



# 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 patriline 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*.

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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*: Percy 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 matrilineal lines 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 patrilineal lines differ significantly in their propensities to develop into a certain worker caste in *Acromyrmex echinator* (Hughes et al. 2003) and *Pogonomyrmex badius* (Rheindt et al. 2005), whereas matrilineal lines 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. echinator*, 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, patrilineal line 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 patrilineal line 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 patrilineal lines. 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 patrilineal lines is expected to be larger than between offspring of the same patrilineal line. Finally, we examine the relative effect of patrilineal line 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 Percy 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 patrilineal line, 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 patrilineal lines of each colony.

### Morphometrics

We first took seven body measurements at a magnification of  $\times 50$  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

with all other body measurements (multiple regression coefficient:  $r = 0.97$ ,  $F_{6,100} = 303.95$ ,  $P < 0.001$ ). Moreover, head width measures showed high levels of precision and repeatability (repeated measures differed on average by only 0.02 mm and were highly correlated ( $r_p = 0.99$ ,  $P < 0.001$ )). Overall, to compare worker polymorphism across colonies, we measured maximum head width of 40 workers (i.e. the first 15 workers leaving the nest after sunrise plus 25 workers randomly chosen) from each of the 12 colonies sampled.

### Genotypic Analyses

To test for a possible influence of patriline on worker size and/or task performance, a sample of workers made of the first 15 individuals leaving each nest in the morning plus 10–20 workers for which head width was measured (mean  $\pm$  SD =  $28.8 \pm 2.9$ ;  $N = 345$  workers), and the queen ( $N = 12$ ) from each colony was genotyped at four polymorphic microsatellite loci (*Ccur-46*, *Ccur-11*, *Ccur-58* and *Ccur-63b*; Pearcy et al. 2004b). In the population under study, these markers show 12, 14, 14 and 12 alleles, respectively, and a level of heterozygosity observed ranging from 0.78 to 0.86.

Individual ant DNA was extracted from fine ground samples by incubating for 90–120 min in 120  $\mu$ l of Chelex (Bio-Rad, Hercules, CA, U.S.A.) 5% at 95°C. Samples were centrifuged for 30 s at 12 000 rpm and 2  $\mu$ l of the supernatant was amplified by PCR following the fluorescent analysis protocols described in Pearcy et al. (2004b), using a MJ-Research PTC-200 thermal cycler (Bio-Rad, Hercules, CA, U.S.A.). The amplified products were separated on ABI Prism 3100 capillary sequencer and sized against Rox-350 standard (Applied Biosystems, Foster City, CA, U.S.A.).

### Statistical Analyses

To estimate worker size polymorphism within colonies, we used the variation index corrected for sample size (Sokal & Rohlf 1995),

$$v^* = \left(1 + \frac{1}{4n}\right) \left(\frac{s \times 100}{M}\right)$$

where  $M$  and  $s$  are the mean and standard deviation of maximum head width, respectively, and  $n$  is the sample size.

We examined whether size polymorphism among workers increases with colony genetic diversity in two ways: by testing if the variation index of maximum head width ( $v^*$ ) was negatively correlated with nestmate relatedness or positively correlated with the number of patrilines. Relatedness coefficients were estimated using the program Relatedness 5.0.8 according to the algorithm described by Queller & Goodnight (1989). The population mean relatedness was estimated by weighting colonies equally and standard errors were obtained by jackknifing over colonies. Standard errors over the relatedness within each colony and 95% confidence intervals were obtained by a jackknife procedure over the four loci. The absolute number of patrilines  $k_a$  in each colony was inferred on the basis of the

queen and workers' genotypes. We assigned individuals to patrilines with the maximum-likelihood methods implemented in the program Colony 1.1 (Wang 2004; <http://www.zoo.cam.ac.uk/ioz/software.htm>), assuming a single multiple-mated queen per nest. All workers sampled, except two, were unambiguously assigned to the queen present in each nest. The two 'odd' workers (one per colony) probably belonged to different colonies and were removed from the data set. Multiple runs of the program assigned consistently the same father to each worker offspring. We also estimated the effective number of fathers  $k_e$  following Nielsen et al. (2003; equation 16),

$$k_e = \frac{(n-1)^2}{\sum_{i=1}^k p_i^2 (n+1)(n-2) + 3 - n}$$

where  $n$  is the sample size and  $p_i$  is the proportional contribution to the brood of the  $i$ th mate.

To assess whether there is a genetic component to worker size, we compared the maximum head width between workers of different patrilines within each colony and between workers from different colonies. We used a nested ANOVA with three hierarchical levels (Lynch & Walsh 1998): size variation between individuals of different colonies, size variation between workers of different patrilines within each colony, and size variation between individuals belonging to the same patriline. Heritability was estimated for haplodiploid organisms following Liu & Smith (2000),

$$h^2 = \frac{2\sigma_p}{\sigma_t}$$

where  $\sigma_p$  is the variance component at the patriline level and  $\sigma_t$  the total variance component.

To sort out the relative effect of patriline and size polymorphism on task specialization (to forage or stay in the nest at sunrise), we tested for an association between the workers' behaviour, patriline and maximum head width with a multivariate analysis of variance (MANOVA).

Deviations of the variable from normality and evidence for homoscedasticity were tested using Kolmogorov–Smirnov and Levene's tests, respectively. Parametric statistics were used when the data showed a normal distribution and equality of variances; otherwise nonparametric statistics were used (Sokal & Rohlf 1995). For multiple comparisons, level of significance was adjusted with the Bonferroni correction (Rice 1989). For statistical reasons, patrilines that were represented by a single worker were omitted from nested ANOVA and MANOVA analyses, as well as from Kruskal–Wallis tests. All statistical tests were bilateral; they were carried out with the computer program SPSS 13.0 (SPSS Inc., Chicago, IL, U.S.A.).

## RESULTS

### Worker Size Polymorphism and Genetic Diversity

Worker size had a unimodal distribution, with head width across colonies ranging from 0.83 mm to 1.85 mm (mean  $\pm$  SD =  $1.28 \pm 0.19$  mm).

Over the 12 colonies sampled, the mean genetic relatedness among workers was 0.27 (jackknife SE = 0.02). The absolute number of patriline per colony ranged from 7 to 14, and was on average  $k_a = 10.08 \pm 2.15$ , and the effective number of patrilines reached a value of  $k_e = 9.44 \pm 3.13$  (range 5.26–15.53). Increased genetic diversity did not result in higher worker size polymorphism. Size variation was not significantly associated with the within-colony relatedness (Spearman rank correlation:  $r_s = 0.14$ ,  $P = 0.966$ ), or with the number of patrilines (absolute:  $r_s = -0.14$ ,  $P = 0.677$ ; effective:  $r_s = 0$ ,  $P = 1$ ). Moreover, mean worker size did not increase significantly with the colony level of genetic diversity (within-colony relatedness:  $r_s = 0.19$ ,  $P = 0.546$ ; absolute number of patrilines:  $r_s = 0.11$ ,  $P = 0.745$ ; effective number of patrilines:  $r_s = -0.05$ ,  $P = 0.871$ ).

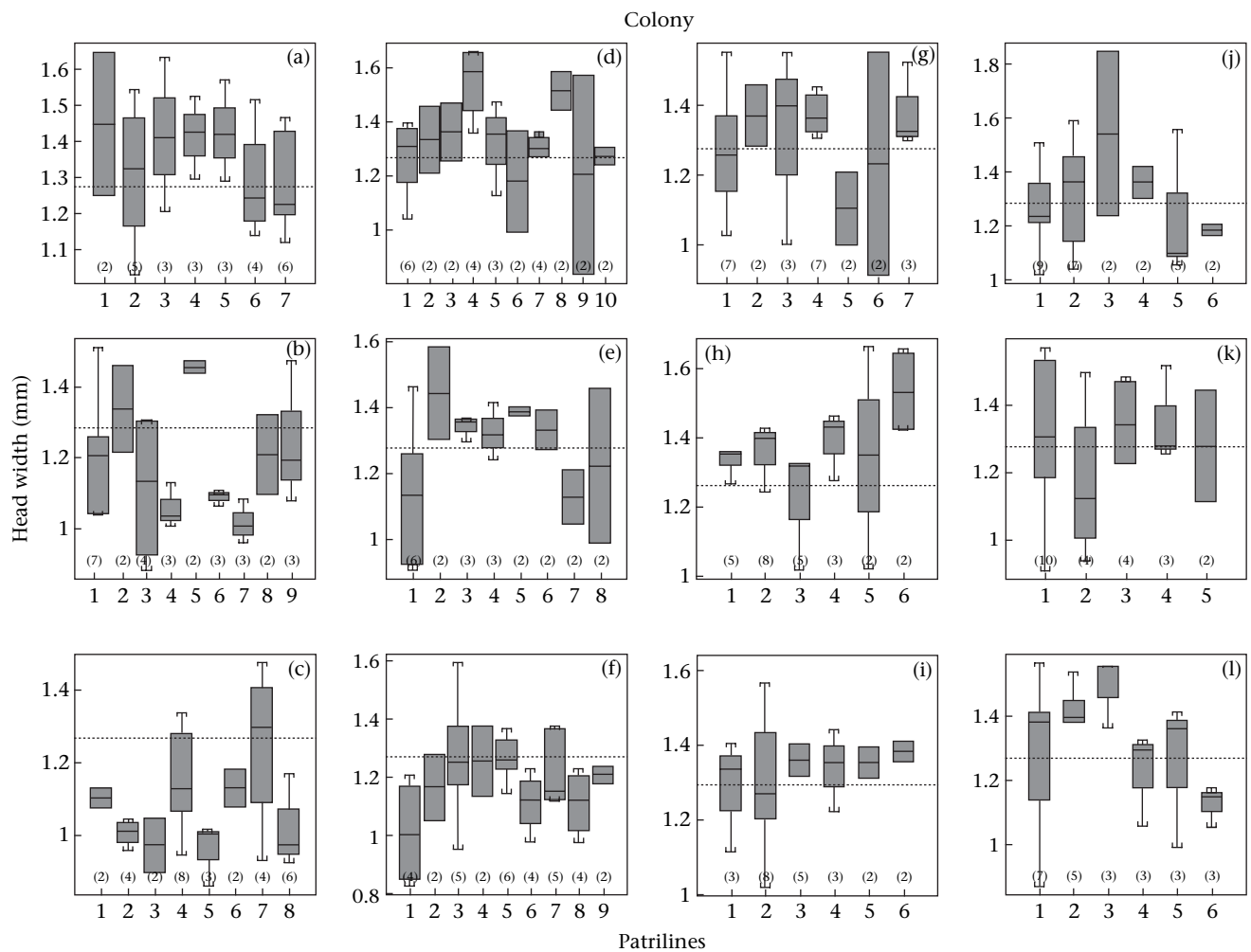
### Heritability of Worker Size

Worker size varied between colonies, but not between patrilines within colonies (nested ANOVA: effect of colony:  $F_{11,298} = 5.86$ ,  $P < 0.001$ ; effect of patrilines in each colony:

$F_{75,234} = 1.16$ ,  $P = 0.206$ ). Worker size variation between colonies indicated that head width differed significantly or was close to significance in two out of the 12 colonies ( $P = 0.002$  and  $P = 0.073$  for colony H and G, respectively), and that workers belonging to certain patrilines differed in head width in three colonies (colony H: patriline 3:  $P = 0.019$ ; colony J: patriline 3:  $P = 0.040$ ; colony L: patrilines 2 and 3:  $P = 0.018$  and 0.011, respectively; Fig. 1). However, when colonies were analysed separately, workers size was not significantly different between patrilines (Kruskal–Wallis tests: all  $P > 0.108$ ). Consistent with these results, the heritability estimate of worker head width was low and equal to  $h^2 = 0.10 \pm 0.09$  (lower limit of the 95% CI = 0.05).

### Effect of Patriline and Size on Task Specialization

The first foragers leaving the nest at sunrise were significantly larger than workers remaining in the nest (mean head width  $\pm$  SD =  $1.30 \pm 0.20$  mm and  $1.24 \pm 0.18$  mm,



**Figure 1.** Size distribution of workers from each patriline within the 12 colonies of *Cataglyphis cursor*. Box plots show median and first and third quartile; whiskers include 95% of all observations. Population mean head width (1.28 mm) is depicted as a dotted horizontal line. Sample size per patriline is given in brackets.

respectively; ANOVA:  $F_{1,308} = 7.12$ ,  $P = 0.008$ ). By contrast, task was not significantly associated with paternal lineage; the genetic relatedness among the first foragers leaving the nest did not differ from the relatedness among the workers in the nest or from the mean within-colony relatedness (ANOVA:  $F_{2,33} = 0.72$ ,  $P = 0.494$ ).

Across the 12 colonies sampled, worker size and patriline have independent effects on task specialization, and worker size only was associated with task preference. When colonies were analysed separately, the association between worker size and task was significant in two colonies (MANOVA:  $F_{1,13} = 8.02$ ,  $P = 0.014$  and  $F_{1,15} = 9.42$ ,  $P = 0.009$  for colonies H and K, respectively), and marginally significant in two others ( $F_{1,18} = 4.32$ ,  $P = 0.054$  and  $F_{1,15} = 4.13$ ,  $P = 0.063$  for colonies J and G, respectively).

## DISCUSSION

Our results show that increased genetic diversity within colonies does not result in more polymorphic workers in the ant *C. cursor*. Most of the colony-wide size variation is present within each patriline and worker head width is not different between patrilines within colonies (Fig. 1). These findings are consistent with other studies showing that increasing the number of breeders does not extend the colony phenotype in ants. Using comparative analyses and simulations models over a large data set of 82 ant species, Brown & Schmid-Hempel (2003) reported no significant association between polyandry and polymorphism after controlling for phylogeny. Similarly, in two species with a continuous (unimodal) worker size distribution like *C. cursor*, namely *Formica truncorum* (Bargum et al. 2004) and *Formica selysi* (Schwander et al. 2005), worker size polymorphism did not increase with intracolony genetic diversity. This suggests that multiple mating or high queen numbers may not be advantageous in terms of increased polymorphism at colony level in these species.

Moreover, we found that the head width of workers had a low heritability ( $h^2 = 0.10$ ). A heritable component to worker size was reported in some ant species. Workers from different patrilines reportedly differ in size in *F. selysi*, with a heritability estimate of 0.26 (Schwander et al. 2005). In *P. badius* (Rheindt et al. 2005) and *A. echinatio* (Hughes et al. 2003), two species with distinct worker castes, larvae of different patrilines within the same colony differ in their propensities to develop into minor or major workers. A genetic influence on worker size was also reported in polygynous colonies, with workers from different matriline differing in size in *F. selysi* (Schwander et al. 2005) and *C. consobrinus* (Fraser et al. 2000). Conversely, no association between worker size polymorphism and paternal lineage was found in *F. truncorum* ( $h^2 = 0.09$ ; Bargum et al. 2004). In the latter species, however, a heritable component for gyne size ( $h^2 = 0.51$ ) was found in one of the study years. These differences between species suggest that the interplay between body size and patriline is complex and may vary greatly among and perhaps within species, possibly according to life history traits and ecological parameters.

The lack of association between mean worker head width and increased genetic diversity found in this study

contrasts with Clémencet & Doums (2006) who reported a positive association of mean worker size with the colony level of polyandry in *C. cursor*. Such a difference indicates that genetic factors may affect individual size but their influence on body size is not always expressed, possibly because environmental factors override these under some circumstances. Several studies indeed showed that heritability estimates vary across years and environments (Falconer & Mackay 1996; Larsson et al. 1997; Ruppell et al. 2001). For example, in *F. truncorum* a high and significant heritability for queen size was found in 1 year but not in the other, a divergence that was attributed to an increase in the total variance component relative to the variance at the patriline level because of environmental variations (Bargum et al. 2004). Our study population of *C. cursor* was located on the border of the Salses-Leucate lake; the site is exposed to the sun and the wind, with low vegetation density. The nests are usually located up to 1 m deep in the sand, right above the water level, and colonies can move frequently in response to climatic conditions or environmental perturbations. Moreover, this species is a scavenger, workers foraging to dead insects whose size and abundance may greatly fluctuate in time. In such conditions, worker size variation could be largely environmentally determined since it may facilitate adjustment of worker size ratios to colony needs (Oster & Wilson 1978).

Our data also show that task specialization is associated with worker size, not patriline, in *C. cursor*. The first foragers leaving the nest at sunrise were significantly larger than workers remaining in the nest, a pattern that is not associated with the genotypic variability within colonies. Thus, genotypic variability does not appear as a necessary condition for intracolony diversity and task specialization in *C. cursor*. This contrasts with the several cases where genetic variability among workers has been shown to influence division of labour in insect societies (honeybee: Frumhoff & Baker 1988; Robinson & Page 1988; Fuchs & Schade 1994; Page et al. 1995; Kryger et al. 2000; termites: Goodisman & Crozier 2003; ants: Stuart & Page 1991; Snyder 1992; Blatrix et al. 2000; Julian et al. 2002; Bargum et al. 2004; Schwander et al. 2005). The lack of genetic polyethism in *C. cursor* also suggests that within-colony genetic diversity is not associated with increased task efficiency and/or colony performance in this species. Empirical tests of the task-efficiency hypothesis still remain rare in social insects, and data are sometimes contradictory. In the honeybee, it has been shown that short-term productivity of small colonies tends to be higher in genetically more diverse colonies (Fuchs & Schade 1994). However, this trend does not occur in larger nests and/or over longer time periods, and it depends on genotypes (Oldroyd et al. 1992; Fuchs & Schade 1994). Genetic diversity was also shown to reduce colony-level variance in task performance (Page et al. 1995) or at maintaining a stable temperature in the nest (Jones et al. 2004). In ants, increased genetic diversity is correlated with higher growth rates and survival of the colonies in the harvester ant, *Pogonomyrmex occidentalis* (Wiernasz et al. 2004), but not with colony productivity in *F. truncorum* (Sundström & Ratnieks 1998) and *Lasius*

*niger* (Fjerdingstad et al. 2003). In a more causal study in which genetic diversity of colonies was experimentally manipulated, Rosset et al. (2005) recently showed that increased genetic diversity had no significant impact on task efficiency (exploration of new territories, food collection, nest moving and corpse removal) and overall colony productivity in the Argentine ant, *Linepithema humile*. Rather, most measures of task efficiency and productivity were strongly correlated with colony size (Rosset et al. 2005), supporting the hypothesis that increased group size may provide direct benefits on group performance independently of genetic diversity (Costa & Ross 2003).

The correlation between size and task found in this study is in agreement with previous observations in *C. cursor* indicating that small individuals have a tendency to stay in the nest and take care of the brood, whereas medium workers take part in foraging and digging, and large individuals specialize in collecting dead insects and nectars (Cagniant 1983). Analogous results were reported in *C. velox*, another polymorphic species belonging to the genus *Cataglyphis*. In this species, only large and medium-sized workers withstand extreme heat conditions and can leave the nest at higher daily temperatures to forage (Cerdá et al. 1998; Cerdá & Retana 2000). The relationship between workers' morphology and the task that she usually does has been documented in several other polymorphic ants (reviewed in: Hölldobler & Wilson 1990; Passera & Aron 2005) and is supposed to confer an ergonomic advantage to the colony through a better and more efficient division of labour among workers (Oster & Wilson 1978; Gordon 1996; Passera et al. 1996; Waser 1998).

The level of polyandry found in this study ( $k_a = 10.08 \pm 2.15$ ;  $k_e = 9.44 \pm 3.13$ ) differed from that previously reported in the same population ( $k_a = 5.56 \pm 1.34$ ;  $k_e = 4.63 \pm 1.35$ ; Percy et al. 2004a). This variation could stem from differences in the mode of sampling. Percy et al. (2004a) determined queen-mating frequency from parent–offspring combinations from laboratory rearing. By contrast, in our study queen-mating frequency was inferred from the genotype of the queen and the workers freshly collected in the field. Hence, it may have been biased by queen replacement, a common phenomenon in *C. cursor* (Percy et al. 2006). Queen's life span is low in this species, and queens may be replaced by sexual daughters arising from worker or queen reproduction through thelytokous parthenogenesis (Lenoir et al. 1988; Percy et al. 2004a, 2006). In the present study, the genotypes of the workers were all consistent with a single matriline in each colony (see Methods). This indicates that workers were produced by queen-produced queens rather than by worker-produced queens. Because sexual daughters of a queen are genetically almost indistinguishable from their mother, colonies appear monogynous but the number of patriline increases at each queen replacement. The lack of association between paternal lineage and task performance (i.e. leaving the nest at sunrise) found in this study also appeals for some caution. Indeed, one may not exclude that paternity influences the propensity of workers to perform other tasks, such as nest defence, nest construction, or brood care. For instance, worker thermal tolerance is size related in *C. cursor*, with larger workers foraging at

temperatures that smaller workers cannot tolerate (Clémencet & Doums 2006). Whether this pattern is also associated with paternal lineage remains, however, unknown.

Overall, our data thus do not support the hypothesis that multiple mating is favoured because increased genetic diversity within colonies results in more polymorphic workers and facilitates genetic division of labour (Page 1980; Crozier & Page 1985). Two alternative hypotheses seem particularly relevant to account for the evolution and maintenance of polyandry in *C. cursor*. First, the 'polyandry versus parasites' hypothesis states that increased genetic diversity within colonies enhances resistance to pathogens (Hamilton 1987; Sherman et al. 1988; Keller & Reeve 1994). This has been shown in the ant *A. echinator* (Hughes & Boomsma 2004, 2006) and the honeybee *Apis mellifera* (Tarpy 2003; Tarpy & Seeley 2006). Also, in the bumblebee *Bombus terrestris* high-diversity colonies have fewer parasites and show greater reproductive success than do low-diversity colonies (Baer & Schmid-Hempel 1999, 2001). That multiple mating evolved to increase colony resistance to severe infections may prove especially relevant for a scavenger ant such as *C. cursor*, where workers are potentially exposed to various pathogens developing on dead arthropods. Furthermore, unlike most other ant species where queen-turnover involves queens that are genetically different, new queens of *C. cursor* are often genetically identical to those replaced owing to thelytokous parthenogenesis (see above). Hence, multiple mating might have been selected since it represents the only source of colony genetic diversity. Second, according to the 'polyandry for social harmony' hypothesis, an increase in genetic diversity within colonies is expected to favour a social harmony by reducing the conflicts between the queen and the workers regarding the maternity of males (Woyciechowski & Lomnicki 1987; Ratnieks 1988). Workers of many social Hymenoptera have functional ovaries and may lay haploid-male eggs. Nevertheless, in most species they do not reproduce and, when they do so, the proportion of worker-produced males is usually weak compared to queen-produced males (Hammond & Keller 2004). Selection for the lack of worker reproduction depends on two variables: the colony-level cost of workers reproducing (Ratnieks 1988; Hammond & Keller 2004) and the relatedness of workers to queen- and worker-produced males (Woyciechowski & Lomnicki 1987; Ratnieks 1988). The relatedness hypothesis predicts that under polyandry, workers should forego reproduction and/or police worker reproduction (worker-policing) by selectively removing worker-laid eggs or by directing aggression towards workers with developing ovaries (Ratnieks 1988). Consistent with these predictions, several empirical studies in ants, bees and wasps have shown that workers police worker reproduction when queen-mating frequency exceeds two (e.g. see review of Ratnieks et al. 2006). In *C. cursor*, unmated workers can produce both males by arrhenotokous parthenogenesis and females by thelytokous parthenogenesis (Cagniant 1973). Yet, workers never reproduce in queenright colonies (Percy & Aron 2006). Genetic analyses showed that workers are on average more related to their reproductive sisters and brothers ( $r = 0.62$  and  $r = 0.32$ , respectively; Percy & Aron 2006) than to the

parthenogenetic daughters and sons of other workers ( $r = 0.42$  and  $r = 0.21$ , respectively). Multiple mating may therefore have been selected in queens of this species to force workers to rear their sibs instead of their own offspring. Whether genetic diversity within colonies has been selected to enhance resistance to parasites and pathogens and/or to favour a social harmony in *C. cursor* awaits further empirical studies.

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