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

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Keywords: parasite; personality; dispersal; aggression; fitness

1. 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 slave-makers 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, owing to an exceptionally warm spring, the nuptial flight of the parasite occurred early that year and about one-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.

2. MATERIAL AND METHODS

(a) Study system, field collection and aggression experiments

In May 2010, we collected and censused 16 mature P. americanus colonies (each containing a parasite queen and at least two slavemaking workers) and 160 T. longispinosus host colonies at the Huyck Preserve, Albany County, NY (42°31′35.3″N, 74°9′30.1″W). Aggression against intruders was determined for all T. longispinosus colonies in a standardized set-up by T.P. and A.P.M. [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 site (dowel [5]) and placed in field enclosures within two days of collection.

(b) Field manipulation

During May–June 2010, we constructed eight enclosures in a homogeneous forest area of about 50 × 50 m. Each of the enclosures was composed of four compartments, two larger ones (9 m²; 3 × 3 m) and two smaller ones (2.25 m²; 1.5 × 1.5 m) that were separated by 40 cm-high aluminium flashing, anchored 10 cm in the ground. The leaf litter and thereby all suitable 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 m⁻² in the dense compartments and
0.6 colonies m$^{-2}$ in the less dense ones. We placed a slavemaker colony in the centre in one of the high- and low-density compartments, creating four different treatments: (i) high density with slavemaker, (ii) high density without slavemaker, (iii) low density with slavemaker, and (iv) 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].

(c) 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 Chi-square-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 percentage of brood captured by the slavemaker queens as the dependent variable and colony aggression as a continuous and observer as a categorical predictor to investigate if more aggressive colonies can escape more brood. To control for potential confounding effects (e.g. host colony growth rates), we conducted additional tests (see electronic supplementary material).

3. RESULTS
The mating flight of P. americanus took place during the manipulation and 21 per cent 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%; $\chi^2 = 13.2, p < 0.0005$), suggesting that about a quarter of all 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 (m$^2 = 10.2, p < 0.02$): while there was no effect of host density (m$^2 = 0.3, p = 0.56$), fewer parasitic founding events occurred in plots in which we released a slavemaker colony (m$^2 = 5.8, p < 0.02$). 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 (Mann Whitney U-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: $F_{1,29} = 4.19, p < 0.05$; figure 1). Observer identity and the interaction did not covary with the percentage of brood captured ($p > 0.05$).

Figure 1. The relationship between brood capture rate of founding Protomognathus americanus ant queens and host colony aggression of Temnothorax longispinosus. Host colony aggression is given as the total number of aggressive interactions. Presented are 33 founding events from a field experiment.

4. 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 in parasite aversion (take-over rate was not influenced by host colony aggression); instead, more aggressive host colonies were able to escape with a higher proportion of brood, which should translate into a fitness benefit. The selection pressure through parasitic nest foundations was found to be high, as 20 per cent 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 fate 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 latter 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 conspecífics [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 defences. Parasite 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].

We thank Adam Caprio and Chad Jemison for their help during fieldwork. This study was funded by the DFG (no. P06-0520.1) and the Huyck Preserve, Rensselaerville, NY, USA. A.P.M was supported by a graduate scholarship of the Universität Bayern eV.

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