Coevolution in host–parasite systems: behavioural strategies of slave-making ants and their hosts

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Recently, avian brood parasites and their hosts have emerged as model systems for the study of host–parasite coevolution. However, empirical studies of the highly analogous social parasites, which use the workers of another eusocial species to raise their own young, have never explicitly examined the dynamics of these systems from a coevolutionary perspective. Here, we demonstrate interpopulational variation in behavioural interactions between a socially parasitic slave-maker ant and its host that is consistent with the expectations of host–parasite coevolution. Parasite pressure, as inferred by the size, abundance and raiding frequency of *Protomognathus americanus* colonies, was highest in a New York population of the host *Leptothorax longispinosus* and lowest in a West Virginia population. As host–parasite coevolutionary theory would predict, we found that the slave-makers and the hosts from New York were more effective at raiding and defending against raiders, respectively, than were conspecifics from the West Virginia population. Some of these variations in efficacy were brought about by apparently simple shifts in behaviour. These results demonstrate that defence mechanisms against social parasites can evolve, and they give the first indications of the existence of a coevolutionary arms race between a social parasite and its host.

Keywords: host–parasite system; coevolution; slave-making ants; social parasites; behavioural defences

1. INTRODUCTION

Coevolution between parasites and hosts has been studied in many biological systems (Allison 1982; Anderson & May 1982; Thompson & Burdon 1992; Gandon *et al.* 1996; Schmid-Hempel 1998), and the arms-race paradigm has proven especially appropriate. Recent work shows that coevolution can also occur between social parasites and their hosts, as exemplified by the charismatic avian brood parasites (e.g. cuckoos and cowbirds; Soler & Møller 1990; Lotem *et al.* 1992; Hill & Sealy 1994; Lotem & Rothstein 1995; Brooke *et al.* 1998; Rothstein & Robinson 1998; Davies 2000). Because avian brood parasites have generation times and population sizes more similar to their hosts than do most ecto- or endoparasites, a stepwise coevolution model fits these host–parasite systems particularly well.

By contrast, coevolutionary studies of the highly analogous insect social parasites and their hosts are conspicuously few (but see Davies *et al.* 1989). Many species of ants, bees and wasps exploit the social systems of other species to raise their own young. Like the avian brood parasites, they have similar generation times and population sizes to their hosts and so fit the model of stepwise coevolution (D'Ettorre & Heinze 2001). Yet, the prevailing view is that hymenopteran social parasites have won the race (Gladstone 1981; Grasso *et al.* 1992). Like cuckoo chicks being fed by foster parents half the chick's size, the apparent complicity of *Leptothorax* ant workers while an *Epimyrma* queen intent on regicide clamps its mandibles around the neck of the workers' mother (Hölldobler & Wilson 1990), suggests that hosts have not evolved to resist their parasites. We now know that avian hosts can evolve cryptic defences against

parasitism (i.e. egg rejection or nest desertion; Lotem *et al.* 1995; Davies *et al.* 1996; Davies 2000). Thus, interactions between insect social parasites and their hosts also deserve to be examined for evidence of ongoing coevolution (Davies *et al.* 1989). Here, we report the first empirical study to do so.

Social parasitism among the Hymenoptera is known for over 150 species (Wilson 1971; Hölldobler & Wilson 1990) divided into two life-history classes. Inquilines are social endoparasites, living inside a host colony without imposing any mortality on adult queens or workers. By contrast, slave-makers, known only in ants, are social exoparasites, living in their own nest but periodically conducting 'slave raids' on nearby host colonies, stealing their larvae and pupae. These raids decimate reproduction and growth in multiple host colonies in a single year (Cool-Kwait & Topoff 1984; Mori *et al.* 1991; Hasegawa & Yamaguchi 1994; Foitzik & Herbers 2001a) and can also lead to the genetic death of the host colonies if the host queen(s) or too many workers are killed (Alloway 1979, 1990; Foitzik & Herbers 2001a). Because selection on hosts can be so strong, we expect to see evidence of hosts evolving resistance to slave-makers.

We used behavioural tests to look for signs of a coevolutionary arms race between the slave-maker *Protomognathus americanus* and its host *Leptothorax longispinosus*. We focused on behavioural adaptations that might have occurred in the context of slave raids, because these have a much greater impact on host-colony fitness than do other slave-maker behaviours (Foitzik & Herbers 2001a). Variation in host-to-parasite ratios across three field sites (West Virginia, New York and Vermont; Herbers & Foitzik 2001) helped us to explore the coevolutionary potential of this system.

We envisioned that coevolution might be either universal or localized. Universal coevolution posits a finite number of solutions that result in effective host or parasite strategies. Thus, all host and parasite populations should

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evolve universal adaptations and counter-adaptations. In any given locale, host–parasite pairs will be engaged in the same arms race as in other locales, but sympatric pairs may evolve at different rates. Across locales, then, we expect to see parasites and hosts engaged at different levels of ‘mobilization’ (in the arms race). The universal-coevolution hypothesis predicts that the effectivenesses of parasites and hosts in sympatry will be correlated. Thus, if parasites from site A are the best raiders then their hosts from the same site will have the best defences against such raids. This hypothesis predicts that parasite and host effectivenesses will be correlated with historical parasite pressure; where the pressure has been stronger, parasite and host will both be more highly mobilized. These parasites and hosts will employ strategies effective against all populations, while parasites and hosts originating from areas where hosts are exploited less severely will perform less effectively.

By contrast, localized coevolution proposes that host and parasite evolve unique strategies within each locale. Each sympatric-population pair will therefore be engaged in a different arms race from every other pair. This hypothesis predicts geographical variation in host and parasite strategies, with little or no overlap between sites. Adaptations of host and parasite might be effective locally, but the hypothesis makes no predictions about the effectiveness of any strategy when allopatric hosts and parasites confront one another. One may, in fact, find a rock–paper–scissors relationship of strategies, with no single strategy obviously superior to the others. Thus, this hypothesis predicts a strong interaction between the effectiveness of a particular strategy and the origin of the population against which that strategy is challenged. Local coevolution does not predict that the hosts and parasites with the most effective strategies will come from the same site, nor does it predict a relationship between parasite or host effectiveness and the historical parasite selection pressure. Our two coevolutionary models may not be mutually exclusive; some strategies might conform to the universal-coevolution model, whereas others might conform to the localized coevolution model.

Our experimental design had five goals: first, to identify behaviours of parasites that increase their raiding efficiency; second, to identify defence mechanisms in host populations; third, to measure geographical variation in the behaviour of either parasites or hosts; fourth, to measure geographical variation in the selection pressure exerted by the social parasite; and fifth, to synthesize the above data in order to test our hypotheses of coevolution. To test for these coevolutionary patterns, we observed the outcome of slave raids in the laboratory between slave-makers and free-living hosts from three different populations of host–parasite pairs that differ from one another in both present and historical parasite selection pressure.

2. MATERIAL AND METHODS

(a) *Study system and field collections*

P. americanus is a widespread slave-maker of the temperate deciduous forests of north-eastern North America, which parasitizes host colonies of three closely related species in the genus *Leptothorax*: *L. longispinosus*, *L. ambiguus* and *L. curvispinosus*. Slave-maker and host colonies are found in leaf litter, inhabiting

cavities in sticks, acorns and nuts. Although individual colonies of both species may occupy more than one nest-site, *P. americanus* colony subunits conduct raids autonomously (Foitzik & Herbers 2001a), so for simplicity we refer to these colony subunits as individual colonies throughout this paper.

We collected colonies of *P. americanus* and its major host species *L. longispinosus* (Herbers & Foitzik 2001) in June 1999 from three different sites. The West Virginia population was located in Watoga State Park (Pocahontas County, WV), the New York population was from the Huyck Preserve (Albany County, NY) and the Vermont population was from Mallett's Bay State Park (Chittenden County, VT). We collected colonies by examining likely colony sites in each location. In the West Virginia and New York sites we stopped collecting host colonies when we had approximately 200; host to parasite ratios are based on data taken to that point, although we collected additional slave-maker colonies when possible.

We transported the ants to the laboratory in Colorado, censused them and allowed them to move into artificial colony sites inside small boxes (10 cm × 10 cm × 1.5 cm) with a moistened plaster floor (Buschinger 1974; Heinze & Ortius 1991). We kept these in an incubator at 20 °C. The ant colonies were fed an artificial diet (Bhatkar & Whitcomb 1970) supplemented with frozen fruitflies twice weekly. As soon as slave-maker workers eclosed from the pupae (around the end of July 1999), we started behavioural trials by moving colonies into the arenas described in § 2(c).

(b) *Genetic analyses*

We analysed the nuclear and mitochondrial relatednesses of enslaved *L. longispinosus* workers in *P. americanus* colonies in order to obtain a surrogate measure of the raiding frequency of slave-maker colonies. The relatedness of the slaves is negatively correlated with the number of different host colonies raided by the slave-maker colony (see Foitzik & Herbers 2001a). To obtain an exact measurement of the raiding frequency we determined the relatedness of worker pupae in free-living host colonies in the vicinity of slave-making colonies. These pupae comprise the slave source for neighbouring social parasitic colonies, and their relatedness divided by the relatedness of enslaved adult workers from a slave-maker colony equals the number of free-living host colonies raided by the slave-maker colony within the last one to two years. This assumes that each host colony raided is equally represented in the adult workers of the slave-maker colony, and that raided host colonies are unrelated to one another. Both these assumptions are conservative; if they are violated we will obtain a lower estimate of raiding frequency than has actually occurred. To estimate relatedness we determined the genotypes of six to eight slaves from between 6 and 18 slave-maker colonies per population at two highly variable microsatellite loci, and we used restriction fragment analysis to determine the mitochondrial haplotypes of the same ants. The complete protocols for DNA extraction, microsatellite and mitochondrial-haplotype analysis can be found in Foitzik & Herbers (2001a,b). We used the program RFLAT 4.2 (Queller & Goodnight 1989) to estimate relatedness coefficients for each class of markers.

(c) *Behavioural observations during raiding trials*

We observed raids in the laboratory using a cross-fostering design. Thus, we induced raids between slave-maker and host colonies both from the same population (i.e. New York versus New York) and from different populations (i.e. New York versus West Virginia and New York versus Vermont). We collected

sufficient numbers of slave-makers only from New York and West Virginia, and each of these was tested against host colonies from all three sites. Thus, we assayed raiding behaviour and defensive behaviour during six types of raids. The inclusion of host colonies from Vermont was critical because it gave us greater power to differentiate between universal and localized coevolutionary patterns.

Both hosts and parasites exhibit different demography (queen numbers, worker numbers and slave numbers) across our three sites (Herbers & Stuart 1998; Herbers & Foitzik 2001), which could confound analysis of raiding behaviour. We therefore selected from each population a series of experimental colonies that exhibited similar distributions of demographic profiles. Thus, the host colonies chosen for this experiment showed no significant between-population variation in queen numbers, worker numbers or the numbers of worker pupae, and slave-maker colonies showed no significant between-population variation in queen numbers or worker numbers (Mann–Whitney *U*-tests or Kruskal–Wallis ANOVA, $p > 0.20$). We were, however, unable to equilibrate slave numbers between populations, as these were smaller in the West Virginia slave-maker colonies (Mann–Whitney *U*-test, $p < 0.005$).

We observed raids in arenas of $0.45\text{ m} \times 0.90\text{ m} \times 0.15\text{ m}$ containing a moistened plaster floor to regulate humidity. We thoroughly cleaned each arena between trials to ensure that residual odours did not influence behaviour in subsequent experiments. Slave-maker colonies were positioned in the arena 12 h prior to the onset of the experiment to allow foragers to acclimatize to the arena. The trial started when we placed a host colony into the arena at a distance of 0.80 m from the slave-maker colony. We provided a second, empty, nest site in the arena to serve as a potential escape location for the host colony. We video-taped or directly observed the interactions within and immediately outside (within 3 cm) the host nest. We terminated each trial 24 h after all the host brood were captured or had been evacuated by their nest-mates. We then censused all ants in each of the three nest sites. We also recorded the numbers of injured and dead individuals of each species and caste.

During raids we recorded the frequency of certain behaviours, including attacks initiated by slave-maker workers, attacks initiated by host workers, placement of guards in the host-nest entrance by either species and the numbers of slave-maker and slave workers recruited on raids. We also recorded the time intervals between the discovery of the host colony by a slave-maker scout and subsequent benchmarks of the raid. These subsequent events included entry into the host nest by slave-makers, escape of the host queen and the removal of the first and the last piece of brood from the nest by host workers.

3. RESULTS

(a) *Estimation of parasite pressure in field populations*

We assessed parasite pressure for each site by the relative abundance, size and raiding frequency of slave-maker colonies. Data addressing the first two measures are presented in detail elsewhere (Herbers & Foitzik 2001); we summarize them here. These combined multi-year data show that West Virginia had the highest (1:7, $n = 362$), New York an intermediate (1:10, $n = 1789$) and Vermont the lowest (1:18, $n = 1200$) parasite-to-host ratio. The demographic data, however, suggest that slave-maker colonies in New York and Vermont have impacts

that are proportionally greater than their relative abundances would suggest. Colonies from New York and Vermont had both significantly more slave-maker workers (35% more) and significantly more slaves (about twice as many) than did those in West Virginia (Herbers & Foitzik 2001). Because colonies with larger slave populations have raided more host colonies (as evidenced by their greater genetic diversity; see Foitzik & Herbers 2001a), we infer that the per-colony impact of slave-makers in New York and Vermont is substantially higher than in West Virginia.

Lastly, we used relatedness estimates to infer the raiding frequencies of parasites from all three sites (Foitzik & Herbers 2001a). The relatedness of slaves in New York was significantly lower than in West Virginia (table 1, Student's *t*-tests based on nuclear and mitochondrial DNA markers, $p < 0.05$), indicating that New York slave-maker colonies raided more host colonies than did slave-makers from West Virginia (table 1). Relatedness of slaves in Vermont slave-maker colonies did not differ significantly from either New York or West Virginia, but more closely resembled the situation in West Virginia. Importantly, we deliberately chose for this analysis *P. americanus* colonies that were similar in size (slave-maker and slave worker numbers) across the populations (Kruskal–Wallis tests, $p > 0.20$), in order to control for the effect of colony size on raiding frequency. Comparison of slave relatedness to relatedness in free-living host colonies allowed us to estimate that New York slave-maker colonies contain the remnants of between 5 and 11 raids (the former estimate comes from the nuclear markers, while the latter is from the mitochondrial markers), whereas their similarly sized counterparts from Vermont and West Virginia contain the remnants of only about two or 2.5 raids (see also Foitzik & Herbers 2001a).

(b) *Behavioural strategies of slave-maker colonies*

The results of the behavioural trials revealed that neither the behaviours exhibited by the ants nor the outcome of the raids differed when the host and the slave-maker came from the same or from different populations. For example, New York slave-makers showed the same behaviours and took the same numbers of pupae whether they raided hosts from New York, West Virginia or Vermont. Similarly, Vermont hosts showed the same behaviours regardless of whether they were raided by slave-makers from New York or West Virginia (table 2, Mann–Whitney *U*-tests, Kruskal–Wallis ANOVAs, $p > 0.20$). The only exception was in the numbers of slave-makers involved in the raids: the significant pattern resulted from more slave-makers being recruited by West Virginia social parasites when raiding allopatric host colonies (Mann–Whitney *U*-test, $p < 0.03$). For simplicity we show only the pooled 'sympatric-population trials' against the pooled 'allopatric-population trials'. Except where noted, we pooled data over the population origin of the host colony for all subsequent analyses in order to increase our statistical power while we analysed the behaviour of the slave-makers, and we similarly pooled data over the population origin of the slave-maker to analyse the behaviour of the hosts.

We found striking differences between slave-makers from New York and West Virginia in their effectiveness

Table 1. *Relatedness (r) of Leptothorax longispinosus slaves in Protomognathus americanus colonies from three different populations using microsatellite and mitochondrial markers*

(The number of host colonies raided shows the range of estimates from both sets of markers. We assume that free-living colonies have a mitochondrial *r* of 1.0 since these colonies have either one queen or a few related queens (Herbers & Stuart 1996).)

	number of colonies	microsatellite <i>r</i>	free-living worker <i>r</i>	mitochondrial DNA <i>r</i>	number of host colonies raided per year
West Virginia	13	0.29 ± 0.06	0.72 ^a	0.51 ± 0.11	2–2.5
New York	18	0.15 ± 0.04	0.76 ^b	0.09 ± 0.05	5–11
Vermont	6	0.28 ± 0.08	0.50 ^a	0.45 ± 0.14	1.8–2.2

^aBased on adult workers in summer (Herbers & Stuart 1996).

^bBased on adult worker pupae in summer from plots also containing slave-makers (Foitzik & Herbers 2001a).

Table 2. *The outcomes of raids between sympatric and allopatric populations of slave-makers and hosts, and the outcomes of raids by slave-makers from West Virginia and New York against hosts from all populations*

(Outcomes that were significantly different from one another are denoted with asterisks (see §3(b) for details). Values are given as mean ± s.e.m. where appropriate.)

	localized coevolution		site-specific slave-maker strategy	
	sympatric slave-maker and host	allopatric slave-maker and host	slave makers from West Virginia	slave-makers from New York
percentage of brood saved	21.6 ± 7.0	19.5 ± 4.4	25.7 ± 7.2*	18.0 ± 4.4*
queen saved	7/11	21/39	9/11*	15/38*
percentage of slave-makers killed	18.8 ± 4.9	19.3 ± 2.8	17.8 ± 4.0	20.0 ± 3.1
percentage of host workers killed	20.1 ± 3.4	21.3 ± 2.0	14.3 ± 2.5*	23.6 ± 2.6*
slave-makers attacked by host	9/14	21/25	10/12	19/26
guard placed by slave-maker	10/12	16/23	5/12*	20/22*
number of slave-makers involved	2.83 ± 0.32*	3.73 ± 0.25*	3.18 ± 0.35	3.69 ± 0.26

during raids. The percentage of brood that host workers were able to remove to a safe location was consistently higher when their natal colony was attacked by slave-makers from West Virginia (table 2, Mann–Whitney *U*-test, $p < 0.04$). Similarly, host queens were significantly more likely to escape from their raided colony when attacked by slave-makers from West Virginia (table 2, χ^2 -test, $p < 0.015$). Thus, slave-makers from New York were more destructive raiders than slave-makers from the West Virginian population.

The speed with which host workers were able to respond to raiding slave-makers also differed depending on the population origin of the slave-makers. The time interval between the entry of the first slave-maker into the nest and the rescue of the first piece of brood by a host worker was shorter when a slave-maker colony from West Virginia was raiding (West Virginia, mean ± s.e.m. = 3.2 ± 0.9 min; New York, mean ± s.e.m. = 27.3 ± 0.9 min, Mann–Whitney *U*-test, $p < 0.05$). The host queen also escaped from the nest site more quickly when the colony was under siege from slave-makers from West Virginia (West Virginia, mean ± s.e.m. = 12.3 ± 9.6 min; New York, mean ± s.e.m. = 48.9 ± 18.3 min, Mann–Whitney *U*-test, $p = 0.05$). Behavioural observations suggest a simple mechanism underlying these differences: slave-maker colonies from New York nearly always positioned a guard ant in the host-nest entrance during a raid, while those from West Virginia placed a guard less than half the time

(table 2, χ^2 -test, $p < 0.002$). This guard ant physically deterred host workers from leaving with brood and sometimes prohibited the escape of the host queen. This difference in the frequency of guard placement did not arise simply from a difference between slave-maker populations in the number of slave-maker workers recruited during raids (Mann–Whitney *U*-test, $p = 0.39$). Rather, the frequency of guard placement reflected an intrinsic difference between the slave-maker populations.

Although we found no between-population differences in the numbers of slave-makers recruited during raids (see above), some within-population differences were apparent. The number of slave-makers recruited was positively correlated with the size of the host colony (Spearman's rank correlation, $r_s = 0.42$, $p < 0.02$), and slave-makers from West Virginia attacking host colonies from West Virginia recruited significantly fewer slave-makers than when they attacked similarly sized hosts from New York or Vermont (Mann–Whitney *U*-test, $p < 0.035$).

We also found that the combined proportion of free-living and enslaved *L. longispinosus* workers that were killed during raids was higher when slave-makers from New York were raiding (table 2, Mann–Whitney *U*-test, $p < 0.02$). Importantly, behavioural observations indicated that slave-maker workers rarely fought with the free-living host workers, whereas enslaved host workers,

Table 3. Influence of aggressiveness of population origin on host colonies

(*Leptothorax longispinosus* workers from Vermont and New York populations attacked slave-makers during raids more frequently. As a result of this aggressive behaviour a higher proportion of *Protomognathus americanus* workers were killed or heavily injured during raids involving host colonies from Vermont and New York (values are given as mean $\pm$ s.e.m.).)

	West Virginia host	New York host	Vermont host
slave-makers attacked by host	5/11	12/15	13/13
percentage of slave-makers killed	12.7 $\pm$ 3.4	18.6 $\pm$ 4.7	26.3 $\pm$ 4.4

which accompanied slave-makers on raids, often fought with conspecifics. Thus, the increased mortality evident in table 2 resulted from aggression between free-living and enslaved hosts. The fraction of *L. longispinosus* workers killed was correlated with the number of free-living host workers (Spearman's rank correlation, $r_s = 0.45$, $p = 0.00002$) but not with the number of slaves in the raiding colony (Spearman's rank correlation: $r_s = 0.03$, $p = 0.74$). We also found no interpopulation differences in the frequency of slaves that accompanied slave-makers on raids (Mann–Whitney *U*-test, $p = 0.21$). Therefore, the higher death toll from New York slave-maker raids did not reflect the higher number of slaves in these experimental colonies.

(c) Behaviour of host colonies

Host colonies from our three sites differed significantly in their responses to raids. Host workers from West Virginia were much less likely to attack and bite slave-maker workers on raids or scouting missions (table 3, Kruskal–Wallis ANOVA, $p < 0.002$). Furthermore, host colonies from West Virginia killed or injured fewer slave-maker workers during raids than did host workers from New York and Vermont (table 3, Kruskal–Wallis ANOVA, $p < 0.03$).

When we considered all raids together, the percentage of *P. americanus* workers injured or killed during a raid was correlated with the proportion of brood that a host colony was able to save (figure 1, Spearman's rank correlation, $r_s = 0.26$, $p < 0.02$). We also found, across all populations, that the percentage of *P. americanus* workers that were injured or killed by host workers during a raid was positively correlated with the likelihood that the host queen survived that raid (Mann–Whitney *U*-test, $p < 0.03$).

4. DISCUSSION

Our data are consistent with the existence of a coevolutionary arms race between the social parasite *P. americanus* and its host, *L. longispinosus* (summarized in table 4). Our results reveal geographical variation both in the behaviours of the social parasite and in the outcome of raids against its host: slave-makers from New York were much more likely than those from West Virginia to guard the

Figure 1. The percentage of slave-makers that were killed or injured during raids was positively correlated with the percentage of brood that *Leptothorax longispinosus* host workers were able to save.

host nest's entrance during raids, and raids conducted by New York parasites captured more of the brood from the host nests than raids by West Virginia parasites. Our data also show geographical variation in host effectiveness in dealing with the slave-makers: colonies of free-living hosts from New York and Vermont exhibited greater aggression against parasite scouts and raiders than did hosts from West Virginia, and more aggressive colonies also saved more of their brood and more often saved the queen than did those colonies with less aggressive workers. This is not only the first documented evidence for the existence of host defences against a socially parasitic ant species but also these strategies showed geographical variation that was consistent with the expectations of the universal-coevolution model.

We did not observe unique behaviours in either parasites or hosts across different populations. All the between-population variation in effectiveness seemed to stem from changes in the frequency of a few key behaviours rather than from the evolution of novel strategies in each population. We suggest that variations in the frequency of guarding behaviour were responsible for most of the variation we observed in the outcomes of raids. Thus, during a raid, the slave-makers from New York would nearly always place a guard ant in the host colony's entrance; this guard physically prevented host ants from leaving the nest while other slave-makers carried host brood back to their own nest. This not only ensured that the slave-makers captured more of the host's brood but also appeared to lead to more destructive raids: hosts raided by New York slave-makers had more queens and adult workers killed during the raids. Ironically, observations indicate that these host ants are probably killed by enslaved conspecifics that accompany *P. americanus* workers on raids, rather than by the slave-makers themselves; the guard ant presumably causes more of these intraspecific fights by preventing the immediate departure of the free-living host workers and queens. Similarly, our observations indicate that variation between populations of host ants in their ability to counter the parasites did not depend on the evolution of unique strategies in different populations. Rather, we found that the variation in host effectiveness was correlated with the variation in aggression that host ants showed against scouting or

Table 4. *Estimates of components of parasite pressure for each population, and site-specific behavioural strategies of the slave-maker and the host*

(Dashes indicate behaviours that could not be tested.)

	West Virginia	New York	Vermont
parasite pressure			
slave-maker frequency	intermediate	high	low
slave-maker colony size	small	large	large
number of slave-makers	low	high	low
number of slaves	low	high	high
relatedness of slaves	high	low	high
raiding frequency per colony	low	high	low
parasite efficacy			
slave-maker guard in entrance	sometimes	always	—
escape of host queen	common	rare	—
brood saved by host colony	some	few	—
host workers killed	few	many	—
host defences			
slave-maker attacks	rare	common	common
slave-makers killed or injured	few	many	many

raiding *P. americanus* workers from any site. These patterns of variation in both parasite and host behaviours are more consistent with the universal than with the localized model of coevolution.

The relative ability of slave-maker populations to raid their hosts was correlated with the ability of their sympatric hosts to counter those raids. That is, one site (New York) had both the most effective slave-makers and the most effective hosts (table 4), which is also consistent with the expectations of the universal-coevolution model. We found moderate support for the third major prediction of the universal-coevolution model, that host and parasite effectiveness will be correlated with parasite selection pressure. The three correlates of parasite pressure (the relative abundance, size and raiding frequency of slave-maker colonies) allow us to obtain a rough overall measure of relative parasite pressure in these three populations. Historically, slave-makers in West Virginia were 40% more abundant than in New York relative to their hosts, although this has clearly changed in recent years. Slave-maker colonies in New York not only raided twice as often as similarly sized colonies from West Virginia, but also were, on average, twice as large and contained twice as many slaves. The combined effects of more frequent raiding and larger colony size in the New York slave-makers indicates that parasite pressure was substantially higher in New York than in West Virginia. This conclusion is further supported by the observation that the presence of slave-maker colonies had demonstrable effects on the demography of nearby host colonies in New York but not in West Virginia (Herbers & Foitzik 2001).

Why is the parasite pressure so much lower in West Virginia? This site showed two key aspects that differed strikingly from New York: the presence of a second host species (*L. curvispinosus*) at relatively high frequencies, and the low density of both host species (Herbers & Foitzik 2001). The presence of a second host may reduce the potential for a close evolutionary arms race between *P. americanus* and *L. longispinosus*. Additionally, the low host nest density might pose a problem for the slave-maker

species, because it has to search for host colonies over a much larger area. These lower host densities could select for *P. americanus* to be a 'prudent parasite' (Hare & Alloway 2001). This is analogous to the evolution of less virulent strains of diseases: less deadly forms of a disease should be more likely to evolve when the disease has a very low transmission rate. Similarly, when the effective number of hosts available to the slave-maker is low, this could select for less destructive slave-makers. Under this scenario, the lack of a guard in the nest entrance, rather than representing an inefficient slave-maker strategy, may be designed to facilitate the escape of the host queen and ensure the continued survival of the host colony. The observation that West Virginia slave-makers invest less in new workers and more in the winged sexuals compared to the New York population (Herbers & Stuart 1998) also suggests that West Virginia slave-makers may have evolved investment strategies, which are favoured by lower host densities. An interesting parallel to this hypothesis has been described in the socially parasitic ant genus *Epimyrma*. In several species of this genus the complete loss of the worker caste has been interpreted as an adaptation to low host density (Buschinger 1989). Strong inference testing is needed to determine whether West Virginia slave-makers have evolved to be more prudent parasites or simply have a less highly derived strategy than slave-makers from New York.

Slave-makers from Vermont were less abundant than those from either New York or West Virginia, raided less frequently than those from New York, and their colonies were about twice as large as those from West Virginia. Thus, parasite pressure was certainly lower in Vermont than in New York, but since we cannot accurately determine the combined effects of large colony size and relative scarcity in Vermont, we cannot unambiguously define the relative strengths of parasite pressures between Vermont and West Virginia. Our overall conclusion, however, that the effects of *P. americanus* on its host are greater in New York than in the other two sites, is mirrored by the results of a long-term ecological study on these three pairs of

populations (Herbers & Foitzik 2001). As expected under the universal-coevolution model, New York hosts and parasites were more effective than those from the West Virginia site. However, even though we estimated parasite pressure to be lower in Vermont than in New York, the *L. longispinosus* hosts from Vermont attacked and killed as many slave-makers as did hosts from New York.

Why are hosts as aggressive in Vermont as they are in New York, when we measure parasite pressure to be considerably higher in New York than in Vermont? An undescribed *Leptothorax* slave-maker species whose biology resembles quite closely that of its congener *Leptothorax duloticus* has also been found in this population (Herbers & Foitzik 2001). If *L. longispinosus* evolves general defences against all slave-maker species, the addition of a second parasite could mean that selection for defensive strategies in the host is stronger than the effects of *P. americanus* alone would indicate. Interpretation of the host's behaviour in Vermont, however, is also complicated by a recent shift in ecology. Herbers & Foitzik (2001) have shown that the host population there has steadily declined over the last 15 years, but that the slave-maker density has remained stable. Thus, the slave-makers in Vermont have become more abundant relative to their hosts in the recent past, and we cannot infer with any certainty what that ecological shift may mean for behavioural coevolution. A closer examination of all three populations in the future will provide us with a better understanding of the evolutionary dynamics of this host-parasite system. Data that would be of particular interest are the relative effectiveness of Vermont slave-makers compared to those from New York and West Virginia. The universal-coevolution model predicts that Vermont slave-makers would be closely matched in effectiveness to their sympatric hosts.

This work shows that experimental analysis can provide considerable insight into the adaptations of social parasites and their hosts. Unlike avian brood parasites and their hosts, slave-making ants can be easily studied in the laboratory with sufficiently large sample sizes to allow for rigorous data analysis. We have shown here that not only are there clear behaviours that both the parasite and the host have evolved but also that the expression of these behaviours varies significantly between populations in a manner that is largely consistent with predictions of coevolution. The fascinating life history of these insects can therefore provide considerable insight into fundamental questions in the field of host-parasite coevolution.

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