

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

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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 m²; 3 x 3 m) and two smaller ones (2.25 m²; 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 m² in the dense compartments and 0.6 colonies per m² 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 χ^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 confounding 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 %; $\chi^2_1 = 13.2$, $p < 0.0005$), 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 ($\chi^2_3 = 10.2$, $p < 0.02$): while there was no effect of host density ($\chi^2_1 = 0.3$, $p = 0.56$), fewer parasitic founding events occurred in plots in which we released a slavemaker colony ($\chi^2_1 = 5.8$, $p < 0.02$). 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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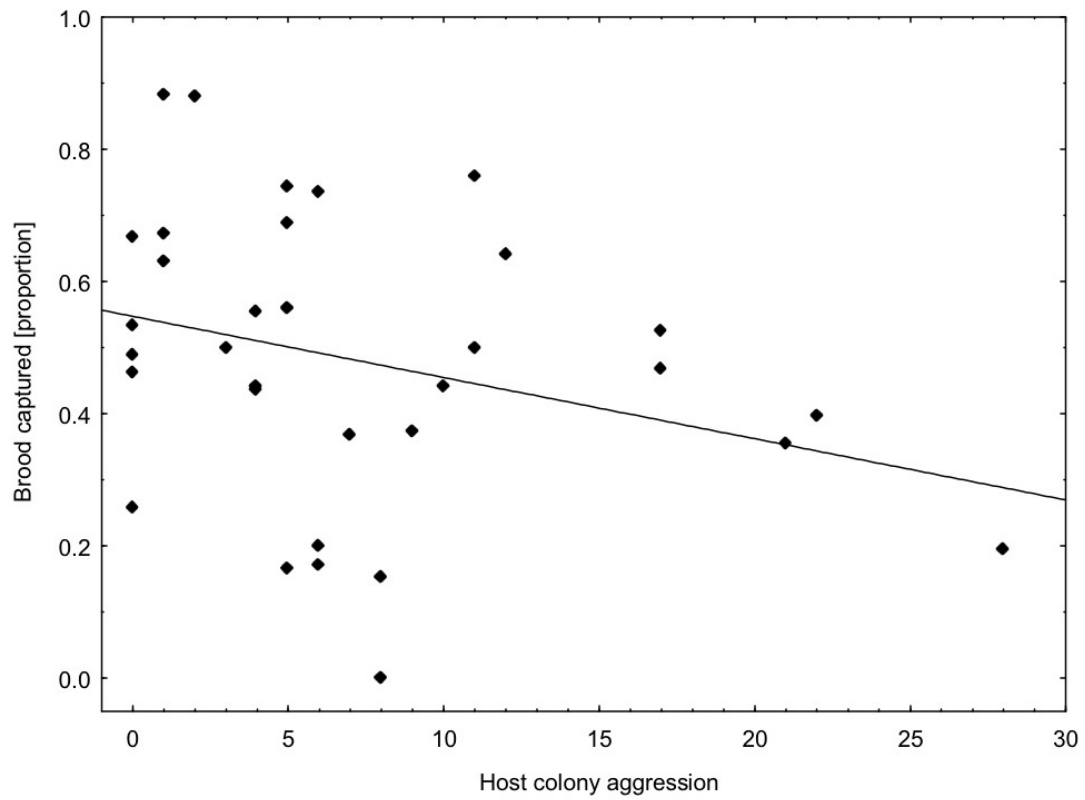
References

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. *Behavioral 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. *Behavioral 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 legends

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.



Supplementary Material

Methods

Aggression tests

We measured colony aggression by introducing a frozen conspecific non-nestmate. The usage of a dead adversary removed any effects of the opponents' behaviour and was shown to reflect host colony aggression against live conspecifics [7]. All interactions of the host colony to the dead opponent were recorded by scan sampling every 20 seconds in the first minute and every 30 seconds for the following 4 minutes resulting in a total of 11 observations. We recorded six behavioural categories: antennation (as a non-aggressive interaction) and mandible spreading (threat display), biting, holding, dragging and stinging as aggressive interactions. An aggression index was calculated as the sum of all aggressive acts within 5 min.

Host colony survival

We analysed whether host colonies survived more often in parasite-free plots using a χ^2 -test. Furthermore, we tested separately for parasitized and unparasitized plots whether surviving colonies were larger or smaller than those which perished by using a t-test based on log-transformed colony size (N of workers).

Slavemaker founding event

We investigated the decision making or invasion success of *P. americanus* founding queens by analysing how the following parameters influence frequency of host colony invasion: slavemaker presence (frequency of founding events within slavemaker treatments), host density and colony size. We performed χ^2 -tests to examine whether founding queens preferentially invaded host colonies of a certain treatment. First, we tested for differences in the frequency of

successful parasitic colony founding between all four treatments in a 2x4 matrix. Then, we compared founding preferences for density and slavemaker presence separately using 2x2 matrices. The effect of colony size and host aggression on whether or not a host colony was invaded was evaluated by a t-test using log-transformed colony size (N of workers) and a Mann-Whitney U test, respectively. These tests included only host colonies that were still present at the end of the manipulation.

Potential relationship between host aggression and growth rate

We correlated aggressiveness with growth rates in *T. longispinosus* colonies that were not overtaken by *P. americanus*. Growth rate was calculated as the number of brood/callows at the end of the experiment divided by the number of brood before the experiment. We performed an ANCOVA that was similar to the one used to calculate take-over success. Accordingly, growth rate was the dependent variable, colony aggression (at the start of the experiment; as in the takeover success analysis) the continuous and observer the categorical predictor. The results revealed that growth rate was not related to colony aggression (ANCOVA: $F_{1,85} = 0.05$, $p = 0.83$). Hence, we were able to exclude the possibility that the observed relationship between host aggressiveness and brood capture rate of the slavemaker queen is due to a correlation between colony growth rates and aggression.

Density treatment after the experiment

Despite the loss of *T. longispinosus* colonies in all treatments, and a consequent drop of host density in all treatments, we recovered the original ratio of 4:1 in host density between high and low density treatments. This indicated that the density differences between treatments were present during the entire experiment.