Nutritional versus genetic correlates of caste differentiation in a desert ant

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Abstract. 1. In many ant species, caste differentiation stems from trophic differences at the larval stage. Adult workers that feed larvae have great control over the allocation of colony resources to growth (production of workers) versus reproduction (production of queens). However, larval caste fate may also be constrained very early on through direct genetic effects or non-genetic maternal effects.

2. Here, we combined isotopic and genetic analyses to study the developmental origin of queens and workers in a desert-dwelling ant, *Cataglyphis tartessica* (Amor & Ortega, 2014). Queens do not found new colonies alone but rather disperse with workers. As the latter are always wingless, selection pressures on specific queen traits such as flight ability have become relaxed. Though the phylogenetically related species, *C. emmae* (Forel, 1909) only produces winged queens much larger than workers, *C. tartessica* produces two types of small queens relative to workers: brachypterous (short-winged) queens and permanently apterous ergatoid (wingless and worker-like) queens.

3. Upon emergence, workers and ergatoids have similar $\delta15N$ isotopic values, which were lower than those of brachypters, suggesting the latter are fed more protein as larvae. Microsatellite analyses indicated that: (i) colonies are mostly monogynous and monandrous; (ii) both ergatoids and brachypters are equally related to workers; and (iii) in the few polyandrous colonies, patrilines were evenly represented across workers, brachypters and ergatoids.

4. Overall, there was no evidence of genetic caste determination. We suggest that, in contrast to brachypters, ergatoids are selfish individuals that escape the nutritional castration carried out by workers and develop into queens in spite of the colony's collective interests.

Key words. Caste differentiation, diet, larval development, stable isotopes.

Introduction

In colonies of social insects, there coexist two castes of females with distinct functions: queens, who dedicate themselves to

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Michael J. Jowers was initially omitted by the authors and has now been added to this version on 12 August 2016 after the article's first online publication.

reproduction, and workers, who are entirely or partially sterile and carry out non-reproductive tasks. In ants, the degree of worker–queen dimorphism largely depends on the mode of dispersal (Peeters & Molet, 2010). In species with independent colony founding, queens are born with functional wings and a strong thoracic musculature, allowing them to fly away from the nest and found new colonies on their own. Workers, in contrast, are generally smaller and wingless. Worker–queen dimorphism tends to be less pronounced in species that disperse

by colony fission (Peeters & Ito, 2001; Cronin *et al.,* 2013). In such systems, young queens are accompanied and protected by a group of workers throughout the colony foundation process. However, the fact that dispersal occurs exclusively by foot promotes diminished flying capacity and the production of brachypterous queens with short vestigial wings or wingless ergatoid queens (Heinze, 1989; Tinaut & Ruano, 1992).

Trophogenesis is responsible for the different developmental fates of workers and queens in many species (Emery, 1896; Marchal, 1898; Brian, 1957; Wilson, 1971; Michener, 1974; Oster & Wilson, 1978; Wheeler, 1986). This suggests that diploid larvae are totipotent and only those given special food become queens. By limiting protein intake, adult workers could castrate diploid larvae, forcing them to develop into workers. This mechanism would grant great power to the workers, allowing them to decide to allocate colony resources to growth (through the production of workers) versus reproduction (through the production of queens). Trophogenic caste determination is well known in the honeybee *Apis mellifera* (Linnaeus, 1758), where only larvae receiving royal jelly develop into queens (Seeley, 2010; Kamakura, 2011). In ants, major differences in the isotopic values of queens versus workers provide evidence for trophogenesis. For example, in *Pogonomyrmex badius* (Latreille, 1802), young unmated queens have much higher $\delta^{15}N$ ratios than do workers, which suggests that the former receive relatively more proteinaceous food (Smith *et al.,* 2008; Smith & Suarez, 2010). Similar patterns have been found in two species of *Aphaenogaster* ants (Caut *et al.,* 2013, 2014), as well as in a eusocial yellowjacket (Schmidt *et al.,* 2012). Nevertheless, these correlations are to be taken carefully and cannot speak to causality.

Some studies suggest that the ability of adult workers to control caste determination may also be limited by the precocious self-determination of the larvae. In blastogenic determination, an individual follows either the worker or the queen developmental pathway starting at the embryonic stage, even before the egg hatches (Passera, 1980; Fersch *et al.,* 2000; Schwander *et al.,* 2008; Schwander & Keller, 2012; Libbrecht *et al.,* 2013). In addition, a larva's fate may be influenced by its genotype. This occurs in the highly polyandrous species *Acromyrmex echiniator* (Forel, 1899), in which some paternal genotypes yield queens at a relatively higher frequency (Hughes & Boomsma, 2008). In another highly polyandrous ant, *Cataglyphis bombycina* (Roger, 1859), soldiers sharing developmental pathways with queens (Molet *et al.,* 2014) were found to be derived from completely different patrilines than workers (Leniaud *et al.,* 2013) although this was not confirmed in another study (Leniaud *et al.,* 2015). Stricter cases of genetic caste determination have also been observed – while gynes are the product of intralineage mating in *Pogonomyrmex* or thelytoky in certain *Cataglyphis* species, in both cases, workers are derived from interlineage mating (Julian *et al.,* 2002; Leniaud *et al.,* 2012; Eyer *et al.,* 2013).

Cataglyphis tartessica (formerly *C. floricola*; Jowers *et al.,* 2014) is a sand-dwelling ant that is distributed throughout southern Spain, and that disperses via colony fission. Interestingly, both brachypterous and ergatoid queens have been observed in this species (Amor *et al.,* 2011). Brachypters are small relative to the macropterous queens of the related species *C. emmae* which
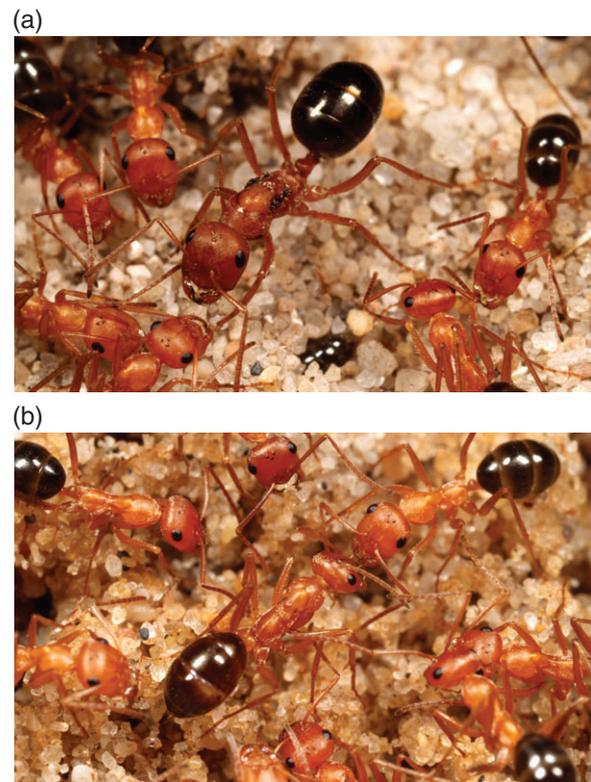
Fig. 1. *Cataglyphis tartessica* brachypterous queen (a) and worker-like ergatoid queen (b). Although brachypters are easily recognisable because of their size and distinct morphology, ergatoids can only be distinguished from workers based on the presence of small thoracic tegulae (indicated by the arrow).

found colonies through independent colony foundation (Jowers *et al.,* 2013). Yet brachypters' thorax is segmented markedly different from the workers. In contrast, ergatoids are only distinguishable from workers by the presence of small tegulae (Fig. 1). Out of nine morphological traits measured by Amor *et al.* (2011), seven were significantly smaller in ergatoids than in brachypters (see also Amor & Ortega, 2014). Although, at the population level, more ergatoids than brachypters are produced, the proportions of colonies headed by each are fairly equal (Amor *et al.,* 2011). Moreover, behavioural observations showed that, during fission, almost all the cocoons containing brachypters but only one-third of those containing ergatoids are transported to the daughter nests. After emergence, these individuals compete to become the next queen of the daughter nest. In contrast, the ergatoid that emerge from the cocoons not transported to the daughter nest are eliminated by the workers probably to maintain monogyny (Amor *et al.,* 2011). This differs from other species (*Ocymyrmex*: Forder & Marsh, 1989; *Eutetramorium*: Heinze *et al.* 1999; *Mystrium*: Molet *et al.,* 2007) in which unmated ergatoids participate to non-reproductive tasks such as nursing and foraging. Therefore, the excess of *C. tartessica* ergatoids that are eliminated at the early adult stage may be viewed as 'useless' by the colony. In a previous study, we proposed that these individuals were selfish individuals that escaped the

'nutritional castration' imposed by adult workers by becoming worker-like queens in spite of what was in the collective interests of the colony (Amor *et al.,* 2011). A similar explanation based on caste conflict has been evoked for *Melipona* bees, in which worker-like queens are produced in excess (Wenseleers *et al.,* 2004, 2005). In ants, social coercion of larval development has been shown in several species with marked worker–queen polymorphism (Brian, 1973; Penick & Liebig, 2012; Villalta *et al.*, 2016b) and is similar in many aspects to what is known of worker policing in species with no specialised queen caste (Bourke & Ratnieks, 1999; Monnin & Ratnieks, 2001).

Here, we tested the hypothesis that though brachypter-fated larvae receive queen-specific food, ergatoids receive the same food as workers. To test this prediction, direct behavioural observations are not possible; we, therefore, analysed the N and C isotopic values of *C. tartessica* workers, brachypters, and ergatoids right when they emerged. In addition, workers, brachypters, and ergatoids were genotyped at nine microsatellite loci to determine the degree of colony polyandry and the degree of relatedness among workers, thus clarifying the possibility of genetic caste determination.

Materials and methods

Sampling

Cataglyphis tartessica colonies were collected in 2010 and 2014 from a population located in Doñana National Park (southern Spain). The distance between colonies ranged from 10 m to 3 km. Shallower chambers were excavated to collect pupae and workers, which were taken back to the laboratory. Cocoons and three adult workers were placed in individual Petri dishes. The ants had access to water, and the dishes were kept at 27 °C (±3 °C). The workers were needed to help the pupae emerge from their casings. The Petri dishes were monitored daily until all the cocoons had emerged. Each new callow – whether the worker, brachypter, or ergatoid – was preserved in 96% alcohol until the isotopic and genetic analyses could be carried out. No food was provided to the ants during the experiment, which means the isotopic values of the callows reflected their nutritional experiences in the field.

Isotopic analyses

Samples were dried at 60 °C for 48 h, ground to a fine powder, weighed in tin capsules, and stored in a desiccator. Isotopic analyses were performed using continuous flow isotope–ratio mass spectrometry. More specifically, a Flash HT Plus elemental analyser was coupled with a Delta V Advantage isotope ratio mass spectrometer via a ConFlo IV interface (Thermo Fisher Scientific, Inc., Bremen, Germany). Isotope ratios are presented as δ values (‰), which are expressed relative to the Vienna Peedee Belemnite (vPDB) standard and atmospheric N$_2$ for carbon and nitrogen, respectively. Stable C and N isotope ratios (δ^{13}C or δ^{15}N) were found using the following equation: $[(R_{sample}/R_{standard}) - 1] \times 1000$, where R is either ^{13}C/^{12}C or ^{15}N/^{14}N. The reference materials used were

IAEA-CH-6 (−10.4‰) and IAEA-N1 (+0.4‰) for δ^{13}C and δ^{15}N, respectively. One hundred replicate assays of internal laboratory standards indicated maximum measurement errors (SD) of ±0.2‰ and ±0.15‰ for stable carbon and nitrogen isotopes, respectively. The C/N ratio was the total percentage of carbon divided by the total percentage of nitrogen.

DNA extraction and analysis

A total of 162 workers, 19 brachypters and 17 ergatoids from 18 colonies were genotyped (mean = 9.0 workers per colony). DNA was extracted from the brain and the surrounding muscular tissue using the HotShot method (Truett *et al.,* 2000); it was then stored at −20 °C. Nine microsatellite markers (Ccur11, Ccur51, Ccur60, Ccur61, Ccur63a, Ccur63b, Ccur89, Ccur99, and Ccur100) developed for use in *C. cursor* (Foscolombe, 1846) (Pearcy *et al.,* 2004a) were employed to study nuclear polymorphism. Polymerase chain reactions (PCRs) were carried out in pairs (i.e. as duplex reactions) or individually. A PCR reaction volume of 20 μl was used; it contained 50 ng DNA, 200 μM of each dNTP, 0.15 μM of each primer, 2 μl of 10× buffer, 2 μl MgCl$_2$, and 0.1 unit of Taq polymerase (Qiagen, Hilden, Germany). The following thermal cycle was employed: an initial denaturation step of 2 min at 94 °C; 35 denaturation cycles of 30 s at 94 °C; an annealing step of 30 s at 52 °C; an extension step of 45 s at 72 °C; and a final extension step of 5 min at 72 °C. Labelled fragments were resolved on an A3130xl Genetic Analyser (Applied Biosystems, Foster City, California). Genotyping errors because of null alleles and allele drop-outs were controlled using Micro-Checker.

Statistical analyses

Statistical analyses were performed using R unless otherwise specified. Isotopic values (δ^{13}C, δ^{15}N, and C/N) were compared using a generalised linear model (lmer function; lme4 library); caste, year, and the caste-by-year interaction were the predictor variables, while the colony of origin was included as a random variable. The effects of the predictor variables were examined using *F*-statistics. A Holm–Bonferroni correction was applied to control the family-wise error rate.

Basic statistics, as well as estimates of linkage disequilibrium and Hardy-Weinberg equilibrium, were obtained using GENEPOP ON THE WEB and the ADEGENET package in R. A subset of data was used – it comprised one randomly chosen individual per colony (to reduce the risk of pseudoreplication owing to the non-independence of ants taken from the same colony). Relatedness coefficients (*r*) were estimated using Relatedness (v. 5.0.8). All colonies were equally weighted, and standard errors were obtained by jackknifing over colonies. Our approach was as follows: first, we estimated mean relatedness among all the individuals within each colony (*n* = 198 individuals across 18 colonies). Then, we estimated the mean relatedness among 45 workers and 17 ergatoids from a subset of seven colonies and among 32 workers and 19 brachypters from a second subset of nine colonies.

Table 1. Results of the generalised linear models used to compare isotopic values among castes and between years.

	δ^{15}N			δ^{13}C			C/N		
	F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Caste	**14.45**	**2,72.14**	**<0.0001**	2.09	2,70.89	0.13	1.04	2,69.90	0.36
Year	0.12	1,12.93	0.73	**16.90**	**1,11.97**	**0.002**	1.10	1,8.54	0.32
Caste × year	1.49	2,72.14	0.23	1.54	2,70.89	0.22	2.38	2,69.90	0.10

Significant values are in bold (i.e. remained significant after the *α* threshold was lowered following the Holm−Bonferroni correction to control the family-wise error rate).

The number of queens and the number of their mating partners were inferred using COLONY. Effective male paternity ($M_{e,p}$), which quantifies the relative contribution of different males to offspring production, was calculated using equation 16 in Nielsen *et al.* (2003). In addition, we estimated the probability that two males bear, by chance, the same combinations of alleles at all loci, as per Boomsma and Ratnieks (1996). In colonies in which the genotyping results suggested the occurrence of polyandry, we tested symmetry in the patriline distribution among castes using a chi-square test with Yates' correction for continuity.

Results

Isotopic analyses

The isotopic analyses revealed that δ^{15}N values differed significantly among castes but not between years (Table 1). δ^{13}C values, in contrast, were higher overall in 2014 than in 2010 but did not differ among castes (Table 1). These differences remained significant even after the Holm−Bonferroni correction was applied to control the family-wise error rate. The significant effect of caste on δ^{15}N was as a result of workers and ergatoids having significantly lower δ^{15}N values than brachypters (Fig. 2; $t_{76.1} = -5.04$, $P < 0.0001$ and $t_{80.9} = -4.17$, $P < 0.0001$, respectively). However, the difference between ergatoids and workers was not significant ($t_{73.1} = -0.53$, $P = 0.59$). The C/N ratio did not differ significantly among castes or between years (Table 1).

Genetic analyses

The nine loci examined displayed between 2 and 15 alleles. The mean observed heterozygosity (*Ho*) was 0.61 (range: 0.22−0.84), and the mean expected heterozygosity (*He*) was 0.63 (range: 0.27−0.84). Genetic descriptive statistics are given in Table 2. Only one locus (Ccur 61) was not at Hardy−Weinberg equilibrium (Table 2). None of the 36 tests of linkage disequilibrium was significant ($\alpha = 0.05$). The mean within-colony genetic relatedness was 0.72 ± 0.03 (Table 3) and did not differ significantly from the expected value of 0.75 (i.e. relatedness among full sisters in a monogynous and monandrous colony; *t*-test: $t = -1.0767$, d.f. = 17, $P = 0.2967$). Relatedness between workers and ergatoids and between workers and brachypters was 0.79 ± 0.04 and 0.69 ± 0.06, respectively. Neither value was significantly different from 0.75
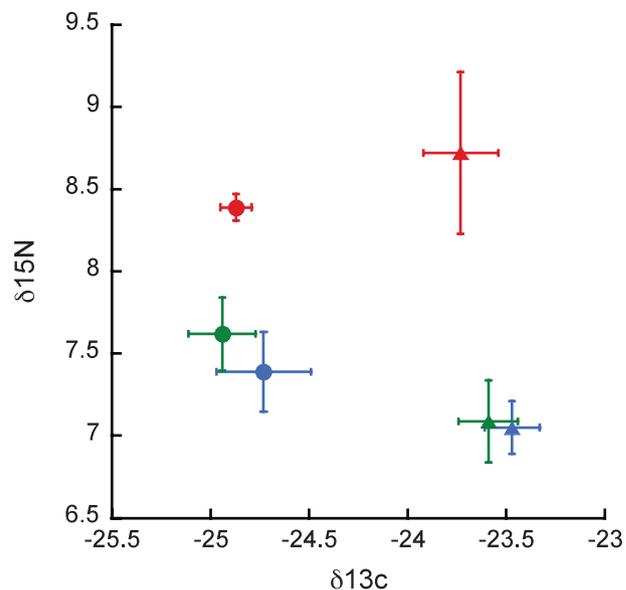

Fig. 2. The mean (+SE) values of carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios for the three castes and the two study years. Values for 2010 and 2014 are represented by circles and triangles, respectively. Workers, brachypters, and ergatoids are represented in green, red, and blue, respectively.

($t = 0.437$, d.f. = 8, $P = 0.67$ and $t = -1.012$, d.f. = 6, $P = 0.35$, respectively).

The COLONY analysis of individual genotypes supported the hypothesis of monogyny for 17 of the 18 colonies (Table 3). However, the individuals collected from one colony (#100712B) were most likely the daughters of two different queens. There was no evidence of polyandry in six of the colonies. In the remaining colonies, the queens had likely been inseminated by two to three males. On average, a colony's workers were inferred to have been sired by 1.79 ± 0.14 males. The mean effective paternity ($M_{e,p}$) was 1.55 ± 0.13. The probability of two males having the same genotype was very low (1.38×10^{-5}). Of the six colonies in which individual genotypes suggested polyandry, the patrilines distributions did not differ significantly among castes (Fig. 3).

Discussion

The evolutionary transition from independent colony founding to fission is associated with the loss of the ability to fly, queen

Table 2. Statistical characterisation of the nine polymorphic loci in *Cataglyphis tartessica* used in this study.

Loci	Basic statistics			Inbreeding		Hardy–Weinberg	
	N_a	H_o	H_e	F_{is}	P	χ^2 (d.f.)	P
Ccur11	5	0.84	0.69	−0.32	0.37	17.15 (6)	0.380
Ccur51	10	0.74	0.84	0.04	0.42	83.03 (36)	0.429
Ccur60	2	0.45	0.40	0.06	1.00	35.62 (1)	1.000
Ccur61	15	0.65	0.89	0.22	0.00	220.71 (105)	0.007
Ccur63a	6	0.73	0.72	−0.02	0.76	170.01 (10)	0.785
Ccur63b	3	0.22	0.27	−0.06	1.00	0.15 (1)	1.000
Ccur89	4	0.43	0.42	−0.11	1.00	71.53 (6)	1.000
Ccur99	6	0.72	0.72	−0.10	0.75	11.57 (10)	0.755
Ccur100	7	0.75	0.68	−0.33	0.63	222.38 (10)	0.583

Allelic richness (N_a), observed heterozygosity (H_o), and expected heterozygosity (H_e) were estimated. Fis is the Weir and Cockerham (1984) inbreeding coefficient, which was calculated by randomly selecting one individual per colony ($n = 18$). Deviation from Hardy-Weinberg equilibrium was examined using a chi-square test.

Table 3. Details of colony-level genetic results

Colony	W	E	B	r_{all}	r_{w-e}	r_{w-b}	M_p	$M_{e,p}$
100601B	22	0	0	0.77	–	–	2	1.10
100601C	16	0	0	0.71	–	–	2	1.14
100602B	5	5	0	0.51	0.48	–	2	1.84
100602C	5	1	3	0.69	0.70	0.69	1	1.00
100604D	5	1	1	0.72	0.88	0.44	2	1.83
100607C	3	0	7	0.75	–	0.65	2	1.84
100614A	4	2	0	0.80	–	–	1	1.00
100614D	5	2	0	0.71	–	–	2	1.83
100614G	5	1	1	0.75	–	0.74	2	2.19
100615A	4	0	2	0.73	–	0.67	2	1.99
100615E	3	0	3	0.88	–	0.86	1	1.00
100617G	4	1	0	0.78	0.85	–	1	1.00
100617H	5	3	0	0.68	0.71	–	1	1.00
100625B	22	0	0	0.78	–	–	2	1.21
100708D	15	0	0	0.60	–	–	3	2.75
100712A	13	0	0	0.81	–	–	3	2.55
100712B	17	0	0	0.38	–	–	2	1.22
100721A	7	1	2	0.88	0.98	0.84	2	1.99

W, E, and B are the sample sizes for the workers, ergatoids, and brachypters, respectively. r_{all}, r_{w-e}, and r_{w-b} are the estimates of mean relatedness among all the individuals found in the colony, between workers and ergatoids, and between workers and brachypters. M_p is absolute paternity, and $M_{e,p}$ is effective paternity. Note that all colonies with the exception of colony 100712B contained a single matriline.

miniaturisation, and a diminished degree of worker–queen dimorphism (Heinze, 1989; Tinaut & Ruano, 1992; Peeters & Ito, 2001). However, the fact that two distinct morphs of non-flying queens have evolved and co-occur raises some interesting questions. We discovered that workers and ergatoids had similar $\delta^{15}N$ values, which were lower than those of brachypters. In contrast, there were no genetic differences among castes. Taken together, these results suggest that polymorphism between brachypters on the one hand and ergatoids/workers, on the other hand, have a nutritional determinism.

The isotope data suggest that, compared to workers and ergatoids, brachypters receive more protein or are better at assimilating it. These results fit with those found in previous studies of other species, where the $\delta^{15}N$ values of queens were higher than those of workers (Smith *et al.*, 2008; Caut *et al.*, 2013, 2014). It is, therefore, likely that N limitations affect an individual's general growth as well as the production of wings and the associated musculature. However, whether the development of reproductive organs is N limited, as proposed by Smith and Suarez (2010), is unclear in *C. tartessica*. Indeed, the higher number of ovarioles in ergatoids compared with workers (12 vs. 2, respectively; Amor *et al.*, 2011) is not related to a greater $\delta^{15}N$ value in the former; in contrast, brachypters, which have about 20 ovarioles, also have more $\delta^{15}N$ than workers and ergatoids. An interesting model system to test N limitation on the development of reproductive organs could be the South American seed-harvester ant *Pogonomyrmex laticeps* (Santschi, 1922) in which ergatoids and brachypters have the same number of ovarioles (Peeters *et al.*, 2012). The large difference in $\delta^{13}C$ values between 2010 and 2014 may have been as a result of changes in plant communities or to a shift in the selection/
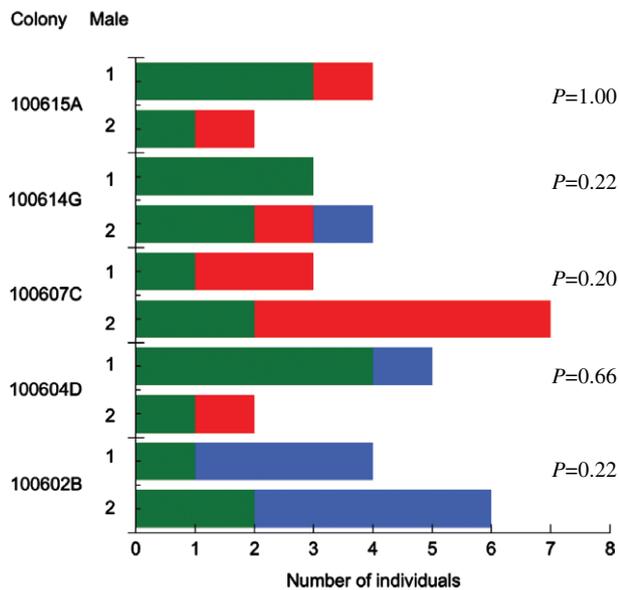
Colony   Male

Fig. 3. Patriline distribution among workers (green), brachypters (red), and ergatoids (blue) in the five polyandrous colonies.

availability of carbon resources between the 2 years (Peterson & Fry, 1987).

One hypothesis to explain the evolutionary origin of ergatoids is that their production allows colonies to produce queens even when the scarcity of N-rich food items prevents the production of brachypters. Although most *Cataglyphis* species are thought to be scavengers that forage on dead insects (Cerdá *et al.,* 1989; Wehner *et al.,* 1992), *C. tartessica* also retrieves large quantities of flower petals (Cerdá *et al.,* 1996) and frequently collects aphid honeydew (F. Amor, unpublished). It is, therefore, possible that larvae that feed mostly on low proteinaceous food items are more likely to become workers or ergatoids, whereas only those that receive animal prey become brachypters. Ergatoids would, therefore, be a sort of second-chance queens, produced in case of insufficient protein input. However, this strategy may seem paradoxical from the colony perspective: if resources are limited, one may expect workers to reduce their allocation of resources to individuals that will neither reproduce nor contribute to colony growth. This situation, therefore, differs from that observed in other species in which unmated ergatoids do participate in domestic tasks (Forder & Marsh, 1989; Heinze *et al.,* 1999; Molet *et al.,* 2007). One way to test the impact of N-limitation on the production of ergatoids would be to raise colonies under various N regimes and record colony-level caste allocation. Unfortunately, this manipulation is not easy in *C. tartessica* which is relatively difficult to raise in laboratory conditions.

Another hypothesis is that ergatoids are selfish individuals that, although receiving a worker diet, develop into queens in spite of the colony interest. Theory predicts that individual larvae may expect to have greater direct fitness if they become queens rather than workers (Bourke & Ratnieks, 1999; Ratnieks *et al.,* 2006). In species with marked worker–queen dimorphism, adult workers may constrain a larva's developmental fate by limiting its protein intake. However, the evolutionary

transition to colony fission reduced the selective advantage of large queens, opening the door for larvae to develop selfishly into small queens. Such a mechanism has been proposed for *Melipona* bees (Wenseleers *et al.,* 2004, 2005) and could also occur in *C. tartessica* (Amor *et al.,* 2011). Although the difference of diet between ergatoids and brachypters is not sufficient to conclude about 'larval selfishness', such difference is a necessary condition for this process to occur. Again, this hypothesis would deserve careful behavioural observations in laboratory conditions to test whether workers differentially feed larvae depending on their caste fate.

Interestingly, there was no evidence of genetic caste determination in the microsatellite data. The genotype patterns of all but one colony were reflective of monogyny, and the number of patrilines was between one and two. As a consequence, workers, brachypters, and ergatoids were full sisters and did not originate from different patrilines. A previous study, in which a large number of nests were excavated, found that colonies were strictly monogynous and contained either a brachypterous or an ergatoid queen (Amor *et al.,* 2011). Thus, the one colony in which worker genotypes were suggestive of two matrilines may have experienced queen replacement, which is frequent in some species that carry out colony fission (Chéron *et al.,* 2009). Although strict monogyny and monandry are probably the ancestral state in ants (Hughes *et al.,* 2008; Boulay *et al.,* 2014), they are relatively rare in *Cataglyphis* species (Cronin *et al.,* 2015): a high level of polyandry has been reported in 9 of 13 species, and only one species is strictly monogynous (*C. hispanica*, Leniaud *et al.,* 2012). Moreover, 5 of 14 species are known to be strictly or facultatively polygynous. A previous study has shown that the closest relative of *C. tartessica* – *C. emmae* – is strictly monogynous and slightly polyandrous ($M_p = 3.04 \pm 1.04$ and $M_{e,p} = 2.74 \pm 1.09$; Jowers *et al.,* 2013), which suggests that monandry was secondarily evolved in *C. tartessica*. Finally, the production of queens via thelytoky has also evolved in a number of *Cataglyphis* species (Pearcy *et al.,* 2004b; Leniaud *et al.,* 2012; Eyer *et al.,* 2013) but has not been observed in *C. tartessica*.

In conclusion, nutrition is likely a major regulator of caste determination in ants and other social insects. This fact gives workers coercive power over larvae – they can force larvae to become workers, who must help the colony and forgo reproducing themselves (Ratnieks & Wenseleers, 2008). However, in species that perform colony fission, where queens no longer need to fly, the door has been left open for the evolution of selfish larvae. The overproduction of selfish queens may constitute a serious cost in the evolutionary transition from independent colony founding to colony fission. In some species, this cost is limited because unmated ergatoids remain in the nest and adopt a 'worker' behaviour (Forder & Marsh, 1989; Heinze *et al.,* 1999; Molet *et al.,* 2007). Yet, larval development may still be constrained by non-genetic maternal effects, such as the amount of vitellogenin provided to the egg, and by the interaction between early determination and subsocial effects (Linksvayer, 2006; Villalta *et al.,* 2016a). Further comparative studies are needed to understand how all these complex factors ultimately generate different adult phenotypes.

Acknowledgements

References

Amor, F. & Ortega, P. (2014) *Cataglyphis tartessica* sp.n., a new ant species (Hymenoptera: Formicidae) in southwestern Spain. *Myrmecological News*, **19**, 125–132.

Amor, F., Ortega, P., Jowers, M.J., Cerdá, X., Billen, J., Lenoir, A. *et al.* (2011) The evolution of worker–queen polymorphism in *Cataglyphis* ants: interplay between individual-and colony-level selections. *Behavioral Ecology and Sociobiology*, **65**, 1473–1482.

Boomsma, J.J. & Ratnieks, F.L.W. (1996) Paternity in eusocial Hymenoptera. *Philosophical Transactions of the Royal Society of London B*, **351**, 947–975.

Boulay, R., Arnan, X., Cerdá, X. & Retana, J. (2014) The ecological benefits of larger colony size may promote polygyny in ants. *Journal of Evolutionary Biology*, **27**, 2856–2863.

Bourke, A.F. & Ratnieks, F.L. (1999) Kin conflict over caste determination in social Hymenoptera. *Behavioral Ecology and Sociobiology*, **46**, 287–297.

Brian, M.V. (1957) Food distribution and larval size in cultures of the ant *Myrmica rubra* (L.) *Physiologia Comparata et Oecologia*, **4**, 329–345.

Brian, M.V. (1973) Caste control through worker attack in the ant *Myrmica*. *Insectes Sociaux*, **20**, 87–102.

Caut, S., Jowers, M.J., Cerdá, X. & Boulay, R.R. (2013) Questioning the mutual benefits of myrmecochory: a stable isotope-based experimental approach. *Ecological Entomology*, **38**, 390–399.

Caut, S., Jowers, M.J., Arnan, X., Pearce-Duvet, J., Rodrigo, A., Cerdá, X. *et al.* (2014) The effects of fire on ant trophic assemblage and sex allocation. *Ecology and Evolution*, **4**, 35–49.

Cerdá, X., Retana, J., Bosch, J. & Alsina, A. (1989) Daily foraging activity and food collection of the thermophilic ant *Cataglyphis cursor* (Hymenoptera, Formicidae). *Vie et Milieu*, **39**, 207–212.

Cerdá, X., Retana, J., Carpintero, S. & Cros, S. (1996) An unusual ant diet: cataglyphis floricola feeding on petals. *Insectes Sociaux*, **43**, 101–104.

Chéron, B., Doums, C., Federici, P. & Monnin, T. (2009) Queen replacement in the monogynous ant Aphaenogaster senilis: supernumerary queens as life insurance. *Animal Behaviour*, **78**, 1317–1325.

Cronin, A.L., Molet, M., Doums, C., Monnin, T. & Peeters, C. (2013) Recurrent evolution of dependent colony foundation across eusocial insects. *Annual Review of Entomology*, **58**, 37–55.

Cronin, A.L., Chifflet-Belle, P., Fédérici, P. & Doums, C. (2015) High inter-colonial variation in worker nestmate relatedness and diverse social structure in a desert ant from Mongolia. *Insectes Sociaux*, **63**, 87–98.

Emery, C. (1896) Le polymorphisme des fourmis et la castration alimentaire. *Compte Rendu des Séances du Troisième Congrès Internationale de Zoologie*, Leyde, The Netherlands, pp. 395–410.

Eyer, P.-A., Leniaud, L., Darras, H. & Aron, S. (2013) Hybridogenesis through thelytokous parthenogenesis in two *Cataglyphis* desert ants. *Molecular Ecology*, **22**, 947–955.

Fersch, R., Buschinger, A. & Heinze, J. (2000) Queen polymorphism in the Australian ant *Monomorium* sp. 10. *Insectes Sociaux*, **47**, 280–284.

Forder, J.C. & Marsh, A.C. (1989) Social organization and reproduction in *Ocymyrmex foreli* (Formicidae: Myrmicinae). *Insectes Sociaux*, **36**, 106–115.

Heinze, J. (1989) Alternative dispersal strategies in a North American ant. *Naturwissenschaften*, **76**, 477–478.

Heinze, J., Hölldobler, B. & Alpert, G. (1999) Reproductive Conflict and Division of Labor in Eutetramorium mocquerysi a Myrmicine Ant Without Morphologically Distinct Female Reproductives. *Ethology*, **105**, 701–717.

Hughes, W.O. & Boomsma, J.J. (2008) Genetic royal cheats in leaf-cutting ant societies. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 5150–5153.

Hughes, W.O.H., Oldroyd, B.P., Beekman, M. & Ratnieks, F.L.W. (2008) Ancestral monogamy shows kin selection is key to the evolution of Eusociality. *Science*, **320**, 1213–1216.

Jowers, M.J., Leniaud, L., Cerdá, X., Alasaad, S., Caut, S., Amor, F. *et al.* (2013) Social and population structure in the ant *Cataglyphis emmae*. *PLoS One*, **8**, e72941.

Jowers, M.J., Amor, F., Ortega, P., Lenoir, A., Boulay, R.R., Cerdá, X. *et al.* (2014) Recent speciation and secondary contact in endemic ants. *Molecular Ecology*, **23**, 2529–2542.

Julian, G.E., Fewell, J.H., Gadau, J., Johnson, R.A. & Larrabee, D. (2002) Genetic determination of the queen caste in an ant hybrid zone. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 8157–8160.

Kamakura, M. (2011) Royalactin induces queen differentiation in honeybees. *Nature*, **473**, 478–483.

Leniaud, L., Darras, H., Boulay, R. & Aron, S. (2012) Social hybridogenesis in the clonal ant *Cataglyphis hispanica*. *Current Biology*, **22**, 1188–1193.

Leniaud, L., Pearcy, M. & Aron, S. (2013) Sociogenetic organisation of two desert ants. *Insectes Sociaux*, **60**, 337–344.

Leniaud, L., Pearcy, M., Taheri, A. & Aron, S. (2015) Testing the genetic determination of the soldier caste in the silver ant. *Insectes Sociaux*, **62**, 517–524.

Libbrecht, R., Corona, M., Wende, F., Azevedo, D.O., Serrão, J.E. & Keller, L. (2013) Interplay between insulin signaling, juvenile hormone, and vitellogenin regulates maternal effects on polyphenism in ants. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 11050–11055.

Linksvayer, T.V. (2006) Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. *Evolution*, **60**, 2552–2561.

Marchal, P. (1898) Le polymorphisme des fourmis et la castration alimentaire. *Année Biologique*, **2**, 249–251.

Michener, C.D. (1974) *The Social Behavior of the Bees: A Comparative Study*. Harvard University Press, Cambridge, Massachusetts.

Molet, M., Peeters, C., Follin, I. & Fisher, B.L. (2007) Reproductive caste performs intranidal tasks instead of workers in the ant *Mystrium oberthueri*. *Ethology*, **113**, 721–729.

Molet, M., Maicher, V. & Peeters, C. (2014) Bigger helpers in the ant *Cataglyphis bombycina*: increased worker polymorphism or novel soldier caste? *PLoS ONE*, **9**, e84929.

Monnin, T. & Ratnieks, F.L. (2001) Policing in queenless ponerine ants. *Behavioral Ecology and Sociobiology*, **50**, 97–108.

Nielsen, R., Tarpy, D.R. & Reeve, H.K. (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. *Molecular Ecology*, **12**, 3157–3164.

Oster, G.F. & Wilson, E.O. (1978) *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton, New Jersey.

Passera, L. (1980) La ponte d'œufs préorientés chez la fourmi *Pheidole pallidula* (Nyl.) (Hymenoptera-Formicidae). *Insectes Sociaux*, **27**, 79–95.

Pearcy, M., Clémencet, J., Chameron, S., Aron, S. & Doums, C. (2004a) Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*. *Molecular Ecology Notes*, **4**, 642–644.

Pearcy, M., Aron, S., Doums, C. & Keller, L. (2004b) Conditional use of sex and parthenogenesis for worker and queen production in ants. *Science*, **306**, 1780–1783.

Peeters, C. & Ito, F. (2001) Colony dispersal and the evolution of queen morphology in social Hymenoptera. *Annual Review of Entomology*, **46**, 601–630.

Peeters, C. & Molet, M. (2010) Colonial reproduction and life histories. *Ant Ecology* (ed. by L. Lach, C. Parr and K. Abbott), pp. 159–176. Oxford University Press, Oxford, U.K.

Peeters, C., Keller, R.A. & Johnson, R.A. (2012) Selection against aerial dispersal in ants: two non-flying queen phenotypes in *Pogonomyrmex laticeps*. *PLoS One*, **7**, e47727.

Penick, C.A. & Liebig, J. (2012) Regulation of queen development through worker aggression in a predatory ant. *Behavioral Ecology*, **23**, 992–998.

Peterson, B.J. & Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, **18**, 293–320.

Ratnieks, F.L. & Wenseleers, T. (2008) Altruism in insect societies and beyond: voluntary or enforced? *Trends in Ecology and Evolution*, **23**, 45–52.

Ratnieks, F.L., Foster, K.R. & Wenseleers, T. (2006) Conflict resolution in insect societies. *Annual Review of Entomology*, **51**, 581–608.

Schmidt, K., Hunt, B. & Smith, C. (2012) Queen, worker, and male yellowjacket wasps receive different nutrition during development. *Insectes Sociaux*, **59**, 289–295.

Schwander, T. & Keller, L. (2012) Sociality as a driver of unorthodox reproduction. *Current Biology*, **22**, R525–R527.

Schwander, T., Humbert, J.-Y., Brent, C.S., Helm Cahan, S., Chapuis, L., Renai, E. *et al.* (2008) Maternal effect on female caste determination in a social insect. *Current Biology*, **18**, 265–269.

Seeley, T.D. (2010) *Honeybee Democracy*. Princeton University Press, Princeton, New Jersey.

Smith, C.R. & Suarez, A.V. (2010) The trophic ecology of castes in harvester ant colonies. *Functional Ecology*, **24**, 122–130.

Smith, C.R., Anderson, K.E., Tillberg, C.V., Gadau, J. & Suarez, A.V. (2008) Caste determination in a polymorphic social insect: nutritional, social, and genetic factors. *The American Naturalist*, **172**, 497–507.

Tinaut, A. & Ruano, F. (1992) Braquipterismo y apterismo en formicidos. Morfologia y biometria en las hembras de especies ibericas de vida libre (Hymenoptera: Formicidae). *Graellsia*, **48**, 121–131.

Truett, G., Heeger, P., Mynatt, R., Truett, A., Walker, J. & Warman, M. (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques*, **29**, 52–54.

Villalta, I., Blight, O., Angulo, E., Cerdá, X. & Boulay, R. (2016a) Early developmental processes limit socially mediated phenotypic plasticity in an ant. *Behavioral Ecology and Sociobiology*, **70**, 285–291.

Villalta, I., Amor, F., Cerdá, X. & Boulay, R. (2016b) Social coercion of larval development in ant species. *The Science of Nature*, **103**, 18.

Weir, B.S. & Cockerham, W.W. (1984) Estimating F-Statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wehner, R., Marsh, A. & Wehner, S. (1992) Desert ants on a thermal tightrope. *Nature*, **357**, 586–587.

Wenseleers, T., Hart, A.G. & Ratnieks, F.L. (2004) When resistance is useless: policing and the evolution of reproductive acquiescence in insect societies. *The American Naturalist*, **164**, 154–167.

Wenseleers, T., Ratnieks, F.L., Ribeiro, M., Alves, D. & Imperatriz-Fonseca, V.L. (2005) Working-class royalty: bees beat the caste system. *Biology Letters*, **1**, 125–128.

Wheeler, D.E. (1986) Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *The American Naturalist*, **128**, 13–34.

Wilson, E.O. (1971) *The Insect Societies*. Harvard University Press, Cambridge, Massachusetts.