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ORIGINAL ARTICLE

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Do these eggs smell funny to you?: an experimental study of egg discrimination by hosts of the social parasite *Polyergus breviceps* (Hymenoptera: Formicidae)

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Abstract Social parasites exploit the behaviours of other social species. Infiltration of host systems involves a variety of mechanisms depending on the conditions within the host society and the needs of the social parasite. For many species of socially parasitic ants, colony establishment entails the usurpation of colonies of other species. This frequently involves the eviction or death of the host colony queen and the subsequent adoption of the invading queen. The social parasite queen achieves host worker acceptance by either manipulating the nest-mate recognition processes of the host or undergoing chemical modification. Little is known, however, about how host workers respond to social parasite eggs or whether host species defend against brood parasitism during parasite invasions. Host species are believed to adopt social parasite offspring because the recent common ancestry between many social parasites and their hosts may grant the sharing of certain characteristics such as chemical cues. Use of multiple host species, however, suggests other processes are needed for the social bond between host and parasite young to form. This study reports the findings of adoption bioassays in which eggs from a slave-maker ant, *Polyergus breviceps*, were offered to workers of two of its host species from unparasitised or newly parasitised nests to determine whether *P. breviceps* eggs generally elicit rearing behaviours from multiple host species. Comparisons of parasite egg survival until adulthood with conspecific egg survival reveal that workers of both host species, free-living or newly enslaved, do not typically accept slave-maker eggs. Both host species thus have sufficient discriminatory power to reject social parasite eggs although our hydrocarbon analysis indicates parasite eggs may be adapted to their local host species. Combined these results suggest that host rearing of *P. breviceps* eggs may reflect an evolutionary equilibrium that is maintained by probability and cost of recognition errors.

Keywords Dependent colony founding · Colony integration · Local adaptation · Co-evolutionary processes · *Formica*

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Introduction

Social parasites, much like endo- and ecto-parasites, exert substantial pressure on their host, inevitably reducing the life-time reproductive output (e.g., Lotem et al. 1995) if not, in some cases, destroying it altogether (e.g., Foitzik and Herbers 2001). Rather than feeding directly on host organs, social parasites siphon energy by diverting behavioural attention and, thus, resources away from the host and towards themselves. This requires the development of appropriate social relations with another species. In the family of ants, approximately 150 species are social parasites of other ant species (Hölldobler and Wilson 1990), many of which share recent ancestry and, therefore, ecological, behavioural and morphological features with their host taxon [Emery's rule (Emery 1909); Bourke

and Franks 1991]. Closely related sympatric species with similar habits and ecology frequently compete heavily for shared resources, and antagonism between these species is typically high (Gause's law, Gause 1935). Social parasites, however, have evolved to circumvent this friction (on one level) and capitalise from the commonalties by exploiting typical nest-mate recognition systems of their host species. Some species of social parasites dupe their hosts by acquiring cuticular chemical cues important for nest-mate recognition (or queen identification) through biosynthesis (Bagnères et al. 1996) or sequestration (Vander Meer et al. 1989; Franks et al. 1990; Breed et al. 1992; Kaib et al.1993; Vienne et al. 1995; Johnson et al. 2001). Other species make use of the learning process and expose hosts to foreign (parasite) odours during critical periods (Goodloe and Topoff 1987). In either instance, the social parasite is able to secure a relationship with its host that appears to be unilaterally advantageous.

The full spectrum of social parasitism can be found in the ant subfamily Formicinae, with parasite relationships being facultative or obligatory, and temporary or permanent (see Hölldobler and Wilson 1990 for a review). Probably the most complex of these social parasites are the obligatory slave makers because an array of strategies is required to maintain a host species cohort at different stages of the life cycle (e.g., D'Ettorre et al. 2002) as well as to retain some sort of effective dispersal (see D'Ettorre and Heinze 2001 for a review). Slave supply replenishment involves raiding host nests, primarily for pupae and larvae. These individuals develop in the parasite nest and become full-functioning members, which provide all the foraging, cleaning and brood care services for their captors. Typically, social insect colonies maintain some semblance of genetic integrity through the expression and recognition of colony specific chemical cues [gestalt odour (Crozier and Dix 1979)]. Non-members are generally rejected based on their epicuticular chemicals that clash with the perceiver's nest-mate recognition template (Vander Meer and Morel 1998), which is shaped by odours experienced during early development (Jaisson and Fresneau 1978; Isingrini et al. 1985; Jaisson 1987). Capturing individuals at the immature stages and prior to the critical learning period guarantees that parasite odour as well as odour of other slaves will be incorporated into the nest-mate recognition template of the host species and, thus, the social parasite will be readily adopted (Goodloe and Topoff 1987). New gynes (young reproductive females) of some facultative slave makers and temporary social parasites also rely on nest-mate recognition development processes to take over nests of other species. Often causing adult inhabitants of an invaded nest to flee, the new queens procure the abandoned brood (Topoff et al. 1990).

Cuticular hydrocarbons are considered the primary mediators of nest-mate recognition (see Singer 1998 for a review; Lahav et al. 1999), as well as an array of other functions (e.g., Wagner et al. 1998; Peeters et al. 1999). New gynes of some obligatory slave maker species modify their cuticular hydrocarbon profiles apparently by acquiring chemicals from a host species queen. This transformation seems to allow them to be perceived as a host species queen to host workers and to effectively take over the nest (Franks et al. 1990; Errard and D'Ettorre 1998; D'Ettorre and Errard 1998; Johnson et al. 2001). It is yet unclear, however, what processes underlie the adoption of her offspring by naive host workers. Virtually nothing is known about initial host responses to social parasite eggs or whether host species can defend against brood parasites in ants. Many social parasites are often close phylogenetic relatives of their host species (Agosti 1994; Savolainen and Vepsäläinen 2003; Sumner et al. 2004). Consequently, the hosts are believed to adopt social parasite offspring because juvenile chemical cues may be the same or similar owing to this recent common ancestry [Emery's rule (Emery 1909); Alloway 1982; Zimmerli and Mori 1993]. Chemical signatures of pupae in a number of social parasites approximate those of host species pupae and are suggested to provoke tending behaviours in the host (e.g., Howard et al. 1990; Yamaoka 1990; Kaib et al. 1993; Akino et al. 1999; Elmes et al. 1999). During colony establishment, however, it is the egg stage that is critical for parasite integration, and behavioural and chemical studies on adoption of social parasite eggs are lacking. Considering the use of multiple host species by many obligate social parasites, further investigation seems warranted.

Many host species appear to lack defensive adaptations. Concomitant rarity of social parasites relative to the host has led to a long-held belief that there is insufficient selective pressure for host counter defence (Hölldobler and Wilson 1990). Recent work, however, has shown that slave raids by leptothoracine social parasites are sufficiently detrimental to provoke host counter adaptations (Foitzik and Herbers 2001, Foitzik et al. 2001). Here, we examine the fate of eggs from the social parasite, *Polyergus breviceps*, when offered to workers of two of its host species (*Formica gnava*, *Formica occulta*) in two different social environments (hosts in free-living colonies and hosts as slaves) to infer both mechanisms of host defence against brood parasitism and parasite integration during colony founding. We also analyse egg and pupa hydrocarbon profiles to reveal potential co-evolutionary and developmental processes at the stage of parasite colony founding.

Methods

Ant collections, housing, and food

The *P. breviceps* from southeastern Arizona, USA, span the elevational range of the Chiricahua Mountains and form two subpopulations, utilising only *F. gnava* at lower elevations and only *F. occulta* at high elevations. We collected colonies of the host species from this region during June, July, and August of 1997. Eighteen queenright colonies of *F. gnava* were collected from the Arizona oak–alligator juniper woodlands of the Southwestern Research Station (SWRS) of the American Museum of Natural History (AMNH) (elevation 1,646 m). Twenty-two queenright colonies of

Table 1 Numbers (mean and total) of eggs presented to worker sub-colonies of two species of *Formica* that are hosts of the slave-maker ant *Polyergus breviceps*

			Eggs presented			
			F. gnava	*F. occulta*	*P. breviceps* (*F. gnava*)	*P. breviceps* (*F. occulta*)
F. gnava	Free-living	Mean	6.09	5.62	5.84	6.43
		SE[a]	0.56	0.33	0.21	0.57
		Sum	67	73	216	45
		n[b]	11	13	37	7
	Enslaved	Mean	6.00	5.00	6.86	6.00
		SE	0.38	0.00	0.43	0.58
		Sum	42	20	151	36
		n	7	4	22	6
F. occulta	Free-living	Mean	5.67	6.19	5.63	6.57
		SE	0.33	0.36	0.24	0.57
		Sum	51	99	135	46
		n	9	16	24	7
	Enslaved	Mean	5.80	5.83	6.58	6.41
		SE	0.80	0.48	0.54	0.32
		Sum	29	35	79	109
		n	5	6	12	17

[a] Standard error of the mean
[b] Number of trials (i.e. number of subcolonies presented with eggs of that species)

F. occulta were collected from an area just east of the Barfoot Peak trailhead (elevation 2,750 m) in Coronado National Forest populated with ponderosa pine. Entire colonies were brought into the laboratory at SWRS and placed in large Tupperware containers lined with Fluon (Northern Products, Warwick, R.I., USA) to prevent the escape of workers, and eventually transported to Hunter College, New York City, where the experiment was completed. Colonies were provided with the Bhatkar and Whitcomb (1970) diet, 1:1 honey/water solution, water when needed, and local insects when at the SWRS or crickets (Fluker Farms) after being transported to Hunter College.

New alate (winged) gynes of *P. breviceps* mate and shed their wings while running alongside worker nest mates that have been recruited to raid a discovered host colony. We collected newly mated, dealate gynes from nests with *F. gnava* slaves (n=9) and from nests with *F. occulta* slaves (n=11) as they approached the *Formica* nest being raided in the field during July 1997 from the same regions as above. Each newly mated queen was placed into an individual 14.79-ml styrene vial (21 ID × 52 mm) containing a moistened ball of cotton and brought into the laboratory. To verify insemination, queens were dissected and their spermathecae examined for the presence of sperm upon termination of the experiment.

Laboratory supply colonies

Nine of the 18 *F. gnava* and 11 of the 22 *F. occulta* queenright nests provided (1) the eggs for the control conditions, and (2) the free-living host workers whose responses to the eggs were to be tested. The remaining 9 and 11 nests were used to create 'incipient' slave maker nests that provided the supply of *P. breviceps* eggs and newly enslaved host workers. To create the incipient slave-maker nests, we placed single *Formica* queens into individual Tupperware containers (20.5×30×9 cm) containing a thin layer of soil and introduced a single newly mated *P. breviceps* queen. The *P. breviceps* queens were allowed to complete their attack on the *Formica* queen and were then placed with the workers of the queen they had killed. *P. breviceps* queens will kill a queen of a host species not found in her natal nest. Because this is a difficult task to achieve, we staged attacks by *P. breviceps* queens only against queens of the host species found in the natal nest.

Five *F. gnava* colonies and five *F. occulta* colonies contained at least two queens. Each of these colonies was fractionated into two, approximately equal queenright parts to also supply host species eggs and free-living host species workers. Any additional queens were discarded. Although no genetic data exist for *F. gnava* or *F. occulta*, it is possible that these queens were sisters and that the workers were closely related. However, both *F. gnava* and *F. occulta* polygyne nests tend to be quite large and at least several years old. A log-linear analysis showed that there were no colony effects on rearing tendencies (L–R χ^2=93.3, df=116, P=0.94), and we believe the behaviour of these workers to be independent of their colony of origin.

Supply nests were examined for eggs every 4 h for the first week after new *P. breviceps* colonies were created. Whereas *F. gnava* and *F. occulta* queens produced eggs regularly, only a few eggs (between 3 and 7) were found in 6 of the newly enslaved nests and 0 were found in the other 14. Subsequent daily nest checks, however, often revealed no eggs. Because the numbers of *P. breviceps* eggs were generally insufficient to conduct a single trial, we did not harvest slave-maker eggs until at least five eggs could be removed.

Experimental colonies and bioassays

To test host worker response to eggs, sub-colonies of 35–40 workers taken from either the free-living or parasitised nests described above were presented with a clump of 5–8 eggs from a foreign conspecific, heterospecific *Formica* or a slave-maker queen (Table 1; Fig. 1). One hour prior to the experiment, host workers were removed from brood-containing areas of a supply nest, placed in a Tupperware container (18×30×9 cm) with Fluon-coated rims and soil-lined bottoms, and allowed to acclimate. In the centre of each Tupperware container was a cloth covered, inverted plastic petri dish (5.5 cm diameter) with two entrances 180° apart that provided a nesting site. Egg clumps were then carefully lifted from their colony with fine-tipped forceps, which had been cleaned with hexane and air dried, and placed 3 cm from one of the nest entrances. The mean and total number of eggs presented to workers in each condition and number of experimental trials are presented in Table 1.

For 15 min after an egg clump was introduced into an experimental box, worker actions (trampling, antennating, hoisting, carrying) and the latency and duration of each action were recorded. Boxes were examined every day thereafter for the presence of immature brood until callow emergence or until no brood was observed for 72 h, at which point the trial was terminated. If eggs hatched into first instar larvae, the numbers of immature individuals (larvae, pupae, callows) present were counted. Each sub-colony was presented with an egg clump only once and workers were

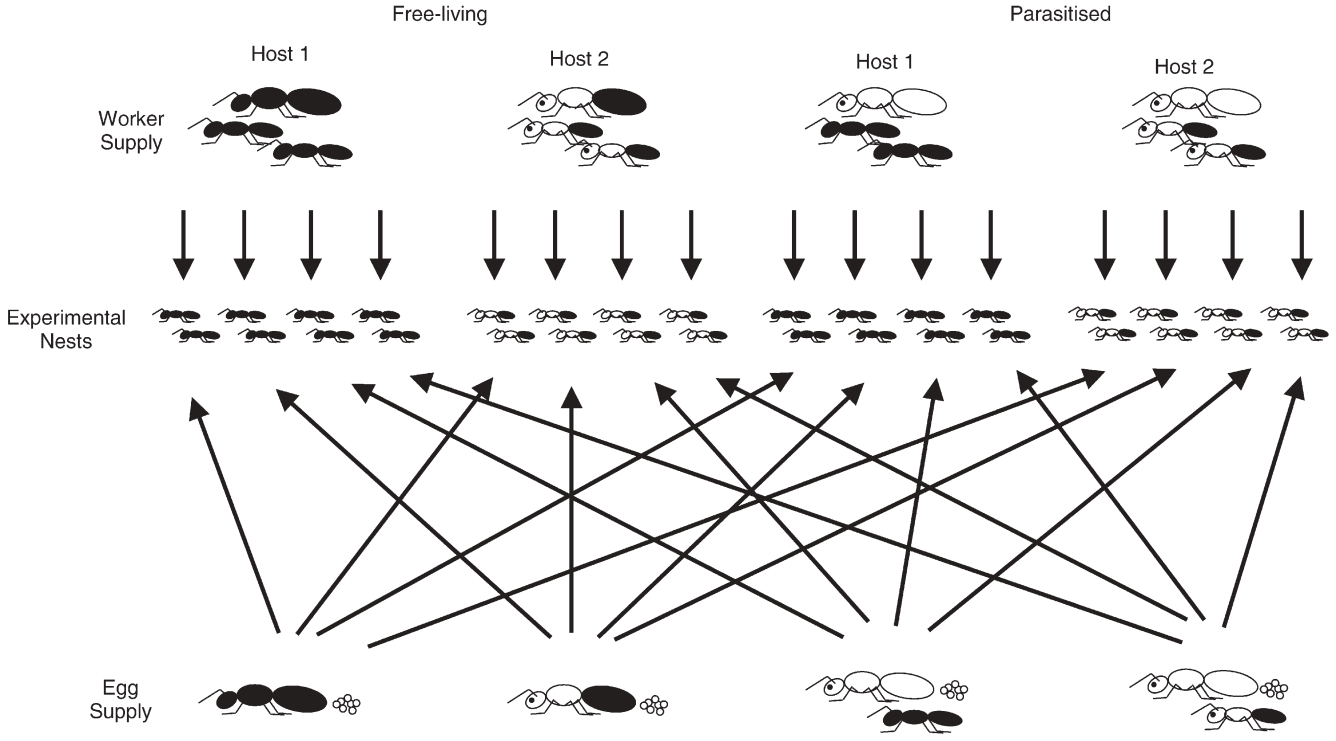

Fig. 1 Schematic of experimental design. Sub-colonies of approximately 35–40 workers taken from natural colonies of two host species (*Formica gnava*, *Formica occulta*) were presented with eggs from (1) conspecific, (2) heterospecific alternative host, or (3) parasite (*Polyergus breviceps*) queens associated with one or the other host species, and the rearing patterns monitored. About half of the sub-colonies consisted of workers from nests that had been newly usurped in the laboratory by a *P. breviceps* queen (parasitised) and the other half consisted of workers taken from queen-right unparasitised nests (free-living)

discarded after the trial was completed, i.e., when all the immature brood disappeared or metamorphosed into adults.

Chemical sampling

Cuticular components were extracted from clumps of 10–20 eggs or from five pupae for each species by immersing them in 2 ml of high purity hexane (B & J, GC^2 grade, Burdick and Jackson, Muskegon, Mich.) for 10 min in 7-ml glass scintillation vials. Solvent extracts were transferred from the sample with a Pasteur pipette to a 2-ml glass scintillation vial, and allowed to evaporate. The evaporated solvent extracts were redissolved with 0.2 ml hexane, vortexed for 1–2 s, applied to a small silicic acid (70–230 mesh, 60 Å, Aldrich Chemical, Milwaukee, Wis.) Pasteur pipette column and then eluted with hexane to isolate the hydrocarbons from other lipids. The elutant, containing purified hydrocarbons, was evaporated to ca. 10 µl under a stream of nitrogen. Samples were analyzed by gas chromatography [Varian 3700 (Varian, Walnut Creek, Calif.) equipped with a split-splitless injector, a capillary column (DB–1, 30 m, 0.32 mm ID, 0.25-μ film thickness; Agilent Technologies, J & W Scientific, Folsom, Calif.) and flame ionisation detector]. The initial injector and detector temperatures were set at 300°C; the oven was programmed from 190° to 290°C at 5°C/min, and then held at 290°C for 5 min. Hydrogen was the carrier gas and nitrogen was the make-up gas. Hydrocarbon standards [C_{24}, C_{26}, C_{28}, C_{30}, C_{32}, n–Parafin mix, no. NP-MIX-H (Alltech Associates, Deerfield, Ill.)] were injected at regular intervals during sample analysis and used to calculate Kovat Indices. Data were analyzed using PE Nelson Turbochrom Navigator 6.1.0.1FO4 (Perkin Elmer, Norwalk, Conn.).

Statistical methods

Behavioural data

Data were analysed using the Cox proportional hazards fit model (Cox 1972) in JMP 4.0.2 (SAS Institute). We compared the survival of eggs from conspecific queens, heterospecific host queens and slave-maker queens that had been presented to host species workers from unparasitised and newly parasitised nests using failure rates—the stage at which immature individuals were no longer present inside experimental nests. The null hypothesis in a Cox regression model assumes that the hazard rate (i.e., probability of failure) at any given time for an individual (here a juvenile) in one group is proportional to the hazard at that time for a similar individual (juvenile) in the other group(s). It is assumed that the fate of any individual is independent of the other individuals within each group. The model included egg species, worker species and enslavement state as variables and was used to calculate the likelihood ratio for the survival rate of the different egg species after allowing for the effects of worker species and social condition. Data are presented as the untransformed mean proportion of immature brood remaining at each developmental stage rather than the hazard function.

Chemical data

The hydrocarbon data were pre-processed for multivariate analysis. The relative proportions of cuticular hydrocarbons were computed by dividing the peak area for each cuticular hydrocarbon by the total integrated peak area of the profile. To assure equal weight in the analysis, each peak was then autoscaled using the following equation:

$$x_{\mathrm{i,new}} = \frac{\left(x_{\mathrm{i,original}} - m_{\mathrm{i,original}}\right)}{\left(s_{\mathrm{i,original}}\right)}$$

where $m_{i,original}$ is the mean of the variable and $s_{i,original}$ is the standard deviation of the variable (Otto 1999). Analysis of principal components was conducted first on 48 normalised variables for eggs (n=33), and then on 47 normalised variables for eggs (n=29) and for pupae (n=17) to reduce the number of variables considered in the discriminant analysis. A plot of the first two principal components was generated to provide an overview of meaningful biological trends in the data. In the combined analysis, eggs from *P. breviceps* from nests with *F. occulta* hosts were excluded due to insufficient quality profiles from pupae of the same species and host association. A stepwise linear discriminant analysis was used to assess whether eggs, and eggs and pupae could be discriminated according to species and species and developmental stage on the basis of the first three principal components. Wilk's λ-test and the percentage of correct assignments were used to assess the strength of the discriminant function. Multivariate analyses were conducted in JMP 4.0.2 and Pirouette (InfoMetrix, Woodinville, Wash.).

Results

Behavioural data

During the 15-min observations, eggs were contacted (trampled, antennated, hoisted) by at least one worker in 70% of the trials (n=193). Nonetheless, in only 45% of these trials (32% of the total number of trials) were eggs reared to a succeeding stage. This shows that there was no relationship between rearing propensity and contact with egg piles just after introduction into the experimental chamber (χ^2=0.071, P=0.79). If eggs were retrieved into the nest during the 15-min observation period, they were retrieved as a clump. Single eggs were never seen outside the nest once the clump had been retrieved.

Significantly more eggs that had been presented to conspecific workers were reared to adulthood than eggs that had been presented to heterospecific workers (Cox regression, Table 2; Fig. 2). Egg rearing was furthermore independent of enslavement state (Cox regression, Table 2; Fig. 2). Whereas free-living and enslaved workers of both host species combined, reared 45% and 26% respectively of conspecific eggs; only 1% and 2% of slave-maker eggs were reared to adulthood. Free-living workers reared 4% of heterospecific *Formica* eggs, but all these individuals emerged in one experimental subcolony. Enslaved workers did not rear any heterospecific *Formica* eggs (Fig. 2).

Chemical data

Hydrocarbon profiles of eggs from *F. gnava*, *F. occulta*, and *P. breviceps* are distinct (Fig. 3). A plot of the first two, and thus most informative, principal components, which accounted for 34% of the total cumulative variance, reveals a general separation between species (Fig. 4a). The *P. breviceps* egg profiles are, furthermore, noticeably different depending on their specific host association (Fig. 3) and, in the principal component plot, tend to cluster near their utilised host species (Fig. 4a). Visual inspection of profiles from *P. breviceps* eggs acquired from queens in nests with *F. gnava* host workers shows two evident 'forms' of *P. breviceps* egg profiles. One form (form 1, Fig. 3d) clearly resembles a common profile of *F. gnava* eggs (Fig. 3e), with numerous peaks present in both host and parasite profiles in similar relative proportions. The other form, although containing shared compounds, is conspicuously different and appears somewhat intermediate between form 1 and the profile of *P. breviceps* eggs from nests with *F. occulta* host workers (Fig. 3g). Discriminant analysis showed that the species are distinguishable on the basis of the first three principal components (Wilk's λ=0.20, df=6, 56, P<0.0001), although some samples were misclassified (Table 3). With the exception of two samples, misclassified eggs were classified incorrectly according to their host association or slave-maker association.

Pupal hydrocarbon profiles of *F. gnava*, *F. occulta*, and *P. breviceps* associated with *F. gnava* are distinct (Fig. 3c, f, h; Fig. 4b), and there is a clear change in quality of compounds from the egg stage. However, the pupal profiles appear simpler than egg profiles, with fewer compounds overall. Furthermore, the pupal profiles are all dominated by several of the same compounds, which differ slightly in their relative proportions. Pupal profiles of *P. breviceps* associated with *F. occulta* are not pictured due to the lack of quality of available samples, and eggs and pupae of these are excluded from the analysis. Discriminant analysis of species and developmental stage based on the first three principal components showed that there was discrimination among the six groups (Wilk's λ=0.06, df=15, 105, P<0.00001), although some individuals were misclassified (Table 4). The most notable incorrect classifications are the samples of *P. breviceps* pupae from nests with *F. gnava* workers, which are classified as either *F. gnava* eggs or *F. gnava* pupae.

Table 2 Likelihood ratio table comparing the survival of eggs of different species when presented to free-living and newly enslaved workers. The survival function is modeled without (*null*) and with egg species (*F. gnava*, *F. occulta*, *P. breviceps* with *F. gnava* host, *P. breviceps* with *F. occulta* host), worker species (*F. gnava*, *F. occulta*), and enslavement state (free-living and enslaved) (*overall*) as factors. The model also tests the effect of egg species, worker species, enslavement state and the relevant interactions (egg species × worker species, worker species × enslavement state, egg species × worker species × enslavement state)

Term	− Log likelihood	χ^2	df	P
Null	934.99			
Overall	958.75	49.52	12	<0.0001
Egg species		12.69	3	0.0053
Worker species		1.96	1	0.16
Enslavement state		2.01	1	0.16
Egg species × worker species		24.03	3	<0.0001
Worker species × enslavement state		0	1	0.95
Egg species × worker species × enslavement state		1.23	3	0.75

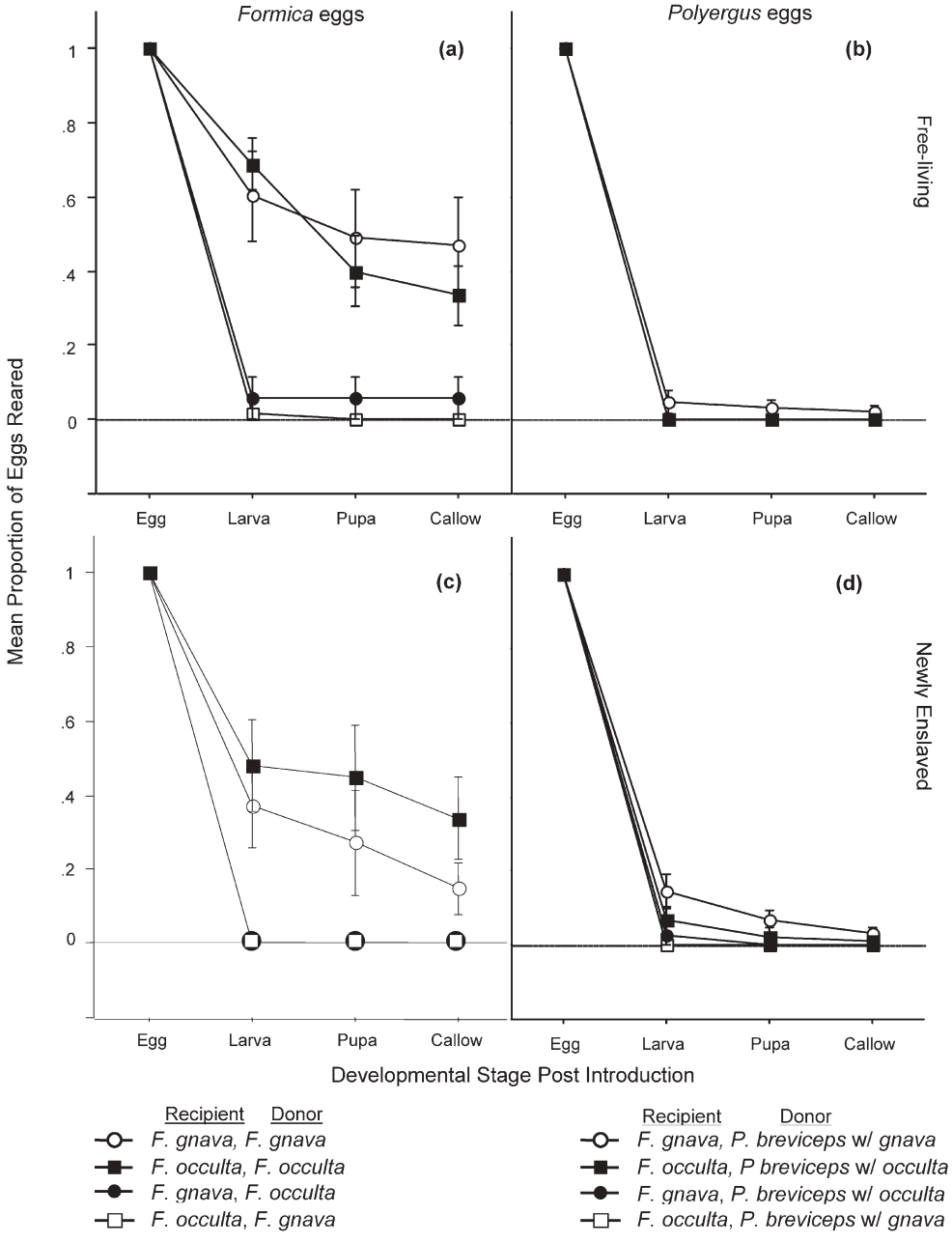

Fig. 2 Mean proportion of eggs presented to experimental colonies that were reared to larval, pupal and callow stages. *Bars* represent standard errors of the mean

Discussion

Our cross-fostering experiment shows that pure clusters of eggs of the slave-maker ant *P. breviceps* do not elicit brood tending behaviours from two of their host species. In other words, workers of the host species *F. gnava* and *F. occulta* are able to discriminate slave-maker eggs and will reject them. Whereas the presence of some slave-maker species prompts host workers to rear brood of other species (Alloway 1982; Schumann and Buschinger 1991), changes in social environment, at least in the 9-month time period of this study, seemed to have no impact on altering the rearing tendencies of host workers. The number of *P. breviceps* reared to adulthood did not differ whether sub-colony workers were from nests that were free-living or had been recently enslaved (Table 2; Fig. 2). There are indications, however, that prolonged exposure to the parasite queen may eventually promote host adoption of slave maker eggs. In trials where enslaved workers were of the same species as the host of the eggs presented, there was a subtle increase in egg-to-larva survival rate (Fig. 2d), and slave makers emerged primarily in later trials. Our hydrocarbon results, on the other hand, revealed a mild convergence of slave-maker egg profiles with the egg profiles of their locally available host species, which were clearly species-specific. Given that slave-maker eggs are obviously reared in natural circumstances, these results are surprising.

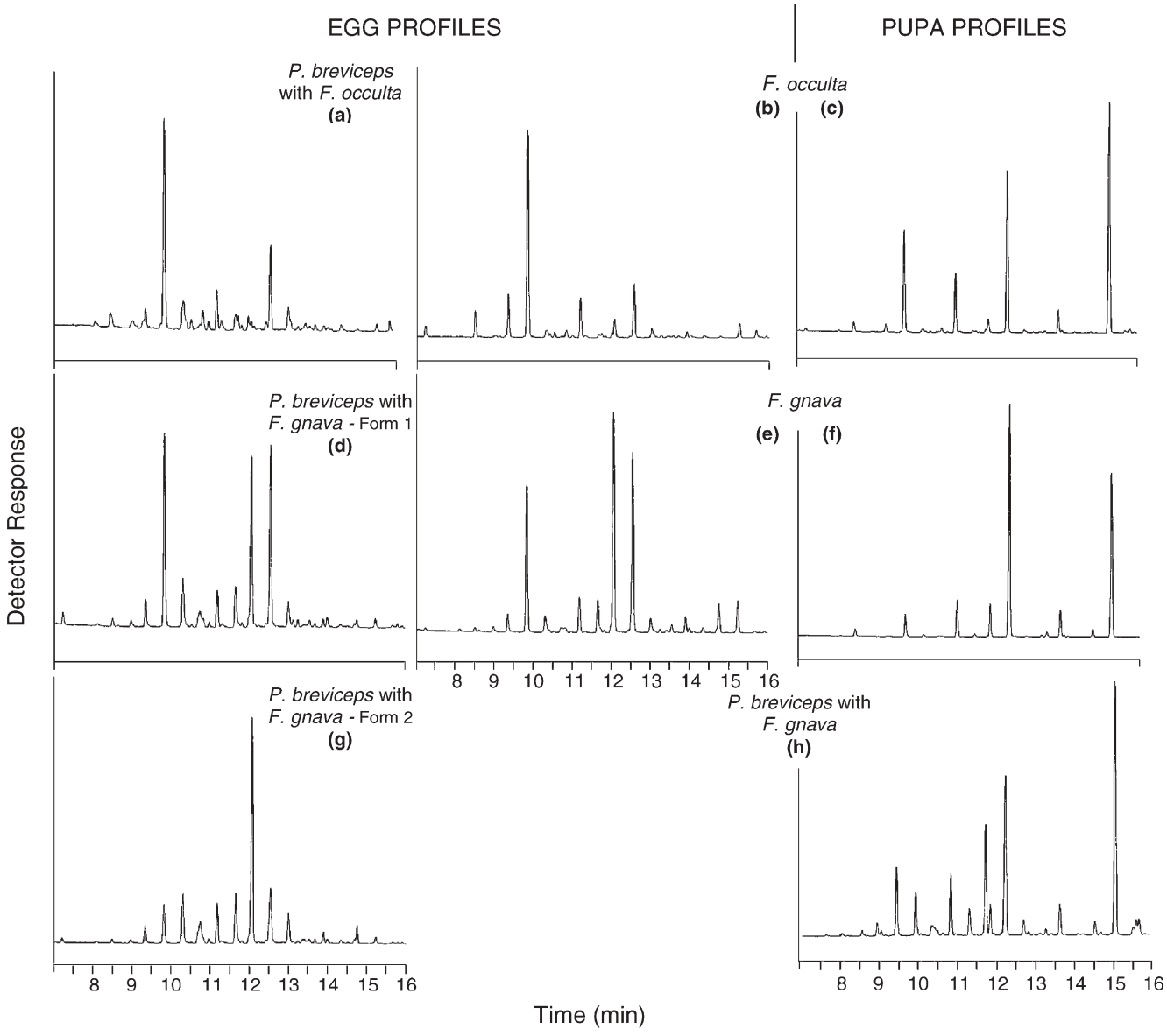

Figure 3 Representative hydrocarbon profiles from eggs and pupae of the social parasite and two of their host species

On the basis of egg hydrocarbon analysis alone, *P. breviceps* eggs can be considered somewhat chemically adapted to its utilised host species, the functional significance of which may be perceived as facilitating the respective integration process. Such host-specific chemical similarity of *P. breviceps* eggs is not particularly surprising given that we have yet to discover an overlap zone of the two host species and the degree to which *P. breviceps* switch host species is unclear. The attractiveness of synthesised sex pheromone of *P. breviceps* females from the lower elevation nests to males from high elevation nests indeed suggests that gene flow between the two populations is possible (L. Greenberg and C. Johnson, personal observation). But, such long-distance dispersal is most likely limited to the males because of the typical mode of female dispersal in this population. Hence, it is easy to envision selective pressure for parasite eggs to resemble eggs of the host species locally available to minimise fatal discriminations.

Nevertheless, the response of host workers to parasite eggs clearly indicates that the particular combination or relative proportion of hydrocarbons from the parasite has a minimal effect on inducing rearing behaviours even though a number of the same hydrocarbons are also found on the host species (Fig. 3). That eggs of the alternative host species were also rejected furthermore suggests that chemical similarity, as a consequence of close phylogenetic relatedness, may be an unlikely explanation for the contemporary adoption of parasite offspring. While it is possible that the chemical similarity reflects chemical tainting by workers prior to our removal of eggs for sampling, we consider such an artifact unlikely because

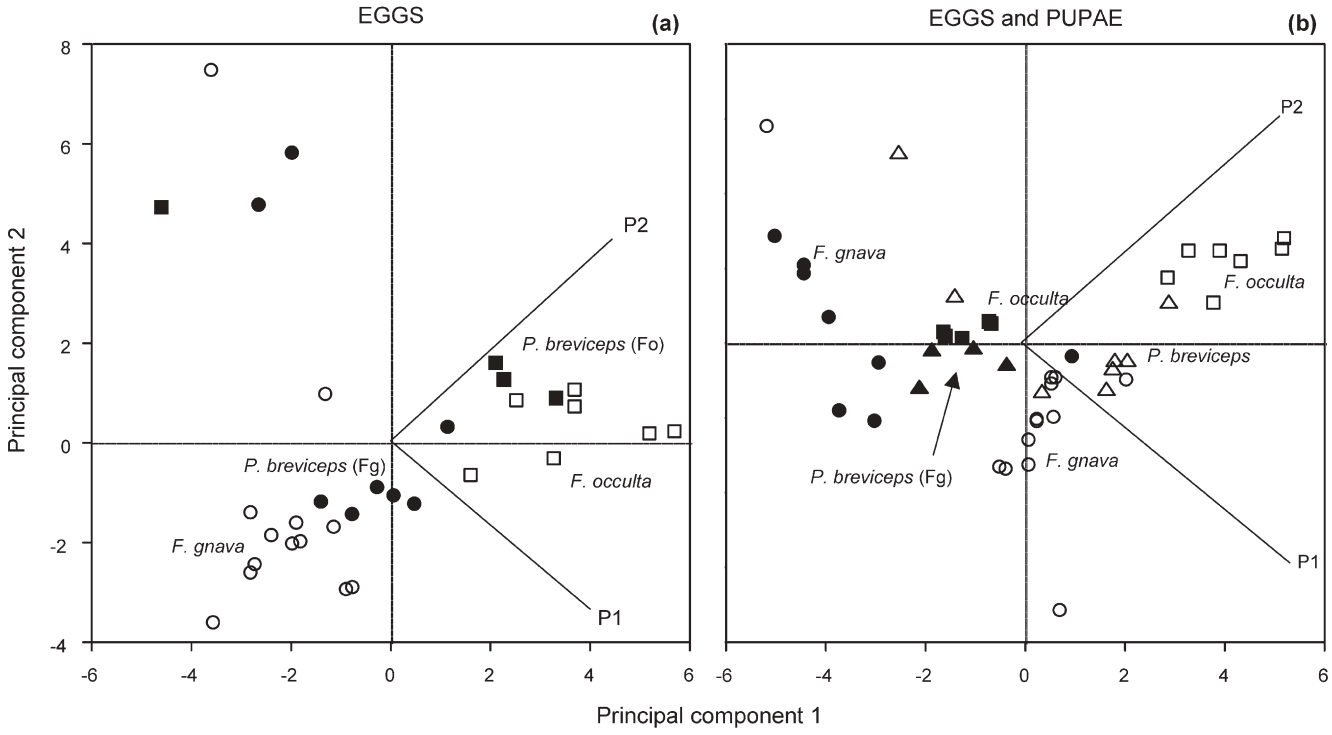

Fig. 4 **a** A plot of the first two principal components of hydrocarbons extracted from eggs. *Opaque circles*, *P. breviceps* from nests with *F. gnava* host workers. *Clear circles*, *F. gnava*. *Opaque squares*, *P. breviceps* from nests with *F. occulta*. *Clear squares*, *F. occulta*. **b** A plot of the first two principal components of hydrocarbons extracted from eggs and pupae of *P. breviceps*, *F. gnava*, and *F. occulta*. *Clear symbols* Eggs, *opaque symbols* pupae. *Triangles*, *P. breviceps*; *circles*, *F. gnava*; *squares*, *F. occulta*

Table 3 Classification matrix of egg species and host association according to the discriminant function analysis of the first three principal component scores. Notice that *P. breviceps* eggs are classified correctly with respect to their specific host association, incorrectly as *P. breviceps* associated with the alternative host species, or incorrectly as their host species

			Eggs (predicted)			
		Percent correct	*P. breviceps* (*F. gnava*)	*P. breviceps* (*F. occulta*)	*F. gnava*	*F. occulta*
Eggs (observed)	*P. breviceps* (*F. gnava*)	75	6	1	1	0
	P. breviceps (*F. occulta*)	50	0	2	0	2
	F. gnava	92.3	1	0	12	0
	F. occulta	87.5	1	0	0	7
	Total	81.8	8	3	13	9

both the egg profiles and the pupa profiles are unmistakably different from worker profiles of all three species (C. Johnson, unpublished data). It may also be possible that the compounds we identified using only Kovat indices and deemed as being the same are structurally different. However, it would seem peculiar that a single species of obligate social parasite would produce hydrocarbon patterns that approximate their particular host species but be constitutively dissimilar so as not to be useful. Moreover, there are countless examples in which juvenile and adult social parasites maintain chemical profiles remarkably similar to its host species (e.g., Howard et al. 1990; Yamaoka 1990; Kaib et al. 1993; Errard and D'Ettorre 1998; Akino et al. 1999; Elmes et al. 1999; Johnson et al. 2001). The incongruence between the behavioural and chemical findings in our study is most likely due to the arms race between host and parasite, and may reflect a co-evolutionary lag behind increased discriminatory ability of the host or host egg modification. Regardless, what can be concluded definitively from these results is that *P. breviceps* currently utilises means other than or in addition to chemical similarity to insure the successful adoption of its eggs.

Formicid social parasites are generally considered rare relative to their hosts, which has led to the assumption that the apparent lack of counter adaptations is a result of weak selective pressures (Gladstone 1981; Davies et al. 1989). It has become recently apparent in some slave maker–host relationships that the host species has evolved defensive reactions to counter or ease the relative impact of slave raids by the social parasite (Foitzik and Herbers 2001; Foitzik et al. 2001). Clearly, rejection of parasite eggs in our study can be considered an adaptive response against social parasites. Oophagy by sisters and daugh-

Table 4 Classification matrix of groups according to the discriminant function analysis on first three principal component scores of the combined egg and pupa chemical data. Notice that *P. breviceps* pupae are misclassified either as *F. gnava* eggs or *F. gnava* pupae, suggesting a potential chemical influence from host workers grooming *P. breviceps* immatures

			Eggs (predicted)			Pupae (predicted)		
		Percent correct	*P. breviceps* (*F. gnava*)	*F. gnava*	*F. occulta*	*P. breviceps* (*F. gnava*)	*F. gnava*	*F. occulta*
Eggs (observed)	*P. breviceps* (*F. gnava*)	62.5	5	2	1	0	0	0
	F. gnava	84.6	1	11	0	0	1	0
	F. occulta	100	0	0	8	0	0	0
Pupae (observed)	*P. breviceps* (*F. gnava*)	0	0	2	0	0	2	0
	F. gnava	75	1	0	0	0	6	1
	F. occulta	100	0	0	0	0	0	5
	Total	76.09	7	15	9	0	9	6

ters, however, is a common threat in ant colonies (Bourke 1994), and linked to reproductive conflicts due to different agendas among colony members (e.g., Hannonen and Sundström 2003). For example, eggs laid by subordinate, virgin or worker individuals are often chemically distinguishable and destroyed [e.g., ants (Monnin and Peeters 1997); bees (Ratnieks 1995) and wasps]. This suggests that discriminatory ability is plesiomorphic and unlikely to be an evolved response to inter-specific parasitism (but see Lorenzi and Filipone 2000), although it can function as an anti-parasite defence and sensitivity to foreign odours may vary relative to parasite pressure.

The ability to discriminate among eggs by odour, thus, is a trait that becomes important in the co-evolutionary arms race between social parasite and host. Current strategy by *P. breviceps* to encourage egg adoption has undoubtedly been shaped by such overwhelming promise for egg rejection during colony takeovers as revealed by our study. That host workers rear parasite eggs under natural circumstances while maintaining an extraordinary ability to distinguish them suggests a balance between the costs of counter defences and the costs of parasitism rather than a lag in the co-evolutionary arms race [rare enemy effect (Dawkins 1982); Lotem et al. 1995; Laws and Marthews 2003]. This is remarkably similar to the behaviour of avian host species that rear parasite brood (Davies and Brooke 1989). In ants, however, queens (at least) may attenuate the discriminatory ability of rivals by mixing their own eggs in amongst eggs of others to prevent agonistic consumption (e.g., Oliveira and Hölldobler 1991; for review, see Ayasse and Paxton 2002). Egg mixing certainly has the potential to increase host risk of making discrimination errors, and the concomitant inability to replace diploid eggs in usurped colonies amplifies the cost of such errors. Collectively, the associated risk and cost of discrimination errors of egg mixing is arguably a strong force behind parasite-egg adoption (Lawes and Marthews 2003). That both host species in our study reared a substantial proportion of foreign conspecific eggs supports the notion that host workers 'bet-hedge' and potentially benefit from rearing a proportion of individuals with 'questionable' (foreign) but reasonable (conspecific) odours. The protective properties of egg mixing are furthermore evident in a study on colony usurpation, in which slave-maker callows emerged in newly formed slave-maker colonies that had been additionally provisioned with host brood (Mori et al. 1995). In the laboratory, a *P. breviceps* queen places herself on the brood pile or is dragged there by host workers after she has finished her attack on the host queen. Most likely under natural circumstances a similar sequence of events occurs, which provides the *Polyergus* queen an opportunity to mix her eggs amongst the younger brood that raiders typically leave behind (Topoff et al. 1988) to secure the adoption of her offspring during colony usurpation.

In general, the value of a developing individual increases in proportion to the energy invested as it ages (Bourke and Franks 1995). We thus find the general pattern of attrition in our experimental sub-colonies and the changes in hydrocarbon chemistry from egg to pupa stages noteworthy. For all species in our study, the primary loss of individuals in the experimental sub-colonies occurred at the egg stage, whereas the decline at any one stage thereafter was minimal. Egg hydrocarbon profiles of the host species and parasite are clearly species- and host-specific respectively, and clearly different from pupa hydrocarbon profiles except for a few outliers (Figs. 3, 4; Table 3, 4). The pupa profiles of all three species, on the other hand, take on a similar hydrocarbon pattern, particularly with respect to two high molecular weight peaks seen at approximately 12.5 and 15.0 min (Fig. 3c, f, h). Lenoir et al. (1999, 2001) suggested that new ants are 'chemically insignificant' or lack external chemical substances for a period just after emergence, which allows them to evade aggression and acquire significant chemicals. Although we do not consider the pupae sampled in this study to be necessarily chemically inconsequential, the predominance of the two high molecular weight peaks is conspicuous. Rather than making pupae insignificant, these compounds may serve to minimise discrimination errors by making pupae generally appealing to workers. The change from a more species-specific hydrocarbon profile at the egg stage to one less so at the pupa stage clearly seems to reflect the increased value of an older individual and may account for the relative ease (as

compared to eggs) with which some species of pupae can be transferred between nests (e.g., Plateaux 1985).

P. breviceps colonies can be proportionately scarce, undoubtedly a consequence of the obstacles the slave maker encounters when attempting to assimilate into a host species colony. A newly inseminated queen must locate a host nest, appease or repel aggressive workers (Topoff et al. 1988; D'Ettorre et al. 2000; Mori et al. 2000a, 2000b), attack the appropriate individual to gain favourable worker attention (Errard and D'Ettorre 1998; Johnson et al. 2001, 2002), and secure the adoption of her offspring. In parasite systems, the pressure to maximise fitness gains (or minimise fitness loss) often leads to a series of counter adaptations both by the host and the parasite, the so-called arms race (Thompson 1994). Successful colony founding by *P. breviceps* translates into the eventual demise of an entire host species colony and is undoubtedly a strong selective pressure, as is the ability of the host to reject the slave maker. Our results demonstrate that two *P. breviceps* host species have a line of defence against brood parasitism—egg rejection. This fact, however, is clouded by the continued establishment of *P. breviceps* colonies in nature, which in itself strongly implies risks and costs are associated with discrimination errors that can select for the displacement of parasite egg rejection. Admittedly, the frequency with which takeovers fail because of offspring rejection rather than denied access to a host queen or insufficient camouflage is unknown. The carnage remnants observed in two *F. gnava* colonies excavated 1 day after being raided, however, indicate a great deal of resistance is directed against invading *P. breviceps* queens prior to the onset of parasite egg laying.

Although marvelled since Darwin's time, relatively little is known about the evolutionary dynamics in social parasite–host systems. The notion of rarity in conjunction with certain evolutionary equilibria may inadvertently lead to an underestimation of social parasite impact on host species. Increasing our understanding of the mechanisms of parasite integration will contribute to a greater understanding of co-evolutionary processes between social parasites and their hosts, as well as potentially reveal various dynamics of many free-living social insect societies.

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