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ORIGINAL PAPER

The role of microgynes in the reproductive strategy of the neotropical ant *Ectatomma ruidum*

Jean-Christophe Lenoir · Jean-Paul Lachaud · Alejandro Nettel · Dominique Fresneau · Chantal Poteaux

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Abstract Miniaturized queens, microgynes, are regarded as an alternative reproductive strategy sparsely present through the ant world. The described roles of miniaturized queens include alternative short-distance dispersal morphs, an adaptation to polygyny and inquiline parasites. Some of these inquiline parasite microgynes have been described as a separate species from their host. In the poneromorph group, miniaturized queens are only reported in two Mexican populations of two Ectatomminae: *Ectatomma tuberculatum*, in which small queens represent an inquiline species (*Ectatomma parasiticum*) and *Ectatomma ruidum*. *E. ruidum* presents apparently facultative polygyny with microgynes. We used mitochondrial DNA markers and newly developed microsatellite loci to investigate the status as well as the role of microgynes in *E. ruidum*. We confirmed that microgynes and macrogynes are from the same species. This species is almost exclusively monogynous and monoandrous, supernumerary dealate queens of both types being actually daughters of the mother queen. An apparently polygynous nest was more often headed by a macrogyne than a microgyne. We didn't find any inbreeding or isolation by distance in the studied population, indicating that new gynes are inseminated by unrelated males and can establish a new nest far from their natal nest. However, re-adoption of daughter queens seems to be the rule and rate of microgyny appears to be linked to nest density and environmental factors.

Keywords Social organisation · Alternative reproductive strategy · Microgyne · Microsatellites · Neotropical area

J.-C. Lenoir (✉) · A. Nettel · D. Fresneau · C. Poteaux
Laboratoire d'Ethologie Expérimentale et Comparée, EA 4443,
Université Paris-Nord,
UFR L.S.H.S, 99, avenue J.-B. Clément,
93430 Villetaneuse, France
e-mail: jean-christophe.lenoir@neuf.fr

J.-P. Lachaud
Centre de Recherches sur la Cognition Animale,
CNRS-UMR 5169, Université Paul Sabatier,
Bât. IVR3, 118 route de Narbonne,
31062 Toulouse cedex 09, France

J.-P. Lachaud
El Colegio de la Frontera Sur, Dpto Entomología Tropical,
Avenida Centenario Km. 5.5, AP 424,
77900 Chetumal, Quintana Roo, Mexico

A. Nettel
Campus del Mar, Universidad de Ciencias y Artes de Chiapas.
Prolongación Juán José Calzada s/n,
Tonalá, Chiapas, Mexico

Introduction

Polygyny, the simultaneous presence of several queens in an ant colony, directly affects relatedness among colony members. As the number of queens producing offspring increases, overall relatedness within colonies tends to decrease. This reduction in relatedness could result in a decrease in the colony members' inclusive fitness and in individual interest to perform altruistic behaviour (Bourke and Franks 1995; Foster et al. 2006). Moreover, the addition of new queens to a colony is associated with changes in a wide range of life history traits that Keller (1993) called the "polygyny syndrome". Briefly, this polygyny syndrome affects colony founding, dispersion of sexual and social behaviours such as nestmate recognition capacity and nepotistic acts.

Polygyny is an alternative strategy for reproduction and dispersal in ants. In general, alate females produced from

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monogynous colonies are relatively large and are costly to produce due to their high level of fat reserves. They usually copulate during a single mating flight before dispersing far away from their natal nest in order to found a new colony on their own (independent foundation). Gynes from polygynous nests are usually smaller in body size and with a far lower relative fat content, reproducing in the vicinity of their maternal nest (if not inside it) where they can be re-adopted. The new queens can then form a new unit of colony by splitting from their natal nest with a part of the worker force, a strategy called budding (dependent foundation; Keller and Passera 1989; Keller 1993; Bourke and Franks 1995; Rosset and Chapuisat 2007).

However, the number of queens per colony, as well as their size and their dispersal tactics may vary among species, among populations and sometimes among colonies within the same population (Bourke and Franks 1995; Crozier and Pamilo 1996; Ross 2001; Chapuisat et al. 2004; Rosset and Chapuisat 2007). Such "social plasticity", described as intraspecific variation of the colony phenotype by Heinze (2008b), depends on both colony size and maturity. This phenomenon has been related to environmental conditions (Molet et al. 2008; McGlynn 2010), differences in genotypes (Keller and Parker 2002) and their interactions. In certain ecological conditions, polygyny can be advantageous. Indeed, unoccupied but stable habitat patches can be rapidly colonized by polygynous ant species (Hölldobler and Wilson 1990; Savolainen and Vepsäläinen 2003).

However, independently from the number of queens, the presence of various dealate queens in a colony is not always the guarantee of a real reproductive status for all of them. Some can be uninseminated or, even if they are inseminated, can suffer some degree of physical or pheromonal inhibition from one or various other queens. In the most extreme cases, it results in functional monogyny, in which only one mated female lays eggs (Buschinger 1968; Heinze and Buschinger 1988; Heinze 1993; Ito 1993, 2005).

Polygyny linked with dependent founding strategies promotes queen miniaturization (Bourke and Franks 1991; Rüppell and Heinze 1999). Young mated queens that are re-adopted by a conspecific or their natal nest, no longer need the large fat reserves that characterize many solitarily founding queens. Therefore two morphological types of queens can be found together in the same colony; miniaturized queens representing reproductive females adapted to dependent foundation called "microgynes" and large queens called "macrogynes". Microgynes are an isometric reduction of the large morph and are relatively frequent in the ant subfamilies Pseudomyrmecinae (Janzen 1973), Formicinae (Heinze and Hölldobler 1993; Sundström 1995a), Myrmicinae (Elmes 1991; McInnes and Tschinkel 1995; Rüppell et al. 2001; Schlick-Steiner et al. 2005; Lenoir et al. 2010), Amblyoponinae (Molet et al. 2007) and Ectatomminae (Lachaud et al. 1999b). In some cases, microgynes, are regarded as a different socially parasitic species (inquiline) (Hora et al. 2005; Savolainen and Vepsäläinen 2003; Vepsäläinen et al. 2009 but see Steiner et al. 2006 and Seifert 2010).

The first case of queen dimorphism in a poneromorph ant species was described in *Ectatomma ruidum* Smith 1958 from two South-Eastern Mexican populations (Schatz et al. 1996; Lachaud et al. 1999b). This neotropical facultative polygynous species presents colonies with macrogynes and/or microgynes (Lachaud et al. 1999a). These microgynes appear to be a 20% isometric body size reduction of macrogynes. Despite reduced potential capacities in reproduction of the small morph compared to the large morph, both macrogynes and microgynes can produce both queen morphs as well as workers and males (Schatz et al. 1996; Lachaud et al. 1999b). This species is known to reproduce most commonly via an independent and non-claustral manner (Lachaud and Fresneau 1987). However, readoption of newly mated queens from the same or a neighbouring colony resulting in secondary polygyny has also been suggested (Lachaud et al. 1999b). Due to the significantly greater "wing surface/body weight" ratio of microgynes compared to macrogynes, Lachaud et al. (1999b) suggested that microgynes would disperse far from their natal nest to mate and then penetrate conspecific nests where they participate to the colony development. Conversely, some authors consider microgynes as neighbourhood-colonizing specialists (Rüppell and Heinze 1999) and disagree with the hypothesis that microgynes' more favourable wing loads imply greater dispersal capacities.

In this paper, we present our fine-scale genetic structure research of an *E. ruidum* Mexican population where microgynes are present (Lachaud et al. 1999b). The aims of our study are: (1) to depict the polygynous status of this species, and define the number of reproducing queens per colony; (2) establish microgynes role within colonies (intraspecific parasitic or not); and (3) understand microgynes' role in the species reproductive strategy.

Materials and methods

Material collection

E. ruidum has microgynes in only two populations located at Izapa and Rosario Izapa (Municipio Tuxtla Chico, Chiapas, Mexico). This study concentrated on Rosario Izapa where 97 nests located on a 400 m^2 area were excavated during April 2008. Distances between nests were measured with a decametre and nests were mapped.

After separation and counting, collected individuals were stored in alcohol for DNA preservation (details in Table 1) and were not suitable for dissection. However, over 226 colonies sampled by Cadena et al. (2001) in the same locality, 99.3% and 94.4% of dealate gynes of both morphs from monogynous and polygynous colonies respectively, presented a full spermatheca (Table 2). Thus, we considered all dealate females, both macro- and microgynes, of a colony as potential reproductive queens.

DNA extraction and cytochrome b analysis

DNA of 11 macrogynes (nine dealates and two alates) and ten microgynes (seven dealates and three alates) found in seven polygynous colonies were extracted from ethanol-preserved tissues (head and thorax) using a DNeasy Blood and Tissue kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's recommendations. A portion of the mitochondrial DNA cytochrome b (cyt b, ~700 bp) was amplified using primers CB1 (5′-TAT-GTA-CTA-CCA-TGA-GGA-CAA-ATA-TC-3′) and tRS (5′-TAT-TTC-TTT-ATT-ATG-TTT-TCA-AAA-C-3′) from Simon et al. (1994). Each PCR was carried out in a 50-µl volume according to standard protocol using a T1 thermal cycler (Biometra). The PCR program consisted of an initial denaturation step (2 min at 94°C) followed by 34 cycles of 30 s at 94°C, 30 s at 48°C, 30 s at 72°C and a final extension of 5 min at 72°C. Amplified products were sequenced using the same primers as used for the amplification, by Genoscreen using an ABI 370 automatic sequencer (Applied Biosystem). Sequences were edited and aligned using the default settings of Clustal X (Thompson et al. 1997; Larkin et al. 2007) and checked by eye.

Microsatellite library and locus amplification

First, microsatellite loci from other Ectatommini ants: *Ectatomma tuberculatum* (Poteaux et al. 2003) and *Gnamptogenys striatula* (Giraud et al. 1999) were tested on *E. ruidum* in order to find polymorphic loci. Over the 32 microsatellite markers tested, only one from *E. tuberculatum* was selected for further analysis.

A microsatellite enriched-library of *E. ruidum* was built using biotin-labelled microsatellite oligoprobes [TG and TC] and streptavidin-coated magnetic beads following modifications as in Giraud et al. (2002). A total of 1,295 clones were screened: 470 gave a positive response and 125 of them were of interesting size (300–600 pb). From the 96 sequenced clones, PCR primers were designed for 20 microsatellite loci (mainly 11 to 29 dinucleotide repeats), using the web computer program Primer3 (http://frodo.wi.mit.edu/primer3/). Finally, 6 of the 20 microsatellite loci revealed a clear pattern of amplification and were polymorphic (Table 3). Sequences of these *E. ruidum* microsatellite loci were deposited in GenBank (accession numbers HM770099—HM770105).

Consequently, we used seven microsatellite loci to genotype 12 workers from monogynous colonies and 24

Table 1 Distribution of both sexual female morphs of *E. ruidum* in colonies sampled at Rozario Izapa, Chiapas, México for this study

Type of colony	Type of reproductive females					Sampling for genetic analyses[a]
	Number of colonies	Macrogyne	Microgyne	Alate macrogyne	Alate microgyne	
Queenless 15 (16.5%)	13	–	–	–	–	
	1	–	–	1	–	
	1	–	–	1	1	1[b]
Monogynous 58 (63.7%)	1	–	1	–	–	
	48	1	–	–	–	8
	6	1	–	1	–	2
	1	1	–	1	1	
	1	1	–	–	1	
	1	1	–	–	2	1[b]
Polygynous 18 (19.8%)	9	2	–	–	–	6
	2	3	–	–		2
	1	3	1	1	2	1
	5	1	1	–	–	5
	1	1	1	1	1	1
Total	91	90	8	11	8	27

[a] 12 workers analysed in monogynous colonies

[b] Colonies for which 24 workers were studied, 24 workers analysed in polygynous colonies

Table 2 Comparison of colony structure between current and previous studies on *E. ruidum* populations from Rozario Izapa, Chiapas, México

	Lachaud et al. 1999a, b, and an unpublished data	This study
Date of collection	1994–1998	2008
Total of investigated colonies	550	91
Queenless colonies	52 (9.5%)	15 (16.5%)
Monogynous colonies	290 (52.7%)	58 (63.7%)
Average nest population	77.6±36.8 workers (n=67 colonies)	71.3±41.0 workers
Polygynous colonies	208 (37.8%)	18 (19.8%)
Average nb. of dealate gynes	3.1	2.2±0.05
Average nest population	101.8±39.2 workers (n=59) (colonies)	96.9±64.3 workers
Density in Rosario Izapa	11,200 nests/ha	≈ 2,500 nests/ha
Microgynes (μQ)	300 dealate μQ in 142 nests (=25.8%)	8 dealate μQ in 8 nests (=8.8%)
Dealates only	=32.1% of the total	=8.2% of the total
Alates+dealates	35.7% of all the females collected in 30.7% of all the colonies	13.7% of all the females collected in 13.2% of all the colonies

workers from polygynous ones, plus all their queens and alate females (both macro- and microgynes) when available. Twenty-four workers from a queenless colony with two alate macro- and microgynes and 24 workers from a colony with a macrogyne and two alate microgynes were also genotyped. These two last colonies were chosen because of their abundant brood, leading us to suspect their production by several but not found reproductive queens. The overall set of individuals is detailed in Table 1 and included 580 genotypes.

For microsatellite analysis, DNA was extracted from the head and thorax using a standard 10% Chelex protocol. PCR were performed individually in 10-μl volumes containing 1 μl of extract of DNA (about 50 ng/μl); 1 μl of 10× polymerase buffer (50 mM KCl, 0.1% Triton X-100, 10 mM Tris-HCl); 0.5 μl of dNTP mix (5 mM); 0.5 μl of each primer (10 μM); 0.1 μl of Taq DNA polymerase (5 U/μl; Promega); 0.6 μl of $MgCl_2$ (25 mM). The locus L92 was amplified using standard PCR program (Poteaux et al. 2003) whereas a touchdown PCR program with a step of temperature decreasing of 0.5°C per cycle from highest to lowest Ta (see Table 3) was used for all other loci.

PCR products were mixed with highly deionized formamide (Hi-Di™ Formamide, Applied Biosystems) in two sets: mix1 (Er5042, Er3157, Er2038) and mix2 (Er2035, L92, Er2050, Er4160). Fragment length was analysed with the internal GeneScan™ 350 ROX™ size standard (Applied Biosystems) by an automated ABi 3,100 Sequencer (Applied Biosystems) and scored using the freeware application Peak Scanner™ v1.0 (Applied Biosystems).

Genetic analysis and population structure

The number of alleles per locus (A), observed (H_O) and unbiased expected (H_E) heterozygosities, and linkage disequilibrium were assessed with Genepop on the web (Raymond and Rousset 1995; Rousset 2008). Linkage equilibrium between loci was tested by a Markov chain method (5,000 steps of dememorisation, 100 batches, 1,000 iterations per batch). As this study was performed at fine scale and to avoid use of non-independent genotypes caused by sampling, we randomly selected one individual for each colony and estimated F_{IS} values and P values for each microsatellite loci for this set of data using Genepop 4.0 (Rousset 2008). We perform this random selection 100 times using a modified SAS procedure of randomization. The overall F_{IS} was computed with GDA (Weir and Cockerham 1984) and a 95% confidence interval was based on 1,000 bootstrapping repetitions. We used the program Matesoft 1.0 (Moilanen et al. 2004) to determine the genotypes of the putative queen and her mate(s) by performing parentage analysis based on worker genotypes. We then compared these putative queen genotypes to those of the females found in each colony in order to verify their status in monogynous colonies or to determine which females participated in worker production in polygynous colonies. In cases of polyandry, Matesoft 1.0 was also used to estimate the reproductive paternity skew.

Since *E. ruidum* has been reported to perform cleptobiosis (i.e. workers steal food from the inside of neighbouring colonies, Breed et al. 1990; De Carli et al. 1998), alien workers in any given colony were likely to be present. Alien and native individuals were determined by comparing their genotypes to the known (or inferred) genotype of the colony queen. Females with at least two alleles at one locus differing from the colony queen's genotype were considered as alien individuals.

Relatedness coefficient values (r) among nestmate workers were estimated using the algorithm implemented in the program RELATEDNESS 5.0.2 (Queller and Goodnight

Table 3 Repeat motif, primer sequences, size range, amplification conditions, used primer label and number of alleles, observed homozygosity (H_O), expected heterozygosity (H_E), F_{IS} P values (H_I=Heterozygote deficit), and GenBank accession number of the six microsatellite loci isolated in *E. ruidum* plus one microsatellite locus (L92) from *E. tuberculatum*

Locus	Repeat sequence	Primer sequence (5′–3′)	Size range	T_a	°C[a]	label of F-primer	Number	No. of alleles	H_o	H_E	F_{IS} P values	GenBank accesion no.
Er5042	(CA) 21–(CG) 4	F: GCATTCATAACGTATTTAGGAGCA R: AGGTTCCCGGTCGGATTTAC	293.7–300.3	TD	71 to 65	6–FAM	575	6	0.544	0.569	0.4834	HM770099
Er2038	(CTT) 9–(CT) 20	F: CACGGACACCTACGACTTGA R: GAGTTTGTAAATCAATTG	294.6–298.4	TD	62 to 56	NED	571	5	0.574	0.508	0.8550	HM770102
Er3157	(GA) 24	F: CTTCCGTCGGTTCACAATT R: CGTCACTTGGCCTTCAATTT	200.1–264.4	TD	62 to 56	HEX	568	9	0.822	0.790	0.4208	HM770104
L92	(GA) 10–(GT) 19[b]	F: GCTTCCCGATAGATAGA R: TTGCTCTCTGATTAACTTTC	250.1–273		50	6–FAM	558	9	0.787	0.808	0.2432	AY332726
Er4160	(CT) 7–(CA) 17	F: AGTGAATCGCGAGGCATAGT R: AATCAGCCAATGGAAATGGT	293.7–321.9	TD	62 to 56	6–FAM	576	5	0.684	0.233	0.6191	HM770100
Er2035	(GA) 29	F: GAGATGTGCTTATGCCGCC R: TTGTAGAAATCGCGAGCTAATG	103.9–122.6	TD	71 to 65	NED	579	7	0.684	0.647	0.6840	HM770103
Er2050	(CT) 18	F: CGTTGATTAGAACCGCTTACG R: TTAGGCACCTGAAACGATCC	108.7–118.4	TD	62 to 56	HEX	579	2	0.257	0.340	0.2556	HM770101

[a] *TD* Touchdown PCR program

[b] Conditions for *Ectatomma tuberculatum* (Poteaux et al. 2003)

1989). Colonies were weighted equally and the standard errors of indices were obtained by jackknifing over colonies.

A pattern of isolation by distance (IBD) between pairs of colonies was assessed by plotting the genetic differentiation [F_{ST}/(1—F_{ST})] coefficients estimated using Genepop on the web (Raymond and Rousset 1995; Rousset 2008) against the matrix of ln-transformed geographical distances (Rousset 1997). IBD was tested using a Mantel test with 10,000 permutations to estimate the level of significance of the obtained Spearman rank correlation coefficients using Genepop on the web (Raymond and Rousset 1995; Rousset 2008). In addition, the spatial genetic structure at this fine scale was also investigated using spatial autocorrelation analysis performed with the program SPAGEDI 1.2 (Hardy and Vekemans 2002). Statistics were calculated for diploid multilocus worker genotypes using the matrix of geographical distances. P values were obtained by performing 10,000 permutations of spatial locations of individuals.

Results

At our collection site, 97 nests were located over a 400 m² area, the estimated nest density was close to 2,500 nests/ha. Three nests were empty and three others were incomplete (due to the presence of rocks) and were not considered for this study. Details of the reproductive females (alate and dealate, micro- and macrogynes) are given in Table 1. The level of polygyny observed here (19.8%) was significantly lower than the rate of 37.8% to 40% found in previous studies at the same site (χ^2=23.128; dl=1; P<0.0001; Table 2; Lachaud et al. 1999a; Lachaud et al. 1999b). Moreover, in our study, only 13.7% of all (alate and dealate) females were microgynes, a much lower value (χ^2=21.967; dl=1; P<0.0001) than that the value of 35.7% previously found for the same population between 1994 and 1998 (Tables 1 and 2).

Worker population was about the same in monogynous, queenless and polygynous colonies (mean ± STD: 71.3±41 (N=58) workers, 78.7±34.5 (N=15) workers and 96.9±64.3 workers (N=18), respectively; Kruskal–Wallis test, N=91, H=2.009, df=2, P=0.366). However, the comparison of the mean colony size in polygynous colonies with microgynes (n=8, mean of individuals= 129.3±66.9) and polygynous colonies without microgynes (n=10, mean of individuals=71±24.3), is marginally significant (t test, t=2.091, p=0.052).

Cytochrome b

The 21 gyne sequences of mtDNA *Cyt b* were identical (GenBank accession number: HM770105), confirming that

macrogynes and microgynes belonged to the same species and that no haplotypic variation was present at this scale.

Microsatellite analysis and population structure

Mean genetic diversity (H_E) in our population was 0.556 (±0.216, SD) with a mean number of alleles of 6.14±2.48 (Table 3). No pair of loci departed significantly ($P \geq 0.05$) from linkage disequilibrium.

Overall F_{IS} was not significantly different from 0 (F_{IS}= 0.008) indicating that the population was at Hardy–Weinberg equilibrium and that inbreeding was absent or not significant in the studied population. Winged females mated with a brother in only 0.32% of their copulations according to the estimation of sib-mating frequency α from Pamilo (1985) or Suzuki and Iwasa (1980): $F_{IS}=\alpha/(4-3\alpha)$.

Over the 511 analysed workers, 19 (3.6%) were identified as foreign individuals that did not belong to the colony they were sampled from. Of the 15 polygynous colonies analysed, from one to four foreign individuals were found in seven polygynous colonies (representing 4.2% to 16.7% of colony's size analysed). Of the 11 monogynous colonies, foreign individuals were found in only one colony where they represented 36.4% of the individuals. In total, the mean percentage of foreign individuals per nest presenting some is 12.4±10.1.

The mean genetic relatedness among nestmate workers, excluding foreign individuals, was $r_{w\text{-}w}$=0.725±0.124. This value was not significantly different from the relatedness of 0.75 expected under monogyny and monandry (*t* test, t= −1.02, N=26, P=0.31). The only exception concerned the most polygynous colony where the relatedness value obtained among workers was rather low ($r_{w\text{-}w}$=0.263± 0.098); among queen relatedness was also rather low for that colony (n=4, $r_{q\text{-}q}$=0.197±0.124). Including this colony in the previous statistical analysis has a mild influence on the results (*t* test, t=−1.45, N=27, P=0.16; mean relatedness among workers lower to 0.708±0.151). According to pedigree analysis and Matesoft simulations, the most parsimonious hypothesis concerning the genetic structure of this colony corresponds to three sets of individuals: one group of 13 workers, all daughters of one inferred queen; a group of 11 individuals (seven workers, two dealate macrogynes and two alate microgynes) all daughters of one genotyped macrogyne; and a group of six individuals (four workers, one dealate macrogyne and one dealate microgyne) all daughters of another inferred queen. From genotype comparison, even if two were inferred, we can assume that these gynes were related since they shared almost one allele at each locus. It is noteworthy that this colony presented the highest colony size (256 workers and abundant brood, especially cocoons).

The structure of functional monogyny with a singly mated queen was confirmed for all the monogynous colonies with one macrogyne, and deduced for the queenless colony. For the 15 polygynous colonies with at least two dealate females, all of them were headed by only one functional queen (macro or microgyne) except in the case of the most polygynous colony described above. Only two of the seven nests with both macro- and microgynes were headed by a microgyne. All these queens from polygynous colonies were singly mated except in three colonies showing workers from two patrilines: two of them headed by a macrogyne and the last one headed by a microgyne. In these three colonies, the paternity skew was 0.58 and 0.61 for both colonies headed by a macrogynous queen and 0.75 for the colony headed by a microgyne. According to pedigree analysis, it appeared that all but one supernumerary dealate females (18 out of 19) found in the 15 colonies containing some were daughters of the functional queen. In the same way, all alate females, both macro- and microgynes, analysed (n=11 out of a total of 19, Table 1) were produced by the colony where they were found.

No pattern of isolation by distance was clearly found between colonies in our population as the correlation between $F_{ST}/(1\text{-}F_{ST})$ and ln(distance) was low and marginally significant (Fig. 1; Spearman rank correlation: N=351, rs=−0.0955, P=0.07). The result was the same with spatial autocorrelation (slope=−0.0142, p=0.33) at a fine geographic scale.

Discussion

Microgynes are hypothesized to be a specialized dispersal strategy morph, a facilitation of dependent colony founding or social parasites. In this study, we depict the role of microgynes in *E. ruidum*. Our results are consistent with previous studies (Lachaud et al. 1999b) that regard *E.*

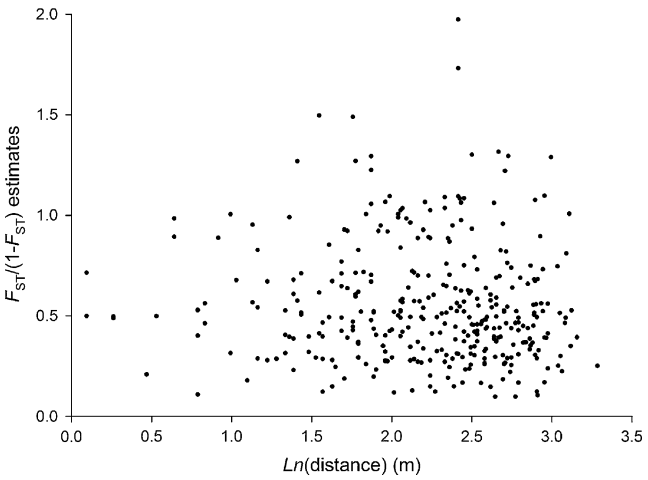

Fig. 1 Isolation by distance of *E. ruidum* colonies sampled showed as $F_{ST}/(1-F_{ST})$ according to *Ln*(distance) between each pair of nest (Rousset 1997)

ruidum macrogynes and microgynes as two morphs of the same species. However, the main result of this study is the mother–daughter genetic relationship found between the functional monogynous queen of any polygynous colony and the other supernumerary dealate gynes (macro- as well as microgynes).

The detected sociogenetic structure in *E. ruidum* is consistent with a monogynous and monandrous ($r_{w\text{-}w}$= 0.725) species. Moreover, our results confirmed, except in one case, functional monogyny when various dealate females were present. In polygynous colonies presenting both types of queens, macrogynes tend to head colonies, except in two digynous colonies in which the microgyne was the mother queen. The functional monogyny confirmed laying queen inhibition over the other dealate females present into the nest as already reported by Cadena et al. (2001). This inhibition is, however, stricter than previously suspected on the basis of the presence of yellow bodies in dealate female ovaries. Only the most populous colony was highly polygynous with three reproductive queens, apparently related. Passera et al. (1994) regarded *E. ruidum* as monandric, a hypothesis confirmed by our results in most of the cases. However, polyandry was not totally absent; double fecundation occurred in three cases (10.3% of all the functional queens) involving both macrogynes and microgynes. Sperm of both males seemed to be used by the females to produce offspring as the paternity skew values were 0.58, 0.61 and 0.75, respectively.

E. ruidum is known to perform independent and non-claustral colony foundation (Lachaud and Fresneau 1987), generally initiated by macrogynes. A previous hypothesis (Lachaud et al. 1999b) suggested that microgynes were likely to be a more effective dispersal form, flying away from their mother colonies and, after mating, either penetrating conspecific colonies where they were adopted or founding a new colony by their own. However, the fact that almost all supernumerary dealate females found in polygynous colonies are daughters of the functionally monogynous queen invalidates this hypothesis. Newly mated gynes of both morphs tend to reincorporate back into their mother nest, probably as reproductive insurance in case of fertility problems or death of the mother queen. Moreover, the lack of inbreeding and field observations suggest that gene dispersion is mainly done by flying males. Classical nuptial flights or sexual swarms seem to be rare and have been reported only once (Passera et al. 1994). This same study also reports that new gynes (both macrogynes and microgynes) generally stayed near their natal nest or flew over short distances. Copulation and wing shedding occur on the ground and some dealate macrogynes can be transported and retrieved to colonies by workers (Lachaud pers. obs.). *E. ruidum* workers are able to distinguish between nestmates and non-nestmates (Jaffé and Marquez 1987; Breed et al. 1990; Breed et al. 1992; Jeral et al. 1997; De Carli et al. 1998; Breed et al. 1999); thus, newly mated females of both morphs could have been recognized by sister workers and re-adopted by their natal nest. In *Myrmica ruginodis* (Elmes 1991), *Leptothorax spinosior* and *Leptothorax rugatulus* (Hamaguchi et al. 1998; Rüppell et al. 2001), queen dimorphism has been attributed to a dispersal polymorphism, with monogynous macrogynous and polygynous microgynous colonies. Our results also reject such a hypothesis of dispersal polymorphism between both morphs of *E. ruidum* since both are essentially re-adopted by their mother colony. Moreover, microgynes are able to found new colonies both in laboratory conditions (Schatz et al. 1996; Cadena et al. 2001) and, even if rare (about 5% of the cases, Lachaud et al. 1999b), in field conditions. All these evidences suggest that microgynes, in *E. ruidum*, represent an example of alternative reproductive low dispersal strategy. However, the lack of viscosity confirming this pattern is certainly due to small scale of this study or the fact that this population is yet in a recovery phase (see below).

Alternative reproductive strategies can also be influenced by ecological constraints (Bourke and Franks 1995; Heinze and Tsuji 1995; Crozier and Pamilo 1996; Ross 2001; Chapuisat et al. 2004; Rosset and Chapuisat 2007). Three environmental factors are supposed to present main influences on the degree of polygyny: competition, food limitation and nest limitation (Hölldobler and Wilson 1977; Bourke and Franks 1995; McGlynn 2010). In certain ecological conditions such as large and stable habitats, a polygynous strategy can be of advantage to rapidly colonize free areas (Hölldobler and Wilson 1990; Savolainen and Vepsäläinen 2003; Zinck et al. 2007). In the present study, the sampling site was located in a cultivated area (coffee plantations) where human practices can lead to exaggerated nest densities. In spring 1998, *E. ruidum* presented a nest density in Rosario Izapa as high as 11,200 nests/ha (Lachaud et al. 1999b; Schatz and Lachaud 2008) which promoted a high intraspecific competition. Under such conditions, dispersal risks made solitary founding costly for this species because places for new nests were almost unavailable. As suggested for other polygynous ant species (Pamilo 1991; Sundström 1995b; Heinze 2008a; McGlynn 2010), nest sites availability is a key factor that favours philopatry among reproductive females, with adoption of newly mated queens by their mother or neighbouring colonies.

At the time of our study, 10 years later, the nest density decreased by five (2,500 nests/ha), with a global decrease of both the polygyny rate and the proportion of microgynes over the total amount of females (see Table 2). The occurrence of microgynes into *E. ruidum* populations seems effectively related to nest density in this site. Producing and reincorporating back a reserve of daughter queens for a very low

energetic cost (about 7.5 times less than for a macrogyne, Lachaud et al. (1999b)) could then be a benefit for their natal colony when the habitat becomes saturated. This hypothesis appears even more likely if we consider that, in spite of their reduced size and their lower reproductive capacity, microgynes under such environmental conditions would not have to found new colonies (the period during which their reproductive weakness is the most obvious, see Schatz et al. (1996)) but only to secure the reproductive continuity of an already mature colony. The observed decreases in polygyny and microgyny could be explained by the fact that *E. ruidum* suffered severe population reductions between the two collection periods due to successive passage of hurricanes (http://stormadvisory.org/map/). One of the most dramatic events was the transformation of the hurricane Mitch (1/11/1998) into a tropical depression near the studied area, resulting in the production of locally heavy rainfall over this region for several days (Guiney and Lawrence 1999). Heavy rains caused flooding and mud flow that damaged our sampling area and could have resulted in the noticed reduction in polygyny and microgynes occurrences compared to Lachaud et al. (1999b). Moreover, during our sampling in April 2008, *E. ruidum* population density was still in a recovery phase, after being once again strongly disturbed by another tropical depression (Stan) in 2005 (http://en.wikipedia.org/wiki/Hurricane_Stan).

Foreign individuals were found in relatively high proportions (12.4%) in almost half of the polygynous colonies, whereas they were abundant in only one of the 11 studied monogynous nests. Unlike most ant species where social closure is well developed and is the cause of strong homospecific competition, the tolerance level between colonies of *E. ruidum* from the same population is high, leading to the overlap of colony territories (Breed et al. 1990; Lachaud 1990; De Carli et al. 1998). This species is also known to display homospecific cleptobiosis, that is, the stealing of food resources from neighbour colonies where they can stay during several hours and sometimes spend the night (De Carli 1997; Lachaud pers. obs.). The presence of heterocolonial workers in a nest could then be easily explained by some occurrences of thief workers before nest excavation. The alternative hypothesis would be polydomy, that is, a complex structure of a colony, with constituent nests functioning as a single social unit by exchanging workers and brood among them (Debout et al. 2007). However, this hypothesis can be rejected here because of: (a) the regular distribution of the colonies (Levings and Franks 1982; Schatz and Lachaud 2008), (b) the monodomous nest structure observed over 450 excavated colonies (Breed et al. 1990; Schatz and Lachaud 2008) and (c) the absence of migration or splitting of colonies reported in the field (Lachaud unpubl. data) which is realistic considering the lack of viscosity found in the present studied population. Moreover, Jaffé and Marquez (1987) and Schatz et al. (1997) reported evidence of territorial marking and territoriality in this species, and workers have been proved to mark their nest entrances with hindgut pheromones deposited in faecal droplets which serve as colony-specific markers (Pratt 1989). Consequently, the presence of heterocolonial workers can be explained by cleptobiosis alone and confirmed the importance of cleptobiotic behaviour in the foraging strategies of this species.

E. ruidum is a functional monogynous/monandrous species that re-adopt a part of their gynes once fecundated—the others dispersing and founding new colonies independently. The supernumerary females remain reproductively inhibited while their mother is still active. Our results are consistent with a scenario where, in dense conditions, the population develops non dispersing re-adopted microgynes as an alternative strategy in order to maximize the chances of the colony to monopolize nest sites, apparently increasing worker force and leading to colony continuity. Recent literature has shown that reproductive conflicts are highly frequent in polygynous ant societies and occur at different levels within a colony. Reproductive conflicts are regulated through pheromones and/or policing behaviours, that is, overt or ritualized aggression and egg cannibalism to prevent nestmate reproduction (Ratnieks et al. 2006). In *E. ruidum*, no such aggressive interactions have been observed in both artificial pleometrotic colony foundations and natural polygynous colonies (Cadena et al. 2001). We hypothesize that chemical regulation of reproduction, based on pheromones produced by reproductives, occurs in *E. ruidum*. Pheromones-based chemical regulation of reproduction has been reported in other social insects (Monnin 2006; Hefetz 2007; Cournault and de Biseau 2009; Smith et al. 2009; Izzo et al. 2010). Chemical characterization of both types of queens should be investigated in this species to address this question.

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