

Original Article

Increased host aggression as an induced defense against slave-making ants

Tobias Pamminger, Inon Scharf, Pleuni S. Pennings, and Susanne Foitzik

Department of Biology II, Ludwig Maximilian University of Munich, Grosshadernerstrasse 2, D-82152 Planegg–Martinsried, Germany

Slave-making 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 toward social parasites, and indeed, we show that host ants react more aggressively toward slavemakers than toward nonparasitic 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 slave-making ants: Cues from the slavemaker *Protomognathus americanus* caused an unspecific but long-lasting behavioral response in *Temnothorax* host ants. A 5-min within-nest encounter with a dead slavemaker raised the aggression level in *T. longispinosus* host colonies. Contrarily, encounters with nonparasitic competitors did not elicit aggressive responses toward non-nestmates. Increased aggression can be adaptive if a slavemaker encounter reliably indicates a forthcoming attack and if aggression increases postraid survival. Host aggression was elevated over 3 days, showing the ability of host ants to remember parasite encounters. The response disappeared after 2 weeks, possibly because by then the benefits of increased aggression counterbalance potential costs associated with it. *Key words*: aggression, behavior, parasites, phenotypic plasticity, social insects. [*Behav Ecol* 22:255–260 (2011)]

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 defenses to prevent parasitic exploitation as well as subsequent proliferation of parasites. Antiparasite adaptations range from general responses, such as behavioral 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 defense systems, such as the vertebrate adaptive immune system. All types of anti-parasite defenses aim to improve host survival and reproduction, but the development and maintenance of these defense 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 defenses in invertebrates as well (Kraaijeveld and Godfray 1997; Moret and Schmid-Hempel 2000). The evolution of host defense mechanisms frequently cause a decreased parasite fitness and consequent counteradaptations 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 antienemy 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 2 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 slave-making ant *Protomognathus americanus* can use 3 different *Temnothorax* species as hosts. This parasite exerts especially strong selection pressures on its main host species *Temnothorax 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). *Protomognathus americanus* colonies are patchily distributed within host

Address correspondence to T. Pamminger. E-mail: pamminger@bio.lmu.de.

Tobias Pamminger and Inon Scharf contributed equally to this work.
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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 slave-making *P. americanus*–*Temnothorax* system, fixed host defenses include enemy recognition and elevated aggression directed toward 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, Heinze, et al. 2005); therefore, host defenses against slave-making 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 2 stages (Alloway 1979): the scouting phase and the raiding event. When a *P. americanus* scout discovers a suitable target, that is, a host colony, it enters the nest site (Pohl and Foitzik, forthcoming) and after inspection, returns to its colony to recruit additional slave-making workers as well as enslaved *Temnothorax* workers to participate at the following raid (Alloway 1979). Therefore, raiding parties include workers of both ant species, that is, slavemaker and host workers (Figure 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. Because 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, nonparasitic 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 toward slavemakers but also to non-nest mate workers of their own species. In line with these expectations, we show that *T. longispinosus* host workers do not only react more aggressively toward slave-making ants than to conspecifics during a first encounter but in addition that a contact with a slavemaker worker induces an aggressive response toward non-nestmate conspecifics. This is the first example of an induced antisocial parasite defense in social insects.

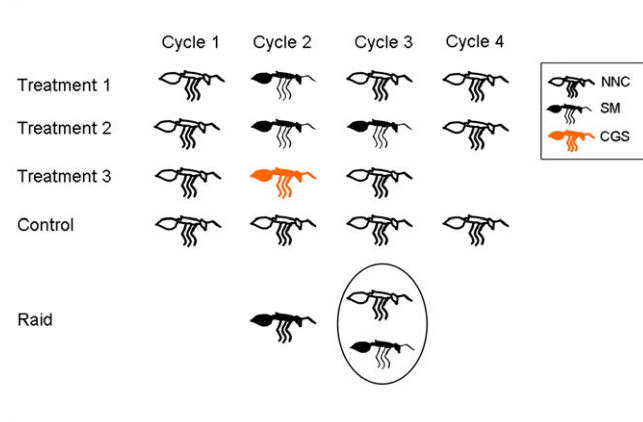


Figure 1

The experimental setup, demonstrating the encounter sequence of the 3 different treatments and the control during the experimental cycles. “NNC” stands for a non-nestmate *Temnothorax longispinosus* conspecific; “SM” stands for a slave-making worker of the species *Protomognathus americanus* and “CGS” stands for congeneric nonparasitic species (*Temnothorax 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 slave-making 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 slave-making worker induces elevated aggression, treatments 1 and 2 were exposed to a non-nestmate conspecific and again to a slave-making 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.

MATERIALS AND METHODS

Study system

The slave-making ant *P. americanus* parasitizes 3 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 (lat 42°31'35.3"N, long 74°9'30.1"W) in March–April 2009. *Temnothorax curvispinosus* colonies, which belong to a congeneric but nonparasitic species, were collected in August 2009 in the Watoga State Park, Pocahontas County, WV (lat 38°06'13"N, long 80°08'59"W). Nests of both species were kept in artificial nests (7.5 × 2.5 × 0.5 cm) in plastic boxes (10 × 10 × 1.5 cm) with a plastered floor in a climate chamber in Munich (day:night temperatures 20:15 °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 setup

We tested whether host colonies demonstrate an inducible response by confronting host nests with a sequence (4 cycles of encounters) of dead ants, simulating the encounter sequence of an actual raiding event in nature (Figure 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 4 experimental groups

(3 treatment groups and a control; Figure 1). Host colonies included different social organizations representing natural variation including queenless, monogynous, and polygynous colonies (see Supplementary Material). The experiment consisted of 4 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 (Figure 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 toward this dead worker was recorded.

Cycle 2

Three days later, treatment 1 and 2 were exposed to a dead slave-making worker simulating the presence of a slavemaker scout within the host nest, whereas 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 Supplementary Material for sample sizes). The experiments with treatment group 3 were conducted 3 months after the original experiment. We included the third 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, Heinze, et al. 2005). 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), whereas treatment 2 was again confronted with a dead slave-making 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 (Figure 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 slave-making workers) were frozen one day before the experiment and stored at -20°C . Due to the small number of slave-making workers present in a nest (Foitzik and Herbers 2001), slave-making workers belonging to 25 nests were pooled and randomly chosen for the experiment. Each opponent was used 3 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 slave-making 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 5 min before reuse.

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 s during the first minute and every 30 s for the following 4 min (11 observations in total). We scored antennation events (*a*) as nonaggressive interactions and 6 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 $\frac{m+b+h+d+s}{a+m+b+h+d+s}$ 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 5-min encounter (the numerator of the above formula). The results did not differ qualitatively (see Supplementary Material). 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 Supplementary Table 1. 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 1-way analysis of variance (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 ($\chi^2 = 5.47$, degrees of freedom [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 analysis of covariance. 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 3 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 first and second cycle, reexamining Alloway's (1990) demonstration that slave-makers 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 because both groups received the same treatment in cycle 2. We used a Fisher least significance difference (LSD) post hoc analysis to test for differences in aggression directed toward 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 4 treatments (control and treatments 1–3) between the first and third 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 treatments 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 toward the slavemaker than the non-nestmate conspecific (control) and the related species (the interaction term cycle \times 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, that is, *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, 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, that is, aggression toward non-nestmate conspecifics was elevated only if colonies had encountered a slavemaker before (the interaction term cycle \times treatment was significant: $F_{3,78} = 17.56$, $P < 0.0001$; post hoc indicated a significant difference between the control and the 2 treatments ($P = 0.005$, $P = 0.004$) but not between treatments 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 cycles 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 slave-maker ant within a host colony), which invariably precedes destructive slave raids, we were able to induce a long-lasting and strong aggressive reaction of host colonies, directed toward non-nestmate conspecifics. After the encounter with a slave-making ant, host colonies reacted toward non-nestmate

conspecific similarly aggressive as toward slavemakers. In addition, we confirmed the findings of Alloway (1990) showing that host colonies treat slave-making 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 toward them. The response toward slavemaker ants is fixed and always aggressive independent of context. This fixed aggressive response makes intuitive sense because 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 by eliminating the scout, by preventing the slave-making raid, or by improving the chance of surviving the raiding event. Behavioral experiments have demonstrated that higher aggression directed toward slavemakers (e.g., number of injured and killed slavemakers) results in a higher proportion of brood being rescued by attacked host colonies (Foitzik et al. 2001).

The response toward 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 toward conspecifics could be adaptive under natural conditions if contact to a single slave-making ant in the nest is a reliable cue that a slave raid is forthcoming and if the consequent lasting aggressive response toward 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 slave-making 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 toward competitors is variable and environment dependent. Colonies originating from high-density areas were found to be more aggressive toward non-nestmate conspecifics than colonies from low-density areas (Modlmeier and Foitzik, in review). Moreover, host colonies were shown to react more aggressively toward enslaved host workers during the raiding season in summer than in spring, when *Protomognathus* colonies never go on raids (Brandt, Foitzik, et al. 2005). In addition to this seasonal adjustment in the aggression level, we provide evidence here that *T. longispinosus* colonies upregulate their aggressive response toward non-nestmate conspecifics in reaction to parasite cues.

We initially expected that host colonies would increase their aggression toward 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 toward non-nestmates, whereas 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 toward 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, whereas a congeneric species, which is chemically more distant, *T. curvispinosus* (Brandt, Heinze, et al. 2005) 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 (Figure 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 3 days. The stimulation followed by a downregulation 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 toward nestmates. Detecting possible costs of induced defense is 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 antipredatory-induced responses is environment dependent. In our system, we speculate that competition with conspecifics colonies is less important than avoiding raids by slave-making 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 (D'Ettoire et al. 2004; Brandt, Foitzik, et al. 2005). Our findings offer a potential proximate explanation: The increased encounter rate of host workers with slave-making 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, whereas a proportion of colonies showed a consistent weak response toward 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.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>.

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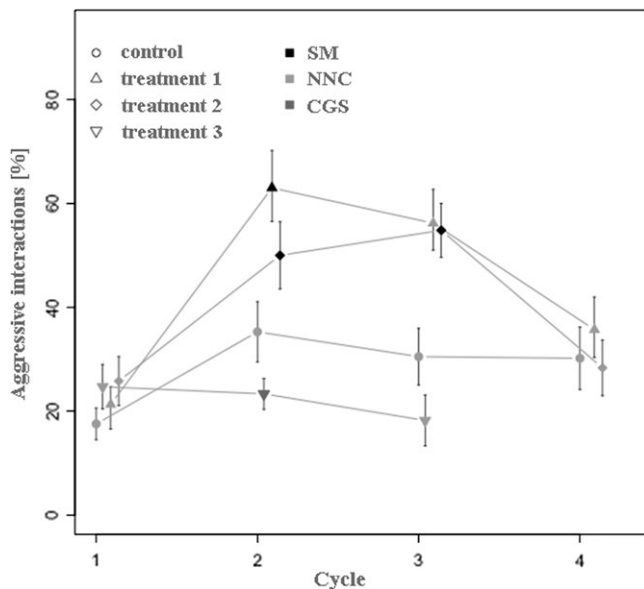


Figure 2
Mean aggression per treatment and cycle. Control: circles; treatment 1: triangles (upwards); treatment 2: diamonds; and treatment 3: triangles (downwards). Non-nestmate *Temnothorax longispinosus* opponent (non-nestmate conspecific, NNC) (gray), slave-making worker of the species *Protomognathus americanus* (slavemaker, SM) (black), and *T. curvispinosus* (congeneric species, CGS) (dark gray). Cycle 2, 3, and 4 took place 3, 6, and 20 days after cycle 1, respectively. Means \pm 1 standard error are presented.

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