

**Geographic distribution of the anti-parasite trait “slave rebellion” and potential costs of the defense**

TOBIAS PAMMINGER<sup>\*,†</sup>, ANNETTE LEINGÄRTNER<sup>†</sup>; ALEXANDRA ACHENBACH<sup>†</sup>,

5 PLEUNI S. PENNING<sup>S#†</sup> AND SUSANNE FOITZIK<sup>\*†</sup>

10 *\* Institute of Zoology, Johannes Gutenberg University of Mainz, Germany*

*† Department of Biology II, Ludwig Maximilian University of Munich, Planegg – Martinsried, Germany*

*# Department of Organismic and Evolutionary Biology, Harvard University, Boston, USA*

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**Running title:** Geographic variation in slave rebellion

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**Correspondence:** Tobias Pamminger, Institute of Zoology, Johannes Gutenberg University of Mainz, Johannes von Müller Weg 6, D-55099 Mainz, Germany

25 **e-mail:** [pamminge@uni-mainz.de](mailto:pamminge@uni-mainz.de)

## Abstract

Social parasites exploit the brood care behaviour of another species and exert strong selection pressures on their hosts. As a consequence, hosts have developed defence mechanisms to prevent parasitism or lower its costs. Recently, a novel post-parasitism defence has been documented in the hosts of a slave making ant. Slave-workers of *Temnothorax longispinosus* are able to lower parasite pressure by regularly killing pupae of the obligatory slavemaking ant *Protomognathus americanus*. Consequently, growth of parasite nests is reduced, which leads to fewer raids and likely increases fitness of neighbouring host colonies. This defence trait has been termed “slave rebellion”. In this study, we investigate its presence, expression and potential costs in three well-studied communities. We report its presence in all communities, document strong variation in its expression in between the different habitats and discuss possible costs of this behaviour potentially limiting its expression and spread.

**Keywords:** coevolution, selection mosaic, parasitism, cost-benefit trade-off, social parasites, slavemaking ants

## Introduction

Parasitism is the most common life style on earth and virtually all organisms are affected by it  
45 (Price 1980). Parasites are organisms 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) up to specialized  
behavioural manipulations of the host which result in increased parasite transmission with an  
50 often lethal outcome for the host (Berdoy 2000; Thomas et al. 2005).

As a consequence of parasite pressure, host species have developed a variety of  
defensive strategies that limit the impact of parasites. Such defensive adaptations on the host  
side can lead to counter-adaptations of parasites, a process which may lock both species in a  
coevolutionary dynamic, potentially escalating in an evolutionary arms race (Dawkins and  
55 Krebs 1979). Interactions of host with brood parasites, in ants usually called social parasites,  
have been suggested as model systems to study such escalating dynamics (Thompson 2005).

Brood parasitism describes the interaction between two species, one of which exploits  
the brood care behaviour of the other species avoiding the costs of rearing their own  
offspring. This form of parasitism has evolved multiple times independently (Beibl et al.  
60 2005; Kruger 2007; Buschinger 2009) in species in which brood care represents a substantial  
investment and can be transferred to individuals other than the parents. In birds, fish and  
insects these requirements are met, and indeed brood parasitism originated in all three  
taxonomic groups (Sato 1986; Rothstein 1990; Davies 1999, Buschinger 2009). In the well-  
studied avian systems (Kruger 2007) as well as in social insects (Hare and Alloway 2001;  
65 Fischer-Blass et al. 2006; Foitzik et al. 2009) severe fitness costs for the host have been  
demonstrated, resulting in strong selection pressures on the host species. In response, they  
have developed defence mechanisms either to avoid parasitic exploitation (Alloway 1990;

Moksnes et al. 1991; Roskaft et al. 2002) or to reduce its costs once parasitized (Langmore et al. 2003; Kruger 2007; Achenbach and Foitzik 2009).

70 In brood parasite systems of birds (e.g. cuckoo and cowbird) hosts exhibit well developed defence mechanisms to prevent brood parasites from laying eggs in their nests. These defence mechanisms often involve mobbing of parasites as soon as they are spotted (Moksnes et al. 1991; Roskaft et al. 2002). In addition, various parasitized species have developed the ability to recognize parasite eggs by their size or colour. In the parasites, on the  
75 other hand, egg mimicry has evolved, which limits egg detection by hosts (Kruger 2007). One might expect that hosts in highly parasitized populations could evolve to become so restrictive in rejecting slightly deviant eggs, that they would occasionally reject some of their own eggs with slightly deviant coloration or size. Indeed, numerous studies found evidence that shifts in the host species recognition thresholds led to the accidental rejection of own eggs with  
80 deviant colouration (Davies et. al. 1996; Kruger 2007). Obviously, rejection of own eggs poses a fitness cost which could limit the spread of the egg rejection behaviour. Such a cost of defence should lead to a geographic pattern, in which parasite egg rejection only occurs in areas where parasite pressure is strong so that the benefit of rejecting parasite eggs outweighs the cost of accidental rejecting own eggs. If parasites are rare, the costs of unintended  
85 rejection of own offspring would otherwise be too high and therefore selected against (Davies et.al.1996).

Slave-making ants are social parasites, which depend on host workers of another species either during the colony founding phase or during their entire life cycle (Buschinger 2009). The workers of slavemaking ants often exhibit a limited behavioural repertoire. They  
90 do not have the ability to perform routine tasks such as foraging, brood care and nest maintenance and depend on enslaved host ants (slaves) to perform these essential tasks. Instead, slavemaker workers are specialized in searching and attacking neighbouring host

colonies during so-called slave raids. During these raids they kill or expel the adult host ants and steal their brood. The brood will develop in the slavemaker nest and become a new  
95 generation of slaves.

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

The behaviour, ecology, chemistry and population genetics of this host-parasite interaction has been studied extensively over the past two decades, revealing several host defence mechanisms. Most studies have focused on adaptations prior to enslavement  
105 including enemy recognition, adjustment of the recognition threshold, fighting abilities, inducible aggression and 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 defence mechanisms of enslaved workers are unlikely to evolve, because it was assumed that slaves cannot escape and  
110 reproduce, hence no behaviour could increase their 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 defence, which has been termed “slave rebellion” (Achenbach and Foitzik 2009; Achenbach et al. 2010). Instead of raising *P. americanus* parasite brood to adulthood, enslaved *Temnothorax* were observed to  
115 kill a large proportion of the slavemaker pupae either by direct attack or by neglect (Achenbach and Foitzik 2009), even though they raised their own brood efficiently under the same conditions. By killing or neglecting slave-making pupae, the slaves limit the growth rate

of a slavemaker colony and reduce parasite pressure on nearby host colonies. If nearby colonies are related to the slaves, and indeed recent genetic analyses suggest this, the  
120 behaviour could spread through kin selection (Metzler et al. unpublished ms).

There are two main differences between egg rejection in birds and pupae killing in the ants we study. First, a bird that correctly identifies a parasite egg and rejects it will benefit directly from this action because the energy not spent on the parasite young can be spend on its own young or its own survival. Ant slaves, on the other hand, that kill a parasite pupae  
125 cannot directly benefit, as they do not reproduce. The only benefit could come from increased survival of related colonies, for example the colony from which they were raided as pupae was raided. Secondly, there is a difference in the recognition mechanism of the parasite brood. In contrast to the avian system where parasites and their eggs are detected visually, ants mainly use chemical clues for nest mate recognition and enemy detection (Hefetz 2007).  
130 Chemical analyses revealed that parasite pupae show a cuticular hydrocarbon profile different from that of the hosts' brood (Achenbach et. al. 2010) and these chemical differences could be used by the slaves to identify parasite brood. Moreover, chemical analyses and cross-fostering experiments demonstrated that the parasite tries to track local host profiles, as expected when parasite pupae which deviate less in their profile from their sympatric hosts are killed at a  
135 lower rate (Achenbach et.al. 2010).

In this study, we investigate the brood care behaviour of the main host species, *T. longispinosus*, both in its own nests and in slavemaker nests. We study hosts from three well-studied communities in New York (NY), West Virginia (WV) and Ohio (OH), which differ in their community composition, but are very similar in parasite prevalence (Herbers  
140 and Foitzik 2002; Brandt and Foitzik 2004). Previous studies have found differences between the three 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 than compared to the other two sites. For example, hosts from NY are able to rescue more brood when their nest is raided 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 approximately 84% - 98% of the host ant community. Secondary hosts occur in low densities and are expected to be not very important for the host-parasite interaction in both habitats (*T. ambiguus* in NY and *T. curvispinosus* in WV). 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), so that both host species are important for the slavemaker. In this study, we focus on *T. longispinosus*, excluding the other hosts from the analysis. We will test the following six hypotheses.

1. We hypothesize, that if the slave rebellion trait is present, the survival rate of *P. americanus* brood will be considerably lower than *T. longispinosus* brood survival in the same habitat.
2. 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, population genetic analyses based on neutral markers detected strong structuring in the host and the parasite (Brandt et al. 2007; Pennings et al. 2011). In addition, geographic differences in other defence behaviours have been documented (Brandt and Foitzik 2004).
3. If the host is under selection to be more restrictive in accepting variation in brood chemical profiles, we expect that hosts under strong selection pressure (e.g. restrictive worker) would kill a high proportion of parasite pupae if they are enslaved. However, as the chemical profile

of host pupae vary, restrictive non-parasitized workers would be at risk to reject a proportion of their own pupae. This would constitute a cost of the rebellion trait. If so, the brood survival rates in host and parasite nests should co-vary geographically.

4. Arms race dynamics are expected to progress in a stepwise fashion and result in an accumulation of host and parasite adaptations (Gandon et. al. 2008), but the outcome at a specific point in time depends on multiple conditions. We anticipate that in communities with strong selection pressures on the host i.e. in NY, enslaved workers should be well able to recognize and eliminate parasite brood.

5. The impact of slavemakers on neighbouring colonies varies with the caste of the slavemaker. The most direct threat to the local host community is posed by slavemaker workers, because they stay in the nest and conduct slave raids. Queens and males usually do not stay in the nest and they do not conduct raids. However virgin queens were observed to be involved in raids in the laboratory and genetic data indicate that slavemaker queens return to the vicinity of the mother colony after the mating flight (Foitzik and Herbers 2001). We therefore hypothesize that slaves benefit most from killing slave-maker worker pupae, followed by queen pupae and no fitness benefits are expected from the destruction of male pupae of the social parasite.

6. Most *P. americanus* nests are small with an average number of slavemaking workers below five, but we occasionally find very large slavemaking colonies containing up to 50 slavemaking workers in the WV population (Pamminger unpublished). We hypothesize that larger colonies contain less rebellious slaves, i.e. slavemaking colonies were only able to grow to these large sizes, because a higher percentage of slavemaking workers survived to adulthood.

## **Material and Methods**



*Study system, collection and housing*

195 *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 a period of four years (2005-2008) we  
collected *P. americanus* nests as well as nests of its preferred host species *Temnothorax*  
*longispinosus* in three different communities. Ants were collected in early summer at the  
200 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)  
and at the Watoga State Park, Pocahontas County, WV (N 38°06'13" W 80°08'59"; in 2007).  
To investigate pupal survival rates in the three communities, the ant colonies were transported  
to Munich in their natural nests (e.g. an acorn or small rotten sticks), counted and transferred  
205 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  
210 consequence of this timing *P. americanus* colonies only contained *P. americanus* brood (as  
they have not performed any raid at this point in the season) and adult enslaved *T.*  
*longispinosus* workers originating from raiding events of previous years. All *P. americanus*  
colonies containing slave species other than *T. longispinosus* were excluded from the analysis.  
Free living *T. longispinosus* colonies only contained their own brood.

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*Survival rate of pupae*

We contrasted the brood rearing success of enslaved *T. longispinosus* workers to that of free-living *T. longispinosus* colonies. 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  
220 eclosion of the pupae to adult ants. As both species belong the subfamily Myrmicinae, the pupae lack cocoons and their caste can be clearly identified by its shape. All pupae decapitated or cut in pieces were counted as dead and all missing pupae and callows (freshly enclosed workers) were assumed to have died as well. For details on the behaviour of pupae killing and the observation protocol we refer to Achenbach and Foitzik (2009). We collected  
225 data from 158 *P. americanus* and 74 *T. longispinosus* colonies from all three communities. This dataset was extended with the data from Achenbach and Foitzik (2009) (51 *P. americanus* and 41 *T. longispinosus* from New York and Ohio). In total, we monitored the brood development in 324 colonies (WV: 16 host and 15 slavemaker colonies, NY: 51 host and 166 slavemaker colonies, OH: 48 host and 28 slavemaker colonies). Most of these  
230 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 = 538 measurements).

### *Statistical analysis*

To analyse the data on pupal survival, we used logistic regressions. To fit the logistic  
235 regressions, we use the “glm” command in the R statistical computing software with a logit link function and with option “family = quasibinomial” to 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*), whether a colony has a queen, community of origin, total number of host  
240 workers and brood items in the nest and caste of the brood. For this analysis, we assumed that survival probabilities of different castes in one nest are independent, although we realize that

this may not be the case. Because we do one analysis with both host and slavemaker brood, we cannot take the number of slavemaker workers per nest into account. We will do this in a subsequent analysis. For model selection, we start with a parameter-rich model, with all possible pairwise interactions and iteratively remove the least significant interaction, covariable or cofactor. Ultimately, we report the minimal adequate model, in which all cofactors and covariables or their interactions have a significant effect.

As we cannot analyse the effect of the number of slavemaker workers in the complete data set, we have to analyse the data from slavemaker nests separately. For the slavemaker dataset we used the same modeling approach as in the main analysis, with the only difference that we now exclude species as a factor but include number of slavemaker workers.

## Results

Using the full data set (with nests of both species) we find that pupae in *P. americanus* nests survive worse than pupae in free-living *T. longispinosus* nests ( $p = 0.001$ , for all p-values, see Table S1). In general, pupal survival varied between communities ( $p < 0.001$  for NY vs OH and  $p = 0.002$  for NY vs WV), with a higher survival in Ohio and West-Virginia than in New York, but this mostly true for the host and less so for the slavemaker (interaction species x OH:  $p < 0.001$ , species x WV:  $p = 0.006$ ).

The number of brood items in a nest was found to have a negative effect on brood survival in general ( $p = 0.001$ ), but this effect disappears in nests with queens (queenright x sum brood :  $p = 0.002$ ) and in slavemaker nests (species x sum brood:  $p < 0.001$ ), leading to a positive effect of brood number in slavemaker nests with queen, but a negative effect in host nests without queen.

Survival is lower for queen pupae ( $p = 0.026$ ) and for pupae in nests with queen ( $p = 0.009$ ), but males survive equally well in nests with queen and without queen (male  $\times$  queenright  $p = 0.026$ ). With increasing number of *Temnothorax* workers (nest size), pupae survived better in New York ( $p = 0.004$ ), but worse in West-Virginia (nest size  $\times$  WV:  $p < 0.001$ ). All results and their graphical representation are given in the supplementary material (Tab.S1, Fig.S1-S4).

When looking at the slavemaker data set separately, we find that slavemaker pupae survive worse in WV ( $p = 0.004$ ) than in the other two communities. When we analyse caste fate, we find that queens ( $p < 0.001$ ) survive worse than workers. We find that the number of brood items in the nest has a positive effect on slavemaker brood survival ( $p < 0.001$ ). We find no significant effect of number of slavemaker workers or number of host workers in the slavemaker dataset. 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 positive effect of worker number on survival rate of pupae ( $p = 0.001$ ), however, this effect is not present in WV ( $p = 0.006$ ). The model also suggests that the presence of a queen has a strong negative effect ( $p=0.023$ ) but this is not the case in large colonies ( $p=0.016$ ). We find no significant effect of the caste of host pupae in the host dataset (Tab. S3).

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## Discussion

In this study, we investigated the geographic variation in offspring survival rates in the obligate social parasite *P. americanus* and its main host *T. longispinosus*. While in both types of nests *T. longispinosus* workers care for the brood, we find severely reduced brood survival rates in *P. americanus* nests compared to *T. longispinosus* nests in all three studied

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communities (Table S1). This phenomenon is intriguing, because the observed difference in brood survival could be caused by a host defence mechanism, named slave rebellion (Achenbach and Foitzik 2009). If slavemaker brood rarely survives, slavemaking colonies remain small and nearby host colonies, which are potentially related to the slaves in the  
295 slavemaker nest, will experience a reduced raiding risk. The severe reduction in parasite brood survival presents an explanation why slavemaker nests of *P. americanus* are exceptionally small compared to nests of related species (Foitzik and Herbers 2002). The strong differences we find in brood survival between the three habitats suggest that these communities are at different stages in a coevolutionary arms race or that the costs/benefit  
300 trade-off for the trait differs between sites.

Before we discuss the details of our findings, we want to reflect on the possibility that the observed effect is not related to the coevolutionary process we are interested in. One potential explanation of the observed low survival of slavemaker pupae could be that it is a laboratory artefact, which is not relevant under natural conditions. However, earlier studies  
305 found very low per-worker-productivity in parasite nests under field conditions (Foitzik and Herbers 2002) indicating similarly low survival rates in the field. 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 this explanation as well, because under laboratory conditions slavemaker larvae develop normally under the care  
310 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 as they ceased feeding. The high survival rate during the larval stage indicates adequate care by slave workers at this developmental stage. If *T. longispinosus* care would simply not be adequate, larvae survival should have been much more affected compared to pupal survival.  
315 Thirdly, we have direct observational evidence that enslaved host workers attack seemingly

healthy parasite pupae and tear them apart (Achenbach and Foitzik 2009). Finally, in the current study we find that the survival rate is reduced to different extents in the different habitats, which provides additional evidence that the effect is not due to a general inadequacy of *T. longispinosus* workers to care for *P. americanus* pupae. We therefore conclude that  
320 neither inadequate feeding nor insufficient hygienic behaviour of enslaved host workers is likely to cause the increased mortality rate in parasite pupae.

The following part of the discussion is organized along the six expectations we stated in the introduction. (1.) We find evidence for a post-enslavement defence to be present in all three study sites, reflected in a severely reduced survival of *P. americanus* pupae compared to  
325 host pupae (Tab.S1, Fig.1). In these communities about half of the parasite pupae do not survive to adulthood. These results confirm the findings of Achenbach and Foitzik (2009) based on a substantially larger data set and an additional community. (2.) Because the populations we study are genetically differentiated and differ in other defence traits, we expected quantitative differences in the rebellion trait expression in different habitats and indeed our data in-  
330 dicates pronounced differences in the expression level between the 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 at the two other communities. We currently do not know what causes survival of slavemaker pupae to be lower in West-Virginia. However, this finding could provide an explanation for the observa-  
335 tion that slavemaker nests in the field are smaller in WV than in other populations.

(3.) We hypothesized that the slave rebellion trait could come with a cost, such as *T. longispinosus* workers may accidentally reject their own offspring in free-living *T. longispinosus* colonies. Indeed we find that host pupal survival is reflected in slavemaker pupal survival. Specifically, in WV where we find exceptionally low parasite survival (33%)  
340 we also find low survival rates of host pupae (75%) but in OH, where parasite survival is

higher (54%) than in WV, host survival is as well (93%) see Figure.1. These results suggest that host populations which kill a large fraction of the parasite pupae when enslaved, might pay a price by erroneously killing own offspring in free-living nests. However, we only have data for three communities, so we cannot formally test whether slavemaker pupal survival and  
345 host pupal survival covary. Additional communities would have to be investigated to see whether the pattern we observe holds.

We expected (4.) to find higher killing rates in host populations under strong parasite pressure or under conditions where rebelling slaves gain a higher indirect fitness benefit from their behaviour. It is not easy to quantify parasite pressure, because it depends on several para-  
350 meters such as parasite prevalence, raid frequency and destructiveness as well as community composition. Previous studies have shown that parasite prevalence is very similar in the three focal communities (Table 1), but slavemaker colonies in NY seem to have a higher frequency of successful raids as compared to WV and OH (Foitzik et al. 2001, Brandt & Foitzik 2004). Parasite pressure is therefore likely to be higher on *T. longispinosus* in NY than at the two  
355 other sites. However, we found the lowest slavemaker survival in WV and not in NY as we had expected (Fig.1). This suggests that the slavemaker might lead the arms race in NY, for example, by closely tracking host pupae profiles. In addition, it is possible that a higher number of raided host nests survive in WV, where slavemaker are less destructive during raids (Foitzik et al. 2001). If so, slaves in WV might be more likely to have nests with relatives in  
360 the vicinity. These related neighboring nests could benefit from the rebellion behavior of the slaves.

We expected an effect of caste on survival rates of the slavemaker (5.) and indeed, survival rates of pupae were found to be caste specific for the parasite, but not for the host *T. longispinosus* (Tab.2). In our previous study (Achenbach and Foitzik 2009), we found a  
365 different pattern with low survival rates for *P. americanus* queens and workers, but very high

survival rates of *P. americanus* male pupae. In both studies queen survival was lowest. Therefore, slave workers apparently target queen pupae, possibly because the larger queen pupae carry more chemical cues, by which enslaved workers can recognize them. In principal, destruction of parasite queens could lower the impact of social parasitism on the local host population if queens return to the neighborhood of their mother nest as genetic analyses indicate (Foitzik and Herbers 2001). However, slavemaking workers, which conduct raids on foot, are certainly more likely to interact with neighboring related host colonies than slavemaking queens are, so that enslaved workers should benefit more by kin selection from the destruction of slavemaker worker pupae than by the destruction of queen pupae. However, if all three castes are detected by the same chemical cues and the actual killing of pupae as such carries no direct fitness costs for the slave - slavemakers have never been observed to “punish” slaves - we would expect that all three castes are targeted. In this scenario, only sensory limitation – the ability to distinguish parasite from host brood - would limit the killing behavior in enslaved workers. As queens carry more identification cues on their cuticle they are more easily recognized and consequently killed. The relatively low survival of parasite queen pupae appears therefore more the result of sensory limitation than differential selection.

We hypothesised (6.) that part of the variation in nest size of *P. americanus* could be explained by variation in killing behaviour between nests. We predicted that only colonies with less rebellious slaves are able to reach large colony size. However, we do not find such an association. Instead, we find that *P. americanus* nests with more pupae show a higher pupal survival rate. This effect (Fig.1) could result in a high variation in nest size, because unproductive nests will remain small due to low survival rates of their offspring, but once they reach a certain nest size survival is better and nests can grow large. Note that this finding, that slavemaker pupal survival is better in nests with large numbers of slavemaker pupae, is



390 another indication that enslaved host workers are fully capable of caring for the slavemaker pupae.

In summary, we demonstrate the presence of the slave rebellion trait in three different communities supporting the results of Achenbach and Foitzik (2009) and the pronounced variation in this trait between the three communities indicates the presence of a geographic  
395 coevolutionary mosaic (Thompson 2005). We also find that the slave rebellion trait may be associated with a cost of accidental killing of the hosts' own pupae. Local parasite pressure did not explain parasite survival, either because the parasite is taking over the lead in the evolutionary arms race or because variation in kin selected benefits are more important for the trait expression. The severely reduced survival rates of *P. americanus* pupae have been  
400 suggested to present an additional defence mechanism, analogous to egg rejection behaviour in avian social parasite systems (Achenbach and Foitzik 2009), in which birds can reject parasite eggs after parasitism has taken place. While many studies on avian brood parasites find a close relation between local parasite pressure and the expression of host defence traits (e.g. Soler and Moller 1990, Stokke et.al. 2008) a recent publication found no such  
405 relationship (Vikan et. al 2010). Vikan et al. documents a lack of variation in the defence trait between populations experiencing different levels of parasite pressure. The authors suggest that strong gene flow between the populations might synchronize the different evolutionary dynamics in the studied populations. In contrast to this study, we find pronounced differences between populations but we still need to explore the driving forces in this coevolutionary  
410 dynamic.

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520 Figure 1.: Survival probabilities of pupae of the slavemaker *P. americanus* and the host *T. longispinosus* in the three communities West Virginia (WV), New York (NY) and Ohio (OH). Presented are means and error bars indicate standard error.

Figure 2.: Survival rates of pupae of different castes of the slavemaker *P. americanus* and the  
525 host *T. longispinosus*. Presented are means and error bars indicate standard error.

Figures S1 & S2.: The association between total number of brood items in a nest and survival of the pupae of different castes (black: workers, red: queens, green: males) in host nests with (S1) and without queen (S2) in all three communities. Lines show the predictions of an  
530 overdispersed logistic regression model. To obtain model predictions, we used for each category (caste, species and community) the median nest size.

Figures S3 & S4.: The association between total number of brood items in a nest and survival of the pupae of different castes (black: workers, red: queens, green: males) in slavemaker  
535 nests with (S3) and without queen (S4) in all three communities. Lines show the predictions of an overdispersed logistic regression model. To obtain model predictions, we used for each category (caste, species and community) the median nest size.

Table 1: Composition of the ant community in the three study sites. Parasite prevalence is given as number of host nests per parasite nest. Species contribution to the ant community is given in percent (Herbers & Foitzik 2002; Brandt & Foitzik 2004).

Community	Prevalance	<i>T. longispinosus</i>	<i>T. curvispinosus</i>	<i>T. ambiguus</i>
New York	10	97.7	0	2.3
West Virginia	8.4	83.6	16.4	0
Ohio	10.2	29	71	0



Figure 1

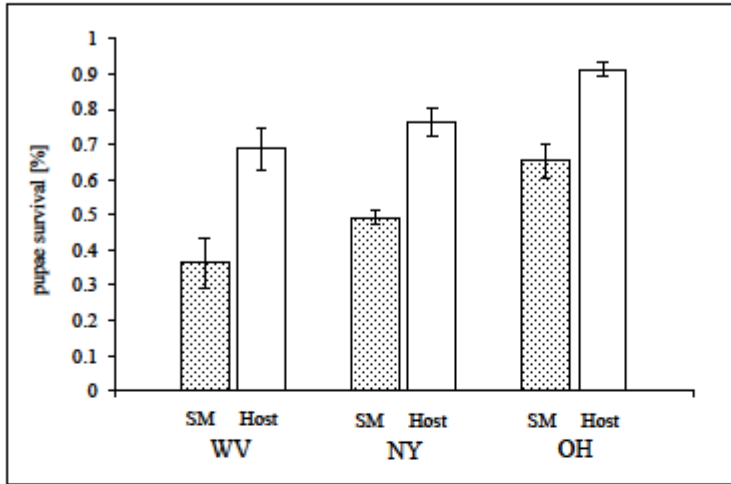
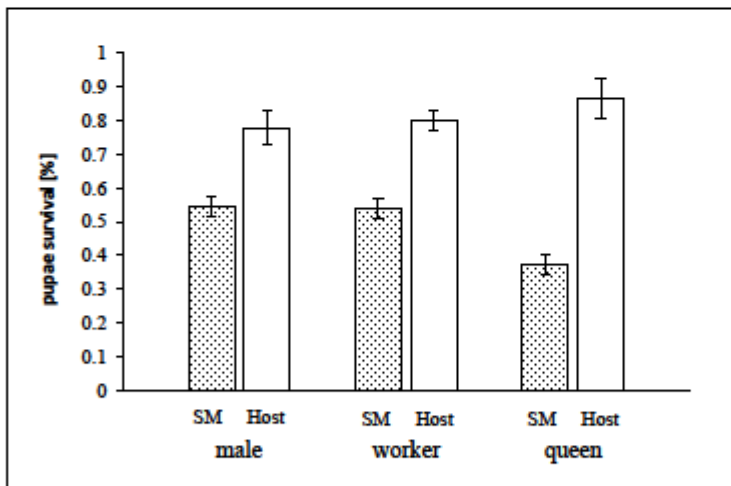


Figure 2



## Supplementary material

### 1. Software used

To fit generalized linear models to our data we used the glm command of the R statistical programming environment. Dispersion parameters were estimated by the glm routine. During  
545 model selection procedures we checked significance of regression coefficients based on approximation with Student's t-distribution as implemented in the R command summary.glm. We used the command anova.glm to perform analyses of deviance.

### 2. Factors influencing the proportion of pupae that survived in complete dataset

550 Using the option family=quasibinomial in the glm command, we applied an overdispersed binomial logistic regression to the complete dataset to test whether the four cofactors species (*P.americanus* or *T.longisinosus*), community (NY, WV, OH), caste (male, worker, queen) and whether a colony has a queen (queenright), and the two covariables sum.brood (total number of brood items present in the nest) items and total nest size (number of host workers) have an  
555 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 is shown in table S1.

Table S1

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	1.249	0.355	3.514	<0.001
<b>Species</b>	<b>-0.999</b>	<b>0.288</b>	<b>-3.473</b>	<b>0.001</b>
<b>CommunityOH</b>	<b>2.220</b>	<b>0.517</b>	<b>4.292</b>	<b>&lt;0.001</b>
<b>CommunityWV</b>	<b>1.728</b>	<b>0.547</b>	<b>3.158</b>	<b>0.002</b>
Male	-0.400	0.211	-1.896	0.059
<b>Queen</b>	<b>-0.718</b>	<b>0.322</b>	<b>-2.232</b>	<b>0.026</b>
<b>Queenright</b>	<b>-0.749</b>	<b>0.286</b>	<b>-2.614</b>	<b>0.009</b>

<b>Nest size</b>	<b>0.018</b>	<b>0.006</b>	<b>2.904</b>	<b>0.004</b>
<b>Sum brood</b>	<b>-0.056</b>	<b>0.018</b>	<b>-3.213</b>	<b>0.001</b>
<b>Species:CommunityOH</b>	<b>-1.634</b>	<b>0.423</b>	<b>-3.865</b>	<b>&lt;0.001</b>
<b>Species:CommunityWV</b>	<b>-1.358</b>	<b>0.492</b>	<b>-2.758</b>	<b>0.006</b>
<b>Species:Sum brood</b>	<b>0.059</b>	<b>0.016</b>	<b>3.722</b>	<b>&lt;0.001</b>
<b>Queenright:Sum brood</b>	<b>0.046</b>	<b>0.015</b>	<b>3.072</b>	<b>0.002</b>
<b>male:Queenright</b>	<b>0.673</b>	<b>0.302</b>	<b>2.231</b>	<b>0.026</b>
queen:Queenright	-0.085	0.455	-0.187	0.852
CommunityOH:Nest size	-0.024	0.013	-1.781	0.076
<b>CommunityWV:Nest size</b>	<b>-0.060</b>	<b>0.016</b>	<b>-3.619</b>	<b>&lt;0.001</b>

560

The prediction of this model is, for example, that the expected proportion of surviving pupae of male caste in a slavemaker nest in Ohio with a queen, and with nest size  $n$  and total brood number  $b$  is  $1/(1+e^x)$ , where

$$x = 1.249 - 0.999 + 2.220 - 0.400 - 0.749 + 0.018*n - 0.056*b - 1.634 + 0.059*b + 0.046*b + 0.673 -$$

565  $0.024*n = 0.36 + 0.049*b - 0.006*n$

The median nest size for nests from Ohio with a queen is 30, and the median number of brood is 6, so  $x = 0.474$  and the survival probability is  $1/(1+e^{-x}) = 0.62$ .

The results are visualized in figure 1.

570 **3. Factors influencing the proportion of pupae that survived in the slavemaker dataset**

Using the option family=quasibinomial in the glm command, we applied an overdispersed binomial logistic regression to the dataset containing only the slavemaker nests, to test whether the three cofactors community, caste and whether a colony has a queen, and the three  
575 covariables total number of brood items, number of slavemaker workers 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 is shown in table S2.

580 Table S2

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.220	0.141	1.554	0.121
CommunityOH	0.281	0.206	1.366	0.173
<b>CommunityWV</b>	<b>-0.881</b>	<b>0.304</b>	<b>-2.897</b>	<b>0.004</b>
Male	-0.274	0.150	-1.828	0.068
<b>Queen</b>	<b>-0.737</b>	<b>0.209</b>	<b>-3.531</b>	<b>&lt;0.001</b>
<b>Sum.brood</b>	<b>0.023</b>	<b>0.006</b>	<b>3.845</b>	<b>&lt;0.001</b>

#### 4. Factors influencing the proportion of pupae that survived in the host dataset

Using the option family=quasibinomial in the glm command, we applied an overdispersed binomial logistic regression to the dataset containing only the host nests, to test whether the three cofactors community, caste and whether a colony has a queen, and the two covariables total number of brood items 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 is shown in table S3.

590

Table S3:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	1.964	1.473	1.334	0.184
CommunityOH	1.065	0.917	1.162	0.247
CommunityWV	0.882	0.849	1.039	0.300
<b>Nest size</b>	<b>0.109</b>	<b>0.031</b>	<b>3.485</b>	<b>0.001</b>
Sum brood	-0.159	0.087	-1.820	0.071
<b>Queenright</b>	<b>-3.083</b>	<b>1.346</b>	<b>-2.291</b>	<b>0.023</b>
CommunityOH:Nest size	-0.038	0.039	-0.967	0.335
<b>CommunityWV:Nest size</b>	<b>-0.165</b>	<b>0.059</b>	<b>-2.793</b>	<b>0.006</b>
<b>CommunityOH:Sum brood</b>	<b>0.093</b>	<b>0.045</b>	<b>2.054</b>	<b>0.042</b>
<b>CommunityWV:Sum brood</b>	<b>0.188</b>	<b>0.082</b>	<b>2.299</b>	<b>0.023</b>

<b>Nest size:Sum.brood</b>	<b>-0.003</b>	<b>0.001</b>	<b>-2.667</b>	<b>0.008</b>
<b>Sum brood:Queenqueen</b>	<b>0.199</b>	<b>0.082</b>	<b>2.430</b>	<b>0.016</b>

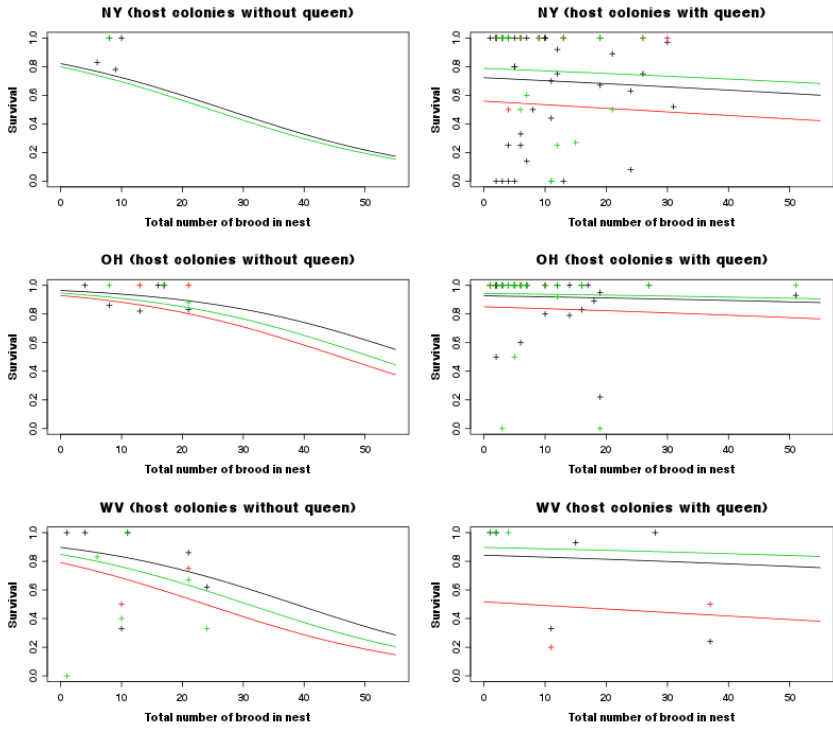


Figure S1

Figure S2

595

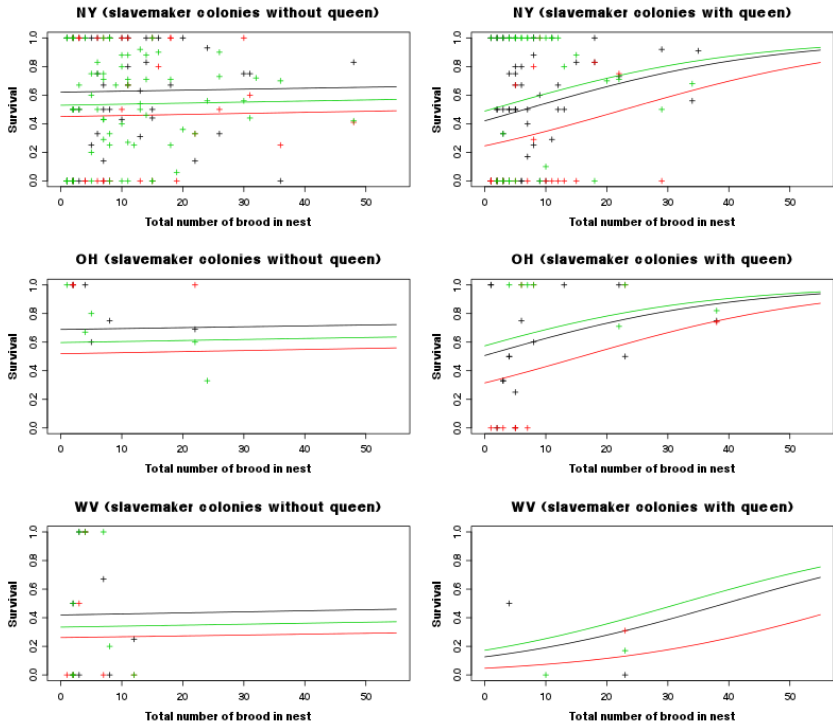


Figure S3

Figure S4