

Worker caste determination in the army ant *Eciton burchellii*

Rodolfo Jaffé^{1,*}, Daniel J. C. Kronauer^{2,†},
F. Bernhard Kraus³, Jacobus J. Boomsma²
and Robin F. A. Moritz¹

¹Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, 06099 Halle/Saale, Germany

²Department of Population Biology, Institute of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark

³El Colegio de la Frontera Sur (ECOSUR), Carretera Antigua

Aeropuerto km 2.5, C.P. 30700 Tapachula, Chiapas, Mexico

*Author for correspondence (rodolfo.jaffe@zoologie.uni-halle.de).

†These authors contributed equally to this work.

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 intracolony 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,

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2007.0257> or via <http://www.journals.royalsoc.ac.uk>.

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 intracolony 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).

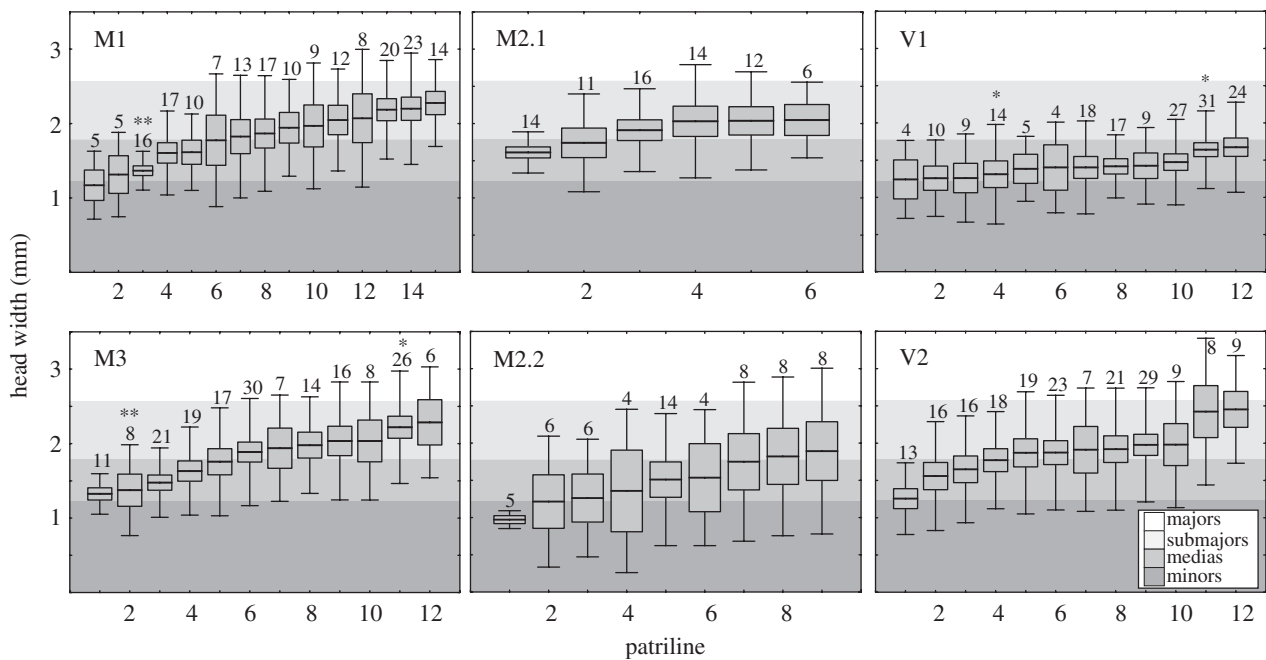


Figure 2. Ranked worker head width variation among the most frequent patriline (865 genotyped workers) of five *E. burchellii* colonies (six matriline). Caste size ranges are indicated by different grey shadings. Overall caste representation among patriline was significantly skewed ($p < 0.01$). Significant biases in caste representation within individual patriline 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 patriline 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 patriline (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 patriline 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 \pm s.e.: 16.7 ± 0.4), with an effective number of matings between 9.4 and 17.8 (harmonic mean \pm s.e.: 11.6 ± 0.2) confirming earlier estimates (Kronauer *et al.* 2006). Overall caste representation among patriline was highly significantly skewed ($\chi^2_{147} = 193.77$, $p = 0.006$) and several patriline 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 intracolony 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 patriline or matriline 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 intracolony 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).

We thank P. Leibe, F. Hesche, D. Kleber, H. Bang Madsen, S. Mathiasen, O. Argüello Najera and E. Rodríguez for their valuable assistance. Financial support was provided by a DAAD-FUNDAYACUCHO scholarship (R.J.) and the Danish Research Training Council (D.J.C.K.).

- Boomsma, J. J. & Ratnieks, F. L. W. 1996 Paternity in eusocial Hymenoptera. *Phil. Trans. R. Soc. B* **351**, 947–975. (doi:10.1098/rstb.1996.0087)
- Bourke, A. F. G. & Franks, N. R. 1995 *Social evolution in ants*. Princeton, NJ: Princeton University Press.
- Crozier, R. H. & Fjerdingstad, E. J. 2001 Polyandry in social Hymenoptera—disunity in diversity? *Ann. Zool. Fenn.* **38**, 267–285.
- Denny, A. J., Franks, N. R. & Edwards, K. J. 2004 Eight highly polymorphic microsatellite markers for the army ant *Eciton burchellii*. *Mol. Ecol. Notes* **4**, 234–236. (doi:10.1111/j.1471-8286.2004.00627.x)
- Falconer, D. S. & Mackay, T. F. C. 1996 *Introduction to quantitative genetics*, 4th edn. New York, NY: Longman.
- Fjerdingstad, E. J. & Crozier, R. H. 2006 The evolution of worker caste diversity in social insects. *Am. Nat.* **167**, 390–400. (doi:10.1086/499545)
- Franks, N. R. 1985 In *Reproduction, foraging efficiency and worker polymorphism in army ants*, vol. 31 (eds B. Hölldobler & M. Lindauer) *Experimental behavioral ecology and sociobiology*, pp. 91–107. Sunderland, UK: Sinauer.
- Fraser, V. S., Kaufmann, B., Oldroyd, B. P. & Crozier, R. H. 2000 Genetic influence on caste in the ant *Camponotus consobrinus*. *Behav. Ecol. Sociobiol.* **47**, 188–194. (doi:10.1007/s002650050010)
- Frumhoff, P. C. & Baker, J. 1988 A genetic component to division of labour within honey bee colonies. *Nature* **333**, 358–361. (doi:10.1038/333358a0)
- Hölldobler, B. & Wilson, E. O. 1990 *The ants*. Cambridge, UK: Belknap Press of Harvard University Press.
- Hughes, W. O. H. & Boomsma, J. J. 2007 Genetic polymorphism in leaf-cutting ants is phenotypically plastic. *Proc. R. Soc. B* **274**, 1625–1630. (doi:10.1098/rspb.2007.0347)
- Hughes, W. O. H., Sumner, S., van Borm, S. & Boomsma, J. J. 2003 Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. *Proc. Natl Acad. Sci. USA* **100**, 9394–9397. (doi:10.1073/pnas.1633701100)
- Julian, G. E., Fewell, J. H., Jürgen, G., Johnson, R. A. & Larrabee, D. 2002 Genetic determination of the queen caste in an ant hybrid zone. *Proc. Natl Acad. Sci. USA* **99**, 8157–8160. (doi:10.1073/pnas.112222099)
- Keller, L. 1993 *Queen number and sociality in insects*. New York, NY: Oxford University Press.
- Kerr, W. E. 1950 Evolution of the mechanism of caste determination in the genus *Melipona*. *Evolution* **4**, 7–13. (doi:10.2307/2405530)
- Kronauer, D. J. C., Schöning, C., Pedersen, J. S., Boomsma, J. J. & Gadau, J. 2004 Extreme queen-mating frequency and colony fission in African army ants. *Mol. Ecol.* **13**, 2381–2388. (doi:10.1111/j.1365-294X.2004.02262.x)
- Kronauer, D. J. C., Berghoff, S. M., Powell, S., Denny, A. J., Edwards, K. J., Franks, N. R. & Boomsma, J. J. 2006 A reassessment of the mating system characteristics of the army ant *Eciton burchellii*. *Naturwissenschaften* **93**, 402–406. (doi:10.1007/s00114-006-0121-2)
- Kronauer, D. J. C., Johnson, R. A. & Boomsma, J. J. 2007 The evolution of multiple mating in army ants. *Evolution* **61**, 413–422. (doi:10.1111/j.1558-5646.2007.00040.x)
- Moilanen, A., Sundström, L. & Pedersen, J. S. 2004 MATESOFT, a program for deducing parental genotypes and estimating mating system statistics in Haplodiploid species. *Mol. Ecol. Notes* **4**, 795–797. (doi:10.1111/j.1471-8286.2004.00779.x)
- Moritz, R. F. A., Lattorff, H. M. G., Neumann, P., Kraus, F. B., Radloff, S. E. & Hepburn, H. R. 2005 Rare royal families in honeybees, *Apis mellifera*. *Naturwissenschaften* **92**, 488–491. (doi:10.1007/s00114-005-0025-6)
- Nielsen, R., Tarp, D. R. & Reeve, H. K. 2003 Estimating effective paternity number in social insects and the effective number of alleles in a population. *Mol. Ecol.* **12**, 3157–3164. (doi:10.1046/j.1365-294X.2003.01994.x)
- Oldroyd, B. P. & Fewell, J. H. 2007 Genetic diversity promotes homeostasis in insect colonies. *Trends Ecol. Evol.* **305**, 402–404. (doi:10.1016/j.tree.2007.06.001)
- Oster, G. F. & Wilson, E. O. 1978 *Caste and ecology in the social insects*. Princeton, NJ: Princeton University Press.
- Page, R. E. & Erber, J. 2002 Levels of behavioral organization and the evolution of division of labor. *Naturwissenschaften* **89**, 91–106. (doi:10.1007/s00114-002-0299-x)
- Page, R. E., Robinson, G. E., Fondrk, M. K. & Nasr, M. E. 1995 Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L.). *Behav. Ecol. Sociobiol.* **36**, 387–396. (doi:10.1007/s002650050161)

- Palmer, K. A. & Oldroyd, B. P. 2000 Evolution of multiple mating in the genus *Apis*. *Apidologie* **31**, 235–248. (doi:10.1051/apido:2000119)
- 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. (doi:10.1111/j.1365-2435.2006.01184.x)
- Rheindt, F. E., Strehl, C. P. & Gadau, J. 2005 A genetic component in the determination of worker polymorphism in the Florida harvester ant *Pogonomyrmex badius*. *Insect. Soc.* **52**, 163–168. (doi:10.1007/s00040-004-0787-4)
- Robinson, G. E. 1992 Regulation of division of labor in insect societies. *Ann. Rev. Entomol.* **37**, 637–665. (doi:10.1146/annurev.en.37.010192.003225)
- Robinson, G. E. & Page, R. E. 1988 Genetic determination of guarding and undertaking in honey-bee colonies. *Nature* **333**, 356–358. (doi:10.1038/333356a0)
- Schneirla, T. C. 1971 *Army ants—a study in social organization*. San Francisco, CA: W. H. Freeman and Company.
- Schwander, T., Rosset, H. & Chapuisat, M. 2005 Division of labour and worker size polymorphism in ant colonies, the impact of social and genetic factors. *Behav. Ecol. Sociobiol.* **59**, 215–221. (doi:10.1007/s00265-005-0027-6)
- Sokal, R. R. & Rohlf, F. J. 1994 *Biometry*. New York, NY: W. H. Freeman and Company.
- Volny, V. P. & Gordon, D. M. 2002 Genetic basis for queen–worker dimorphism in a social insect. *Proc. Natl Acad. Sci. USA* **99**, 6108–6111. (doi:10.1073/pnas.092066699)