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Worker caste determination in the army ant *Eciton burchellii*

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Elaborate division of labour has contributed significantly to the ecological success of social insects. Division of labour is achieved either by behavioural task specialization or by morphological specialization of colony members. In physical caste systems, the diet and rearing environment of developing larvae is known to determine the phenotype of adult individuals, but recent studies have shown that genetic components also contribute to the determination of worker caste. One of the most extreme cases of worker caste differentiation occurs in the army ant genus *Eciton*, where queens mate with many males and colonies are therefore composed of numerous full-sister subfamilies. This high intracolonial genetic diversity, in combination with the extreme caste polymorphism, provides an excellent test system for studying the extent to which caste determination is genetically controlled. Here we show that genetic effects contribute significantly to worker caste fate in *Eciton burchellii*. We conclude that the combination of polyandry and genetic variation for caste determination may have facilitated the evolution of worker caste diversity in some lineages of social insects.

Keywords: division of labour; polyandry; multiple mating; worker polymorphism; social insects

1. INTRODUCTION

Division of labour increases efficiency and has contributed significantly to the ecological success of insect societies (Oster & Wilson 1978; Bourke & Franks 1995). Worker specialization in colonies requires behavioural or morphological differentiation among nest members, which can be achieved either by assuming temporary roles or by the evolution of permanently differentiated morphological castes (Oster & Wilson 1978).

The most fundamental physical caste differentiation in social insects is the reproductive division of labour between workers and queens. However, in termites and approximately 15% of the ant genera, the worker collective can also be differentiated into multiple castes as a result of differences in allometric growth during larval development (Oster & Wilson 1978; Hölldobler & Wilson 1990). Traditionally, environmental factors have been assumed to be the main drivers of caste differentiation (Oster & Wilson 1978; Robinson 1992), but genetic effects on queen–worker caste determination are known for some bees and ants (Kerr 1950; Julian *et al.* 2002; Volny & Gordon 2002; Moritz *et al.* 2005). Furthermore, genetic components in the determination of worker polymorphism have been demonstrated in some ant species, where queens are either inseminated by many males (polyandry) or where colonies are headed by several reproducing queens (polygyny). In these cases, the workers of different families or subfamilies within a colony develop under very similar conditions, allowing for the detection of genetic effects on caste differentiation. Two studies on polygynous ant species have reported that worker size is influenced by the genotypes of mother queens and their mates (Fraser *et al.* 2000; Schwander *et al.* 2005). However, it is difficult to exclude differences in rearing environment between the offspring of different queens as a confounding factor in studies of polygynous species (Fraser *et al.* 2000). Two further studies on ant species with polyandrous queens in single queen colonies have demonstrated a genetic effect on worker differentiation into either small or large workers (Hughes *et al.* 2003; Rheindt *et al.* 2005). However, a species providing both extreme intracolonial genetic variation via polyandry, and extreme morphological worker polymorphism, would be the most suitable model to test the generality of genetic effects on caste differentiation in insect societies.

Eciton burchellii, an army ant of New World tropical forests, shows an extreme worker polymorphism with four recognized physical worker castes (Franks 1985; figure 1). Moreover, *E. burchellii* queens typically mate with 10–20 males, resulting in a large number of worker patrilines (full-sib families with the same mother and father) in each colony (Kronauer *et al.* 2006). Taking advantage of this combination of social traits, we show that genes have a significant effect on worker caste determination.

Figure 1. Extreme worker polymorphism of the army ant *E. burchellii*. Here, a major with ivory-coloured head and sabre-shaped mandibles guards a raiding column in which smaller workers carry prey items (photograph by D. J. C. Kronauer).

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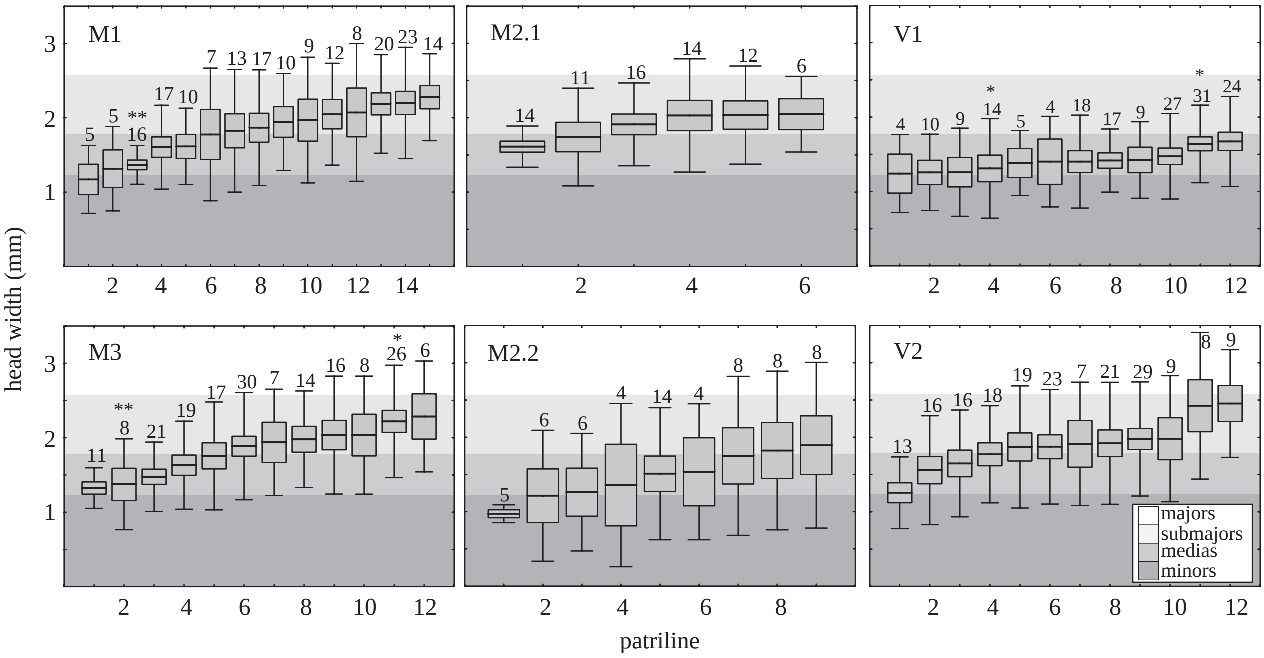

Figure 2. Ranked worker head width variation among the most frequent patrilines (865 genotyped workers) of five *E. burchellii* colonies (six matrilines). Caste size ranges are indicated by different grey shadings. Overall caste representation among patrilines was significantly skewed ($p<0.01$). Significant biases in caste representation within individual patrilines are indicated by $^{*}p<0.05$ and $^{**}p<0.01$, while mean values, standard errors and standard deviations are represented by horizontal bars, grey boxes and whiskers, respectively. Sample sizes are given above the whiskers.

2. MATERIAL AND METHODS

(a) *Genotyping*

We genotyped a total of 1143 workers from three *E. burchellii* colonies collected near Tapachula, Chiapas, Mexico (M1: $n=264$, M2: $n=209$ and M3: $n=271$), and two colonies collected in Parque Nacional Henri Pittier, Venezuela (V1: $n=200$ and V2: $n=199$), for different combinations of seven polymorphic DNA microsatellites (Denny *et al.* 2004). DNA extractions were performed following a Chelex protocol and PCR products were run on an ABI377 or a MegaBACE 1000 capillary sequencer.

(b) *Patriline assignment*

Workers were assigned to patrilines using the program MateSoft v. 1.0 (Moilanen *et al.* 2004), as described by Kronauer *et al.* (2006). The probability that two males could not be distinguished in our analysis was very low (between 0.01 and 3.7×10^{-5}; Boomsma & Ratnieks 1996). Since colony M2 consisted of offspring from two queens (presumably due to a recent colony fission event), workers were assigned to the deduced queens following the most parsimonious explanation based on Mendelian inference (Kronauer *et al.* 2004).

(c) *Analyses*

Sample size corrected effective queen mating frequencies were calculated according to Nielsen *et al.* (2003), with standard errors from jackknifing over colonies. In order to quantify the phenotypic variability, we measured maximum head width in 991 individuals (all those with more than three successfully amplified loci, unambiguously assigned into a patriline containing more than one individual). Repeating the measurements for 20 individuals (five per caste) and performing a linear regression, we showed that our head width measurements were highly repeatable ($r^2=0.99$, $p<0.001$). We grouped individuals into four distinct castes following Franks (1985): minors (less than 1.16 mm); medias (1.27–1.72 mm); submajors (1.84–2.51 mm); and majors (above 2.63 mm). We employed a χ^2 analysis to test whether the distribution of worker castes deviated from homogeneity across patrilines (Sokal & Rohlf 1994). In three colonies, some castes were merged due to their low representation among the sampled workers (electronic supplementary material). Workers with head widths in between the specified ranges of caste values were omitted from this analysis, except when castes were merged. In order to estimate heritability of worker size, we determined the variance components of worker head width through a hierarchical analysis of variance (ANOVA), nesting patrilines within colonies (Falconer & Mackay 1996). The standard error of the estimate and lower 95% confidence limit was obtained by jackknifing over colonies.

3. RESULTS

We found that queens had mated with 13–25 males (arithmetic mean±s.e.: 16.7±0.4), with an effective number of matings between 9.4 and 17.8 (harmonic mean±s.e.: 11.6±0.2) confirming earlier estimates (Kronauer *et al.* 2006). Overall caste representation among patrilines was highly significantly skewed ($\chi^2_{147}=193.77$, $p=0.006$) and several patrilines were significantly biased towards producing certain worker morphs (figure 2; electronic supplementary material). This demonstrates a considerable genetic component influencing caste determination. The variance component estimation resulted in an index of head width heritability of $h^2=0.15$ (±0.01 s.e.; lower 95% confidence limit$=0.13$), providing further evidence for a significant genetic component to caste determination.

4. DISCUSSION

As in other social Hymenoptera with a demonstrated genetic component for caste determination (Fraser *et al.* 2000; Page & Erber 2002; Hughes *et al.* 2003; Rheindt *et al.* 2005; Schwander *et al.* 2005; Hughes & Boomsma 2007), the genetic control in *E. burchellii* is plastic rather than hard-wired. Most males were able to sire daughters of all worker castes (figure 2; electronic supplementary material) and the additive genetic variance accounted for only 15% of the total observed polymorphism, the remaining 85% being probably due to environmental effects and maternal genes. The mechanisms by which genetic effects translate into worker phenotypes of different sizes remain unknown. However, as body size variation appears already at early larval stages (Schneirla 1971), they may involve direct effects on the rate of larval development, larval hunger signals or larval attractiveness towards the nursing workers, i.e. the key variables that determine body size.

Size-specific allometries would then produce the observed caste differences (Powell & Franks 2006).

Irrespective of the adaptive value of polyandry and polygyny (Keller 1993; Palmer & Oldroyd 2000; Crozier & Fjerdingstad 2001), both have important consequences for colony composition in that they enhance intracolonial genetic variance. Genetically determined thresholds for the development of a larva into a specific morphological caste, or for an adult to perform a certain task, will lead to a non-random association of castes with patrilines or matrilines within a colony. The details of these associations have been well studied in polyandrous honeybees, where gene effects are known to determine the response thresholds of individuals to task-associated stimuli. As a result, behavioural specialists are differentially represented across the different patrilines (Frumhoff & Baker 1988; Robinson & Page 1988; Page *et al.* 1995; Page & Erber 2002; Oldroyd & Fewell 2007).

So far, a genetic influence on worker caste determination has only been detected in polyandrous or polygynous species, which are most amenable to such studies because different subfamilies develop under similar environmental conditions in the same colony (Fraser *et al.* 2000; Hughes *et al.* 2003; Rheindt *et al.* 2005; Schwander *et al.* 2005). However, this does not imply that strictly monandrous and monogynous species completely lack a genetic component to worker caste. A recent study has shown that polyandry occurs both in army ants with monomorphic and polymorphic workers (Kronauer *et al.* 2007), suggesting that multiple mating did not evolve to promote greater morphological variability among workers *per se*. Nevertheless, worker polymorphism and intracolonial genetic diversity appear to be positively correlated across ant taxa (Fjerdingstad & Crozier 2006), possibly because high genetic variation combined with a genetic component to worker caste determination increases homeostasis in systems with complex division of labour (Fjerdingstad & Crozier 2006; Hughes & Boomsma 2007; Oldroyd & Fewell 2007).

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