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Polymorphic social organization in an ant

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Identifying species exhibiting variation in social organization is an important step towards explaining the genetic and environmental factors underlying social evolution. In most studied populations of the ant *Leptothorax acervorum*, reproduction is shared among queens in multiple queen colonies (polygyny). By contrast, reports from other populations, but based on weaker evidence, suggest a single queen may monopolize all reproduction in multiple queen colonies (functional monogyny). Here we identify a marked polymorphism in social organization in this species, by conclusively showing that functional monogyny is exhibited in a Spanish population, showing that the social organization is stable and not purely a consequence of daughter queens overwintering, that daughter queen re-adoption is frequent and queen turnover is low. Importantly, we show that polygynous and functionally monogynous populations are not genetically distinct from one another based on mtDNA and nDNA. This suggests a recent evolutionary divergence between social phenotypes. Finally, when functionally monogynous and polygynous colonies were kept under identical laboratory conditions, social organization did not change, suggesting a genetic basis for the polymorphism. We discuss the implications of these findings to the study of reproductive skew.

Keywords: colony structure; functional monogyny; *Leptothorax acervorum*; multiple queen; polygyny; reproductive skew

1. INTRODUCTION

Animal societies vary greatly in their social organization and explaining why such variation exists and to what extent genetic change underpins this variation are important goals of evolutionary biology. Reproductive skew, the degree to which reproduction is partitioned among individuals within social groups, is a fundamental aspect of social organization and a large body of theory has been developed to explain variation in skew (e.g. Emlen 1982; Vehrencamp 1983*a*,*b*; Reeve & Ratnieks 1993; Reeve *et al.* 1998; Johnstone 2000). Eusocial Hymenopteran species with multiple queen colonies have become popular models to investigate variation in skew and to test the predictions of skew theory (e.g. Keller & Reeve 1994; Reeve *et al.* 1998; Bourke 2001; Reeve & Keller 2001; Hammond *et al.* 2006).

Skew within multiple queen colonies is known to vary widely among species (see Keller 1993; Bourke & Franks 1995; Keller 1995), although relatively little variation has been found within species (e.g. Field *et al.* 1998; Reeve *et al.* 2000; Fournier & Keller 2001; Seppa *et al.* 2002; Sumner *et al.* 2002; Hannonen & Sundstrom 2003; Nonacs *et al.* 2004; Liebert & Starks 2006). In ants with multiple queen colonies, the majority of species have low skew as reproduction is partitioned fairly evenly among queens; a situation known as polygyny. However, there are species in which a single queen monopolizes all reproduction in multiple queen colonies. This rare social organization, termed functional monogyny (Buschinger 1968), has been reported in just a handful of ant species: *Formicoxenus hirticornis* (Buschinger 1979); *Formicoxenus nitidulus* (Buschinger & Winter 1976); *Formicoxenus provancheri* (Buschinger 1980; Heinze *et al.* 1993); *Leptothorax gredleri* (see Heinze *et al.* 1992; Lipski *et al.* 1992); *Leptothorax* species A (see Heinze & Buschinger 1989; Heinze & Smith 1990) and *Leptothorax sphagnicolus* (see Buschinger & Francoeur 1991).

Functional monogyny has also been reported in multiple queen colonies of the ant *Leptothorax acervorum* (Ito 1990; Seppa *et al.* 1995; Felke & Buschinger 1999). This is intriguing as studies of UK and central European populations show multiple queen colonies to be polygynous (low skew) based on strong and comprehensive evidence, including data on egg maternity (Hammond *et al.* 2006), low nestmate relatedness (Douwes *et al.* 1987; Stille *et al.* 1991; Chan & Bourke 1994; Heinze *et al.* 1995*a*,*b*; Bourke *et al.* 1997; Hammond *et al.* 2001), queen ovary development, and behaviour (Buschinger 1968; Bourke 1991, 1993; Heinze *et al.* 1995*b*). By contrast, the evidence for functional monogyny is weaker. Ovary dissections and observations of colonies suggest that a single queen is reproductive in multiple queen colonies from a population in Spain (Felke & Buschinger 1999) and that reproduction is heavily biased towards one queen in multiple queen colonies from Japan (Ito 1990). In addition, two genetic studies using allozyme data revealed high relatedness among workers in populations from Finland (Seppa *et al.* 1995) and Spain (Heinze *et al.* 1995*a*), but crucially queen number and mating status in the studied colonies was unknown. It has been suggested that the presence of non-reproductive queens may be because newly mated queens are simply overwintering before dispersing, rather

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than a stable social organization, and that functionally monogynous populations may be a different species (Felke & Buschinger 1999).

Altogether these studies tentatively suggest that *L. acervorum* may exhibit a marked polymorphism in social organization, with low skew in some populations because queens share reproduction equally (polygyny) and high skew in others because reproduction is monopolized by a single queen (functional monogyny). The identification of such a polymorphism would be very interesting because it would have important implications for our understanding of the evolution of social organization and the genetic underpinning of such variation, but more solid data supporting functional monogyny are needed. Furthermore, a polymorphism in such an important aspect of social organization as reproductive skew has not been described in any other animal species.

The aim of this study is fourfold. First, to confirm functional monogyny in the Spanish population described by Felke & Buschinger (1999) using a detailed genetic analysis of colony kin structure. Second, to investigate whether functional monogyny is temporally stable in the field. Third, to investigate environmental influences on colony social organization (polygyny and functional monogyny) by keeping field collected colonies in a common laboratory environment. Finally, to infer the evolutionary history of social organization by investigating the genetic relationships between populations described as polygynous and putatively functionally monogynous.

2. MATERIAL AND METHODS

(a) *Colony collection and maintenance*

Leptothorax acervorum populations consist of both single queen and multiple queen colonies. We sampled colonies from a potentially functionally monogynous population: Orihuela del Tremendal, Sierra de Albarracin, Spain, in 2004 (OT04) and 2006 (OT06). In 2004, we sampled in June, before eclosion of sexual offspring, and in 2006, we sampled in October, after eclosion of sexual offspring and mating. We also sampled colonies from a known polygynous population in Sherwood Forest, UK, in March and October 2007. To increase the geographical spread of populations sampled for our study of genetic relationships among populations, we collected colonies from an additional five populations: Valdelinares, Spain (V); Solvorn, Norway (SO); Umea, Sweden (UM); Tvarminne, Finland (TV) and Vaasa, Finland (VN). We also used workers previously collected from colonies in Santon Downham, UK (SD) (Hammond *et al.* 2006).

Colonies were found in cavities in partially decayed twigs on the ground of coniferous forests and were removed from twigs within 5 days. As whole twigs were collected, it was likely that all queens and the vast majority of workers were collected. Scandinavian (SO, UM, TV, VN) colonies were stored in 75 per cent ethanol for later genetic analysis. Spanish (OT) and UK (SF) colonies were transferred to laboratory nests, censused (see electronic supplementary material, table S1), kept in identical conditions in environmental chambers (Sanyo MLR-351H) and fed chopped meal worms and dilute honey solution two to three times per week. OT and SF colonies collected in October were kept in autumn conditions (light/dark: 14 h/10 h, temperature: 20°C/10°C, humidity: 80/70%) for eight weeks, winter conditions (light/dark: 13 h/11 h, temp.: 10°C/0°C, humidity: 60%) for six weeks, then transferred to spring conditions (light/dark: 14 h/10 h, temp.: 20°C/10°C, humidity: 80/70%) for eight weeks (A. Buschinger 2003, personal communication). SF colonies collected in March did not overwinter. During spring, we monitored colonies (OT: $n = 44$, SF (October): $n = 5$, SF (March): $n = 9$) to determine the number of queens showing reproductive activity once egg-laying began. We classified queens with enlarged (physogastric) abdomens and occupying a central position among nestmates as reproductive.

(b) *Colony sampling*

From the OT04 collection, four workers from each of 19 colonies (13 multiple queen and six single queen) were removed and frozen (−20°C). In the OT samples, we classed multiple queen colonies as those with multiple dealate queens. From the OT06 collection, 15 colonies (11 multiple queen and four single queen) were randomly selected and frozen immediately after removal from the twig to provide a snap-shot of colony social structure upon collection (referred to as 'snap-shot' colonies). From the remaining OT06 colonies we removed and froze (−20°C) samples of workers (range = 4–12 per colony) and larvae (range = 3–8 per colony) from 60 colonies (42 multiple queen, 17 single queen and one queenless) for genetic analysis of colony social structure. Larvae were categorized as being small (first instar to half-grown larvae) or large (fully grown larvae to pre-pupae). To investigate genetic relationships among populations using mtDNA and nDNA, we sampled one worker per colony from a sample of colonies (colonies sampled per population, mtDNA: OT = 7, V = 6, SF = 8, SD = 7, SO = 5, UM = 5, VN = 6, TV = 3; nDNA: OT = 7, V = 3, SF = 6, VN = 3, TV = 2).

(c) *Dissection*

In the 11 snap-shot multiple queen colonies, we dissected the ovaries of all dealate queens ('queens' from hereon; $n = 81$ queens, range = 2–16 per colony). Mated queens had an opaque spermatheca (sperm filled), whereas unmated queens had a transparent spermatheca. We classified ovarian development into: A = elongated ovarioles each with large yolk-filled eggs and large numbers of corpora lutea; B = shorter ovarioles with less than five yolk-filled eggs and some corpora lutea; C = short ovarioles, small eggs and no corpora lutea; and D = very short ovarioles with no yolky eggs and no corpora lutea. The length of ovaries was scored relative to the size of the spermatheca (see electronic supplementary material, figure S2).

(d) *Molecular methods*

We extracted DNA by grinding ants in 200 µl (queens, workers and large larvae) or 50 µl (small larvae) of 10 per cent Chelex solution (10 mM Tris–HCl, pH 7.5) followed by heating for 10 min at 100°C.

(i) *Microsatellite genotyping*

Individuals were genotyped at three (OT04, not LXAGA2) or four (OT06) polymorphic microsatellite loci: LXAGT1, LXAGA1, LXAGA2 (Bourke *et al.* 1997) and L18 (Foitzik *et al.* 1997) with allele sizes determined by reference to an internal standard (GenomeLab standard-400) using a Beckman Coulter CEQ 8000. Only individuals genotyped at two or more (OT04 cols) and three or more (OT06 cols) loci were analysed (OT04/OT06: 100/86% of individuals; mean

number of loci per individual = 2.65/3.85). From OT04, four workers per colony from 19 colonies were genotyped. From OT06, individuals from 75 colonies (53 multiple queen, 21 single queen and one queenless) were genotyped with an average of 7.3 workers per colony ($n = 70$ colonies; range = 3–11) and 5.0 larvae per colony ($n = 55$ colonies, range = 1–12). In the majority of colonies (50/75), both workers and larvae were genotyped. Larval sex was determined by ploidy with individuals having one allele at all genotyped loci classified as male. The likelihood of misclassifying diploids as haploids was low as only 1.4 per cent of diploids (workers: $n = 511$) were homozygous at three loci, and none were homozygous at four loci. We found 72 per cent of larvae were diploid ($n = 54$ colonies; diploids: mean = 3.6 per colony; range = 0–11; haploids: mean = 1.4 per colony; range = 0–6). From the 11 snap-shot multiple queen colonies, all queens were genotyped ($n = 81$; mean = 7.4 queens per colony; range = 2–16).

(ii) *Siblingship, relatedness analysis and queen turnover*

We investigated the siblingship of all workers and larvae genotyped from OT06 colonies ($n = 75$) using the program Colony (Wang 2004) to group individuals into fullsibling families assuming that queens mate singly (Hammond *et al.* 2001). In this analysis, we set the level of allelic dropout and genotyping errors equal to 0.05 for each locus. We checked whether maternal genotypes generated by Colony matched observed queen genotypes for each fullsibling family in the 11 snap-shot multiple queen colonies.

We calculated regression relatedness (Queller & Goodnight 1989) between various parties from OT colonies using the program Relatedness 5.08 (available from: http://www.gsoftnet.us/GSoft.html). We estimated population allele frequencies with individuals weighted equally and allele frequency bias corrected by colony. Standard errors were estimated by jackknifing over colonies. Statistical significance between relatedness estimates were analysed using Mann–Whitney U tests and between relatedness estimates and expected point values by seeing if expected point values fell outside 95 per cent confidence limits.

From OT06 colonies, we estimated queen turnover by comparing relatedness, within and between, small diploid larvae and adult workers using equation four in Pedersen & Boomsma (1999). Only colonies (21 multiple queen and eight single queen) with multiple small diploid larvae (multiple queen: mean = 3.5 per colony; range = 2–7; single queen: mean = 4 per colony; range = 2–6) and multiple workers (multiple queen: mean: 7.5 per colony; range = 4–10; single queen: mean = 7.5 per colony; range = 6–8) were used.

(iii) *Genetic relationship among populations*

We PCR amplified (see electronic supplementary material, S3) a region of the mitochondrial cytochrome *b* gene (cyt*b*) using primers CB1 and tRs (Simon *et al.* 1994). We also PCR amplified (see electronic supplementary material, S3) a region of the nuclear encoded cGMP-activated protein kinase gene (*foraging*) using primers designed from published sequences (Ingram *et al.* 2005, GenBank: AY800387). *Foraging* sequence trace files were inspected for heterozygotes and sorted into alleles. As the majority of individuals in all populations were homozygous for *foraging* allele H1 (see §3), we inferred other alleles (H2–H5) by subtracting the H1 allele. We aligned sequences using ClustalW in MEGA 4.0 (Tamura *et al.* 2007). For mtDNA data, we constructed neighbour-joining trees using MEGA 4.0 and investigated the robustness of tree topology using 1000 bootstrap re-samples of the data.

3. RESULTS

(a) *Dissection*

Ninety-six per cent of queens in the 11 snap-shot multiple queen colonies were mated (70/73, eight undetermined because of dissection errors) with an average of 6.4 mated queens per colony (range = 2–14). In all colonies, only one queen per colony had type A ovarian development and all such queens were mated. All remaining queens had either type C or D ovaries (none possessed type B ovaries).

(b) *Molecular analysis*

(i) *Siblingship*

In all 75 colonies, the majority of workers and larvae (range = 50–100%) were full sisters (figure 1) as they were assigned to the same fullsibling family ('the majority fullsibling family' from hereon). An average of 90 per cent of workers and larvae per colony grouped into the majority fullsibling family with a mean of 1.5 fullsibling families per colony (range = 1–5). Importantly, there was no significant difference between multiple and single queen colonies in the proportion of workers and larvae assigned to the majority fullsibling family (figure 1: Fisher's exact test: d.f. = 4; $p = 0.90$), showing that multiple and single queen colonies have the same colony sibling structure.

In nine of the 11 snap-shot multiple queen colonies the observed genotype of the type A queen matched the maternal genotype predicted by Colony for the majority fullsibling family (table 1). In colony A09_1910, the type A queen's genotype matched the predicted maternal genotype of a single larva, whereas in colony B13_1710 the type A queen's genotype matched no genotyped colony member. All queens with type C or D ovaries did not match the predicted maternal genotype of any worker or larvae, in fact, 86 per cent of these queens were assigned to the majority fullsibling family. In colonies A09_1910, B13_1710, B17_1810 and B19_1810, a number of type C or D queens (two, three, one and two queens per colony) were full sisters of the type A queen (table 1).

(ii) *Relatedness*

Within colony relatedness ($r \pm$ s.e.) was high in OT samples (see electronic supplementary material, table S4). In OT04 multiple queen colonies, the average relatedness among workers (0.83 ± 0.05, $n = 13$ colonies) was not significantly different from 0.75, nor different to worker relatedness in single queen colonies (0.83 versus 0.76; $U = 30$, $n_1 = 14$, $n_2 = 6$, $p = 0.46$). In OT06 multiple queen colonies, the average relatedness among workers (0.64 ± 0.02, $n = 48$ colonies/349 ind.) was significantly lower than 0.75, whereas relatedness among larvae (0.70 ± 0.02, $n = 38$ colonies/151 ind.) was not significantly different from 0.75, but there was no significant difference between worker and larvae relatedness (0.64 versus 0.70; $U = 747$, $n_1 = 48$, $n_2 = 38$, $p = 0.15$). Like OT04, importantly there was no difference in worker relatedness in OT06 multiple and single queen

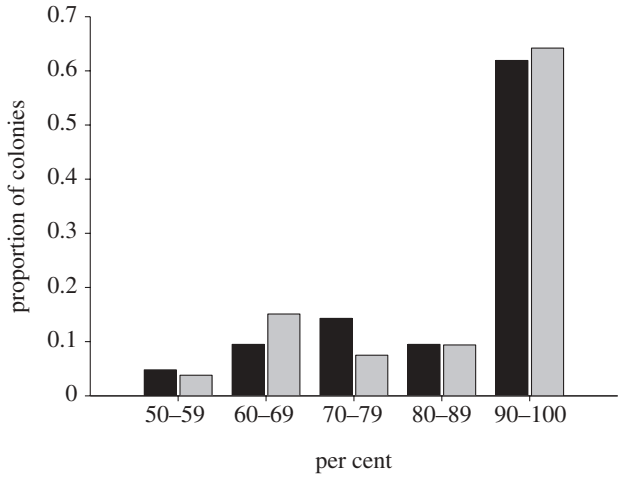


Figure 1. Variation between multiple queen (black bars, $n = 53$) and single queen (grey bars, $n = 21$) colonies in majority fullsibling family assignment. Categories are the percentage of workers and larvae in each colony assigned to the majority fullsibling family.

colonies (0.67 versus 0.64; $U = 404$, $n_1 = 48$, $n_2 = 21$, $p = 0.19$) or larvae (0.66 versus 0.70; $U = 217$, $n_1 = 38$, $n_2 = 12$, $p = 0.81$). In OT06 multiple queen colonies, the average relatedness of workers to larvae (0.65 ± 0.02, $n = 37$ colonies/274W-132L) was significantly lower than 0.75, a difference most probably explained by the few workers and larvae that did not belong to the majority fullsibling family.

In the snap-shot multiple queen colonies, the average within colony relatedness among mated queens was 0.59 ± 0.05 ($n = 11$ colonies/70 ind.). Siblingship analysis indicated that most type C or D queens were full sisters and daughters of the type A queen. Accordingly, one would expect the average relatedness among nestmate type C or D queens to approach that between full sisters ($r = 0.75$) and relatedness between type C or D queens and type A queens to approach that expected for mother–offspring ($r = 0.5$). Observed values agreed with these predictions as relatedness between type C or D queens (0.64 ± 0.06; $n = 9$ colonies/57 ind.) did not differ significantly from 0.75 and the relatedness of type C or D queens to type A queens (0.41 ± 0.04; $n = 11$ colonies/59Q^{CD}- 11Q^{A}) did not differ significantly from 0.5, but the two values differed significantly from each other (0.64 versus 0.41; $U = 3$, $n_1 = 8$, $n_2 = 10$, $p = 0.019$). The average relatedness of workers to type A queens (0.39 ± 0.08; $n = 9$ colonies, 68W-9Q^{A}) and larvae to type A queens (0.41 ± 0.06; $n = 11$ colonies, 51L-11Q^{A}) was not significantly different from that expected between daughters and mothers ($r = 0.5$). The average relatedness of workers to type C or D queens (0.66 ± 0.03; $n = 9$ colonies, 68W-48Q^{CD}) and larvae to type C or D queens (0.65 ± 0.05; $n = 11$ colonies, 51L-59Q^{CD}) was significantly higher than 0.5 but lower than 0.75. The lower than expected value is most likely explained because of the few workers and larvae that did not belong to the majority fullsibling family.

(iii) *Queen turnover*

Queen turnover was 19.7 per cent in multiple queen colonies ($n = 21$), 11.3 per cent in single queen colonies ($n = 8$), and 17.3 per cent for multiple and single queen colonies combined. Given that small larvae most likely arise from eggs laid in the year of collection, these queen turnover estimations are directly comparable to those reported in Hammond *et al.* (2006). Our estimation of queen turnover is considerably less than that reported by Hammond *et al.* (2006) and by Bourke *et al.* (1997) in a low skew UK population of *L. acervorum*.

(iv) *Genetic relationship among populations*

Alignment of 685 bp of cyt*b* from 47 workers revealed 17 unique haplotypes (figure 2). Tree building showed that the majority of haplotypes (76%) grouped into a single clade with a high bootstrap support. Within this clade, branch lengths were short with an overall uncorrected distance of just 0.5 per cent, and importantly the haplotypes from the OT population were scattered throughout this clade with no evidence that high and low skew populations were genetically distinct from one another. In fact, one haplotype was found both in known low skew populations (SD and SF) and in the high skew population (OT). Furthermore, the largest distance between a high skew OT haplotype and a haplotype from a well-studied low skew population, SD (Hammond *et al.* 2006), was the same as that between the two most divergent haplotypes within the low skew population, SD (both 0.6%). Alignment of a 287 bp fragment of the *foraging* gene from 21 workers revealed five alleles which differed by a maximum of four substitutions. In all populations, the same allele (H1) was at a high frequency (range 0.667–0.917) with every individual having at least one copy of this allele (see electronic supplementary material, table S5). In each geographical area (Spain, UK and Finland), we found area specific alleles, but these differed by only one to three substitutions from H1. The sharing of the same allele in all populations, and the minimal sequence differences among all alleles therefore provide additional evidence for a close genetic relationship between populations.

(c) *Colony observations*

Out of 44 OT multiple queen colonies overwintered in the laboratory (mean number of queens per colony = 10.7 ± 1.6; range = 2–30), 37 colonies had just a single queen that showed signs of reproductive activity in the eight weeks of observation. In the remaining seven colonies, no queens showed evidence of reproductive activity and laying was not observed. By contrast, in all 14 SF multiple queen colonies (mean number of queens per colony = 6.0 ± 1.0; range = 2–13), more than one queen per colony showed signs of reproductive activity during the eight weeks of observation (average percentage of queens showing reproductive activity = 80%; range = 24–100%).

4. DISCUSSION

Our data show that the Spanish population of *L. acervorum* studied is functionally monogynous. Dissections showed that in colonies with multiple dealate queens, most queens were mated but only one of them had developed ovaries and showed signs of recent egg laying (type A queens). Confirming this, workers and larvae within multiple queen colonies were highly related,

Table 1. Siblingship analysis of the snap-shot multiple queen colonies. (Genotypes of queens (Q), workers (W) and larvae (L) grouped into fullsibling families. In 'fullsibling family membership', the number of each type is in brackets (e.g. L(4) = 4 larvae) and letter superscripts show ovarian class of queens (e.g. Q^A) (see text). 'Q^A genotype match' shows which family's predicted maternal genotype matches the observed Q^A genotype.)

	number genotyped			fullsibling family membership					
colony	Q	W	L	majority fullsibling family	2	3	4	5	Q^A genotype match
A08_1710	7	—	6	Q^C(2), Q^D(4), L(4)	Q^A	L(2)			majority fullsibling family
A09_1910	8	8	5	Q^C(2), Q^D(3), W(8), L(4)	Q^A, Q^C, Q^D	L			family 3
A10_1910	2	8	2	Q^C, W(7), L(2)	Q^A	W			majority fullsibling family
A14_1910	4	8	5	Q^C, Q^D(2), W(8), L(5)	Q^A				majority fullsibling family
B02_1710	9	7	6	Q^C(4), Q^D(4), W(7), L(6)	Q^A				majority fullsibling family
B04_1910	9	8	5	Q^C(3), Q^D(4), W(8), L(5)	Q^A	Q^C			majority fullsibling family
B11_1810	11	7	6	Q^C(10), W(6), L(6)	Q^A	W			majority fullsibling family
B13_1710	7	—	6	Q^C, L(4)	Q^A, Q^C(2), Q^D, L	Q^D	Q^C	L	none
B17_1810	16	8	11	Q^C(2), Q^D(12), W(4), L(11)	Q^A, Q^D, W(4)				majority fullsibling family
B18_1710	5	6	3	Q^C(2), Q^D(2), W(4), L(2)	Q^A	W(2), L			majority fullsibling family & 3
B19_1810	3	8	6	W(6), L(6)	Q^A, Q^C, Q^D, W	W			majority fullsibling family

and siblingship analysis showed that the majority of colony members (including type C and D queens) grouped into a single 'majority fullsibling family'. Furthermore, the type A queen was, in most cases, genetically compatible with being the mother of the majority fullsibling family (exceptions discussed below), and no type C or D queen was compatible with being the mother of any other colony member. These data confirm a previous report of functional monogyny (Felke & Buschinger 1999).

Our data also reveal that functional monogyny is temporally stable and not solely the consequence of daughter queens overwintering before dispersal (Felke & Buschinger 1999). First, both worker relatedness and dealate queen number were high in samples collected in both early summer (OT04) and late autumn (OT06). Second, in two colonies (B17_1810 and B19_1810), the type A queen was a member of a fullsibling family (table 1) that included other queens and workers. Interestingly, in both colonies, the type A queen was also the mother of the majority fullsibling family, which included, at least in one case (B17_1810), mated daughter queens. Given that it takes 2 years for queens to develop from egg to adulthood (see Heinze *et al.* 1995*b*), this means that mated queens can remain non-reproductive within their natal colonies for at least 2 years. Third, queen turnover (19.7%) was lower than the rate estimated in polygynous *L. acervorum* populations (Bourke *et al.* 1997; Hammond *et al.* 2006), and other ant species (e.g. Pedersen & Boomsma 1999; Bargum *et al.* 2007). Finally, the siblingship analyses showed that in the majority of snap-shot multiple queen colonies (7/11), the type A queens were assigned to a fullsibling family containing no other individuals and were the mother of the majority fullsibling family which also contained most type C or D queens. In addition to confirming a low level of queen turnover and a reproductive tenure of multiple years, this finding suggests daughter queen re-adoption is frequent.

The genetic data also showed that a very small number (13/210, 6%) of workers, larvae and non-reproductive queens did not belong to the type A queen fullsibling family and were not the offspring of any queen within the colony. Adult members of these families might be the offspring of queens lost because of queen turnover events (e.g. death or colony budding), or perhaps have drifted into non-natal nests, a credible explanation given the high population densities (up to four nests per square metre). It is less obvious, however, why a small number of larvae could not be attributed to either fullsibling family (mismatches at multiple loci discount genotyping errors and mutations). One possibility is that these are brood left behind during colony emigrations and later collected by workers of another colony (Hare 1996).

(a) *Social polymorphism in* Leptothorax acervorum

The social organization of our studied population strongly contrasts (table 2) with that reported from other polygynous *L. acervorum* populations (Stille *et al.* 1991; Chan & Bourke 1994; Heinze *et al.* 1995*a*,*b*; Bourke *et al.* 1997; Hammond *et al.* 2001, 2006). For example, in a UK population, Hammond *et al.* (2006) showed that in the majority of nests (70% of colonies, $n = 17$), skew was not significantly different from that expected if all queens reproduced equally. Moreover, our estimates of worker relatedness (0.64 and 0.83), which agreed with a previous estimate based on allozyme data from a Spanish population ($r = 0.72$; Heinze *et al.* 1995*a*), were much higher than the values calculated with microsatellites reported from multiple queen colonies in polygynous populations (e.g. UK: $r = 0.26$, Bourke *et al.* 1997; $r = 0.28$, Hammond *et al.* 2006; Germany: $r = 0.49$, Heinze *et al.* 2001).

We found limited genetic differentiation between the Spanish and UK populations at both mitochondrial and nuclear markers, suggesting that the two populations share a common history in the recent past. For the *foraging* gene (nDNA), we found an allele that was frequent in all populations, and in cyt*b* (mtDNA), we found no evidence

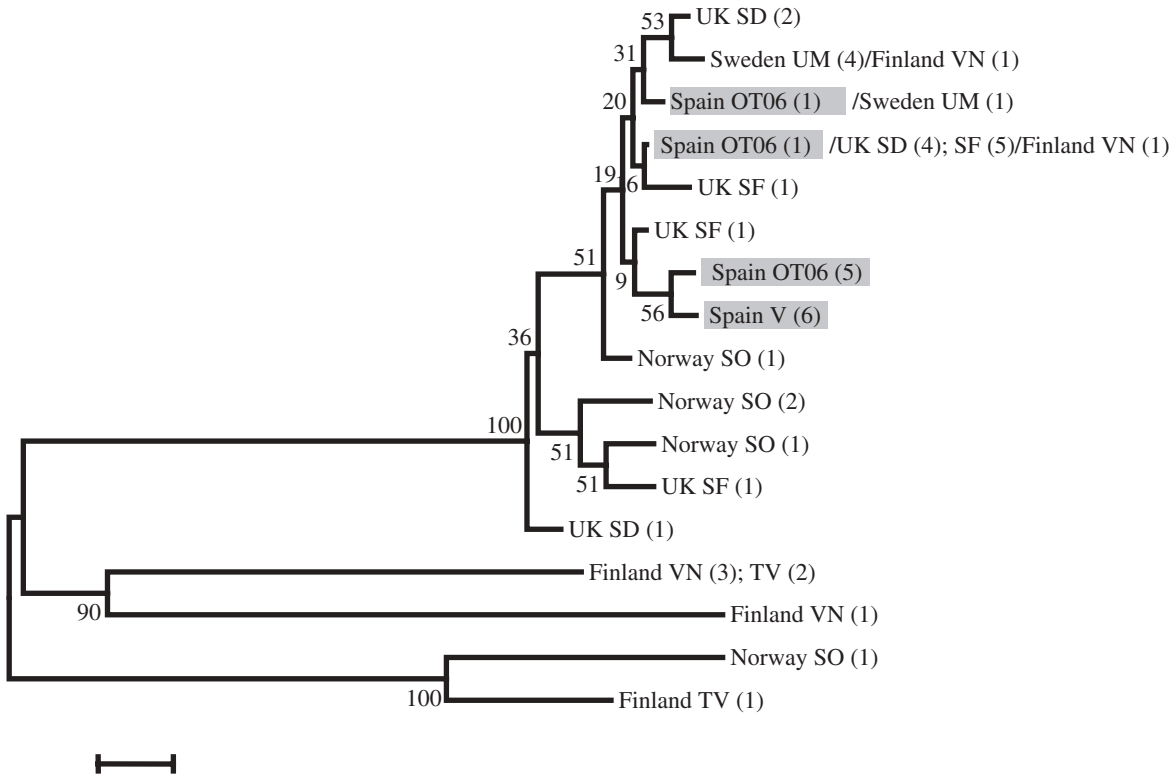


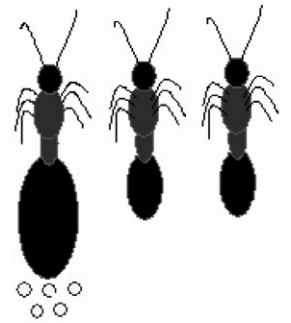
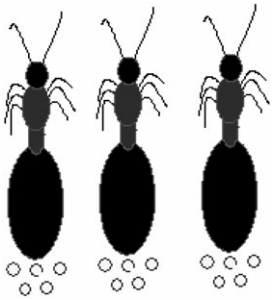
Figure 2. Neighbour-joining tree of the 17 haplotypes recovered from 685 bp of cytochrome *b*. Populations are: OT, Orihuela del Tremendal, Spain; V, Valdelinares, Spain; SD, Santon Downham, UK; SF, Sherwood Forest, UK; SO, Solvorn, Norway; UM, Umea, Sweden; TV, Tvarminne, Finland; VN, Vaasa, Finland. For each haplotype, we show the population(s) and in brackets the number of individuals in which it was found. The Spanish high skew populations are highlighted in grey and the scale bar shows 0.1% sequence divergence.

of haplotypes sorting into groups concordant with social organization or geography. Lack of mtDNA differentiation is particularly telling as mtDNA is sensitive to differentiation by drift because Ne is low on account of uniparental inheritance and haploidy. Furthermore, in ants with queen re-adoption, female dispersal is limited and so gene flow will probably have only a weak homogenizing effect on mtDNA haplotype frequencies, supporting recent shared history as the most likely explanation of limited differentiation between populations.

Two lines of evidence suggest that this social polymorphism is owing to genetic differences rather than plasticity in social phenotype. First, populations appear to show exclusivity in social phenotype, as in polygynous populations multiple queens always reproduce in nests containing several mated queens (Hammond *et al.* 2006), whereas we found only a single queen reproduced in nests containing several mated queens in the Spanish population. In addition, as the local environment almost certainly varied within the Spanish population, our finding of just functional monogyny further supports a genetic rather than plastic response. Second, in our common garden experiments both OT (functionally monogynous) and SF (polygynous) colonies were kept in a common laboratory environment during and after overwintering but this did not lead to a convergence in social organization. In none of the OT colonies did more than one queen reproduce after overwintering; by contrast, in all SF colonies, multiple queens showed signs of reproductive activity. Such stability does not support the hypothesis that social organization tracks current environmental cues, but points to a genetic difference. There are few cases of genetically based differences in social organization, but in the fire ant *Solenopsis invicta*, a single genomic element, marked by the odourant-binding protein gene *Gp-9*, is responsible for the existence of two distinct social forms (Keller & Ross 1998; Krieger & Ross 2002). This shows that a complex social phenotype can have a simple genetic basis, so a variation at a single genetic region or a quantitative genetic effect, are both possible explanations for the contrasting social organization in *L. acervorum*. That said, we cannot completely rule out complex explanations such as maternal effects, or social organization being environmentally influenced early in colony development in a fashion similar to that seen in the process of caste determination. Breeding studies are needed to show conclusively that polygyny and functional monogyny are heritable.

So far an important limitation of studies on reproductive skew has been the relatively low variance in reproductive skew within and between populations which reduces the power to identify social or ecological factors that affect skew (e.g. Field *et al.* 1998; Magrath & Heinsohn 2000; Sumner *et al.* 2002; Nonacs *et al.* 2004; Hammond *et al.* 2006; Liebert & Starks 2006). Ecological constraints on dispersal (Emlen 1982) have been considered important both in the evolution of polygyny, *per se* (Keller 1993), and in determining the level of skew among queens within colonies (Bourke & Heinze 1994; Keller & Reeve 1994; Reeve & Keller 2001). In our study, functionally monogynous colonies were restricted to sites above 1500 m in altitude, and nest density appeared patchy (R. J. Gill & R. L. Hammond 2006, personal observation). This seems to suggest that

Table 2. Comparison of the fundamental differences between a functionally monogynous Spanish population (this study) and well-studied polygynous UK populations.

population	present study: Spain	UK
social organization	functional monogyny	polygyny
skew	high (complete skew)	low
three-queen scenario		
description	single queen monopolizes all reproduction in a multiple queen colony	more than one queen shares reproduction in a multiple queen colony
worker relatedness	0.83 and 0.64	0.28[a]; 0.26[b]; 0.28[c]; 0.44[d]
queen relatedness	0.59	0.26[a]; 0.48[a]; 0.48[b]; 0.26[c]; 0.17[d]; 0.28[e]
queen turnover	19.7%	43–67.2%[a]

[a]Hammond *et al.* 2001.
[b]Bourke *et al.* 1997.
[c]Heinze *et al.* 1995*a*.
[d]Chan & Bourke 1994.
[e]Hammond *et al.* 2006.

constraints on dispersal are indeed high and so at least partly explain functional monogyny (Bourke & Heinze 1994). However, high ecological constraints should also select for the re-adoption of all daughter queens owing to high costs associated with solitary nest founding. We would therefore expect a higher proportion of multiple queen colonies in functionally monogynous populations (high skew) than in polygynous (low skew) populations. However, we found that the proportion of multiple queen colonies (61%) is within the range found in low skew UK populations (21–69%, Chan & Bourke 1994), suggesting no great difference in ecological constraints. In our experience, colonies in polygynous populations are, like in the functionally monogynous population, distributed patchily.

Concession models predict that skew should positively correlate with relatedness between potential reproductives (Vehrencamp 1983*a*; Reeve & Ratnieks 1993; Reeve & Keller 1997). In line with this prediction, we found that queen relatedness is higher in the functionally monogynous population than in polygynous populations (Chan & Bourke 1994; Heinze 1995; Hammond *et al.* 2001, 2006). Our data also fit Reeve & Keller's (1995) prediction that skew should be higher in societies comprising the mother and her offspring than in colonies comprising sisters as we found that non-reproductive queens are generally the daughters of the reproductive queen. However, queens in polygynous colonies are also related because of daughter queen re-adoption (Hammond *et al.* 2001), yet in these colonies re-adopted queens reproduce (Hammond *et al.* 2006). It therefore remains to be investigated whether the relationship of skew and relatedness is a consequence of skew rather than a cause. The contrast in skew between populations may be explained if a future breeding component is incorporated into skew models (Kokko & Johnstone 1999; Ragsdale 1999), and such models predict queuing for a reproductive position when individual survivorship is high (Kokko & Johnstone 1999). In line with this, daughter queens do supersede their mother in functionally monogynous colonies (table 2) and queen turnover is lower than in polygynous colonies, suggesting that differences in survivorship may underlie differences in skew.

More fundamentally, transactional skew models, which include concession models, assume that there is a social contract between group members. Thus, when model parameters such as constraints on solitary breeding vary the behaviour of group members is predicted to change. For instance, in concession models, if ecological constraints on solitary breeding reduce dominants they should concede more reproduction to subordinates (Reeve & Ratnieks 1993). However, the lack of variation in skew in the functionally monogynous Spanish population, despite almost certain variation in constraints on solitary breeding within populations, and that skew was not obviously changed when both functionally monogynous and polygynous colonies were kept in a common and importantly novel laboratory environment, suggests that behavioural adjustments are not made. Furthermore, the likely genetic polymorphism suggests that the level of skew is an evolved response rather than a behavioural one, an important issue that has previously been highlighted (Kokko 2003).

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