

How ecology shapes caste evolution: linking resource use, morphology, performance and fitness in a superorganism

S. POWELL*†

*Department of Biology, University of Missouri – St Louis, MO, USA

†Instituto de Biologia, Universidade Federal de Uberlândia, Uberlândia, Brazil

Keywords:

adaptive evolution;
Cephalotes;
colony reproduction;
ecological performance;
soldier.

Abstract

Caste evolution is a central process in the adaptive diversification of insect superorganisms. Nevertheless, how ecology shapes adaptive caste evolution remains poorly understood. Recent work with the ant genus *Cephalotes* has provided new comparative evidence that ecological specialization may drive adaptive caste specialization. Here, three key predictions of this adaptive hypothesis are supported, using a representative of the highest level of ecological specialization and the most specialized soldier phenotype. First, soldier defensive performance was maximal for the specific nesting resource used most often in nature. Second, colonies only used a specialized subset of available nesting resources and preferred the specific resource that maximizes soldier performance. Third, soldier performance and its limitations on resource use were found to have both direct and indirect consequences for colony reproduction. These findings suggest that the most specialized soldier phenotype in *Cephalotes* is indeed an adaptation to ecological specialization on a narrowly defined subset of available nesting resources.

Introduction

A central challenge of evolutionary biology is to understand how ecology shapes adaptive phenotypic diversification (reviewed in Schluter, 2000). The colony, or 'superorganism', is the principal unit upon which selection acts in derived social insect species (e.g. Seeley, 1995; Queller & Strassmann, 2002). Yet understanding of how ecology shapes phenotypic change at this level of biological organization remains relatively limited. The primary path by which the phenotype of superorganisms can change over evolutionary time is by the diversification of the individuals that make up the whole (Oster & Wilson, 1978). This is particularly true for the usually sterile worker forms in social insect colonies. These individuals can be seen as functionally analogous to nonreproductive phenotypic characters at the whole organism level (Powell, 2008). Both worker size range

and overall workforce size can be targets of selection, but the evolution of physical castes has produced the most significant patterns of phenotypic diversification (reviewed in Oster & Wilson, 1978; Hölldobler & Wilson, 1990).

Physical castes (castes hereafter) are subsets of individuals that are morphologically specialized for particular tasks within a colony's broader ecological niche (e.g. a soldier caste specialized for defence). They have evolved independently in the ants (Hölldobler & Wilson, 1990; Baroni Urbani, 1998), aphids (Stern, 1994), polyembryonic wasps (Cruz, 1981), termites (Thorne *et al.*, 2003; Thompson *et al.*, 2004; Inward *et al.*, 2007) and thrips (McLeish & Chapman, 2007) and multiple times within at least three of these lineages. Castes therefore represent an important convergent pattern in the phenotypic diversification of insect superorganisms, but what factors influence the evolution of this phenomenon?

Kin selection theory provides a powerful explanation for how specialized, sterile forms can evolve within a society (Queller & Strassmann, 2002; Foster *et al.*, 2006; Strassmann & Queller, 2007). However, caste evolution is likely to be constrained by a number of forces,

Correspondence: Scott Powell, Department of Biology, University of Missouri – St Louis, R223 Research Building, One University Boulevard, St Louis, MO 63121-4499, USA.
Tel.: +1 314 445 9912; fax: +1 314 516 6233;
e-mail: scottpowell@mac.com

including intracolony conflict, the timing of queen-worker divergence, developmental mechanisms and the level of genetic diversity (e.g. Franks & Norris, 1987; Wheeler, 1991; Bourke, 1999; Fjerdingstad & Crozier, 2006; Smith *et al.*, 2008). Nevertheless, in lineages where these constraints are not present, caste diversification can be pronounced (e.g. de Andrade & Baroni Urbani, 1999; Schöning *et al.*, 2005; Powell & Franks, 2006; Pie & Traniello, 2007; Moreau, 2008). The interspecific differences in these lineages can be cautiously interpreted as adaptations to different ecology (Powell, 2008) but how ecology shapes adaptive caste evolution is not well understood. This aspect of caste evolution will be the focus of the present study.

The classic theoretical framework for studying the ecology of caste evolution is based on optimization theory (Oster & Wilson, 1978). The central assumption of this theory is that natural selection should optimize the ergonomic efficiency of colony structure and organization to a local fitness maximum set by the environment (Oster & Wilson, 1978, p. 175). The ideas and approaches of this theory have dominated the literature on the organization of social insect colonies. However, critical empirical tests of the fit between different aspects of caste structure and local ecological condition have been few and yielded mixed levels of support for the theory (e.g. Walker & Stamps, 1986; Beshers & Traniello, 1994, 1996; Kaspari & Byrne, 1995). Full application of the ergonomic optimization theory of caste evolution requires detailed quantification of both caste structure and the distribution of resources used by the colony (Oster & Wilson, 1978, p. 26). This task is prohibitively difficult for many social insect taxa, which may explain why the theory has not been more widely tested. Although Oster and Wilson's work remains an inspirational treatise on the ecology of caste evolution, alternative empirical approaches are likely to offer valuable new insights into the issue.

The comparative method (Harvey & Pagel, 1991) and experimental studies of organismal performance (Arnold, 1983) represent powerful and complementary approaches to studying adaptation (Schluter, 2000; Irschick, 2002). Comparative analyses can provide correlative evidence of evolutionary relationships between particular aspects of ecology and phenotype within lineages (Harvey & Pagel, 1991; Schluter, 2000). These relationships may suggest new adaptive hypotheses or serve as tests of existing ones. Performance studies, on the other hand, allow direct, experimental tests of putative adaptive relationships between ecology and phenotype. Ideally, such studies should address whether particular phenotypic characters produce maximal performance under commonly encountered ecological conditions, whether these conditions are actively selected by the organism, and whether the ecology–performance relationships yield fitness benefits (Schluter, 2000; Irschick, 2002, 2003). Support for these causal links

between an organism's ecology (e.g. resource use), morphology, performance and fitness provides compelling evidence for the process of adaptive evolution by natural selection (Schluter, 2000; Irschick, 2002, 2003). To date, comparative analyses of the relationships between ecology of caste evolution have been limited (e.g. Schöning *et al.*, 2005; Powell & Franks, 2006; Moreau, 2008; Powell, 2008) and performance studies that are informed by comparative patterns are rarer still (e.g. Powell & Franks, 2005).

Of focus here is the recent comparative evidence for an evolutionary relationship between ecological specialization and soldier specialization in *Cephalotes* ants (Powell, 2008). *Cephalotes* nest in pre-existing arboreal cavities, which they defend by blocking the cavity entrances with their armoured heads (de Andrade & Baroni Urbani, 1999; Powell, 2008; details below). Soldiers specialize in this task when present. However, extant species span four steps in the evolution of this caste, from the ancestral condition of no soldier through three discrete soldier phenotypes of increasing morphological specialization (de Andrade & Baroni Urbani, 1999; details below). Powell (2008) showed that a quantitative increase in specialization on entrances matching the head area of one ant is associated with each of the three major transitions to a more specialized soldier phenotype. This positive relationship between ecological specialization and caste specialization is significant because it is the reverse of a general prediction of the ergonomic theory of caste (Powell, 2008). However, it is concordant with often tight relationships between ecological and morphological specialization at the whole organism level (Futuyma & Moreno, 1988; Ferry-Graham *et al.*, 2002; Irschick *et al.*, 2005). Ecological specialization may therefore drive phenotypic specialization at the levels of both the whole organism and the superorganism (Powell, 2008). Building from this comparative evidence in *Cephalotes*, the next crucial step is to test the adaptive nature of the relationship between ecological specialization and soldier specialization. This can be achieved by addressing how the known relationships between resource use and soldier morphology are causally linked to soldier performance, the selection of new resources and colony fitness.

The highest level of ecological specialization in *Cephalotes* is the consistent use of cavity entrances that match the head area of one ant. This ecology correlates with the most specialized and derived soldier phenotype (Powell, 2008), which includes a disc-like structure on the dorsal surface of the head (de Andrade & Baroni Urbani, 1999; details below). If the most specialized *Cephalotes* soldier phenotype is an adaptation to highly specialized nesting ecology, the causal links that will exist between resource use, morphology, performance and fitness provide three specific predictions. First, soldier performance should be maximal for the entrance size used most often in the focal population, i.e. entrances matching the head area of

one soldier. Second, the known entrance-size specialization in the focal population should represent an evolved pattern of resource use, or 'fundamental niche' and the specific size that maximizes soldier performance should be preferred. Third, entrance-dependent differences in soldier performance, and any limitations that this places on the use of new cavities, should have consequences for colony fitness. Here, these predictions are tested using field studies of *Cephalotes persimilis*, a representative of the highest level of ecological specialization and the most specialized soldier phenotype (Powell, 2008; details below).

Materials and methods

Relevant *Cephalotes* biology

Most, if not all, members of the New World ant genus *Cephalotes* nest in the canopy or under-storey and occupy pre-existing cavities in woody stems (Creighton, 1963; Creighton & Nutting, 1965; de Andrade & Baroni Urbani, 1999; Powell, 2008). These cavities are usually the abandoned tunnels of wood-boring beetles and have relatively fixed dimensions, because *Cephalotes* cannot excavate in hard wood (de Andrade & Baroni Urbani, 1999; Powell, 2008). Each cavity is thus selected as-is from those available in the environment. Existing data on *Cephalotes* colony structure (de Andrade & Baroni Urbani, 1999; present study) suggest that a colony is typically founded and headed by a single queen (monogynous), and expands into multiple cavities soon after the colony-founding stage (polydomous). Each colony therefore consists of repeated subunits, with each cavity containing workers, soldiers (if present in the species) and brood. The brood is brought in as eggs or early-instar larvae from the one cavity that also houses the queen.

The pre-existing cavities occupied by *Cephalotes* are also used by most other arboreal ants and are generally a limited resource (Carroll, 1979; Philpott & Foster, 2005). Consequently, competition over open and occupied cavities can be intense, with frequent cavity usurpation (e.g. Davidson *et al.*, 1989; Hasegawa, 1993; Vasconcelos, 1993; Stanton *et al.*, 1999, 2002; Palmer, 2004). Nevertheless, each cavity provides a highly defensible shelter that is only accessible via a small entrance hole (i.e. the exit hole of the original occupant; Creighton, 1963; Powell, 2008). *Cephalotes* defend their cavity entrances by blocking them with their armoured heads, both individually and co-operatively, and extant species span four discrete character states in the evolution of a soldier caste specialized for this task (Creighton, 1963; Creighton & Nutting, 1965; Baroni Urbani, 1998; de Andrade & Baroni Urbani, 1999; Powell, 2008). These character states, in the order of increasing morphological specialization are: (1) no soldier (ancestral), (2) soldiers with a simple domed head, (3) soldiers with an incomplete head disc that retains characteristics of the domed head and (4)

soldiers with an elaborate and complete head disc (Baroni Urbani, 1998; de Andrade & Baroni Urbani, 1999).

Study site and focal species

Fieldwork was conducted at the reserve of Clube Caça e Pesca Itororó, Ublerlândia, Brazil, which is dominated by neotropical savannah or 'cerrado'. At this site, cerrado physiognomy ranges from *cerrado sensu stricto* to *cerrado denso* (30–50% canopy cover; Oliveira-Filho & Ratter, 2002), with a canopy height of 3–6 m. The focal species, *C. persimilis*, has a soldier caste with a complete head disc, the most specialized and derived general soldier phenotype in the genus *Cephalotes* (Baroni Urbani, 1998; see above).

Cephalotes persimilis colonies were located by placing nitrogen-rich baits on all trees on 5 × 50 m² transects. Trees with *C. persimilis* were marked for later use. Trees that lacked *C. persimilis* but had resident *Azteca* sp. and *Cephalotes pusillus* colonies were also marked for use in the soldier performance experiments (see below). High-density baiting was then used on the home trees of some *C. persimilis* colonies to identify all occupied cavities. Recruitment to baits was strong; so, occupied cavities were located by visually tracking home-bound workers. Preliminary studies established that workers from different colonies fight vigorously when in close contact. The lack of aggression among ants from different cavities was therefore used as an indicator of membership to the same colony. If ants from the different cavities on a focal tree did not interact as a result of the baiting, pairwise aggression tests were staged in a small arena. Colonies that had their cavities mapped in this way were used as source colonies for soldier performance experiments, or collected to assess the relationship between nest number, colony size and reproductive output (see below).

Natural resource use in the focal population

Cavity use by *C. persimilis* in the focal population was quantified in detail in an earlier study (Powell, 2008). The mean entrance area for the focal population is 2.4 mm². This is equivalent to the head area of one soldier (i.e. 1.0 head areas, calculated by dividing the entrance area by maximum head area of the soldier caste). This, the most commonly used entrance size, is called a 'small' entrance in the present study. Three other entrance sizes are recognized here, based on the relative frequency with which they are used by the focal species. These are as follows: 'medium', equal to the upper quartile of the distribution of entrance sizes used by the focal population (1.5 head areas or 3.8 mm²); 'large', near the maximum entrance size for the focal population (2.8 head areas, or 7.1 mm²) and 'oversized', 6% larger than the maximum entrance size in focal population (3.4 head areas or 8.6 mm²).

Resource use and soldier performance

Experimentally established *C. persimilis* cavities were used to address how cavity entrance size impacts soldier defensive performance. Twenty-four experimental cavities (Fig. 1a) were each populated with 50 workers, 10 soldiers, 15 larvae, 10 worker pupae and a small egg cluster of about 10 eggs. This composition matched the population of an average cavity in colony collections (see below), and thus mimicked a natural colony subunit. Half of the cavities were given a 'small' entrance (see above; Fig. 1b), equal to the mean entrance size in the focal population. The other half had a 'large' entrance (see above; Fig. 1c) near the maximum entrance size in the population. Cavities were held and fed in the laboratory for 5 days before the start of the field experiment. Each cavity was then placed on a naturally isolated tree that lacked an existing *C. persimilis* colony but had at least two common cavity competitors of *C. persimilis* (see above for how trees were located). The two known cavity competitors were a highly aggressive *Azteca* sp. and *C. pusillus*, a cavity generalist with cavity use that overlaps that of *C. persimilis* (Powell, 2008). Cavities were monitored ever 4 days for 80 days. Failed cavity defence was recorded when a cavity no longer contained *C. persimilis*. Any new cavity occupant was identified, and the tree was baited to determine if the cavity's original *C. persimilis* occupants had been killed outright (i.e. no trace on the tree) or had emigrated to a natural cavity. Any occupied natural cavities were collected and the contents censused (see below for

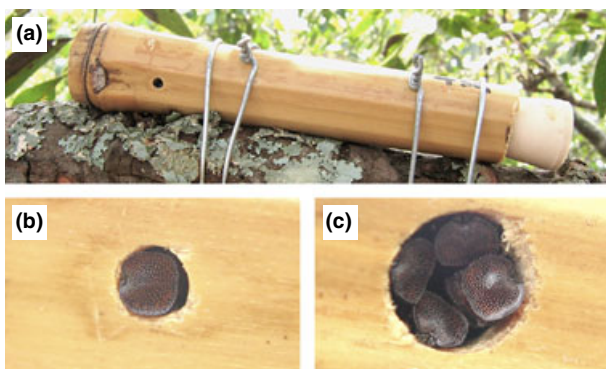


Fig. 1 Experimental cavities made from bamboo. (a) Typical cavity attachment and orientation. Bamboo sections were 70 mm long with an internal cavity diameter of 9–11 mm. The natural node was left intact at one end, whereas the other end was sealed with a latex stopper. A drilled entrance hole of controlled size was provided near the node (left) and positioned to the side. In one experiment, the entrance hole was provided in the soft wood of the node. (b) A cavity with a 'small' entrance (1.0 head areas) blocked by a single *Cephalotes persimilis* soldier and (c) a cavity with a 'large' entrance (2.8 head areas) blocked by four *C. persimilis* soldiers.

collection method). Univariate survival analysis was used to statistically analyse the differences in survival rates between entrance size treatments.

It is important to note that this experiment used cavity survival as an indirect measure of soldier performance, because no meaningful direct measure was possible. Unlike, say, running speed as a measure of locomotor performance, performance in the ecological task of cavity defence must necessarily be measured as endurance against a range of natural enemies over time. As attempts to observe the moment of defensive failure for all experimental nests would be futile, the persistence of *C. persimilis* in experimental cavities was censused regularly. This indirect measure is, however, likely to be a reliable measure of soldier performance. First, only soldiers engage in entrance defence in *C. persimilis* (Powell, 2008). Second, all experimental trees were isolated, preventing emigrations to another tree via the canopy. Third, *C. persimilis* is strictly arboreal; so, the long-distance, over-ground dispersal necessary to reach another tree would be highly uncharacteristic.

Soldier performance and selection of new resources

Experiments that provided empty cavities to wild *C. persimilis* colonies were used to test three key issues related to the selection of new resources. The first experiment served as a preliminary test of whether *C. persimilis* colonies readily select newly available cavities, providing an indication that nesting cavities are a limited resource. Ten empty cavities with small entrances (1.0 head areas) were distributed throughout the crown of each of five trees with an established *C. persimilis* colony. These cavities used an initial 'soft wood' design, with the entrance hole drilled in the soft node closing one end of the bamboo section (Fig. 1). Cavity use was then recorded weekly for 1 month by checking for individuals guarding the entrances. Empty cavities were verified as such by quickly removing the stopper to check inside. A Wilcoxon signed rank test was used to assess whether cavity selection was significantly different from zero.

The second experiment addressed whether observed cavity use in the focal population was representative of an evolved pattern of resource use, or 'fundamental niche'. Ten cavities were distributed throughout the crown of each of 20 trees with an established *C. persimilis* colony. All cavities used a final 'hard wood' design, with the entrance drilled through the hard side wall of the bamboo. Cavities with small entrances (1.0 head areas) were placed on 10 trees, whereas the other 10 trees received cavities with an oversized entrance (3.4 head areas; see above for description). Treatments were assigned randomly. Cavity use was recorded after 1 month. A Wilcoxon rank sum test was used to statistically analyse the differences in cavity selection between the two entrance size treatments.

The third experiment addressed the preferences of *C. persimilis* colonies for different entrance sizes within the natural range. Nine cavities (hard wood design) were placed throughout the crown of each of 10 trees with established *C. persimilis* colonies. Three cavities had small entrances (1.0 head areas), three had medium entrances (1.5 head areas; see above for description) and three had large entrances (2.8 head areas; see above for description). Cavity use was recorded every 4 days for 32 days. Each cavity was collected and the occupants censused at the end of the experiment (see below for collection method). Sign tests were used to statistically analyse the differences in cavity selection by each colony at the start and end of the experiment.

Soldier performance and colony reproductive output

Complete, wild colonies of *C. persimilis* were collected and censused to understand how the number of cavities held by a colony (i.e. the net outcome of cavity defence and new cavity use) is related to colony size and reproductive output. Stratified sampling was used to ensure even coverage of the colony size range. Ten *C. persimilis* colonies were collected and censused over the first 18 months of the study to assess the relationship between cavity number and colony size (i.e. total workers and soldiers). These and additional noncensused collections provided the ants for the soldier performance experiments (see above), and established that a single reproductive brood is raised each year. In mid-July, this reproductive output reaches a state of final-instar larvae, pupae and callow adults that are all still contained within a colony's cavities. Ten additional colonies were then collected in this period to establish the relationship between cavity number and reproductive output. Gynes and males are essentially the same size in *C. persimilis* (de Andrade & Baroni Urbani, 1999); so, sexing of the reproductive brood was not needed to determine the total investment of resources in reproductives. Each colony was collected by removing all branches containing occupied cavities and sealing the cavity entrances. Collections were made at 06:00–08:00 hours, when *C. persimilis* does not forage. This procedure ensured that all colony members were collected and held in their respective cavities. Cavity contents were censused in the laboratory. The relationships between nest number, colony size and reproductive output were analysed statistically with Spearman's rank correlations.

Results

Resource use and soldier performance

Soldier defensive performance was significantly higher for small cavity entrances than for large entrances (Fig. 2; univariate survival analysis with log rank test, $\chi^2 = 4.64$, d.f. = 1, $P = 0.03$). Four small-entrance cavities and nine

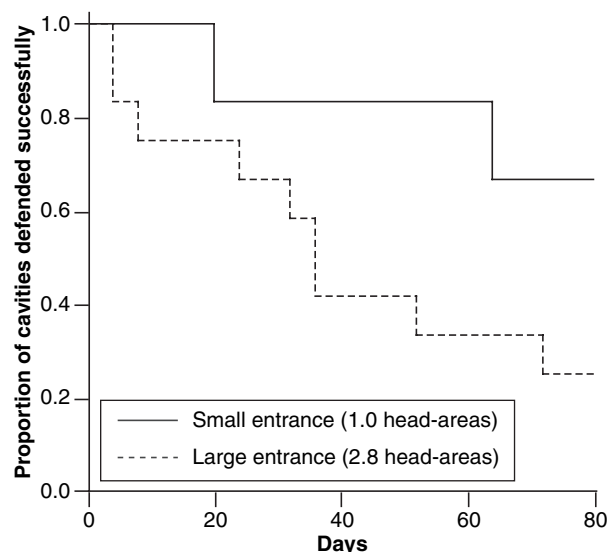


Fig. 2 The proportion of experimental cavities successfully defended by *Cephalotes persimilis* soldiers over time (product-limit survival fit plots)

large-entrance cavities were not successfully defended during the experiment. No signs of the original occupants were found for 11 of these 13 cavities, whereas the occupants of the remaining two moved to low-quality natural cavities. A different ant species replaced *C. persimilis* in 11 of 13 cavities, with two taken over by *Azteca* sp., and the remaining nine taken over by *C. pusillus*. For the two cases where the *C. persimilis* occupants moved, the experimental cavity had been taken over by another ant species and the natural cavity contained no brood and a 30% and 90% loss of adults respectively. All soldiers were lost in one case. All cavities that had been successfully defended at the end of the experiment had workers, soldiers and brood. Attacks by foreign ants were seen against all cavities (i.e. biting the heads of defending soldiers and trying to chew open the entrance hole) over the course of the experiment. At each census (20 total), all cavities that were still occupied by *C. persimilis* had soldiers in a blocking position, suggesting continuous commitment to the task of defence.

Soldier performance and selection of new resources

In the first resource selection experiment, the use of newly introduced cavities was rapid, with at least one cavity (mean 2, SD ± 0.7) used on all five experimental trees after just 1 week (Wilcoxon signed rank test, null expectation of 0 cavity use, $V = 15$, $P = 0.05$). After 1 month, the soft wood entrances of all cavities initially occupied by *C. persimilis* had been chewed open to a larger size and were occupied by larger ant species. In the

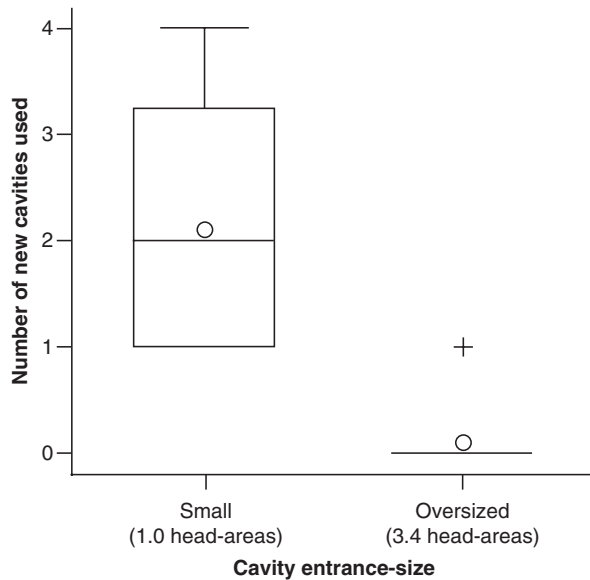


Fig. 3 Boxplots of the number of new cavities used by colonies of *Cephalotes persimilis* after 1 month. Ten colonies were assigned to each treatment and each colony initially had access to 10 unoccupied cavities. In each boxplot, the box encompasses the interquartile range, a line is drawn at the median, a circle represents the mean, whiskers extend to the nearest value within 1.5 times the interquartile range and outliers are marked with a plus sign.

second experiment, cavity occupation for small-entrance cavities was significantly higher than for oversized-entrance cavities (Wilcoxon rank sum test, $W = 2$, $P = 0.0001$; Fig. 3). Empty cavities remained on all trees. In the third experiment, no large-entrance cavities were used by *C. persimilis*. Use of small-entrance cavities was significantly higher than for medium-entrance cavities, for both the first cavities used by the colonies (sign test, $n = 10$, $P = 0.02$; Table 1) and at the end of the experiment (sign test, $n = 10$, $P = 0.02$; Table 1). Six of the 10 colonies were using some of the new cavities after 4 days (Table 1). Six of the 10 colonies subsequently experienced more than one cavity change (cavity gains and

losses), and all colonies were using at least one cavity by the end of the experiment (Table 1). At least one empty cavity of each entrance size remained on most of the 10 trees at the end of the experiment, except for the small entrance size on two trees (Table 1). Fourteen nests were occupied in total, and those with a population of only one or two individuals contained workers only (Table 1).

Soldier performance and colony reproductive output

Different colonies never occupied the same tree, and each colony had a single queen. Colonies ranged from 1 to 20 cavities, and cavity number positively correlated with both colony size (Spearman's rank correlation; $\rho = 0.83$, $P < 0.00001$) and reproductive output (Spearman's rank correlation; $\rho = 0.86$, $P = 0.001$). Moreover, reproduction was limited to colonies with greater than three nests. In the colonies of reproductive size (6 of 10 collected in the reproductive season), a mean of 75% (SD \pm 22) of the cavities contained reproductive brood.

Discussion

This study has addressed the adaptive nature of the putative relationship between ecological specialization and caste specialization in *Cephalotes* ants. This was carried out by addressing the causal links between resource use, morphology, performance and fitness in a representative of the most specialized nesting ecology and soldier morphology seen in the genus. Soldier performance, measured as cavity survival rate, was shown to be the greatest for the specific cavity entrance size used most often in the focal population, a size that matches the head area of one soldier. Colonies readily occupied newly available cavities, but they did not use cavity entrance sizes outside the narrowly defined natural range of the focal population. Moreover, within this range, colonies preferred the entrance size that maximizes soldier performance. Finally, examination of natural colony structure showed that the number of cavities that a colony holds, the net outcome of soldier performance and new cavity use, is directly and indi-

Table 1 Cavity gains over time in *Cephalotes persimilis*. Each colony was given access to three small-entrance cavities (s), three medium-entrance cavities (m) and three large-entrance cavities (l). At the end of the experiment, the individuals in each occupied cavity were categorized as workers (W), soldiers (S), larvae (L) or eggs (E).

| Colony | Days until first cavity gain | Cavity gains and losses | Final cavity gains | Final individuals in gained cavities | Empty cavities remaining |
|--------|------------------------------|-------------------------|--------------------|--------------------------------------|--------------------------|
| 1 | 8 (1s) | 1 | 1s | 3W | 2s, 2m, 3l |
| 2 | 16 (1s) | 1 | 1s | 2W, 1S | 2s, 2m, 2l |
| 3 | 24 (1s) | 1 | 1s | 3W | 1s, 3m, 1l |
| 4 | 4 (3s, 1m) | 6 | 2s | 10W, 6S, 4L, 6E; 1W, 3S | 1s, 2m, 2l |
| 5 | 4 (1s) | 11 | 2s, 1m | 1W, 2S; 2W; 1W | 0s, 2m, 2l |
| 6 | 4 (2s) | 2 | 2s | 2W; 1W | 0s, 1m, 1l |
| 7 | 4 (1m) | 3 | 1m | 3W, 2S | 1s, 1m, 3l |
| 8 | 4 (2s) | 5 | 1s | 2W, 2S | 2s, 2m, 2l |
| 9 | 8 (1s) | 1 | 1s | 3W, 1S | 2s, 2m, 3l |
| 10 | 4 (1s) | 7 | 1s | 2W, 1S | 2s, 2m, 3l |

rectly tied to colony reproductive output. These findings suggest that the most specialized soldier phenotype in *Cephalotes* is an adaptation to specialized use of cavity entrances that match the head area of one ant. More broadly, they also provide the first experimental support for the general hypothesis that ecological specialization shapes adaptive caste specialization (Powell, 2008).

In general terms, if an adaptive fit exists between an organism's environment and phenotype, performance should be maximized for the environment and specific resource that the organism typically uses (Schluter, 2000; Irschick, 2003). Soldier performance in *C. persimilis* was therefore examined within the general competitive context of the canopy, and with respect to entrance sizes used with different frequency within the focal population. The results indicate that soldier performance is indeed significantly higher for the entrance size used most often (equal to the head area of one soldier) than it is for larger entrances used infrequently. For most apparent cases of failed cavity defence, no signs of *C. persimilis* were found, suggesting that they had died outright, and the cavities were occupied by either *Azteca* sp. or *C. pusillus*. For the two cavities where the original *C. persimilis* occupants had moved to a natural cavity, they had lost all brood and significant numbers of adults. These findings indicate that *Cephalotes* nests are under intense and potentially fatal usurpation pressure and that specialized use of a particular cavity entrance size significantly increases the performance of soldiers in holding nests.

In interpreting these results, it is important to note that the absence of *C. persimilis* from a cavity was used as an indirect measure of soldier performance. It is possible that the absence of *C. persimilis* from a cavity could have occurred for reasons other than poor soldier performance. However, all evidence suggests that this is highly unlikely. Voluntary emigration to another tree via the canopy was prevented by using naturally isolated trees, and over-ground emigration would not be expected for a strictly arboreal species. The two emigrations recorded on experimental trees appeared to be driven by cavity usurpation, and they resulted in significant brood and adult mortality regardless of the cause. The complete absence of *C. persimilis* from the experimental trees is therefore best explained by death. Only soldiers perform entrance defence in *C. persimilis* (Powell, 2008; present study); so, death from cavity invasion or usurpation can only be explained by poor soldier performance. Vertebrate predation did not occur because all cavities remained intact. A pathogen is therefore the only alternative for *C. persimilis* death, but no ant remains or signs of a pathogen were seen. All considered, the time until the loss of *C. persimilis* from an experimental cavity is likely to be a highly reliable indirect measure of soldier defensive performance.

A second crucial test of the adaptive fit between environment and phenotype is whether organisms

actively select the resources that maximize performance (Irschick, 2002). For *C. persimilis*, this issue was addressed at two levels. The first was whether the narrow range of nest entrances used in nature is an evolved characteristic, or 'fundamental niche'. The second was whether the species has an evolved preference for the specific size that maximizes soldier performance. Cavities with entrances within the natural range were readily used by *C. persimilis*. However, only one cavity with an entrance outside the natural range was used, despite the strong evidence of cavity limitation (rapid invasion of suitable cavities; Philpott & Foster, 2005). Moreover, given a choice of entrance sizes within the natural range, colonies preferentially used the size that is typically used most often in the focal population and that maximized soldier performance. Interestingly, not all used cavities had workers, soldiers and brood, and the smallest populations were workers only. This suggests that workers initially select new cavities, even though the preferred entrance properties appear to be determined by soldier morphology. Crucially, unoccupied cavities of all entrance sizes remained on almost all trees. This indicates that *C. persimilis* was almost never competitively excluded from using more cavities with a particular entrance size. Consequently, observed cavity use can be reliably interpreted as evidence of a narrow fundamental niche and an evolved preference for the entrance size that maximizes performance.

By using general approaches to testing adaptation, the performance and resource-use studies provide strong support for an adaptive relationship between ecological specialization and caste specialization. However, understanding how these processes are likely to affect colony fitness will provide more certainty to an adaptive interpretation. The examination of wild colonies established that both colony size and reproductive output increase with cavity number. It also established that only colonies with more than three cavities reproduced but that reproductive brood is raised in most cavities of reproductively mature colonies. It is not surprising that more space (cavities) is associated with more growth and reproduction. However, cavities are clearly hard to keep and to acquire. Outside the reproductive season, lower soldier performance and less effective selection of new cavities should therefore limit nest number, with indirect consequences for colony growth and, ultimately, reproduction. In addition, cavity loss from poor soldier performance during the reproductive season will directly impact reproductive output, because most cavities rear reproductive brood. Soldier performance and the process of selecting new cavities are therefore likely to have both direct and indirect consequences for colony fitness.

The general approach taken here has been to use natural ecological context and patterns of resource use to conduct better informed caste performance studies, and to link these to colony fitness. Studies of 'ecological

performance' (performance within a natural ecological context) are generally scarce, and although all performance studies assume that higher performance increases fitness, few verify that this assumption holds (Irschick, 2003). One benefit of studying ecological performance is that maximal performance in the laboratory may not correspond to performance in nature (Irschick, 2003). This issue may be particularly relevant for laboratory-based studies of caste performance. For instance, two meticulous studies of caste behaviours in *Cephalotes varians* did not record soldier defensive behaviours under normal laboratory conditions (i.e. without extreme agitation; Wilson, 1976; Cole, 1980). Indeed, the seemingly obvious blocking function of *Cephalotes* soldiers was not successfully elicited under artificial conditions for many years (reviewed in de Andrade & Baroni Urbani, 1999). By contrast, the present study has shown that in the natural canopy environment, soldiers are dedicated to the task of entrance blocking, and that this behaviour is only relevant to a specific and narrow range of entrance sizes. This demonstrates the importance of establishing both the general and specific ecological context for eliciting and measuring caste performance.

Although field studies of caste performance are not uncommon, they typically use only the general ecological context of the focal species, not the specific context of the resource distributions they use (but see Powell & Franks, 2005). Such studies often compare performance among different castes within the colony (e.g. Wilson, 1980; Franks, 1985, 1986; Roschard & Roces, 2003) or assess the colony-level impacts of losing the performance capabilities of a caste or worker size class (e.g. Wilson, 1983; Porter & Tschinkel, 1985; Foster, 1990; Hasegawa, 1993; Billick, 2002; Billick & Carter, 2007). Higher performance of a particular caste for a particular task can identify the caste's specialized function and how its presence benefits the colony. Caste removal experiments, on the other hand, provide insights into how the colony is affected by the loss of a performance specialist. However, these kinds of studies do not yield any direct information on what ecological contingencies exert selective pressure on a caste, and to what extent it is adapted to a particular set of conditions. For insights into these issues, caste performance must be quantified under a range of ecological conditions faced by the species. This approach has provided evidence that for *C. persimilis*, selection exerted by cavity competition has resulted in an adaptive fit between a specific nesting resource and a highly specialized soldier phenotype. More generally, these findings suggest that a caste can be a fine-tuned adaptation, specialized not only to a particular task, but also to a remarkably narrow subset of resources. An important focus for future work will be to contrast this finding against comparable data for *Cephalotes* that use a wider range of resources and have a less specialized soldier phenotype (e.g. *C. pusillus*; Powell, 2008).

Regardless of the focus of performance studies, the underlying assumption is that measured differences in performance are relevant to colony fitness. As is true for the broader performance literature, studies of caste performance rarely address this assumption. This is problematic because measurable performance gains by a caste could easily be invisible to the selection acting on the colony. For instance, greater transport efficiency by a foraging caste may not increase fitness if colony nutrition, and thus reproductive output, is limited by factors other than resource delivery rates (Dornhaus & Powell, in press). Indeed, in the leaf-cutting ant *Atta colombica*, the colony-level rate of leaf tissue processing is greatest when individual forager performance (i.e. size of transported leaf fragments) is suboptimal (Burd & Howard, 2008). For *C. persimilis*, it seems clear that differences in soldier performance, and the related task of selecting new cavities, will determine the number of discrete cavities held by the colony, and thus their maximum reproductive output. The ideal test of the relationship between performance and fitness would be to manipulate the conditions of caste performance through a full year-long reproductive cycle. Although challenging, such a test would provide valuable new detail on how the performance of a small number of specialists directly impact the fitness of the larger superorganism.

Broadly, this study demonstrates the utility of addressing the causal links between resource use, morphology, performance and fitness. Although all components of this causal chain are rarely integrated into performance studies (Irschick, 2002, 2003), the approach offers valuable insights into the process of adaptation. Here, it has provided strong evidence that the evolution of one of the most striking examples of a highly specialized caste is an adaptation to highly specialized nesting ecology. It is important to note, however, that these studies were not conducted in isolation. They were informed by a comparative evidence for a broader evolutionary relationship between ecological specialization and caste specialization in *Cephalotes*. This not only allowed more precise tests of soldier performance and resource use in the focal species, it allows the present study to serve as a critical test of the causal nature of the broader pattern. Comparative analyses and ecological performance studies thus offer complementary and highly informative tools for studying adaptive caste evolution, which, to date, have been largely unused.

Acknowledgments

I thank Beatriz Baker, Kleber Del Claro, Heraldo L. Vasconcelos and Alan Nilo da Costa for logistical help. I also thank two anonymous reviewers for valuable comments on an earlier version of this manuscript. This research was funded by an 1851 Research Fellowship

from the Royal Commission for the Exhibition of 1851, UK and by a Research Grant from the Association for the Study of Animal Behaviour, UK.

References

- de Andrade, M.L. & Baroni Urbani, C. 1999. Diversity and adaptation in the ant genus *Cephalotes*, past and present. *Stuttg. Beitr. Naturkd. Ser. B (Geol. Paläontol.)* **271**: 1–889.
- Arnold, S.J. 1983. Morphology, performance and fitness. *Am. Zool.* **23**: 347–361.
- Baroni Urbani, C. 1998. The number of castes in ants, where major is smaller than minor and queens wear the shield of the soldiers. *Insectes Soc.* **45**: 315–333.
- Beshers, S.N. & Traniello, J.F.A. 1994. The adaptiveness of worker demography in the attine ant *Trachymyrmex septentrionalis*. *Ecology* **75**: 763–775.
- Beshers, S.N. & Traniello, J.F.A. 1996. Polyethism and the adaptiveness of worker size variation in the attine ant *Trachymyrmex septentrionalis*. *J. Insect Behav.* **9**: 61–83.
- Billick, I. 2002. The relationship between the distribution of worker sizes and new worker production in the ant *Formica neorufibarbis*. *Oecologia* **132**: 244–249.
- Billick, I. & Carter, C. 2007. Testing the importance of the distribution of worker sizes to colony performance in the ant species *Formica obscuripes* Forel. *Insectes Soc.* **54**: 113–117.
- Bourke, A.F.G. 1999. Colony size, social complexity, and reproductive conflict in social insects. *J. Evol. Biol.* **12**: 245–257.
- Burd, M. & Howard, J.J. 2008. Optimality in a partitioned task performed by social insects. *Biol. Lett.* **4**: 627–629.
- Carroll, C.R. 1979. A comparative study of two ant faunas: the stem-nesting ant communities of Liberia, West Africa and Costa Rica, Central America. *Am. Nat.* **113**: 551–561.
- Cole, B.J. 1980. Repertoire convergence in two mangrove ants, *Zacryptocerus varians* and *Camponotus (Colobopsis)* sp. *Insectes Soc.* **27**: 265–275.
- Creighton, W.S. 1963. Further studies on the habits of *Cryptocerus texanus* Santschi (Hymenoptera: Formicidae). *Psyche* **70**: 133–143.
- Creighton, W.S. & Nutting, W.L. 1965. The habits and distribution of *Cryptocerus rohweri* Wheeler (Hymenoptera, Formicidae). *Psyche* **72**: 59–64.
- Cruz, Y.P. 1981. A sterile defender morph in a polyembryonic hymenopterous parasite. *Nature* **294**: 446–447.
- Davidson, D.W., Snelling, R.R. & Longino, J.T. 1989. Competition among ants for myrmecophytes and the significance of plant trichomes. *Biotropica* **21**: 64–73.
- Dornhaus, A. & Powell, S. 2009. Foraging and defence strategies in ants. In: *Ant Ecology* (L. Lach, C. Parr & K. Abbot, eds), in press. Oxford University Press, Oxford.
- Ferry-Graham, L., Bolnick, D.L. & Wainwright, P.C. 2002. Using functional morphology to examine the ecology and evolution of specialization. *Int. Comp. Biol.* **42**: 265–277.
- Fjerdingstad, E.J. & Crozier, R.H. 2006. The evolution of worker caste diversity in social insects. *Am. Nat.* **167**: 390–400.
- Foster, W.A. 1990. Experimental evidence for effective and altruistic colony defence against natural predators by soldiers of the gall-forming aphid *Pemphigus spyrothecae* (Hemiptera: Pemphigidae). *Behav. Ecol. Sociobiol.* **27**: 421–430.
- Foster, K.R., Wenseleers, T. & Ratnieks, F.L.W. 2006. Kin selection is the key to altruism. *Trends Ecol. Evol.* **21**: 57–60.
- Franks, N.R. 1985. Reproduction, foraging efficiency and worker polymorphism in army ants. In: *Experimental Behavioral Ecology and Sociobiology: In Memoriam Karl von Frisch, 1886–1982*, Vol. 31 (B. Hölldobler & M. Lindauer, eds), pp. 91–107. Sinauer Associates, Sunderland, MA.
- Franks, N.R. 1986. Teams in social insects: group retrieval of prey by army ants (*Eciton burchelli*, Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **18**: 425–429.
- Franks, N.R. & Norris, P.J. 1987. Constraints on the division of labour in ants: D'Arcy Thompson's cartesian transformations applied to worker polymorphism. In: *From Individual to Collective Behavior in Social Insects*, Vol. 54 (J.M. Pasteels & J.L. Deneubourg, eds), pp. 253–270. Birkhauser, Basel.
- Futuyma, D.J. & Moreno, G. 1988. The evolution of ecological specialization. *Annu. Rev. Ecol. Syst.* **19**: 207–233.
- Harvey, P.H. & Pagel, M.D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.
- Hasegawa, E. 1993. Nest defense and early production of the major workers in the dimorphic ant *Colobopsis nipponicus* (Wheeler) (Hymenoptera, Formicidae). *Behav. Ecol. Sociobiol.* **33**: 73–77.
- Hölldobler, B. & Wilson, E.O. 1990. *The Ants*. Harvard University Press, Cambridge, MA.
- Inward, D.J.G., Vogler, A.P. & Eggleton, P. 2007. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Mol. Phylogenet. Evol.* **44**: 953–967.
- Irschick, D.J. 2002. Evolutionary approaches for studying functional morphology: examples from studies of performance capacity. *Integr. Comp. Biol.* **42**: 278–290.
- Irschick, D.J. 2003. Measuring performance in nature: implications for studies of fitness within populations. *Integr. Comp. Biol.* **43**: 396–407.
- Irschick, D., Dyer, L. & Sherry, T.W. 2005. Phylogenetic methodologies for studying specialization. *Oikos* **110**: 404–408.
- Kaspari, M. & Byrne, M.M. 1995. Caste allocation in litter *Pheidole*: lessons from plant defense theory. *Behav. Ecol. Sociobiol.* **37**: 255–263.
- McLeish, M.J. & Chapman, T.W. 2007. The origin of soldiers in the gall-inducing thrips of Australia (Thysanoptera: Phlaeothripidae). *Aust. J. Entomol.* **46**: 300–304.
- Moreau, C.S. 2008. Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). *Mol. Phylogenet. Evol.* **48**: 224–239.
- Oliveira-Filho, A.T. & Ratter, J.A. 2002. Vegetation physiognomies and woody flora of the cerrado biome. In: *The cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna* (P.S. Oliveira & R.J. Marquis, eds), pp. 91–120. Columbia University Press, New York.
- Oster, G.F. & Wilson, E.O. 1978. *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, NJ.
- Palmer, T.M. 2004. Wars of attrition: colony size determines competitive outcomes in a guild of African acacia-ants. *Anim. Behav.* **68**: 993–1004.
- Philpott, S.M. & Foster, P.F. 2005. Nest-site limitation in coffee agroecosystems: artificial nests maintain diversity of arboreal ants. *Ecol. Appl.* **15**: 1478–1485.
- Pie, M.R. & Traniello, J.F.A. 2007. Morphological evolution in a hyperdiverse clade: the ant genus *Pheidole*. *J. Zool.* **271**: 99–109.
- Porter, S.D. & Tschinkel, W.R. 1985. Fire ant polymorphism: the ergonomics of brood production. *Behav. Ecol. Sociobiol.* **16**: 323–336.

- Powell, S. 2008. Ecological specialization and the evolution of a specialized caste in *Cephalotes* ants. *Funct. Ecol.* **22**: 902–911.
- Powell, S. & Franks, N.R. 2005. Caste evolution and ecology: a special worker for novel prey. *Proc. R. Soc. B* **272**: 2173–2180.
- Powell, S. & Franks, N.R. 2006. Ecology and the evolution of worker morphological diversity: a comparative analysis with *Eciton* army ants. *Funct. Ecol.* **20**: 1105–1114.
- Queller, D.C. & Strassmann, J.E. 2002. The many selves of social insects. *Science* **296**: 311–313.
- Roschard, J. & Roces, F. 2003. Fragment-size determination and size-matching in the grass-cutting ant *Atta vollenweideri* depend on the distance from the nest. *J. Trop. Ecol.* **19**: 647–653.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schöning, C., Kinuthia, W. & Franks, N.R. 2005. Evolution of allometries in the worker caste of *Dorylus* army ants. *Oikos* **110**: 231–240.
- Seeley, T.D. 1995. *The Wisdom of the Hive: The Social Physiology of Honey Bee Colonies*. Harvard University Press, Cambridge, MA.
- Smith, C.R., Toth, A.L., Suarez, A.V. & Robinson, G.E. 2008. Genetic and genomic analyses of the division of labour in insect societies. *Nat. Rev. Gen.* **9**: 735.
- Stanton, M.L., Palmer, T.M., Young, T.P., Evans, A. & Turner, M.L. 1999. Sterilization and canopy modification of a swollen thorn acacia tree by a plant-ant. *Nature* **401**: 578–581.
- Stanton, M.L., Palmer, T.M. & Young, T.P. 2002. Competition-colonization trade-offs in a guild of African Acacia ant-plants. *Ecol. Monogr.* **72**: 347–363.
- Stern, D.L. 1994. A phylogenetic analysis of soldier evolution in the aphid family Hormaphididae. *Proc. R. Soc. Lond. B* **256**: 203–209.
- Strassmann, J.E. & Queller, D.C. 2007. Insect societies as divided organisms: the complexities of purpose and cross-purpose. *Proc. Natl Acad. Sci. U S A* **104**: 8619–8626.
- Thompson, G.J., Kitade, O., Lo, N. & Crozier, R.H. 2004. On the origin of termite workers: weighing up the phylogenetic evidence. *J. Evol. Biol.* **17**: 217–220.
- Thorne, B.L., Breisch, N.L. & Muscedere, M.L. 2003. Evolution of eusociality and the soldier caste in termites: influence of intraspecific competition and accelerated inheritance. *Proc. Natl Acad. Sci. U S A* **100**: 12808–12813.
- Vasconcelos, H.L. 1993. Ant colonization of *Maieta guianensis* seedlings, an Amazon ant-plant. *Oecologia* **95**: 439–443.
- Walker, J. & Stamps, J. 1986. A test of optimal caste ratio theory using the ant *Camponotus (Colobopsis) impressus*. *Ecology* **67**: 1052–1062.
- Wheeler, D.E. 1991. The developmental basis of worker caste polymorphism in ants. *Am. Nat.* **138**: 1218–1238.
- Wilson, E.O. 1976. A social ethogram of the Neotropical arboreal ant *Zacryptocerus varians* (Fr. Smith). *Anim. Behav.* **24**: 354–363.
- Wilson, E.O. 1980. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). II. The ergonomic optimization of leaf cutting, *Atta sexdens*. *Behav. Ecol. Sociobiol.* **7**: 157–165.
- Wilson, E.O. 1983. Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). III. Ergonomic resiliency in foraging by *A. cephalotes*. *Behav. Ecol. Sociobiol.* **14**: 47–54.

Received 24 November 2008; revised 16 January 2009; accepted 20 January 2009