

# Ecology and the evolution of worker morphological diversity: a comparative analysis with *Eciton* army ants

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## Summary

1. Certain ants have considerable morphological diversity within the workforce, with individuals physically specialized for particular jobs. The ecological conditions that select for this phenomenon are still poorly understood.

2. We use a comparative analysis to address the hypothesis that prey type has selected for greater morphological diversity in the small, monophyletic army ant genus *Eciton*. These ants are nomadic group-predators and transport loads in the limited space below the body, so bulkier prey types should select for more diverse worker morphology.

3. We show that five *Eciton* species differ in the presence or absence of a distinct ‘submajor caste’, the morphological exaggeration of submajors when present, and the allometric scaling of standard workers over a broad size range. Crucially, the presence and morphological exaggeration of submajors is associated with the variety of awkwardly shaped prey types that the species takes, and this caste specializes in prey transport.

4. Our findings support the importance of dietary composition in the evolution of worker morphological diversity in *Eciton* and demonstrate the utility of our general approach. Future critical tests of this hypothesis are discussed, as well as how our approach can contribute to a broader understanding of the relationship between ecology and the evolution of worker morphological diversity.

**Key-words:** allometry, caste, Ecitoninae, polymorphism, prey transport

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## Introduction

Specialization within the workforce is central to the sophisticated organization of eusocial animal societies. This is particularly apparent when the specialized roles that ‘workers’ adopt are based on their size and shape, with examples seen in such distantly related taxa as ants (Hölldobler & Wilson 1990), aphids (Rhoden & Foster 2002; Kurosu *et al.* 2006), termites (Badertscher, Gerber & Leuthold 1983; Gerber, Badertscher & Leuthold 1988; Noirot & Darlington 2000) and thrips (Perry *et al.* 2004). A morphologically diverse workforce is thought to evolve because the matching of worker size and shape with task should benefit organizational efficiency and therefore colony fitness (Oster & Wilson 1978; Bourke & Franks 1995). Yet worker morphological diversity (‘worker diversity’ hereafter) is not common. For instance, only about 20% of ant genera display some

degree of worker polymorphism (allometric scaling of body parts over a broad size range) and distinct worker types or castes are rarer still (Oster & Wilson 1978). The evolution of worker diversity is probably constrained by certain life-history characters and intracolony conflict (Fjerdingstad & Crozier 2006). However, to understand this phenomenon fully we must also know what ecological conditions select for a more diverse workforce. The relationship between ecology and worker diversity remains a poorly understood aspect of social evolution (Hölldobler & Wilson 1990; Bourke & Franks 1995).

The relationship between worker diversity and behavioural specialization within ant colonies has been firmly established by many studies (e.g. Hölldobler & Wilson 1990; Hasegawa 1993; Franks, Sendova-Franks & Anderson 2001; Roschard & Roces 2003a; Powell & Clark 2004; Schöning, Kinuthia & Franks 2005). Nevertheless, one cannot assume that all aspects of a worker’s size and shape are adaptations to the role they usually perform. This is because developmental constraints can limit the range of possible morphologies (Oster & Wilson 1978; Franks & Norris 1987; Wheeler 1991), and many characters can be conserved within

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lineages and are therefore not necessarily adaptations to current behaviour (Harvey & Pagel 1991). Thus, while studies of worker allometry and size-based specialization address the relationship between morphology and colony organization, they provide little insight into which aspects of worker diversity are adaptations to which aspects of the species' ecology.

The adaptive relationship between ecology and worker diversity can be tested in a general way by altering the size frequency distribution within colonies and assessing its impact on some measure of colony fitness. Studies of this kind have provided crucial evidence that worker diversity and ecology can interact in some way to improve colony fitness (Porter & Tschinkel 1985, 1986; Hasegawa 1993; Billick 2002). However, a deeper understanding of the relationship between ecology and worker diversity should be possible by investigating the evolutionary relationship between particular ecological and morphological variables. We argue that investigations of this kind require two key steps, and that comparative analyses are vital. The first step is to identify a pattern of correlated evolutionary change in particular ecological variables and morphological characters associated with worker diversity. This is necessary to identify a potentially adaptive relationship between ecology and worker diversity. The second step is to demonstrate that the interaction between the focal ecological and morphological variables improves the efficiency and fitness of the colony. This is necessary to address whether the identified relationship between ecology and worker diversity is causal. This paper is focused on a comparative analysis for tackling the first step of this process.

Considerable worker diversity has evolved a number of times independently in ants (Hölldobler & Wilson 1990; Baroni Urbani 1998), permitting studies that address convergent patterns in worker diversity. However, the phylogenetic relationships among taxa need to be known, to control for similarities shared by common descent (Fjerdingstad & Crozier 2006), and this information is not always available. An alternative is to address the correlated evolutionary changes in ecology and worker diversity within a lineage, which is possible because the workers of closely related species (e.g. congeners) can differ significantly in size and shape. This approach has the advantage that the confounding influences of common descent are automatically controlled when only differences are addressed (Harvey & Pagel 1991). Consequently, correlated change in ecology and worker diversity among close relatives can be tentatively interpreted as an adaptive pattern, and although a well-supported phylogeny can improve this interpretation, useful insights can be gained without one. To our knowledge, only one study to date has used a comparative analysis to address the relationship between ecology and worker diversity among closely related taxa (Schöning *et al.* 2005). Specifically, this study addressed the relationship between substantial differences in habitat use and worker diversity among

members of the six subgenera of the diverse Old World army ant genus *Dorylus*. Our study will compare differences among more closely related species that differ more subtly in basic ecology, which is a common pattern within lineages with well-developed worker diversity.

Our focal taxon is the monophyletic New World army ant genus *Eciton* (Brady 2003; Brady & Ward 2005), which contains only nine species (three additional species described from males only, Watkins 1976). *Eciton* is particularly well suited for studying the evolutionary relationship between ecology and worker diversity within a lineage. First, workers vary considerably in size and shape within each species, and although direct comparisons have not been made, worker diversity also appears to differ among species (Rettenmeyer 1963; Franks 1985; Feener, Lighton & Bartholomew 1988). Second, detailed behavioural and morphological studies of *Eciton burchellii* have shown that leg length and head width are strongly associated with the size of the prey item that individuals transport (Franks 1985, 1986; Franks *et al.* 1999). Unlike most ants, *Eciton* workers transport prey beneath the body. This permits fast and mechanically efficient transport (Bartholomew, Lighton & Feener 1988), but prey has to be held at the tip, requiring a powerful bite, and prey size is limited by the space defined by the legs, resulting in an unusually strong relationship between ant size and load size (see Franks 1986; Wetterer 1994; Kaspari 1996; Morehead & Feener 1998; Roschard & Roces 2003b). This suggests a potential evolutionary relationship between the ecology of prey transport and leg length and head width, providing excellent focal variables for comparison among species. Third, *Eciton* has pronounced queen-worker dimorphism (Schneirla 1971; Brady 2003) and high genetic variation within colonies (Denny *et al.* 2004; Kronauer *et al.* 2006), and both of these characteristics are thought to reduce constraints on the evolution of worker diversity (Fjerdingstad & Crozier 2006). This increases the likelihood of significant adaptive differences in worker diversity among species. Finally, although all army ants are nomadic and obligate group predators (Brady 2003), *Eciton* species are unique in that colony emigrations and raids are conducted entirely on the surface (Rettenmeyer 1963; S. Powell unpublished data). *Eciton* foraging ecology is therefore unusually accessible, which is important for future experimental studies that address the causal relationship between the focal ecological and morphological variables.

Here we compare the allometric scaling of back-leg length and head width as a function of body size, diet and prey-transport behaviour among five species of *Eciton* army ants. Our goal is to assess the importance of diet in the evolution of worker morphological diversity within the genus. Our central hypothesis is that prey types that are more awkward to transport, via the characteristic below-body method used by army ants, have selected for greater morphological diversity in the workforce.

## Materials and methods

### STUDY SITE AND STUDY SPECIES

Fieldwork was conducted on Barro Colorado Island (BCI), Panama. All five *Eciton* species presently found on BCI are included in this study. These are as follows: *Eciton burchellii foreli* (Mayr), *Eciton dulcium crassinode* (Borgmeier), *Eciton hamatum* (Fabricius), *Eciton mexicanum panamense* (Borgmeier) and *Eciton vagans mutatum* (Borgmeier). Names follow Bolton (1995).

### SAMPLING AND MEASUREMENTS

A large random sample of workers was taken directly from the nest or emigration column of each colony, ensuring all worker sizes were initially collected. Measurements were then taken from a smaller stratified subsample spanning the full worker size range. Three colonies were sampled for each species. A subsample of 60 individuals was taken for the first colony of each species. This showed that the pattern of worker diversity could be adequately described from fewer individuals, so a subsample of 30 was used for the remaining colonies. For each specimen, the following three measurements were recorded: head width (line through the centre of the eyes) to the nearest 0.03 mm, right back-leg length (last tarsal segment, excluding tarsal claw, to trochanter) to the nearest 0.13 mm, and constant dry weight to the nearest 0.001 mg. Dry weight was considered the best comparative measure of body size because it is independent of morphology. Carl Rettenmeyer provided one large colony sample for *E. vagans* (collected on BCI in 1956), and Scott Powell collected all other samples between February 2001 and October 2003.

### MORPHOLOGICAL COMPARISONS AND STATISTICAL ANALYSIS

Worker morphology was compared with natural-log transformed bivariate plots of head width, back-leg length, or ratio of these measures, on the cube-root of dry weight (cube-root transformation converts raw dry weight to the one-dimensional scale of the linear morphological measurements). Preliminary analysis revealed that all bivariate relationships were best explained by complex curvilinear fits, precluding standard methods for comparing linear allometric relationships. Statistical comparisons were therefore made using a similar method to Schöning *et al.* (2005). For each focal bivariate relationship, the data for all species were combined and the best-fitting least squares regression line was found (see later). The relative residuals (residuals divided by predicted values) from this common fit were then grouped by colony or species, depending on the analysis, such that the pattern of worker diversity for each was represented by a single distribution of relative residuals. The distribution means were then compared with Kruskal–Wallis tests (Wilcoxon rank tests for pairwise

comparisons), with significant differences indicating that the pattern of worker diversity differed among the colonies or species being compared. Differences in body size (dry weight) were assessed with Kruskal–Wallis tests and Wilcoxon rank tests for pairwise comparisons.

The best-fitting polynomial least-squared regression line was defined as the most complex fit for which all coefficients were significantly different from zero (Zar 1996). This procedure was used to find the best common fit in the statistical analysis, and to better define the visual pattern of worker diversity for each species in figures. Some authors recommend alternative parametric fitting techniques for allometry studies, but when the data are well described by the best-fitting line, the line-fitting technique is relatively unimportant (Martin, Genoud & Hemelrijk 2005). Least squared regression provided an extremely close fit to our data, and alternative fitting techniques produced best fits that were not significantly different. The computationally more straightforward least squares regression technique was therefore justified and used throughout.

### SAMPLING OF PREY

Prey was sampled to identify key differences in diet. For *E. burchellii*, *E. dulcium* and *E. mexicanum*, 10 workers transporting an adult prey ant were collected whenever a foraging column was encountered. Prey type is unlikely to vary with time of day because all *Eciton* raid in an approximately straight line during a set foraging period each day, encountering appropriate prey at random as the raid advances. Sampling biases were avoided by collecting the next prey-transporter to pass a predetermined point, after processing the previous sample. These species take a mixture of adults and brood from prey colonies (Rettenmeyer 1963; Rettenmeyer *et al.* 1983), so this procedure provided a good estimate of the relative abundance and diversity of the prey species taken by each. *E. burchellii* takes ants and non-ant arthropod prey (Franks 1983), and for *E. dulcium* and *E. mexicanum* the foraging columns were visually scanned for any non-ant prey during the collection of ant prey.

*E. hamatum* take predominantly or exclusively brood from the ants they raid, and they frequently prey upon the leaf-cutting ant *Acromyrmex octospinosus* on BCI (Rettenmeyer *et al.* 1983; S. Powell personal observation). *A. octospinosus* has distinctive larvae and pupae (Wheeler & Wheeler 1976) that can be distinguished reliably from other prey. For seven *E. hamatum* colonies, total prey items and *A. octospinosus* prey items were recorded in 3-min bouts. For each colony, counts were taken every 20 min on all columns going into the nest during a 3-h observation period. This procedure was repeated on each of four consecutive days of a nomadic phase starting at dawn, mid-morning, noon, or early afternoon, with the order randomized for each colony. *E. hamatum* takes large wasp prey in addition to ants (Rettenmeyer *et al.* 1983), and the foraging columns were scanned

for any nonhymenopteran prey during the observation periods. It was not possible to collect any diet data for *E. vagans* because the nocturnal foraging columns of this species were extremely rare.

PREY-TRANSPORT SAMPLES

Prey-transport samples were collected as independent samples of the size distribution of workers, and to determine the relative involvement of workers of different sizes in prey-transport. One hundred prey-transport samples (ant plus prey item) were collected from each of four colonies of *E. burchellii*, *E. hamatum* and *E. dulcium*. Fifty prey-transport samples were also collected from each of two *E. mexicanum* colonies (more and larger samples were not possible because of sensitivity to the collection procedure). Sampling biases were avoided as before. No *E. vagans* samples were collected for reasons already stated.

Results

THE MAJOR CASTE

*Eciton* majors are the largest individuals in the colony and are clearly identifiable by their sickle-shaped mandibles that are specialized for defence (Rettenmeyer 1963). No majors were involved in prey-transport in the four species for which prey and prey-transport samples were collected (Table 1). This supports previous quantitative studies of the behaviour of majors in some species, and observations made on others (Rettenmeyer 1963; Franks 1985). Given that the present study addresses the relationship between prey transport and worker diversity, the rest of the analysis was focused on the non-major workers, which do transport prey. The body-size range for the majors of each species was as follows:

*E. burchellii* 9.3–11.4 mg; *E. dulcium* 15.3–18.3 mg; *E. hamatum* 8.5–10.3 mg; *E. mexicanum* 5.2–6.8 mg; *E. vagans* 10.7–15.3 mg.

NON-MAJOR WORKERS

This part of the analysis addressed the degree of morphological variation in non-major workers among colonies of the same species and among species, as well as the body-size range for the non-major workers. The scaling relationship of head width as a function of body size (RHW) did not differ significantly among colonies for three of five species (comparison of relative residuals grouped by colonies; *E. burchellii*,  $\chi^2 = 3.7$ , d.f. = 2,  $P = 0.16$ ; *E. dulcium*,  $\chi^2 = 0.4$ , d.f. = 2,  $P = 0.82$ ; *E. hamatum*,  $\chi^2 = 1.8$ , d.f. = 2,  $P = 0.41$ ; *E. mexicanum*,  $\chi^2 = 13.0$ , d.f. = 2,  $P = 0.002$ ; *E. vagans*,  $\chi^2 = 16.4$ , d.f. = 2,  $P = 0.003$ ). The scaling relationship of back-leg length as a function of body size (RBL) did not differ significantly among colonies for two of five species (comparison of relative residuals grouped by colonies; *E. burchellii*,  $\chi^2 = 4.6$ , d.f. = 2,  $P = 0.10$ ; *E. dulcium*  $\chi^2 = 8.0$ , d.f. = 2,  $P = 0.02$ ; *E. hamatum*  $\chi^2 = 1.9$ , d.f. = 2,  $P = 0.38$ ; *E. mexicanum*  $\chi^2 = 35.8$ , d.f. = 2,  $P < 0.0001$ ; *E. vagans*,  $\chi^2 = 46.6$ , d.f. = 2,  $P < 0.0001$ ). Visually, the body-size range and shape of the curvilinear scaling relationships were very similar among colonies of each species, even though there were statistical differences among some colonies of some species. However, differences in the scaling relationships for head width and back-leg length as a function of body size were marked among species, both in terms of statistical differences (comparison of relative residuals grouped by species; RHW,  $\chi^2 = 141.0$ , d.f. = 4,  $P < 0.0001$ ; RBL,  $\chi^2 = 217.6$ , d.f. = 4,  $P < 0.0001$ ), and the apparent shape of the relationships. All subsequent analyses addressed in detail these more significant interspecific

**Table 1.** The prey, size range of prey-transporters, and the proportion of prey-transporters that were submajors for five *Eciton* species. For ant prey, percentages are given for the most common ant genera, along with the number of independent samples and the total number of prey items sampled. Diet information for *E. vagans*\* is based on prey collected from foraging columns by other authors (Chadab 1980; Rettenmeyer *et al.* 1983; Longino 2005). ‘Within range’ indicates that, for the particular species, the prey-transporters were within the size range of non-major individuals identified in the morphological analysis. Members of the major caste were not involved in prey-transport in any of the species

	<i>E. mexicanum</i>	<i>E. dulcium</i>	<i>E. vagans</i>	<i>E. hamatum</i>	<i>E. burchellii</i>
Submajor present/absent	Absent	Absent	Present	Present	Present
Ant prey	<i>Ectatomma</i> 68% <i>Pachycondyla</i> 18%	<i>Odontomachus</i> 53% <i>Pachycondyla</i> 47%	<i>Aphaenogaster</i> <i>Odontomachus</i> <i>Pheidole</i> *	<i>Acromyrmex</i> 23%	<i>Camponotus</i> 96%
Ant prey sampling	10 samples 100 items	45 samples 450 items	–	704 samples 38 872 items	38 samples 380 items
Polymorphic ant prey?	No	No	Yes	Yes	Yes
Non-ant prey	None	None	None	Social wasps	Social wasps/other arthropods
Size range of prey-transporters	1.4–4.5 mg (within range)	0.9–10.3 mg (within range)	–	1.1–8.5 mg (within range)	0.7–8.7 mg (within range)
Prey-transporters that were submajors	No submajors	No submajors	–	27%	20%



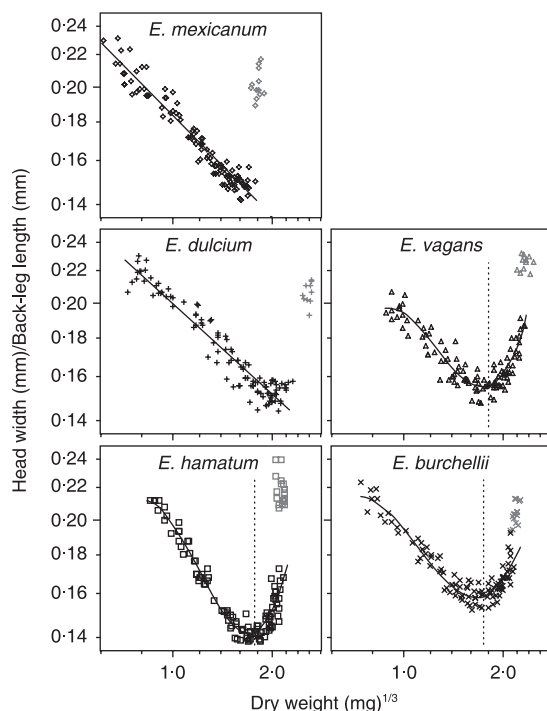
differences (see subsequent sections), and we used the data from all three colonies per species so that these species-level analyses were more inclusive of colony-level variation in worker diversity.

The body-size range of non-major workers of each species was as follows: *E. burchellii* 0.4–10.0 mg; *E. dulcium* 0.4–12.3 mg; *E. hamatum* 0.6–10.0 mg; *E. mexicanum* 0.2–5.7 mg; *E. vagans* 0.7–12.5 mg. There was a significant difference in body size of non-major workers among species ( $\chi^2 = 61.0$ , d.f. = 4,  $P < 0.0001$ ). However, after removing *E. mexicanum* from the analysis, there was no significant difference among the remaining four species ( $\chi^2 = 2.6$ , d.f. = 4,  $P = 0.46$ ), indicating that the size range of non-major workers was significantly smaller in *E. mexicanum* than in the other four species.

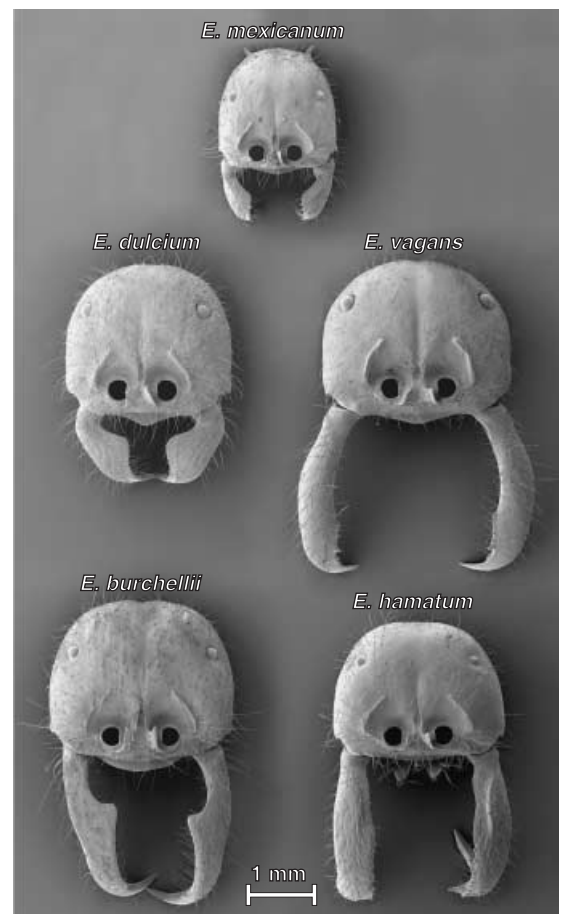
#### THE PRESENCE OR ABSENCE OF A SUBMAJOR CASTE

This part of the analysis addressed whether a submajor caste, previously described for *E. burchellii* (Franks 1985), was present in other *Eciton* species. The *E. burchellii* submajor has been defined as having disproportionately longer legs, wider head, and enlarged mandibles compared with smaller non-major workers. Initial

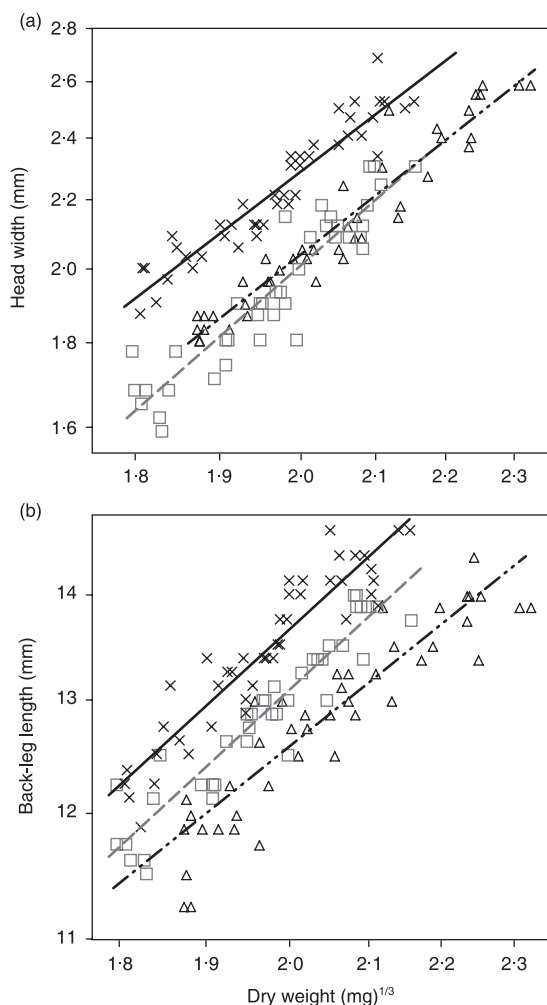
analysis indicated that while all five species had similar allometric scaling of back-leg length as a function of body size, three of five species showed a marked upturn in the allometric scaling of head width as a function of body size at about 6 mg dry weight. This key dichotomy was best illustrated with a ratio of head width to back-leg length on body size (Fig. 1). In *E. dulcium* and *E. mexicanum*, back-leg length increased more rapidly than head width across the full size range of non-major workers, so that the ratio of head width to leg length declined (linear line of best-fit in both species; Fig. 1). In *E. burchellii*, *E. hamatum* and *E. vagans*, back-leg length also increased more rapidly than head width, but only for body sizes between the minimum and about 6 mg. In larger individuals, head width then increased at an accelerated and more rapid rate than back-leg length, so that the ratio of head width to back-leg length switched to an increasing relationship (curvilinear line of best-fit in all species; Fig. 1). Thus, in the largest workers in *E. burchellii*, *E. hamatum* and *E. vagans*, head width scaled differently than in smaller workers, resulting in a different overall shape, and this change is not seen at all in *E. dulcium* and *E. mexicanum*. There was also a dramatic and synchronized change in mandible morphology in the largest workers of *E. burchellii*, *E. hamatum* and *E. vagans* (Fig. 2).



**Fig. 1.** The relationship between the ratio of head width to back-leg length and body size for non-major workers in five species in the army ant genus *Eciton*. The best-fitting regression line is plotted for each species. Majors in each species are shown by grey symbols for illustrative purposes only, and are therefore not included in the regression fit. The dashed vertical lines represent the approximate lower size limit of the submajor caste in *E. vagans* ( $1.87 \text{ mg}^{1/3} = 6.5 \text{ mg}$ ), *E. hamatum* ( $1.79 \text{ mg}^{1/3} = 5.7 \text{ mg}$ ), and *E. burchellii* ( $1.78 \text{ mg}^{1/3} = 5.6 \text{ mg}$ ) and were placed where the slope of the best-fitting line for each relationship became positive.



**Fig. 2.** Representatives of the largest non-major workers in five species in the army ant genus *Eciton*.

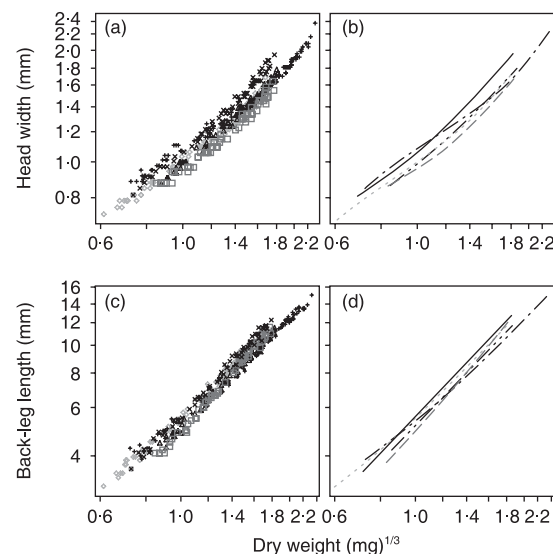


**Fig. 3.** The relationship between head width and body size (a) and back-leg length and body size (b) for the submajor caste in *E. burchellii* (dark grey exes and solid line), *E. hamatum* (grey squares and dashed line), and *E. vagans* (black triangles and dashed/double-dotted line). In both relationships, the best-fitting regression line is plotted for each species.

Defining distinct castes can be inappropriate in continuous worker size distributions that are best described by curvilinear fits (Feener *et al.* 1988). However, here worker morphology changes significantly at a narrow transitional region in three species, and there is a clear presence vs. absence dichotomy among the five congeners in this study. For these reasons, we feel it is justified and useful to define the uniquely shaped size class in *E. burchellii*, *E. hamatum* and *E. vagans* as a 'submajor caste', a term previously limited to *E. burchellii*.

#### THE SUBMAJOR CASTE

We then addressed if the submajors of *E. burchellii*, *E. hamatum* and *E. vagans* differed in the degree of morphological exaggeration (see Fig. 1 for cut-off point). Pairwise comparisons showed that in *E. burchellii*, submajor head width was significantly greater relative to body size than in the submajors of *E. vagans* ( $\chi^2 = 61.1$ , d.f. = 1,  $P < 0.0001$ ; Fig. 3a), which had wider



**Fig. 4.** The relationship between head width and body size (a,b) and back-leg length and body size (c,d) for the standard worker caste in *E. burchellii* (dark grey exes and solid line), *E. dulcium* (black pluses and dashed/dotted line), *E. hamatum* (grey squares and dashed line), *E. mexicanum* (light grey diamonds and dotted line) and *E. vagans* (black triangles dashed/double-dotted line). For each relationship, the left panel shows the raw data points, and the right panel shows the best-fitting regression line with the data points removed for clarity.

heads relative to body size than the submajors of *E. hamatum* ( $\chi^2 = 7.8$ , d.f. = 1,  $P = 0.005$ ; Fig. 3a). Pairwise comparisons also showed that *E. burchellii* submajors had longer legs relative to body size than the submajors of *E. hamatum* ( $\chi^2 = 42.9$ , d.f. = 1,  $P < 0.0001$ ; Fig. 3b), which had longer legs relative to body size than the submajors of *E. vagans* ( $\chi^2 = 24.53$ , d.f. = 1,  $P < 0.0001$ ; Fig. 3b). This indicated that *E. burchellii* submajors had the most exaggerated head and back-leg morphology among the three species that had this caste.

#### THE STANDARD WORKER CASTE

The final part of the morphological analysis addressed whether the morphology of standard workers differs among species. There were few clear separations among species, with the scaling relationships for head width and back-leg length relative to body size in each species intercepting those of the other species at numerous points along their length (Fig. 4). However, head width and back-leg length relative to body size were significantly greater in *E. burchellii* standard workers than in *E. hamatum* standard workers throughout their respective size ranges (RHW,  $\chi^2 = 29.7$ , d.f. = 1,  $P < 0.0001$ ; RBL,  $\chi^2 = 53.2$ , d.f. = 1,  $P < 0.0001$ ), resulting in no intercept points in either relationship (Fig. 4).

#### PREY-TRANSPORTERS AND DIET

These results addressed whether the differences in worker diversity among species were associated with clear differences in diet and prey-transport behaviour. *E. dulcium*

and *E. mexicanum*, the species without submajors, took exclusively monomorphic poneroid ant prey (Table 1). In contrast, *E. burchellii*, *E. hamatum* and *E. vagans*, the species with submajors, all took polymorphic ants, while *E. burchellii* and *E. hamatum* also took bulky non-ant prey. *E. burchellii*, the species with the most exaggerated submajor caste, was the only species that also took nonhymenopteran arthropod prey (Table 1), which results in irregular and awkwardly shaped loads. In the four species for which prey-transporter samples were collected, individuals were within the size range of non-major workers identified by the morphology study (Table 1), supporting independently that *E. dulcium* and *E. mexicanum* lack a submajor caste. *E. burchellii* submajors comprise approximately 3% of the workforce (Franks 1985) and *E. hamatum* has a similar worker size frequency distribution (Schneirla 1971), but this caste transported about one-quarter of all prey in both species (Table 1), indicating that they are extreme prey-transport specialists.

## Discussion

We have demonstrated key differences in worker morphological diversity among five members of the small, monophyletic army ant genus *Eciton*. Specifically, we have shown that species differ in the presence or absence of a distinct submajor caste, the degree of morphological exaggeration of this caste when present, and the allometric scaling of head width and back-leg length relative to body size in the standard worker caste. Crucially, we have also established that the presence and degree of morphological exaggeration of a submajor caste is associated with a diet that includes a variety of awkwardly shaped prey types. The most exaggerated submajor morphology is seen in *E. burchellii*, the only species with a diet that includes nonhymenopteran arthropod prey. Finally, we have confirmed that *E. burchellii* submajors specialize in prey-transport (Franks 1985) and have shown for the first time that *E. hamatum* submajors are also prey-transport specialists.

A complete and well-supported *Eciton* phylogeny is not currently available, preventing detailed phylogenetic analysis of the relationship between diet and worker diversity. However, Brady (2003) placed the five species included in our study in the following arrangement: (*E. mexicanum*, ((*E. vagans*, *E. dulcium*), (*E. hamatum*, *E. burchellii*))). These hypothesized relationships suggest some very tentative but interesting evolutionary patterns with respect to diet and submajors. As we have shown, *E. mexicanum* takes only monomorphic ants and lacks a submajor. Thus, with this species sister to the clade containing the other four, it suggests that a diet including polymorphic ants and non-ant prey and a submajor caste may be derived character states within the genus. We have also shown that *E. dulcium* takes only monomorphic ants and lacks a submajor. With this species as sister to *E. vagans*, while *E. hamatum* is sister to *E. burchellii*, this suggests that a submajor

caste may have evolved more than once, or alternatively was secondarily lost in *E. dulcium*. While the phylogenetic patterns for diet and worker diversity remain unclear, our study does demonstrate that among congeners there is a clear and consistent association between a greater variety of prey types and greater morphological diversity. These findings provide good initial support for the hypothesis that prey types that are more awkward to transport have selected for greater worker diversity in *Eciton*. It is important to stress, however, that while comparative analyses can identify new and potentially adaptive evolutionary scenarios, as seen here, they cannot address causation. Consequently, experimental studies will be needed to address the causal relationship between diet and worker diversity in *Eciton*. To identify appropriate experimental studies, we must first explore how dietary changes could have driven the evolution of a more diverse workforce.

The common load among *Eciton* species is their own larvae, which are slender (Wheeler & Wheeler 1976, 1984) and fit neatly below workers' bodies during colony emigrations (Schneirla 1971). Possible selection on worker diversity associated with transporting different prey types should therefore be considered relative to larva transport. Prey types that closely approximate the shape of *Eciton* larvae, such as the slender poneroid prey of *E. dulcium* and *E. mexicanum*, should be easy for workers to transport. In contrast, some of the prey taken by the other three species differs significantly from the shape of *Eciton* larvae and should therefore be more difficult to fit below the body, resulting in reduced prey-transport efficiency. Lower prey-transport efficiency is likely to strongly impact colony fitness in *Eciton*, because of their unusual foraging ecology and brood rearing method. Colonies forage for a set period each day before moving to a new location, and reduced transport efficiency can result in a significant decrease in overall prey delivery (S. Powell unpublished data). Within the nest, a large synchronized cohort of developing larvae are fed on the fresh prey, without any stored from day to day, but a queen may lay many more eggs than are usually reared to adulthood (Rettenmeyer 1963; Schneirla 1971; S. Powell unpublished data). This suggests that the size of new worker cohorts is limited by daily prey intake, and that reduced prey intake will have an unusually strong affect on colony growth. The result of this is likely to be strong selection for adaptations that improve efficiency. Under such conditions, longer legs should be favoured because they increase the loading space below the body, while exaggerated head and mandible morphology should be selected because they improve grasping and handling of awkward loads.

If the positive relationship between prey variety and worker diversity is indeed causal, it is possible to make two general and testable predictions about the functional relationship between diet and worker diversity in contemporary species. First, more awkwardly shaped prey, such as polymorphic ants, should reduce transport efficiency compared with slender monomorphic ants

and *Eciton* larvae. Efficiency may be affected in terms of the biomass individuals can transport, transport speed, or both. Support for these predictions would suggest that selective pressure for greater efficiency can result from transporting awkwardly shaped prey. Second, we can predict that the individuals with the most exaggerated morphology, and particularly submajors, will specialize in transporting the most awkward prey types, and that they will transport these loads more efficiently than other colony members. Support for these predictions will suggest that the focal changes in worker diversity are indeed adaptations to the reduced transport efficiency associated with awkward prey types. Ultimately it will also be necessary to test whether reduced efficiency negatively impacts colony fitness in *Eciton*. However, without studies that first address how the functional relationship between prey type and worker diversity affects efficiency, evidence that reduced efficiency impacts colony fitness provides no insight on the importance of ecology in the evolution of worker diversity.

The general predictions outlined above can be made more specific for any of the detailed patterns we have identified here. For example, we have shown that *E. burchellii* is the only species to take nonhymenopteran arthropod prey, and that this is associated with the most exaggerated submajor morphology. We can predict therefore that nonhymenopteran arthropod prey has a greater negative impact on transport efficiency than other prey types, that *E. burchellii* submajors specialize in transporting this type of prey, that they transport it more efficiently than nonsubmajors, and that they transport items with this shape more efficiently than the less exaggerated submajors of other species. Based on our findings here, we have already tested some of these predictions, yielding compelling support for a causal relationship between the unique diet of *E. burchellii* and their uniquely exaggerated submajor caste (Powell & Franks 2005).

At this stage, it is also worth acknowledging that hypothetically the evolutionary origin and elaboration of submajors could have resulted from selection unrelated to diet, and that presence of this caste secondarily allowed a wider range of prey types. However, this appears to be very unlikely based on existing knowledge of *Eciton* biology. In all *Eciton*, their preferred prey is captured and subdued by a raid front populated primarily by mid-sized standard workers (T. Brown, personal communication S. Powell unpublished data). Once large prey has been subdued, or a cache of prey ants has formed, submajors then start to arrive in significant numbers to help with retrieval (S. Powell unpublished data). In short, submajors are not responsible for capturing the prey that they specialize in transporting. It is also worth noting that submajors are not known to specialize on any task other than prey transport. This suggests that if selective forces unrelated to diet drove the evolution of this caste, then these forces are no longer of any significance to colony fitness in extant species. Generally,

then, the biology of *Eciton* indicates that a greater variety of prey types, determined primarily by the behaviour of standard workers in the raid front, selected for a specialized submajor caste and not vice versa. Future experimental and phylogenetic studies should have the power to firmly establish the causal direction in the relationship between diet and submajor evolution.

Generally, modern comparative analyses have been used rarely in the study of ecology and worker morphology. Noteworthy exceptions to this rule are relatively recent studies addressing the 'size grain hypothesis'. Briefly, this hypothesis suggests that smaller ants tend to inhabit more cramped spatial niches, such as crevices in the soil, and that this selects for shorter legs (Kaspari & Weiser 1999). This reasoning has helped explain interspecific differences in worker morphology (Kaspari & Weiser 1999; Yanoviak & Kaspari 2000; Espadaler & Gomez 2001; Parr, Parr & Chown 2003; Farji-Brener, Barrantes & Ruggiero 2004), as well as worker diversity in one recent study (Schöning *et al.* 2005). Nevertheless, many ant lineages with pronounced worker diversity use very similar spatial niches. For instance, there are striking differences in worker diversity within *Cephalotes*, the most speciose ant genus limited to the New World, but all species nest in arboreal cavities (De Andrade & Baroni Urbani 1999). Indeed, all *Eciton* forage and emigrate on the surface, but our analysis has shown that there are still clear differences in worker diversity and that they are associated with ecological differences unrelated to spatial niche. Thus, while studies addressing the size grain hypothesis can identify broad patterns in worker morphology among taxa that differ significantly in spatial niche, our approach can identify important patterns in ecology and worker diversity within lineages that share a common spatial niche. The two approaches are therefore complementary ways of studying the relationships between ecology and worker diversity in eusocial taxa.

Broadly, our study demonstrates that relatively straightforward comparative analyses can identify new and potentially adaptive relationships between particular ecological variables and key aspects of worker morphological diversity. Greater use of such analyses, combined with subsequent experimental studies to address causation, therefore promise valuable new insights on an important and poorly understood aspect of social evolution.

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