



The evolution of ant worker polymorphism correlates with multiple social traits

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Received: 27 April 2021 / Revised: 22 June 2021 / Accepted: 25 June 2021

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Abstract

In eusocial insects, worker polymorphism is shaped by several factors, including colony size, queen mating frequency, and the timing of queen-worker differentiation during larval development. In a comparative study of 18 species of *Cataglyphis* desert ants representing a wide range of worker sizes, we used phylogenetically controlled analyses to examine correlations between worker head width variation (i.e., worker polymorphism) and multiple social traits, namely, mature colony size, mean worker head width, queen head width, queen-worker head width dimorphism, and within-colony genetic relatedness, resulting from multiple mating by queens. We found that worker polymorphism was positively correlated with mature colony size, mean worker head width, and queen head width. In contrast, worker polymorphism was not correlated with queen-worker dimorphism and within-colony genetic relatedness. These results underscore that evolution of worker polymorphism and social traits are correlated. They also illustrate that additional research using multivariate approaches is needed to further clarify the evolution of insect societies.

Significance statement

In eusocial insects, worker morphological variation (i.e., worker polymorphism) is tightly linked to division of labor. Multiple factors are supposed to shape the evolution of worker polymorphism. Using phylogenetically controlled analyses of worker head width variation from 18 species of *Cataglyphis* desert ants, we show that worker polymorphism positively correlates with mature colony size, mean worker head width, and queen size. These results highlight that the evolution of worker polymorphism and social traits are correlated. Identifying the mechanisms underlying these relationships could provide major insights into the development and evolution of insect societies.

Keywords Worker polymorphism · Head width · Colony size · Polyandry · Queen-worker dimorphism · Evolution

Communicated by L. Keller.

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Introduction

Eusocial insects (ants, termites, social bees, and wasps) display marked variation in morphology within colonies (Wilson 1971; Oster and Wilson 1978; Hölldobler and Wilson 1990). Reproductive queens are typically larger than workers, which care for the brood and forage. Within the worker caste, further specialization often occurs and can be associated with morphological variation (hereafter, worker polymorphism). The emergence of such a worker polymorphism is one of the most intriguing phenomena in the evolutionary biology of social insects (Oster and Wilson 1978; Hölldobler and Wilson 1990; Traniello and Rosengaus 1997). Yet, despite decades of research devoted to exploring its genetic and developmental origins, we remain far from understanding the evolution and maintenance of this life history trait.

In ants, worker polymorphism has reached extreme levels of complexity with considerable variation in worker morphology (Oster and Wilson 1978). Polymorphism has appeared repeatedly and seems to be an evolutionarily labile trait (Blanchard and Moreau 2017), although only 15% of all ant genera exhibit some degree of worker polymorphism (Hölldobler and Wilson 1990). A number of factors have been suggested to affect worker polymorphism in ants. They can be classified into colony extrinsic and intrinsic (Wills et al. 2018). Comparative studies have provided insight into how extrinsic constraints such as diet (Wetterer 1999; Powell and Franks 2005, 2006) and habitat use (Schöning et al. 2005; Powell 2008, 2016; Powell et al. 2020) may drive worker polymorphism. Intrinsic factors known to correlate with worker polymorphism can be further classified into social environment (e.g., colony size or mean worker size), heritable factors (e.g., paternal identity), and developmental factors (e.g., timing of caste determination) (Wills et al. 2018). However, the functional and evolutionary mechanisms behind these relationships remain poorly studied. Worker polymorphism may more readily evolve in species with larger mature colony sizes because (i) the costs associated with morphologically specialized castes may only be borne by large colonies (Oster and Wilson 1978; Wheeler 1991) and (ii) workers obtain fewer advantages from reproducing, relaxing constraints on worker morphology (Bourke 1999). Worker polymorphism could be negatively correlated with mean worker size (Karsai and Wenzel 1998; Bourke 1999); as workers specialize in their “helping” role, selection on the maintenance of worker reproductive traits would be relaxed, leading to a reduction in mean worker size and to an increase in worker polymorphism (Bourke 1999). As for heritable factors, worker polymorphism is correlated with genetic diversity in colonies headed by queens with multiple mates; a significant paternal effect on worker size has been found in certain polyandrous species (e.g., Rheindt et al. 2005; Jaffé et al. 2007; Evison and Hughes 2011). Finally, it has been hypothesized that queen-worker dimorphism is shaped by the timing of caste determination: when caste is determined early on, workers have more time to differentiate in size, leading to higher worker polymorphism (Wheeler 1986). Moreover, when queen-worker dimorphism is large, workers may obtain fewer advantages from reproducing, relaxing constraints on worker morphology and favoring the evolution of worker polymorphism (Bourke 1999).

Factors affecting worker polymorphism, however, do not operate independently; instead, they interact in multifarious ways (e.g., Smith et al. 2008; Lillico-Ouachour and Abouheif 2017; Wills et al. 2018). So far, only three studies have investigated the joint effect of multiple factors on the evolution of worker polymorphism in ants, and they have yielded somewhat contradictory results. A study looking at 35 ant species across different genera found that worker

size variation was positively associated with within-colony genetic diversity (owing to polyandry) and with queen-worker size dimorphism but not with colony size (Fjerdingstad and Crozier 2006). In contrast, worker polymorphism has been linked to colony size in fungus-growing ants (Ferguson-Gow et al. 2014; Attine subfamily). Additionally, research on the morphological evolution of multiple traits in ants found that herbivory was correlated with a higher probability of both gaining and losing worker polymorphism, while carnivory was correlated with a higher probability of gaining worker polymorphism (Blanchard and Moreau 2017). The differences in findings among these studies may stem from the different phylogenetic scales and evolutionary contexts considered.

In this study, we used a phylogenetic continuous character mapping approach to investigate patterns of correlated evolution between worker polymorphism and intrinsic social traits, across 18 *Cataglyphis* desert ant species. We investigated the effect of four intrinsic social traits (Wills et al. 2018): (i) mature colony size (social environment), (ii) mean worker head width (social environment), (iii) within-colony genetic relatedness owing to polyandry (heritable factor), and (iv) queen-worker head width dimorphism (developmental factor). We also explored the effect of (v) queen head width on worker polymorphism in case there was a positive association between worker and queen head width. Our comparative study is original because of its finer phylogenetic scale. Past research has explored correlations among ant species from a single subfamily (Ferguson-Gow et al. 2014) and multiple subfamilies (Fjerdingstad and Crozier 2006) as well as among ant genera (Blanchard and Moreau 2017). In contrast, we focused on species from a single genus because of their greater shared similarities in genomes, physiological constraints, and/or developmental processes. The ant genus *Cataglyphis* is an excellent study system for exploring such relationships because the traits considered are variable across species (Agosti 1990; Aron et al. 2016; Boulay et al. 2017). First, the genus exhibits a high degree of variation in worker size and worker polymorphism within colonies and among species. Second, colony size is highly variable across species: mature colonies can contain between 150 and 3,000 individuals. Third, species employ a diversity of mating systems (i.e., anything from monandry to obligate polyandry), resulting in dramatic differences in within-colony genetic relatedness.

We used this system to address a series of hypotheses regarding worker polymorphism. First, we expected that, for the reasons mentioned above, worker polymorphism would increase with colony size. Second, we predicted that worker polymorphism decreases with mean worker head width due to relaxing selection on reproductive traits. Third, given possible paternal effects on worker size variability, we hypothesized that worker polymorphism would be positively associated with decreasing colony genetic relatedness resulting from polyandry.

Fourth, we posited that worker polymorphism should increase with queen-worker dimorphism because of the early determination of caste fate. Finally, because larger queens are expected to have higher levels of fecundity (e.g., Wickman and Karlsson 1989; Karlsson and Wickman 1990; Roff 2002; Pincheira-Donoso and Tregenza 2011; Pincheira-Donoso and Hunt 2017), we predicted that worker polymorphism should increase with queen head width; thus, large queens may produce large colonies, which may favor worker polymorphism.

Methods

Sampling

Between 2009 and 2019, we collected specimens of 18 *Cataglyphis* species from different geographical zones (Table 1, Figure S1). We sampled both foragers and intranidal workers to more fully represent the colony. To control for variation in worker polymorphism due to colony ontogeny (Tschinkel 1998; Wetterer 1999; Smith and Tschinkel 2006), we only sampled mature colonies, defined as colonies that were producing sexuals. To control for the potential effects of queen number on worker polymorphism (Frumhoff and Ward 1992; Goodisman and Ross 1996), we focused on populations displaying monogyny (one reproductive queen/colony). There were two exceptions: *C. anaescens*, in which the study population contained both monogynous and polygynous colonies, and *C. mauritanica*, a species in which all populations studied to date have been polygynous. In *C. anaescens*, worker polymorphism does not vary between monogynous and polygynous colonies (Cronin et al. 2016a). In *C. mauritanica*, queens are parthenogenetically produced; they usually stay in the natal nest, which means that nestmate queens are genetically identical (Eyer et al. 2013b). They use sexual reproduction to produce workers. Consequently, workers in a given colony have the same maternal genome but belong to different patrines, which is the same situation as in colonies headed by a single queen that has mated multiple times (polyandry).

For each species, we selected a single study population in which we collected data on worker head width, colony size, within-colony genetic relatedness, queen head width, and DNA sequences. It was crucial to take this approach because previous research has found intraspecific geographical variation in these traits in ants (e.g., Kaspari and Vargo 1995; Clémencet and Doums 2007).

Study traits

Worker polymorphism, worker, and queen head width

To quantify worker polymorphism, we used worker maximum head width (eyes included; HW), a meaningful trait for it is widely used, it varies considerably in polymorphic ants,

and it is highly repeatable (e.g., Jaffé et al. 2007; Fournier et al. 2008). We used a sample-size-corrected coefficient of variation (CV*) estimated by the ratio of the median absolute deviation of worker HW (MAD) to median worker HW (Mdn) (Marmolejo-Ramos and Ospina 2019):

$$CV^* = \left(1 + \frac{1}{4N}\right) \times \frac{MAD}{Mdn}$$

CV* values climb as worker polymorphism increases. This CV* estimator performs better than others when dealing with non-normal distributions (Marmolejo-Ramos and Ospina 2019), which was the case in this study (see Results).

We measured HW for 40 randomly chosen workers (i.e., foragers and intranidal workers) per colony, which is the number of workers at which CV* plateaued (Figure S2). We collected these data for four colonies per species. We also measured HW for one queen per species, except in the case of *C. israelensis* and *C. aenescens*, for which we had no queens. All measurements were performed to the nearest 0.01 mm using a MZ6 stereomicroscope (Leica Microsystems, Wetzlar, Germany).

We quantified queen-worker dimorphism using the metric QWD, which was estimated using the following equation (Ferguson-Gow et al. 2014):

$$QWD = 100 \times \left(\frac{2 \times (\text{queenHW} - \text{meanworkerHW})}{\text{queenHW} + \text{meanworkerHW}} \right)$$

Possible values of QWD ranges from -200 to 200, and higher estimates of QWD reflect higher levels of queen-worker dimorphism.

Colony size

For each species, the mean number of workers per colony was obtained from the literature or from our own field research (Table 1). Because data for *C. aenescens* were not available, we estimated mean colony size (600 workers) using the mean number of workers per colony for the other four species in the same phylogenetic group (i.e., the *cursor* group) (Peeters and Aron 2017).

Within-colony genetic relatedness

To measure the degree of within-colony genetic relatedness, we used mean genetic relatedness among nestmate workers (Queller and Goodnight 1989). The values of this metric were obtained from the literature, when available (Table 1). No genetic data existed for *C. holgerseni*, *C. cubica*, and *C. viatica*. Therefore, in these species, we estimated mean genetic relatedness among workers ourselves, utilizing microsatellite markers (Appendix S3A and Table S4).

Table 1 Statistics for colony size, within-colony genetic relatedness, worker head width, the coefficient of variation for worker head width, queen head width, and queen-worker dimorphism in *Cataglyphis* ant species. For each species, the table indicates the latitude and longitude (decimal degrees) for the locations where each species was sampled, the mean number of workers per colony \pm SD (colony size), mean within-colony genetic relatedness \pm SE_{jackknife} (r_{workers}), mean worker head width (HW; mm), the mean sample-size-corrected coefficient of variation for worker HW (CV*), queen HW (mm), and queen-worker dimorphism. For colony size, the range is given in brackets, and the sample size is in

Group	Species	Latitude	Longitude	Colony size	r_{workers}	HW	CV*	Queen HW	Queen-worker dimorphism
<i>cursor</i>	<i>C. piliscapa</i>	42.793	2.984333	752.4 \pm 602.4 [78–2658] (57) ^a	0.42 \pm 0.02 ^a	1.37 \pm 0.20	0.15 \pm 0.03	1.75	24.53
	<i>C. cretica</i>	35.51533	23.988262	521.1 \pm 283.6 [41–1139] (25) ^b	0.45 \pm 0.03 ^b	1.38 \pm 0.20	0.12 \pm 0.04	1.98	35.46
	<i>C. aenescens</i>	44.89167	110.13667	600 ^c	0.48 \pm 0.06 ^m	1.07 \pm 0.24	0.17 \pm 0.09	-	-
<i>altisquamis</i>	<i>C. mauritanica</i>	33.42483	-5.140333	1500 [200–3000] (32) ^d	0.41 \pm 0.03 ⁿ	1.78 \pm 0.40	0.27 \pm 0.01	2.33	26.42
	<i>C. velox</i>	37.42143	-2.862164	783.8 \pm 340.8 [220–1300] (16) ^d	0.38 \pm 0.02 ^o	1.79 \pm 0.27	0.14 \pm 0.02	1.91	6.23
	<i>C. hispanica</i>	37.30667	-6.676333	600 [300–1500] (68) ^e	0.66 \pm 0.04 ^p	1.81 \pm 0.36	0.24 \pm 0.12	2.36	26.57
	<i>C. altisquamis</i>	33.00231	35.480917	650 [400–800] (34) ^f	0.47 \pm 0.03 ^f	1.60 \pm 0.35	0.25 \pm 0.04	2.16	29.35
<i>bombycinus</i>	<i>C. bombycina</i>	30.6895	-6.208	3000 [800–5000] (20) ^e	0.31 \pm 0.05 ^q	1.62 \pm 0.27	0.15 \pm 0.03	2.30	34.58
	<i>C. sabulosa</i>	31.77739	34.657356	185.7 \pm 95.4 [47–369] (29) ^e	0.49 \pm 0.05 ^e	1.05 \pm 0.11	0.11 \pm 0.01	1.94	59.17
<i>emmae</i>	<i>C. emmae</i>	31.08667	-7.293358	702.5 \pm 78.5 (21) ^h	0.43 \pm 0.03 ^h	1.02 \pm 0.23	0.16 \pm 0.10	1.96	62.59
	<i>C. tartessica</i>	36.99056	-6.444444	182.8 \pm 130.6 (106) ⁱ	0.72 \pm 0.03 ^r	1.24 \pm 0.05	0.04 \pm 0.01	1.53	21.06
<i>bicolor</i>	<i>C. holgerseni</i>	30.65333	35.237333	3000 (5) ^e	0.37 \pm 0.02 ^e	2.12 \pm 0.40	0.18 \pm 0.06	2.40	12.19
	<i>C. savignyi</i>	31.25317	35.156667	536.2 \pm 55.94 (13) ^j	0.26 \pm 0.03 ^j	2.04 \pm 0.52	0.22 \pm 0.12	2.88	33.99
	<i>C. israelensis</i>	32.61296	35.007528	720.0 \pm 400.4 [435–1306] (4) ^e	0.3 \pm 0.02 ^s	2.17 \pm 0.49	0.24 \pm 0.04	-	-
	<i>C. viatica</i>	32.00123	-6.725797	385.8 \pm 143.3 [206–565] (6) ^e	0.34 \pm 0.03 ^e	1.92 \pm 0.31	0.16 \pm 0.04	2.63	31.35
<i>albicans</i>	<i>C. livida</i>	32.12167	34.784667	146.8 \pm 98.2 [37–333] (17) ^k	0.42 \pm 0.05 ^k	1.21 \pm 0.14	0.09 \pm 0.02	1.92	45.44
	<i>C. theryi</i>	33.42467	-5.140667	300 [100–500] (12) ^l	0.56 \pm 0.06 ^q	1.41 \pm 0.18	0.14 \pm 0.04	2.17	42.68
	<i>C. cubica</i>	32.00123	-6.725797	600 [500–1000] (10) ^e	0.51 \pm 0.02 ^e	1.50 \pm 0.17	0.11 \pm 0.03	2.08	32.04

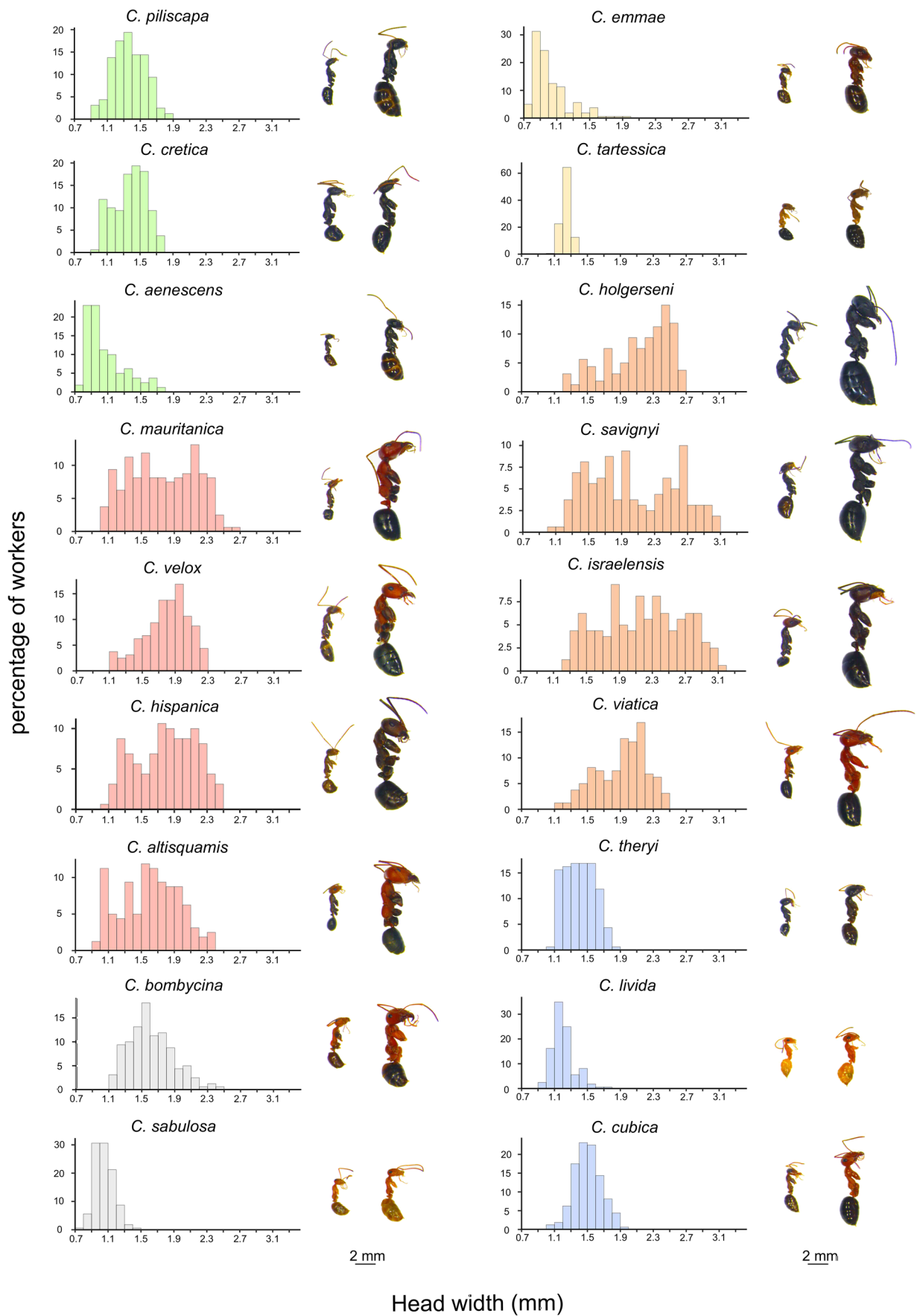
^a Percy and Aron (2006); ^b Kuhn (personal communication); ^c estimated from Peeters and Aron (2017); ^d Eyer (2014); ^e this study; ^f Kuhn (2013); ^g Timmermans et al. (2008); ^h Jowers et al. (2013); ⁱ Amor and Ortega (2014); ^j Leniaud et al. (2011); ^k Timmermans et al. (2010); ^l Leniaud (personal communication); ^m Cronin et al. (2016a); ⁿ Aron et al. (2013); ^o calculated using the data in Darras et al. (2019); ^p Darras (2010); ^q Leniaud et al. (2013); ^r Amor et al. (2016); ^s Reiner Brodetzki and Hefetz (2018)

Phylogenetic inference and patterns of trait co-evolution

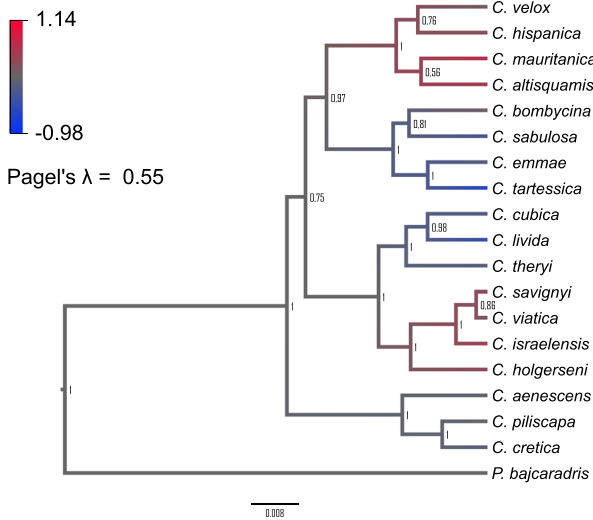
We used the software package BEAST (v. 1.10.5) (Suchard et al. 2018) to carry out two kinds of analyses at once: (i) a phylogenetic inference based on sequences for one mitochondrial gene (COI) and three nuclear genes (Wingless, Wg; abdominal-A, AB; and longwave rhodopsin, LR) (Appendix S3B) and (ii) independent continuous character mapping analyses (Lemey et al. 2010) for each continuous trait under investigation (worker polymorphism, mean worker head width, colony size, within-colony genetic relatedness, queen-worker dimorphism, queen head-width). The continuous character mapping analyses employed an anisotropic relaxed random walk (RRW) diffusion model to infer

parentheses. Colony size was obtained by counting workers. For *C. aenescens*, colony size was estimated using data from four other species in the *cursor* group (see Materials and Methods). Mean worker HW and the mean sample-size-corrected coefficient of variation for worker HW were calculated based on measurements for 40 workers from 4 colonies for each species. Queen HW was obtained from one queen per species. Queen-worker dimorphism was calculated based on queen HW and mean worker HW (see Materials and Methods for further details). For each species, all the data were collected within the same study population

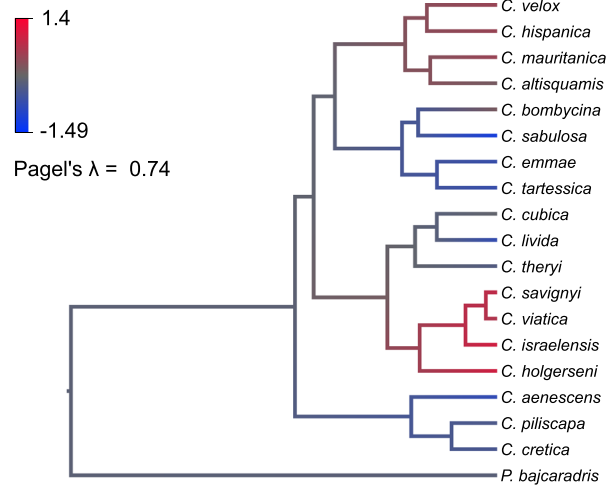
Fig. 1 Worker head width distributions for the 18 *Cataglyphis* species studied. Worker head width distributions were determined for each species using measurements from 160 workers (40 workers/colony, 4 colonies/species). Next to each graph are pictures of a small and a large worker; the scale is the same for all the pictures. Each species group is depicted using a different color: *C. cursor* (green), *C. altisquamis* (red), *C. bombycinus* (gray), *C. emmae* (yellow), *C. bicolor* (orange), and *C. albicans* (blue). Worker head width was normally distributed in *C. piliscapa* (Shapiro test, $P=0.47$), *C. sabulosa* ($P=0.07$), *C. tartessica* ($P=0.07$), and *C. cubica* ($P=0.51$). Distributions were platykurtic in *C. mauritanica* (excess kurtosis $k=-1.10$), *C. hispanica* ($k=-1.05$), *C. israelensis* ($k=-1.04$), and *C. theryi* ($k=-0.86$); bimodal in *C. savignyi* (Hartigans' dip test for unimodality/multimodality, $P=0.05$); left skewed in *C. velox* (coefficient of skewness, $S=-0.55$), *C. holgerseni* ($S=-0.49$), and *C. viatica* ($S=-0.43$); and right skewed in *C. aenescens* ($S=1.08$), *C. bombycina* ($S=0.56$), *C. emmae* ($S=1.63$), and *C. livida* ($S=1.03$). Other distribution types were seen in *C. cretica* and *C. altisquamis*



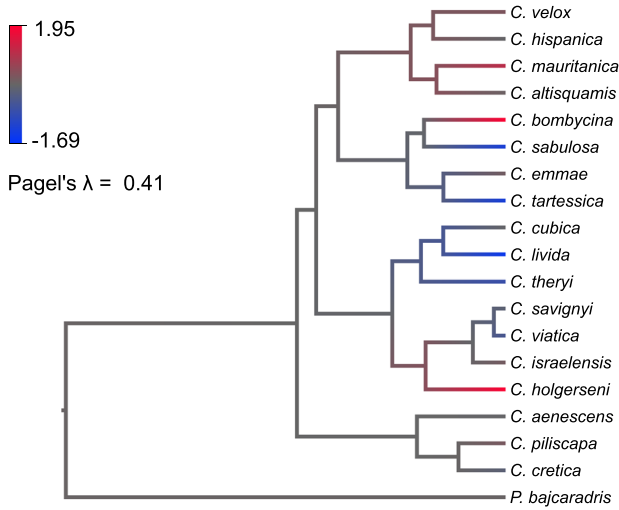
A) Coefficient of worker head width variation



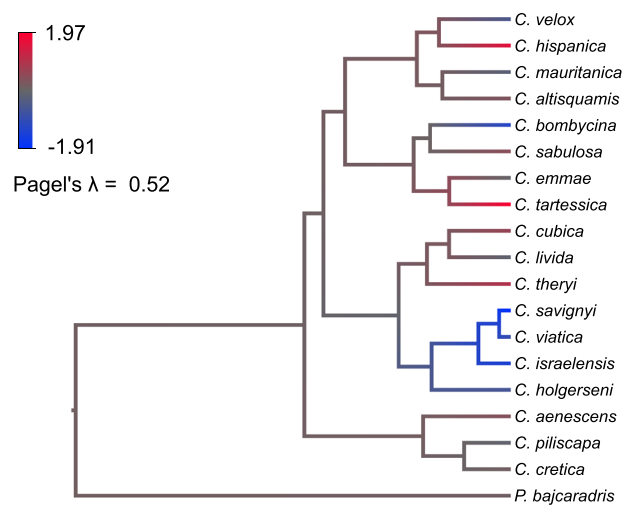
B) Worker head width



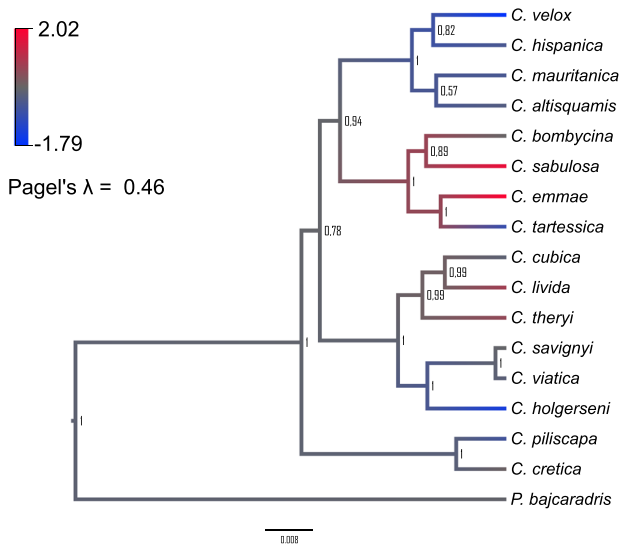
C) Colony size



D) Within-colony genetic relatedness



E) Queen-worker dimorphism



F) Queen size

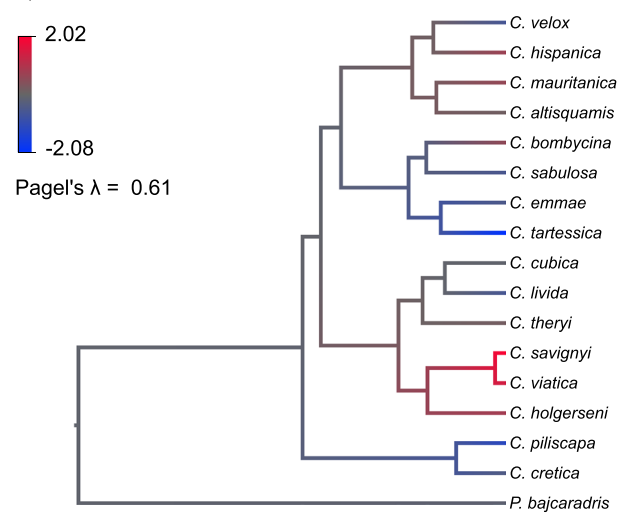


Fig. 2 Evolution of social traits in *Cataglyphis* ant species. The phylogenies show the evolutionary relationships among the *Cataglyphis* species examined in this study (analysis 1, all 18 species [A–D], and analysis 2, the 16 species for which queens were available [E–F]). Bayesian maximum clade consensus trees were inferred using sequences from one mitochondrial gene (COI) and three nuclear genes (Wg, LR, and AB); phylogenetic analysis was performed with BEAST (v. 1.10.5). The posterior probability values for the trees in A–D are indicated next to the nodes of tree A; those for the trees in E–F are next to the nodes of tree E. The branches are colored based on the continuous character mapping analysis performed for each trait. For each trait, we also report Pagel's λ values indicating the degree of associated phylogenetic signal

continuous trait values at ancestral tree nodes (Pybus et al. 2012). Contrary to a Brownian diffusion model that assumes constant diffusion rate across the phylogenetic tree, the RRW diffusion model accommodates diffusion rate heterogeneity by rescaling a precision matrix in a branch-specific manner (Lemey et al. 2010).

Specifically, our joint phylogenetic and continuous character mapping analysis was based on a strict molecular clock model, and a speciation birth–death model as the tree prior (Gernhard 2008), and a RRW diffusion model using an underlying gamma distribution to represent among-branch heterogeneity (Lemey et al. 2010). Bayesian inference through Markov chain Monte Carlo (MCMC) was run for 100 million iterations and sampled every 100,000 iterations. The first 2% of trees sampled from the posterior distribution were discarded as burn-in. We inspected MCMC convergence and mixing properties using the program Tracer (v. 1.7.1; Rambaut et al. 2018) to ensure that effective sample size (ESS) values associated with estimated parameters were all > 200 . Finally, a maximum clade credibility (MCC) tree was then retrieved from the posterior distribution of the trees and annotated using TreeAnnotator (v. 1.10.5) from the BEAST package (Suchard et al. 2018).

All trait values were log-transformed (except queen-worker dimorphism, where transformation did not improve trait distribution), centered to have a mean of 0, and scaled to obtain a standard deviation of 1. For worker polymorphism (CV*) and mean worker head width, we used multiple values for each species, allowing the analysis to account for intraspecific variability in these traits. When including repeated measures in this way, the continuous character mapping analysis considers the trait value at the tip to be an unknown mean of a normal distribution. Then, the repeated measures and the phylogenetic diffusion model are both used to determine the mean and variance of the normal distributions at the tips (Vrancken et al. 2015). We lacked sufficient data on the distributions of colony size, within-colony genetic relatedness, queen-worker dimorphism, and queen head width to account for intraspecific variation in these traits. Because we lacked queens for two species, we ran two analyses: one included the 16 *Cataglyphis* species for

which queens were available and all the traits, and the other included the 18 *Cataglyphis* species and all the traits except queen-worker dimorphism and queen head width.

We investigated the phylogenetic signal associated with each trait by estimating Pagel's λ value (Pagel 1999) for each continuous trait under investigation. When λ values are close to 0, the traits are likely to have evolved independently of phylogeny. Alternatively, λ values close to 1 indicate that traits display a strong phylogenetic signal and arise according to a classic Brownian diffusion model in a manner that is perfectly consistent with the phylogenetic relationships of the organisms from which the trait measurements were taken (Lemey et al. 2010; Vrancken et al. 2015; Monjane et al. 2020). Finally, we also used continuous character mapping reconstructions to estimate pairwise correlations among traits extracted from the precision matrix (Vrancken et al. 2015), i.e., correlation values that thus accounted for the phylogenetic relatedness of *Cataglyphis* species.

Results

Worker polymorphism

In total, we obtained measurements for 3,060 workers from 72 mature colonies of 18 *Cataglyphis* species. Distributions of worker HW varied greatly among species (Fig. 1, Table S5). Similarly, both mean worker head width and worker polymorphism varied considerably across species (Table 1): mean worker head width ranged from 0.87 to 2.38 mm, and worker CV* ranged from 0.03 to 0.29.

Phylogenetic inference and analyses of past trait values

Our inferred phylogenetic tree (Fig. 2) is consistent with previous trees built for the same genus (Aron et al. 2016; Kuhn et al. 2019). The six *Cataglyphis* groups (*cursor*, *altisquamis*, *bombycinus*, *emmae*, *bicolor*, and *albicans*) appear to be reciprocally monophyletic and were supported by high Bayesian posterior probabilities.

The analysis of past trait evolution found that all six traits displayed a strong phylogenetic signal (Fig. 2), with mean λ values ranging from 0.41 to 0.74. The λ values were highest for mean worker head width and queen head width; they were lowest for colony size and queen-worker dimorphism.

The results show that worker polymorphism evolution was positively correlated with mature colony size and mean worker head width; in contrast, worker polymorphism was not associated with within-colony genetic relatedness (Table 2). Worker polymorphism was also found to be positively associated with queen head width but not with queen-worker dimorphism (Table 3).

With regard to other trait relationships, our analysis including all 18 species found a positive correlation between mean worker head width and mature colony size, as well as a negative correlation between colony size and within-colony genetic relatedness (Table 2). The analysis with the 16 species found that mean worker head width was negatively correlated with queen-worker dimorphism and positively associated with queen head width. Furthermore, queen head width was positively correlated with colony size and negatively correlated with within-colony genetic relatedness (Table 3).

Discussion

Our study supports the hypotheses that the evolution of worker polymorphism correlates with mature colony size, queen head width (a proxy of queen size), and mean worker head width (a proxy of worker size) in *Cataglyphis* ant species. However, worker polymorphism was not associated with queen-worker dimorphism and within-colony genetic relatedness.

The positive association between worker polymorphism and mature colony size lends credence to the idea that the costs associated with producing a morphologically diverse worker force are more easily borne by species with large colonies (Oster and Wilson 1978; Wheeler 1991). Furthermore, it fits with the assumption that worker polymorphism, colony size, and worker reproduction are positively associated (Bourke 1999). In large colonies, the reproductive potential of workers is expected to be lower since a worker's relative contribution to the pool of worker-laid eggs is smaller (Bourke 1999). Hence, selection on workers to retain reproductive traits should be weak, allowing a morphologically variable worker force to evolve. The few studies that have explored the relationship between worker polymorphism and mature colony size in ants have generally found a positive relationship between the two variables (e.g., Anderson and McShea 2001; Ferguson-Gow et al. 2014; but see Fjerdingstad and Crozier 2006). However, whether

worker polymorphism and colony size are associated with worker reproduction in *Cataglyphis* and other members of Formicidae remains unstudied.

Our results also show that worker polymorphism was positively correlated with queen head width in *Cataglyphis*. Interestingly, queen head width was also positively associated with colony size, and queen head width and colony size were both negatively associated with increasing genetic relatedness within colonies. Thus, species with larger queens also had higher levels of polyandry (lower within-colony genetic relatedness) and larger colony sizes. These findings suggest that, at the species level, larger queens have more mates and can produce larger colonies; greater colony size then favors the evolution of worker polymorphism (see above). Consistent with this scenario, a fecundity–body size relationship has been seen in various ant species (Hölldobler and Wilson 1990; Wiernasz and Cole 2003). Moreover, in social insects, multiple mating allows queens to accumulate larger stores of sperm, resulting in larger colony size and lower within-colony genetic relatedness (Cole 1983; Boomsma and Ratnieks 1996; Fjerdingstad and Boomsma 1998; Kraus et al. 2004; den Boer et al. 2009). In turn, the presence of a genetically diverse worker force can enhance colony-level fitness because the division of labor is more efficient and/or resistance to pathogens is boosted (Schmid-Hempel 1998; Oldroyd and Fewell 2007; Mattila and Seeley 2007), which may then allow the evolution of larger colony sizes. In *C. piliscapa* (previously, *C. cursor*), patriline identity has been shown to affect task preference, suggesting that higher levels of polyandry improve the division of labor (Fournier et al. 2008; Eyer et al. 2013a). However, it remains unknown whether this latter colony feature increases colony size or productivity in *Cataglyphis*.

Our results also reveal that species displaying greater levels of worker polymorphism had larger colony sizes, larger queens, and larger mean worker sizes. The positive correlation between worker polymorphism and mean worker size is consistent with the observations that (i) small workers are similarly sized in all the species sampled and (ii) large workers in the more polymorphic species are larger than large workers in less polymorphic species (Fig. 1). Furthermore, among several models of trait evolution examined (Brownian motion, Ornstein Uhlenbeck with one optimum and two optima, early burst, constant trend, and white noise with two optima), the Ornstein Uhlenbeck model applied with a “large versus small mean worker size” dichotomy gives the better fit of the evolution of worker polymorphism in *Cataglyphis* (Table S6). Thus, species with large mean worker size evolve towards more polymorphic workers compared to species with small mean worker size. Altogether, these data suggest that, in *Cataglyphis*, worker polymorphism evolved through increasing worker head width, possibly in a positive feedback with

Table 2 Correlations among social traits in *Cataglyphis* ant species

Colony size				1
Worker HW				0.69
CV*	1			0.6
Relatedness	1	–0.4	–0.4	–0.51
	Relatedness	CV*	Worker HW	Colony size

For each pair of traits, we report the correlation accounting for phylogenetic relatedness of *Cataglyphis* species. Correlation estimates are provided for all 18 *Cataglyphis* species. Significant correlations are in bold. A correlation was considered to be significant if the 95% highest posterior density interval of the estimate's posterior distribution excluded zero

Table 3 Correlations among social traits in *Cataglyphis* ant species

Q-W dim						1
Queen HW					1	0.22
Colony size			1	0.47		−0.38
Worker HW		1	0.68	0.53		− 0.64
CV*	1	0.44	0.59	0.78		0.17
Relatedness	1	−0.47	−0.35	− 0.51	− 0.48	−0.01
	Relatedness	CV*	Worker HW	Colony size	Queen HW	Q-W dim

For each pair of traits, we report the correlation accounting for phylogenetic relatedness of *Cataglyphis* species. Correlation estimates are provided for the 16 species for which queens were available. Significant correlations are in bold. A correlation was considered to be significant if the 95% highest posterior density interval of the estimate's posterior distribution excluded zero

colony size and queen size. This contrasts with previous empirical (Karsai and Wenzel 1998) and theoretical (Bourke 1999) studies pointing to a reduction of worker size with increasing queen size, colony size, and worker polymorphism in social insects. Such reduction in the mean worker size is predicted since, as workers specialize in their role of helpers, selection on the maintenance of reproductive traits is relaxed (Karsai and Wenzel 1998; Bourke 1999). Our findings show, however, that a reduction of worker size with increasing queen size, colony size, and worker polymorphism cannot be generalized to all social insects. Further studies are needed to test these relationships and to identify the factors affecting their directions.

No association was observed between worker polymorphism and queen-worker size dimorphism. In ants, the degree of worker polymorphism and queen-worker dimorphism is both expected to be influenced by the developmental timing of caste determination: the earlier the differentiation between workers and queens, the more time individuals have to differentiate in size (Wheeler 1986). In our study, the lack of a relationship between worker polymorphism and queen-worker dimorphism suggests that the timing of caste differentiation does not critically affect worker head width. We found no association between worker polymorphism and the degree of genetic relatedness within colonies; the latter is determined by queen mating frequency. Thus, across multiple *Cataglyphis* species, there were apparently no significant paternal genetic effects on worker head width. These results are consistent with those from previous species-specific studies, which found that the association between worker size variation and within-colony genetic relatedness or patriline identity was weak to absent in the ants *C. piliscapa* (Fournier et al. 2008) and *C. bombycina* (Leniaud et al. 2015) (but see Cronin et al. 2016b, who discovered that patriline affected worker size in *C. cursor*). Overall, it seems there is weak selection on polyandry to increase the morphological diversity of workers in *Cataglyphis* ant species.

Conclusion

This study shows that the evolution of worker polymorphism correlates with multiple intrinsic social traits in the ant genus *Cataglyphis*. We found evidence of positively reinforced relationships among worker polymorphism, mature colony size, and queen-specific characteristics (i.e., boosted fertility resulting from greater queen size and mating frequency). In addition, our results suggest that worker polymorphism arose via increases in maximum worker head width. Identifying the mechanisms underlying these relationships could provide major insights into the development and evolution of insect societies.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00265-021-03049-6>.

Acknowledgements We thank Xim Cerdá, Hugo Darras, Abraham Hefetz, Alexandre Kuhn, Laurianne Leniaud, Rémy Perez, and Quentin Willot for their help on the field; ICTS-RBD for providing us fieldwork facilities; Claudie Doums for kindly providing us with samples of *C. aenescens*; J. Pearce-Duvet for her language editing services; and two reviewers for their constructive comments on the manuscript.

Author contribution NL and SA conceived the study; NL and SA collected field data; NL carried out the lab work; NL and SD performed the data analysis; NL and SA drafted the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Funding NL, SD, and SA are supported by the Belgian *Fonds National pour la Recherche Scientifique* (FRS-FNRS) (Grant No. J.0063.14, J.0151.16, and T.0140.18 to SA).

Data availability The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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