Insectes Sociaux
https://doi.org/10.1007/s00040-019-00697-w

Insectes Sociaux

RESEARCH ARTICLE

Population structure and sociogenetic organisation in a species with ergatoid queens, the desert ant *Ocymyrmex robustior*

N. Lecocq de Pletincx[1] · A. Kuhn[1] · S. Aron[1]

Received: 28 December 2018 / Revised: 13 March 2019 / Accepted: 27 March 2019

Abstract
In ants, reproductive division of labour is typically associated with queen-worker dimorphism. In some species with ergatoid queens (wingless worker-like queens), this polymorphism is drastically reduced and virgin queens may integrate the worker force. While ergatoid queens have been described in several species, their colony and population genetic structure remain largely unstudied. Here, we investigated the population structure and sociogenetic organisation of the desert ant *Ocymyrmex robustior*. All *Ocymyrmex* species have only ergatoid queens that are worker-sized. Workers, queens, and males from a large population were genotyped at ten polymorphic microsatellite loci. Our results show that the study population is genetically structured, consistent with dependent colony foundation. Genetic analyses revealed that 17.6% of the males were diploid; diploid males are fertile, siring triploid females. Nests were typically headed by a single queen, and queens were strictly monandrous. However, several nests in the population shared matrilines, indicating polygyny, polydomy, dependent colony foundation, serial polygyny, or a combination of these processes. Dissections reveal that workers lay eggs in both queenright and queenless nests, while virgin ergatoid queens lay eggs in queenright nests only. However, our genetic analyses show that male offspring in queenright nests are all queen-produced, suggesting worker policing and/or trophic egg laying.

Keywords Population genetics · Colony structure · Ergatoid queens · Worker reproduction · *Ocymyrmex*

Introduction

The major organising principle of social insect colonies is reproductive division of labour, whereby one or a few individuals (the queens) specialize in reproduction, whereas the others (the workers) forego their own reproductive effort to participate in cooperative tasks (e.g., building the nest, collecting food, rearing the young, and defending the colony) (Hölldobler and Wilson 1990). Lifetime monogamy is recognized as an essential condition for the evolution of a sterile worker caste (Boomsma 2007, 2009, 2013). This is because monogamy is associated with a fixed average relatedness among siblings ($r=0.5$), so that the sibling/offspring relatedness ratio is equal to 1. In this situation, inclusive fitness theory (Hamilton 1964, 1972) predicts that a slight cost–benefit advantage ($c/b>1$) suffices to create an evolutionary transition to irreversible social breeding (Boomsma 2007). Consistent with the monogamy hypothesis, monogyny (one queen per colony) and monandry (one mating per queen) likely constitute the ancestral reproductive systems in social insects (Thorne 1997; Boomsma 2009; Hughes et al. 2008a).

In ants, a great diversity of mating systems and social structures evolved secondarily. Both polygyny (co-occurrence of several reproductive queens) and polyandry (multiple mating by queens) have been documented in a large number of species (e.g., Keller 1993; Bourke and Franks 1995; Crozier and Pamilo 1996; Heinze and Keller 2000; Crozier and Fjerdingstad 2001; Hughes et al. 2008a, b). The evolution of polygyny and polyandry involves multiple interacting selective pressures. Polygyny could be selected by ecological constraints responsible for a weak rate of success of dispersion under independent foundation (Herbers 1986; Bourke and Heinze 1994; Keller 1995; Foitzik and Heinze 1998; McGlynn 2010). It may also have been favoured

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00040-019-00697-w) contains supplementary material, which is available to authorized users.

✉ N. Lecocq de Pletincx
nlecocqd@ulb.ac.be

[1] Evolutionary Biology and Ecology, Université libre de Bruxelles, 50, CP 160/12 avenue FD Roosevelt, 1050 Brussels, Belgium

Published online: 08 April 2019

thanks to the benefits of an increase in long-term colony survival (Nonacs 1988; Keller 1995), intra-colonial genetic diversity (Crozier and Page 1985; Schmid-Hempel and Crozier 1999), and group productivity (Kokko et al. 2001). Similarly, several hypotheses have been proposed to explain the evolution of polyandry in Hymenoptera; they stress the benefits of increased genetic diversity in the offspring and of receiving a sufficient sperm reserve (Crozier and Page 1985; Crozier and Fjerdingstad 2001; Strassmann 2001; Brown and Schmid-Hempel 2003; Boomsma et al. 2009). In support to the genetic diversity hypothesis, experimental studies have shown that multiple mating and multiple colony queens enhance resistance to pathogens, and raise the efficiency of the colony and its overall productivity (Baer and Schmid-Hempel 1999; Brown and Schmid-Hempel 2003; Julian and Fewell 2004; Mattila and Seeley 2007).

In the Formicidae, reproductive division of labour is typically associated with dramatic morphological differences between queens and workers. In a number of species, however, this polymorphism is weak or even absent. A striking example concerns ergatoid queens, i.e., wingless worker-like queens. Ergatoid queens evolved several times independently in lineages characterized by winged queens (Peeters 2012); they have been documented in 55 genera belonging to 15 of the 21 ant subfamilies. In some species, ergatoid queens co-exist with large winged queens or brachypterous queens (e.g., *Cataglyphis tartessica*, Amor et al. 2016; *Hypoponera opacior*, Foitzik et al. 2010). In the ant genera *Eutetramorium, Proceratium*, and *Ocymyrmex*, ergatoid queens and workers are similar in size. They can be differentiated based on their physiology, with queens having a functional spermatheca and a larger number of ovaries than workers (Peeters 2012; but see Ravary and Jaisson 2004). Ergatoid queens being wingless, they probably mate in or close to their natal nest and, together with nestmate workers, disperse on foot to found a new colony nearby (dependent colony foundation; Peeters 2012; Cronin et al. 2013).

Whereas a substantial number of ant species are characterized by ergatoid queens, their population structure and sociogenetic organisation remain unstudied. Here, we investigated the mating system and the genetic structure of a large population of the ant *Ocymyrmex robustior* in western Namibia. The thermophilic ant genus *Ocymyrmex* is restricted to savannah and deserts of the southern part of the Afrotropical realm and is composed of 37 morphologically described species (Marsh 1985a, b; Bolton and Marsh 1989). In all *Ocymyrmex* species studied so far, winged queens have totally disappeared and were replaced by ergatoid queens that are similar in size to workers (Bolton 1981; Forder and Marsh 1989; Bolton and Marsh 1989). In *O. foreli, O. sphinx, O. picardi*, and *O. flaviventris*, ergatoid queens represent up to 20% of the females in a colony; however, only a single ergatoid queen is inseminated and reproductively active (Forder and Marsh 1989). Mating takes place at the entrance of the mother nest, ergatoid queens probably attracting males by sexual pheromones, and new nests are founded by dependent nest foundation (Bolton and Marsh 1989). Moreover, workers of *Ocymyrmex* retain ovaries (Forder and Marsh 1989). Whether unmated, ergatoid queens and workers contribute to reproduction is nevertheless unknown.

First, we developed a genomic library of microsatellite markers and used 10 polymorphic loci to detail the genetic structure of the nests and the population. We estimated inbreeding and genetic differentiation between nests. We also inferred the dispersal strategy by testing the relationship between genetic differentiation and geographical distance between nests. Second, by combining genetic analyses and morphological observations, we determined the number of reproductive queens per nest and their mating frequency. Third, we investigated whether virgin ergatoid queens (VEQs) and workers do reproduce in queenright and queenless nests of *O. robustior*. To our knowledge, this is the first study to provide molecular insights into the sociogenetic organisation of desert ants of the genus *Ocymyrmex* and, more generally, in a species with only ergatoid queens.

Materials and methods

Field collection and sampling

Nests of *O. robustior* inhabit gravel plains and sand dunes of Namibia and South Africa. Ergatoid queens, workers, and winged males are produced all year long (Forder and Marsh 1989). Fifty-six nests from a single population were excavated in April 2017, in the National Park of Namib-Naukluft (Namib desert, Namibia; Fig. 1; Table 1). All adults (males, workers, and queens) and brood at various stages of development (eggs, larvae, and pupae) were collected. Males (when produced) and all individuals from 34 nests were directly stored in 98% ethanol for subsequent analyses. The 22 remaining nests were maintained under laboratory conditions (26 ± 2 °C and 12h:12 h light/dark photoperiod) and fed with sugar water and cockroaches. Nests were surveyed twice a week and emerging adults were collected and stored at − 20 °C in 98% ethanol.

The numbers of workers and ergatoid queens per nest ($N = 20$), and the proportion of ergatoid queens in each nest ($N = 15$) were counted immediately after samples were brought back to the laboratory. The caste of females (worker or ergatoid queen) was determined on the basis of the cephalic structure (Bolton 1981; Fig. 2) and, when necessary, confirmed by ovarian dissection.

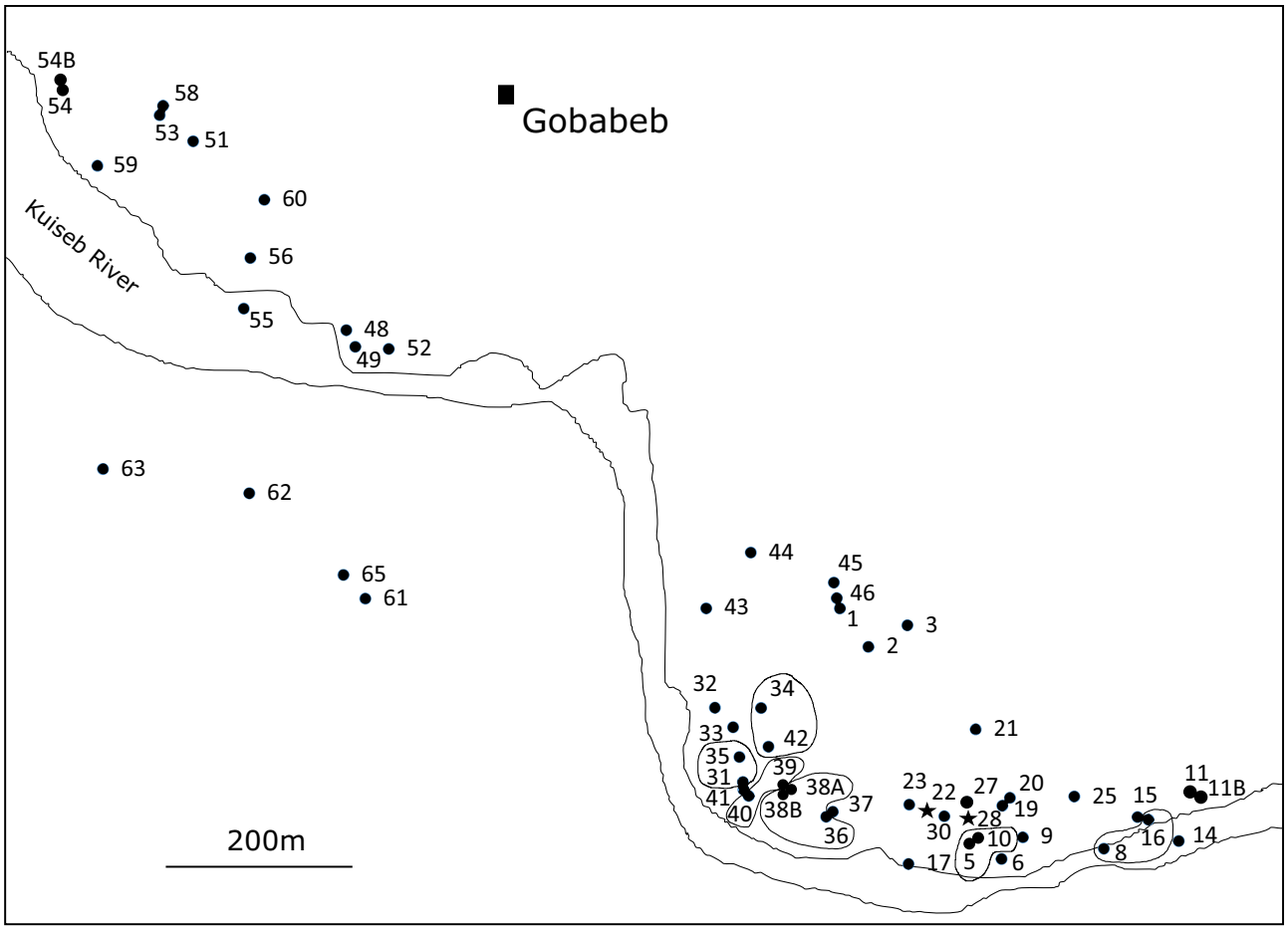

Fig. 1 Map of *O. robustior* sampled nest localities in the Namib desert (Namibia). Fifty-six nests were sampled at Gobabeb (23°33′42″S; 15°2′28″E) along the Kuiseb River. Clusters of nests sharing matriline lineages are delimited (5–10, 8–16, 31–35, 34–42, 39–40, and 36–38A–38B). The two nests represented by a star, 22 (only the queen was genotyped) and 28 (producing triploid workers only), were excluded from the population genetic analyses

Genetic analyses

Microsatellite markers and genotyping

For genomic library development, the total genomic DNA was extracted from a pool of nine individuals using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocol. Non-enriched genomic libraries were constructed (Rohland and Reich 2012; Mariac et al. 2014) and the DNA was sequenced using Illumina MiSeq (GIGA platform, Liège, Belgium). The resulting paired-end reads were aligned using PANDAseq v2.10 (Masella et al. 2012), providing 549,856 reads. Microsatellite motifs were identified using QDD v3.1 (Meglécz et al. 2014). We obtained 5,904 (1.1%) sequences containing at least one nSSR motif. We selected 48 candidate loci using the following criteria: (1) a minimum 20-pb distance between the primer and the nSSR motif; (2) a minimum of 9 nSSR repetitions; (3) only pure microsatellite (a single motif present in the fragment); (4) a weak alignment score (i.e., weak complementarity) between the primers and the amplicon; and (5) expected PCR product size between 100 and 300 pb.

For genotyping, DNA was extracted using the Chelex extraction process (Bio-Rad®, Hercules, CA, USA; Walsh et al. 1991). For adults and pupae, 2 legs were collected, while large larvae were clipped in three parts and the middle part was used. Samples were ground 1 min at 20 Hz in 100 µl of 5% Chelex and then incubated 1h30 at 85 °C. After 3 min of centrifugation at 12,000 rpm, 40 µl of the supernatant were collected into a 1.5 ml tube. Small larvae and eggs were fully extracted in 20 µl of 5% Chelex. To test microsatellite amplification and polymorphism, a fluorochrome was assigned to each microsatellite marker following a three primers PCR approach (Blacket et al. 2012). The 48 markers were then tested on seven *O. robustior* workers (each from a different nest) in standard simplex PCR conducted using *MyTaq DNA polymerase* (Bioline) following

Table 1 Nest size, number of ergatoid queens, queen mating frequency, and relatedness values in *O. robustior* nests

	Nest	Size	NE (proportion)	Genetic analyses									
				E	W	M	B	R	L	NML	MF	r^*_{n-n}	r^*_{q-q}
Field	1	–	–	5	9	–	–	–	–	1	1	0.93	–
	2	–	–	5	10	–	–	–	–	2	1	0.39	0.35
	3	–	–	5	10	1	–	–	–	2	1	0.26	0.06
	6	–	–	5	10	3	–	–	–	1	1	0.73	–
	10	–	–	5	10	–	–	–	–	2	1	0.67	0.86
	11	–	–	5	10	5	–	–	–	3	1	0.75	NA
	14	–	–	4	11	–	–	–	–	1	1	0.79	–
	15	–	–	5	10	1	–	–	–	1	1	0.80	–
	16	–	–	5	10	–	–	–	–	1	1	0.76	–
	17	–	–	4	11	4	–	–	–	3	1	0.12	0.03
	21	–	–	5	10	5	–	–	–	1	1	0.70	–
	30	–	–	5	10	–	–	–	–	1	1	0.60	–
	31	–	–	5	10	5	–	–	–	4	1	0.26	− 0.09
	32	–	–	7	15	–	–	–	–	1	1	0.75	–
	34	–	–	6	10	–	–	–	–	3	1	0.50	0.18
	36	–	–	5	10	1	–	–	–	1	1	0.59	–
	38B	–	–	5	10	3	–	–	–	1	1	0.56	–
	39	–	–	5	10	–	–	–	–	2	1	0.26	− 0.18
	40	–	–	5	10	6	–	–	–	2	1	0.34	− 0.18
	43	–	–	5	10	–	–	–	–	1	1	0.66	–
	44	–	–	5	10	2	–	–	–	1	1	0.76	–
	45	–	–	5	10	–	–	–	–	1	1	0.76	–
	46	–	–	3	11	1	–	–	–	1	1	0.68	–
	48	–	–	5	10	1	–	–	–	1	1	0.55	–
	49	–	–	2	10	4	–	–	–	1	1	0.81	–
	53	–	–	5	10	5	–	–	–	1	1	0.55	–
	54	–	–	4	10	4	–	–	–	1	1	0.70	–
	54B	–	–	2	13	10	–	–	–	1	1	0.82	–
	55	–	–	5	10	2	–	–	–	1	1	0.63	–
	56	–	–	4	10	2	–	–	–	1	1	0.80	–
	58	–	–	5	10	–	–	–	–	1	1	0.75	–
	61	–	–	5	10	–	–	–	–	1	1	0.74	–
	62	–	–	5	10	4	–	–	–	3	1	0.44	0.28
	63	–	–	5	10	2	–	–	–	1	1	0.63	–

Table 1 (continued)

	Nest	Size	NE (proportion)	Genetic analyses									
				E	W	M	B	R	L	NML	MF	r^*_{n-n}	r^*_{q-q}
Laboratory	5	272	71 (0.26)	7	14	6	5	–	5	1	1	0.85	–
	8*	100	11 (0.11)	3	4	5	–	14	–	1(1)	1(1)	0.78	–
	9*	219	24 (0.11)	5	5	15	5	–	–	2(1)	1(1)	0.49	0.63
	11B	–	–	1	14	3	–	–	–	1	1	0.71	–
	19*	166	28 (0.17)	6	27	–	5	–	13	1(1)	1(1)	0.75	–
	20*	416	58 (0.14)	1	10	–	–	15	–	1(1)	1(1)	0.71	–
	22*	–	–	–	–	–	–	–	–	1(1)	1(1)	NA	–
	23*	195	49 (0.25)	2	5	–	5	10	–	1(1)	1(1)	0.73	–
	25	177	–	3	9	–	–	–	11	1	1	0.83	–
	27*	382	53 (0.14)	5	10	10	5	10	–	1(1)	1(1)	0.71	–
	28*	242	22 (0.09)	1	10	1	5	–	–	1(1)	1(1)	NA	–
	33	529	–	–	–	–	5	–	15	1	1	0.69	–
	35*	396	0 (0)	2	10	–	5	10	–	1(1)	1(1)	0.67	–
	37*	474	43 (0.09)	5	10	–	5	10	–	1(1)	1(1)	0.87	–
	38A	292	41 (0.14)	5	9	–	–	–	13	1	1	0.55	–
	41*	281	39 (0.14)	4	8	12	–	15	–	1(1)	1(1)	0.78	–
	42*	630	145 (0.23)	–	18	1	–	15	–	1(1)	1(1)	0.68	–
	51	346	48 (0.14)	4	8	–	–	–	–	1	1	0.59	–
	52*	626	106 (0.17)	3	12	–	5	13	–	1(1)	1(1)	0.80	–
	59	678	–	4	10	3	5	–	–	1	1	0.73	–
	60	148	–	2	12	–	–	–	11	1	1	0.85	–
	65	382	–	5	10	–	–	–	–	1	1	0.69	–
	Mean	347.6	49.2 (0.15)	4.32	10.46	4.23	5.31	12.44	11.33	1.37	1	0.66	0.20
	SD	170.5	36.7 (0.07)	1.40	3.12	3.48	0.48	2.40	3.44	0.76	0	0.17	0.34

The total number of females per nests (size) and the number of ergatoid queens (NE) are given, when available, for nests maintained under laboratory conditions. The number of ergatoid queens (E), workers (W), males (M), and brood individuals (B) genotyped is provided for each nest. The number of individuals produced and genotyped in queenright (R) and in queenless (L) nests under laboratory conditions is supplied. The number of maternal lineages per nest (NML) inferred from the genotype of field and lab (in parentheses) workers and VEQs as well as mating frequency (MF) inferred from field and lab (under laboratory parent-offspring combination and sperm genotyping; in parentheses) are given. The relatedness among nestmates (r^*_{n-n}) and among queens from a single nest (r^*_{q-q}) is provided. Field and laboratory maintained nests are separated. Nests found queenright are marked with an asterisk

the manufacturer's protocol in a 12.5 µl volume and a 58 °C annealing temperature. Finally, 12 polymorphic microsatellite loci were retained for subsequent genetic analyses (Table S1). We designed two multiplex of six markers each using the Multiplex Manager v1.0 program (Holleley and Geerts 2009; Table S1). The two 6-plex PCR reactions were carried out using a *QIAGEN Type-it Microsatellite PCR kit* (10 µl reactions following manufacturer's protocol with a 58 °C annealing temperature). All reactions were conducted using the VWR Thermal Duo-Cycler (VWR, Berntsen, Denmark).

Linkage disequilibrium and Hardy–Weinberg equilibrium

From the 56 nests sampled, two were excluded from our population genetic analyses: nest 28, in which only triploid workers were produced, and nest 22 for which only the queen and its spermathecal contents were genotyped (see "Results"). We first tested whether allelic frequencies differed between ergatoid queens and workers from the field ($N = 54$ nests). To this aim, we compared allelic frequencies at each locus with a *G* test using GENEPOP v4.7.0 (Rousset 2008); a global test across all loci was then carried out using Fisher's method. Allelic frequencies did not differ between the female castes, either when considering each individual locus [range of *P* values: (0.19–0.97)] or for the global test (*G* test, Fisher's method, $P = 0.82$). Hence, the two castes were treated equally in the following analyses.

Second, we tested linkage disequilibrium (LD) and deviation from Hardy–Weinberg equilibrium (HWE) across the 54 nests using GENEPOP v4.7.0 (Rousset 2008). Because some nests contained several matrilines (i.e., lineages of offspring from different mothers; see "Results"), the composite LD test (Weir 1996) was conducted separating matrilines

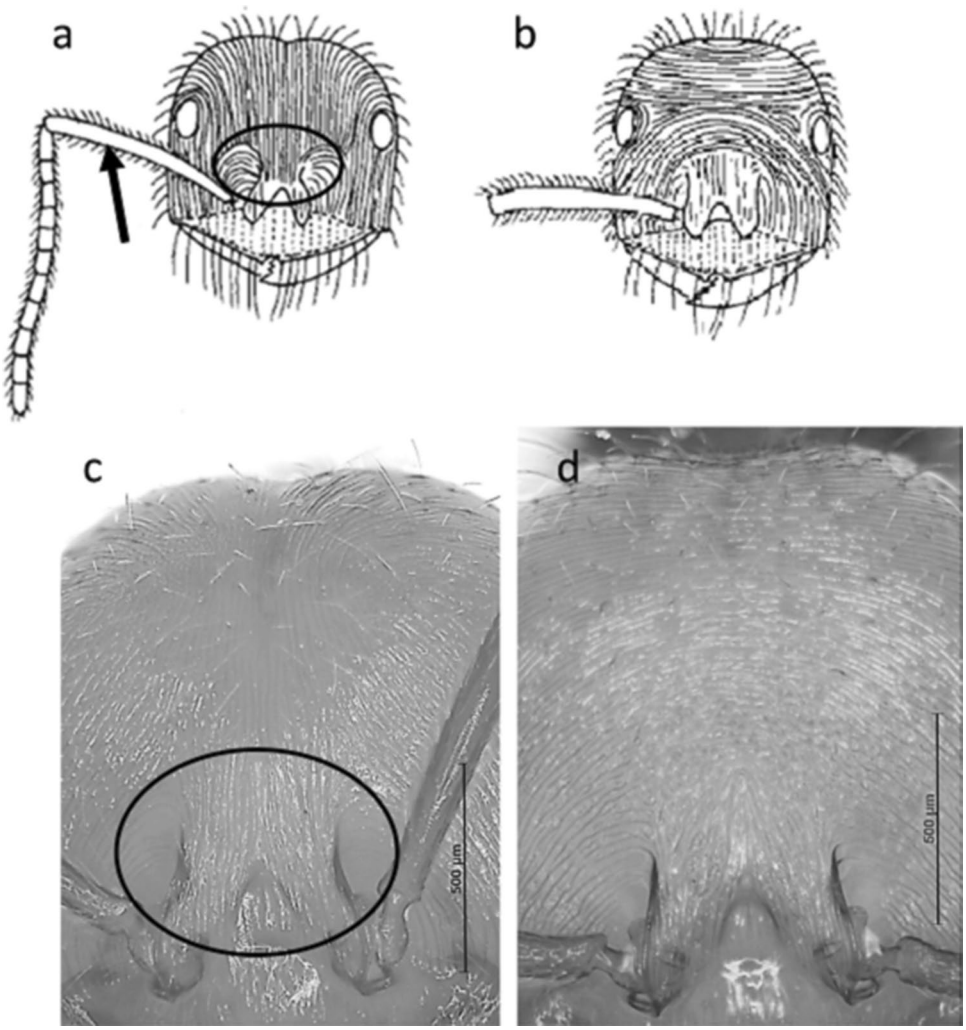

Fig. 2 Illustrations and pictures depicting cuticular sculpturing on the head of *O. robustior* workers (**a, c**) and ergatoid queens (**b, d**). Cuticular sculpturing is vertical for workers and horizontal for ergatoid queens, the proximal part of the frontal lobes (circle) is narrower on his proximal part for workers, and the antennal scape (arrow) is thinner and longer for workers. Drawings modified from Bolton and Marsh (1989)

($N=67$) instead of nests ($N=54$). In several instances, different nests shared the same matriline(s); this resulted in clusters of nests with the same maternal lineage(s) (see Results). Because multiple genotypes from one matriline but also from clusters of nests are not independent, a resampling procedure was performed to test for deviations from HWE (Vargo 2003): a single individual per nest (in case of cluster of nests, one individual per cluster) was selected at random; the procedure was repeated 20 times ($N=47$ individuals per replicate). Two tests were applied on each replicate: the exact test of HWE (Weir 1996) and the *U* test for heterozygote deficiency (Raymond and Rousset 1995). For each, the 20 *P* values were combined using a modified Lancaster procedure (generalisation of the Fisher's method) to deal with the dependency between the replicates (Dai et al. 2014). Control for genotyping errors due to null alleles and allele dropouts was performed using the *Expectation–Maximization* (*EM*) algorithm implemented in INEst (Chybicki and Burczyk 2008), using the 20 replicates generated to test for HWE. This approach allows to estimate null allele frequencies accurately in a population experiencing inbreeding, which is the case in our study.

Population genetic structure

The number of alleles per locus, allelic frequencies, observed heterozygosity (H_O), and expected heterozygosity (H_E) was estimated based on the 20 replicates (i.e., 47 individuals × 20 replicates) generated to test for HWE, using SPaGeDi v1.5.a (Hardy and Vekemans 2002). Individuals in ant colonies do not represent independent samples, because they are related. Therefore, the F_{IT} coefficient was calculated using the 20 replicates generated to test for HWE and its significance was estimated by 1 000 permutations of the alleles between individuals using SPaGeDi v1.5.a. The mean F_{IT} accounting for all 10 loci was finally calculated and the *P* values were combined through a modified Lancaster procedure (Dai et al. 2014).

To test whether different nests correspond to different colonies, the genetic differentiation between

nests (mean number of individuals genotyped per nest ± SD = 15.94 ± 4.68, $N = 54$ nests) was estimated using F_{ST} coefficients calculated through a three-level hierarchical F-analysis of variance (alleles within individuals, individuals within nests, and nests within population) implemented in SPaGeDi v1.5.a. The standard error was obtained by jackknifing over all loci and the significance was estimated through 1 000 permutations of the individuals between nests.

Isolation-by-distance was investigated by plotting the coefficients $[F_{ST}/(1 - F_{ST})]$ between pairs of nests against the logarithm (ln) of geographical distances (Rousset 1997). The significance of the regression slope was estimated by 1 000 permutations of the locations of the nests using SPaGeDi v1.5.a.

Relatedness coefficients among nestmates (r_{n-n}), queens and their mate (r_{q-m}), nestmate queens (r_{q-q}), and laboratory-reared offspring (r_{o-o}) were estimated using the algorithm of Queller and Goodnight (1989) implemented in the program RELATEDNESS (version 5.0.8). Nests were weighted equally. The inbreeding corrected coefficient of relatedness r^* was calculated according to the equation of Pamilo (1985):

$$r^* = \left[\mathrm{r} - 2F_{IT}/\left(1 + F_{IT}\right)\right]/\left[1 - 2F_{IT}/\left(1 + F_{IT}\right)\right].$$

Queen number and mating frequency

Nest queen number

The number of reproductive queen(s) per nest was determined from both laboratory and field data. A total of 89 ergatoid queens from 18 laboratory nests were dissected (mean number of ergatoid queens per nest ± SD = 4.94 ± 3.99), and their ovarian activity (see below) as well as the status of the spermatheca (empty or with sperm) were examined. Moreover, the minimum number of queen(s) per nest was inferred from the pedigree of field individuals ($N = 54$ nests), assuming that queens are single-mated (see "Results"). Individuals were assigned to different matrilines if they did not share an allele for at least one locus. Assignment of individuals to different matrilines was confirmed with the maximum-likelihood method implemented in the program COLONY v2.0.6.4 (Jones and Wang 2010). When a reproductive queen was not sampled, its genotype was inferred from that of nestmates workers and VEQs.

Queen mating frequency

Queen mating frequency was estimated based on mother-offspring genetic combinations under laboratory conditions, as well as on the genotype of the sperm stored in the queen spermatheca ($N = 13$). The absolute number of matings per queen (M_P), i.e., the minimum number of males inferred from the genotype of the offspring produced, was determined. This was easily achieved, as our laboratory nests were headed by a single reproductive queen. Then, the 13 queens were dissected and the sperm was collected. Sperm DNA was extracted using the Chelex extraction procedure (Kuhn et al. 2017) and genotyped at all 10 loci. We calculated the consanguinity corrected probability of two males bearing the same allele at each locus by modifying the equations of Pamilo (1993) and Boomsma and Ratnieks (1996):

$$d = \prod_j \left(F_{IT} + \left(1 - F_{IT}\right) \times \sum_i {p_{ij}}^2 \right),$$

where p_{ij} is the population frequency of allele i at the locus j.

Workers and virgin ergatoid queens' reproduction

Workers having retained ovaries, they can potentially contribute to reproduction by laying haploid eggs that result in males. We examined whether virgin females (workers and VEQs) do reproduce in queenright and queenless nests in two ways: (1) by examining ovary development and (2) by genetic analyses aimed at determining the maternity of males.

Queenright nests

To assess ovarian activity, 176 workers (mean ± SD = 22 ± 9.84) and 45 VEQs (mean ± SD = 5.63 ± 5.13) from 8 male-producing nests were dissected. Their ovarian activity was characterized according to the following scale of development: (1) reduced ovarioles; (2) expanded ovarioles with at least one oocyte; and (3) expanded ovarioles and yellow bodies.

For genetic analyses, 142 males from 31 nests were genotyped (mean ± SD = 4.58 ± 3.63): 90 collected from the field ($N = 24$ nests) and 52 produced under lab conditions ($N = 7$ nests). Their genotypes were compared to those of the queen(s) and workers. Sons of queens must carry a queen-derived allele at all loci, and as a group, they should not display more than two alleles at a single locus. Sons of virgin females may carry with equal probability an allele derived from either the mother or the father of the workers and VEQs. Any male that carries a non-queen allele is the son of a worker or of a VEQ. However, because such sons may carry queen alleles at all loci by chance, the probability of detecting a male produced by a worker or a VEQ was estimated following Foster et al. (2001) as

$$P = \sum_1^n p_i \times \left(1 - 0,5^{l_i}\right),$$

where n is the number of patrilines in the nest, p_i is the proportional contribution of the ith father to the brood, and l_i is the number of informative loci analysed at the ith patriline.

An informative locus is one, where the queen and her mate have different alleles, so that the workers carry an allele that the queen does not.

Queenless nests

To assess ovarian activity, 132 workers (mean ± SD = 22 ± 3.69) and 13 VEQs (mean ± SD = 2.17 ± 1.94) from 6 orphaned nests (5 queenless nests from the field and 1 nest experimentally orphaned in the lab) were dissected, and their ovarian activity was characterized as described above.

For genetic analyses, 68 eggs from 6 orphaned nests were genotyped (mean ± SD = 11.33 ± 3.44). The genotypes of the eggs were compared to those of the queen and its sexual partner (inferred from spermathecal content), as well as to the genotypes of the workers and the VEQs of the nest.

Results

In *O. robustior*, the mean number of workers plus ergatoid queens per nest ± SD was 347.6 ± 170.5 ($N = 20$). The proportion of ergatoid queens per nest ranged from 0 to 26%, averaging 14.5%.

Population genetic structure

A significant linkage disequilibrium was found for 3 pairs of microsatellite loci; two were, therefore, excluded from the genetic analyses, leaving us with 10 polymorphic markers (Table S1). These markers showed no null alleles. The exact test of HW indicated a significant departure from HWE for 2 markers (Or19 and Or38). Furthermore, the *U* test revealed significant heterozygote deficiency for five markers (Or02, Or10, Or19, Or 22, and Or38). The number of alleles per marker ranged from 3 to 16, with a multi-locus mean observed heterozygosity $H_O = 0.64$ (range 0.47–0.88) and a multi-locus mean expected heterozygosity $H_E = 0.67$ (range 0.45–0.89). The inbreeding coefficient F_{IT} was low though significantly different from 0 in the study population ($F_{IT} = 0.038 \pm 0.019$; permutation test, $P = 0.0003$). Inbreeding was also supported by the weak but significant relatedness between the queens and their mates $r_{q-m} = 0.087$ ($SE_{Jackknife} = 0.049$; 95% IC: − 0.012–0.186; student *t* test: $t = 3.19$, $df = 63$, $P = 0.002$).

We observed a high level of genetic differentiation between nests ($F_{ST} = 0.36 \pm 0.01$; permutation test, $P = 0$). However, both pedigree analyses and the maximum-likelihood method implemented in the software COLONY indicated that some nests shared matrilines. The nests sharing the same matrilines were grouped in clusters. Especially, we identified 6 clusters of nests: 5 clusters made of 2 nests (5–10, 8–16, 31–35, 34–42, 39–40) and one cluster of 3 nests (36-38A-38B) (Fig. 1; Table 2). The mean geographic distance ± SD between the nests belonging to a given cluster was 37.46 ± 22.74 m (ranges 1.65–61.49), consistent with the distances of relocation or multiplication by dependent nest foundation previously reported for the species by Bolton and Marsh (1989).

Table 2 Clusters of nests

Cluster	Nests	Geographic distance (m)	NML	NMLe	r^*_{n-n}	r^*_{q-q}
5–10	5	6.69	1	2	0.72	0.86
	10		2			
8–16	8	61.49	1	1	0.75	NA
	16		1			
31–35	31	28.66	4	4	0.41	− 0.09
	35		1			
34–42	34	44.98	2	2	0.48	0.18
	42		1			
36–38A–38B	36	53.77	1	1	0.59	NA
	38A		1			
	36	55.33	1			
	38B		1			
	38A	1.65	1			
	38B		1			
39–40	39	47.14	2	2	0.29	− 0.18
	40		2			

For each cluster of nests, the geographic distance (meters) between nests of a cluster, the number of maternal lineages within each nest (NML) and each cluster of nests (NMLe), the mean relatedness among nestmates (r^*_{n-n}) and among queens (r^*_{q-q}) of a cluster are given

There was low but significant genetic structure of the population among nests (isolation-by-distance; linear regression: slope ± $SE_{Jackknife}$ = 0.035 ± 0.009; permutation test, $P = 0.004$; $N = 54$ nests).

The average genetic relatedness among nestmates (workers + VEQs) was $r_{n-n} = 0.68$ ($SE_{Jackkniffe}$ = 0.02; 95% IC: 0.63–0.73; $N = 54$ nests) and the consanguinity corrected relatedness was $r^*_{n-n} = 0.66$, significantly different from 0.75 expected under monogyny and monandry (Student t test: $t = -3.95$, $df = 53$, $P = 0$). This indicates the occurrence of multiple mating and/or multiple matrilines in a proportion of nests of *O. robustior*.

Queen number and mating frequency

Nest queen number

We dissected all ergatoid queens from 18 nests freshly excavated; 13 contained a single inseminated ergatoid queen, while the remaining 5 nests held only VEQs. Reproductive queens were characterized by an engorged spermatheca, well-developed ovarioles and yellow bodies. In contrast, VEQs showed an empty spermatheca, reduced ovarioles and no yellow bodies.

We also inferred the number and the genotype of reproductive queen(s) from each nest based on the genotype of field collected individuals (workers and VEQs). In 43 out of 54 nests (79.6%), the genotype of the workers and VEQs indicated that they were produced by a single, once-mated queen. In the 11 remaining nests (20.4%), workers and VEQs could not be assigned to a single matriline (assuming that queens are single-mated); the number of matrilines per nest ranged from 2 to 4 (mean number of matrilines per nest ± SD = 2.55 ± 0.69). The mean relatedness among (inferred) reproductive queens from a single nest was $r_{q-q} = 0.31$ ($SE_{Jackniffe}$ = 0.10; 95% IC: 0.10–0.52), while the consanguinity corrected relatedness was $r^*_{q-q} = 0.25$ (range 0.0–0.86). The proportion of workers from each matriline in the 11 nests ranged from 0.04 to 0.9 (Table S2). Relatedness among nestmates was $r_{n-n} = 0.49$ ($SE_{Jackkniffe}$ = 0.06; 95% IC: 0.36–0.62) and the consanguinity corrected relatedness was $r^*_{n-n} = 0.45$ (range 0.12–0.93). We also genotyped the brood from 5 of the 11 multi-matriline nests; in 1 nest, a single matriline was detected, while in 4 nests, several matrilines were identified in the brood. For the 5 nests, the matriline(s) found in the brood was always shared with workers and VEQs of the same nest.

These results show that nests of *O. robustior* are typically headed by a single queen, but that several potentially unrelated matrilines can co-exist in about 20% of the nests.

Queen mating frequency

The 13 inseminated ergatoid queens (see above), their seminal fluid, and 97 offspring (mean number of offspring per queen ± SD = 9.33 ± 5.99) were genotyped. Mother-offspring genetic combination analyses were consistent with all queens being mated once; hence, the absolute number of matings per queen $M_P = 1$. Consistent with this result, sperm typing showed a single allele per locus for 12 queens. In one nest, the queen carried sperm with 2 alleles for 5 loci. All her offspring (workers and VEQs) were triploid; their genotype at each locus was compatible with those of the queen and the sperm. Thus, this queen was mated with a diploid male-producing diploid sperm and fathering triploid offspring. The alternative hypothesis that two males bore the same alleles at each locus in the population studied was $d = 1.76E{-}05$. The mean relatedness among laboratory-reared offspring was $r_{o-o} = 0.74$ ($SE_{Jackkniffe}$ = 0.02; 95% IC: 0.72–0.76) and the consanguinity corrected relatedness was $r^*_{o-o} = 0.71$, a value not different from 0.75 (Student t test: $t = -1.19$, $df = 7$, $P = 0.274$). Overall, these results demonstrate that queens of *O. robustior* are strictly singly mated.

Workers and virgin ergatoid queens' reproduction

Queenright nests

Dissections showed that in the 8 queenright nests producing males, workers were characterized by ovarian development of types 1–3. Among these, 28.4% had ovaries of type 2 (expanded ovarioles with at least one oocyte) and 5.4% had ovaries of type 3 (expanded ovarioles and yellow bodies). In contrast, all VEQs ($N = 45$) had type 1 (reduced) ovaries, suggesting that they were reproductively inactive in the presence of a mated queen.

All 52 males produced under laboratory conditions ($N = 7$ nests) and 90 males collected on the field ($N = 24$ nests) carried only queen-derived alleles. The average detection probability of a male being produced by a worker or a VEQ was 0.92 (SD = 0.12). From the 142 males genotyped, 25 (17.6%) were diploid. Diploid males were found in 5 nests; in 3 of them, all the males were diploid.

Thus, in the presence of the reproductive queen, workers (but not VEQs) of *O. robustior* do lay eggs. However, these eggs apparently do not reach adulthood.

Queenless nests

Workers from the 6 queenless nests sampled showed also all types of ovarian development: 22.7% had ovaries of type 2 and 14.4% had ovaries of type 3. Whatever the number of VEQs in a nest (from 1 to 5), only one had type 3 ovaries per nest; the other VEQs were all characterized by undeveloped

ovaries of type 1. The VEQ with developed ovaries had also a swollen (but empty) spermatheca, contrarily to the other VEQs. The proportion of workers with developed ovaries (type 2 + 3) did not differ between queenless and queenright nests (Fisher's exact test: $P = 0.55$). However, queenless nests contained a higher proportion of workers with type 3 ovaries (Fisher's exact test: $P = 0.008$).

All 68 eggs genotyped were haploid (mean number of eggs per nest ± SD = 11.33 ± 3.44; $N = 6$ nests). Their genotype was compatible with those of workers and VEQs of the nest. In 5 of the 6 nests, the eggs laid carried 3 alleles at several loci, indicating that they were produced by at least 2 individuals. In one nest, the genotype of the five eggs available was compatible with their production by the VEQ-bearing ovaries of type 3. Whether these eggs can reach adulthood is unknown.

Thus, in the absence of the reproductive queen, a single VEQ develops ovaries. This VEQ and the workers lay haploid, male eggs by arrhenotokous parthenogenesis.

Discussion

Our results show that in the desert ant *Ocymyrmex robustior*, nests are typically headed by a single queen and that queens are strictly monandrous. All the ergatoid queens in a nest, except one, are virgin and do not reproduce in the presence of the reproductive queen. In the absence of the reproductive queen, a single VEQ develops ovaries and starts laying eggs. Workers, however, lay eggs in both queenright and queenless nests.

Ergatoid queens represent 14.5% of the nest worker force. A substantial proportion of ergatoid queens per nest was also documented in other ants, such as *Eutetramorium mocquerysi, Leptogenys diminuta*, and some species of the genus *Mystrium* (Heinze et al. 1999; Ito and Ohkawara 2000; Molet et al. 2009). In species with 'multi-purpose' ergatoid queens (i.e., where ergatoid queens can contribute to colony reproduction or work force), it has been suggested that such queens constitute an adaptation to the unpredictability of the environment (Forder and Marsh 1989; Ito and Ohkawara 2000). In deserts, favourable conditions for nest foundation are intimately linked to rainfall, which are unpredictable (Hölldobler and Bartz 1985). In *O. robustior*, the continuous production of ergatoid queens may have been selected to respond rapidly to foundation opportunities. The reduced size of ergatoid queens (identical to the size of workers) and the capacity of VEQs to integrate the worker force reduce the cost associated with their production.

Our data reveal that workers and VEQs originate from multiple matrilines in about 20% of the nests sampled. This contrasts with observations from ovary dissections (Forder and Marsh 1989; this study), showing that a single ergatoid queen is inseminated per nest. Although one may not completely exclude the co-existence of multiple reproductive queens in some nests, polygyny seems unlikely in *O. robustior*. At least 4 alternative explanations, not mutually exclusive, may account for the occurrence of several matrilines in the worker force. First, there may be successive queen replacement (i.e., serial polygyny; André et al. 2001), with workers produced by a previous queen that had remained in the nest. Second, workers may drift from their natal nest to a host nest due to navigation errors and/or misidentification of their own nest location (Katzerke et al. 2006). This may be particularly the case for matrilines represented by very few workers. Third, colonies may be polydomic structures, whereby spatially separated nests remain socially connected with exchanges of workers and brood between them (Robinson 2014). Our data indeed show that some monogynous nests form clusters sharing the same matrilines. Fourth, multiple matrilines within a nest may result from a recent event of dependent nest foundation, with co-occurrence of workers from the mother nest and workers produced by the queen of a new nest. Dependent nest foundation in *O. robustior* is supported by field observations, showing that nests reproduce by budding (Bolton and Marsh 1989) and the resulting population genetic structure (this study). In addition, workers from the cluster of nests 36–38A–38B have genotypes consistent with their production by the same queen (the low relatedness value among workers results from particular allele combinations and their frequencies in the clustering nests). Given the limited dispersal distances of the queens (60 m, Table 2; Bolton and Marsh 1989), the weak genetic structure observed suggests that males of *O. robustior* ensure genetic mixing within populations of *O. robustior*.

Our genetic analyses indicate that the population studied is inbred. Inbreeding reduces sex allele diversity and is expected to increase the frequency of diploid males in hymenopteran populations with single-locus complementary sex determination (*sl-CSD*; Cook 1993; Van Wilgenburgh *et al*. 2006; Heimpel and de Boer 2008; Harpur et al. 2013). We found diploid males in 5 of 30 nests (16.7%); in total, 25 of the 142 males genotyped (17.6%) were diploid. In ants, diploid males have been documented in a number of species belonging to different subfamilies (reviewed in Cournault et al. 2009; Kureck et al. 2012; Doums et al. 2013). The proportion of nests producing such diploid males varies from 3.5 to 39% according to the species and the population studied. In one nest of *O. robustior*, all the workers and VEQs were triploids. This indicates that diploid males of this species can produce fertile diploid sperm and father triploid offspring. Triploid females fathered by fertile diploid males were previously reported in *Solenopsis invicta* (Krieger et al. 1999), *Tapinoma erraticum* (Cournault et al. 2009), *Hypoponera opacior* (Kureck et al. 2012), and *Cataglyphis cursor* (Doums et al. 2013).

Both workers and VEQs can potentially reproduce by arrhenotokous parthenogenesis. VEQs do not lay eggs in the presence of the inseminated queen; in its absence, a single VEQ develops her ovaries and starts laying haploid, male eggs. The selection of the VEQ destined to become the new reproductive queen remains unknown. Two hypotheses may be proposed: (1) the occurrence of a social hierarchy among ergatoids, with the dominant VEQ developing her ovaries in the absence of the reproductive queen (e.g., *Leptothorax* sp.A, Heinze and Smith 1990; *Diacamma* sp., Peeters and Tsuji 1993; *Dinoponera quadriceps*, Monnin and Peeters 1999) and (2) after the disappearance of the reproductive queen, a new VEQ emerges with developed ovaries and inherits the nest (e.g., *Cardiocondyla argyrotricha*, Schmidt et al. 2017). In contrast to the VEQs, workers of *O. robustior* do lay eggs in both queenright and in queenless nests, as evidenced by the presence of yellow bodies. This is consistent with the previous observations in other *Ocymyrmex* species (Forder and Marsh 1989), showing that workers' ovaries can contain eggs even in the presence of the reproductive queen. However, in queenright nests, the 142 males that we genotyped harboured a queen-derived allele at all loci and no adult males could be assigned to workers (or VEQs). This suggests worker policing (Ratnieks 1988; Foster and Ratnieks 2000, 2001; Oldroyd et al. 2001) and/or laying of trophic eggs. Worker policing can result from genetic and colony productivity factors. In *O. robustior*, monogyny and monandry result in workers being more closely related to their sons ($r=0.5$) and nephews ($r=0.375$) than to their brothers ($r=0.25$). Workers are, therefore, selected, to rear sons and nephews instead of brothers. Thus, worker policing should not find its origin in genetic factors. Rather, worker policing in *O. robustior* is best explained by the cost of worker reproduction to nest productivity (Ratnieks 1988; Pamilo 1991; Hartmann et al. 2003). This hypothesis deserves to be tested. As for trophic egg laying, it has been documented in various taxa (Perry and Roitberg 2006) including ants (Gobin et al. 1998). It is, therefore, plausible that the yellow bodies found in the workers' ovaries are due to workers laying such eggs.

Over the last decade, unusual reproductive systems have been reported in several phylogenetically widespread ant genera inhabiting arid and semi-arid environments. For example, queens of several *Cataglyphis* species were shown to simultaneously harvest the benefits of sexual and asexual reproduction, by producing workers by sexual reproduction and new reproductive queens through thelytokous parthenogenesis (Pearcy et al. 2004; Aron et al. 2016). Similarly, four ant genera occupying arid ecological conditions have independently evolved an atypical genetic system called "social hybridogenesis", wherein queen-worker caste determination is primarily shaped by genetic factors (*Pogonomyrmex*: Cahan and Vinson 2003; *Cataglyphis*: Leniaud et al. 2012; *Messor*: Norman et al. 2016; Romiguier et al. 2017). In hybridogenetic species, two interdependent genetic lineages co-exist within populations. Queens of each lineage mate with males originating from the co-occurring lineage to produce F1 hybrid workers. By contrast, new reproductive queens arise from intra-lineage mating or parthenogenesis, hence maintain pure-lineage genomes. It has been suggested that idiosyncratic environmental conditions, such as those found in arid habitats, may have favoured the evolution of singular reproductive strategies in ants (Boulay et al. 2017). Like *Cataglyphis*, species of the desert ants *Ocymyrmex* are highly thermophilic (Wehner 1987; Muser et al. 2005). Ants of these two genera are exposed to extreme conditions and evolved a number of morphological, physiological, and behavioural adaptations in response to the ecological pressures of their habitat (Marsh 1985a, b; Wehner and Wehner 2011; Sommer and Wehner 2012; Willot et al. 2016). This "thermophilia syndrome" led Wehner and Wehner (2011) to consider *Ocymyrmex* as the ecological equivalent in the southern hemisphere of the genus *Cataglyphis*. Despite these common adaptations, the present study shows that *Ocymyrmex robustior* evolved a classical reproductive system, with workers and queens arising from sexual reproduction.

Acknowledgements We thank Quentin Willot for his help on the field and Erik T. Frank for providing us the samples used for genomic library development. We are grateful to the Ministry of Environment and Tourism of Namibia (Permit No. 2268/2017) and Gobabeb Research and Training Center for granting us collection permits. This work was supported by PhD fellowships (ASP fellowship to N.L., FRIA fellowship to A.K.) as well as several grants (S.A.) from the Belgian Fonds National pour la Recherche Scientifique (FRS-FNRS).

Data accessibility The microsatellite data set supporting this article is available on the online version of this paper, as part of the electronic supplementary material (ESM_3).

References

Amor F, Villalta I, Doums C, Angulo E, Caut S, Castro S, Jowers MJ, Cerdá X, Boulay R (2016) Nutritional versus genetic correlates of caste differentiation in a desert ant. Ecol Entomol 41:660–667

André JB, Peeters C, Doums C (2001) Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*. Behav Ecol Sociobiol 50:72–80

Aron S, Mardulyn P, Leniaud L (2016) Evolution of reproductive traits in *Cataglyphis* desert ants: mating frequency, queen number, and thelytoky. Behav Ecol Sociobiol 70:1367–1379

Baer B, Schmid-Hempel P (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature 397:151–154

Blacket MJ, Robin C, Good RT, Lee SF, Miller AD (2012) Universal primers for fluorescent labelling of PCR fragments—an efficient and cost-effective approach to genotyping by fluorescence. Mol Ecol Resour 12:456–463

Bolton B (1981) A revision of six minor genera of Myrmicinae (Hymenoptera Formicidae) in the Ethiopian Zoogeographical Region. Bull Br Mus Nat Hist Zool, Entomol ser (UK)

Bolton B, Marsh AC (1989) The Afrotropical thermophilic ant genus *Ocymyrmex* (Hymenoptera: Formicidae). J Nat Hist 23:1267–1308

Boomsma JJ (2007) Kin selection versus sexual selection: why the ends do not meet. Curr Biol 17:673–683

Boomsma JJ (2009) Lifetime monogamy and the evolution of eusociality. Philos Trans R Soc Lond B: Biol Sci 364:3191–3207

Boomsma JJ (2013) Beyond promiscuity: mate-choice commitments in social breeding. Philos Trans R Soc Lond B: Biol Sci 368:20120050

Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. Philos Trans R Soc London Ser B 351:947–975

Boomsma JJ, Kronauer DJC, Pedersen JS (2009) The evolution of social insect mating systems. In: Gadau J, Fewell J (eds) Organization of insect societies. Harvard University press, Cambridge, pp 3–25

Boulay R, Aron S, Cerdá X, Doums C, Graham P, Hefetz A, Monnin T (2017) Social life in arid environments: the case study of *Cataglyphis* ants. Annu Rev Entomol 62:305–321

Bourke AF, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton, 550 pp

Bourke AF, Heinze J (1994) The ecology of communal breeding: the case of multiple-queen leptothoracine ants. Phil Trans R Soc Lond B 345:359–372

Brown MJ, Schmid-Hempel P (2003) The evolution of female multiple mating in social Hymenoptera. Evolution 57:2067–2081

Cahan SH, Vinson SB (2003) Reproductive division of labor between hybrid and nonhybrid offspring in a fire ant hybrid zone. Evolution 57:1562–1570

Cahan SH, Parker JD, Rissing SW, Johnson RA, Polony TS, Weiser MD, Smith DR (2002) Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. Proc Royal Soc B 269:1871–1877

Chybicki IJ, Burczyk J (2008) Simultaneous estimation of null alleles and inbreeding coefficients. J Hered 100:106–113

Cook JM (1993) Sex determination in the Hymenoptera: a review of models and evidence. Heredity 71:421

Cournault L, Aron S (2009) Diploid males, diploid sperm production, and triploid females in the ant *Tapinoma erraticum*. Naturwissenschaften 96:1393

Cronin AL, Molet M, Doums C, Monnin T, Peeters C (2013) Recurrent evolution of dependent colony foundation across eusocial insects. Annu Rev Entomol 58:37–55

Crozier RH, Fjerdingstad EJ (2001) Polyandry in social Hymenoptera—disunity in diversity? Ann Zool Fenn 38:267–285

Crozier RH, Page RE (1985) On being the right size: male contributions and multiple mating in social Hymenoptera. Behav Ecol Sociobiol 18:105–115

Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Oxford University Press, Oxford, 314 pp

Dai HD, Leeder JS, Cui Y (2014) A modified generalized Fisher method for combining probabilities from dependent tests. Front genet 5:32

Doums C, Ruel C, Clémencet J, Fédérici P, Cournault L, Aron S (2013) Fertile diploid males in the ant Cataglyphis cursor: a potential cost of thelytoky? Behav Ecol Sociobiol 67:1983–1993

Foitzik S, Heinze J (1998) Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*. Behav Ecol 9:367–375

Foitzik S, Kureck IM, Rüger MH, Metzler D (2010) Alternative reproductive tactics and the impact of local competition on sex ratios in the ant *Hypoponera opacior*. Behav Ecol Sociobiol 64:1641–1654

Forder JC, Marsh AC (1989) Social organization and reproduction in *Ocymyrmex foreli* (Formicidae: Myrmicinae). Insectes Soc 36:106–115

Foster KR, Ratnieks FL (2000) Social insects: facultative worker policing in a wasp. Nature 407:692–693

Foster KR, Ratnieks FL (2001) Convergent evolution of worker policing by egg eating in the honeybee and common wasp. Proc Royal Soc B 268:169–174

Foster KR, Ratnieks FL, Gyllenstrand N, Thorén PA (2001) Colony kin structure and male production in *Dolichovespula wasps*. Mol Ecol 10:1003–1010

Gobin B, Peeters C, Billen J (1998) Production of trophic eggs by virgin workers in the ponerine ant *Gnamptogenys menadensis*. Physiol Entomol 23:329–336

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Harpur BA, Sobhani M, Zayed A (2013) A review of the consequences of complementary sex determination and diploid male production on mating failures in the Hymenoptera. Entomol Exp Appl 146:156–164

Hartmann A, Wantia J, Torres JA, Heinze J (2003) Worker policing without genetic conflicts in a clonal ant. PNAS 100:12346–12840

Herbers JM (1986) Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. Behav Ecol Sociobiol 19:115–122

Heimpel GE, de Boer JG (2008) Sex determination in the Hymenoptera. Annu Rev Entomol 53:209–230

Heinze J, Keller L (2000) Alternative reproductive strategies: a queen perspective in ants. Trends Ecol Evol 15:508–512

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

Heinze J, Smith TA (1990) Dominance and fertility in a functionally monogynous ant. Behav Ecol Sociobiol 27:1–10

Holldobler B, Bartz SH (1985) Sociobiology of reproduction in ants. In: Holldobler B, Lindauer M (eds) Experimental behavioral ecology and sociobiology. Sinauer and Associates, Sunderland, pp 237–257

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge, 746 pp

Holleley CE, Geerts PG (2009) Multiplex Manager 1.0: a cross-platform computer program that plans and optimizes multiplex PCR. Biotechniques 46:511–517

Hughes WOH, Ratnieks FLW, Oldroyd BP (2008a) Multiple paternity or multiple queens: two routes to greater intracolonial genetic diversity in the eusocial Hymenoptera. J Evol Biol 21:1090–1095

Hughes WOH, Oldroyd BP, Beekman M, Ratnieks FLW (2008b) Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science 320:1213–1216

Ito F, Ohkawara K (2000) Production and behavior of ergatoid queens in two species of the Indonesian ponerine ant genus *Leptogenys* (diminuta-group) (Hymenoptera: Formicidae. Ann Entomol Soc Am 93:869–873

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555

Julian GE, Fewell JH (2004) Genetic variation and task specialization in the desert leaf-cutter ant, *Acromyrmex versicolor*. Animal Behav 68:1–8

Julian GE, Fewell JH, Gadau J, Johnson RA, Larrabee D (2002) Genetic determination of the queen caste in an ant hybrid zone. Proc Natl Acad Sci USA 99:8157–8160

Katzerke A, Neumann P, Pirk CW, Bliss P, Moritz RF (2006) Seasonal nestmate recognition in the ant *Formica exsecta*. Behav Ecol Sociobiol 61:143–150

Keller L (1993) Queen number and sociality in insects. Oxford University Press, Oxford, 456 pp

Keller L (1995) Social life: the paradox of multiple-queen colonies. Trends Ecol Evol 10:355–360
Kokko H, Johnstone RA, Clutton-Brock TH (2001) The evolution of cooperative breeding through group augmentation. Proc. Royal Soc. B 268:187–196
Krieger MJ, Ross KG, Chang CW, Keller L (1999) Frequency and origin of triploidy in the fire ant *Solenopsis invicta*. Heredity 82:142–150
Kuhn A, Bauman D, Darras H, Aron S (2017) Sex-biased dispersal creates spatial genetic structure in a parthenogenetic ant with a dependent-lineage reproductive system. Heredity 119:207–213
Kureck IM, Jongepier E, Nicolai B, Foitzik S (2012) No inbreeding depression but increased sexual investment in highly inbred ant colonies. Mol Ecol 21:5613–5623
Leniaud L, Darras H, Boulay R, Aron S (2012) Social hybridogenesis in the clonal ant *Cataglyphis hispanica*. Curr biol 22:1188–1193
Mariac C, Scarcelli N, Pouzadou J, Barnaud A, Billot C, Faye A et al (2014) Cost-effective enrichment hybridization capture of chloroplast genomes at deep multiplexing levels for population genetics and phylogeography studies. Mol Ecol Resour 14:1103–1113
Marsh AC (1985a) Microclimatic factors influencing foraging patterns and success of the thermophilic desert ant, *Ocymyrmex barbiger*. Insectes Soc 32:286–296
Marsh AC (1985b) Thermal responses and temperature tolerance in a diurnal desert ant, *Ocymyrmex barbiger*. Physiol Zool 58:629–636
Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD (2012) PANDAseq: paired-end assembler for illumina sequences. BMC bioinform 13:31
Mattila HR, Seeley TD (2007) Genetic diversity in honey bee colonies enhances productivity and fitness. Science 317:362–364
McGlynn TP (2010) Polygyny in thief ants responds to competition and nest limitation but not food resources. Insectes Soc 57:23–28
Meglécz E, Pech N, Gilles A, Dubut V, Hingamp P, Trilles A et al (2014) QDD version 3.1: a user-friendly computer program for microsatellite selection and primer design revisited: experimental validation of variables determining genotyping success rate. Mol Ecol Resour 14:1302–1313
Molet M, Fisher BL, Ito F, Peeters C (2009) Shift from independent to dependent colony foundation and evolution of 'multi-purpose' ergatoid queens in *Mystrium* ants (subfamily Amblyoponinae). Biol J Linnean Soc 98:198–207
Monnin T, Peeters C (1999) Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. Behav Ecol 10:323–332
Muser B, Sommer S, Wolf H, Wehner R (2005) Foraging ecology of the thermophilic Australian desert ant, *Melophorus bagoti*. Austral J Zool 53:301–311
Nonacs P (1988) Queen number in colonies of social Hymenoptera as a kin-selected adaptation. Evolution 42:566–580
Norman V, Darras H, Tranter C, Aron S, Hughes WO (2016) Cryptic lineages hybridize for worker production in the harvester ant *Messor barbarus*. Biol lett 12:20160542
Oldroyd BP, Halling LA, Good G, Wattanachaiyingcharoen W, Barron AB, Nanork P et al (2001) Worker policing and worker reproduction in *Apis cerana*. Behav Ecol Sociobiol 50:371–377
Pamilo P (1985) Effect of inbreeding on genetic relatedness. Hereditas 103:195–200
Pamilo P (1991) Evolution of colony characteristics in social insects. II. Number of reproductive individuals. Am Nat 138:412–433
Pamilo P (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. Heredity 70:472–480
Pearcy M, Aron S, Doums C, Keller L (2004) Conditional use of sex and parthenogenesis for worker and queen production in ants. Science 306:1780–1783
Peeters C (2012) Convergent evolution of wingless reproductives across all subfamilies of ants, and sporadic loss of winged queens (Hymenoptera: Formicidae). Myrmecological News 16:75–91
Peeters C, Tsuji K (1993) Reproductive conflict among ant workers in *Diacamma* sp. from Japan: dominance and oviposition in the absence of the gamergate. Insectes Soc 40:119–136
Perry JC, Roitberg BD (2006) Trophic egg laying: hypotheses and tests. Oikos 112:706–714
Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275
Ratnieks FL (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am Nat 132:217–236
Ravary F, Jaisson P (2004) Absence of individual sterility in thelytokous colonies of the ant *Cerapachys biroi* FOREL (Formicidae, Cerapachyinae). Insectes Soc 51:67–73
Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283
Robinson EJH (2014) Polydomy: the organisation and adaptive function of complex nest systems in ants. Curr Opin Insect Sci 5:37–43
Rohland N, Reich D (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. Genome Res 22:939–946
Romiguier J, Fournier A, Yek SH, Keller L (2017) Convergent evolution of social hybridogenesis in *Messor* harvester ants. Mol Ecol 26:1108–1117
Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228
Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103–106
Schmidt CV, Heimbucher A, Bernadou A, Heinze J (2017) First come, first served: the first-emerging queen monopolizes reproduction in the ant *Cardiocondyla "argyrotricha"*. J Ethol 35:21–27
Sommer S, Wehner R (2012) Leg allometry in ants: extreme long-leggedness in thermophilic species. Arthropod Struct Dev 41:71–77
Strassmann J (2001) The rarity of multiple mating by females in the social Hymenoptera. Insectes soc 48:1–13
Thorne BL (1997) Evolution of eusociality in termites. Annu Rev Ecol Evol Syst 28:27–54
Van Wilgenburg E, Driessen G, Beukeboom LW (2006) Single locus complementary sex determination in Hymenoptera: an "unintelligent" design? Front Zool 3:1
Vargo EL (2003) Hierarchical analysis of colony and population genetic structure of the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. Evolution 57:2805–2818
Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513
Wehner R (1987) Spatial organization of foraging behavior in individually searching desert ants, *Cataglyphis* (Sahara Desert) and *Ocymyrmex* (Namib Desert). In JM Pasteels and J-L Deneubourg (ed) *From Individual to Collective Behavior in Social Insects*. Birkhäuser Basel, Switzerland, pp. 15–42
Wehner R, Wehner S (2011) Parallel evolution of thermophilia: daily and seasonal foraging patterns of heat-adapted desert ants: *Cataglyphis* and *Ocymyrmex* species. Physiol Entomol 36:271–281
Weir BS (1996) Genetic Data Analysis II. Sinauer and Associates, Sunderland
Willot Q, Simonis P, Vigneron JP, Aron S (2016) Total internal reflection accounts for the bright color of the Saharan silver ant. PloS One 11:e0152325