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## Knowing your enemies: seasonal dynamics of host-social parasite recognition

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**Abstract** Despite its evolutionary significance, behavioural flexibility of social response has rarely been investigated in insects. We studied a host-social parasite system: the slave-making ant *Polyergus rufescens* and its host *Formica rufibarbis*. Free-living host workers from parasitized and from unparasitized areas were compared in their level of aggression against the parasite and alien conspecifics. We expected that a seasonal change would occur in the acceptance threshold of *F. rufibarbis* workers from a parasitized area towards the parasite, whereas *F. rufibarbis* workers from an unparasitized area would not show substantial changes connected with the parasite’s peak in activity (raiding and colony-founding season). The results showed a significant adaptive behavioural flexibility of host species workers and are consistent with the acceptance threshold model’s (Reeve 1989) prediction that recognition systems are not fixed but context-dependent. In particular, host workers from the unparasitized area were highly aggressive towards the parasite regardless of the season, whereas host workers from the parasitized area significantly increased their aggression towards the parasite during its raiding and colony-founding season. Being able to detect and possibly kill a *Polyergus* scout searching for host nests can be an effective strategy for a *Formica* colony to avoid being raided or usurped by a parasite queen.

### Introduction

In social insects, nestmates are characterized by a distinct label (colony odour), determined by the interaction of genetic and environmental cues. Recognition occurs when the label of an incoming individual matches the internal template of a discriminating individual (see Crozier and Pamilo 1996; Lenoir et al. 1999). The occurrence of acceptance and rejection errors is a central problem in recognition processes. These errors are inevitable when desirable and undesirable individuals have overlapping cues. The acceptance threshold model (Reeve 1989) analyses the maximum tolerated amount of dissimilarity between template and label and predicts that the action component of a recognition system should not be fixed but should be context-dependent (see Sherman et al. 1997). The threshold is expected to vary with the cost of accepting an undesirable individual, the benefit of accepting a desirable individual, and the frequencies with which these individuals are encountered.

Despite its evolutionary significance, such a flexibility of response thresholds has rarely been investigated in insects. Downs and Ratnieks (2000) showed the occurrence of an adaptive shift in the acceptance threshold of honeybee guards in a fluctuating environment. The acceptance threshold of guards became more permissive as nectar conditions improved, providing strong support for Reeve’s model. Similarly, hosts of brood parasitic cuckoos showed an adaptive behavioural flexibility depending on variations in parasite pressure over a period of 10 years (Brooke et al. 1998) and their rejection rates of non-mimetic eggs decreased with a seasonal decline in parasitism (Davies et al. 1996; Brooke et al. 1998).

The aim of the present study was to investigate the seasonal dynamics of the action component of recognition in another host parasite system: the slave-making ant *Polyergus rufescens* and its host *Formica rufibarbis*. *Polyergus* relies on workers of the genus *Formica* for foraging and colony maintenance. The slave-makers periodically conduct group raids to pillage the brood of neighbouring host colonies. This brood will eventually

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eclose in the parasitic nest and serve as a workforce (see Hölldobler and Wilson 1990).

Newly mated *P. rufescens* queens are not capable of independent foundation but will usurp a host nest and kill the resident queen to successfully found a new colony (Mori et al. 1995; D'Ettorre et al. 1997).

We compared, over a period of 6 months, the level of aggression towards the parasite by host species workers from one parasitized and one unparasitized area. According to the acceptance threshold model, we expected that the aggression level of free-living *F. rufibarbis* workers from a parasitized area would rise during the parasite's peak in activity (raiding and colony-founding season), whereas the aggression level of *F. rufibarbis* workers from an unparasitized area would not show substantial changes connected with the parasite's activity.

## Materials and methods

Workers from free-living *F. rufibarbis* colonies, one from a parasitized area (University Campus, Würzburg, Germany) and one from an unparasitized area (University Campus, Regensburg, Germany), were compared in their level of aggression against the slave-maker and alien conspecifics (control).

In Würzburg, workers from a free-living colony of *F. rufibarbis* (F1P), located at about 10 m from a *P. rufescens* colony (P1), were used as test individuals both for encounters with P1 and for encounters with *F. rufibarbis* workers from F2P, another free-living host colony located approximately 50 m from F1P. Presumably, F1P had already had contact with the parasite during the previous raiding seasons. The study area was monitored weekly to assess the level of activity of *P. rufescens*.

Workers from the test *F. rufibarbis* colony (F1U) in Regensburg were similarly confronted both with parasitic workers (P1) and workers from another *F. rufibarbis* colony (F2U), located approximately 80 m apart. We can safely assume that the F1U workers did not have contact with the parasite during the previous raiding seasons, as *P. rufescens* does not occur in this area.

Workers of *F. rufibarbis* were tested in dyadic encounters over a period of 6 months (11 April to 8 October). We carried out 13 sessions of hetero- and homospecific encounters both in Würzburg and in Regensburg. Each type of encounter was usually replicated 10 times for each session (the exact sample size is indicated in Fig. 1). The total number of encounters was 502, of which 252 were in Würzburg and 250 in Regensburg.

Tests were carried out during sunny days, in the field, soon after collecting the tested ants at their nest entrance. In this way, the climatic conditions were natural and the manipulation minimal. *P. rufescens* workers were collected from their colony at the beginning of each experimental session and kept in a plastic box with about 50 slaves and nest material until the experiment started. P1 workers had therefore experienced the same artificial environment before the experiment regardless of whether they were confronted with workers from the neighbouring host colony F1P or the 200 km distant colony F1U. The tested *F. rufibarbis* worker and the opponent were placed into a neutral arena (8 cm diameter plastic box, lined with a filter paper changed after each test) and allowed to acclimatize for 5 min by isolating them in glass tubes. The trial started by removing the tubes so that the ants could make contact and lasted for 3 min. The behaviour of the tested ant (*F. rufibarbis*, F1P or F1U) was recorded continuously with an observation transcription tool (EthoLog).

Aggression was scored using the following index: 0 = inspection and antennal contact; 1 = threat displays (mandibular opening); 2 = biting; 3 = biting with gaster flexing (with spray of formic

acid). The overall aggression exhibited in each encounter was calculated using the following formula (Errard and Hefetz 1997):

$$AI = \frac{\sum_{i=1}^n AI_i * t_i}{T}$$

where  $AI_i$  and  $t_i$  are the aggression index and the duration of each act respectively, and  $T$  is the total interaction time (sum of times in which ants were in physical contact). An individual ant was never used in more than one encounter.

## Statistical analysis

Variation in the level of aggression of the host (*F. rufibarbis*, F1P and F1U) towards the social parasite (*P. rufescens*, P1) in and out of the raiding season (end of June – end of August vs. April – mid-June and beginning of October), in the two areas (Regensburg and Würzburg) and for the different dates (Table 1) were compared using a generalized linear model (GLM; Neter et al. 1996). Variations in the amount of aggression between homospecific alien workers (different colonies of *F. rufibarbis*) were analysed in the same way. Statistical analyses were carried out using Statsoft Statistica 6.0.

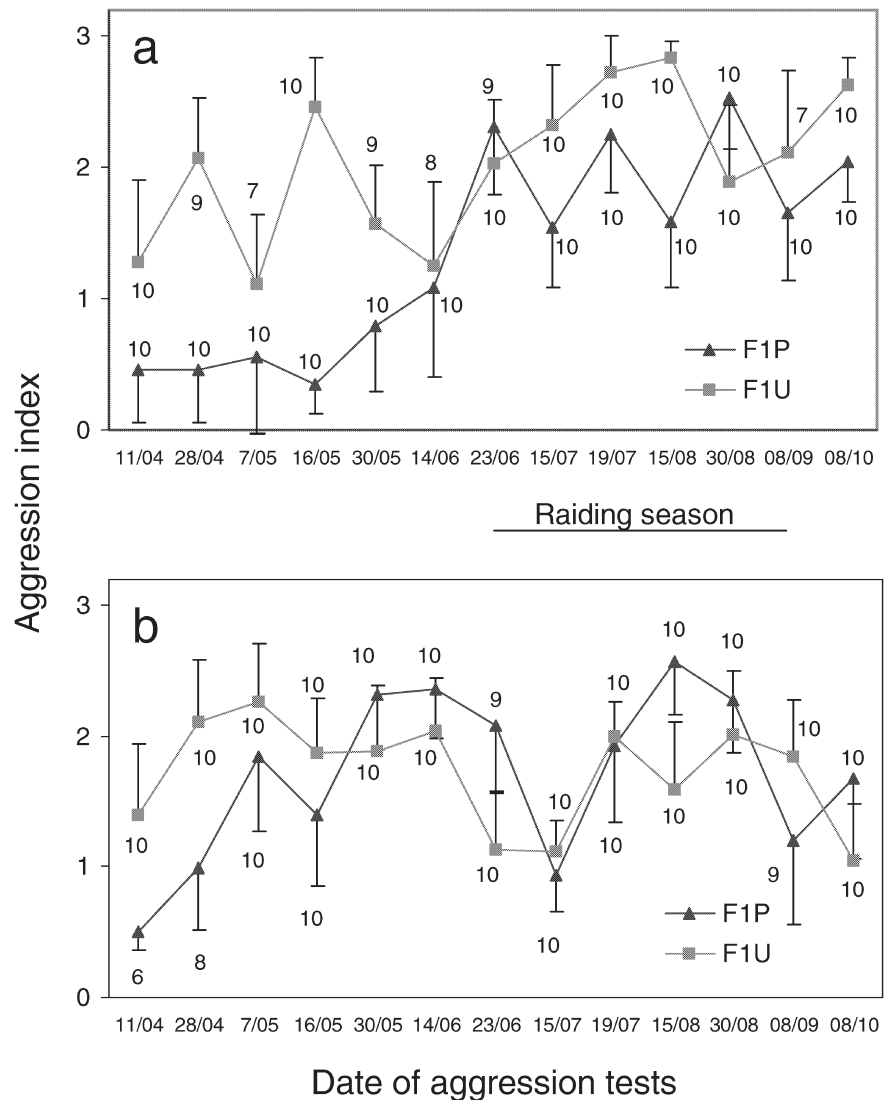
## Results

In Würzburg, the first parasitic workers were found to be active at the nest entrance in early June, but the typical scouting and raiding activity started only during the second half of June and continued until September. In this period, the *P. rufescens* study colony (P1) was repeatedly observed conducting successful slave-raids.

Aggression between F1P and P1 workers was very low from April to mid-June, as shown by an aggression index (AI) close to zero for the first six series of encounters (11 April–14 June, Fig. 1a). In these encounters before the raiding season, F1P workers did not overtly attack P1 workers, but only showed antennation, threat displays, and rarely biting. In contrast, from the beginning of the raiding season onwards, F1P workers became very aggressive during the encounters with the parasite (from 23 June, Fig. 1a), with frequent threat displays, biting and gaster flexing, during which formic acid was sprayed at the opponent. In contrast, in the unparasitized area (Regensburg), *F. rufibarbis* workers (F1U) were overtly aggressive in their encounters with P1 throughout the 6-month study (Fig. 1a). In both study areas the level of aggression varied among the different months but the increase in aggression in the raiding season was significantly stronger for F1P workers than for F1U workers (GLM, Table 1a).

The dynamics of the aggressive encounters between free-living *F. rufibarbis* colonies in Würzburg and Regensburg is depicted in Fig. 1b. Both F1P and F1U workers were almost always aggressive towards homospecific alien workers and there was no specific pattern connected with the progress of season (Table 1b).

**Fig. 1** Variation in the aggressive response shown by *Formica rufibarbis* workers during the study. The aggression index (mean and standard error) of workers from the parasitized (F1P) and the unparasitized area (F1U) is compared (the sample size is indicated for each series of encounters): **a** heterospecific host–social parasite interactions (*F. rufibarbis* – *P. rufescens*); **b** homospecific host–host interactions (*F. rufibarbis* – *F. rufibarbis*)



**Table 1** Variation in the level of aggression across the season for heterospecific host–social parasite and homospecific host–host interactions in the host–parasite system *Polyergus rufescens* and *Formica rufibarbis*. Generalized linear model, type III decomposition

Effect	Effect	Heterospecific interactions			Homospecific interactions		
		df	F	P	df	F	P
Intercept	Fixed	1	218.58	<0.001	1	212.02	<0.001
Population <sup>a</sup>	Fixed	1	24.17	<0.001	1	0.40	0.527
Season <sup>b</sup>	Fixed	1	11.42	0.006	1	0.09	0.768
Population×season	Random	1	5.53	0.019	1	6.49	0.011
Date (season)		11	3.67	<0.001	11	3.77	<0.001

<sup>a</sup> Parasitized or unparasitized population

<sup>b</sup> In raiding season or not

## Discussion

We investigated the seasonal dynamics of aggressive interactions in a host–social parasite system: the slave-making ant *P. rufescens* and its host *F. rufibarbis*. According to the acceptance threshold model (Reeve 1989), we expected a behavioural flexibility in the aggression level of the host towards the parasite, expressed as a seasonal change in the acceptance threshold of the host linked to the parasite's peak in activity (raiding and col-

ony-founding season). The results of the present study, although based on only a single colony of the rare and threatened slave-maker *P. rufescens*, show that the aggressiveness of free-living *F. rufibarbis* workers from a parasitized area (Würzburg) against the slave-maker rose during this period, whereas the aggression-level of *F. rufibarbis* from an unparasitized area (Regensburg) did not show substantial changes connected with the parasite's peak in activity. Such a seasonal variation was not found when the same host, from the parasitized area, was

confronted with alien homospecifics: *F. rufibarbis* was almost always aggressive against homospecific workers from an alien potential competitor colony. This also holds for *F. rufibarbis* from the unparasitized area. Host workers from different areas did not differ in their aggression level towards homospecifics. Interestingly, workers from the unparasitized area were almost always aggressive towards the unfamiliar parasite. This could either be the result of an arms race won in the past by the now unparasitized population against the parasite (Dawkins and Krebs 1979; Foitzik et al. 2003), or simply a strong reaction to an unknown potential enemy. Our data do not allow discrimination between these two hypotheses since there is no reliable information on the presence of *P. rufescens* in Regensburg in the past (J. Heinze, personal communication).

Slave-making workers are active outside the nest only in summer. During the early afternoon of sunny days between end of June and mid-September, *P. rufescens* scouts start searching individually for potential host nests and eventually lead a raiding swarm of hundreds of workers to the target nest (e.g. Le Moli et al. 1994). In a parasitized area, the frequency of encounters between *F. rufibarbis* workers and slave-makers increases during the raiding season due to the high number of scouts searching for target colonies and the occurrence of slave-raids. Thus, the cost for a potential host of not attacking *P. rufescens* workers increases as well. The increase in these two variables might produce a shift in the acceptance threshold of the host, which becomes less permissive (see Reeve 1989). Being able to detect and possibly kill *Polyergus* scouts can be an effective strategy for a *Formica* colony to prevent slave-raids. This preserves the host brood and, at the same time, helps to prevent the founding of a parasitic colony by *P. rufescens* queens, which usually follows the raids to find host nests to usurp (Mori et al. 1994).

Acceptance of social parasites could be the result of an adaptive cost-benefit balance of establishing defences: acceptance errors should persist if the costs of correct discrimination exceed the benefits (Zahavi 1977; Lotem 1993). The cuticular chemical profile of *P. rufescens* is very similar to that of the host species (D'Ettorre et al. 2002). Having a strict acceptance threshold is costly for the host and could produce recognition errors, in which nestmates would be rejected (see Sherman et al. 1997). It is unlikely that free-living *F. rufibarbis* workers from the parasitized area would encounter the parasite outside the raiding and colony-founding season. An adaptive behavioural flexibility, as suggested by the present study, would allow a more permissive acceptance threshold during the rest of the year, which probably decreases rejection errors.

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