offspring.

1.1 Reproduction by slaves

In order to investigate whether and to which extend *T. longispinosus* slave workers care able to reproduce once they outlived their oppressors, we screened our genetic data for potential former slavemaker nests. Such a nest would be characterised by:

1. Low within-nest relatedness resembling the relatedness between slaves in the slavemaker nest.
2. The absence of a queen (*P. americanus* or *T. longispinosus*).
3. No *P.americanus* workers, but possibly some parasite brood
4. The presence of *T. longispinosus* males.

We computed the relatedness between pairs of free-living *T. longispinosus* nests and between free-living *T. longispinosus* nests and enslaved workers in *P. americanus* nests and the relatedness within workers of free *T. longispinosus* nests and within *P. americanus* nests according to equation (10) in Queller and Goodnight (1989). The coefficients of relatedness were calculated relative to the microsatellite allele frequencies in the Huyck Preserve (NY) and Watoga State Park (WV).

1.2 Results

2. DIRECT FITNESS BENEFITS

After screening 241 host colonies we did not find a single nest exhibiting reliable signs of reproducing slaves. A major problem with this analysis is caused by the unexpectedly wide distribution of within-nest relatedness values in *T. longispinosus* nests (Fig.2). We expected within-nest relatedness of free-living *T. longispinosus* nests to be well separated from and much higher than within-nest relatedness of enslaved *T. longispinosus* workers. Indeed, we find that slaves on average are less closely related to each other compared free living host workers, but the relatedness distribution in free-living *T. longispinosus* nests widely overlaps with slave relatedness (Fig.2). In addition successful slave reproduction becomes more unlikely if you consider the small window of opportunity slaves have to produce males that would get the chance to reproduce. Like many ant species *T .longispinosus* reproduces in mating swarms and all sexuals of a given area have to leave the nest at the same time. In order for sexuals to mature at the right time they have to be layed sortly after colonies come out of hibernation and only if no slavemaker would be present at the time. Given these facts we conclude that, we have no evidence for slave reproduction at this time but it is unlikely that this is an event occurring on a frequent basis and would contribute substantially to slaves fitness.

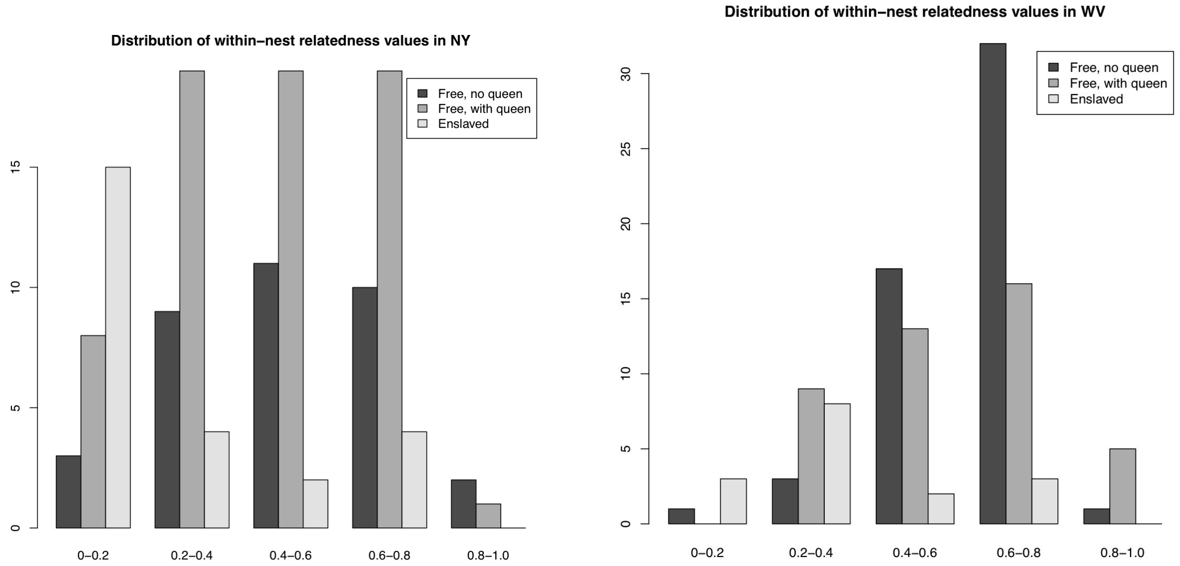

Figure 2: Illustrating the distribution of within nest relatedness of free living host colonies (with and without queen) and the relatedness of slaves in slavemaker nests in both habitats under

investigation. The height of the bars represents the number of colonies with a within colony relatedness in a certain range (indicated on the x-axis). Relatedness values are calculated according to Queller and Goodnight (1989) equation (10) relative to the allele frequencies in the habitat.

2. Indirect fitness benefits

In order for slave rebellion to provide an indirect fitness benefit for rebellious slaves, their behaviour must benefit related colonies within the area threatened by a raiding event of the slavemaker nest they are living in. A modeling/simulation based approach suggests that a slave rebellion allele could be maintained/spread in a population via indirect fitness benefits, if the host population is sufficiently structured within the raiding range of the slavemaker nest (Metzler et al. unpublished). In order to determine the relevant scale, we calculated the approximate raiding distance slavemaker nests within one season. Because we only had the required data (raiding frequencies and average host density) for one of the two habitats (NY) we will assume a similar raiding range for WV.

2.1 Raiding distance

We calculated the approximate area threatened by raids of a slavemaker nest by dividing the average number of successful raids a slavemaker nest conducts each season by the average density of host colonies in parasitized areas (Foitzik and Herbers 2001b, Herbers and Foitzik 2002). We assume that both host and slavemaker nests stay in one location during the raiding season and that empty nest sites are not recolonized by other *T .longispinosus* nests, therefore all raided nests must have been within raiding range at the start of the season. This results in the minimum area raided by a slavemaker nest per season (ignoring unsuccessful raids). Assuming no directional preference of raiding slavemaker nest we estimate the radius of the affected area using the formula $r = \sqrt{A/\Pi}$. with r being the radius of the area (A) threatened.

Slavemaker colonies in NY conduct between 5 and 6 success full slaveraids per season (Foitzik and Herbers 2001b). The compiled data of 20 years sampling suggests an average host nest density of approximately 0.69 host nests/m^2 (Herbers and Foitzik 2002). Assuming six raids per season we calculated the area threatened by a single slavemaker colony to be 8.7m^2 or a radius of 1.7 m around the slavemaker nest. This means that nests within 1.7 m of a slavemaker nest have a very high probability to be raided in a given season.

2.2 Relatedness of slaves to nearby free nests within raiding range (RR)

After estimating the raiding range, we were interested to find out whether nests in the raiding range of a slavemaker nest are more closely related to the slaves in this nest compared to other nests in the same population. This is important because it determines whether a slave's behavior can impact its indirect fitness by changing the raiding risk for related colonies. To test whether nearby nests are related to slaves, we computed the coefficient of relatedness of slaves to surrounding free nests in the same transect. The test statistic is then the average of these values over all cases where the free nest is closer than 1.7 m to the slavemaker nest in which the slaves were found. We then performed a permutation test similar to a Mantel test with 1000 repetitions (Mantel 1967). In each repetition we shuffled the nests within each transect of a sampling area (NY or WV) for the computation of distances and computed the test statistic using these permuted distances (and the original coefficients of relatedness). The p-value is then the relative rank of the original value of the test statistic within those from the 1000 nest permutations. We computed the Queller-Goodnight relatedness values and the permutation test in R (version 2.13.0, R Development Core Team, 2009). Our R script is available upon request.

2.3 Results

The average relatedness of slaves to free-living host nests found closer than 1.7 m to the slavemaker nest was 0.055 in the Huyck Preserve (NY) and 0.067 in Pocahontas County (WV). Both values are significantly higher than the relatedness of slaves to host nests further than 1.7 m away in the same transect (mean relatedness between slaves and nests more than 1.7 m away 0.021 in NY and 0.019 in WY, permutation test $p = 0.01$ in NY and $p < 0.001$ WV, see Fig. 3). The coefficients of relatedness are (on average) clearly increased in the close vicinity of the parasite nest but drop to values close to the population average when the distance becomes larger than 2m.

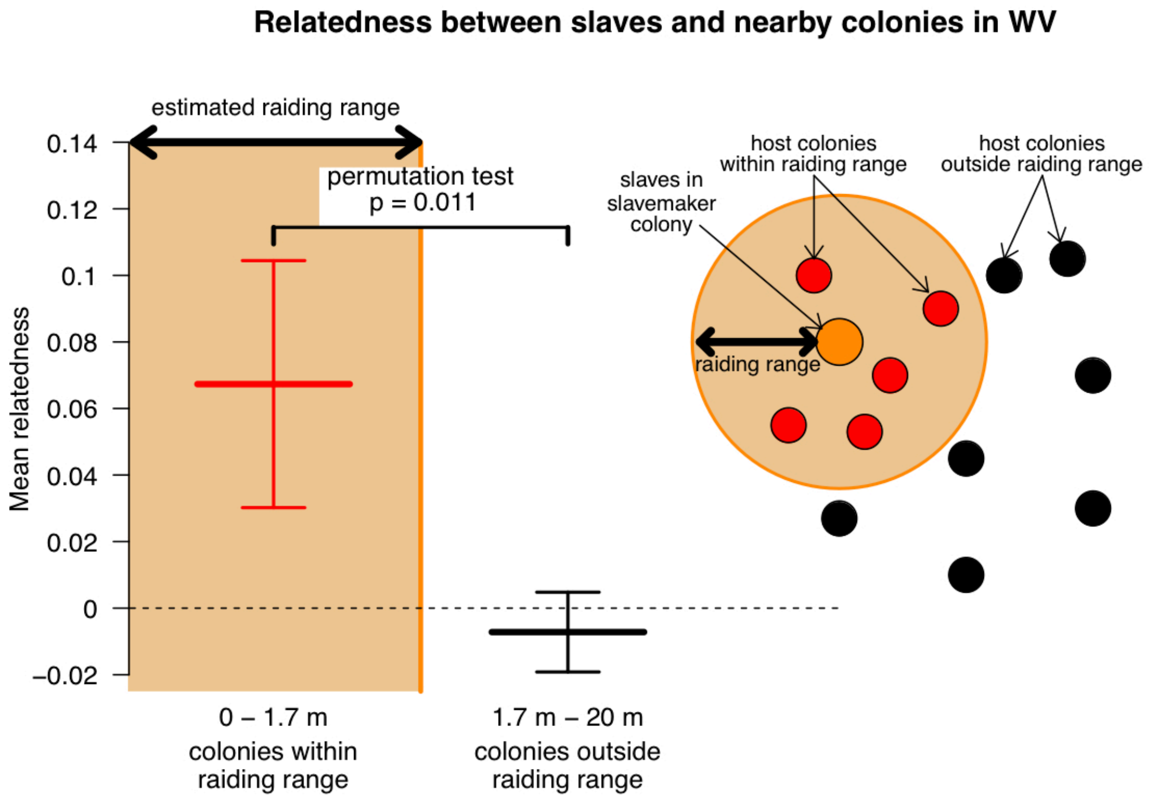

Figure 3: Presenting the average relatedness of slaves to host colonies within raiding range (red) in contrast to host colonies further away (black) in WV. Presented are mean relatedness and error bars indicate standard error.

3. Why are slaves related to nearby nests?

In the next step we investigated why enslaved host worker exhibit elevated relatedness levels with nests in the close proximity of their slave maker nests. In order to find the origin of the observed increase in relatedness we had a closer look at the free living host population to see if we can detect a similar small scale structure.

Results

In the first step we analysed if the host population had similar elevated relatedness levels to close neighbours similar to that of slaves to surrounding host colonies. Using the same test statistic as with the slaves we analysed the between nest relatedness of free living host nests. Indeed, we find that the host population is structured on a small scale in all habitats similar to the slave structure (Fig.5). Nests that are closer than 1.7 m are more related than nests that are further apart than 1.7 m (NY $p<0.001$ WV $p<0.001$)). In NY, the local population structure is similar in the free-living colonies as compared to slaves (mean relatedness within 1.7 meters distance 0.07 in free-living colonies vs 0.05 for slaves) but in WV the effect is somewhat clearer in free-living colonies (0.14 for free-living colonies and 0.06 for slaves in WV, difference not significant), see figure 5.

3.1 Source of host population structure

A potential source for the observed small scale population structure in *T.longispinosus* population is polydomy, i.e., the occupation of several nest sites by a single colony. If one part of a polydomous colony is raided, the new slaves will still be related to the other part of the colony, which likely resides in a nearby nest. We analysed genetic structure between free-living colonies to see if polydomous colonies could be identified and to determine whether polydomy is common enough to explain the observed relatedness between slaves and neighboring colonies.

In order to identify polydomous nest pairs we went through several steps. We assume that polydomous nests originate when a random subset of the individuals in a colony move to a new nest site. The resulting relatedness between such polydomous nests can be determined by splitting up the 5 sampled individuals from free-living colonies in a group of two individuals and a group of three individuals, and calculating the relatedness between those two groups according to Equation (10) in Queller and Goodnight (1989), which is what we did for all free-living colonies for which we had 5 sampled individuals. We then fitted a beta distribution (whose domain was stretched to the interval between -1 and 1 by linear transformation) to those relatedness values and we will refer to this distribution as the "distribution D_{within} of relatedness within nests" (see figure 4a). This distribution has a mode of 0.55 in NY and 0.65 in WV. Next, we took pairs of nests that were more than 50 meters apart and calculated relatedness between two random individuals from one nest and three from the other nest. We used two and three individuals to keep the same sample sizes as previously, again using Equation (10) of Queller and Goodnight (1989). Then we fitted a second beta distribution (on the interval between -1 and 1), which we will refer to as the "distribution D_{indep} of relatedness between independent nests" (see figure 4b). This distribution has a mode very close to 0 in both locations.

In a second step we divided the nest pairs in six distance segments <1m, 1-2m, 2-3m, 3-5m, 5-10m and >10m. For each segment we modeled the distribution of relatedness values found with this segment as a mixture between the distribution D_{within} of relatedness within polydomous nests and the distribution D_{indep} of relatedness between related nest. This means that for each pair of nests we assumed that with probabilty p its relatedness comes from the distribution D_{within} and with probability 1-p it comes from the distribution Dindep. The mixture coefficient p is fitted to the relatedness data of nests in the distance segment (see figure 4c). We interpret p as the probability that two nests found with such a distance belong to the same polydomous colony.

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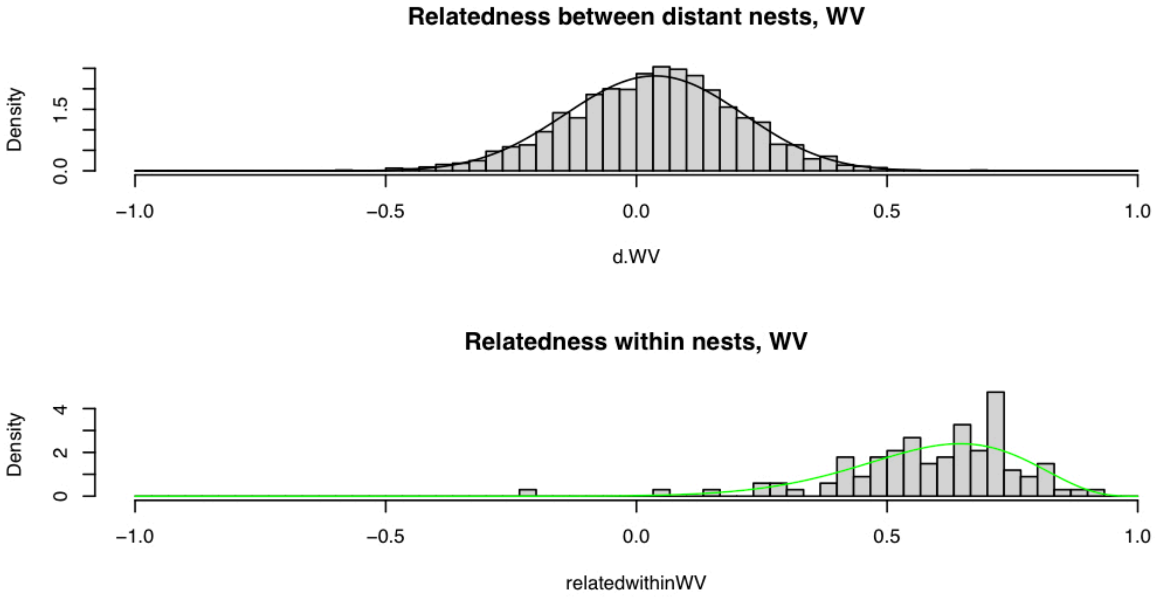

Fig 4a, 4b, The distribution of relatedness within nests D_{within} (black) and the distribution of relatedness between independent nests D_{indep} (green) for West-Virginia.

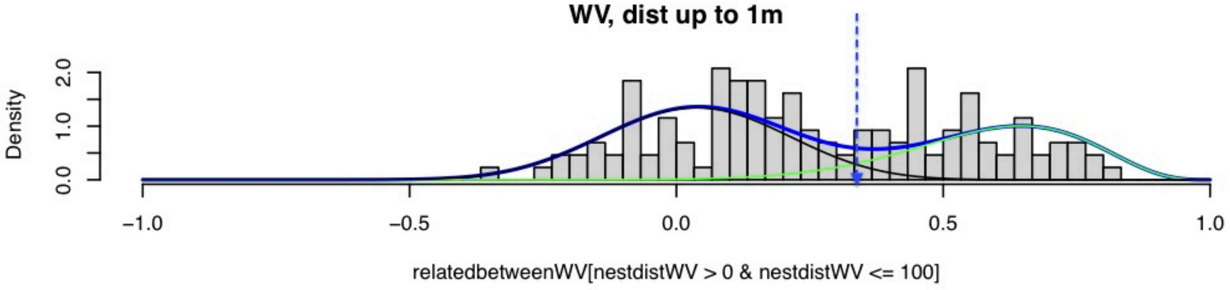

Fig 4c, The fitted mixture (blue) of D_{within} (black) and the D_{indep} (green) for West-Virginia. The threshold above which nest pairs are more likely to be polydomous than not is indicated with a blue dashed line.

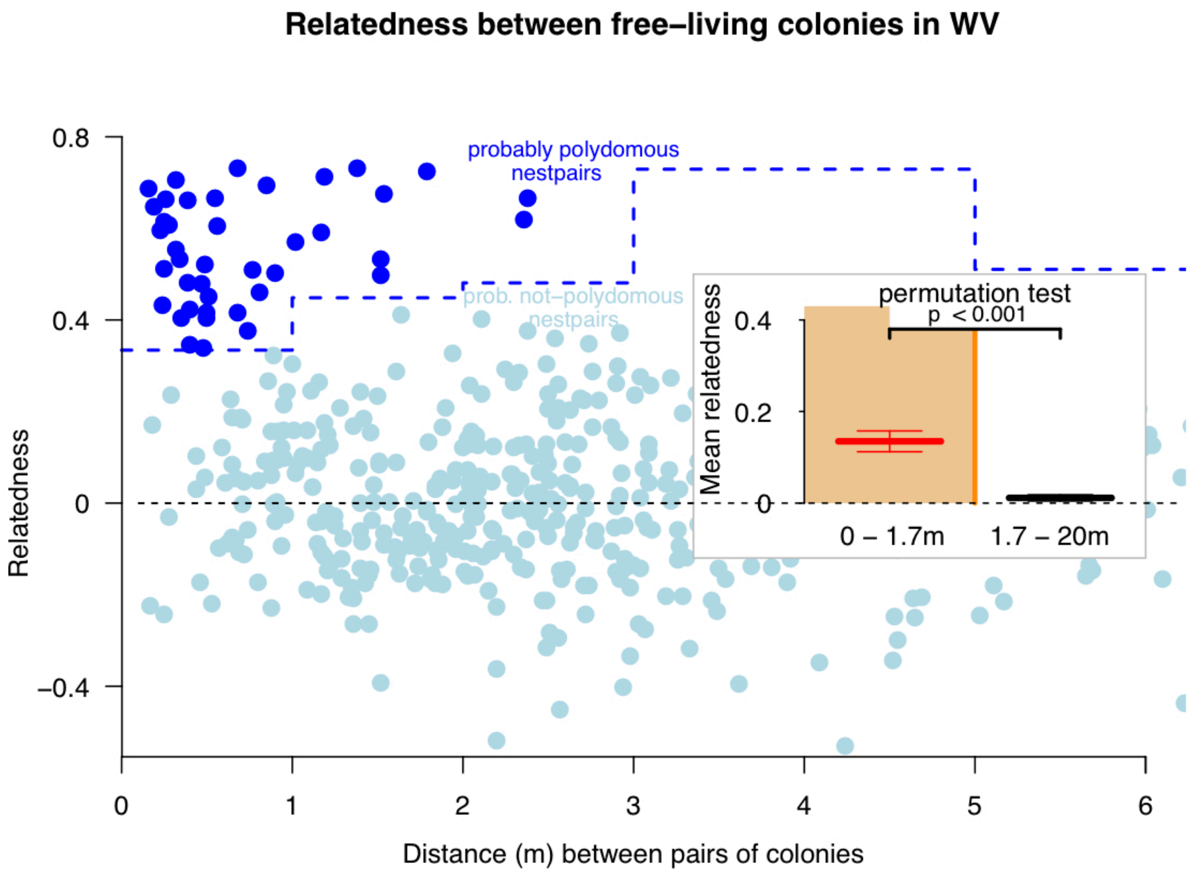

Figure 5: Presented is the relatedness between nests of freeliving host colonies in WV indicating elevated relatedness when colonies are close to each other. The colonies above the dashed blue line are likely to be polydomous exhibiting high degrees of relatedness in close proximity to each other.

For each of the distance bins, the weights of the two distributions define a threshold, the relatedness value where the distribution D_{within} of relatedness within nests rises above the distribution D_{indep} of relatedness between independent nests (indicated by the blue dotted line in figure 4c). Colony pairs within the distance bin with a relatedness value higher than this threshold are more likely to be polydomous colonies than not. We have indicated the thresholds in figure 5. At more than 2 meters distance, most colony pairs are not polydomous, but at closer distances there is a significant number of probably polydomous colonies. These probably polydomous nest pairs cause a significant elevated average relatedness between pairs of colonies that are less than 1.7 meters apart (see fig 5). This local structure is sufficient to explain why slaves are more related, on average, to colonies that are less than 1.7 meters from their slavemaker colony. In order to see if the observed small scale population structure is caused by the nest pairs which are probably polydomous, we eliminated these nest pairs, and determined again the average relatedness between nests at less than 1.7 m distance. After eliminating polydomous nest sites from the data set we no longer detect a significant population structure (NY mean relatedness near: 0.04, far: 0.02, p=0.6 WV near: 0.004, far: 0.01, p=0.8). Our results indicate that polydomy is sufficient to explain the small scale population structure in *T.longispinosus* populations.

Discussion

Is it possible that a trait evolves if the individuals carrying it do not reproduce and do not directly interact with related reproducing individuals? This was the central question of this investigation. It has been argued that once host workers are enslaved by their parasites they would have no behavioural option to increase their fitness, hence selection could not operate on post enslavement host defence traits, trapping enslaved workers in an evolutionary dead end (Gladstone 1981). The discovery of the first post enslavement slave rebellion trait in the *P.americanus* - *T. longispinosus* system was somewhat puzzling from an evolutionary perspective (Achenbach and Foitzik 2009). We proposed an alternative explanation offering an escape from the apparent evolutionary trap.

We argued that slaves could increase their indirect fitness by lowering the parasite pressure on surrounding host colonies if they would prevent a proportion of parasite brood from hatching. Such behaviour, first described by Achenbach and Foitzik (2009) and confirmed as a wide spread trait by Pamminger et al. (2012), would result in a reduction of slavemaker nest size and consequently in a lower raiding frequency of affected parasite nests (Foitzik et al. 2001). In order for slave rebellion to result in an increase of the indirect fitness of enslaved host workers their behaviour must benefit related nests that are within the raiding distance of the slavemaker nest. Or more formally, slaves must have higher probability to increase the survival chance of related host nests than unrelated host colonies.

To test if these conditions are fulfilled we calculated the approximate raiding distance with data from the literature, and concluded that a slavemaker colony will threaten host colonies within a 1.7m radius. We subsequently tested if slaves are on average more closely related to host colonies within a 1.7m radius than to host colonies further away. Indeed, we found a significant elevation of slave relatedness to surrounding host colonies, in both habitats investigated, making it possible for the slave rebellion trait to result in an a potential indirect fitness benefit. Our data show that slaves are indeed more related to nearby nests than to nests further away.

The increased relatedness of slaves to nearby nests is clearly significant although it is a small effect (Fig 3). The observed relatedness of slaves to surrounding hosts colonies similar in WV and NY indicating similar potential benefits in both habitats. However, one has to remember that the actual benefit of the slave rebellion trait not only depends on the relatedness with nearby colonies, but also various other factors, such as the raiding frequency, the realized reduction in raiding frequency, the actual raiding distance and potential costs associated with this behaviour (Metzler et al, unpublished manuscript). The goal of the current study was not to quantify the fitness benefit directly but rather show that, in principal, the conditions for an indirect fitness benefit are fulfilled. We conclude that the essential conditions for the indirect fitness benefit of slave rebellion is fulfilled in both habitats.

In order to determine the source of the increased relatedness of slaves to nearby host colonies we tested if the local host population was characterised by similar population characteristics. Indeed, we found that the host population structure shows a similar, structure to the one found for slaves in both habitats. We hypothesized that polydomy is the main source for the observed structure and estimated the probability that a pair of nests forms a polydomous colony. We concluded that polydomy is sufficient to explain the observed small scale population structure. If a portion of free-living nests is polydomous, this will automatically lead to relatedness between slaves and surrounding host colonies. The structure occurs if a slavemaker raids one sub-nest of a polydomous host colony and the surviving part nest staying in the area for some time. If slavemakers raid all surrounding nests, or if surviving nests are likely to flee, the local structure will disappear. The latter effects could explain why slaves are on average less related to nearby colonies than free-living ants are.

Like the host species, the parasites are also known to be polydomous (Foitzik and Herbers 2001a). So we can not rule out completely that we misclassified some of the host colonies in close proximity with high relatedness with slaves. However we think that the proportion of such colonies should be small, because only if neither slavemakers nor slavemaker brood was present at the time of collecting we would have mistaken them for freeliving host colonies.

One surprising result of this study was our inability to rule out the possibility that slaves could increase their fitness directly by surviving their slavemakers and producing males. Genetic data seem unfit to identify potential former slavemaker nests because *T. longispinosus* exhibit an unexpectedly wide distribution of inner nest relatedness (Fig. 2) overlapping with the relatedness between slaves. We suggest that one way to resolve are laboratory experiments. One could remove all parasites from a slavemaker colony under laboratory conditions and track the reproductive activity of the slaves. However, we believe that strong direct fitness benefits are unlikely under natural conditions due to the small window of opportunity to produce male offspring which have a realistic chance to mate. Like many hymenoptera *T. longispinosus* probably reproduces in highly synchronised mating swarms. Only if slave decedent males are produced at the right time of the year they have realistic chance. Considering the limited lifespan (approximately up to three years), and reproductive potential, of an average worker it seems unlikely that direct fitness benefits can explain the evolution of the slave rebellion trait.

In summary we find the necessary conditions for the indirect fitness benefit of slave rebellion fulfilled in both habitats under investigation and conclude that polydomy is a likely cause for the observed local population structure. By keeping parasite nest small, slaves can apparently increase the survival probability of related nests. This finding offers a potential escape from the evolutionary dead-end slaves where thought to be trapped in. In addition, this study high-lights the strong interdependence of population genetic structure and host resistance. Population structure in the host will impact the coevolutionary arms race between host and parasite leading to potential alternative outcomes. This study provides empirical evidence for the conclusions of Best et al. (2011) that population structure can substantially influence the outcome of host resistance.

References:

Achenbach, Alexandra and Foitzik, Susanne (2009). First evidence for slave rebellion: enslaved ant workers systematically kill the brood of their social parasite Protomognathus americanus. *Evolution*, *63*(4), 1068-1075.

Alloway, Thomas M. (1979). Raiding behaviour of two species of slave-making ants, Harpagoxenus americanus (Emery) and Leptothorax duloticus Wesson (Hymenoptera: Formicidae). *Animal Behaviour* 27: 202-210.

Alloway, Thomas M. (1990). "Slave-species ant colonies recognize slavemakers as enemies." *Animal Behaviour*

Bennett, Richard N, and Roger M Wallsgrove. 1994. Tansley review no. 72. Secondary metabolites in plant defence mechanisms. *New Phytologist*: 617-633.

Best, Alex et al. "Host resistance and coevolution in spatially structured populations." *Proceedings of the Royal Society B: Biological Sciences* 278.1715 (2011): 2216-2222.

Booth, David T, Dale H Clayton, and Barbara A Block. 1993. Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 253, no. 1337: 125-129.

Boots, Michael, and Yoshihiro Haraguchi. "The evolution of costly resistance in host-parasite systems." *The American Naturalist* 153.4 (1999): 359-370.

Boots, M, PJ Hudson, and A Sasaki. "Large shifts in pathogen virulence relate to host population structure." *Science* 303.5659 (2004): 842-844.

Bremermann, Hans J, and John Pickering. "A game-theoretical model of parasite virulence." *Journal of Theoretical Biology* 100.3 (1983): 411-426.

Brunham, Robert C, Francis A Plummer, and Richard S Stephens. "Bacterial antigenic variation, host immune response, and pathogen-host coevolution." *Infection and immunity* 61.6 (1993): 2273.

Buschinger, Alfred. "Social parasitism among ants: a review (Hymenoptera: Formicidae)." *Myrmecological News* 12.September (2009): 219-235.

Daszak, Peter, Andrew A Cunningham, and Alex D Hyatt. "Emerging infectious diseases of wildlife--threats to biodiversity and human health." *Science* 287.5452 (2000): 443.

Foitzik, Susanne, and Joan M Herbers. (2001) a. Colony structure of a slavemaking ant. I. Intracolony relatedness, worker reproduction, and polydomy. *Evolution* 55, no. 2: 307-315.

Foitzik, S., & Herbers, J. M. (2001) b. Colony structure of a slavemaking ant. II. Frequency of slave raids and impact on the host population. *Evolution*, *55*(2), 316-323.

Gladstone, Douglas E. "Why there are no ant slave rebellions." *The American Naturalist* 117.5 (1981): 779-781.

Gross, Paul. 1993. Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology* 38, no. 1: 251-273.

Hamilton, William D, Robert Axelrod, and Reiko Tanese. "Sexual reproduction as an adaptation to resist parasites (a review)." *Proceedings of the National Academy of Sciences* 87.9 (1990): 3566.

Heinze, J, W Puchinger, and B Hölldobler. 1997. Worker reproduction and social hierarchies inLeptothoraxants. *Animal Behaviour* 54, no. 4: 849-864.

Heinze, J, and B Oberstadt. 1999. Worker age, size and social status in queenless colonies of the ant Leptothorax gredleri. *Animal behaviour* 58, no. 4: 751-759.

Herbers, Joan M. 1986. Nest site limitation and facultative polygyny in the ant Leptothorax longispinosus. *Behavioral Ecology and Sociobiology* 19, no. 2: 115-122.

Herbers, Joan M, and Susanne Foitzik. 2002. The ecology of slavemaking ants and their hosts in north temperate forests. *Ecology* 83, no. 1: 148-163.

Kilner, Rebecca M, and Naomi E Langmore. "Cuckoos versus hosts in insects and birds: adaptations, counter‐adaptations and outcomes." *Biological Reviews* 86.4 (2011): 836-852.

Konrad, Matthias, Tobias Pamminger, and Susanne Foitzik. 2012. Two pathways ensuring social harmony. *Naturwissenschaften*: 1-10.

Mantel, Nathan. 1967. The detection of disease clustering and a generalized regression approach. *Cancer research* 27, no. 2 Part 1: 209.

Minchella, DJ. 1985. Host life-history variation in response to parasitism. *Parasitology* 90, no. 01: 205-216.

Morran, Levi T et al. "Running with the Red Queen: host-parasite coevolution selects for biparental sex." *Science* 333.6039 (2011): 216.

Sato, Tetsu. 1986. A brood parasitic catfish of mouthbrooding cichlid fishes in Lake Tanganyika 323, no. 6083: 58-59.

Pamminger, Tobias et al. "Increased host aggression as an induced defense against slave-making ants." *Behavioral Ecology* 22.2 (2011): 255-260.

Pamminger, Tobias et al. "Geographic distribution of the anti-parasite trait “slave rebellion”." *Evolutionary Ecology*: 1-11 (2012)

Petrie, M, and AP Møller. "Laying eggs in others' nests: intraspecific brood parasitism in birds." *Trends in Ecology & Evolution* 6.10 (1991): 315-320.

Price, Peter W. *Evolutionary biology of parasites*. Princeton Univ Pr, 1980.

Queller, David C, and Keith F Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution*: 258-275.

Rothstein, Stephen I. 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics* 21: 481-508.

Sheldon, Ben C, and Simon Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* 11, no. 8: 317-321.

Scharf, Inon, Tobias Pamminger, and Susanne Foitzik. "Differential Response of Ant Colonies to Intruders: Attack Strategies Correlate With Potential Threat." *Ethology* (2011).

Stuart, Robin J, and Thomas M Alloway. 1985. Behavioural evolution and domestic degeneration in obligatory slave-making ants (Hymenoptera: Formicidae: Leptothoracini). *Animal behaviour* 33, no. 4: 1080-1088.

Suni, SS, and DM Gordon. 2009. Fine-scale genetic structure and dispersal distance in the harvester ant Pogonomyrmex barbatus. *Heredity* 104, no. 2: 168-173.

Wommack, K Eric, and Rita R Colwell. "Virioplankton: viruses in aquatic ecosystems." *Microbiology and Molecular Biology Reviews* 64.1 (2000): 69-114.

Electronic Supplemental Material (ESM)

DNA extraction, PCR, fragment analysis and allele binning

DNA of five workers per non-parasitized T. longispinosus colony and 20 enslaved T. longispinosus workers of the P. americanus colonies was extracted using the standard protocol of the Puregene DNA extraction kit (Gentra System®). Six highly variable microsatellite loci were amplified in two multiplex PCR assays using the primers L5, L18 (Foitzik et al., 1997), LXA GT1 (Bourke et al., 1997) , LX GT218 and LX GT223 (Hamaguchi et al., 1993) and Myrt 3 (Evans, 1993). For PCR reactions the QIAGEN Multiplex PCR Kit® was used. The first multiplex contained the primers L18, LXA GT1, LX GT218 and LX GT223. The 20 µl reaction mix included 10 µl QIAGEN Multiplex PCR mastermix, 1 µl Primer mix (containing equal amounts of all primer pairs [10pmol]), 1 µl DNA solution and 8 µl H2O. The reaction protocol consisted of an initial 15 min denaturation step at 95◦C followed by six PCR cycles (95◦C 30sec, 48◦C 60 sec, 72◦C 90 sec) with a annealing temperature increment of 1◦C/cycle, followed by 23 PCR cycles (95◦C 30sec, 54◦C 60 sec, 72◦C 90 sec). The 2nd multiplex included the primer L4 and Myrt3.

The 15 µl reaction mix included 7.5 µl QIAGEN Multiplex PCR mastermix 0.75 µl Primer mix (containing equal amounts of both primer pairs [10pmol]) 0.75 µl DNA and 6 µl H2O. The reaction protocol consisted of an initial 15 min denaturation step at 95◦C followed by six PCR cycles (95◦C 30sec, 47◦C 60 sec, 72◦C 90 sec) with an annealing temperature increment of 0.5◦C/cycle, followed by 23 PCR cycles (95◦C 30sec, 50◦C 60 sec, 72◦C 90 sec). Genotyping was performed on a ABI 3730 capillary sequencers and fragments were analyzed using the

GenMapper® Software. The observed allele lengths were rounded to integers (binned) using the software TANDEM (Matschiner and Salzburger, 2009).

"It is said that if you know your enemies and know yourself, you will not be defeated in a hundred battles; if you do not know your enemies but do know yourself, you will win one and lose one; if you do not know your enemies nor yourself, you will be defeated in every single battle."

Sun Tzu the art of war

General discussion

Alongside the intensively studied avian brood parasites (e.g. Payne 1977, Rothstein 1990), the *Temnothorax* sp. - *Protomognathus americanus* system is one of the few host - brood parasite arms race dynamics (Cervo 2006) to have received increasing attention in the last two decades (Killner et al. 2011, Foitzik et al. 2001c, Foitzik and Herbers 2001 a, b, Brandt 2005, Pennings 2010). In contrast to brood parasites in birds, the scientific community studying social parasites, in particular the *T. longispinosus* - *P. americanus* system, is small and, consequently, the data available is limited. Choosing the *T. longispinosus – P. americanus* study system to investigate coevolutionary dynamics has both advantages and disadvantages.

On one hand the small size of the host and parasite colonies in combination with their simple housing requirements make these ants ideal objects for experimental investigation under laboratory- and semi-controlled field conditions (see. Foitzik et al. 2001, Brandt et al. 2004, Foitzik et al 2009, Pohl and Foitzik 2011). It is possible to keep them in large numbers in the laboratory all year around and set up experiments with considerable sampling size and high statistical power. The fact that most of the host-parasite interactions are staged on the behavioural level (Alloway 1990, Foitzik et al. 2001c, Achenbach and Foitzik 2009, Publication 1), makes it easy to relate to this system and communicate new findings and their importance to non-experts and the general public.

On the other hand, the long generation times of more than 10 years, the inability to breed them under laboratory conditions, the lack of appropriate genetic tools (but the same is true for most non-model systems) and limited data on different communities limit the potential questions (approaches) which can be addressed using this system.

One of the most interesting aspects of this study system, separating it from avian brood parasites, is the fact that most of the host defense mechanisms are centered on collective (colony), rather than individual defense mechanisms (Killner et al 2011). This collective level adds a challenging layer of complexity, requiring information on the individual behavior in order to understand how complex defensive strategies are generated on the colony level. In publication 1 we were interested in such a colony level defensive response.

The aggressive afford of a single worker would probably be insufficient to deny a parasite worker access to a host colony. We investigated if host colonies are able to utilize the information of a parasite threat to prepare for an upcoming raiding event by adjusting the colony aggression. We found that host colonies always respond with high aggression against parasites, confirming the findings of Alloway (1990), independent of how often they encounter a parasite. In addition, colonies can adjust their aggression level following a parasite encounter against non-nestmate conspecifics. Surprisingly, a short (5 min) within-nest encounter with a parasite increases the colony aggression against non-nestmates conspecifics for at least three days. We interpreted this elevated aggression as an induced defense mechanism, which should provide better defense during a raiding event, where non-nestmate conspecifics are known to participate and steal pupae. The finding that a short encounter with their parasite results in a lasting response in the colony raises the interesting question of memory in insects. Although insects do posses a relatively small brain, many species are capable of solving complicated tasks relevant to their ecology (Menzel et al. 2006, Laeadbeater and Chittka 2007). Recent experiments indicate that some insects appear to have robust long-term memory used to identify other individuals (Sheehan and Tibbets 2008). This, in turn, raises the question if the inducible effects found in our experiments were also based on individual memory and, if so, whether this information could be communicated in between individuals. These findings could provide a potentially fruitful future research direction in our system. As a first step, it is necessary to identify whether the same individuals that encountered the parasites were the individuals responsible for the increased aggression observed days later. If so, we could investigate whether these individuals are able to transfer the information to other individuals of the colony. An experiment testing this hypothesis could start on the individual level and test if individuals adjust their aggression level after a parasite encounter and how long this increased aggression lasts. If individuals were identified it should be tested whether these were the ones responsible for the elevated aggression after a parasite encounter by marking the individuals involved in parasite defense. In a last step, to test if such information is transferable, we could separate (and mark) the individuals encountering parasites and subsequently transfer them back to the colony. After some time these animals should be removed and it could be measured if the colony responded with increased aggression.

As mentioned earlier, aggression as a defense mechanism can only be effective on a collective level. Aggression in *Temnothorax* ants involves a variety of different behavioral options, ranging from states of alertness (threat displays) and biting up to stinging. It has been assumed that these behavioral options follow a sequence (see publication 2). However, we found strong variation in the defense behavior, indicating that this sequence can be radically altered on an individual level. As aggression has a genetic (heritable) component in many animals (e.g. Rushton et al. 1986, Miczek et al. 2001, Van Oers et al. 2005), and, as ants posses the ability to clearly separate different intruders (Alloway 1990), we were interested if *T. longispinosus* had evolved different defense strategies. If evolution shaped these defensive strategies, we would expect these strategies to match the potential selection pressure (threat) of the intruder in a qualitative and quantitative way (publication 2). We found that host aggression is indeed a non-uniform process. Not only does the overall aggression level match the potential threat of the intruding ants (parasites being the most dangerous), but also colonies apply a variety of different defensive behaviors specific to certain intruders. This study demonstrates that colony level aggression is not a simple all or nothing process in *T. longispinosus*. The intensity and the type of defensive strategies used can be fine-tuned in response to various threats, indicating that selection can shape the aggressive response on a colony level against attackers independent of each other.

Considering the apparently complex genetic architecture of aggression in insects (Zwarts et al. 2011), it would be interesting to have a closer look at the genetic basis of aggression in *T. longispinosus*. The repeatedly demonstrated increased aggression *T. longispinosus* displays when confronted with *P. americanus*, appears a promising future research direction. The specificity of this elevated aggression makes it a good candidate for a trait evolved in this host-parasite context (Thompson 2005). Therefore it is likely that this trait is under strong selection. However, it is possible that aggression is a multi locus trait, a simple gene by gene interaction would be unlikely. But, through screening for associated QTLs (Quantitative Trait Loci) the identification of candidate genes involved in the detection of the parasite could be facilitated. The specificity and robustness of this behavioral reaction are a good physiological foundation for finding underlying genomic regions, since the behavioral phenotype can easily and reliably be determined. Such candidate genes could open the door to investigate this host-parasite interaction on a genetic level similar to the well-studied newt - snake arms race in California (Geffeney et al. 2002).

In publication 3 we had the unique opportunity to investigate a critical step in the parasite's life cycle. During nest foundation mortality of young parasitic queens is suspected to be high in some species, while other parasites are relatively successful at this stage under laboratory conditions (Topoff et al. 1988, Topoff et al. 1990, Mori et al. 1995). As a result of our field experiment we were able to investigate factors (host colony demographic and behavioral traits), which determine parasitic nest foundation success under more natural conditions. At the founding stage, parasite queens not only need to infiltrate a host colony, but also secure enough brood (the first slave generation) to start a new colony (see Brandt et al. 2005 for a review). Analyzing the takeover pattern, we discovered that more aggressive host colonies were able to rescue a higher proportion of their brood and, moreover,, that parasites do not settle in areas where *P. americanus* is already present. We concluded that this strong selection event (locally a fourth of the colonies where wiped out by the founding queens) should select for high aggression in host colonies, shaping this behavioral trait on a colony level. The high take over rate supports the findings of Foitzik and Herbers (2001b), indicating that *P. americanus* exerts strong selection pressure on its host species during the entire live cycle. It would be interesting to investigate, if colony aggression was an important factor of host defense during colony raiding events as well. If so, we would expect strong selection on host colony aggression during the entire season. As a consequence, *T. longispinosus* colonies should express high levels of aggression in areas where the parasite is present (directional selection). In contrast, we found pronounced variation in colony aggression within and in between all sampling areas (Pamminger unpublished data), indicating that another evolutionary mechanism was maintaining the observed variation. A potential mechanism maintaining trait variation in a population is balancing selection. The most likely form of balancing selection would be variation in the fitness optimum (temporal or spatial variation in selection pressure) and, consequently, different optimal levels of aggression. It is likely that high colony aggression carries direct or indirect fitness costs, because highly aggressive workers are more likely to get injured or killed (Pamminger unpublished data). High mortality rates should translate into a fitness decrease (see Modlmeier and Foitzik 2011). Accordingly, high aggression levels would be favored in parasitized areas, while different levels of the same trait should result in a disadvantage where parasites are absent (Modlmeier and Foitzig 2011). Whether balancing selection was a potential cause for the maintenance of trait variation in colony aggression would be another promising research approach. The maintenance of variation of a particular trait is of prime interest in biology. As many processes (e.g. drift and purifying selection) tend to remove variation from the gene pool, active processes have to be at work in order to preserve variation. If, indeed, context dependent cost benefit trait-offs (one form of balancing selection) are present in this system, it could explain the strong variation in colony traits and help to understand the forces at work which preserve variation in animal personality traits in general.

In publication four and five we investigated different aspects of the post-enslavement trait “slave rebellion”. Combining the data of six years, we concluded that slave rebellion is a common phenomenon, present in all populations, as indicated by a severely reduced survival rate of parasite brood in all habitats under investigation (publication 4). We found that the expression level of the traits varies (difference between host and parasite brood survival) in between populations indicating different selective regimes, which is consistent with the predictions of the MTC (Thompson 2005). This publication supports the findings of Achenbach and Foitzik 2009, demonstrating that slave rebellion is indeed a wide spread phenomenon. Our study highlightens the problem of working with coevolutionary systems, where the organisms involved have long generation cycles. Such systems are inaccessible for experimental evolution, so it was impossible to follow the trajectory of the coevolutionary interaction during a reasonable amount of time. In order to investigate the progress of this interaction, a variety of different habitats assumed to be at different stages in the arms race dynamic had to be sampled. However, the approach created a different set of problems inherent to a coevolutionary process. As all investigated habitats differed in some aspect, the coevolutionary processes were influenced by different factors (abitoic and biotic) in all locations. This high level of noise in the data made it difficult to actually link trait expression levels to coevolutionary dynamics (publication 4). One solution would be to substantially increase sampling size and include a large number of habitats, in order to document trends persistent in all habitats. The strong variation in trait expression raises the question why some populations appear to be less protected against parasite attacks compared to others. Considering individual traits, this apparent lack of host defense seems not uncommon, as reflected in host resistance traits in birds (Bristkie et al. 1992, Takasu 1997). Based on theoretical considerations, Britton et al. (2007) suggested that if multiple defense strategies were utilized in a host-parasite system, the presence of one effective defense strategy would relax selection for alternative defenses. Our study adds to the growing data available on the expression of host defense and opens future research possibilities looking at defense suites. The interaction of multiple anti-parasite defense traits is an important addition to the study of host-parasite interactions in general and could help explain why some populations seem exceptionally well defended while others clearly lack any form of parasite defense.

In publication 5 we investigated a potential proximate mechanism enabling the evolution and spread of the defense trait slave rebellion. We hypothesized that enslaved host workers were able to increase their indirect fitness by increasing the survival chance of surrounding host colonies within the raiding range of the slavemaker colony. If these host colonies were related slaves, they could potentially help close relatives, hence increase their fitness indirectly. As a matter of fact, we found that the host population is structured within the raiding range of slavemaker nests in both communities investigated. Consequently, slave rebellion could spread via indirect (kin) selection benefits. Furthermore, we were able to track down the origin of this small scale population structure and concluded that mainly polydomy (the occupation of more than one nestsite by a single colony) is the most likely origin of the observed small scale structure. Our study indicated that polydomy provided a two-fold benefit. First, if a colony occupies two nest sites the chances of a complete loss due to a raiding event are halved, and second, if polydomous nest sites were the most important cause for the observed small scale structure, an additional indirect fitness benefit - via kin selection - should be provided. If ecological conditions were not restricting the nest splitting, we would expect higher levels of polydomy in areas with strong parasite pressure. In order to investigate this hypothesis, more unparasitized populations would have to be sampled in order to compare them to parasitized areas and get meaningful results. Traditionally, models dealing with host-parasite interactions assume well-mixed populations (Bremermann et al. 1983, Boots et al.1999). In the past decade researchers have realized that local population structure can influence parasite virulence (Boots et al. 2004) as well as host resistance traits (Best et. al. 2011). In our system, without local host population structure, slaves could not increase the survival of close relatives and slave rebellion would probably not have evolved, providing empirical evidence for the findings of Boots et al.

(2002) and Best et al. (2011) This study adds to the growing realization, that population structure can severely influence the outcome of host-parasite interactions.

In addition, our study high lightens the problem of matching scales of population structure and life history parameters of the participants. In our case the effective range of the parasite is rather small (1.7m). Consequently, structure in this range only can create a potential benefit for the host, while population structure on a large scale (Pennings et al. 2011) should not affect the outcome of this interaction directly. The problem of scale matching should apply to different host-parasite systems as well and must be considered when analyzing the outcome of host-parasite interactions.

Future research

Recapulatory, the *T. longispinosus* - *P. americanus* system is a fascinating but challenging system to address questions related to escalating coevolutionary dynamics. However, researchers have to be careful to ask the correct questions (which the system can answer) and be aware of the limitations of the study system in order to minimize unsuccessful experiments. In my opinion, the most promising future research directions are centered around three fundamental areas:

1. **Truly coevolved traits** (Thompson 2005). A good candidate for a trait not co-opted in this coevolutionary dynamic but directly evolved in response to the parasite is the highly re-conditioned mechanism of the hosts. It will be interesting to see if this trait is “hard wired” into the host species, already present at birth without any contact to parasites (learning). In that case it should be investigated whether variation is found in this trait or if it has already gone to fixation in the majority of the populations. I believe that this trait could be a potential candidate to progress to the genetic level and further the general understanding of coevolutionary dynamics.

2. **The collective defense**: One unique advantage of this system is the combination of individual- and collective levels of host defense. The question how individual behavior variation can generate complex coordinated processes on the colony level, without any central leadership, is an urgent question in biology. The defense strategies in *T. longispinosus* provide an attractive study system to address some of these questions.
3. **Defensive suites**: The interaction in between different defense traits. Most populations investigated in this system vary in the defense mechanisms they express and/or the combination of traits present. It would be interesting to investigate how these suites of defensive traits are distributed and if they can influence each other. It seems likely that successful defense strategies can alter selection on alternative defensive traits.

Acknowledgements

[redacted]

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Concluding remark

At some point during my studies I started thinking about biology as a way to join a conversation hidden from most people. All living things exchange information, but their way of communication is so vastly different that our limited sensory equipment is unfit to join the conversation. Biology is a way to close this gap a little bit. It’s the tedious process of trial and error to get into a conversation with a different organism, which does not necessarily want to be talked to. But, if successful, you start an intimate conversation and it’s amazing what stories most organisms are able to tell. In a way, the first years of studying biology can be compared to learning a completely new language, composed of an uncountable number of weird accents. Studying this accent jungle can be frustrating and confusing at some points (yes, there still is a lot of confusion), but with time you find accents that suit you and things become a little easier. In the end it was worth it! The last 3 years felt like the first real and intimate conversation (more like the babbling of an infant in my case) I had with an organism other then a human being.

July 14th, 2012 Barcelona

Tobias Pamminger

References

Achenbach, Alexandra, and Susanne Foitzik. 2009. First evidence for slave rebellion: enslaved ant workers systematically kill the brood of their social parasite Protomognathus americanus. *Evolution* 63, no. 4: 1068-1075.

Achenbach, Alexandra, Volker Witte, and Susanne Foitzik. 2010. Brood exchange experiments and chemical analyses shed light on slave rebellion in ants. *Behavioral Ecology* 21, no. 5: 948-956.

Allies, Anthony B, Andrew FG Bourke, and Nigel R Franks. 1986. Propaganda substances in the cuckoo antLeptothorax kutteri and the slave-maker *Harpagoxenus sublaevis*. *Journal of chemical ecology* 12, no. 6: 1285-1293.

Alloway, Thomas M. 1979. Raiding behaviour of two species of slave-making ants, Harpagoxenus americanus (Emery) and Leptothorax duloticus Wesson (Hymenoptera: Formicidae). *Animal Behaviour* 27: 202-210.

Alloway, Thomas M. 1982. How the slave-making ant Harpagoxenus americanus (Emery) affects the pupa-acceptance behavior of its slaves. *The biology of social insects. Westview, Boulder, Colo*: 261-265.

Alloway, Thomas M. 1990. Slave-species ant colonies recognize slavemakers as enemies. *Animal Behaviour*.

Antonovics, Janis, and Mike Edwards. 2011. Spatio-temporal dynamics of bumblebee nest parasites (Bombus subgenus Psythirus ssp.) and their hosts (Bombus spp.). *Journal of Animal Ecology*.

Beibl, J, RJ Stuart, J Heinze, and S Foitzik. 2005. Six origins of slavery in formicoxenine ants. *Insectes sociaux* 52, no. 3: 291-297.

Benkman, Craig W. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepole pine. *the american naturalist* 153, no. S5: S75-S91.

Benus, RF, B Bohus, JM Koolhaas, and GA Van Oortmerssen. 1991. Heritable variation for aggression as a reflection of individual coping strategies. *Cellular and Molecular Life Sciences* 47, no. 10: 1008-1019.

Best, Alex et al. "Host resistance and coevolution in spatially structured populations." *Proceedings of the Royal Society B: Biological Sciences* 278.1715 (2011): 2216-2222.

Bolton, Barry. 2003. Synopsis and classification of Formicidae.

Boots, Michael, and Yoshihiro Haraguchi. "The evolution of costly resistance in host□parasite systems." *The American Naturalist* 153.4 (1999): 359-370.

Boots, M, PJ Hudson, and A Sasaki. "Large shifts in pathogen virulence relate to host population structure." *Science* 303.5659 (2004): 842-844.

Brandt, Miriam, and Susanne Foitzik. 2004. Community context and specialization influence coevolution between a slavemaking ant and its hosts. *Ecology* 85, no. 11: 2997-3009.

Brandt, Miriam, Susanne Foitzik, Birgit Fischer□Blass, and Jürgen Heinze. 2005. The coevolutionary dynamics of obligate ant social parasite systems—between prudence and antagonism. *Biological Reviews* 80, no. 2: 251-267.

Bremermann, Hans J, and John Pickering. "A game-theoretical model of parasite virulence." *Journal of Theoretical Biology* 100.3 (1983): 411-426

Briskie, James V, Spencer G Sealy, and Keith A Hobson. 1992. Behavioral defenses against avian brood parasitism in sympatric and allopatric host populations. *Evolution*: 334-340.

Britton NF, Planque R, Franks NR. 2007. Evolution of defence portfolios in exploiter-victim systems. *Bulletin of Mathematical Biology* vol.69 no.3 957-988

Brodie Jr, Edmund D, BJ Ridenhour, and ED Brodie III. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. *Evolution* 56, no. 10: 2067-2082.

Burdon, JJ, and PH Thrall. 1999. Spatial and temporal patterns in coevolving plant and pathogen associations. *The American Naturalist* 153, no. S5: S15-S33.

Buschinger, Alfred. 1974. Experimente und Beobachtungen zur Gründung und Entwicklung neuer Sozietäten der sklavenhaltenden AmeiseHarpagoxenus sublaevis (Nyl.). *Insectes sociaux* 21, no. 4: 381-406.

Buschinger, Alfred. 1986. Evolution of social parasitism in ants. *Trends in Ecology & Evolution* 1, no. 6: 155-160.

Buschinger, Alfred. 2009. Social parasitism among ants: a review (Hymenoptera: Formicidae). *Myrmecological News* 12, no. September: 219-235.

Cervo, Rita. 2006. Polistes wasps and their social parasites: an overview. *Annales Zoologici Fennici*. Helsinki: Suomen Biologian Seura Vanamo, 1964-.

Cool-Kwait, E, and H Topoff. 1984. Raid organization and behavioral development in the slave-making antPolyergus lucidus Mayr. *Insectes Sociaux* 31, no. 4: 361-374.

Daszak, Peter, Andrew A Cunningham, and Alex D Hyatt. 2000. Emerging infectious diseases of wildlife--threats to biodiversity and human health. *Science* 287, no. 5452: 443.

Davies, NB, Andrew FG Bourke, and M De L Brooke. 1989. Cuckoos and parasitic ants: interspecific brood parasitism as an evolutionary arms race. *Trends in Ecology & Evolution* 4, no. 9: 274-278.

Dawkins, Richard, and John R Krebs. 1979. Arms races between and within species. *Proceedings of the Royal Society of London. Series B. Biological Sciences* 205, no. 1161: 489-511.

D'Ettorre, Patrizia, and Jürgen Heinze. 2001. Sociobiology of slave-making ants. *Acta ethologica* 3, no. 2: 67-82.

Friedmann, Herbert. 1965. The history of our knowledge of avian brood parasitism. *Centaurus* 10, no. 4: 282-304.

Foitzik, Susanne, and Joan M Herbers. 2001a. Colony structure of a slavemaking ant. I. Intracolony relatedness, worker reproduction, and polydomy. *Evolution* 55, no. 2: 307-315.

Foitzik, Susanne, and Joan M Herbers. 2001b. Colony structure of a slavemaking ant. II. Frequency of slave raids and impact on the host population. *Evolution* 55, no. 2: 316-323.

Foitzik, Susanne, Christopher J DeHeer, Daniel N Hunjan, and Joan M Herbers. 2001c. Coevolution in host–parasite systems: behavioural strategies of slave–making ants and their hosts. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 268, no. 1472: 1139-1146.

Foitzik, Susanne, Birgit Fischer, and Jürgen Heinze. 2003. Arms races between social parasites and their hosts: geographic patterns of manipulation and resistance. *Behavioral Ecology* 14, no. 1: 80-88.

Foitzik, Susanne, Alexandra Achenbach, and Miriam Brandt. 2009. Locally adapted social parasite affects density, social structure, and life history of its ant hosts. *Ecology* 90, no. 5: 1195-1206.

Forde, Samantha E, John N Thompson, and Brendan JM Bohannan. 2004. Adaptation varies through space and time in a coevolving host–parasitoid interaction. *Nature* 431, no. 7010: 841-844.

Geffeney, Shana, Brodie Jr, Edmund D, Ruben, Peter C, and Brodie III, Edmund D. 2002. Mechanisms of Adaptation in a Predator-Prey Arms Race: TTX-Resistant Sodium Channels. *Science.* Vol. 297 no.5585 1336-1339

Gladstone, Douglas E. 1981. Why there are no Ant Slave Rebellions. *American Naturalist*. Vol.117 no.5 779-781

Gomulkiewicz, Richard, Devin M Drown, Mark F Dybdahl, et al. 2007. Dos and don'ts of testing the geographic mosaic theory of coevolution. *Heredity* 98, no. 5: 249-258.

Gross, Paul. 1993. Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology* 38, no. 1: 251-273.

Hamilton, William D, Robert Axelrod, and Reiko Tanese. 1990. Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences* 87, no. 9: 3566.

Hanifin, Charles T, and Edmund D Brodie. 2008. Phenotypic mismatches reveal escape from arms-race coevolution. *PLoS biology* 6, no. 3: e60.

Herbers, Joan M, and Susanne Foitzik. 2002. The ecology of slavemaking ants and their hosts in north temperate forests. *Ecology* 83, no. 1: 148-163.

Hölldobler, Bert, and Edward O Wilson. 1990. *The ants*. Belknap Press.

Kilner, Rebecca M., and Langmore Naomi E. 2011. Cuckoos *versus* hosts in insects and birds: adaptations, counter-adaptations and outcomes. *Biological Reviews.* Vol. 86 no.4 836-852

Korb Judith and Heinze Jürgen 2004. Multilevel selection and social evolution of insect societies. *Naturwissenschaften* vol. 91 no. 6 291-304

Krüger, Oliver 2007. Cuckoos, cowbirds and hosts: adaptations, trade-offs and constraints. *Philosophical transactions of the royal society series B* vol. 362 no. 1486 1873-1886

Leadbeater, Ellouise, and Lars Chittka. 2007. Social learning in insects--from miniature brains to consensus building. *Current Biology* 17, no. 16: R703-R713.

Lenoir, Alain, Christian Malosse, and Ryohei Yamaoka. 1997. Chemical mimicry between parasitic ants of the genus Formicoxenus and their host Myrmica (Hymenoptera, Formicidae). *Biochemical Systematics and Ecology* 25, no. 5: 379-389.

Lyon, Bruce E. 2003. Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature* 422, no. 6931: 495-499.

Menzel, Randolf, Gérard Leboulle, and Dorothea Eisenhardt. 2006. Small brains, bright minds. *Cell* 124, no. 2: 237-239.

Miczek, Klaus A, Stephen C Maxson, Eric W Fish, and Sara Faccidomo. 2001. Aggressive behavioral phenotypes in mice. *Behavioural brain research* 125, no. 1-2: 167-181.

Modlmeier Andreas P, Liebmann Julia E and Foitzik Susanne. 2012. Diverse societies are more productive: a lesson learned from ants. *Proceedings of the royal society series B.* doi: 10.1098/rspb.2011.2376

Modlmeier Andreas P,and Foitzik Susanne. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behavioural Ecology.* Vol.22 no.5 1026-1032

Mori, n A, P D'Ettorre, and F Le Moli. 1995. Host nest usurpation and colony foundation in the European amazon ant, Polyergus rufescens Latr.(Hymenoptera: Formicidae). *Insectes sociaux* 42, no. 3: 279-286.

Morran, Levi T, Olivia G Schmidt, Ian A Gelarden, Raymond C Parrish, and Curtis M Lively. 2011. Running with the Red Queen: host-parasite coevolution selects for biparental sex. *Science* 333, no. 6039: 216.

Payne, Robert B. 1977. The ecology of brood parasitism in birds. *Annual Review of Ecology and Systematics* 8: 1-28.

Pennings, Pleuni S, Achenbach, Alexandra and Foitzik Susanne. 2011. Similar evolutionary potentials in an obligate ant parasite and its two host species. *Journal of Evolutionary Biology* 24, no. 4: 871-886.

Pohl, Sebastian and Foitzik Susanne. 2011a. Slave-making ants prefer larger, better defended host colonies. *Animal behavior.* Vol. 81 no. 1 61-68

Pohl, Sebastian, Witte, Volker, and Foitzik Susanne. 2011b. Division of labor and slave raid initiation in slave-making ants. *Behavioural Ecology and Sociobiology* vol. 65 no.11 2029-2036

Price, Peter W. 1980. *Evolutionary biology of parasites*. Vol. 15. Princeton Univ Pr, May 1.

Rothstein, Stephen I. 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics* 21: 481-508.

Rudgers, Jennifer A, and Sharon Y Strauss. 2004. A selection mosaic in the facultative mutualism between ants and wild cotton. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271, no. 1556: 2481-2488.

Rushton, J Philippe, David W Fulker, Michael C Neale, David KB Nias, and Hans J Eysenck. 1986. Altruism and aggression: the heritability of individual differences. *Journal of personality and social psychology* 50, no. 6: 1192.

Sato, Tetsu. 1986. A brood parasitic catfish of mouthbrooding cichlid fishes in Lake Tanganyika 323, *Nature*. 6083: 58-59.

Takasu, Fugo. 1998. Why do all host species not show defense against avian brood parasitism: evolutionary lag or equilibrium?. *The American naturalist* 151, no. 2: 193-205.

Schmid-Hempel, Paul. 1998. *Parasites in social insects*. Princeton Univ Pr.

Thompson, John N. 1997. Evaluating the dynamics of coevolution among geographically structured populations. *Ecology* 78, no. 6: 1619-1623.

Thompson, John N. 1999. Specific hypotheses on the geographic mosaic of coevolution. *the american naturalist* 153, no. S5: S1-S14.

Thompson, John N. 2005. *The geographic mosaic of coevolution*. University of Chicago Press, June 15.

Topoff, Howard, Stefan Cover, Les Greenberg, Linda Goodloe, and Peter Sherman. 1988. Colony Founding by Queens of the Obligatory Slave□making Ant, Polyergus breviceps: The Role of the Dufour's Gland1. *Ethology* 78, no. 3: 209-218.

Topoff, Howard, Tom Weickert, and Ellen Zimmerli. 1990. A comparative study of colony takeover between queens of facultative and obligatory slave-making ants (Hymenoptera: Formicidae). *Journal of insect behavior* 3, no. 6: 813-817.

Van Oers, Kees, Gerdien De Jong, Arie J Van Noordwijk, B Kempenaers, and Pieter J Drent. 2005. Contribution of genetics to the study of animal personalities: a review of case studies. *Behaviour, 142* 9, no. 10: 1185-1206.

Wesson, Laurence G. 1939. Contributions to the natural history of Harpagoxenus americanus Emery (Hymenoptera: Formicidae). *Transactions of the American Entomological Society (1890-)* 65, no. 2: 97-122.

Winfree, Rachael. 1999. Cuckoos, cowbirds and the persistence of brood parasitism. *Trends in Ecology & Evolution* 14, no. 9: 338-343.

Wommack, K Eric, and Rita R Colwell. 2000. Virioplankton: viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* 64, no. 1: 69-114.

Zangerl, AR, MC Stanley, and MR Berenbaum. 2008. Selection for chemical trait remixing in an invasive weed after reassociation with a coevolved specialist. *Proceedings of the National Academy of Sciences* 105, no. 12: 4547.

Zwarts, Liesbeth, Michael M Magwire, Mary Anna Carbone, et al. 2011. Complex genetic architecture of Drosophila aggressive behavior. *Proceedings of the National Academy of Sciences* 108, no. 41: 17070-17075.

Authors contributions

Publication	original idea	work planned	laboratory/ field work	analysis	manuscript	manuscript finalised
1	TP, IS, SF	TP, IS, SF, PPS	TP, IS	IS, TP	TP	TP, IS, SF, PPS
2	IS	IS, TP	IS, TP	IS	IS	IS, TP, SF
3	TP, APM	TP, APM, SF	TP, APM, SS	TP, APM	TP	TP, APM, SF
4	TP, PPS, SF	TP, PPS	AL, AA, IK	TP	TP	TP, PPS, SF
5	SF, PPS	SF, PPS, TP	TP	TP, PPS, DM	TP	TP, PPS, SF, DM

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2009 Field work in Sommerhausen (Germany) one week

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2010 Poster presentation At the international IUSSI (Copenhagen) Title: ”Increased host aggression as an induced defense against slavemaking ants”.

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Talks scientific:

2011 Talk at the 2ed Central European Meeting of the IUSSI. Title: ”Parasites, aggression and why it matters”

2010 Graduate college behavioural ecology: „Science and relationships a possibility?”

2009 – 2011 Three talks at DFG Forschergruppe 1078, “Natural selection in structured populations”

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Soft skills:

2010 An introduction to R

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Awards:

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Publications:

Pamminger, T., Scharf, I., Pennings, P., Foitzik, S. 2011. Increased host aggression as an induced defence against slavemaking ants. **Behavioural Ecology,** 22: 255-260.

Scharf, I., Pamminger, T., Foitzik, S. 2011. Differential response of ant colonies to intruders: attack strategies correlate with potential threat. **Ethology**, 117: 731-739.

Pamminger, T., Leingärtner A., Achenbach A., Kleeberg I., Pennings P.S., Foitzik S. Geographic distribution of the anti-parasite trait “slave rebellion. **Evolutionary Ecology**, in press

Pamminger, T, Modlmeier, A.P., Suette S., Foitzik S. Raiders from the sky: slavemaker founding queens select for aggressive host colonies. **Biology Letters**, in press.

Konrad, M., Pamminger, T, Foitzik S. Two pathways ensuring social harmony. **Naturwissenschaften**, in press. (shared first)