

Original Article

Genetic polyethism in the polyandrous desert ant *Cataglyphis cursor*

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Several genetic and nongenetic hypotheses have been formulated to account for the evolution and maintenance of multiple mating by females (polyandry) in social Hymenoptera. A major hypothesis argues that polyandry allows production of genetically diverse workers varying in their inclination to perform different tasks, thereby enhancing division of labor and colony task efficiency. We tested the relationship between patriline, worker size, and task specialization in the ant *Cataglyphis cursor*, a species showing natural variation in queen mating frequency. Our results reveal a significant association between patriline and task preference: workers belonging to different patrilines differ in their propensity to perform a given task (foraging, nest construction, waste management, or food storage). Furthermore, we found that worker size is closely associated with task specialization but not with paternal origin. Overall, these results show that task performance is at least partly genetically influenced in the ant *C. cursor*, which is a key prerequisite for polyandry to improve division of labor in social insects. **Keywords:** division of labor, genetic polyethism, polyandry, polymorphism, social insect. [*Behav Ecol*]

INTRODUCTION

Multiple mating by females (polyandry) has long intrigued biologists because it is associated with ecological costs in terms of time, energy, increasing predation risks, and exposure to sexually transmissible diseases (Reynolds 1996; Hosken and Stockley 2003). Polyandry can be directly beneficial to females: sufficient sperm to fertilize their eggs, nutrient-rich ejaculates, or nuptial gifts. It may also bring females indirect benefits, such as an increase in genetic diversity among offspring (Choe 1995; Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Cornell and Tregenza 2007; Gowaty et al. 2010; Slayter et al. 2012).

In social Hymenoptera, multiple mating is a derived trait that appeared independently in 14 genera (Hughes et al. 2008). Several genetic and nongenetic hypotheses have been proposed to account for its evolution in ants and in social bees and wasps (reviewed in Crozier and Fjerdingstad 2001; Strassmann 2001; Brown and Schmid-Hempel 2003; Boomsma et al. 2009). Nongenetic explanations suggest that polyandry could be selected to avoid sperm depletion (Cole 1983), to improve postcopulatory sperm competition (Parker 1970; Simmons 2001), or because avoiding extra copulations is costlier than conceding to mate (Alcock et al. 1978; Thornill and Alcock 1983). On the other hand, genetic hypotheses stress the benefits of increased genetic diversity in the offspring: improves colony-level resistance to pathogens (Hamilton 1987; Sherman et al. 1988; Schmid-Hempel 1998; Baer and Schmid-Hempel 1999; Seeley and Tarpay 2007); dilutes genetically incompatible matings, which may result in production of nonviable offspring and/or sterile diploid males (Cook and Crozier 1995; Tarpay and Page 2002; Michalczyk et al. 2011); or alleviates reproductive conflicts among colony members

(Trivers and Hare 1976; Woyciechowski and Lomnicki 1987; Ratnieks 1988; Ratnieks and Boomsma 1995).

A prominent genetic hypothesis for the evolution of polyandry in social Hymenoptera posits that mating with different males allows a more efficient division of labor by increasing the production of genetically diverse workers varying in their inclination to perform different tasks (Crozier and Page 1985). Genetically mediated polyethism could emerge through variation among workers in their response thresholds to task-specific stimuli and hence in their propensity to engage in a given task (Robinson and Page 1989; Bonabeau et al. 1996, 1998; Oldroyd and Fewell 2007). By increasing the number of response thresholds in the worker force, polyandry would, therefore, optimize colony efficiency by allocating an adequate number of workers in each task, thereby allowing rapid responses to environmental changes. The genetic polyethism hypothesis for a more efficient division of labor has been tested successfully in the honeybee *Apis mellifera*, where queens can mate with up to 20 males (Tarpay et al. 2010). The response threshold model was supported by several empirical works showing that multiple mating raises the efficiency of the colony and its overall productivity (Frumhoff and Baker 1988; Robinson and Page 1988; Kryger et al. 2000; Jones et al. 2004; Chapman et al. 2007; Mattila and Seeley 2007).

In ants, evidence for genetic polyethism remains much more limited. In polygynous species (i.e., with colonies headed by several queens), differences among workers from different matrilines in overall task performance were reported in the ponerine ant *Gnamptogenys striatula* (Blatrix et al. 2000) and the leaf-cutting ant *Acromyrmex versicolor* (Julian and Fewell 2004) (but see, e.g., Rosset et al. 2005 for the lack of association between colony genetic diversity and task efficiency in the Argentine ant *Linepithema humile*). However, such differences can be due to various factors, including maternal effects, differences in rearing conditions, or genetic effects (Waddington et al. 2010). To date, a patriline effect

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on worker task performance has been reported in 2 species only, the leaf-cutting ant *Acromyrmex echinator* (Waddington et al. 2010) and the weaver ant *Oecophylla smaragdina* (Schlüns et al. 2011).

Task allocation in ants is often associated with morphological adaptations. This is particularly obvious in species characterized by morphologically distinct worker castes, with large workers (soldiers or *major*) tending to specialize in nest defense or foraging and small workers (*minor*) in brood care (Hölldobler and Wilson 1990; Wetterer 1999). Such a task–size association is also often found in species with continuous size polymorphism (Waser 1998). Worker morphological caste and size determination have long been assumed to result from environmental cues (Hölldobler and Wilson 1990). Recently, however, a genetic component to worker size has been documented in some species (*Camponotus consobrinus*, Fraser et al. 2000; *A. echinator*, Hughes et al. 2003; *Formica selysi*, Schwander et al. 2005; *Pogonomyrmex badius*, Rheindt et al. 2005; *Eciton burchellii*, Jaffé et al. 2007; *Atta colombica*, Evison and Hughes 2011). These studies suggest that division of labor in ants may be affected by a complex interaction between genetic influences and worker size polymorphism.

Polyandry for a more efficient division of labor was proposed as one of the major explanations to account for the occurrence of multiple mating by queens in the genus *Cataglyphis* (Percy et al. 2004, 2009; Timmermans et al. 2008; Lenoir et al. 2009). Here, we examine whether patriline influences worker size and task preference in the desert ant *Cataglyphis cursor*, a species with continuous worker size distribution (Cagniant 1983). Colonies of *C. cursor* are headed by a single, multiple-mated queen. Queens show natural variations in their mating frequency (range: 2–8 different males; Percy et al. 2004, 2009). A previous field study revealed no genetic influence on worker size and task specialization in *C. cursor* (Fournier et al. 2008). However, this work was based on a comparison of relatedness values between 2 groups of workers only: the 15 first foragers leaving the nest at sunrise and a sample of workers staying in the nest. Here, we perform a more detailed study on the relationship between patriline, worker size, and task preference in this species. We investigate the patriline distribution of workers engaged in 4 different tasks under laboratory conditions: foraging, nest construction, waste management, and food storage. We analyze whether the representation of patrilines in workers engaged in the 4 tasks differs from the overall representation of patrilines in the colony. We also test for a possible relationship between the size of workers, their paternal origin, and their behavioral task.

MATERIALS AND METHODS

Four monogynous colonies of *C. cursor* were collected in early April 2011 in Argeles-sur-Mer, Southern France (42°34'22N; 3°02'39E). At the end of excavation, all the workers from each nest were placed in a plastic box, and a sample of 50–60 workers per colony was chosen at random and stored in 98% ethanol. These workers were used for subsequent morphologic and genetic analyses aimed at determining the overall representation of patrilines in worker population from each nest; they are hereafter called “random workers.” Ants were installed in artificial nests made of water tubes covered by a cardboard box and placed in large sand-filled arenas (1 × 1 m). Colonies were maintained under laboratory conditions (27 ± 2 °C and natural photoperiod 12:12 h light:dark) and fed daily with cockroaches and sugar water.

Task sampling

After 2 months of acclimation period, we marked workers engaged in foraging, nest construction, and waste management with task-specific colors. The ants were removed, cooled down on ice, and marked with a dot of paint on the abdomen; they were kept apart for 5 min before being replaced in their natal colony. Workers observed to execute a second task were marked with a second dot of paint on the thorax. For each colony, we collected and marked workers for 5 h per day for 3 successive days. After this 15-h observation period, replete workers (i.e., workers staying in the nest and characterized by a visibly corpulent gaster between tergites due to abdominal distension by food storage) from each colony were collected. The paint-marked individuals and the replete workers were used for morphometric and genetic analyses to determine if worker size and patriline influence task preference.

Morphometric analyses

All the marked, replete, and random workers were measured to the nearest 0.01 mm using a MZ6 stereomicroscope (Leica Microsystems, Wetzlar, Germany). Maximum head width (eyes included) was used as estimate of size because of its high representation of body measurement, good level of precision, and repeatability (Fournier et al. 2008).

Genetic analyses

Individual ant DNA was extracted from ant legs incubated for 120 min in 100 µl of 5% Chelex at 85 °C (Bio-Rad, Hercules, CA, USA). Samples were centrifuged for 5 min at 12 000 rpm, and 2 µl of the supernatant was amplified by PCR at 8 microsatellite loci (Ccur26, Ccur89, Ccur46, Ccur63b, Ccur99, Ccur58, Ccur76, and Ccur11; Clémencet et al. 2005) using a TProfessional Thermo-cycler (Biometra). The amplified products were separated on ABI 3730 capillary sequencer (Applied Biosystems, Foster City, CA, USA) and sized against MapMarker 400 sizing standards (BioVentures, Murfreesboro, TN, USA). Male genotypes were inferred from the queen and worker's genotypes; each worker was assigned to a given patriline with the maximum-likelihood method implemented in the software COLONY 1.1 (Wang 2004). Workers that could not be unambiguously assigned to the mother queen or to one of the patrilines in each colony ($n = 9$ from 3 colonies), due to failed PCR amplification or because they shared no allele with the colony queen, were excluded from the analyses. Overall, 601 workers were successfully assigned to the colony queen and one of the queen's mates (Table 1).

Statistical analyses

Parametric statistics were used after verification of nondeviation of the variables from normality (Kolmogorov–Smirnov test) and evidence for homoscedasticity (Bartlett's test). To assess the effect of task preference and genetic influence on worker size, we compared the head width between workers performing different tasks, between workers from different colonies, and between workers of different patrilines within each colony. We used a nested ANOVA with 2 hierarchical levels (Lynch and Walsh 1998): size variation between individuals of different colonies and size variation between workers of different patrilines within each colony.

To study the effect of paternal origin on task preference, we compared the distribution of workers belonging to each patriline in each task (foraging, waste management, nest construction, or food storage) to the distribution of

Table 1

Colony size, number of workers engaged in a specific task (number of workers faithful to their task during the observation period), number of random workers sampled, mean worker size, and absolute number of mating per queen (Mp) for the 4 *C. cursor* colonies studied

Colony	Colony size	Tasks-specific marked workers (workers faithful to their task)	Random workers	Worker size (mm), mean \pm SD	M_p
A	1387	135 (126)	55	1.32 \pm 0.18	6
B	1037	130 (115)	55	1.23 \pm 0.19	5
C	738	68 (66)	52	1.32 \pm 0.22	5
D	536	51 (49)	55	1.31 \pm 0.17	6
Total		384 (356)	217	1.29 \pm 0.19	

Tasks-specific marked and random workers were all used for both morphometric and genetic analyses ($n = 601$).

workers from each patriline at the colony level (i.e., the overall distribution of patrilines among random workers), using χ^2 tests. For multiple comparisons, level of significance was adjusted with Holm's sequential Bonferroni correction (Holm 1979). The strength of the association between patrilines and tasks was estimated by measuring the effects sizes, using Cramer's V test (0 = no association, 1 = maximum association; Acock and Stavig 1979).

RESULTS

The 4 colonies sampled were each headed by single queens mated with 6, 5, 5, and 6 males, respectively (Table 1). Of the 384 paint-marked workers, 356 (i.e., 92.7%) remained faithful to their tasks during the 3 days of behavioral observations. Twenty-eight workers executed 2 different tasks: 9 workers (4.4%) in Colony A, 15 (8%) in Colony B, 2 (1.5%) in Colony C, and 2 (1.9%) in Colony D. No waste management activity was observed in Colonies C and D.

Genetic influence on worker size

Worker head width showed a unimodal distribution, ranging from 0.79 to 1.75 mm ($X \pm SD = 1.29 \pm 0.19$ mm, $n = 573$). Head size varied significantly between colonies and between patrilines within colonies (nested ANOVA—colony: $F_{3,532} = 6.67$, $P < 0.001$; patriline: $F_{18,532} = 3.22$, $P < 0.001$). The workers from Colony B were significantly smaller than those from Colonies A and C (Tukey HSD post hoc test, $P < 0.003$ for all comparisons) but not from the

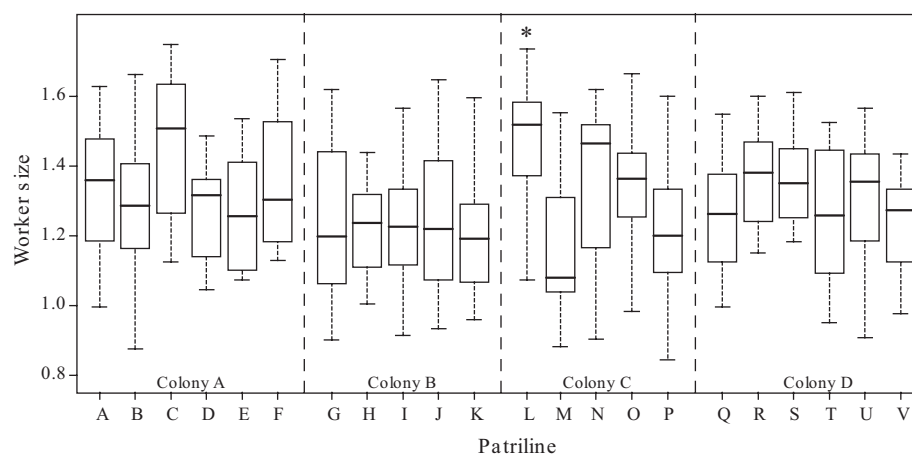
workers of Colony D ($P = 0.508$). Of the 22 patrilines found across the 4 colonies, workers from patriline L appeared significantly larger than those belonging to patrilines G, I, K, M, Q, and S (Tukey HSD post hoc test, $P < 0.05$ for all comparisons). Apart from workers from patriline L, worker size did not differ between the other 21 patrilines (Figure 1).

Effect of task specialization on worker size

No significant interaction between patrilines and tasks on worker's size was detected ($F_{65,463} = 1.16$, $P = 0.195$). However, we found a strong effect of task specialization on worker's size ($F_{4,528} = 90.08$, $P < 0.001$; Figure 2). Nest builders were significantly smaller than both workers engaged in foraging and food storage and random workers (Tukey HSD post hoc test, $P < 0.001$ for all comparisons). They were also marginally smaller than waste managers ($P = 0.071$). Conversely, repletes were significantly larger than both workers performing the others tasks and random workers ($P < 0.001$ for all comparisons). The size of workers assigned to waste management was significantly smaller than that of random workers ($P = 0.016$) but not that of foragers ($P = 0.285$); the latter did not differ from random workers ($P = 0.113$).

Effect of patriline on task specialization

The representation of patrilines in workers engaged in the 4 tasks varied markedly (Figure 3). Comparison between the distribution of workers belonging to each patriline in each task and the distribution of workers from each

**Figure 1**

Size distribution of workers from each patriline in 4 colonies of *Cataglyphis cursor*. Box plots show median and 1st and 3rd quartile; whiskers include 95% of all observations. * $P < 0.05$ (ANOVA and Tukey HSD tests).

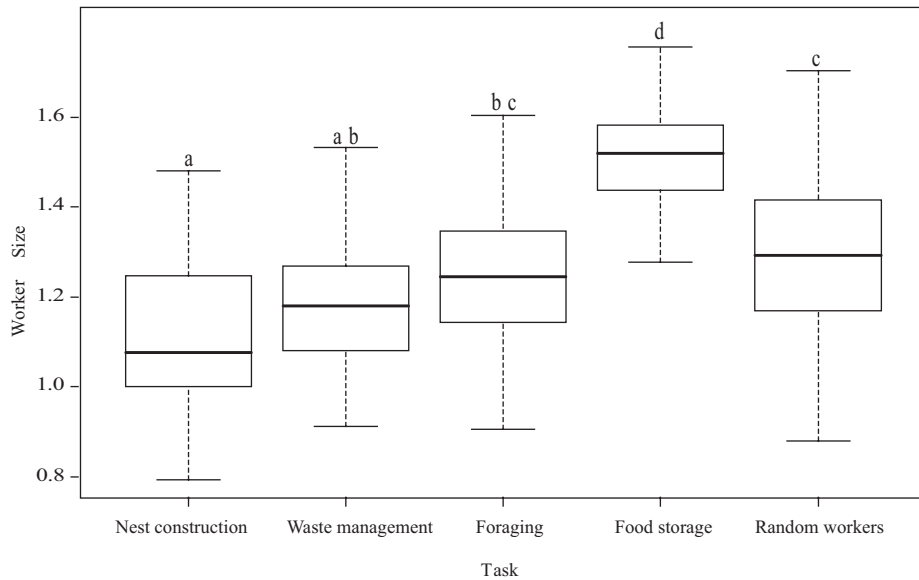


Figure 2 Size distribution of workers engaged in nest-construction, waste management, foraging, and food storage (replete), and of random workers for the 4 colonies of *Cataglyphis cursor*. Box plots show median and 1st and 3rd quartile; whiskers include 95% of all observations. Worker size with different lower case letters differed significantly (ANOVA and Tukey HSD tests, $P < 0.05$).

patriline among random workers (i.e., the overall distribution of patrilines in each colony) revealed significant differences in 3 out of 4 colonies. Contribution of patrilines to workers engaged in nest construction differed significantly from that observed in random workers in Colonies A (chi-square

test, $\chi^2 = 20.59$, $P = 0.002$, Cramer's $V = 0.49$), C ($\chi^2 = 12.35$, $P = 0.029$, $V = 0.39$), and D ($\chi^2 = 34.70$, $P < 0.001$, $V = 0.89$). Furthermore, a significant difference occurred for replete workers in Colonies A ($\chi^2 = 34.10$, $P < 0.001$, $V = 0.44$), C ($\chi^2 = 21.48$, $P < 0.001$, $V = 0.47$), and D ($\chi^2 = 11.67$, $P = 0.039$,

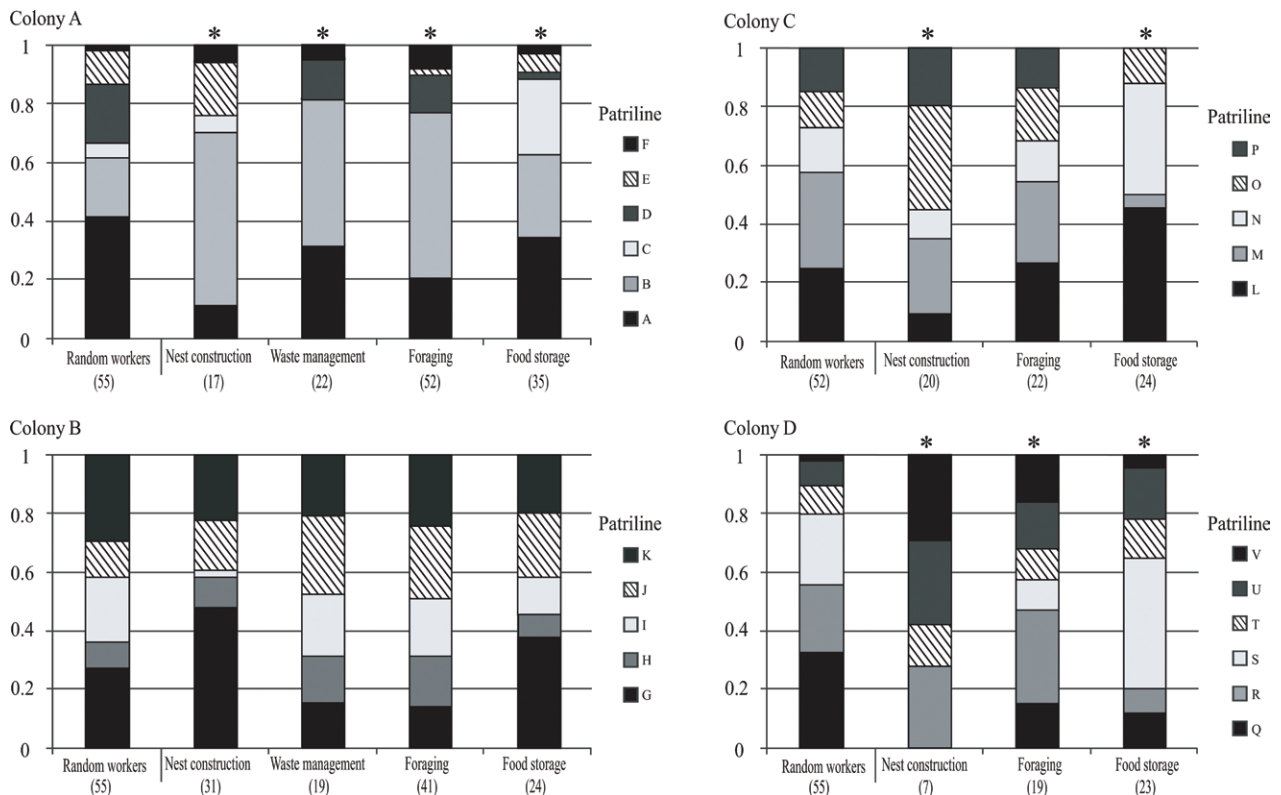


Figure 3 Proportion of workers of each patriline sampled per task. Random workers give, for each colony, the overall contribution of each patriline to the worker force. Asterisks represent a significant difference in patriline contribution between task-specialized workers and random workers (chi-square tests with Holm's sequential Bonferroni correction, $P < 0.05$).

$V = 0.32$), for foragers in Colonies A ($\chi^2 = 58.01$, $P < 0.001$, $V = 0.48$) and D ($\chi^2 = 22.49$, $P < 0.001$, $V = 0.51$), and for workers engaged in waste management in Colony A ($\chi^2 = 14.47$, $P = 0.012$, $V = 0.36$). In Colony B, the distribution of patriline was not different between task-specialized workers and random workers. Though nonsignificant, slight differences in patriline distributions among nest builders ($\chi^2 = 10.72$, $P = 0.089$, $V = 0.29$) and foragers ($\chi^2 = 10.06$, $P = 0.078$, $V = 0.24$) were found in Colony B.

DISCUSSION

The present study reveals a great fidelity in task performance by workers of *C. cursor*, with more than 90% of individuals being faithful to their task over the 3 days of observation. Our data show that task inclination is at least partly genetically influenced. Workers of different paternal origin differ in their propensity to engage in a task in 3 of the 4 colonies sampled. Apart from Colony B, unequal contribution of patriline occurred for all tasks studied in Colony A (nest construction, foraging, waste management, and food storage) and Colony B (nest construction, foraging, and food storage), and for 2 tasks in Colony D (nest construction and foraging) (Figure 3). Such a genetic polyethism, with workers belonging to different patriline showing different propensities to perform distinct tasks, has been largely documented in honeybees (Robinson and Page 1988; Oldroyd et al. 1994; Page et al. 1995; Jones et al. 2004; Chapman et al. 2007; Oldroyd and Fewell 2007; Oxley et al. 2010; Kraus et al. 2011). By contrast, such an effect of patriline on worker task performance remains poorly studied in ants. To the best of our knowledge, it has been unambiguously shown only in the leaf-cutting ant *A. echinator* (Waddington et al. 2010). In this species, a genetic division of labor was concluded from a different representation of patriline between workers engaged in 2 tasks, waste management and foraging, in 2 of the 3 colonies sampled. Genetic polyethism was also reported in the ant *O. smaragdina* (Schlüns et al. 2011). However, in this study, queens were found to be multiple mated in 2 out of 5 colonies only, and no genetic polyethism was observed in one of them. Our data show that, in the desert ant *C. cursor*, there is an unequal investment of workers according to their paternal origin in 4 tasks in 3 of the 4 colonies examined.

It seems unlikely that our results are confounded by the combination of age polyethism and changes in sperm use from the queen's mates over time, which may be responsible for an unequal patriline distribution of workers among the different tasks (Wiernasz and Cole 2010; Holman et al. 2011). First, comparison between parent-offspring combination genetic analyses and genotyping of the content of queen spermatheca showed that queens of *C. cursor* do not use sequentially the sperm of their mates: the number of patriline detected in the worker force (at the adult or larval stages) and in the spermatheca are identical (Percy et al. 2009). A paternity skew among workers was found in a small proportion of colonies (22%), but it was most likely explained by a variation in sperm contribution of the different fathers. Furthermore, a temporal change in sperm use seems rare in ants and has been documented in 2 species only, with highly populous colonies from several thousands to several millions of workers (*P. occidentalis*, Wiernasz and Cole 2010; *Formica truncorum*, Sundström and Boomsma 2000). By contrast, colonies of *C. cursor* are quite small, usually comprising hundreds of workers (range: 78–2658; $n = 57$ colonies; Percy and Aron 2006). Second, workers of *C. cursor* have a relatively short life span, not exceeding more than a couple of months (Percy et al. 2006); thus, they do not vary much in age.

Our results contrast with those previously reported by Fournier et al. (2008) in the same species, who reported no association between task performance and patriline. However, the experimental design used by these authors differed greatly from that used in the present work. The study of Fournier et al. (2008) was carried out in the field and was based on the single comparison between patriline of 2 groups of workers, individuals staying in the nest and the 15 first foragers leaving the nest at sunrise. Our work was performed under laboratory conditions, allowing the analysis of a larger number of tasks and observation of worker behavior over a much longer time (5 h per day for 3 successive days; see Waddington et al. 2010; Waddington and Hughes 2010 for a similar observation period).

To date, the response threshold model is considered as the main explanation for a genetic component in the division of labor in social insects (reviewed in Beshers and Fewell 2001; Bertram et al. 2003; Oldroyd and Fewell 2007). This model assumes that division of labor stems from variation among nestmate workers in their threshold for responding to task-specific stimuli. In performing a particular task, individuals with lower thresholds become specialists for this task and reduce the likelihood that the same task will be carried out by individuals with higher thresholds. By increasing the genetic diversity among workers within colonies, multiple mating would augment the number of response thresholds in the worker force. In line with this hypothesis, our data show that workers of *C. cursor* belonging to different patriline differ in their tendency to achieve different tasks, suggesting variations in their response threshold. However, despite the strong association between the patriline and the task performed, all the individuals of a particular patriline are not restricted to achieving the same task. This was also reported in other studies on the genetic influence on worker task in ants (Hughes et al. 2003; Rheindt et al. 2005; Jaffé et al. 2007; Waddington et al. 2010). Thus, genetically mediated polyethism and the associated response threshold model do not completely explain division of labor in ants. Other factors like worker age (Camargo et al. 2007), individual experience (Ravary et al. 2007), worker position in the nest (Jandt and Dornhaus 2009; Johnson 2010; Jandt and Dornhaus 2009), environmental cues, or colony's needs probably also interact to determine task performance.

We found a strong relationship between task specialization and worker size in *C. cursor*. The smaller workers engage in nest construction and waste management, intermediate workers tend to forage, and the largest workers store food as repletes. Such a size-based division of labor was also observed in the field (Fournier et al. 2008), with the first foragers leaving the nest at sunrise being significantly larger than workers remaining in the nest. It has been suggested that worker polymorphism represents an adaptive way of achieving better exploitation of external environmental conditions by thermal specialists such as *Cataglyphis* desert ants (Cerdà 2001). Worker thermal tolerance is indeed size related in *C. cursor*, with large workers foraging at temperatures that smaller workers cannot tolerate (Clémencet et al. 2010). Analogous results were reported for the highly polymorphic species *C. velox*, where only large and medium workers can tolerate extreme daily temperatures (Cerdà et al. 1998; Cerdà and Retana 2000). The association between worker size and task allocation has been documented in several ant species (Hölldobler and Wilson 1990). In *Atta* and *Acromyrmex* species, for example, the largest workers usually engage in foraging, because of their greater efficiency at cutting vegetation, whereas smaller individuals engage in waste management (Wilson 1980; Ballari et al. 2007; Waddington and Hughes

2010; Muscedere et al. 2011; but see Hart and Ratnieks 2002). A positive association between worker size and colony size has long been reported in ants, suggesting that larger colonies can better afford the production of large workers (Brian 1957; Wood and Tschinkel 1981; Porter and Tschinkel 1985; Wetterer 1994; Kaspari and Byrne 1995). Consistent with a higher investment in large workers by larger colonies, the mean worker size is positively correlated with colony size in *C. cursor* (Clémencet and Doums 2007).

No significant worker size difference was found in 21 out of 22 patrines. This is consistent with the study of Fournier et al. (2008), who showed that worker size polymorphism is not correlated with the number of patrines or intracolony genetic diversity in colonies of *C. cursor*. Most of the colony-wide size variation is overwritten by intrapatrine size variation (Fournier et al. 2008). Whether an increase in colony genetic diversity results in extended colony-wide phenotypes in ants remains ambiguous. A large-scale analysis of 82 ant species revealed no association between queen mating frequency and worker size polymorphism, after controlling for phylogeny (Brown and Schmid-Hempel 2003). Similarly, increased intracolony genetic diversity does not result in a more polymorphic worker force in *F. truncorum* (Bargum et al. 2004) and *F. selysi* (Schwander et al. 2005), 2 species showing continuous unimodal size distribution, like *C. cursor*. On the other hand, detailed genetic analyses showed that in the polygynous species *F. selysi* (Schwander et al. 2005) and *C. consobrinus* (Fraser et al. 2000), workers belonging to different matrines differ significantly in size. Genetic effects were also shown to contribute to worker caste fate in leaf-cutting ants *A. echinatior* (Hughes et al. 2003) and *A. colombica* (Evison and Hughes 2011), the harvester ant *P. badius* (Rheindt et al. 2005), and the army ant *E. burchellii* (Jaffé et al. 2007), with larvae from different patrines having different propensities to develop into major or minor worker castes.

In *C. cursor*, new queens (gynes) are usually produced by thelytokous parthenogenesis and are almost genetically identical to their mother queen, whereas workers arise from sexual reproduction (Percy et al. 2004). Genetic recombination associated with sexual reproduction and multiple mating, therefore, represents the only source of colony genetic diversity. Our results show that worker task behavior is to some extent genetically mediated in the ant *C. cursor*. Genetic polyethism is a prerequisite for polyandry to improve division of labor in social insects. Whether polyandry increases the number of thresholds in the worker force and hence improves colony efficiency in *C. cursor* awaits further studies.

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