

RESEARCH ARTICLE

Analysis of ants' rescue behavior reveals heritable specialization for first responders

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ABSTRACT

In colonies of *Cataglyphis cursor* ants, a single queen mates with multiple males, creating the foundation for heritable behavioral specializations. A novel and unique candidate for such specializations is rescue behavior, a precisely delivered form of altruism in which workers attempt to release trapped nestmates and which relies on short-term memory of previous actions to increase its efficiency. Consistent with task specialization, not all individuals participate; instead, some individuals move away from the victim, which gives rescuers unrestricted access. Using a bioassay to identify rescuers and non-rescuers, coupled with paternity assignment via polymorphic microsatellite markers, we not only show that rescue behavior is heritable, with 34% of the variation explained by paternity, but also establish that rescue, heretofore overlooked in analyses of division of labor, is a true specialization, an ant version of first responders. Moreover, this specialization emerges as early as 5 days of age, and the frequency of rescuers remains constant across ants' age ranges. The extremely broad range of these ants' heritable polyethism provides further support for the critical role of polyandry in increasing the efficiency of colony structure and, in turn, reproductive success.

KEY WORDS: Ants, *Cataglyphis cursor*, Polyandry, Behavioral genetics, Division of labor

INTRODUCTION

In colonies of eusocial Hymenoptera, individuals specialize in performing particular tasks, a division of labor that greatly increases the efficiency with which those tasks are performed (Hölldobler and Wilson, 1990; Passera et al., 1996; Ferguson-Gow et al., 2014; Yang et al., 2004; Beshers and Traniello, 1994; Arnan et al., 2011) and represents a major transition in evolution (Smith and Szathmari, 1997). This task specialization plays a crucial role in the phenomenal reproductive success enjoyed by hymenopteran species (Hölldobler and Wilson, 2009). In ants, some of the most diverse and abundant eusocial species (Brady et al., 2006), behavioral specializations vary greatly within and between species and include such common tasks as foraging, brood care, nest maintenance, waste management and defense of the colony (Hölldobler and Wilson, 1990; Robinson, 1992). Over the last few decades, a variety of increasingly sophisticated tools, from

behavioral genetic techniques (Frumhoff and Baker, 1988) to molecular genetic markers (Grover and Sharma, 2016), have been used to demonstrate the heritability of task specialization in eusocial hymenopterans (Robinson and Page, 1988; Gordon, 2015; Friedman and Gordon, 2016; Robinson et al., 2005; Smith et al., 2008; Schlüns et al., 2011; Gotzek and Ross, 2007; Waddington et al., 2010; Jaffe et al., 2007; Leniaud et al., 2013; Julian and Fewell, 2004; Hughes et al., 2003; Wiernasz and Cole, 2010; Kwapich et al., 2017). Although most studies have focused on honeybees – indeed, some researchers have argued that behavioral genetic analyses of ants are much needed (Friedman and Gordon, 2016) – genetic tools have been able to illuminate the critical role of polyandry in creating heritable task specializations and, thus, have helped solve an intriguing evolutionary puzzle. That is, polyandry, in which a queen mates with multiple males, introduces obvious costs for the queen, for example, the risk of sexually transmitted pathogens and the risk of injury from greater exposure. Polyandry also produces less closely related workers that should be, according to kin selection theory, less likely to cooperate with one another than workers sharing both maternal and paternal genes. If, however, polyandry is an adaptation for imparting genetic diversity into the colony and thereby generating heritable task specializations (Robinson, 1992; Timmermans et al., 2008), amongst other potential benefits (Kraus and Moritz, 2010; Crozier and Fjerdingstad, 2001), then the advantages of this reproductive strategy could be expected to outweigh the many costs.

The desert-dwelling formicine ant, *Cataglyphis cursor* (Fonscolombe 1846), is an important model species for studying polyandrous mating strategies. *Cataglyphis cursor* queens leave the protection of the nest repeatedly, during only a brief period of time, to mate with nearby males (Lenoir et al., 2009). These multiple matings produce an average of 10 different patrilines (Fournier et al., 2008), groups of half-siblings that have different fathers but share the same mother. Ants are haplodiploid, with haploid males and diploid females, and thus diploid female offspring share an average of 75% genetic identity with others in the same patriline, but only 25% genetic identity with their half-sisters from other patrilines. Because this mating strategy carries a substantial risk of mortality for the queen, its presence suggests that polyandry imparts a particularly valuable increase in fitness for *C. cursor* colonies (Lenoir et al., 2009).

Individual *C. cursor* workers specialize in performing different tasks within the colony, and recent research has demonstrated that at least some of worker ants' task specializations are heritable and distributed differentially among patrilines within the same colony (Eyer et al., 2012). A unique candidate for task specialization, and one that suggests we should think differently about ants' division of labor, is rescue behavior. Documented in only a small fraction of ant species (Czechowski et al., 2002; Nowbahari et al., 2009; Hollis and Nowbahari, 2013; Taylor et al., 2013; Miler, 2016; Frank et al., 2017; Kwapich and Hölldobler, 2019), rescue requires a worker to

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respond to a call for help from an entrapped nestmate and to attempt to free that victim with precisely targeted behavior that avoids injuring the victim (Nowbahari et al., 2009; Hollis and Nowbahari, 2013). When, for example, *C. cursor* workers encounter a trapped and partially buried nestmate, they begin by systematically uncovering it. Relying on short-term memory of previous actions to increase efficiency and greatly reduce energy expenditure (Duhoo et al., 2017), rescuers excavate around the victim, transport substrate away from the victim as needed to permit greater access, and then tug on the victim's exposed legs and body parts. Although rescue efforts are vigorous and concerted, rescuers avoid the fragile antennae, which, if pulled, would result in serious injury. Once whatever is restraining the victim has been revealed, rescuers precisely target that object (Fig. 1). The precision with which workers attempt to free the victim is revealed in both laboratory and field simulations of entrapment. For example, in several species, including *C. cursor*, rescuers identified and bit at an inert snare that held the victim in place (Nowbahari et al., 2009; Hollis and Nowbahari, 2013; Taylor et al., 2013). When, however, the victim was captured by a predatory antlion, *Tetramorium* ant rescuers attempted to sting it instead, eschewing all other rescue behavior components, such as digging at the sand on the walls of the antlion's pit trap, that would have put themselves at even greater risk of sliding down into the pit (Taylor et al., 2013). Importantly, *C. cursor* rescuers do not discriminate amongst their nestmates, and not all *C. cursor* workers deliver such aid (Nowbahari et al., 2009). Instead, some ants immediately move away from the victim and refrain from engaging in any form of the rescue attempt. This clear divergence in behavior amongst workers led us to wonder whether

C. cursor workers might be genetically specialized for rescue behavior.

As a task specialization, rescue behavior differs substantially from all other tasks for which *C. cursor* is specialized. First, it is the only specialization that involves a dynamic social interaction between adult nestmates: rescue not only requires that the victim calls for help (Nowbahari et al., 2009), but also depends upon a constantly changing social interaction between rescuer and victim (Duhoo et al., 2017). Other genetically mediated tasks that have been identified in this species – nest construction, waste management, foraging and food storage (Eyer et al., 2012) – make no such demands upon the worker. Second, unlike other task specializations in *C. cursor*, rescue meets the strict definition of an altruistic act, that is, behavior that increases the fitness of another individual while reducing the individual fitness of the actor. *Cataglyphis cursor* rescuers risk a reduction of individual fitness when they attempt to rescue a nestmate because, unlike the sterile worker females of most ant species, *C. cursor* workers are capable of reproduction. *Cataglyphis cursor* workers have the unusual capacity to produce diploid female eggs (known as thelytokous parthenogenesis), which, under the right conditions, may become colony queens (Lenoir et al., 2009). Thelytokous parthenogenesis is likely an adaptation to compensate for the high mortality of roaming polyandrous queens (Lenoir et al., 2009); indeed, genetic analyses reveal that a large proportion of *C. cursor* colonies possess queens produced by workers. In one study, for example, the proportion of colonies headed by worker-produced replacement queens was estimated to be more than 60% (Pearcy et al., 2006). Thus, unlike most other ant species, *C. cursor* workers are not necessarily

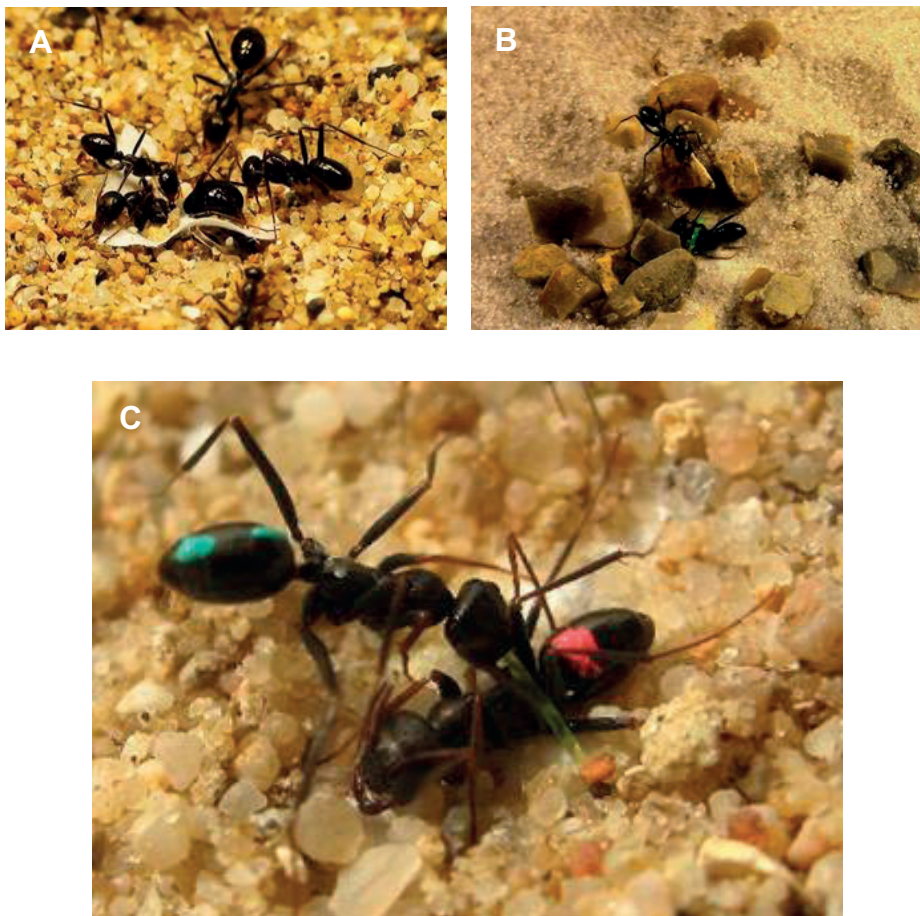


Fig. 1. Rescue behavior in *Cataglyphis cursor*. (A) Several rescuers dig sand away from the victim, exposing the filter paper to which the victim had been tied using a nylon thread snare. (B) A rescuer removes a sand particle, held in its mandibles, from atop the victim and transports it some distance away from the victim. (C) A rescuer (marked with green) bites at the nylon thread holding the victim (marked in red) in place. Photo credit: Paul Devienne.

reproductive dead-ends. If a *C. cursor* worker engages in rescue behavior, it is performing an act that, at least potentially, reduces its own reproductive prospects – not only from obvious energetic costs but also from the risk of its own entrapment (Taylor et al., 2013) – while increasing the fitness of the individual it is helping. Rescue behavior in *C. cursor* is thus an act of individual altruism.

To determine whether rescue behavior is a genetically mediated task specialization in *C. cursor*, we investigated whether workers belonging to different patriline would differ in their propensity to participate in a rescue attempt, either engaging in rescue behavior or refraining from doing so. We first conducted a behavioral bioassay to identify and sample equal numbers of rescuers and non-rescuers from each of four colonies of *C. cursor*. Second, we assigned each of these individuals to a colony-specific patriline based on polymorphic microsatellite markers (Percy et al., 2004). We then examined whether an individual ant's paternity was a significant predictor of the propensity to engage in rescue behavior. Finally, to exclude the potential confounding effects of developmental polyethism on the expression of rescue behavior among workers – the possibility that workers might express rescue behavior simply as a function of age rather than paternity – we conducted a follow-up experiment in which we marked individual ants as they emerged from their cocoons, and then tested them over their first 20 days to determine when rescue behavior was expressed.

MATERIALS AND METHODS

Collection and housing

Colonies of *C. cursor*, each with a queen and brood, were collected in April 2013 and April 2014 from different habitats in Menerbes and Bellegarde, located in Vaucluse, France, and housed in the laboratory at Université Paris 13. Each colony was maintained separately: a cylindrical closed nest box (15 cm diameter) was connected via a 20 cm plastic tube to an open foraging area, namely a plastic tray (28×27.5×8.5 cm, length×width×height) covered with a thin layer of sand. Ants were fed mealworm larvae and an apple–honey mixture biweekly. The colony room was maintained at 28±2°C, 20 to 40% humidity, with a 12 h:12 h light:dark cycle.

Behavioral bioassay

We collected rescuers and non-rescuers from each of the four colonies in October 2015 by conducting test trials of rescue behavior used previously with *C. cursor* (Nowbahari et al., 2009; Duhoo et al., 2017). Briefly, a worker ant (the ‘victim’) at least 5 days old – which could be determined by the lighter color of newly eclosed ants (Nowbahari and Lenoir, 1989) – was selected at random from the foraging area and tied to a small piece of filter paper with nylon thread, a procedure that simulates entrapment in the wild, either by predatory antlions or by falling dirt and debris (see Hollis and Nowbahari, 2013). The victim then was placed inside a plastic ring (6.5 cm diameter×5.5 cm high), which was inserted in the sand near the nest entrance. Five worker ants, each of which was also at least 5 days old, were selected at random from the foraging area of the same colony and placed inside the ring; their behavior in the foraging area was observed for 5 min. Workers that contacted the victim and performed one or more of the rescue behavioral patterns (Table 1) for at least 60 s were transferred to a second ring, where another nestmate victim from the same colony was located. Workers that again engaged in rescue behavior for at least 60 s in this second test were selected as rescuers. Workers that made contact with the victim but did not engage in any form of rescue behavior in both of the two tests were selected as non-rescuers. Ants identified as rescuers and non-rescuers were

Table 1. Operational definitions of rescue behavior performed by *Cataglyphis cursor* ants, used in scoring behavior during the behavioral bioassays

Rescue behavior	Operational definition
Sand digging	Ant positions itself within 2 cm of the ensnared victim and flicks sand backward, away from the victim, using its anterior legs.
Limb pulling	Ant grabs limb of victim with mandibles and drags backward with frequent antennation.
Sand transport	Ant picks up sand particle in contact with victim, filter paper or snare using mandibles and moves it at least 5 mm from the snare.
Snare biting	Ant bites and tugs at the nylon snare using mandibles.

Adapted from Nowbahari et al. (2009).

ethanized and stored individually in 98% ethanol for subsequent genetic paternity analysis. Equal numbers of rescuers and non-rescuers were collected from each colony ($n=80$ for each of colonies A, B and C; $n=70$ for colony D) to produce a balanced sampling design with a null expectation of equal frequencies of rescuers and non-rescuers (0.5) for all patrilines.

To ascertain the potential effects of worker age on the expression of rescue behavior, we performed a follow-up experiment. A total of 64 ants, sampled randomly from seven colonies, including the four colonies used in our genetic analysis, were marked within 24 h of eclosion. Marked individuals were tested in behavioral bioassays for the expression of rescue behavior, exactly as described above, at either 5, 10 or 20 days post-eclosion, and a Chi-square test was used to determine whether the frequency of rescuers and non-rescuers differed across the three age groups.

Genetic paternity analysis

A single leg was removed from each sampled individual, and DNA was extracted from the leg using a Qiagen DNEasy Blood and Tissue Kit according to the manufacturer's protocol. Each sample was amplified by PCR at all eight polymorphic microsatellite loci described by Percy et al. (2004) (Table 2), and the size of all PCR products was determined via fragment analysis on an ABI-3130XL capillary sequencer. Amplicon sizes were scored using GeneScan software (Applied Biosystems) and validated by eye. Amplicon sizes were statistically binned, and alleles were assigned using the program TANDEM (Matschiner and Salzburger, 2009). The resulting multilocus genotypes were used to infer the paternity of each sampled worker using a maximum-likelihood method implemented by the program COLONY (v2.0.6.3) (Wang, 2004). To evaluate the association between paternal inheritance and rescue behavior, we performed a generalized linear mixed-effects model with ‘patriline’ as a fixed effect and ‘colony’ as a random effect using the R package lme4 (Bates et al., 2015).

RESULTS

Assignment to patrilines

Microsatellite markers amplified reliably and exhibited diversity commensurate with previously published values (Table 2, Table S1) (Percy et al., 2004). All sampled workers were successfully assigned to colony-specific patrilines with a low probability of detection error, and we detected no evidence of multiple queens. Of the four colonies, colonies A and C each contained 10 patrilines, whereas colonies B and D each contained nine patrilines. The size of all four colonies corresponds well with previously published values (10 patrilines, range 7–14; Eyer et al., 2012). The identified

Table 2. Microsatellite markers and associated diversity and paternity details

Colony	Percent of sampled workers amplified								Number of patriline inferred	Non-detection error
	Ccur63b (13, 0.85)	Ccur58 (17, 0.84)	Ccur46 (14, 0.82)	Ccur11 (13, 0.71)	Ccur26 (12, 0.89)	Ccur 76 (14, 0.89)	Ccur89 (15, 0.89)	Ccur99 (16, 0.84)		
A	100	100	100	100	100	95	100	99	10	4.8×10^{-5}
B	100	99	100	100	100	98	99	98	9	2.9×10^{-5}
C	100	100	100	100	99	95	100	98	10	6.9×10^{-6}
D	100	100	100	100	100	97	100	99	9	6.3×10^{-5}

Markers were first described by Pearcy et al. (2004). Parenthetical values after each marker name indicate number of alleles observed and gene diversity (expected heterozygosity), respectively. Non-detection errors are calculated as in Boomsma and Ratnieks (1996).

patrilines contained anywhere from 4% to 21% of the sampled ants in a given colony (Table 3).

Statistical analyses

Our results reveal a significant association between patriline and rescue behavior (Fig. 2). The generalized linear mixed-effects model with ‘patriline’ as a fixed effect and ‘colony’ as a random

effect was highly significant ($\chi^2_{38}=146.5$, $P < 0.0001$), accounting for 34% of the observed variation in rescue behavior across colonies. Underlining this large effect was the outcome in nine of the 38 total patrilines, in which either all individuals in that patriline were rescuers (six patrilines) or all individuals in that patriline were non-rescuers (three patrilines; Fig. 2).

In our follow-up experiment to exclude the possibility of age-related task specialization confounding our results, we saw no significant variation in the frequency of rescuers and non-rescuers among marked individuals over time ($\chi^2_4=0.6412$, $P=0.725699$), indicating that developmental polyethism cannot explain the correlation we observed between genotype and phenotype (see Table 4).

Table 3. Composition of the four sampled colonies

Patriline	Total	Rescuers	Non-rescuers	Rescuer frequency
A1	6	6	0	1.0
A2	5	5	0	1.0
A3	7	6	1	0.86
A4	3	2	1	0.67
A5	4	2	2	0.50
A6	10	5	5	0.50
A7	14	6	8	0.43
A8	12	5	7	0.42
A9	12	2	10	0.17
A10	7	1	6	0.14
B1	13	12	1	0.92
B2	7	6	1	0.86
B3	5	4	1	0.80
B4	6	4	2	0.67
B5	5	2	3	0.40
B6	8	3	5	0.38
B7	12	4	8	0.33
B8	17	4	13	0.24
B9	7	1	6	0.14
C1	3	3	0	1.0
C2	8	7	1	0.88
C3	6	5	1	0.83
C4	6	5	1	0.83
C5	9	5	4	0.56
C6	6	3	3	0.50
C7	16	6	10	0.38
C8	14	5	9	0.36
C9	3	1	2	0.33
C10	9	0	9	0
D1	6	6	0	1.0
D2	8	8	0	1.0
D3	9	9	0	1.0
D4	12	6	6	0.50
D5	7	3	4	0.43
D6	6	2	4	0.33
D7	6	1	5	0.17
D8	6	0	6	0
D9	10	0	10	0

Colony A had 40 rescuers, 40 non-rescuers and 10 patrilines; colony B had 40 rescuers, 40 non-rescuers and 9 patrilines; colony C had 40 rescuers, 40 non-rescuers and 10 patrilines; and colony D had 35 rescuers, 35 non-rescuers and 9 patrilines.

Note: owing to the balanced sampling design, the expected null frequency of rescuers for all patrilines is 0.5.

DISCUSSION

Taken together, the results of our genetic and developmental analyses clearly demonstrate that aiding victims in distress is heritable in the ant *C. cursor*. Our results extend the findings of Eyer et al. (2012), revealing the extremely broad range of these ants’ heritable task specializations and underscoring the critical role of polyandry in their division of labor. Moreover, rescue behavior is, to our knowledge, the only instance of an altruistic specialization that involves dynamically driven social interactions between individuals in a eusocial insect colony.

Although such common activities as nest construction and foraging would seem obvious tasks comprising the division of labor in ant species, rescue behavior might at first appear less central to the success of a colony. We would argue, however, that rescue – the function of which is a relatively new area of investigation in ants (Czechowski et al., 2002; Nowbahari et al., 2009; Hollis and Nowbahari, 2013; Taylor et al., 2013; Frank et al., 2017; Kwapich and Hölldobler, 2019; Hollis, 2017) – may be equally critical, especially in sand-dwelling species such as *C. cursor*. As in several other sand-dwelling rescuer species (Hollis and Nowbahari, 2013), rescue in *C. cursor* occurs in at least two circumstances. One, nest collapse, and the ensuing entrapment by falling sand and debris, is not uncommon in sandy soils (Hollis and Nowbahari, 2013); indeed, we often have observed rescue behavior when we have disturbed nests while collecting ants for study. Thus, as rescuers work to free trapped nestmates, they may play as important a role in re-building the colony as workers specialized in nest construction. Two, sand-dwelling species live in close proximity to a very common ant predator, trap-building antlion larvae, which construct conical pits in the sand and wait motionless at the bottom for prey to stumble inside (Hollis, 2017). Rescue from antlion pits is known to occur in three other ant species, *Formica sanguinea* (Czechowski et al., 2002), *F. cinerea* (Miler, 2016) and *Tetramorium* sp. E (Taylor et al., 2013).

The potential importance of this antipredator tactic to the overall success of the colony is suggested by two recent studies. In a species

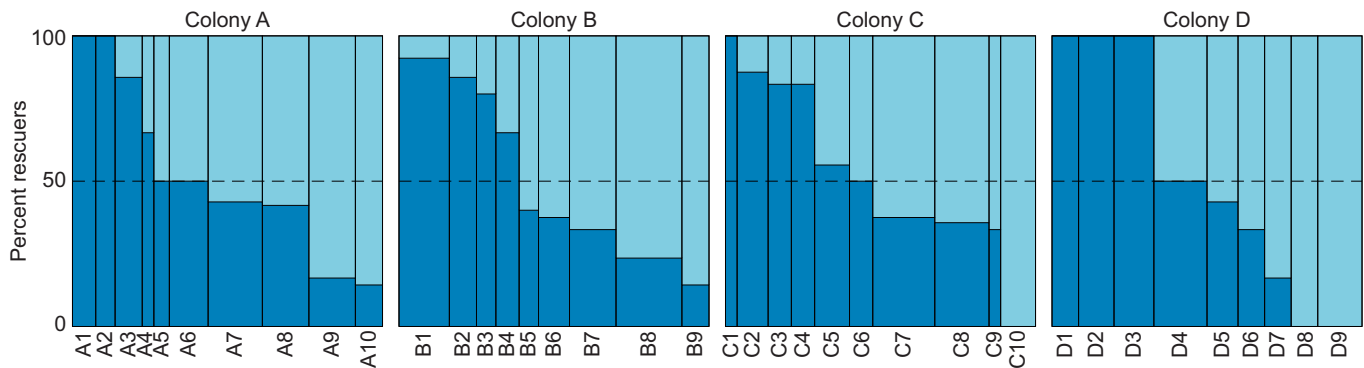


Fig. 2. Rescuers and non-rescuers in colony patriline. Mosaic plot depicting percent rescuers (dark blue bars) and non-rescuers (light blue bars) in each patriline of the four sample colonies: A, B, C and D. Width of bars is scaled by sample size. Dashed horizontal lines indicate the null expected frequency of rescuers for all patrilines (0.5). Colonies A and C each had 10 different patrilines whereas colonies B and D each had nine patrilines.

of termite-raiding ant, *Megaponera analis*, individuals injured during the raid were carried back to the nest, preventing mortality in approximately 32% of these injuries and enabling maintenance of a 28.7% larger colony size, critical to the success of the colony (Frank et al., 2017). In a second study (Kwapich and Hölldobler, 2019), foragers of the granivorous desert ant, *Veromessor pergandei*, not only rescued nestmates ensnared in spider webs, taking the victims back to the nest where the spider silk was removed, but also dismantled all spider webs in which victims were found. Echoing the importance of colony size in the study by Frank et al. (2017), Kwapich and Hölldobler (2019) hypothesize that rescue behavior in *Veromessor pergandei* enables a critical number of ants to maintain the steady supply of seeds, essential to the success of their large colonies.

In *C. cursor*, foragers would be especially vulnerable to trap-building predators, such as antlions, because they travel individually, in the absence of an ant trail that would guarantee them successful return to the colony. As in both *M. analis* and *V. pergandei*, the role of rescuers in *C. cursor* could well be to help maintain a critical number of workers. That is, any risks incurred by individual rescuers could improve the survival and reproduction of the colony, thereby increasing a rescuer's inclusive fitness and rendering the behavior selectively advantageous.

It is important to note that mechanisms of heritability other than simple additive genetic effects may underlie the patterns observed here. For example, social behaviors are known to be under epigenetic control in multiple species of eusocial insect (Opachaloemphan et al., 2018), and division of labor in other species appears to be mediated in part by genomic compatibility, as evidenced by significant interaction effects between maternal and paternal background in caste determination (Libbrecht and Keller, 2013; Libbrecht et al., 2011; Schwander and Keller, 2008). Although it is not possible at this time to speculate what the

mechanistic basis may be for rescue behavior variance in *C. cursor*, each of the above mechanisms would result in some degree of heritability of task specialization and could thus provide an adaptive explanation for polyandry.

An alternative explanation that might have accounted for our results is developmental (or temporal) polyethism. That is, it is not uncommon for workers in some eusocial insect colonies to transition among multiple tasks as they age (Wilson, 1971), and queens of certain ant species have been found to use sperm from multiple males differentially over time, resulting in a temporal 'patriline shift' (Wiernasz and Cole, 2010). If different patrilines of *C. cursor* tend to be different ages, and, critically, if rescue behavior is differentially expressed throughout workers' lives, then developmental differences in rescue behavior could have been mistakenly interpreted as genetically determined. Although *C. cursor* does not exhibit age polyethism – indeed, variations from this classical pattern are typical of most *C. cursor* workers (Retana and Cerda, 1991) – we performed a follow-up experiment to ascertain the potential for developmental polyethism to explain our results. Using exactly the same criteria to establish rescuers and non-rescuers as were used in our genetic analysis, we observed no difference in the number of rescuers and non-rescuers across age groups (Table 4). Thus, the alternative explanation that developmental polyethism can account for our results is extremely unlikely. Moreover, the very early and developmentally consistent expression of rescue behavior revealed by our follow-up experiment provides even further support for our hypothesis that rescue is under genetic control.

Given that the propensity to engage in rescue behavior appears to be both heritable and polymorphic, an open topic for future inquiry is what forces govern the frequency of rescue behavior within and among natural colonies of *C. cursor*. If, as we speculate here, rescue behavior provides important benefits to the colony, why then do not all workers rescue? Does an excess of rescuers somehow compromise colony function? Conversely, if rescuers do, in fact, incur more risk than non-rescuers, what prevents the eventual loss of rescue behavior from colonies? Although the specific costs and benefits of this very elaborate form of altruistic behavior in *C. cursor* are yet to be quantified, the establishment of its heritability demonstrates not only the importance of polyandry in task specialization, but also that rescue needs to be considered alongside other commonly investigated task specializations.

Table 4. Results of a developmental bioassay determining the number of rescuers and non-rescuers at 5, 10 and 20 days

Age (days)	Rescuers	Non-rescuers	Row totals
5	9 (10.50) [0.21]	12 (10.50) [0.21]	21
10	9 (8.50) [0.03]	8 (8.50) [0.03]	17
20	14 (13.00) [0.08]	12 (13.00) [0.08]	26
Column totals	32	32	64 (grand total)

Note: Each cell contains the observed cell totals; expected cell totals are in parentheses and the chi-square statistic for each cell is in square brackets. $\chi^2=0.6412$, $P=0.725699$. The result is not significant at $P<0.01$.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.P.A., K.L.H., E.N.; Methodology: J.P.A., K.L.H., K.A.C., G.C., E.N.; Software: J.P.A., K.L.H.; Validation: J.P.A., K.L.H., E.N.; Formal analysis: J.P.A., K.L.H., K.A.C., G.C., E.N.; Investigation: J.P.A., K.L.H., K.A.C., G.C., E.N.; Resources: J.P.A., K.L.H., E.N.; Data curation: J.P.A., K.L.H., E.N.; Writing - original draft: J.P.A., K.L.H., E.N.; Writing - review & editing: J.P.A., K.L.H., E.N.; Visualization: J.P.A., K.L.H.; Supervision: J.P.A., K.L.H.; Project administration: J.P.A., K.L.H.; Funding acquisition: K.L.H., K.A.C., G.C., E.N.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.212530.supplemental>

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