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ORIGINAL ARTICLE

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Evidence for differential selection and potential adaptive evolution in the worker caste of an inquiline social parasite

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Abstract Social parasites exploit the socially managed resources of social insect colonies in order to maximise their own fitness. The inquilines are among the most specialised social parasites, because they are dependent on being fully integrated into their host's colony throughout their lives. They are usually relatives of their host and so share ancestral characteristics (Emery's rule). Closely related inquiline-host combinations offer a rare opportunity to study trade-offs in natural selection. This is because ancestral adaptations to a free-living state (e.g. the production of a worker caste) become redundant and may be replaced by novel, parasitic traits as the inquiline becomes more specialised. The dynamics of such processes are, however, unknown as virtually all extant inquiline social parasites have completely lost their worker caste. An exception is *Acromyrmex insinuator,* an incipient permanent social parasite of the leaf-cutting ant *Acromyrmex echinatior.* In the present study, we document the size distribution of parasite and host workers and infer how selection has acted on *A. insinuator* to reduce, but not eliminate, its investment in a worker caste. We show that the antibiotic producing metapleural glands of these parasite workers are significantly smaller than in their host counterparts and we deduce that the metapleural gland size in the host represents the ancestral state. We further show experimentally that social parasite workers are more vulnerable to the general insect pathogenic fungus *Metarhizium* than are host workers. Our findings suggest that costly disease resistance mechanisms are likely to have been lost early in inquiline evolution, possibly because active selection for maintaining these traits became less when parasite workers had evolved the ability to exploit the collective immune system of their host societies.

Keywords Inquilinism · Metapleural gland · Disease resistance · *Metarhizium* · Leaf-cutting ant

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Introduction

In the same way as viruses, bacteria and other (non-social) parasitic organisms, social parasites are selected to manipulate host resources to maximise their own fitness at their host's expense. However, social parasites differ from their non-social counterparts in that they exploit the socially managed resources of societies, rather than just an individual. Furthermore, they often share common ancestry with their host (Emery 1909; Ward 1989; Bourke and Franks 1991). Selective forces acting on the evolution of social parasitism can therefore be unravelled by comparing evolutionarily young social parasites with their sister species hosts.

Social parasites are commonly found in the social Hymenoptera, e.g. bees (Pedersen 1996), wasps (Cervo and Dani 1996) and ants (Buschinger 1986), where they specialise in parasitising a single or few host species (Wilson 1971). Inquiline social parasites show the greatest degree of specialisation because, unlike temporary (colony founding) and slave-maker social parasites, most of their life-cycle depends on the continued functioning of their host's society. They therefore have no need to retain many of the characteristics required for independent colony life and so costly traits whose functions are otherwise fulfilled by the host will no longer be maintained by natural selection. By investing less in traits that are essential for free-living species, the parasite may redirect resources into processes that enhance host manipulation, offspring colony growth, and rate of reproduction, i.e. allocation processes that make a more substantial contribution to its reproductive success. In inquiline systems where this evolutionary development has been completed, we thus expect a reduction or degradation of traits associated with resource acquisition, nest building, brood care and defence, relative to the host (e.g. Hölldobler and Wilson 1990; Aron et al. 1999). In evolving away from a free-living state, however, a parasite is likely to become more vulnerable in the face of unpredictable events like disease. The fact that almost all known inquiline social parasites have completely lost their worker caste supports this hypothetical scenario (Hölldobler and Wilson 1990), but has at the same time prevented direct tests of the evolutionary dynamics of the process. In this paper, we examine an exceptional inquiline social parasite of leaf-cutting ants that has retained part of its worker caste and we assess the order and degree to which an important free-living trait associated with disease defence has been lost.

Leaf-cutting ants live in obligatory mutualism with a clonal fungus that provides them with nourishment (Weber 1972). Because neither ant nor fungus can survive without the other, the system is vulnerable to diseases and parasites. Queen and worker leaf-cutting ants have a complex suite of defence strategies, one of which is the pair of metapleural glands which secrete a mix of compounds with antibiotic, fungicidal and herbicidal characteristics (Hölldobler and Engel-Siegel 1984; Beattie et al. 1986; Ortius-Lechner et al. 2000a; Bot et al. 2002). Metapleural glands are ancestral in ants, having only been lost secondarily in a few genera (Hölldobler and Engel-Siegel 1984; Hölldobler and Wilson 1990), and are well developed in all the species of leaf-cutting ants so far examined: *Acromyrmex octospinosus*, *Acromyrmex echinatior*, *Acromyrmex balzani*, *Acromyrmex rugosus*, *Acromyrmex subterraneus subterraneus*, *Atta cephalotes*, *Atta colombica*, *Atta sexdens* (Wilson 1980; Bot and Boomsma 1996; W.O.H. Hughes unpublished data). The glands are positioned at the posterior end of the mesosoma such that their secretions can flow out over the cuticle or be actively applied by the ants (Bot et al. 2001; Murakami and Currie 2002). The role of the secretions in defence against disease is well documented in ants generally (Maschwitz et al. 1970; Veal et al. 1992) and is thought to be of further importance in leaf-cutting ants for controlling pathogens and micro-organisms in the fungus garden (North et al. 1997; Murakami and Currie 2002). Recently, Poulsen et al. (2002) provided experimental in vivo evidence for the hygienic significance of the gland secretions of workers in *Acromyrmex* colonies and showed that the metabolic expenses of metapleural gland secretions amount to 10–20% of the basal metabolic rate of workers. The costliness of this defence system implies that functional aspects of metapleural gland secretions are likely to be lost when selection for disease resistance is relaxed.

Acromyrmex insinuator Schultz, Bekkevold and Boomsma 1998 is a social parasite of the leaf-cutting ant *Acromyrmex echinatior* Forel 1899. It is one of the rare inquiline parasites that produce workers: a variable number of minor (small) workers (Bekkevold and Boomsma 2000) and rarely, a few major (medium-large) workers are produced (this study). Together with the parasite sexuals, the workers closely resemble their host counterparts in size and morphology and were previously identifiable only through molecular analysis (Schultz et al. 1998). Such a close resemblance between a social parasite and its host is generally thought to be characteristic of incipient social parasites that represent an early stage in the evolution of inquilinism (Hölldobler and Wilson 1990). Morphological data and DNA sequence data indicate that *A. insinuator* and *A. echinatior* are probably sister species, sharing a common ancestor as predicted by Emery's rule (Schultz et al. 1998; S. Sumner et al., unpublished data).

In spite of its inferred incipient state and minor morphological modifications, earlier work showed strong modifications in essential life history traits of *A. insinuator*, such as a semelparous mode of reproduction (Bekkevold and Boomsma 2000). Here we examine the degree to which an important worker function has been lost or modified by a social parasite. In particular, we test the prediction that *A. insinuator* invests less in costly metapleural gland secretions than its host. The external diameter of the reservoir of the metapleural gland (the bulla) is a good indicator of the number of secretory cells it contains (Bot et al. 2001). We therefore investigated whether parasite workers have smaller glands that produce less secretion relative to their hosts and whether they are consequently less resistant to disease. We compared the gland sizes of parasite and host workers and examined their relative disease susceptibility using *Metarhizium anisopliae* as a model parasite. This is a generalist entomopathogenic fungus with which these leaf-cutting ant species have regular contact. It can be highly pathogenic to the ants (Kermarrec et al. 1986; Jaccoud et al. 1999; Hughes et al. 2002) whose main defence mechanism is through metapleural gland secretions (Poulsen et al. 2002).

Methods

Parasitised colonies of *Acromyrmex echinatior* were collected from secondary rain-forest in Gamboa, Panama in April 2000 and 2001. Live colonies, complete with fungus, workers and queens of host and parasite were transported to the laboratory and maintained at a constant temperature (25°C) and humidity (75% relative humidity) on a diet of bramble leaves and rice.

Metapleural gland size

Samples of 50 minor (small) workers (<5 mm long) from two field colonies (colonies 102 and 113) were taken for DNA analysis. Larger workers (>5 mm long) were also sampled from colony 113, although parasite workers of this size are rarely produced in the field, having been found in only 2 out of 21 host colonies investigated (J.J. Boomsma, unpublished data). At the time of collection colonies 102 and 113 were in an advanced state of the parasitic life-cycle, producing many parasite sexuals. DNA was extracted from a single leg from each worker using the DNA extraction kit DNeasy (Qiagen) and amplified at the microsatellite locus Ech3385 (Ortius-Lechner et al. 2000b). Reactions were performed in 10 μl volumes of 1× reaction buffer, 0.2 mM dNTPs, 0.5 μM primers, with 0.25 U of Taq polymerase. Amplification proceeded with an initial denaturing step of 96°C for 2 min, followed by 25 cycles of 94°C for 30 s, an annealing temperature of 50°C for 30 s, and an elongation step at 72°C for 30 s. A final elongation step of 72°C for 10 min completed the amplification process. Products were run on 2% agarose gels and were stained with ethidium bromide. Amplification products for parasites and hosts at locus Ech3385 are known to differ by at least 50 base-pairs in size (Ortius-Lechner et al. 2000b) and this difference can easily be seen on 2% agarose gels when run with the size standard $\varphi\chi174$.

Twenty parasite and 20 host minor workers identified by molecular analysis were selected from each of the two colonies for morphometric analysis. Likewise, 13 parasite and 24 host major workers were selected from colony 113. The sample size for larger parasite workers was limited because they are rarely produced. We also measured 20 host gynes and 20 parasite gynes sampled from five colonies, in order to compare the relative size of the metapleural glands across all female castes. Gynes of parasites and hosts were distinguished morphologically following the criteria listed by Schultz et al. (1998). The abdomen and rear legs were removed from each ant and the mesosoma was mounted for measurement. This allowed each ant to be similarly positioned to maximally expose the bulla of the metapleural gland. The bulla was viewed through an Olympus SZX9 binocular microscope at 57× magnification and a photograph of the image was taken with an Olympus DP-10 camera and viewed on a computer screen. Three measurements were taken using the computer package Olympus DP-Soft 3.0: (1) the diameter of the bulla (following methods of Bot and Boomsma 1996), (2) the width of the ventral side of the pronotum, to standardise for body size variation, (3) the shortest distance from the bulla edge to the nearest spiracle, which has a comparable position in parasite and host workers and so is an independent measure of relative gland size when plotted against body size.

For the comparison of bulla width against pronotum width we included major workers. Because the castes differ in their allometric slopes, pronotum widths were log transformed to obtain a linear relationship (Bot and Boomsma 1996). In the examination of bulla-spiracle distance, only minor workers were included and so both variables were log(x+1) transformed. ANCOVA was performed on bulla width measurements and on the distance between the spiracle and bulla using species and colony as factors and pronotum width as a covariate.

Defence against the entomopathogenic fungus, Metarhizium anisopliae

In order to examine whether parasite and host workers differed in their resistance to disease, we challenged them with an entomopathogenic fungus, *M. anisopliae* var. *anisopliae*. The strain of fungus used had been isolated from soil at the colony collection site in Gamboa, Panama, and is pathogenic to *A. echinatior* (Hughes et al. 2002). It was cultured on selective media plates [65 g Sabouraud dextrose agar, 1 ml 10% dodine (*N*-dodecylguanidine monoacetate), 1 ml 10% streptomycin sulfate, 1 ml of 5% chloramphenicol, per litre of water] and spores were harvested from one plate to make up a stock suspension in a 0.05% Triton-X solution. Conidial spore viability was determined by plating on to Sabouraud dextrose agar and recording the proportion of spores germinating after 18–24 h at 25°C. Spore counts were made using a haemocytometer and the spore suspension was serially diluted to give concentrations of 5.0×10^6 (high) 5.0×10^5 (medium) and 5.0×10^4 (low) viable spores per ml.

Minor workers were taken from four *A. echinatior* colonies known to be parasitised with *A. insinuator* (colonies 111a, 112b, 146a and 131b). Based on the unambiguous correspondence between DNA typing and metapleural gland morphology obtained in the analyses described above, host and parasite workers were this time identified based on the morphology of their metapleural glands. A single mid-leg was, however, removed to allow species identification to be verified through DNA analysis, which always confirmed the morphological criteria. Each ant was then placed in an individual pot (diameter: 2.5 cm, height: 4 cm) and maintained at 25°C, 75% relative humidity with an ad libitum supply of distilled water and 10% sugar water throughout the experiment. Twelve hours after removal from their colonies, 0.5 μl of one of the three *Metarhizium* spore suspensions or the 0.05% Triton-X control solution was applied to the thorax of each ant with a micropipette. The ant was then replaced in its individual pot and its survival monitored over the following 10 days. Any ants that died were surface sterilised (Lacey and Brooks 1997), stored under humid conditions and monitored for the appearance of spores diagnostic of a *M. anisopliae* infection. The survival distributions were examined with a Cox regression analysis with species, dose and colony as variables.

Results

Metapleural gland size

PCR products for *Acromyrmex insinuator* were approximately 220 bp in size and those of *A. echinatior* were around 160 bp. A total of 48% (24/50) of minor workers sampled in colony 102 and 40% (20/50) of those in colony 113 were parasites. In colony 113, 33% of the larger workers were parasites. Bulla size was significantly reduced in parasite workers throughout their size range (Fig. 1a): the regression lines were almost exactly parallel (differences among slopes: $F_{1,73}$=0.0000003, P=0.9996), but the difference in intercept between the species was significant ($F_{1,73}$=24.45, P<0.001). The pattern found in colony 102 for minor workers was essentially the same (Fig. 1b). As only minor workers were available here, the pattern was less clear, but there was no significant difference between regression slopes ($F_{1,35}$=2.31, P=0.14) while there was a difference between the species-specific intercepts ($F_{1,35}$=7.38, P=0.01). Comparing the minor workers of both colonies in a single analysis showed that the overall species difference in intercept was significant ($F_{1,72}$=172.26, P<0.001). The effect of colonies on gland size was not significant ($F_{1,72}$=0.002, P=0.967) and neither was there any difference among slopes ($F_{1,72}$=0.018, P=0.893) nor a statistically significant interaction between colony and species ($F_{1,72}$=1.81, P=0.183). In contrast to the workers, there was no difference in relative metapleural gland size of parasite and host queens ($F_{1,36}$=2.56, P=0.118), with the slopes of the regressions being homogenous ($F_{1,36}$=2.33, P=0.136).

The distance from spiracle to bulla relative to pronotum width differed significantly in the minor workers of host and parasite (t=31.53, df=38, P<0.0001), with the bulla in parasite workers being further from the spiracle than in host workers (Figs. 2 and 3). There was also a significant difference in how bulla-spiracle distance varied with pronotum size between the two species (interaction of species, pronotum and spiracle-bulla distance $F_{1,74}$=16.95, P<0.0001). With increasing pronotum size, spiracle-bulla distance increased in parasite workers, but remained constant in host workers. Furthermore, the variance of spiracle-bulla distance was higher in

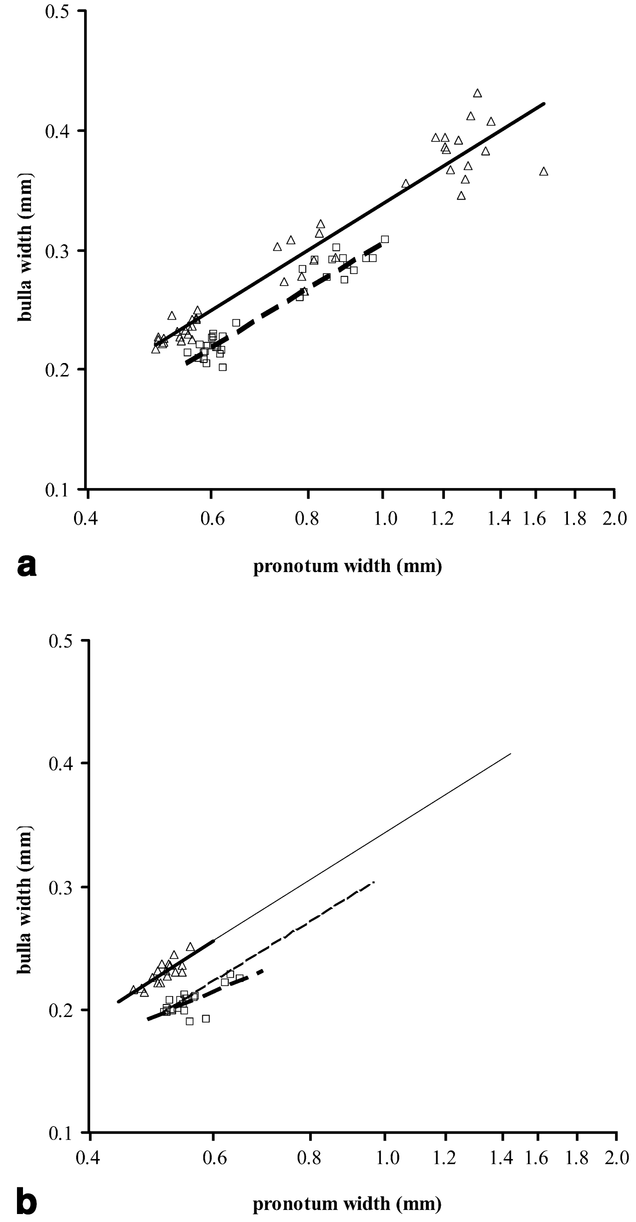

Fig. 1a, b Bulla width as a function of pronotum width with the latter log-transformed to linearise the relationship across all worker castes. *Triangles* represent host workers and *squares* parasite workers. **a** (colony 113) represents the rare situation in which *Acromyrmex insinuator* produces workers >5 mm long so that the full size range of host workers is plotted for comparison. *Lines* are fitted regressions (*solid lines* for hosts, *dashed lines* for parasites). **b** (colony 102) represents the normal situation in which the parasite only produces minor workers (<5 mm long). **b** includes both fitted regressions and the regression lines from **a** to allow direct comparison. Regression lines for **a** are y=0.394x+0.341 (r^2=0.933) and y=0.394x+0.310 (r^2=0.928) for hosts and parasites, respectively. For **b**, the lines fitted through the data for minor workers are y=0.402x+0.351 (r^2=0.633) and y=0.256x+0.275 (r^2=0.513) for hosts and parasites, respectively

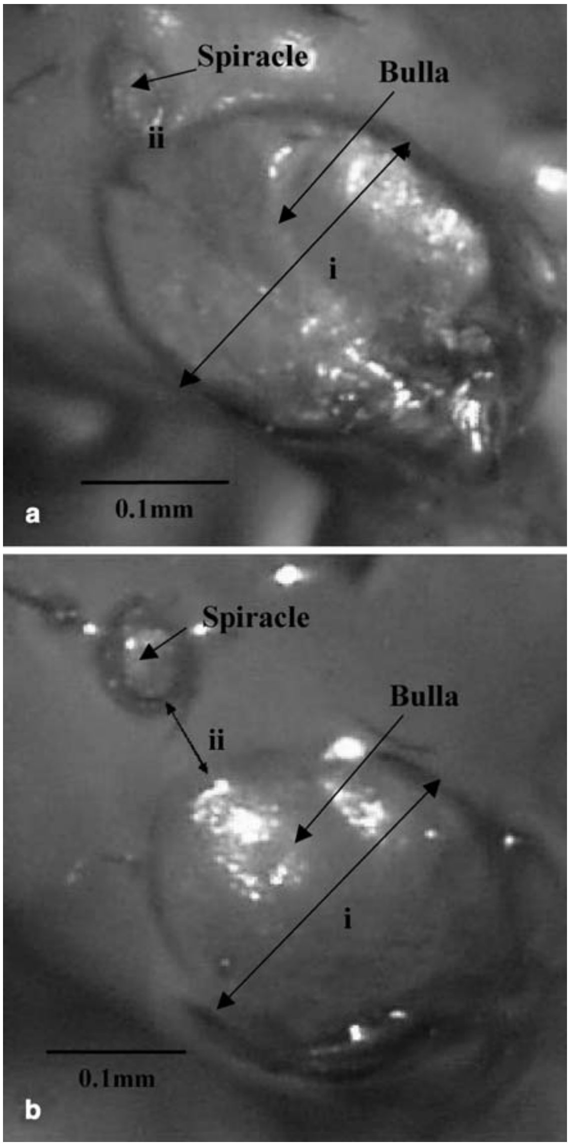

Fig. 2 Metapleural glands of **a** *Acromyrmex echinatior* and **b** *A. insinuator*. Bulla width is shown at (*i*) and the distance between the spiracle and bulla is shown at (*ii*)

parasites than in host workers ($F_{41,36}$=8.76, P<0.0001), and this difference remained significant after adjusting for variation in pronotum width (F-ratio=6.11, P<0.0001). There was no statistical effect of colony on the distance between bulla and spiracle ($F_{1,74}$=1.51, P=0.287).

Defence against the entomopathogenic fungus, *Metarhizium anisopliae*

Both species showed a significant dose-response relationship with increased dose being associated with decreased survival (Wald statistic=15.1, *df*=3, P=0.002) and there was no significant difference between the species in this relationship (Wald statistic=3.05, *df*=3, P=0.384). Species was a significant overall predictor of ant survival (Wald

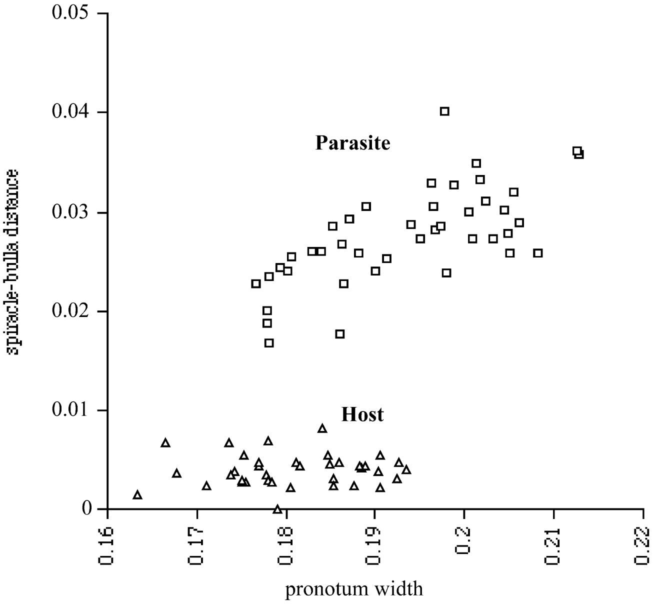

Fig. 3 The distance between the spiracle and bulla as a function of pronotum width for minor workers from colonies 102 and 113. Both variables (mm) were log(*x*+1) transformed. *Symbols* are as in Fig. 1

statistic=12.1, *df*=1, *P*<0.001). Parasite workers had hazard of death ratios that were 3.4, 3.2 and 1.6 times those of host workers at the high, medium and low doses of *Metarhizium,* respectively (Fig. 4). The source colony from which the ants were collected had no significant effect on their subsequent response to the treatments (Wald statistic=0.001, *df*=3, *P*>0.99).

Discussion

We have found that the metapleural glands of minor workers of the social parasite *Acromyrmex insinuator* are significantly smaller than those of its host *A. echinatior.* As shown earlier by Bot et al. (2001) in the closely related species *Acromyrmex octospinosus*, the external diameter of the gland is a reliable indicator of the internal gland size and number of secretory cells present. The difference in bulla size between host and parasite therefore indicates that parasite workers produce smaller quantities of metapleural gland secretions than host workers. Our experiment on disease susceptibility demonstrated that parasite workers suffered greater mortality than host workers when exposed to a pathogen. This could be related to differences in several possible resistance mechanisms, for example degenerative grooming behaviour in the parasite. However, the particular importance of the metapleural gland in leaf-cutting ant disease defence is now well established (Poulsen et al. 2002), and it seems likely that the difference in mortality relates largely to the reduced glands of parasite workers. The results indicate that the cost to parasite workers in having smaller glands is their reduced resistance to pathogens when exposed in the absence of host workers.

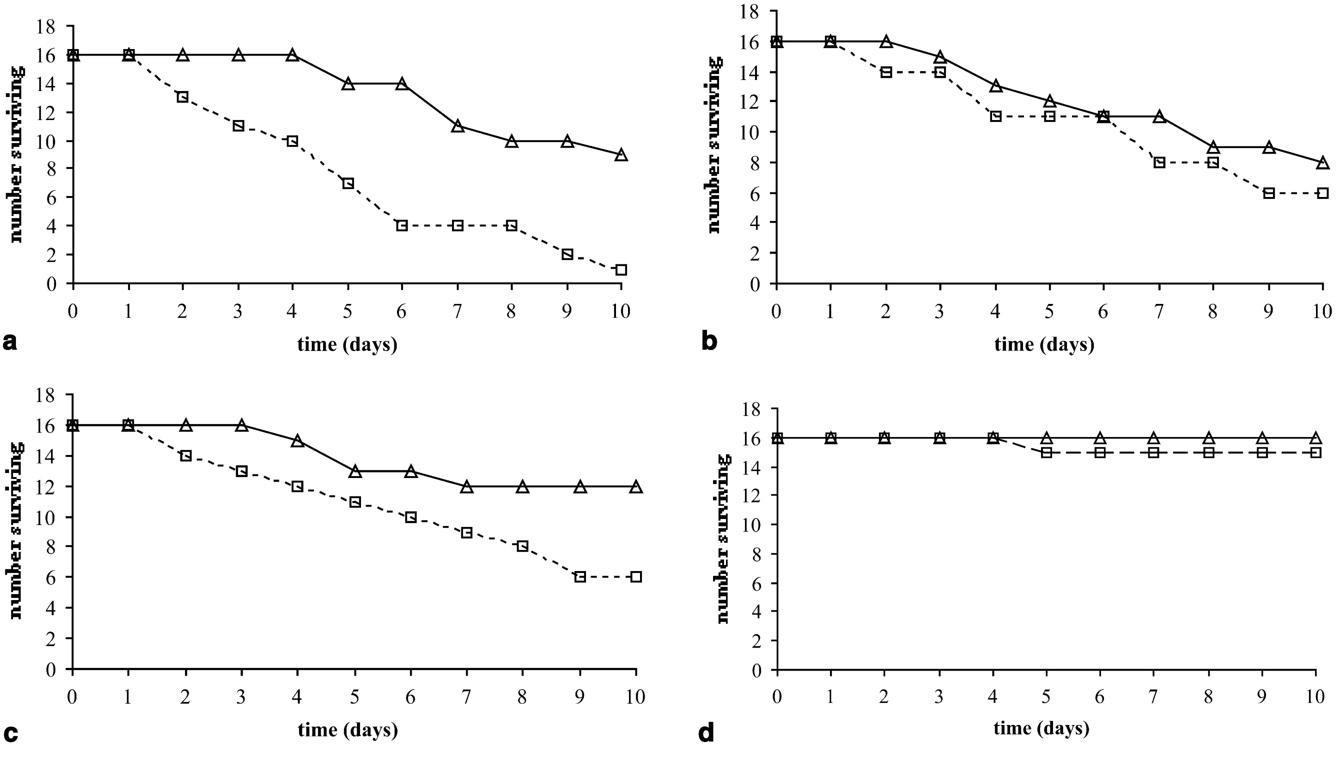

Fig. 4 Survivorship curves for parasite (*squares*) and host workers (*triangles*) for **a** high, **b** medium **c** low doses of *Metarhizium*, and **d** control

We found that the distance from the edge of the bulla of the metapleural gland to the nearby spiracle is on average 11.8% of pronotum width in parasite minor workers, whilst in host minor workers this distance is on average only 1.7% of pronotum width (Fig. 3). This difference can be seen clearly by eye (Fig. 2) and will prove very useful in future studies on this social parasite-host system, since the two worker species can now be distinguished without DNA analysis. This distance did not change with body size in host minor workers, whereas it did in parasite minor workers. This may indicate that maximal gland size in the host is constrained by the position of the spiracle, so that bullae are generally as large as they can physically be. In the parasites the distance between the bulla and the spiracle increased with pronotum width (body size), consistent with the idea that selection favours less than maximal or even minimal investment in this defence function. The difference in species-specific variance of the bulla-spiracle distance among workers supports this interpretation.

Metapleural gland secretions are crucial for *Metarhizium* resistance by free-living *Acromyrmex* ants (Poulsen et al. 2002). The cost of these secretions is substantial but is likely recouped through the fitness gained in belonging to a colony of low vulnerability to disease. For the parasite, the benefits of investing in disease defence are smaller if they are less exposed to infections than host workers or if they are able to compensate their lack of personal defences by exploiting the collective immune system of their host.

Although the exact mechanism of speciation for social parasites is still under debate, Emery's rule (Emery 1909), which states that social parasites and their original hosts are sister species, is well regarded (Wilson 1971; Ward 1989; Buschinger 1990; Bourke and Franks 1991; Lowe et al. 2002) and the species pair *A. insinuator* and *A. echinatior* has been considered a prime illustration of the strict version of Emery's rule (Schultz et al. 1998; S. Sumner et al. unpublished data). Knowledge of their shared ancestry enables us to draw some important conclusions about the selective forces acting in inquiline evolution. Workers of all *Acromyrmex* species examined to date have similarly large metapleural glands to workers of *A. echinatior*. Most importantly, workers of *A. octospinosus*, the sister species to *A. echinatior*+*A. insinuator* (S. Sumner et al. unpublished data), have large glands and the allometry of bulla diameter (metapleural gland size) and pronotum width for *A. octospinosus* and *A. echinatior* follows the same trajectory throughout the total size range of workers (Bot and Boomsma 1996). This clearly indicates that large gland size is the ancestral state of the *A. echinatior*+*A. insinuator* sister group. Moreover, this pattern holds at a larger scale throughout free living *Acromyrmex* species in Latin America (W.O.H. Hughes unpublished data). Our findings thus indicate that metapleural gland size in *A. insinuator* has been secondarily reduced since speciation from the host lineage, and that this is likely due to changes in selection pressures imposed during the transition from a free-living to a parasitic life style.

The differences in gland size that we observed across queen and worker castes of *A. insinuator* demonstrate an adaptive phenotypic plasticity in inquiline evolution (West-Eberhard 1989). Parasite queens have metapleural glands of similar size to host queens, whilst parasite workers have dramatically smaller metapleural glands than their host counterparts. This may have arisen through differential selection on queen and worker castes of the parasite. It has previously been noted that the queens of some workerless inquiline species exhibit atrophied metapleural glands (e.g. *Teleutomyrmex schneideri*, Brown 1968), whilst others have well developed metapleural glands, as we observe in *A. insinuator* (e.g. *Myrmecia inquilina* and the inquiline leaf-cutting ant *Pseudoatta argentina*, Brown 1968). The comparable gland sizes of queen species in our study suggests that *A. insinuator* queens are exposed to similar infection risks as host queens, perhaps prior to and during invasion of the host colony. In contrast, parasite workers are unlikely to leave the colony and are thus less exposed to pathogens and/or may rely on the collective immune system of host workers (see above). Recently, it was shown that groups of *A. echinatior* workers are more resistant to *Metarhizium* than are individual workers (Hughes et al. 2002). *A. insinuator* workers may therefore be exploiting the disease defence system of its host as a collective group.

Inquiline workers are often perceived as being the vestigial remains of an ancestral free-living state, having no constructive role, and that in due course will be removed by natural selection (Wilson and Brown 1958; Wilson 1984; Hölldobler and Wilson 1990; Nonacs and Tobin 1992). In support of this contention, most inquiline ants have completely lost their worker caste and of those that have not, workers perform poorly in typical worker functions (Hölldobler and Wilson 1990). However, recent work suggests that workers of inquiline parasites may sometimes perform special tasks that could augment the inquiline's fitness, such as selective brood carrying (Maschwitz et al. 2000) and suppression of host reproduction (Sumner et al. 2003). The association between degeneration of free-living behaviours and the emergence of special parasitic behaviours has been observed in other social parasites such as the workers of slave-maker ants, which contribute little through domestic duties but are specialised to raid neighbouring host colonies for obtaining slaves (Stuart and Alloway 1985). Although non-adaptive morphological degeneration could explain the small bulla size in *A. insinuator* workers, this seems unlikely given that similar degeneration has not occurred in any other morphological features of the workers: aside from their metapleural glands, parasite and host workers are morphologically identical (Schultz et al. 1998). In contrast to this morphological stasis, evolutionary changes in life history and behaviour (Bekkevold and Boomsma 2000) and caste expression (Schultz et al. 1998; this study) have been substantial. A more advanced end point of inquiline evolution with a completely workerless state has been documented in *Pseudoatta* (=*Acromyrmex*; Hölldobler and Wilson 1990; S. Sumner et al., unpublished data), a morphologically derived inquiline parasite of South American *Acromyrmex* species (Santschi 1926; Gallardo 1929).

Our study documents some important differences in the morphologies of an inquiline parasite and its host and their relative abilities to defend against disease. For *A. insinuator* workers, the pressure for parasitic behaviour (and small metapleural glands) may be stronger than the drive for maintaining rigorous disease resistance via large metapleural glands. By cutting back on costly metapleural gland functions, parasite workers may be able to invest more energy in other tasks of a more direct benefit to their survival or reproductive success. An example of such a task in *A. insinuator* may be their role in suppressing host reproduction such that parasite sexual reproduction is promoted (Sumner et al. 2003). *A. insinuator* has apparently been selected not only to minimise its investment in worker production, but also to produce a worker caste that expresses parasitic traits, independent of those expressed by their queen caste. It remains to be seen whether this is a widespread phenomenon in other inquiline social parasites.

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References

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

Beattie AJ, Turnbull CL, Hough T, Knox RB (1986) Antibiotic production: a possible function for the metapleural glands of ants (Hymenoptera: Formicidae). Ann Entomol Soc Am 79:448–450

Bekkevold D, Boomsma JJ (2000) Evolutionary transition to a semelparous life history in the socially parasitic ant *Acromyrmex insinuator.* J Evol Biol 13:615–623

Bot ANM, Boomsma JJ (1996) Variable metapleural gland size—allometries in *Acromyrmex* leaf-cutting ants (Hymenoptera: Formicidae). J Kans Entomol Soc 69 [Suppl 4]:375–383

Bot ANM, Obermayer ML, Hölldobler B, Boomsma JJ (2001) Functional morphology of the metapleural gland in the leaf-cutting ants *Acromyrmex octospinosus*. Insectes Soc 48:63–66

Bot ANM, Ortius-Lechner D, Finster K, Maile R, Boomsma JJ (2002) Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. Insectes Soc 49:363–370

Bourke AFG, Franks NR (1991) Alternative adaptations, sympatric speciation and the evolution of parasitic inquiline ants. Biol J Linn Soc 43:157–178

Brown WLJr (1968) An hypothesis concerning the function of the metapleural glands in ants. Am Nat 102:188–191

Buschinger A (1986) Evolution of social parasitism in ants. Trends Ecol Evol 1:155–160

Buschinger A (1990) Sympatric speciation and radiative evolution of socially parasitic ants—heretic hypotheses and their factual background. Z Zool Syst Evol-Forsch 28:241–260

Cervo R, Dani FR (1996) Social parasitism and its evolution in *Polistes*. In: Turillazzi S, West-Eberhard MJ (eds) Natural history and evolution of paper-wasps. Oxford University Press, Oxford, pp 98–112

Emery C (1909) Über den Ursprung der dulotischen parasitischen und myrmekophilen Ameisen. Biol Centralbl 29:352–362

Gallardo A (1929) Note sur les moeurs de la fourmi *Pseudoatta argentina*. Rev Soc Entomol Argent 2:197–202

Hölldobler B, Engel-Siegel H (1984) On the metapleural gland of ants. Psyche 91:201–224

Hölldobler B, Wilson EO (1990) The ants. Springer, Berlin Heidelberg New York

Hughes WOH, Eilenberg J, Boomsma JJ (2002) Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. Proc R Soc Lond B 269:1811–1819

Jaccoud DB, Hughes WOH, Jackson CW (1999) The epizootiology of a *Metarhizium* infection in mini-nests of the leaf-cutting ant *Atta sexdens rubropilosa*. Entomol Exp Appl 93:51–61

Kermarrec A, Febvay G, Decharme M (1986) Protection of leaf-cutting ants from biohazards: is there a future for microbiological control? In: Lofgren CS, Vander Meer RK (eds) Fire ants and leaf-cutting ants: biology and management. WestviewPress, Boulder, Colo., pp 339–356

Lacey LA, Brooks WM (1997) Initial handling and diagnosis of diseased insects. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, London, pp 1–16

Lowe RM, Ward SA, Crozier RH (2002) The evolution of parasites from their hosts: intra- and interspecific parasitism and Emery's rule. Proc R Soc Lond B 269:1301–1305

Maschwitz U, Koob K, Schildknecht H (1970) Ein Beitrag zur Funktion der Metathorakaldrüse der Ameisen. J Insect Physiol 16:387–404

Maschwitz U, Dorow WHO, 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

Murakami T, Currie CR (2002) Evolution of garden hygiene behaviours in agriculture by ants. XIV International Congress of International Union for the Study of Social Insects, Proceedings. Hokkaido University, Sapporo, p 204

Nonacs P, Tobin JE (1992) Selfish larvae: development and the evolution of parasitic behaviour in the Hymenoptera. Evolution 46:1605–1620

North RD, Jackson CW, Howse PE (1997) Evolutionary aspects of ant-fungus interactions in leaf-cutting ants. Trends Ecol Evol 12:386–389

Ortius-Lechner D, Maile R, Morgan ED, Boomsma JJ (2000a) Metapleural gland secretions of the leaf-cutter ant *Acromyrmex octospinosus:* new compounds and their functional significance. J Chem Ecol 26:1667–1683

Ortius-Lechner D, Gertsch PJ, Boomsma JJ (2000b) Variable microsatellite loci for the leaf-cutting ant *Acromyrmex echinatior* and their applicability to related species. Mol Ecol 9:114–116

Pedersen BV (1996) A phylogenetic analysis of cuckoo bumblebees (*Psithyrus* Lepeletier) and bumblebees (*Bombus* Latreille) inferred from sequences of the mitochondrial gene cytochrome oxidase I. Mol Phylogenet Evol 5:289–297

Poulsen M, Bot ANM, Nielsen MG, Boomsma JJ (2002) Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. Behav Ecol Sociobiol 52:151–157

Santschi F (1926) Deux nouvelles fourmis parasites de l'Argentine. Folia Myrmecol Termit 1:6–8

Schultz TR, Bekkevold D, Boomsma JJ (1998) *Acromyrmex insinuator* new species: an incipient social parasite of fungus-growing ants. Insectes Soc 45:457–471

Stuart RJ, Alloway TM (1985) Behavioural evolution and domestic degeneration in obligatory slave-making ants (Hymenoptera: Formicidae: Leptothoracini). Anim Behav 33:1080–1088

Sumner S, Nash DR, Boomsma JJ (2003) The adaptive significance of inquiline parasite workers. Proc R Soc Lond B (in press)

Veal DA, Trimble JE, Beattie AJ (1992) Antimicrobial properties of secretions from the metapleural glands of *Myrmecia gulosa* (the Australian bull ant). J Appl Bacteriol 72:188–194

Ward PS (1989) Genetics and social changes associated with ant speciation. In: Breed MD, Page RE (eds) The genetics of social evolution. Westview Press, Boulder, Colo., pp 123–148

Weber NA (1972) Gardening ants: the attines. Mem Am Philos Soc Philadelphia 92:1–146

West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. Annu Rev Ecol Syst 20:249–278

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge, Mass.

Wilson EO (1980) Caste and division of labour in leaf-cutting ants (Hymenoptera: Formicidae: *Atta*) I. The overall pattern in *A. sexdens.* Behav Ecol Sociobiol 7:143–156

Wilson EO (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 EO, Brown WL (1958) The worker caste of the parasitic ant *Monomorium metoecus* Brown and Wilson with notes on behaviour. Entomol News 33–38