Alternative reproductive tactics in the queen-size-dimorphic ant Leptothorax rugatulus (Emery) and their consequences for genetic population structure

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Abstract:

We report the results of a comprehensive investigation of the queen size dimorphism in the North American ant *Leptothorax rugatulus*. Employing allozymes and microsatellites as genetic markers, we found no evidence that the gene pools of large (macrogynes) and small (microgynes) queens are distinct. Queens in polygynous colonies are related to each other, supporting the hypothesis that colonies with more than one queen commonly arise by the adoption of daughter queens into their natal colonies. The higher fat content of macrogynes, their predominance in monogynous societies and in small founding colonies, and their greater flight activity favor the view that macrogynes predominantly found colonies independently, while microgynes are specialized for dependent colony founding by readoption. When comparing the genetic structure of three different subpopulations, we found that the alternative life histories had no significant effect on population viscosity at the scale investigated.

Keywords. Size polymorphism - Microgynes - Reproductive tactics - Life history evolution - Population structure

Article:

INTRODUCTION

Body size is a crucial parameter for the physiology (Schmidt-Nielsen *1990*), life history (Roff *1992*; Stearns *1992*), and fitness (Darwin *1871*; Brown et al. *1993*) of all organisms. In eusocial insects, and ants in particular, the size dichotomy between reproductive females (queens) and workers is related to their different roles in the colony (Wilson *1971*; Oster and Wilson *1978*). Worker size is selected for efficiency in e.g., brood caring, colony maintenance, and foraging, while the almost invariably larger queens are specialized for mating, dispersal, colony initiation, and egg production (Hölldobler and Wilson *1990*). The latter two features probably select for large body size in queens, given that egg production is strongly correlated with female body size in insects (Thornhill and Alcock *1983*). Colony founding typically requires extensive body reserves (Keller and Passera *1989*, *1990*; Wheeler and Buck *1995*), and thus a comparatively large body size (Stille *1996*), because most queens rear their first workers in complete isolation

(claustral, independent mode of colony founding) (Wilson *1971*; Hölldobler and Wilson *1990*; Bourke and Franks *1995*).

However, some ant species have alternative modes of reproduction that bypass solitary nest founding (Wheeler *1933*; Hölldobler and Wilson *1990*; Keller *1991*; Heinze and Tsuji *1995*). Young queens establish themselves with the help of the work force of existing colonies (dependent colony founding), either by seeking adoption into their natal colony (secondary polygyny) or by trying to enter and exploit unrelated colonies (inter- or intraspecific social parasitism) (Hölldobler and Wilson *1990*; Heinze and Tsuji *1995*; Rüppell and Heinze *1999*; Heinze and Keller *2000*). The queens of such species do not require large body reserves and consequently can be smaller (Keller and Passera *1989*; Stille *1996*). Thus, intraspecific size polymorphism of ant queens could evolve into social parasitism or a more pronounced dispersal polymorphism, i.e., wing dimorphism (Rüppell and Heinze *1999*). In general, intraspecific polymorphism gives the opportunity to study current selection pressures little affected by evolutionary latency. However, investigations along this line are scarce, especially in the interesting case of queen size polymorphism, in which behavioral, morphological, and life history traits seem to be linked.

Apart from a reduction in queen body size, secondary polygyny entails a syndrome of life history adaptations (Keller *1991*; Bourke and Franks *1995*), including colony budding for short-range dispersal. This commonly leads to clusters of related colonies and thereby increases population viscosity (Stille and Stille *1993*; Seppä and Pamilo *1995*; Chapuisat et al. *1997*).

In a number of taxonomically unrelated species, queen size appears to be bimodally distributed (Rüppell and Heinze *1999*). In most cases this has been interpreted as evidence for different modes of colony founding (e.g., *Myrmica ruginodis*: Elmes *1991*; *Solenopsis geminata*: McInnes and Tschinkel *1995*; *Pseudomyrmex veneficus*: Janzen *1973*). However, we still do not know how closely behavior is linked to morphology in these cases, and the potential consequences for life histories, mating structure, socio- and population genetics await clarification.

Furthermore, few studies have addressed the question as to whether queen size polymorphism is a stable phenomenon in itself, or whether it constitutes a transitory phase in the evolution of inquilinism (Bourke and Franks *1991*) or a more pronounced dispersal polymorphism with wing reduction (Heinze and Hölldobler *1993*). In the genus *Leptothorax*, a taxon comprising many social parasites and wing-dimorphic species, these questions are of particular interest (Bourke and Franks *1991*). Moreover, queen size dimorphism seems to be more common in the genus *Leptothorax* than in other genera (Rüppell and Heinze *1999*).

The aim of this study was to investigate the widespread queen size dimorphism in the facultatively polygynous ant *Leptothorax rugatulus* (Rüppell et al. *1998*). We addressed five main questions. (1) Do large queens (macrogynes) and small queens (microgynes) belong to the same species or are their gene pools separated? (2) What is the evidence for the hypothesis that microgynes found colonies dependently while macrogynes disperse and initiate new colonies independently? (3) If microgynes start reproduction dependently, do they employ social parasitism or secondary polygyny? (4) What environmental factors are ultimately affecting the

balance between macro- and microgynes? (5) How is the genetic population structure of this queen-dimorphic species affected by the alternative life histories?

In this study, we tried to answer these questions by evaluating social, physiological, and behavioral data and by genetic analyses.

METHODS

Field methods

L. rugatulus occurs throughout most of western North America (Creighton *1950*). We collected 1,310 colonies (244 microgynous, 669 macrogynous, 231 mixed, and 166 queenless), predominantly in mixed coniferous forests, in Arizona, New Mexico, and Colorado in the summers 1996-1999. Voucher specimens have been deposited in the collection of P. Ward (University of California, Davis).

In most colonies, winged male and female sexuals, workers, and queens were counted immediately, and from colonies that showed sexual activity within 2 days after collection, one male and/or one gyne was collected for determination of fat content according to the method of Keller and Passera (*1989*). To investigate ecological correlates of queen morphology, the following parameters were determined for collection sites with more than 20 colonies: geographical location, altitude, vegetation type, nesting substrate, sun exposure, and an estimate of colony density (Table 1).

Table 1 Collection data and ecological parameters from the major subpopulations (collection sites with $n>20$). In two populations (*CH* Chiricahua Mountains, *MZ* Manzano Mountains), we sampled several subpopulations; in all other cases, subpopulation and population labels are identical (*Mono* monogynous, *Poly* polygynous)

Subpopulation	Location	Altitude (m)	Vegetation	Nest sites	Number of microgynes/ macrogynes	Number of colonies						Density	Average queen number	Average worker number
						Queenless	Microgynous		Macrogynous		Mixed			
							Mono	Poly	Mono	Poly				
Barfoot Lookout (BL in CH)	32°00′ N, 109°08′ W	2,630	Mixed oak-pine fir forest	Rock crevices	391/54	5	3	34	10	5	11	High	6.9	89.3
Huachuca Mountains (HU)	31°36′ N, 110°25′ W	2,400	Pine forest	Under stones	30/72	6	1	5	17	17	5	High	2.3	55.7
Magdalena Mountains (MA)	34°25′ N, 107°30′ W	2,270	Coniferous mixed forest	Under stones	18/158	9	0	1	30	19	5	Low	2.7	107.6
Tajique (TA in MZ)	34°45′ N, 106°20′ W	2,040	Open pine-oak forest	Rock crevices	348/373	8	16	31	20	31	50	High	4.9	80.7
Manzano Way (Z in MZ)	34°45′ N, 106°32′ W	2,060	Pine-oak forest	Rock crevices	35/57	0	3	6	8	2	9	Low	3.4	69.3
Mapping Area (Map in CH)	32°03′ N, 109°05′ W	2,300	Coniferous mixed forest	Under stones	52/142	28	4	5	69	26	8	High	1.4	68.1
Monte Vista (MV in CH)	31°47′ N, 109°24′ W	1,850	Mixed oak forest	Under stones	0/24	9	0	0	12	3	0	Low	1.0	106.2
Monte Vista Peak (BVP in CH)	31°47′ N, 109°24′ W	2,700	Low pine forest	Rock crevices	127/57	1	1	8	4	7	7	High	6.6	88.6
Mount Graham (GR)	32°42′ N, 109°45′ W	2,100	Low oak-pine forest	Under stones	7/36	1	0	2	13	3	3	Low	2.0	?
Mount Lemmon (LE)	32°30′ N, 111°00′ W	2,250	Mixed forest	Under stones	29/58	10	0	4	33	6	2	High	1.6	83.1
North Barfoot (NBL in CH)	32°01′ N, 109°08′ W	2,540	Mixed bushes (deciduous)	Rock crevices	678/105	6	8	76	28	9	19	High	5.3	54.1
Onion Saddle (OS in CH)	32°02′ N, 109°05′ W	2,320	Mixed oak-coniferous forest	Under stones	132/196	21	9	17	63	31	22	High	2.2	90.1
Pinery Camp (PC in CH)	32°02′ N, 109°08′ W	2,120	Mixed pine-fir forest	Under stones	7/65	4	1	1	24	8	4	Low	2.3	140.8
Roosevelt Forest (CO)	40°40′ N, 105°20′ W	1,800	Pine forest	?	2/32	2	0	1	12	10	0	?	1.4	54.4
West Turkey Creek (WTC in CH)	31°50′ N, 109°25′ W	1,880	Coniferous mixed forest	Under stones	0/96	10	0	0	64	11	0	High	1.1	62.9

We investigated the relationship between the morphology of a colony's queens and its social structure (queen number) and the correlation across sample sites between the frequency of macrogynes in polygynous colonies (i.e., the tendency for dependent founding by macrogynes) and the relative abundance of microgynes. The relationship between colony size, queen morphology, and queen number was also analyzed. Despite deviations of the data from normality, parametric statistics were most appropriate for these analyses (Lindman *1974*; StatSoft *1994*).

During the mating period, we trapped alates in the Chiricahua Mountains, Cochise County, Ariz., at three sites (subpopulations OS, NBL, and BL) that differed in their frequency of macro- and microgynes (Table 1). In each location, one Malaise trap (2x1.4 m capturing area), three aerial eclectors (Mühlenberg *1993*), and 20 sticky traps (30x20 cm foil-covered cardboard with brushed-on insect glue) were set up from 15 June to 20 August 1998 and checked on average every 4 days.

A systematic subpopulation comparison was performed in 1998 by sampling every visible potential nesting site and mapping colony positions at three sample sites (Fig. 1): West Turkey Creek Valley (WTC; Chiricahua Mountains, Cochise County, Ariz., 800 m^2;), Magdalena Mountains (MA; Socorro County, N.M., 800 m^2), and Tajique Valley (TA; Manzano Mountains, Torrance County, N.M., 200 m^2). Colonies were immediately stored in 96% ethanol for later investigation of colony contents, exact size measurement of the queens (Rüppell et al. *1998*), and genetic analysis of all queens or one arbitrary worker in queenless colonies.

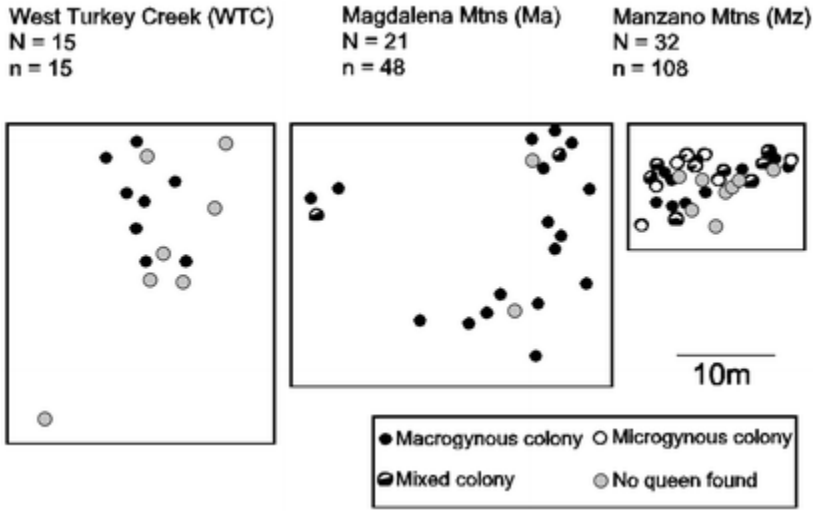

Fig. 1. Colony density, colony composition, and genetic structure of *Leptothorax rugatulus* were compared in three subpopulations. Relative colony position and queen content for these sites are shown

Laboratory methods

For the allozyme analysis, ten different enzymes were initially screened using polyacrylamide gels and cellulose acetate plates following the protocols given in Heinze et al. (*1995*). Glucose-6-phosphate dehydrogenase, hexokinase, lactate dehydrogenase, phosphogluconate dehydrogenase, and xanthine dehydrogenase showed no scorable alleles and isocitrate dehydrogenase, malate dehydrogenase, and malic enzyme were monomorphic. Only glucose-6-phosphate isomerase (GPI) and phosphoglucomutase-1 (PGM-1) were polymorphic and could be scored consistently on polyacrylamide gels. Subsequently, the genotypes of 467 workers at the GPI locus and 473 at the PGM locus were determined from 191 macro- or microgynous colonies.

For a more detailed genetic analysis, we attempted to adopt 15 primers for microsatellite loci in other *Leptothorax* species (Hamaguchi et al. *1993*; Bourke et al. *1997*; Foitzik et al. *1997*) using various PCR protocols. Four loci (L18 from *L. nylanderi*, and LXGT104, LXGT218, and LXGT228 from *L. spinosior*) were finally used, with slightly different PCR conditions for each

locus, to genotype 471 queens (263 macrogynes, 160 microgynes, 48 unclassified) from 195 colonies (technical details of DNA extraction, PCR conditions, and allele identification are available from the first author on request). Furthermore, for 18 similarly sized, monogynous colonies (6 microgynous and 6 macrogynous colonies from the predominantly microgynous sample sites NBL and TA, and 6 macrogynous ones from the macrogynous site WTC), we checked whether the distribution of queen and six worker genotypes was compatible with the hypothesis of independent colony founding (i.e., all workers are daughters of the present queen). Colonies were assigned to "independent founding" when, beside the queen's alleles, only one other allele was present, to "multiple mating" when worker genotypes were compatible with the present queen assuming multiple mating (which is unlikely but could not be ruled out), and to "dependent founding" when at least one of the six workers did not share alleles with the present queen at one or more loci.

Genetic data analysis

The computer program GDA (Lewis and Zaykin *1999*) was used to calculate the number of alleles, and observed and expected heterozygosity for each allozyme and microsatellite locus. To investigate the genetic separation between macro- and microgynes, the allozyme and the microsatellite data were subjected to a hierarchical analysis of molecular variance (AMOVA). One hundred new data sets were generated by drawing at random one individual from each analyzed colony because genotypes within a colony are not independent. Subsequently, each resampled data set was subjected to a three-level AMOVA using GDA and values averaged over the 100 resamples. As there was no a priori reason for nesting morphs within populations (K.G. Ross, personal communication), both possible hierarchies ("individuals within morphs within populations" and "individuals within populations within morphs") were analyzed.

The "morph-groups" (all individuals from either a macro- or a microgynous colony) were clustered with the program TFPGA (Miller *1998*) in a UPGMA tree based on Reynolds coancestry coefficient (Reynolds et al. *1983*). Clustering by Wright's modification of Roger's distance (Wright *1978*) and Nei's *D* (Nei *1972*), as well as using the GDA UPGMA and neighbor-joining algorithms, or excluding rare alleles did not change the results.

Average within-colony relatedness estimates for queens from polygynous colonies were obtained from all microsatellite genotypes by the method of Queller and Goodnight (*1989*), using the computer program Relatedness 5.02 (Goodnight and Queller *1998*). Subpopulation allele frequencies were calculated from individual genotypes with bias correction by colony. Relatedness values were averaged over individuals, and the 95% confidence interval and SE were obtained by jackknifing over colonies.

To assess potential effects of queen morphology and the correlated life history on the genetic population structure, we investigated population viscosity in three subpopulations (Fig. 1): relatedness coefficients and spatial distance between colonies were correlated using Mantel tests (with TFPGA).

In the two polygynous subpopulations, TA and MA, the hypothesis that queens within the same colony were unrelated to each other was evaluated against two null hypotheses, namely a mother-daughter and a full-sister relationship using Kinship 1.1.2 (Goodnight and Queller *1999*).

As a conservative estimate, only those queens were scored as unrelated to the colony for which all intranidal comparisons resulted in a significant rejection of the two null hypotheses. To avoid undefined likelihood ratios (Kinship 1.1.2 Manual), the theoretical relatedness coefficient of 1 (for paternal relatedness of full-sisters and maternal relatedness of mother-daughter) was approximated by 0.99.

RESULTS

Field data

Social colony structure (queen number) and the frequencies of macro- and microgynes varied strongly among sample sites, even within a few kilometers (Table 1). These two parameters were strongly correlated: only 51 of 2,056 microgynes were found in monogynous colonies compared to 472 of 1,665 macrogynes (x^2=509.6, *df*=1, *P*<0.0001). 58.6% (472 of 806) of colonies with macrogynes were monogynous, while this fraction for microgynes was only 12.9% (51 of 394) (x^2=224.0, *df*=1, *P*<0.0001). These overall contingencies became even stronger when subpopulation effects were accounted for by multiway frequency analyses: $x^2_{indiv.}$(maximum likelihood)=3,086.6, *df*=78, *P*<0.0001 and x^2_{colony} (maximum likelihood)=302.7, *df*=66, *P*<0.0001).

The size of a colony (number of workers) was strongly dependent on its social structure (monogynous/polygynous) and on the morphology of its queens (two-way ANCOVA: $F_{social(1,927)}$=27.5, *P*<0.0001; $F_{morph(1,927)}$=26.1, *P*<0.0001, $F_{interaction(1,927)}$=2.4, *P*=0.12; "subpopulation" as covariate). Polygynous colonies were larger than monogynous colonies [91±85 (SD) vs 65±42] and colony size decreased from mixed colonies to macrogynous to microgynous ones (122±125 vs 79±62 vs 65±58). Nevertheless, we found 20 very small colonies (putative founding colonies with one founding queen and less than five workers) headed by a macrogyne and only one such colony with a microgyne. Colony size was more strongly correlated to the number of macrogynes in the colony than to the number of its microgynes (partial correlation: r_{macro}=0.66, r_{micro}=0.39, *n*=954, *P*<0.0001). When the total number of queens was accounted for, the partial correlation coefficient of the number of microgynes became slightly negative (*r*=-0.11, *n*=954, *P*<0.001), while that of macrogynes was insignificant (*r*=0.05, *n*=954, *P*=0.12).

The trapping yielded 5/0, 4/1 and 2/1 macro-/microgynes at OS, BL, and NBL, respectively. We compared these data to the values that would be expected under the null hypothesis that queen morphs did not differ in their reproductive behavior and thus were trapped according to their subpopulation frequencies. The combined probability test of the three binomial tests (Sokal and Rohlf *1995*) indicated that macrogynes were significantly more likely to be trapped (Cp=22.4, *df*=6, *P*<0.005).

When comparing different sample sites, we found a significant positive correlation between the proportion of macrogynes in polygynous colonies and the relative abundance of microgynes (Spearman's rank correlation: r_s=0.69, *n*=15, *P*=0.004). Angular-transformed microgyne frequency was neither correlated with latitude (partial correlation coefficient r_p=0.17, t_{15}=0.23, *P*=0.82) nor with altitude (r_p=0.48, t_{15}=2.04, *P*=0.06), or sun exposure (north-facing slopes vs south facing slopes; r_p=-0.35, t_{15}=-1.41, *P*=0.19), but there was a significant relationship with

nest density (high vs low; r_p=0.51, t_{15}=2.22, P=0.04; e.g., Fig. 1) and nest site stability (in the soil under a stone=low vs in rock crevices=high; r_p=0.60, t_{15}=2.82, P=0.01).

Fat content

Males contained the smallest amount of fat in absolute [10.1±9.0 (SD) µg, n=40] and relative (6.2±5.0%) terms, and their size was not correlated to their relative fat content (Pearson's correlation: r_p=-0.10, P=0.52). In female sexuals however, more fat was stored by macrogynes ($fat_{absolute}$: 466.4±146.9 µg, n=35; $fat_{relative}$: 47.7±8.1%) than by microgynes ($fat_{absolute}$: 51.4±12.3 µg, n=10, $fat_{relative}$: 23.5±5.4%). Differences were significant in both absolute (Mann-Whitney's $U_{10,35}$=350.0, P<0.0001) and relative ($U_{10,35}$=339.0, P<0.0001) terms. Relative fat content was not correlated with head width in microgynes (r_p=-0.19, P=0.60), but was in macrogynes (r_p=0.42, P=0.01).

Allozymes

For both loci, GPI and PGM, over all populations, five alleles could be distinguished electrophoretically, but allele frequencies were highly skewed and consequently both loci were only weakly polymorphic (Table 2). The genetic differentiation between morphs was only significant when they were nested within populations, whereas populations had significantly different allele distribution in both hierarchical AMOVA orders (Table 3). There was no indication of inbreeding. The amount of information gained from the two loci was insufficient to derive any significant clustering of morph groups or meaningful intracolonial relatedness coefficients.

Table 2. Descriptive statistics of genetic data from allozymes and microsatellites over all populations of *Leptothorax rugatulus*

Locus	Overall sample size	Allele number	H_{exp}/H_{obs}
Glucose-6-phosphate isomerase	464	5	0.184/0.183
Phospoglucomutase	470	5	0.373/0.283
LXGT104	390	9	0.653/0.438
L18	410	32	0.894/0.824
LXGT218	409	9	0.528/0.528
LXGT228	370	31	0.942/0.900

Table 3. Results of the three-level hierarchical analyses of molecular variance for allozyme and microsatellite data. 95% confidence intervals (CIs) are given in *parentheses* and significant positive F-values (P<0.05) are *italicized*

AMOVA	F_{IS}	F_{IT}	$F_{(population)}$	$F_{(morph)}$
Allozymes				
Morphs nested in populations	0.090 (-0.003-0.154)	*0.167* (0.100-0.220)	*0.066* (0.032-0.106)	*0.085* (0.057-0.115)
Populations nested in morphs	0.090 (-0.003-0.154)	*0.144* (0.079-0.200)	*0.059* (0.038-0.084)	-0.031 (-0.043 - -0.021)

AMOVA	F_{IS}	F_{IT}	$F_{(population)}$	$F_{(morph)}$
Microsatellites				
Morphs nested in populations	0.040 (-0.005-0.072)	*0.146* (0.048-0.285)	*0.114* (0.051-0.234)	*0.110* (0.044-0.235)
Populations nested in morphs	0.040 (-0.005-0.072)	*0.121* (0.034-0.243)	*0.084* (0.031-0.188)	-0.025 (-0.048 - -0.012)

Microsatellites

The four employed microsatellite loci were more polymorphic than the allozymes (Table 2). Parallel to the allozyme analysis, the three-level AMOVA indicated for both hierarchies, a significant differentiation at the population level, but differentiation between morphs was only significant when morphs were nested in populations (Table 3). The mating structure did not deviate significantly from random mating.

The cluster analyses further supported the view that the genetic separation was stronger between populations than between morphs because the different "morph-groups" of the same population strictly clustered together (Fig. 2).

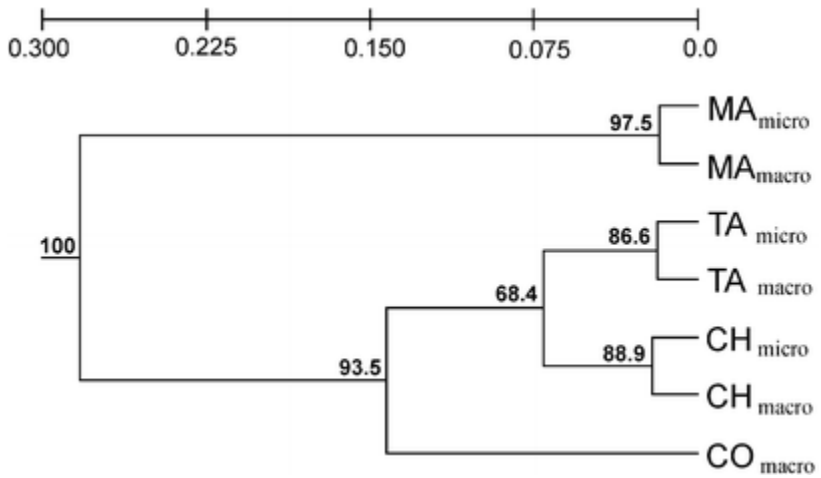

Fig. 2. Cluster analysis (UPGMA) of the different morphs (macro- and microgynes) in different populations based on Reynold's coancestry distance computed from allele frequencies at four microsatellite loci. *Numbers* over the nodes indicate bootstrapping values in percent

Significant genetic structuring was found at the colony level: overall, the average queen-queen relatedness within colonies was r=0.400±0.036 (SE). The relatedness coefficient among macrogynes (r=0.438±0.038) was slightly higher than the relatedness coefficient among microgynes (r=0.365±0.089) or between macro- and microgynes (r=0.351±0.079) but differences were not significant (Table 4).

Table 4. Queen-queen relatedness in colonies of *L. rugatulus*. 95% CIs were obtained by jackknifing over colonies (*n* number of colonies, *N* number of individuals)

Relatedness between	Coefficient of relatedness	95% CI	*n*	*N*
All queens	0.400	0.329-0.471	102	379

Relatedness between	Coefficient of relatedness	95% CI	*n*	*N*
Macrogynes	0.438	0.3626-0.514	59	190
Microgynes	0.365	0.183-0.547	30	134
Macrogynes and microgynes	0.351	0.188-0.514	25	122

Intercolonial relatedness was not correlated to spatial distance in any of the three compared subpopulations (WTC: r_{matrix}=-0.07, n=15, P>0.1; MA: r_{matrix}=-0.08, n=21, P>0.1; TA: r_{matrix}=0.001, n=32, P>0.1) at the scale investigated (Fig. 1).

The tests whether queens were more likely to be unrelated than related as mother-daughters or full-sisters to the rest of their colony were significant for 4 macrogynes (out of 40) and no microgyne (out of 4) in MA, and for 1 macrogyne (out of 50) and 5 microgynes (out of 51) in TA. The ratios of macrogynes to microgynes did not differ significantly from the expected values derived from the subpopulation frequencies (x^2_{MA}=0.82, P=0.36; x^2_{TA}=1.68, P=0.19; combined probability: P>0.1).

The genetic composition of monogynous colonies revealed strong differences between the three compared groups ($x^2_{max.lik.}$=13.8, df=4, P<0.01; Table 5): the genotypes of workers could not be explained by the present queen (as single foundress) in any of the six microgynous colonies, whereas this was the case in at least half of the macrogynous colonies from WTC. As we cannot rule out multiple mating in this species, macrogynous colonies from NBL and TA are intermediate.

Table 5. The most parsimonious explanations of worker genotypes suggest different modes of colony founding in macrogynous and microgynous colonies

Worker genotypes compatible with	Microgynous colonies from NBL and TA	Macrogynous colonies from NBL and TA	Macrogynous colonies from WTC
Independent founding	0	0	3
Multiple mating	0	2	2
Dependent founding	6	4	1

DISCUSSION

Species consistency

Allozyme and microsatellite allele frequencies both indicate that the separation of the gene pools between macro- and microgynes of *L. rugatulus* is weaker than that between different populations. This supports our hypothesis that both morphs belong to the same species, the hypothesis being initially based on the production of similar males and workers by macro- and microgynes and their overall morphological resemblance (Rüppell et al. *1998*). Thus, queen size dimorphism in *L. rugatulus* is one of the rare cases of alternative reproductive morphotypes in females of one species (Gross *1996*; Rüppell and Heinze *1999*).

Reproductive polymorphism commonly leads to reproductive isolation and thus sympatric speciation (Coyne and Orr *1998*) which has also been proposed for the case of microgyny in ants (Buschinger *1990*; Bourke and Franks *1991*). Given species identity in *L. rugatulus* (and consequently the nesting of "morph-groups" within populations), a slight but significant genetic differentiation between morphs was apparent. This indicates the potential for sympatric speciation in this case, but gene flow by males may maintain species integrity. Comparisons between mitochondrial and nuclear genetic structures of ant populations have been successfully employed to identify male gene flow and separation between alternative social forms (Ross and Shoemaker *1997*; Ross et al. *1997*), whereas studies confined to the nuclear level have mostly failed to find any differentiation (e.g., Seppä *1994*). Thus, to evaluate the prospects for speciation further, investigations of mtDNA population structure combined with determination of the heritability of queen morphology are needed.

Alternative tactics

Direct observations of colony-founding behavior are difficult to obtain for most ant species. Consequently, only for *M. ruginodis* (Brian and Brian *1955*) is there direct evidence that microgyny is linked to dependent colony founding. In all other queen-size-polymorphic species, the conclusions about alternative reproductive tactics are drawn from indirect evidence (e.g., Janzen *1973*; McInnes and Tschinkel *1995*; DeHeer and Tschinkel *1998*). This study provides five separate lines of indirect evidence, which together strongly support the view that microgynes and macrogynes are specialized for and employ predominantly alternative reproductive tactics:

1. Relatively more macrogynes than microgynes were found in monogynous colonies. As monogynous colonies mostly originate by independent colony founding of one self-contained queen, and polygynous colonies arise mainly by adoption of additional queens into existing colonies (Hölldobler and Wilson *1977*, *1990*; Bourke and Franks *1995*), the correlation between queen morphology and social colony type provides evidence that macrogynes establish independent colonies more frequently. This was further supported by the genetic investigations of monogynous colonies which suggested that microgynes become queens in monogynous colonies only by budding or the death of their nestmate queens, whereas at least some of the macrogynes found independently. However, we want to stress that the behavior of macrogynes seems to be plastic or at least uncoupled from their morphology to some extent.
2. The ratio of 20 putative founding colonies with a macrogyne to only 1 with a microgyne cannot be explained by the higher abundance of macrogynes. Thus, the most parsimonious explanation is that macrogynes are more likely to initiate colonies independently, or that they are more successful in doing so.
3. Although sample sizes were small, there is evidence that macrogynes are more likely than microgynes to be trapped in flight traps during swarming. This suggests that macrogynes show higher flight activity during mating and/or dispersal flights. Both are typically correlated with each other and with independent colony founding (Bourke and Franks *1995*), while readoption into the mother nest requires restricted flight activity (e.g., Sundström *1995*), as relocating the natal nest over distance may be extremely difficult after swarming.
4. Generally, polygyny is a good indicator of dependent colony founding (Hölldobler and Wilson *1977*; Keller *1991*; Bourke and Franks *1995*). Thus, the degree of polygyny of

macrogynes at a given sample site identifies the tendency of *L. rugatulus* queens at this site to engage in dependent colony founding, irrespective of body size. This behavioral decision may be regarded as an adaptive response to environmental conditions. Its positive correlation with the frequency of microgynes indicates that both dependent colony founding and microgyny are favored by similar environmental conditions. This corresponds well to the hypothesis that microgynes constitute an adaptation to a dependent mode of colony founding.

5. Macrogynes are physiologically adapted to independent colony founding because they harbor more body reserves prior to mating and colony establishment. In a cross-species comparison (Keller and Passera *1989*) macrogynes of *L. rugatulus* classify as independent foundresses, while the relative fat content of microgynes is comparable to species that found dependently. Purely allometric effects can be ruled out on the basis of the fact that in neither males nor within microgynes are relative fat content and body size correlated.

Dependent colony founding

Some of the arguments employed above have implied that microgynes are specialized for secondary polygyny by readoption. However, we have so far only argued for their dependent mode of colony founding which could involve intraspecific social parasitism or secondary polygyny by readoption or both. In *S. invicta*, microgyny has been viewed as a parasitic tactic (Tschinkel *1996*) and for *L. rugatulus* also, we have argued previously (Rüppell et al. *1998*) that microgynes might be efficient "searchers" of unrelated host colonies. Their low flight activity in the field contradicts this hypothesis,and, in general, *L. rugatulus* resembles more the facultatively polygynous species for which the link between microgyny and readoption has been shown (*Camponotus yamaokai*: Satoh et al. *1997*) or suggested (*M. ruginodis*: Elmes *1991*). The high intracolonial queen-queen relatedness revealed in our study supports the view that most dependently founding macro- and microgynes are readopted into their natal colonies. Although occasional adoptions of unrelated queens seem to occur as in other facultatively polygynous ant species (e.g., Stille and Stille *1992*), no significant differences in relatedness were found between macro- and microgynes. Thus, microgyny seems not to be a special adaptation to intraspecific social parasitism.

Nevertheless, the slightly negative correlation between colony size and number of microgynes (controlling for total queen number) can be interpreted as negative effects of microgynes on colony fitness. While the term "social parasite" seems too strong in this case, microgynes might be selfishly pursuing their own interests in conflict with the rest of the colony, an idea that has been suggested on theoretical grounds (Bourke and Ratnieks *1999*). However, adaptive differences in colony dynamics (e.g., budding) provide an alternative explanation.

Ecological parameters

This study is the first to investigate systematically social and genetic structure in relation to queen morphology in a queen-size-dimorphic ant species in several populations. As morph frequencies and the social structure of *L. rugatulus* colonies varied strongly among subpopulations, we conclude that studying a number of populations that differ in their ecological conditions (Travis *1994*) is very important for understanding the evolutionary ecology of any species.

Dispersal polymorphism in female ants is linked to habitat patchiness (Heinze and Buschinger *1989*; Heinze *1993*; Heinze and Tsuji *1995*) and in *L. rugatulus*, this patchiness is twofold: at a large scale (>50 km), mixed forests on mountain ranges that constitute suitable habitats are patchily distributed in the semideserts of southwest North America ("sky islands"; Heald *1951*), and at a finer scale (<5 km), patches with suitable nest sites seem to be separated by largely uninhabitable areas (Rüppell et al.; unpublished data). Presumably, macrogynes are better colonizers, whereas microgynes may establish new colonies more competitively within high-density patches by budding, and profit from stable nesting substrate (rocky outcrops) which makes potential inheritance of the mother colony (Nonacs *1988*) more rewarding. At the metapopulation level, the polymorphism may be balanced by the contrasting selection within and between populations (Olivieri et al. *1995*) and different ecological conditions in different locations.

The sharp clines in morph frequencies without dispersal barriers in *L. rugatulus*, and other species (e.g., Hamaguchi et al. *1998*), indicate strong differential selection, probably by mutual competitive exclusion.

Consequences for population genetics

A clear effect of the alternative reproductive tactics and correlated social colony structure was expected on population viscosity. Increased viscosity in polygynous ants has been demonstrated in other species at a larger spatial scale (Seppä and Pamilo *1995*; Chapuisat et al. *1997*) but in our study no isolation-by-distance effects were detected in any of the three subpopulations. This homogenous population structure might be due to long-range budding or gene flow via males. We favor the latter explanation because *Leptothorax* ants are not very mobile on foot (Herbers *1984*; Heinze et al. *1996*) and the random breeding structure allows for substantial gene flow via the males if females are philopatric. Similar weak nuclear differentiation between social forms opposed to strong mitochondrial differentiation has also been reported from *L. acervorum* (Stille et al. *1991*; Stille and Stille *1993*) and *S. invicta* (Ross and Shoemaker *1997*).

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