The adaptive significance of inquiline parasite workers

Seirian Sumner*, David R. Nash and Jacobus J. Boomsma

Department of Population Ecology, Zoological Institute, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

Social parasites exploit the socially managed resources of their host's society. Inquiline social parasites are dependent on their host throughout their life cycle, and so many of the traits inherited from their free-living ancestor are removed by natural selection. One trait that is commonly lost is the worker caste, the functions of which are adequately fulfilled by host workers. The few inquiline parasites that have retained a worker caste are thought to be at a transitional stage in the evolution of social parasitism, and their worker castes are considered vestigial and non-adaptive. However, this idea has not been tested. Furthermore, whether inquiline workers have an adaptive role outside the usual worker repertoire of foraging, brood care and colony maintenance has not been examined. In this paper, we present data that suggest that workers of the inquiline ant *Acromyrmex insinuator* play a vital role in ensuring the parasite's fitness. We show that the presence of these parasite workers has a positive effect on the production of parasite sexuals and a negative effect on the production of host sexuals. This suggests that inquiline workers play a vital role in suppressing host queen reproduction, thus promoting the rearing of parasite sexuals. To our knowledge, these are the first experiments on inquiline workers and the first to provide evidence that inquiline workers have an adaptive role.

Keywords: reproductive suppression; social parasitism; *Acromyrmex insinuator*; adaptation

1. INTRODUCTION

Social parasites capitalize on the socially managed resources of their hosts, rather like the distant relative who takes up an offer of a bed for the night and never leaves. While the unwanted guest exercises strategies to exploit the resources of the host's home, the host must respond by detecting and ultimately evicting them. This study is concerned with the adaptive strategies of the unwanted guest, the social parasite.

Social parasitism is common throughout the animal kingdom; for example, cuckoos parasitize the brood-caring abilities of another species by laying their eggs in the nests of their hosts (Brook & Davies 1988). Social parasites are common in the hymenopteran societies, and they have evolved repeatedly in the bees, wasps and ants (Wcislo 1987). In some cases, hymenopteran social parasites produce a worker caste that clearly has an adaptive function (e.g. workers of slave-making ants are crucial for raiding other host colonies; Hölldobler & Wilson 1990). However, most permanent hymenopteran social parasites produce sexuals but no workers, and the selection pressure for the loss of the worker caste is likely to be strong if the social parasite can successfully exploit the worker functions of its host.

Permanent social parasites, or parasitic inquilines, are dependent on their host's society throughout their life because they do not kill the host queen. Most inquiline species have lost their worker caste, since they rely on a constant supply of host workers (e.g. *Pseudomyrmex leptosus*; Ward 1996). If the fitness gained by a parasite from producing only sexual brood is greater than that

gained from producing both sexuals and workers, then a workerless state is expected to evolve quickly (Nonacs & Tobin 1992). However, a handful of inquilines do invest in a worker caste (nine out of 67 species listed in Hölldobler & Wilson (1990)). This has been interpreted as incomplete adaptation to inquilinism by parasites at a primitive stage in their evolution (Wilson 1984; Hölldobler & Wilson 1990; Nonacs & Tobin 1992).

There is little evidence that inquiline workers are effective in performing tasks that are typical of free-living workers. They are apparently unmotivated in brood rearing and transportation (e.g. *Kyidris yaleogyna*; Wilson & Brown 1956) and are ineffective hunters, foragers and nest builders (*K. yaleogyna*; Wilson & Brown 1956; *Polyrhachis lama*; Maschwitz *et al.* 2000). There is only one account from a single colony of *Monomorium metoecus* in which inquiline workers appear to forage effectively (Wilson & Brown 1958). However, inquiline workers may perform some adaptive function that falls outside the normal worker repertoire, as is observed, for example, in slave-making ants. These workers show a regression of typical worker functions such as brood caring, foraging and nest-building abilities (Alloway 1979; Hölldobler & Wilson 1990) but are highly adapted nest raiders (Foitzik & Herbers 2001). We know of only one description of inquiline workers performing an adaptive function: *P. lama* parasite workers appear able to care selectively for parasite brood, albeit less effectively than their host counterparts (Maschwitz *et al.* 2000).

In this paper, we introduce the idea that the production of workers may be a fitness insurance policy for incipient inquilines. We suggest that they may help to suppress host-queen reproduction in incipient systems where more complex methods of control have not evolved. We present a set of predictions from the hypothesis and test them with

* Author and address for correspondence: Smithsonian Tropical Research Institute, Balboa, Panama, Republic of Panama (sumners@naos.si.edu).

field data and manipulation experiments on an inquiline socially parasitic fungus-growing ant.

(a) *A hypothesis for the maintenance of adaptive inquiline workers*

Inquiline parasites are selected to increase their own fitness by redirecting the resources of the host colony. One way in which they do this is by suppressing the host queen's reproduction, so allowing resources to be invested in parasite rather than host sexuals (e.g. Wilson & Brown 1956; Passera *et al.* 2001). We propose that parasite workers of incipient inquilines may play a key role in redirecting host resources, by aiding the suppression of host reproduction and promoting the production of parasite sexuals. This hypothesis makes several predictions about how selection acts on incipient inquilines.

First, the presence of parasite workers will be associated with the presence of parasite sexuals. As a corollary, if host resources are diverted via suppression of host reproduction, a negative relationship between parasite worker production and host sexual production is expected (prediction 1).

The number of parasite and host queens present in a colony can affect parasite worker production. If, in addition to workers, parasitic queens play a direct role in the redirection of host resources, then fewer parasite workers will be required for sexual reproduction when the number of parasite queens is high. Furthermore, in polygynous host colonies we expect the combined number of parasite workers and queens required for effective suppression to be greater than in monogynous colonies (prediction 2).

To assess the success of resource distribution and to determine when to start rearing parasite sexuals, parasite workers must be capable of distinguishing between host and parasite brood (prediction 3). This feedback mechanism assumes that caste determination is at least partly under worker control. This is consistent with the current view of caste determination in ants, whereby gyne development may be switched on by queen pheromones, but is ultimately determined by the nutritional level provided by workers (Wheeler 1986, 1991).

As with workers of slave-making social parasites, workers of incipient inquilines that are adaptive in one respect will still be under selection to reduce their investment in functions fulfilled by the host workers, such as brood rearing (prediction 4), disease defence (prediction 5) and foraging (prediction 6). These last three predictions are also consistent with the null hypothesis that workers are vestigial, and so cannot be considered mutually exclusive to the alternative hypothesis that workers are adaptive. However, under this alternative hypothesis it is important to establish that parasite workers have been selected to regress their typical worker functions as well as to evolve novel roles.

(b) *An empirical test of the hypothesis*

We test the predictions detailed in § 1a using the inquiline leaf-cutting ant (*Acromyrmex insinuator*), which parasitizes colonies of *A. echinaior* (Schultz *et al.* 1998), its closest relative (Sumner *et al.* 2003a). This species is a true inquiline in that it is never found in the field without some host workers present in the nest. It does, however,

produce workers that, like the queens, closely resemble their host counterparts (Schultz *et al.* 1998; Bekkevoeld & Boomsma 2000). Parasite workers can be distinguished from the hosts by their relatively smaller metapleural glands (Sumner *et al.* 2003b), facilitating *in vivo* manipulation experiments. Colonies of *A. echinaior* consist of several thousand workers (Weber 1972; Bekkevoeld & Boomsma 2000). Sexual reproduction by the parasite is essentially semelparous and can occur in host colonies of any size, with sexual broods (male and female) varying from two to 326 individuals (Bekkevoeld & Boomsma 2000). After the emergence of parasite sexuals, the host colony declines and eventually dies, putatively owing to the suppression of host worker production (Bekkevoeld & Boomsma 2000). *Acromyrmex insinuator* and *A. echinaior* are both facultatively polygynous, with up to four host and nine parasitic queens in a single colony (Bekkevoeld *et al.* 1999; this paper), and colonies of different gyny are of similar size (see § 3b). The effect of queen numbers on the production of parasite workers and sexuals is, therefore, easily tested using data from field colonies.

Although there is little data available, observations on *A. insinuator* already indicate that its investment in the worker caste has been reduced from that of a free-living state and that the workers are not fully functional. First, *A. insinuator* produces minor workers prior to sexual production, and these constitute on average 25% of the total worker force in colonies where they are produced. They only rarely invest in large workers that would be able to forage, probably when host foragers are in short supply, as suggested by prediction 6 (Sumner *et al.* 2003b). Second, *A. insinuator* workers fare poorly in disease defence relative to their host counterparts (Sumner *et al.* 2003b), as in prediction 5. However, these observations are also consistent with the null hypothesis that workers are vestigial and functionless.

In this paper, we address the remaining four hypotheses. We use data from field colonies collected over 10 years to assess the influence of parasite worker production on sexual production (prediction 1) and how production of parasite workers is influenced by queen number (prediction 2). We also use controlled laboratory experiments to test directly the ability of parasite workers to discriminate between brood species (prediction 3) and to rear brood (prediction 4).

2. MATERIAL AND METHODS

Parasitized *A. echinaior* colonies were collected by complete excavation from a secondary forest in Gamboa, Panama, between 1993 and 2002. Host and parasitic queens were counted, and samples of workers and, if present, winged sexuals (male and female) were stored in 96% alcohol directly after nest collection. Queens, fungus and several hundred workers from each colony were set up as laboratory colonies under controlled conditions (25 °C, 70% relative humidity (r.h.)).

(a) *Predictions 1 and 2: relationship between parasite worker production and queen number, and their effect on sexual (male and female) production*

We tested the predicted effects of parasite and host queen number and parasite worker production on parasite sexual pro-

duction using 22 colonies that produced sexuals (18 field and four laboratorial colonies). Colonies that did not produce sexuals of either species have no bearing on the hypothesis being tested. The field colonies were all collected just before the mating flight (April–May), before the winged parasite sexuals left and invaded other colonies. The proportion of the worker force that was parasitic was estimated from a random sample of 50–100 small workers taken at the time of collection. Parasite and host workers in this sample were distinguished *post hoc* based on the relative sizes of their metapleural glands (Sumner *et al.* 2003b).

For the analysis, the production of parasite sexuals was treated as a binary variable, with sexuals being either present or absent in each colony. The correlates of parasite sexual production were examined using a generalized linear model with binomial errors and logit link (Crawley 1993). The number of parasite queens per colony was highly skewed, with most colonies having a single queen but a few containing many queens. Colonies were therefore categorized as either monogynous (one parasite queen present) or polygynous (two to nine parasite queens present) for the analysis. The number of host queens was less variable (mean $\pm$ s.e. = 1.05 ± 0.047) and more evenly distributed across colonies, so this was included as a continuous variable. Other explanatory variables included were the proportion of parasite workers present in the worker sample, whether the colony was collected in the field or raised in the laboratory, and the estimated volume of the fungal garden of each colony. The volume of the fungal garden (estimated in cubic centimetres on collection of field colonies or sampling of laboratory colonies) was used as a measure of colony size, and was log-transformed before the analysis to normalize the data. Owing to the relatively sparse data, no two-way or higher-order interactions could be included in the model. The significance of each term was assessed by stepwise elimination of terms from the full model, with significance of the contribution of each term being assessed by comparing the change in scaled deviance with a χ^2 -distribution, until the minimal adequate model was obtained. Statistical modelling was carried out using GLMSTAT v. 5.7 (Beath 2002).

(b) Prediction 3: discriminatory brood rearing

Parasite and host workers were separately presented with a choice of tending a parasite larva or a host larva in a choice chamber. A central mesh was used to divide vials (diameter of 2.5 cm, height of 4 cm) into two equally sized chambers. Three holes cut at the top of the mesh were large enough to allow workers to move between the compartments but too small for larvae to be carried through. Each chamber contained a small piece of wet cotton wool and a leaf fragment so that workers could obtain leaf sap.

Sexual larvae (60 parasite and 60 host larvae of undetermined sex) were taken from laboratory colonies that had contained either only a parasitic queen (colony 134b) or only a host queen (colony 109) for at least 13 months. Each parasite larva was paired with a host larva of similar mass in separate chambers of a single vial (paired *t*-test for differences in mass of paired larvae: $t = 0.885$, $n = 60$ pairs, $p = 0.38$). Parasite and host workers were obtained from colony 157, which had been collected from the field one month previously. In this colony, 21% of workers were parasites. Two workers (either parasite or host) were placed in each vial. Workers therefore chose to rear either conspecific or heterospecific brood that were not from their own nest. Vials were kept in darkness at 25 °C and 70% r.h. They were exam-

ined five times over the following 65 hours. At each observation we noted the number of ants tending the larva of each species. An ant was recorded as 'tending' when it was in contact with a larva.

As each ant was observed to be carrying out one of three activities (tending the host larva, tending the parasite larva or tending neither larva), the data from this experiment were analysed using a generalized linear model with multinomial error structure (Crawley 1993, pp. 297–300), implemented using GLMSTAT v. 5.7 (Beath 2002). The proportion of time spent in each activity was assessed as the sum of the observations for the two worker ants (the multinomial numerator) relative to the total sum of the observations (the multinomial denominator, which was thus always 10). The model was corrected for slight overdispersion (Crawley 1993), and simplified by the stepwise elimination of terms and the combination of categories. If the change in deviance caused by combining categories was significant at the 0.05 level (as assessed by comparison with a χ^2 -distribution), the separate categories were retained in the model, if not, the combined category was used.

(c) Prediction 4: brood-rearing ability

We examined larval survivorship under the care of each worker species. The set-up was the same as for testing prediction 3 except that vials were not divided into chambers and each vial contained only one larva, either host or parasite. Six treatments with different combinations of host and parasite workers and larvae, and one control treatment of untended larvae, were set up (see table 1). Parasite workers were nest-mates, but not necessarily siblings, obtained from laboratory colony 134b, which contained 94% parasite workers. 134a and 134b were laboratory colonies obtained by splitting the single polygynous field colony 134 during collection. Queens of *A. insinuator* were present in both 134a and 134b, and they were maintained separately in the laboratory for more than a year before experiments commenced. Host workers for treatments 3–5 were siblings obtained from two unparasitized laboratory colonies derived from a single field colony. We tested between-colony differences in brood-rearing ability by setting up two treatments of host–host associations using host workers from both colonies 134a and 134b from treatments 4 and 5. In all but one treatment, workers were given one non-nest-mate (alien) larva, of either parasite or host. In treatment 6, workers from an unparasitized colony were given one nest-mate larva, to examine any differences in kin (treatment 6) versus non-kin caring (treatments 4 and 5). Although ants can feed their larvae with the sugars they obtain from the sugar water provided, the usual diet for attine larvae is fungus. If fungus had been provided, leaf material would also have been required so that the ants could feed the fungus, making it impossible to standardize the resources available to the larvae. Fungus was not provided, and so larvae were expected eventually to die from starvation. Ant tending of larvae was recorded every day at 17.00 for 20 days. Any ants that had died were replaced each day. A replicate was terminated if its larva died.

The survival of larvae in the different treatments was examined using the Cox proportional hazard model of survival, based on the day of death of each larva. Any larvae still alive at the end of the experiment were included in the analysis as censored data. Worker and larval species and the interaction between them were included in the model as main effects. Treatments 4, 5 and 6 were included in the model by nesting them within the host-worker-with-host-larva treatment. The control treat-

Table 1. Treatments used to test prediction 4, showing the different combinations of workers and larvae and the number of replicates in each case. (The identity assigned to each source colony when collected is given in parentheses for each treatment.)

treatment	worker species	larval species	number of replicates
1	parasite (134b)	parasite (134a)	20
2	parasite (134b)	host (48)	20
3	host (134a)	parasite (134b)	20
4	host (134a)	host (48)	20
5	host (134b)	host (48)	20
6	host (48)	host (48)	20
control	none	host (48)	20

Table 2. The analysis of variables correlated with the presence of *A. insinuator* sexuals in each colony. (The results of the stepwise elimination of variables from a generalized linear model with binomial errors.)

variable	change in deviance	d.f.	<i>p</i>
eliminated variables			
number of host queens	0.000 0025	1	0.999
colonial origin (field or laboratory)	0.000 0023	1	0.999
host sexuals (present or absent)	0.000 0071	1	0.998
ln of fungus-garden size	0.000 0004	1	0.999
retained variables			
number of parasite queens (1 or > 1)	11.79	1	0.0006
proportion of parasite workers	27.49	1	< 0.0001

Figure 1. Relationship between the presence or absence of parasite sexuals in infested colonies and the proportion of parasite workers in a random sample of the worker force, plotted separately for colonies with a single parasite queen (filled circles) and multiple parasite queens (open circles). Lines represent the fitted values from a generalized linear model with binomial errors and a logit link function. Where several colonies share the same values the circles are offset for clarity.

ment was not included in the main model, but was compared with the other treatments in a separate analysis.

3. RESULTS

The minimal adequate model for the production of parasite sexuals contained only two terms: the proportion of parasites in the worker sample and whether there was a single or several parasitic queens (table 2, figure 1).

(a) *Prediction 1: production of sexuals depends on parasite worker production*

Out of the 22 colonies examined, seven contained parasite sexuals; all of these colonies had parasite workers. For both single and multiple parasite queens, there was a striking threshold proportion of parasite workers in the worker sample above which parasite sexuals were produced, and below which there was no production of sexuals (figure 1). Furthermore, there was a significant negative association between the production of host sexuals and the proportion of parasite workers (Fisher’s exact test (one-tailed): *p* = 0.0053).

(b) *Prediction 2: production of parasite workers depends on queen number*

The threshold proportion of parasite workers required for the production of parasite sexuals was significantly lower for colonies with multiple parasite queens (between 4% and 7%) than for colonies with a single parasite queen (between 30% and 39%) (figure 1, table 2). This suggests that additional queens lower the threshold proportion of workers required for host suppression. The number of host queens present did not correlate with the production of parasite sexuals. This indicates that the parasites do not adjust their level of combined suppression according to the number of host queens to be suppressed. The numbers of host and parasite queens present when colonies were collected in the field did not affect colony size, measured as the logarithm of fungal-garden volume (hosts: monogynous versus polygynous (all colonies): *t*₂₉ = −0.276, *p* = 0.785; parasites: monogynous versus polygynous (infested colonies only): *t*₂₁ = −0.331, *p* = 0.744), nor was there any relationship between colonial size and the production of parasite sexuals (table 2).

Figure 2. The proportion of observations in which parasite and host workers were seen tending parasite and host larvae. The error bars represent the standard errors of the mean. The different letters above the bars indicate combinations of worker and larval species that show significantly different levels of tending.

(c) **Prediction 3: parasite workers can discriminate between parasitic and host brood**

The experiment examined whether host and parasite workers tended conspecific larvae, alien larvae or did not tend at all. The data are illustrated in figure 2 as the average proportion of workers' time spent tending each larval species. Both host and parasite species tended larvae of the other species at the same low level (combining the categories led to an insignificant change in scaled deviance: $\Delta D = 0.275$, d.f. = 1, $p = 0.600$), and tended larvae of their own species significantly more ($\Delta D = 54.02$, d.f. = 2, $p < 0.001$), suggesting that they are able to recognize and choose selectively to rear conspecific brood when presented with the choice. *Acromyrmex echinator* workers spent a greater proportion of time tending *A. echinator* larvae than *A. insinuator* workers spent tending *A. insinuator* larvae ($\Delta D = 33.63$, d.f. = 2, $p < 0.001$), meaning that parasite workers are less likely overall to tend brood.

(d) **Prediction 4: parasite workers perform poorly in brood rearing**

Figure 3 shows larval survivorship curves for larvae cared for by parasite and host workers and for untended larvae. Results of the survivorship analysis are shown in table 3. Tending by workers of any species (treatments 1–6) led to a highly significant increase in survival compared with that of untended control larvae. All untended larvae had died by day 7, at which point over 60% of larvae in all the other treatments were still alive. For tended larvae, those tended by *A. insinuator* (treatments 1 and 2) died significantly earlier than those tended by *A. echinator*, and were all dead by day 16 (figure 3). There was no difference in the pattern of survivorship between parasite and host larvae, and there was also no difference in the survivorship

Figure 3. Survivorship curves for *Acromyrmex* larvae. The treatments are those given in table 1. The open symbols represent survivorship of *A. insinuator* larvae, and the filled symbols represent that of *A. echinator* larvae. The solid curves represent the survivorship of larvae tended by *A. echinator* workers, the dotted lines represent that of larvae tended by *A. insinuator* workers and the dashed curve represents that of untended larvae.

of host larvae tended by host workers from different colonies, including their own colony.

4. DISCUSSION

To our knowledge, this is the first study to examine the association of production of sexuals by an inquiline parasite with worker production and queen number, and the first explicitly to examine task performance by inquiline parasite workers. We interpret the results of this study as evidence that the worker caste of the inquiline *A. insinuator* is adaptive, and postulate that this may be a general phenomenon that occurs during the transitional stage of many incipient inquiline parasites.

First, we found that a threshold proportion of parasite workers in the worker force of the colony is essential for the production of parasite sexuals, suggesting that parasite workers play a major role in facilitating parasite sexual reproduction. This means that parasite queens who produce no workers, or fail to reach this threshold, will have zero fitness, whereas those that do will produce at least some sexual offspring. The threshold proportion of workers needed for the production of sexuals is lower if there is more than one parasite queen present. This suggests that it is the combined suppressive power of parasite queens and workers that ultimately determines the timing of takeover and the reproductive success of the parasite. The alternative explanation, that parasite workers are merely a failed sexual brood, can be excluded because a positive rather than a negative correlation with queen number would be expected. Surprisingly, the threshold proportion of parasite workers required for parasite sexual production is independent of host queen number, suggest-

Table 3. The survivorship analysis of larvae of *A. insinuator* and *A. echinator* tended by *A. insinuator* or *A. echinator* workers. (The analysis is based on the Cox proportional hazard model of survivorship.)

source	d.f.	likelihood ratio	<i>p</i>
larval species	1	0.810	0.368
worker species	1	5.26	0.022
larval species $\times$ worker species	1	0.001 42	0.970
nested treatments (4–6)	2	0.404	0.817
control versus treatments	1	37.81	< 0.0001

ing that the combination of parasite queens and workers is equally effective at suppressing single and multiple queens.

The relationship shown in figure 1 suggests that there is a trade-off between queen number and worker number. Multiple invasions of parasite queens into host colonies mean that fewer parasite workers need to be produced and the reproductive phase can be achieved more quickly. Genetic data from field colonies indicate that multiple unrelated parasite queens divide reproduction between them (S. Sumner, unpublished data). Therefore, at low queen numbers females will ultimately achieve a higher proportion of the reproductive output of the colony, but at a considerable cost in terms of worker production and with an ensuing time delay. Polygyny will be adaptive when the total fitness gain from fast reproduction and low individual investment in worker production exceeds the fitness loss from sharing reproduction. Polygyny among parasite queens may even result in intraspecific hyperparasitism, where some queens cheat by not producing workers and parasitizing the suppressive worker force produced by other queens. Likewise, changes in allocation strategies based on local resource competition between parasite queens may occur. Genetic studies of offspring assignment in polygynous colonies are required to determine the details of reproductive strategies.

The second important observation from our study is that parasite workers can discriminate between parasite and host larvae (prediction 3). This means that they may be capable of assessing the degree to which host queen reproduction has been suppressed. This feedback mechanism enables workers to determine when to start rearing parasite brood as sexuals. Because host and parasite workers were equally adept at brood discrimination, selective rearing of parasite brood by parasite workers is unlikely to explain host suppression. However, it would be interesting to examine whether parasite workers are capable of sexualizing diploid broods better than host workers, and whether host workers selectively eliminate parasite brood. In the laboratory it has been repeatedly observed that large parasite larvae are dumped among the waste outside the nest, particularly when several parasite queens are reproducing within a single small fungal garden (J. J. Boomsma, unpublished data). This may indicate that selective elimination of parasites by hosts is possible, but only in the late stages of development. Our results also indicate that the parasite has not evolved cryptic brood, as has been observed in many social parasites whose brood mimic the chemical or physical signatures of their host (e.g. Brook & Davies 1988; Maschwitz *et al.* 2000). So long as *A. insinuator* workers continue to play a key role in queen

suppression, selection for completely cryptic parasite brood in *A. insinuator* will be weak because of their need to assess host suppression.

In concordance with previous observations (Sumner *et al.* 2003b), *A. insinuator* workers showed a regression in their performance of typical worker tasks, in that the survivorship of parasite-tended larvae was significantly lower than that of host-tended larvae (prediction 4). The survivorship of larvae cared for by nest-mate workers did not differ significantly from that of larvae cared for by non-nest-mate workers, suggesting either that worker brood care is not motivated by kinship, or that kin and non-kin cannot be distinguished in the absence of choice.

The suppression of host queen reproduction by parasitic inquiline is a general phenomenon. Observations on the inquiline *K. yaleogyna* indicate that host sexual production is completely suppressed (Wilson & Brown 1956), although the mechanism of suppression is unknown. Ovarian development in host queens of *Polistes biglumis bimaculatus* is suppressed by the presence of the inquiline *P. atrimandibularis*, probably through physical aggression (Cervo & Lorenzi 1996). In the workerless inquiline *Plagiolepis xene*, sexual brood have evolved to be similar in size to host worker brood, so that parasite sexuals are raised even when the host is actively preventing its own sexual reproduction (Aron *et al.* 1999). The same species is also known to reduce host worker production through oophagy (Passera *et al.* 2001). Control over host reproduction is also likely to occur through chemical suppression of host queen ovulation, in a similar way to the suppression of ovarian development that occurs commonly in intraspecific conflicts over reproduction in ants. Chemical analyses of social parasites make it clear that they produce a range of chemicals that have many uses, e.g. mimicry, camouflage and repellents (reviewed by Lenoir *et al.* (2001)). Future research may uncover whether any of these chemicals play a role in actively suppressing host queen reproduction.

The mechanism by which workers of incipient inquilines such as *A. insinuator* inhibit host queen reproduction is not yet known, although it is likely to be physical or through a simple form of chemical inhibition. The size of sexuals cannot account for the apparent suppression of *A. echinator*, since *A. insinuator* sexuals are only slightly smaller than host sexuals and are much larger than host workers (Schultz *et al.* 1998). Furthermore, parasite sexuals are produced at approximately the same time as host sexuals in the field, and so host workers cannot be deceived into raising parasite sexuals, as in *P. xene*. Consumption of host brood by *A. insinuator* workers was never observed in our experiments, but selective oophagy

remains possible. Both physical and chemical mechanisms of suppression represent independent potentially adaptive tactics: incipient inquilines such as *A. insinuator* are likely to use physical force, but will not necessarily evolve chemical suppression. However, subsequent selection for a more complex mechanism of (chemical) suppression that does not require a worker caste is likely to be strong, as that fraction of the parasite's resources invested in worker production, the equivalent of 'somatic tissue', could instead be invested in sexual reproduction.

The findings of this study and previous work on *A. insinuator* (Schultz *et al.* 1998; Bekkevoeld & Boomsma 2000) indicate that there have been at least two independent selection pressures involved in its evolution. First, newly mated parasite queens must invade host colonies without detection. There is ample evidence that wasp and ant social parasites are able to acquire the chemical recognition profile of their host's colony and thus go undetected (Lenoir *et al.* 2001; Sledge *et al.* 2001). *A. insinuator* queens often bury themselves in the fungus garden, where they are likely to acquire some of the nest odour passively. A second step is the suppression of host-queen reproduction such that an increased proportion of the colony's resources can be channelled into the parasite's sexual reproduction. Multi-step selection has also been identified in other forms of parasitism. For example, in avian brood parasites, cuckoo eggs have been selected to mimic the appearance of host eggs (Brook & Davies 1988). Following hatching, the cuckoo chick must monopolize the resources provided by the host. This is not done via visual mimicry of the host young, but by mimicking the calls of the host chick (Davies *et al.* 1998). Just as in *A. insinuator*, this process involves selection in at least two contexts: visual mimicry and auditory mimicry.

We suggest that adaptive workers may represent a common transitional phase in many lineages of inquiline social parasites. Although cases in which inquiline parasite workers have been identified are apparently rare, it is quite possible that incipient inquiline species (which have a higher likelihood of retaining a worker caste) have been overlooked owing to their morphological similarity to the host species. *Acromyrmex insinuator* is a case in point, as both the sexuals and the cryptic worker caste were identified for the first time through genetic analysis and not morphology (Schultz *et al.* 1998). New inquilines are likely to lose their worker caste rapidly through natural selection if parasite queens evolve less costly mechanisms of suppression. For such inquilines, we predict that any workers produced will have little or no adaptive role (e.g. *Plagiolepis grassei*; Passera *et al.* 2001), and that the morphological divergence of host and parasite is likely to be more pronounced. However, when the evolution of chemical host suppression by parasite queens is constrained, the worker caste may be modified through natural selection (e.g. as in *A. insinuator*). An independent lineage of advanced and workerless *Pseudoatta* social parasites has evolved within the genus *Acromyrmex* (Sumner *et al.* 2003a). However, fungus rearing may generally constrain the evolution of chemical suppression by social parasites, as colony odour is likely to be acquired by an invading queen from the fungus garden (Lenoir *et al.* 2001). Further study on the worker castes of other inquiline species and on the behaviour and chemical mimicry

of *Pseudoatta* queens is encouraged to ascertain whether the phenomenon is indeed widespread.

The authors thank J. Bot, M. Brown, S. Van Borm, B. Baer, W. Hughes, M. Poulsen and M. Dijkstra for help in colony collection, and S. Mathiasen for help in rearing laboratorial colonies. They also thank A. Bourke, M. J. West-Eberhard and N. Isaac for valuable contributions to an earlier version of the manuscript. They thank the Smithsonian Tropical Research Institute for providing facilities in Gamboa, and the Instituto Nacional de Recursos Naturales Renovables for permission to export ant colonies to Denmark. This work was supported by the European Commission's programmes on Training and Mobility of Researchers (FMRX-CT960072) and Improving Human Potential (HPRN-CT-2000-00052).

REFERENCES

- Alloway, T. M. 1979 Raiding behaviour of two species of slave-making ants, *Harpagoxenus americanus* (Emery) and *Leptothorax duloticus* Wesson (Hymenoptera: Formicidae). *Anim. Behav.* **27**, 202–210.
- Aron, S., Passera, L. & Keller, L. 1999 Evolution of social parasitism in ants: size of sexuals, sex ratio and mechanisms of caste determination. *Proc. R. Soc. Lond. B* **266**, 173–177. (DOI 10.1098/rspb.1999.0618.)
- Beath, K. J. 2002 GLMSTAT 5.7.0—generalised linear modelling software. See <http://members.ozemail.com.au/~kjbeath/glmstat.html>.
- Bekkevoeld, D. & Boomsma, J. J. 2000 Evolutionary transition to a semelparous life history in the socially parasitic ant *Acromyrmex insinuator*. *J. Evol. Biol.* **13**, 615–623.
- Bekkevoeld, D., Frydenberg, J. & Boomsma, J. J. 1999 Multiple mating and facultative polygyny in the Panamanian leaf-cutter ant, *Acromyrmex echinator*. *Behav. Ecol. Sociobiol.* **46**, 103–109.
- Brook, M. D. L. & Davies, N. B. 1988 Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. *Nature* **335**, 630–632.
- Cervo, R. & Lorenzi, C. 1996 Inhibition of host queen reproductive capacity by the obligate social parasite *Polistes atrimandibularis* (Hymenoptera, Vespidae). *Ethology* **102**, 1042–1047.
- Crawley, M. J. 1993 *GLIM for ecologists*. Oxford: Blackwell Scientific.
- Davies, N. B., Kilner, R. M. & Noble, D. G. 1998 Nestling cuckoos, *Cuculus canorus*, exploit hosts with begging calls that mimic a brood. *Proc. R. Soc. Lond. B* **265**, 673–678. (DOI 10.1098/rspb.1998.0346.)
- Foitzik, S. & Herbers, J. M. 2001 Colony structure of a slave making ant. I. Intracolony relatedness, worker reproduction and polydomy. *Evolution* **55**, 307–315.
- Hölldobler, B. & Wilson, E. O. 1990 *The ants*. Berlin: Springer.
- Lenoir, A., D'Ettorre, P. & Errard, C. 2001 Chemical ecology and social parasitism in ants. *A. Rev. Entomol.* **46**, 573–599.
- Maschwitz, U., Dorow, W. H. O., Buschinger, A. & Kalytta, G. 2000 Social parasitism involving ants of different subfamilies: *Polyrhachis lama* (Formicinae) an obligatory inquiline of *Diacamma* sp. (Ponerinae) in Java. *Insectes Soc.* **47**, 27–35.
- Nonacs, P. & Tobin, J. E. 1992 Selfish larvae: development and the evolution of parasitic behaviour in the Hymenoptera. *Evolution* **46**, 1605–1620.
- Passera, L., Gilbert, M. & Aron, S. 2001 Social parasitism in ants: effects of the inquiline parasite *Plagiolepis xene* St. on queen distribution and worker production of its host *Plagiolepis pygmaea* Latr. *Insectes Soc.* **48**, 74–79.
- Schultz, T. R., Bekkevoeld, D. & Boomsma, J. J. 1998 *Acromyrmex insinuator* new species: an incipient social parasite of fungus-growing ants. *Insectes Soc.* **45**, 457–471.

- Sledge, M. F., Dani, F. R., Cervo, R., Dappoerto, L. & Turillazzi, S. 2001 Recognition of social parasites as nest-mates: adoption of colony-specific host cuticular odours by the paper wasp parasite *Polistes sulcifer*. *Proc. R. Soc. Lond. B* **268**, 2253–2260. (DOI [10.1098/rspb.2001.1799](https://doi.org/10.1098/rspb.2001.1799).)
- Sumner, S., Aanen, D. K., Delabie, J. & Boomsma, J. J. 2003a The evolution of social parasitism in *Acromyrmex* leaf-cutter ants: a test of Emery's rule. *Insectes Soc.* (Submitted.)
- Sumner, S., Hughes, W. O. H. & Boomsma, J. J. 2003b Evidence for differential selection and potential adaptive evolution in an inquiline social parasite. *Behav. Ecol. Sociobiol.* (Submitted.)
- Ward, P. S. 1996 A new workerless social parasite in the ant genus *Pseudomyrmex* (Hymenoptera: Formicidae), with a discussion of the origin of social parasitism in ants. *Syst. Entomol.* **21**, 253–263.
- Wcislo, W. 1987 The roles of seasonality, host synchrony, and behaviour in the evolution and distribution of nest parasites in Hymenoptera (Insecta), with special reference to bees. *Biol. Rev.* **62**, 515–543.
- Weber, N. A. 1972 Gardening ants: the attines. *Mem. Am. Phil. Soc. Philadelphia* **92**, 1–146.
- Wheeler, D. E. 1986 Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *Am. Nat.* **128**, 13–34.
- Wheeler, D. E. 1991 The developmental basis of worker cast polymorphism in ants. *Am. Nat.* **138**, 1218–1238.
- Wilson, E. O. 1984 Tropical social parasites in the ant genus *Pheidole*, with an analysis of the anatomical parasitic syndrome (Hymenoptera: Formicidae). *Insectes Soc.* **31**, 316–334.
- Wilson, E. O. & Brown, W. L. 1956 New parasitic ants of the genus *Kyidris*, with notes on ecology and behaviour. *Insectes Soc.* **3**, 439–454.
- Wilson, E. O. & Brown, W. L. 1958 The worker caste of the parasitic ant *Monomorium metoecus* Brown and Wilson, with notes on behaviour. *Entomol. News* 33–38.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.