

It's good to be queen: classically eusocial colony structure and low worker fitness in an obligately social sweat bee

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Abstract

Lasioglossum malachurum, a bee species common across much of Europe, is obligately eusocial across its range but exhibits clear geographic variation in demography and social behaviour. This variation suggests that social interactions between queens and workers, opportunities for worker oviposition, and patterns of relatedness among nest mates may vary considerably, both within and among regions. In this study, we used three microsatellite loci with 12–18 alleles each to examine the sociogenetic structure of colonies from a population at Agios Nikolaos Monemvasias in southern Greece. These analyses reveal that the majority of colonies exhibit classical eusocial colony structure in which a single queen mated to a single male monopolizes oviposition. Nevertheless, we also detect low rates of multiqueen nest founding, occasional caste switching by worker-destined females, and worker oviposition of both gyne and male-producing eggs in the final brood. Previous evidence that the majority of workers show some ovarian development and a minority (17%) have at least one large oocyte contrasts with the observation that only 2–3% of gynes and males (the so-called reproductive brood) are produced by workers. An evaluation of the parameters of Hamilton's Rule suggests that queens benefit greatly from the help provided by workers but that workers achieve greater fitness by provisioning and laying their own eggs rather than by tending to the queen's eggs. This conflict of interest between the queen and her workers suggests that the discrepancy between potential and achieved worker oviposition is due to queen interference. Comparison of relatedness and maternity patterns in the Agios Nikolaos Monemvasias population with those from a northern population near Tübingen, Germany, points to a north–south cline of increasingly effective queen control of worker behaviour.

Keywords: Halictidae, kin selection, parental manipulation, social evolution, worker reproduction

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Introduction

The term 'social evolution', as applied to invertebrates, has two very different meanings that are not always clearly distinguished. On one hand, it refers to the evolutionary processes involved in transitions between solitary and social behaviour, which includes the evolutionary origins of sociality in ancestrally solitary lineages and reversions to solitary behaviour in social lineages. On the other hand,

'social evolution' also refers to processes that occur after transitions to sociality, especially processes that modulate or maintain sociality (Bourke & Franks 1995). The difference between these two meanings is critical: to understand the origins of social behaviour, it is necessary to study animals at incipient stages of social evolution, not those whose social behaviour may represent adaptations to evolutionary pressures that arose in response to their very sociality (Packer 1992). In particular, if we wish to understand the evolution of altruism, it is important to study species in which prospective altruists have at least the potential to exercise behavioural options other than altruism.

A group of insects that admirably fulfils these criteria are the social sweat bees (Hymenoptera, Halictidae). The

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Halictidae are arguably the most socially labile of all animal taxa, having experienced multiple origins of sociality from solitary ancestors, as well as multiple reversions to solitary behaviour within eusocial lineages (Danforth 2002; Danforth *et al.* 2003). This group even includes socially polymorphic species that revert to solitary behaviour in regions with short or harsh breeding seasons (Sakagami & Munakata 1972; Eickwort *et al.* 1996). The social lability evident in many halictid lineages suggests that sociality is an adaptation to varying environmental pressures (Wcislo 1997; Wcislo & Danforth 1997), although some recent studies of socially polymorphic species suggest a genetic component to social phenotype (Plateaux-Quénu *et al.* 2000; Soucy & Danforth 2002). A defining characteristic of halictid sociality is post-imaginal caste determination: all adult females have the potential to assume any of the reproductive roles typifying a particular species, although the likelihood that they will act as either queens or workers is strongly influenced by their body size, nutritional state, time of year, and social interactions with nest mates (especially the queen) (Yanega 1989; Kukuk & May 1991; Richards & Packer 1994). In other words, in eusocial sweat bees, every newly emerged adult female has the potential to become either a queen or a worker. This makes them ideal models for studying the ecological and evolutionary forces that mould helping behaviour by individuals, as well as interactions that shape colony social organization.

Lasioglossum (Evylaeus) malachurum is a well-studied social halictid and one of the commonest bees in Europe. Although obligately eusocial across its entire range, it exhibits marked geographic variation in colony demography and social organization that may result from adaptation to local environmental conditions. In northern populations, where colonies produce only a single worker brood before producing the final brood of males and gynes (future queens), workers often mate but rarely have developed ovaries (Knerer 1973, 1992). In southern Europe, where colonies produce two or three worker broods and reach much larger sizes, workers rarely mate but often have developed ovaries (Richards 2000; Wyman & Richards 2003). Analyses of this geographic variation in *L. malachurum* social behaviour and demography have led to contradictory predictions about how the strength of eusociality varies across Europe (Wyman & Richards 2003). Low rates of worker ovarian development in northern populations suggest high reproductive skew favouring queens and strong eusociality, while high rates of worker ovarian development in southern colonies suggest lower reproductive skew and weaker eusociality. However, circumstantial evidence suggests that worker ovarian development is a poor predictor of worker oviposition and maternity (Packer 1992; Packer & Owen 1994; Wyman & Richards 2003). Moreover, increased colony size is usually considered to be a correlate of stronger, not weaker, eusociality (Michener 1974).

Clearly, genetic analyses of maternity by queens and workers are required to properly understand colony social organization. Recently, Paxton *et al.* (2002) analysed nest mate relatedness and patterns of maternity of female offspring in colonies from a northern population in Germany. In this study, we investigate patterns of genetic relationship within colonies of a previously studied population in Greece (Richards 2000; Wyman & Richards 2003). Our results reveal several surprises about the nesting biology of this bee and allow us to compare the sociogenetics of two populations that may exemplify northern and southern types of colony cycles.

Methods

Description of colony cycle and social behaviour in southern Greece

The following descriptions and data were derived from Wyman & Richards (2003). In spring (usually in April), overwintered foundresses (queens) establish nests and produce the first worker brood (Brood 1), which consists only of workers. The majority of nests contain only a single foundress-queen. After the Brood 1 workers emerge in May, they provision a second, more numerous brood (Brood 2), which also consists only of workers. These in turn raise a third brood (Brood 3), which emerges in June and consists of a few workers, new queens or 'gynes', and males. After mating, young gynes enter hibernation to begin the colony cycle as foundresses the following spring. Newly emerged workers and gynes exhibit nonoverlapping size distributions, but a few foundress-queens are worker sized, indicating that worker-destined females occasionally overwinter and become foundresses. The vast majority of foraging workers remain unmated, but levels of worker ovarian development vary throughout the colony cycle, suggesting that many could lay eggs. In 2000, average brood sizes were about 7.0 workers per colony in Brood 1, 33.4 workers in Brood 2, and 48.3 workers, males and gynes (young foundresses) in Brood 3 (Wyman & Richards 2003). Therefore, while males and gynes are being produced, the daily average number of active workers in a nest is probably about 30–35.

Field methods

Nest mates from a total of 52 colonies in two aggregations (ANM and MALA1) situated less than 1 km apart near Agios Nikolaos Monemvasias in southern Greece, were collected using two different methods, nest excavation and collections of foragers as they departed from their nest entrances. Nests were excavated in their entirety as far as possible, depending on the size of the boulders that they were entwined with. During production of Brood 2, eight

colonies were excavated, yielding 55 adults (queens and workers), 32 pupae (workers), 104 larvae (workers), and 12 pollen provision masses with or without eggs. An additional 94 foraging workers were collected from 20 nests. Thus a total of 297 bees were collected during the Brood 2 rearing period, of which 289 were genotyped (6 queens and 283 workers). During production of Brood 3, nine nests were excavated, yielding 50 adults (queens and workers), 94 pupae (males, workers, and gynes), 152 larvae (males, females assumed to be gynes, and unknown sex), and 20 pollen masses (unknown sex). An additional 78 foraging workers were collected from 15 more nests. Thus a total of 390 bees were collected during the Brood 3 rearing period, of which 303 were genotyped (including 8 queens and 125 workers). Eggs and larvae smaller than about 2–3 mm in length were rarely collected intact and so were not genotyped.

Genetic analysis

Genetic analyses were carried out only on colonies for which we were confident that the nest occupants had not been confused with those from other nests. Total genomic DNA was isolated from the thoraces or heads of adults and pupae, or from whole larvae using the DNeasy Tissue Kit (QIAGEN Kit #69504), after initially freezing and crushing the tissue in liquid nitrogen. In the initial phase of this study, we assessed six microsatellite loci, *mala01*, *mala04*, *mala06*, *mala07*, *mala09*, and *mala10* (Paxton *et al.* 2002, 2003). Three of these were difficult to amplify or score reliably, so we based our analyses on the genotypes for *mala01*, *mala06*, and *mala07*. These three loci were amplified in separate 20- μ L reactions with 2.5 mM MgCl₂, 75 μ M dGTP, 75 μ M dCTP, 75 μ M dTTP, 6 μ M dATP, 0.125 μ Ci [α ³²P]-dATP, 2.0 μ M of each primer, 1.2 U *Taq* DNA polymerase (MBI Fermentas), 2 μ L *Taq* reaction buffer (MBI Fermentas, supplied with the enzyme), and 1 μ L of genomic DNA (about 1/200th of the total extraction). Polymerase chain reactions (PCR) were heated to 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 60–63 °C for 30 s, and 72 °C for 45 s, followed by a further 72 °C for 10 min. Negative controls using sterile water as a template were carried out regularly to detect contamination. Amplification products were separated in 45 cm, 7% polyacrylamide-urea sequencing gels, and visualized using a Fujichrome phosphorimager.

Genotypes for each locus were assigned based on visual inspections of gel images and comparison with size markers for each locus. Genotypes were scored by two people independently; individuals with unclear genotypes were re-amplified. We used the computer program ARLEQUIN 2.001 (Schneider *et al.* 2000) to carry out tests for linkage disequilibrium and Hardy–Weinberg equilibrium. These tests were based on a subsample of one randomly selected worker per family and any unrelated ‘alien’ workers found

within colonies ($n = 15$ workers from aggregation MALA1 and 46 workers from ANM). When queens had not been collected, their genotypes were inferred from those of their offspring. Using both observed and inferred queen genotypes for each family, we inspected pedigrees to distinguish the following possibilities in each colony: presence of a single vs. multiple egg-laying queens, single vs. multiple mating by queens, presence of ‘alien’ (unrelated) adults in a colony, and egg laying by workers.

Values of relatedness among different classes of nest mates were calculated directly from pedigrees (Crozier & Pamilo 1996; Paxton *et al.* 2001). When colony genotypes indicated that queens had mated more than once and fathers had uneven contributions to the paternity of brood, the effective queen mating frequency was calculated as

$$k_E = 1/(\sum^n y_i^2) \quad (\text{eqn 1})$$

where n is the number of males contributing to the brood and y_i is the proportion of the daughters sired by male i (Starr 1984). Similarly, when multiple maternity was detected in colonies (because of egg laying by a second queen or by workers), the effective polygyny was calculated as

$$Q_E = 1/(\sum^n q_i^2) \quad (\text{eqn 2})$$

where n is the number of egg-laying females and q_i is the proportion of offspring produced by female i (Crozier & Pamilo 1996, p. 110).

Worker-produced males are detectable at a particular locus if they inherit at least one allele different from the queen’s alleles. Thus the probability of detection for worker-produced males is $1-0.5^i$, where i represents the number of loci with distinct maternal and paternal alleles (Foster & Ratnieks 2000; Palmer *et al.* 2002); the probability varies from 0.5 to 0.875 for one to three informative loci, respectively. Worker-produced gynes are detectable at a particular locus if their genotypes rule them out as the queen’s daughters; the probability of detection is influenced both by inheritance of distinct alleles from the queen and by the probability that a worker mates with a male carrying alleles distinct from those of the worker’s parents. Details of the various scenarios are illustrated in Fig. 1.

Sex ratios and investment costs

Most pupae and adults were sexed based on morphology, but larvae and badly damaged pupae were sexed according to genotype, hemizygotes at all three loci being diagnosed as male and heterozygotes at one or more loci as female. The probability that a female will be homozygous at all loci (Kukuk & May 1990; Paxton *et al.* 2000) is

$$R = 1 - \prod_{i=1}^L (\sum p_{ij}^2) \quad (\text{eqn 3})$$

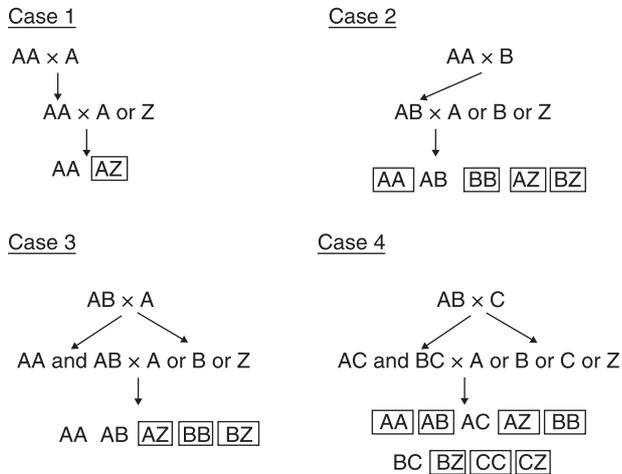


Fig. 1 Distinguishing worker-produced from queen-produced gynes depends on inheritance of distinct grandparental alleles and the mating patterns of reproductive workers. All the daughters produced by a worker whose mate carries an allele distinct from her parents' alleles at that locus, will be detectable. However, even worker-produced gynes without distinct alleles may be detected if their genotypes exclude them as daughters of the queen. In these diagrams, the possible genotypes of a queen and her mate, the queen's daughters (gynes or reproductive workers), and her granddaughters (gynes produced by workers) are represented as diploid or haploid genotypes, with boxes indicating detectable worker-produced daughters. Distinct alleles in the grandparents are represented by the letters A, B, or C with frequencies a , b , and c , respectively, while distinct alleles in workers' mates are represented by Z. Detectable worker-produced, granddaughter genotypes are indicated with a box. Case 1: Neither the queen nor her mate carries any distinct alleles. Assuming random mating, the probability that a worker mates with a male carrying an allele distinct from a is $1 - a$. All her daughters will be detectable as worker produced, so the probability of detection = $1 - a$. Case 2: Alleles of the queen and her mate are distinct, but the queen is homozygotic. When workers mate with males carrying either grandparental allele (mating frequency = $a + b$), half of worker-produced gynes are undetectable. Therefore, the probability of detection is = $1 - 1/2(a + b)$. Case 3: Only one of the queen's alleles is distinct from her mate's allele. If workers mate with males carrying the queen's nondistinct allele (a), none of the daughters are detectable, while if they mate with males carrying the queen's distinct allele (b) three-quarters are undetectable. Therefore the probability of detection = $1 - a - 3/4b$. Case 4: All alleles of the queen and her mate are distinct. If workers mate with males carrying any of the three grandparental alleles, half of their daughters will be undetectable, so the probability of detection = $1 - 1/2(a + b + c)$. Note the similarity of this outcome to that from Case 2.

where i = the i th allele, j = the j th locus, L = the number of unlinked loci, and H_i is the observed heterozygosity at each locus. In our sample of 430 queens and workers which were sexed morphologically, we found none to be homozygous at all three loci; only 0.26% of diploid larvae were expected to be homozygous at all three loci. Since Brood 3

includes a few workers, as well as gynes and males, the reproductive brood sex ratio was calculated as the ratio of total female to male brood in excavated nests. However, many of Brood 3 were eggs or very small larvae that were not collected intact and so were not genotyped. Therefore, we calculated three different values for the Brood 3 sex ratios based on the following assumptions: that all unsexed brood were female, that all unsexed brood were male, or that the sex ratio among unsexed brood was the same as that in sexed brood. Given that Brood 3 was protandrous [males were produced earlier than gynes (Wyman & Richards 2003)], most of the young, unsexed brood were probably female.

Rates of investment in brood of each caste or sex (worker, gyne, or male) were calculated by multiplying the number of brood of each type by the appropriate average dry weight at emergence. Total investment in Broods 1, 2, and 3 was then calculated by summing over all relevant classes of offspring to obtain the rates of investment by the foragers provisioning each of Broods 1, 2, and 3 as well as the investment sex ratio for Brood 3. The average number of individuals in each brood and average dry weights at emergence (workers 5.00 mg, males 4.44 mg, gynes 9.18 mg) were taken from Wyman & Richards (2003). Estimates of investment based on brood body sizes are strongly correlated with those based on relative provisioning effort by adult females (Danforth 1990), but likely underestimate the true investment rate since they do not take into account other types of parental effort such as the energetic costs of egg production and nest construction.

Results

Microsatellite variability

We genotyped 592 individuals from 52 colonies. Assignment of genotypes was straightforward in all but three colonies. In two of these colonies, female genotypes at *mala01* and *mala07* suggested that the bearers were most likely the daughters of one male and one female, whereas the *mala06* genotypes suggested that there were two sets of homozygotes in the colony with no alleles in common, minimally requiring two homozygous mothers each mated to a male with the same allele. This contradiction was resolved by the more parsimonious interpretation of a paternally inherited null allele at *mala06* in each of these two colonies. In a third colony from which six workers were genetically analysed, genotypes at *mala06* and *mala07* suggested that they were full sisters, but at *mala01*, the putative paternal allele of one worker was (CT)₄₀, whereas that of the other five workers was (CT)₄₁. The odd female could have been an 'alien' worker from another colony, but based on observed allele frequencies, the frequency in the population of females with her particular genotype would

Table 1 Colony genetic structure and inferred social structure for nests of *Lasioglossum malachurum* at Agios Nikolaos Monemvasias in 2000. Numbers in parentheses denote colonies for which five or more individuals were genotyped. Colonies that appear in more than one category of social structure are identified in the footnotes

Colony genetic structure	Colony social structure	No. brood 2 colonies	No. brood 3 colonies	Total
1 matriline, 1 patriline	Once-mated queen lays all eggs	22 (16)	19 (12)	41 (28)
1 matriline, 2 patrilines	Twice-mated queen lays all eggs	1 (1)	0	1 (1)
> 1 matriline	Two, once-mated queens share oviposition	1 likely* (1)	1 certain† (1)	2* + (1)
	Unrelated workers present	5* (5)	4‡ § ¶ (3)	9* ‡ § ¶ (8)
	Unrelated males present		1‡ (1)	1‡ (1)
	Detectable worker maternity of male brood	0	2† ¶ (2)	2† ¶ (2)
	Detectable worker maternity of female brood	0	2§ (1)	2§ (1)
	Number of colonies genotyped	28 (22)	24 (17)	52 (39)

*Nest 76; †nest 378; ‡nest 426; § nest 376; ¶ nest 427.

have been 2.6×10^{-5} . Alternatively, the six workers could have been daughters of the same mother but different fathers with matching alleles at *mala07* and *mala06*; the probability that two randomly sampled males would match for these alleles would be 0.004. A third possibility, suggested by the size similarity of the alleles at *mala01*, is that the six workers were indeed full sisters and that the (CT)₄₀ allele represents a mutation of a paternal (CT)₄₁ allele. Based on assessments of 1085 three-locus brood haplotypes, this suggests a microsatellite mutation rate of about 1/1085 or about 1×10^{-3} .

The 592 bees genotyped in this study exhibited 18 alleles at locus *mala01*, 15 alleles at *mala06*, and 12 alleles at *mala07*. There was no evidence of deviation from Hardy–Weinberg equilibrium at any locus in either nesting aggregation (*mala01*: aggregation ANM1: $H_O = 0.73, H_E = 0.90, P = 0.19$; aggregation MALA1: $H_O = 0.91, H_E = 0.89, P = 0.78$; *mala06*: ANM1: $H_O = 0.73, H_E = 0.90, P = 0.19$; MALA1: $H_O = 0.91, H_E = 0.89, P = 0.78$; *mala07*: ANM1: $H_O = 0.73, H_E = 0.90, P = 0.19$; aggregation MALA1: $H_O = 0.91, H_E = 0.89, P = 0.78$). There was no evidence of linkage disequilibrium among the loci (10 100 permutations per pair of loci: *mala07* vs. *mala06*: $\chi^2 = 69.351, d.f. = 110, P = 0.999$; *mala07* vs. *mala01*: $\chi^2 = 75.661, d.f. = 121, P = 0.999$; *mala06* vs. *mala01*: $\chi^2 = 64.347, d.f. = 110, P = 0.999$).

Details of colony genetic structure based on genotype inspection

We scored the genotype arrays of 52 colonies, 39 represented by at least five genotypes, to determine the minimum number of matrilines and patrilines in each colony (Table 1). As expected, in most colonies ($n = 41$), there was only a single matriline and a single patriline, consistent with the hypothesis that all the brood were the offspring of a single queen mated to a single male. In the remaining 11 colonies,

we detected several types of deviation from this family structure, including pleometrosis (two egg-laying queens), worker oviposition of gynes and males, and the presence of unrelated (alien) workers and males in nests.

Monogyny vs. polygyny. Based on field data (Wyman & Richards 2003), the rate of pleometrotic nest founding at Agios Nikolaos Monemvasias was about 8.3% (1/17), but the genetic analyses raised this rate to 11.7% (2/17). Pleometrosis had already been detected in one Brood 3 nest that contained one normal-size queen and one worker-size queen (Wyman & Richards 2003). These turned out to have identical genotypes at *mala06* and to share an allele at *mala07*; they may have been half-sisters (same mother, different fathers). The worker-sized queen must have been a foundress-queen rather than a replacement queen as she produced diploid eggs before the onset of male production, and therefore must have mated the previous year. Despite their size difference, both queens produced offspring equally; among the genotyped brood from this colony, one queen was the mother of 6 workers, 8 males and 13 gynes, and the other was the mother of 7 workers, 8 males and 13 gynes. In addition, this colony contained two worker-produced males, so the effective maternity was $Q_E = 2.14$ (eqn 2).

Genetic analyses suggested that a second colony, which was collected in May as Brood 1 workers were provisioning Brood 2, probably also had two queens, although neither was found when the nest was excavated. This nest contained four adult Brood 1 workers, one of which was an unrelated alien, and 45 worker larvae, of which 26 were genotyped. Three of the youngest larvae in the nest had genotypes that suggested they were the offspring of a singly mated foundress who was not the mother of any of the other brood or the adult workers. The larvae could not have been worker produced as they were present before male emergence.

Frequency of single vs. multiple mating by queens. We assessed the genotypes of 14 queens directly by genotyping and of 23 queens indirectly by inspection of brood genotypes, in 37 colonies with at least five genotyped offspring. Of these, 36 queens (97%) had brood whose genotypes were consistent with the queen having only a single mate. In one colony the queen had mated twice (3%). Paternity was not evenly distributed among her offspring: of 32 genotyped worker brood, 7 (21.9%) were sired by one father and 25 (78.1%) by the other ($\chi^2 = 10.1$, d.f. = 1, $P < 0.01$). The effective number of fathers in this nest was $k_E = 1.519$ (eqn 1).

Egg laying by workers. Worker maternity of gynes and males was detected in four of seven (57%) colonies fully or partially excavated during production of Brood 3. All four of these nests contained viable queens who could be identified morphologically and genetically. In one colony, one worker-produced gyne had inherited her mother's paternal allele at locus *mala07*, and so could only have been the queen's granddaughter, not her daughter. In a second colony, a worker-produced gyne could have been the queen's daughter, but only if the queen had mated twice and paternity was extremely skewed, as the other 23 genotyped, female brood in the colony all shared one father. When all seven colonies excavated during production of Brood 3 are considered, the rate of worker maternity of gynes was 2/87 (2.3%) and for males was 3/80 (3.8%), for an overall worker-maternity rate of 5/167 (3.0%). The average probability of detection for worker-produced males was 79.7%, so the real worker maternity rate for males may be as high as 4.7%. As the average probability of detection for worker-produced gynes was 98.9%, the rate for gynes would still be about 2.3%.

Presence of unrelated adults in nests. Workers whose genotypes at one or more loci indicated that they could not have been produced by the queen or queens that produced their nest mates were categorized as 'aliens' (Table 1). We identified 11 alien workers in nine colonies. Two additional colonies probably also contained alien workers, but small sample sizes did not allow us to exclude the possibility of multiple maternity of brood. In one nest excavated during the production of the final brood, we found two alien males. Since neither male was found in a brood cell and their genotypes excluded maternity by either the queens or the workers, they had probably entered the nest seeking mates.

Estimates of relatedness and the reproductive brood sex ratio

As the great majority of colonies exhibited only one matriline and one patriline (Table 1) and since most brood were produced by singly mated queens even in those colonies with more than one matriline, average levels of

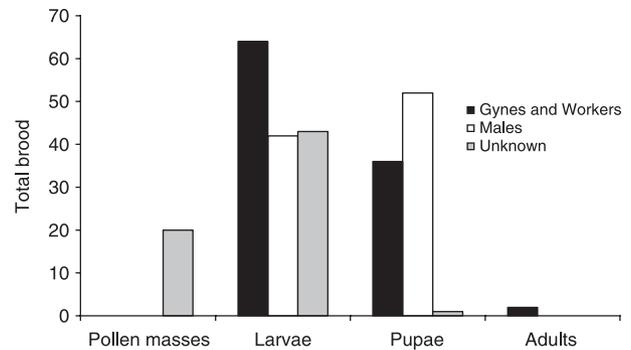


Fig. 2 Numbers of male, female (gyne and worker) and unknown-sex brood from 10 nests excavated during the period of reproductive brood production (Brood 3) at Agios Nikolaos Monemvasias.

relatedness among different classes of nest mates were close to the values expected when oviposition is monopolized by a single queen with a single mate (Table 2).

Inspection of genotypes allowed us to sex about half of the brood that could not be sexed morphologically. Brood 3 was confirmed to be protandrous, as there were proportionally more male than female pupae, but proportionally more female than male larvae (Fig. 2, Likelihood-ratio chi-squared, $G = 7.34$, d.f. = 1, $P = 0.007$). If the remaining unsexed brood, which were mostly eggs and very small larvae, were all female then the numerical sex ratio was 1.8 (F/M) and the investment ratio 3.6. At the other extreme, if the unsexed brood were all male, then the numerical sex ratio was about 0.6 and the investment ratio was about 1.3. If the proportions of males and females were the same in sexed and unsexed brood, then the numerical sex ratio was about 1.1 and the investment ratio was about 2.2. The most likely scenario is that most of the unsexed brood were female.

Discussion

Social variability in Lasioglossum malachurum

The social biology of *Lasioglossum malachurum* has been studied at several locations across Europe. Early studies (Knerer 1973, 1992; Packer & Knerer 1985) contained insufficient detail to detect significant social variation within populations, but certainly indicated a north-south cline of longer colony cycles with more worker broods, increasing colony size, increasing worker ovarian development, and decreasing rates of worker mating. Recent studies in Germany (Paxton *et al.* 2002; Strohm & Bordon-Hauser 2003) and Greece (Richards 2000; Wyman & Richards 2003; this study) have begun to detail the extent of intrapopulation social variation possible in this species. This study adds to the evidence that, despite its reputation as the paradigmatic eusocial sweat bee, there is considerable variability in colony social organization within

and between populations due to pleometrotic nest founding, multiple mating by queens, and worker oviposition. However, these phenomena occur at such low frequencies that they have little overall impact on the genetic structure of colonies — most brood in most colonies are the offspring of a single queen mated to a single male.

In this study we genetically confirmed an earlier contention (Wyman & Richards 2003) that pleometrosis can and does occur in *L. malachurum*. The digynous colony with one normal-size and one worker-size queen is probably a rare example of cooperative nest founding. This is expected to be quite unusual because spring foundresses are normally extremely intolerant of, and aggressive towards, other adult females (Smith & Weller 1989). Since nesting aggregations at Agios Nikolaos Monemvasias may persist in the same spot for several years (M.H. Richards, personal observation), it seems likely that overwintered gynes establish new nests near or directly above their hibernacula. The similar genotypes of these two foundresses suggest that they were half-sisters that overwintered together, established mutual tolerance, and then cofounded the nest. Such events apparently explain polygynous nest founding in another obligately eusocial sweat bee, *Halictus ligatus* (Richards & Packer 1998), as well as in the European paper wasp, *Polistes dominulus* (Dapporto *et al.* 2004). The second digynous colony did not contain a queen, but the adult workers and most of the older brood were the daughters of one mother and father, while the younger brood were the daughters of a second mother and father. In *L. malachurum*, some spring foundresses are kleptoparasitic, attempting to usurp nests rather than establishing their own (Smith & Weller 1989). Successful nest usurpation after the commencement of oviposition would result in exactly the type of maternity pattern observed here.

In *L. malachurum*, virgin gynes are highly attractive to males, but they very rapidly lose their attractiveness when they mate (Ayasse *et al.* 1990, 1993, 1999). It was no surprise, then, to find that multiple mating, like pleometrotic nest founding, is rare in the Agios Nikolaos Monemvasias population and unlikely to have much influence on the genetic structure of colonies.

Field studies of *L. malachurum* social behaviour have indicated marked differences in colony cycle between northern and southern populations (Packer & Knerer 1985; Wyman & Richards 2003). Mediterranean populations like the one at Agios Nikolaos Monemvasias produce two (and possibly three) worker broods followed by the final, reproductive brood. Colonies are large, and queens must contend with dozens of workers by the time the reproductive brood is produced. Few workers mate because males are not produced in the worker broods, but many workers have developed ovaries. More northerly populations exhibit the opposite spectrum of demographic and social characteristics: only one brood of workers is produced, and this

brood is few in number (seven or fewer). As the worker brood includes a few males, mated workers are common, but worker ovarian development is rare. Genetic studies further confirm important social and behavioural differences between southern and northern populations. At Agios Nikolaos Monemvasias, only 1 of 37 queens was multiply mated, whereas in a population from Tübingen, Germany, 3 of 8 (37%) *L. malachurum* queens were multiply mated, with as many as four different fathers being detected in a single brood (Paxton *et al.* 2002). At Agios Nikolaos Monemvasias, only 3% of the reproductive brood develop from worker-laid eggs whereas in Tübingen, workers, especially alien workers, often reproduce even in the presence of the queen (Paxton *et al.* 2002).

The sociogenetic contrasts between the Tübingen and Agios Nikolaos Monemvasias populations suggest that queens at the latter site are much more successful at controlling worker behaviour: they virtually monopolize oviposition despite having to deal with many more workers than northern queens. Behavioural observations and adult dissections at another aggregation in Germany (Würzburg) have shown that the proportion of workers with developing ovaries was greater in colonies with more workers, with queen control becoming markedly less effective 'at surprisingly small colony sizes (four to six workers)' (Strohm & Bordon-Hauser 2003, p. 551), far fewer than the average 33.4 workers present in each Agios Nikolaos colony at the time of Brood 3 production. Thus within populations, *L. malachurum* exhibits a pattern hitherto considered typical of eusocial halictines: as colony sizes grow, queens become less able to dominate their workers and prevent them from laying eggs (Richards *et al.* 1995). Yet the differences between northern and southern populations suggest the opposite pattern, that queen control of worker behaviour co-evolves with colony size (Bourke 1999).

Queen vs. worker fitness at Agios Nikolaos Monemvasias

We combine our estimates of relatedness and the Brood 3 sex ratio with previously collected data on brood productivity and body size at Agios Nikolaos Monemvasias (Wyman & Richards 2003) in order to compare the investment strategies of queens and workers in terms of the energetic costs of provisioning each of the three broods. Then we compare these initial investment costs to the genetic pay-offs received at the end of the colony cycle, in terms of overall colony brood productivity weighted by investor relatedness to brood (Table 2).

The first, rather surprising, result of these calculations, is that the average queen works harder at provisioning brood than does the average worker. In fact, queens constitute only about 2.6% of the total workforce in a colony but provide about 6.5% of the investment in brood. Second, per capita investment costs decline with each successive brood:

Table 2 Investment costs and fitness benefits for queens and workers. Relatednesses denoted by g are life-for-life estimates assuming monandry and monogyny. Subscripts on relatedness estimates refer to the sex of the brood. Values in parentheses refer to calculations based on **R**. Other components of Hamilton's rule are identified as **C** (number of reproductive brood produced by a solitary female), **B** (number of reproductive brood produced by an average worker), r_0 (relatedness of mother to brood)

Investment calculations	Queens	Brood 1 workers	Brood 2 workers
No. of foragers provisioning brood per colony	1.1	7.0	33.4
No. of brood provisioned per colony	7.0 workers	33.4 workers	30.8 females* 17.5 males*
Total dry weight of brood provisioned (mg)	35.0	167.0	331.3
Average investment per adult female (mg)	31.8	23.9	9.9
Fitness calculations			
No. of brood produced if female was solitary, C	4.6	3.5	1.4
Fitness if females were solitary, r_0 C	2.3	1.75	0.7
Total no. of reproductive brood attributable to worker investment (per colony)		30.8–2.3 = 28.5 females 17.5–2.3 = 15.2 males	
Average no. of reproductive brood produced per worker, B		28.5/40.4 = 0.71 females per worker 15.2/40.4 = 0.38 males per worker Total 1.09 reproductive brood per worker	
Relatedness of investors to brood, g	$g_f = 0.5, g_m = 0.5$	$g_f = 0.75, g_m = 0.25$	
Fitness in eusocial colony, g B	24.2	0.63	

*Brood 3 sex ratio based on the assumption that unsexed brood were female and that worker-sized females will overwinter.

the average queen gathers provisions for 31.8 mg (dry weight) of brood, a Brood 1 worker provisions 23.9 mg, and a Brood 2 worker only provisions 9.9 mg (Table 2, based on Wyman & Richards 2003), suggest that a queen does about three times as much work provisioning Brood 1 as do the Brood 2 workers that provision the gynes and males of Brood 3. A second, not so surprising, result of these calculations is that queens are able to produce far more offspring with the help of their workers than they would working alone. For an average queen investment of 31.8 mg (brood dry weight), the return is 48.3 offspring, which represents an absolute fitness of about 24.2 gene copies, at a cost of only 1.31 (mg of investment per gene copy). In fact, the brood productivity of queens is approximately 10 times greater due to the help of their workers, than it would be if they raised their offspring alone. Eusociality is clearly advantageous to queens.

Is eusociality also advantageous to workers? According to Table 2, a colony with 40.3 workers eventually produces 48.3 brood. If we assume that the queen could have produced 4.6 brood working alone, then worker investments produce an additional 43.7 brood, or about 1.09 reproductive brood per worker on average. This number is considerably lower than the 1.4–3.5 brood that we estimate a worker could raise if she used the pollen she gathered to provision her own offspring. Based on these numbers the fitness of worker females that only provisioned their own brood would vary from 0.7 to 1.75, whereas the fitness of workers that provision siblings seems to be somewhat lower (0.6).

These fitness estimates are necessarily imprecise but suggest that if they can get away with it, *L. malachurum* workers would do better to provision their own offspring rather than the queen's offspring. That workers do not often get away with laying their own eggs is evidenced by the fact that at Agios Nikolaos Monemvasias, only 2–3% of gynes and males are produced by workers, yet during egg-laying periods, as many as 17% of workers have at least one oocyte developed to at least 3/4 of its final size (Wyman & Richards 2003). Halictine eggs are large and must be costly to produce, so the discrepancy between rates of worker ovarian development and rates of worker oviposition require explanation. Policing of worker-laid eggs seems likely, especially by queens. However, queen oophagy of worker-laid eggs, as occasionally observed in *Lasioglossum (Dialictus) zephyrum* (Michener & Brothers 1974), seems unlikely in *L. malachurum*. Oophagy is easily detected when it occurs in social halictines by dissecting the bees' abdomens (for instance Packer (1986) found evidence of oophagy in 7 queens and 13 workers in Florida *Halictus poeyi*), but we have dissected hundreds of adult female *L. malachurum*, and have never found anything resembling egg in their guts. We suspect *L. malachurum* queens police worker oviposition using more subtle, pre-emptive tactics. Halictine queens can inhibit worker oviposition simply by behaving aggressively to them (Pabalan *et al.* 2000), and experimental evidence shows that in *H. ligatus*, aggression results in significantly reduced worker ovarian development after only 1.5 h, whereas queens remain

unaffected for almost 10 h (Pabalan 1998; Pabalan & Packer, unpublished).

Kin selection and parental manipulation

Our data and fitness analyses support a neglected explanation for the spread of worker altruism, queen manipulation of worker behaviour (Michener 1974; Charnov 1978). While kin selection suggests that the primary force driving social evolution is selection on worker-altruism genes, a new version of the parental manipulation theory suggests that the primary force driving social evolution in primitively eusocial bees like *L. malachurum* is selection on maternal-effect genes in queens (Wade 2001). In fact, models suggest that eusociality spreads through populations more easily when selection is on queens to exploit daughters who help to raise their siblings, than when selection is on workers to raise siblings rather than their own offspring (Wade 2001). Field studies of nesting aggregations of *L. malachurum* around the village of Agios Nikolaos Monemvasias have underscored the importance of queen control of worker behaviour, not only with respect to queen prevention of worker oviposition. First, queenless colonies have high rates of worker ovarian development, early production of males, and low colony brood productivity (Richards 2000), suggesting not only that queens prevent worker oviposition, but that queens somehow control and promote worker activity and efficiency. Second, queens need workers: Table 2 suggests that working alone, queens provision only about 32 mg of brood (which under a 1:1 investment sex ratio would produce about 1.7 gynes and 3.6 males), whereas the workers of Brood 3 bring in provisions for about 330 mg of brood, 10 times as much as the queen alone. In fact, observations of an aggregation in Würzburg, Germany, in which some colonies produce two worker broods, suggest that queens must produce at least four to six workers in the first brood if nests are to successfully produce males and gynes in later broods (Strohm & Bordon-Hauser 2003), and hints at the reason why there should be strong selection on queens to produce and manipulate workers to their own ends.

We suggested above that, in *L. malachurum*, larger colony sizes in southern populations may be co-evolving with increasingly effective queen control of worker behaviour. Kin selection should also promote worker acquiescence (Wenseleers *et al.* 2004). However, even acquiescent, sterile, altruistic workers may have ways of resisting queen control, most notably by manipulation of the investment sex ratio (Trivers & Hare 1976). At Agios Nikolaos Monemvasias, the evident female bias in the Brood 3 sex ratio suggests that workers are at least somewhat successful in resisting queen control of the reproductive brood sex ratio. Unfortunately, sex ratio information for northern *L. malachurum* populations is unavailable, so no comparison with Agios

Nikolaos Monemvasias is possible. How halictine workers might manipulate the sex ratio among queen-produced brood is unclear, as the sequence of events preceding laying of any particular egg suggests that the sex of the offspring is determined long before the egg is actually laid. Perhaps workers can bias the sex ratio simply by constructing large or small brood cells containing large or small provision masses on which queens then deposit a fertilized or unfertilized egg. Evidence for worker manipulation of the sex ratio has been found in *Lasioglossum laevisimum*, a species in which queens monopolize oviposition (Packer & Owen 1994). The only other eusocial halictine for which sex ratio studies have been undertaken is *Augochlorella striata* (Mueller 1991) in which queen removal resulted in more male-biased sex ratios. This experiment implies that viable queens prevent worker oviposition and that workers are capable of modifying the sex ratio given the opportunity.

Conclusions

A well-known pattern in eusocial sweat bees is that queens are more successful at monopolizing oviposition when they have fewer, smaller workers (Kukuk & May 1991; Richards *et al.* 1995; Richards & Packer 1996), thus stronger eusociality and greater reproductive skew is generally associated with smaller colony sizes. Comparisons between populations of *Lasioglossum malachurum* suggest the opposite pattern, that stronger eusociality and greater reproductive skew is associated with larger colony sizes. In this study, we found almost complete queen monopolization of oviposition in the large colonies typical of Mediterranean populations, whereas in much smaller colonies in Germany, workers obtain a much greater share of oviposition (Paxton *et al.* 2002). The opposing patterns observed within and between populations suggest that the ability of queens to dominate their workers is the most important determinant of colony social organization. Moreover, it would suggest that the ability to dominate workers is a genetically based trait that varies geographically. Thus, we hypothesize that improved queen control of worker behaviour is a prerequisite for the evolution of large colony sizes in this and other eusocial sweat bees, a prediction that should be tested using phylogenetic methods.

A puzzle that remains in our studies of *L. malachurum* is why workers sometimes produce daughters ($r = 0.5$) instead of sisters ($r = 0.75$). In *H. ligatus* (Richards *et al.* 1995), worker production of gynes seems to be a response to protandry of the reproductive brood and early mortality of queens. Thus reproductive workers do not replace sisters with daughters, instead producing daughters with high reproductive value in a male-biased world. More detailed analyses of halictine reproductive brood sex ratios will perhaps reveal how the timing of male vs. gyne production affects sex allocation decisions of altruistic vs. reproductive workers.

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