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Genetic diversity, worker size polymorphism and division of labour in the polyandrous ant *Cataglyphis cursor*

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Understanding the adaptive significance of multiple mating (polyandry) by females has long been a challenge in evolutionary biology. Several genetic and nongenetic benefits have been proposed to explain the evolution and maintenance of polyandry. In eusocial Hymenoptera, a prominent hypothesis is that increased genetic diversity within colonies results in more polymorphic workers and facilitates division of labour. We analysed the genetic basis of worker size (i.e. worker head width) and task preference in *Cataglyphis cursor*, an ant showing natural variations in queen-mating frequency. Our data show that increased genetic diversity within colonies does not result in more polymorphic workers. Moreover, worker head width is not different between patrilines within colonies. Consistent with these findings, worker size has a low heritable component. Moreover, task performance is not correlated with patriline. By contrast, it is significantly associated with worker size: the first foragers leaving the nest at sunrise are significantly larger than workers remaining in the nest. Overall, these results do not support the hypothesis that multiple mating is favoured because increased genetic diversity within colonies translates into more polymorphic workers and facilitates genetic polyethism. We discuss other hypotheses to account for the evolution of polyandry in *C. cursor*.

Keywords: ants; *Cataglyphis cursor*; genetic variability; polyandry; polyethism; size polymorphism; social insects

In many animal species females do not mate once but several times (i.e. polyandry). Social Hymenoptera (ants, bees, wasps) are no exception to this rule and queens of several species mate multiply. Multiple mating is prominent in eight genera, the honeybee (*Apis*: Palmer & Oldroyd 2000), the yellow jacket wasps (*Vespula*: Goodisman et al. 2002), the seed harvester ants (*Pogonomyrmex*: Rheindt et al. 2004), the higher leaf-cutting ants (*Atta* and *Acromyrmex*: Boomsma et al. 1999; Sumner et al. 2004), the army ants (*Dorylus* and *Eciton*: Kronauer et al. 2004; Kronauer et al. 2006) and the desert ant (*Cataglyphis*: Pearcy et al. 2004a). This suggests that polyandry has benefits to queen fitness.

One proposal for the evolution of polyandry in social Hymenoptera is that increased genetic diversity among worker offspring translates into a more efficient division of labour (polyethism), so raising the efficiency of the colony and its overall productivity (Crozier & Page 1985; Robinson & Page 1995; Mattila & Seeley 2007; Oldroyd & Fewell 2007). By mating multiply, queens produce genetically diverse workers that carry different genes from their respective fathers. This provides the colony with a spread of workers' genotypes fitted to perform different tasks. This genetically based polyethism would result from genetic variation in the response thresholds to task-related stimuli, leading to some individuals having a greater ability to perform certain tasks. While the effect of genetic diversity on worker task efficiency and colony productivity in social insects still remains debated (see Rosset et al. 2005 and references therein), several empirical tests of the hypothesis have shown that genetic variability increases polyethism. For instance, workers belonging to certain patrilines show a higher tendency to perform certain tasks such as foraging, recruiting, guarding, stinging, or nest-cleaning in the honeybee (Frumhoff & Baker 1988; Robinson & Page 1988, 1989; Page et al. 1989, 1995; Oldroyd et al. 1994), wasps (O'Donnell 1998), and in a few ant species (Stuart & Page 1991; Hughes

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et al. 2003). Similarly, a significant genetic component to division of labour has been reported in ant colonies headed by multiple queens (polygyny), with workers belonging to different matrilines showing a different propensity to perform distinct tasks (Snyder 1992; Blatrix et al. 2000; Julian et al. 2002).

In ants, task preference among workers is often associated with morphological adaptations (Oster & Wilson 1978; Hölldobler & Wilson 1990; Gordon 1996; Passera & Aron 2005). Some species evolved morphologically distinct worker castes, with larger individuals ('majors' or 'soldiers') typically having large heads and specializing in defence of the colony, whereas smaller workers ('minors') specialize in brood tending or foraging. However, the association between the phenotype and the performance of different tasks also commonly occurs in species with no discrete morphological castes, but where worker size is continuous (Oster & Wilson 1978; Porter & Tschinkel 1985; Waser 1998). Environmental cues have long been recognized as key factors responsible for inducing a developmental pathway leading to a certain caste or size (Wilson 1971; Hölldobler & Wilson 1990). Recently, however, a genetic basis of worker polymorphism within colonies has been documented in some species with distinct morphological castes. Workers of different patrilines differ significantly in their propensities to develop into a certain worker caste in *Acromyrmex echinatior* (Hughes et al. 2003) and *Pogonomyrmex badius* (Rheindt et al. 2005), whereas matrilines influence worker caste determination in *Camponotus consobrinus* (Fraser et al. 2000). This lends support to the hypothesis that increased genetic diversity within colonies results in more polymorphic workers, which in turn could facilitate genetic polyethism by increasing phenotypic plasticity at the colony level (Crozier & Page 1985). Yet, the genetic basis of worker polymorphism and its influence on the division of labour in social insects remain largely unexplored. To our knowledge, two such studies were performed in ants. In the leaf-cutting ant, *A. echinatior*, the genetic component to worker caste polymorphism described above is associated with a division of labour, with small workers specializing in intranidal tasks and large workers in foraging (Hughes et al. 2003). A significant genetic component to worker size was also reported in the wood ant, *Formica selysi*, a species with no distinct morphological castes (Schwander et al. 2005). However, higher colony genetic diversity is not associated with increased worker size polymorphism. In addition, patriline and worker size are often correlated with tasks independently of each other, suggesting that division of labour is modulated by multiple factors.

In this study, we examine the relationship between worker size, task performance and patriline in the ant *Cataglyphis cursor*, a species with continuous worker size distribution (Cagniant 1983). Colonies are headed by single, multiple-mated queens showing natural variations in their mating frequency (Pearcy et al. 2004a). A remarkable feature of this species is that queens use alternative modes of reproduction for the production of reproductive and nonreproductive offspring. While workers are produced by sexual reproduction from fertilized eggs, new queens are almost exclusively produced by thelytokous parthenogenesis (Pearcy et al. 2004a). Thus, although *C. cursor* queens do not require mating to produce diploid offspring, they have retained sexual reproduction to produce workers. This, combined with a level of polyandry lying on the high end of the continuum of mating frequencies reported in ants, suggests that sexual reproduction in this species has important benefits for colony function. We first test if size polymorphism among workers increases with the number of patrilines. Second, we look for a genetic component of worker body size. If there is a heritable component in worker size, the extent of size variation between worker offspring of different patrilines is expected to be larger than between offspring of the same patriline. Finally, we examine the relative effect of patriline and size polymorphism on task specialization among workers.

METHODS

Sampling

The study population of *C. cursor* is located at St-Hippolyte, Southern France (42.47°N–2.59°E). The population sampled was the same as that previously studied by Pearcy et al. (2004a). Genetic studies formerly showed that colonies contain a single queen mated with several males contributing equally to the worker brood (Pearcy et al. 2004a).

Twelve colonies, whose entrances were located >1 m from each other so as to prevent mixing individuals from different nests were selected at random in the population in early July 2005. To determine a possible association between patriline, task and size, we collected the very first 15 workers leaving each colony after sunrise, and moving at least 30 cm away from nest entrance. They were immediately stored in EtOH 98% for subsequent morphometric and genetic (paternal identity) analyses. The colonies were then excavated; adults (the queen and workers) as well as brood at various stages (eggs, larvae and worker pupae) were collected and brought into the laboratory for other purpose. A sample of workers from each nest was taken and stored at −80°C for determination of the genetic structure and number of patrilines of each colony.

Morphometrics

We first took seven body measurements at a magnification of ×50 to the nearest 0.01 mm using a MZ6 stereomicroscope (Leica Microsystems, Wetzlar, Germany) on a subsample of 108 workers from nine colonies. We measured the maximum head width (eyes included), head width between eyes, scape length, thorax length, tibia and femur lengths of the hindleg, and width of the pronotum. To test for repeatability, the seven criteria were measured twice on 40 individuals. Repeated measures were highly correlated (Pearson correlation: $r_p > 0.83$, $P < 0.042$). The seven measures varied isometrically with each other, with correlation coefficients ranging between 0.87 and 0.97 (ANOVA of multiple regression: all $P < 0.001$). Maximum head width was therefore used as a single estimate of size, because of its high correlation