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Colony social organization of *Lasioglossum malachurum* Kirby (Hymenoptera, Halictidae) in southern Greece

L.M. Wyman and M.H. Richards*

Department of Biological Sciences, Brock University, St. Catharines, Ontario L2S 3A1, Canada, e-mail: Miriam.Richards@brocku.ca

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Summary. We studied the nesting and social biology of two aggregations of the obligately eusocial halictine bee *Lasioglossum malachurum* at Agios Nikolaos Monemvasias (ANM) in southern Greece. Observations and nest excavations carried out from May to June 2000, revealed social and demographic variation between aggregations and years at ANM, as well as notable differences between these and other European populations. In southern Greece, the colony cycle includes multiple broods: the first two broods comprise only workers, whereas the third brood comprises workers, males, and gynes. Although pleometrosis is unknown in other populations, in the ANM region, as many as 20% of nests have more than one foundress. Newly emerged workers and gynes exhibit non-overlapping size distributions, but a few queens are worker-sized, indicating that workers occasionally overwinter and become foundresses. Although the vast majority of workers are unmated and most exhibit no ovarian development, an increase in worker ovarian development at the time of male production suggests that many males may develop from worker-laid eggs. Worker reproduction seems to be inhibited by the presence of queens, and annual variation in queen mortality may underlie annual variation in worker ovarian development. Across Europe, the major demographic and social differences among *L. malachurum* populations are in the number of worker broods and the extent of worker ovarian development. This contrasts with the results of a principal components analysis of social traits among 15 social *L. (Evylaeus)* populations, which shows that interspecific social variation is defined by the proportion of males in the early brood, the proportion of workers mated, queen-worker size dimorphism, gyne overwintering locale, and the proportion of workers with developed ovaries.

Key words: Social behaviour, eusocial, Halictidae, *Evylaeus*, geographic variation

Halictine sweat bees are well known for their incredible social diversity. Closely related species may exhibit very similar or widely divergent types of social behaviour (Danforth, 2002; Packer, 1998). This extreme social diversity between species is enhanced by a high degree of social diversity within species, most prominently in the facultatively solitary species, but also within the obligately eusocial species. What ecological and evolutionary factors promote such social diversity? And do the same factors associated with intraspecific social variation also explain interspecific patterns? Until very recently, there was very little detailed information on intraspecific social diversity in halictine bees, so this question could only be addressed in general terms. However, the accumulation of data on intraspecific colony social organization in different places is providing us with the baseline information required to begin to address these questions.

Geographic patterns of intraspecific social variation in social halictines seem to depend on whether species are obligately or facultatively social (Richards, 2000). In facultatively solitary species like *Halictus rubicundus* (Soucy, 2002; Eickwort et al., 1996) and *Lasioglossum calceatum* (Sakagami and Munakata, 1972), harsher environmental conditions are associated with reversion to solitary nesting, with the omission of the worker brood. The existence of both solitary and eusocial nests in the same populations (Yanega, 1989; Yanega, 1993) suggests that population adjustment to local environmental conditions could be mediated by the decisions of individual foundresses to produce workers or gynes, or even workers versus males (Packer, 1990). Obligately social halictines such as *H. ligatus* (Kirkton, 1968; Litte, 1977; Michener and Bennett, 1977; Packer, 1986) and *L. malachurum* (Knerer, 1992) are apparently unable to omit production of a worker brood, but still exhibit considerable geographic variation in colony social organization. The general pattern is one of increasing eusociality (measured as increased queen-worker reproductive skew) under harsher conditions or in areas with shorter breeding seasons. This seems to be because harsh environmental conditions necessitate increas-

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* *Author for correspondence.*

ed worker cooperation in nest maintenance and brood production.

Lasioglossum malachurum is the best studied social halictine in Europe, where it exhibits clinal variation in social behaviour (Knerer, 1992). In northern areas it produces only a single worker brood and then a gyne brood, whereas in southern Europe it produces as many as three worker broods before the gyne brood. In southern populations, workers have higher rates of ovarian development, suggesting that queen-worker reproductive skew may be lower than in northern populations. Richards (2000) suggested that queen lifespan might be the key to declining eusociality in regions with longer breeding seasons, because in a population in southern Greece, it appeared that few queens survived to the time of gyne production, whereas many workers were mated by this time. Thus the long, southern breeding season would allow colonies to produce more broods, but workers might often lay the last eggs, specifically those developing into gynes and last-brood males. However, this hypothesis requires further investigation because the nesting aggregation studied by Richards (2000) may not have been typical. It was in the middle of an olive orchard subject to herbicide and insecticide spraying, and although there was no direct evidence that the poisons killed bees, the observed high queen mortality, small brood sizes, and high rates of worker reproduction observed in 1998 were anomalous in comparison with other aggregations in the area, as presented in the current study.

In the summer of 2000 we carried out a larger field study of two other aggregations of *L. malachurum* in the same area of southern Greece. These aggregations were in areas less affected by pesticide spraying (although few olive-growing areas in southern Greece are not subject to some spraying). As some observations from 1998 were probably atypical, in this study we provide a better outline of colony social organization in this area. With this information in hand, we then are in a position to compare intraspecific patterns of social variation with those in social *Evylaeus* as a whole.

Methods

Nest excavations and bee collections

The study sites were located in the village of Agios Nikolaos Monemvasias, near the southeastern tip of mainland Greece. Field studies were carried out at two nesting aggregations from 10 May to 25 June 2000. Dates are reported in terms of weeks (week 1: 7–13 May, week 2: 14–20 May, week 3: 21–27 May, week 4: 28 May–3 June, week 5: 4–10 June, week 6: 11–17 June, week 7: 18–24 June, week 8: 25 June–1 July). Note that nests must have been established in March or April.

The two aggregations, MALA1 and ANM, were separated by a distance of less than 1 km. In both aggregations, nests were clearly visible because of the tumulus of red soil around each nest entrance. Nests were individually marked with numbered flags made of tape and souvlaki sticks, and nearest neighbour distances were measured as the straight-line distance from one nest entrance to the closest nest entrance.

MALA1 was originally found in 1998, when it consisted of 42 nests in a dirt road on a hillside above Agios Nikolaos. This section of the nesting area was destroyed in the spring of 1999, apparently by road grading. However, in 2000, a few nests were again established beside the road, along with numerous nests on the three olive terraces directly below the road. The terraces are approximately 4 m wide and 112 m long, and had 3–4 m vertical walls on the inside, with red clay soil filled with rocks and boulders. The olive terraces are apparently plowed each spring to remove weeds that may be a fire hazard; they are also occasionally sprayed with herbicides and pesticides. In 2000, the MALA1 aggregation contained at least 133 widely spaced *L. malachurum* nests, and the mean nearest neighbour distance was 49.5 ± 118 cm (n = 75).

The second aggregation, ANM, was located in the middle of the village in an unused garden that contained two large olive trees situated on one edge of the site. The red clay soil contains rocks of all sizes, including small boulders up to 1m in diameter. The site had not been plowed recently although sheep regularly walked through it. Insecticides were sometimes sprayed on the olive trees to kill various homopteran pests. In 2000, the aggregation contained at least 280 nests in an area of about 25 × 6 m. At ANM, the nests were mostly found in the upper half of the garden, and were much more densely packed than at MALA1, with an average nearest neighbour distance of only 14.8 ± 11.3 cm (n = 157). Note that nests excavated in 1998 (Richards, 2000) were not in the MALA1 aggregation, but in a third aggregation about 1.5 km from MALA1 and about 0.5 km from the ANM aggregation.

Nest excavations (three nests at MALA1 in weeks 2 and 17 at ANM in weeks 2–7) were carried out using previously described techniques (Richards, 2000; Richards, 2001). Fully excavated nests (n = 12) were dug to approximately 5 cm below the end of the nest to ensure that all nest contents were found, and were 90–100 cm deep. Eight nests were only partially excavated because we were unable to remove some large boulders buried in the soil. All results refer to fully excavated nests unless otherwise stated. Most nest occupants (e.g. adults, early stage larvae, parasites) were immediately stored in 95% ethanol. Undamaged pupae and larvae that had consumed their entire provision masses were collected in wax-lined petri dishes and raised to adulthood, then stored in 95% ethanol.

The number of active foragers in nests was estimated by capturing foragers at nest entrances by placing a clear plastic cup over the nest entrance, catching the bees using a hand net, and storing them in 95% ethanol. The cup was removed to allow arriving bees to enter the nest. The number of nests with guards was estimated by walking through aggregations and checking nest entrances.

Dissections and measurements of adult females

Adult females were dissected and measured to determine their reproductive status and to confirm caste identifications. *Lasioglossum malachurum* queens and gynes can be reliably distinguished from their own workers based on their larger body sizes (Knerer, 1992; Richards, 2000). In addition, *L. malachurum* queens are expected to be mated, have high rates of ovarian development, and tend to have heavily worn wings and mandibles. Gyne pupae and imagos can also be distinguished from workers by their larger size. We note that in eusocial halictines, the terms queen and gyne are not necessarily interchangeable: although gynes are destined to become queens, not all queens are former gynes, as workers may become nest foundresses if circumstances allow them to diapause (Richards and Packer, 1994). In this study, we use the term queen for nest foundresses with workers and the term gyne for queen-destined pupae collected prior to diapause. To our knowledge, gyne-sized workers (i.e. workers as large as their queens) have never been observed in *L. malachurum*, although there are scattered reports of worker-sized queens (see Knerer, 1992 and results below).

Matedness was determined by the presence or absence of sperm in the spermatheca. Total ovarian development was assessed by summing the fractional sizes of all developing oocytes that were at least $^1/_4$ of their full size (i.e. $^1/_4$, $^1/_2$, $^3/_4$, 1). A score of zero was assigned to females with completely undeveloped ovaries, and a score of 0.1 to females whose ovaries were slightly thickened or which had oocytes too small to score (including those with degraded ovaries).

The degree of mandibular wear is expected to reflect activities such as digging brood cells, while wing wear is expected to reflect activities such as foraging. Mandibular wear was scored on a scale from 0 (mandibles sharp with a well-defined subapical tooth) to 5 (mandibles very blunt with little or no sub-apical tooth). Wing wear was also scored on a scale from 0 (forewings with the distal edge completely undamaged) to 5 (forewings with distal edge mostly or completely torn away by nicks and tears). Total wear represents the sum of wing and mandibular wear for each individual. Although Richards (2000) found very little evidence of wing wear in adult females, this was not the case in the current study, so we present analyses in terms of total wear.

Adult body size was measured in terms of head width (the distance across the widest part of the head, including the compound eyes) and length of the subcostal wing vein, using a binocular dissecting microscope fitted with an ocular micrometer at 32X magnification. For a few newly emerged males, workers, and gynes, we also measured body mass as the dry weight after being desiccated at 37 °C for at least 48 h.

The degree of caste size dimorphism between queens and workers was calculated by comparing each queen's size (i.e. head width) to the average size of her own workers, according to the formula

$$\frac{\text{Queen size} - \text{mean worker size} \times 100}{\text{Queen size}}$$

Interspecific comparisons

The colony social organization of *L. malachurum* at Agios Nikolaos Monemvasias in 1998 and 2000 was compared to that of four other populations of *L. malachurum* and nine populations of other eusocial *Evylaeus* using principal components analysis with the SAS statistical programming language (Proc Factor and Proc Princomp). We did not include *L. marginatum* in the analysis because caste determination and social organization are unique in this species and extremely different from all other social halictines (Plateaux-Quénu, 1962). In principal components analyses, the correlations among a large number of variables are reduced into fewer, independent variables known as principal components or factors. The degree to which each variable contributes to defining each factor is given by its factor loading score, and is considered significant if the loading factor exceeds 0.70 (Dillon and Goldstein, 1984).

Eight nesting and social traits were used in the analysis:

1. The type of nest founding by queens in the spring (0: no pleometrosis, 1: majority of nests haplometrotic, 2: majority of nests pleometrotic).
2. The number of worker broods.
3. The mean number of first brood workers.
4. The proportion of males in the first brood(s) (excluding the gyne-producing or reproductive brood).
5. The proportion of workers with developed ovaries (note that for species or populations with multiple worker broods, this number may include workers from both early and late worker broods).
6. The proportion of workers mated (the same proviso applies as for the previous trait).
7. The mean proportional size difference between queens and workers.
8. The overwintering location of gynes (0: natal nest, 1: elsewhere).

Results

Sociobiology of L. malachurum *at ANM*

Colony development

The age structure of broods excavated in weeks 2–7 is indicated in Figure 1. Quiescent periods lasting about 1 week, during which no foraging activity was observed, occurred prior to the emergence of Broods 2 and 3. Fully excavated nests contained *L. malachurum* pollen masses, larvae, pupae,

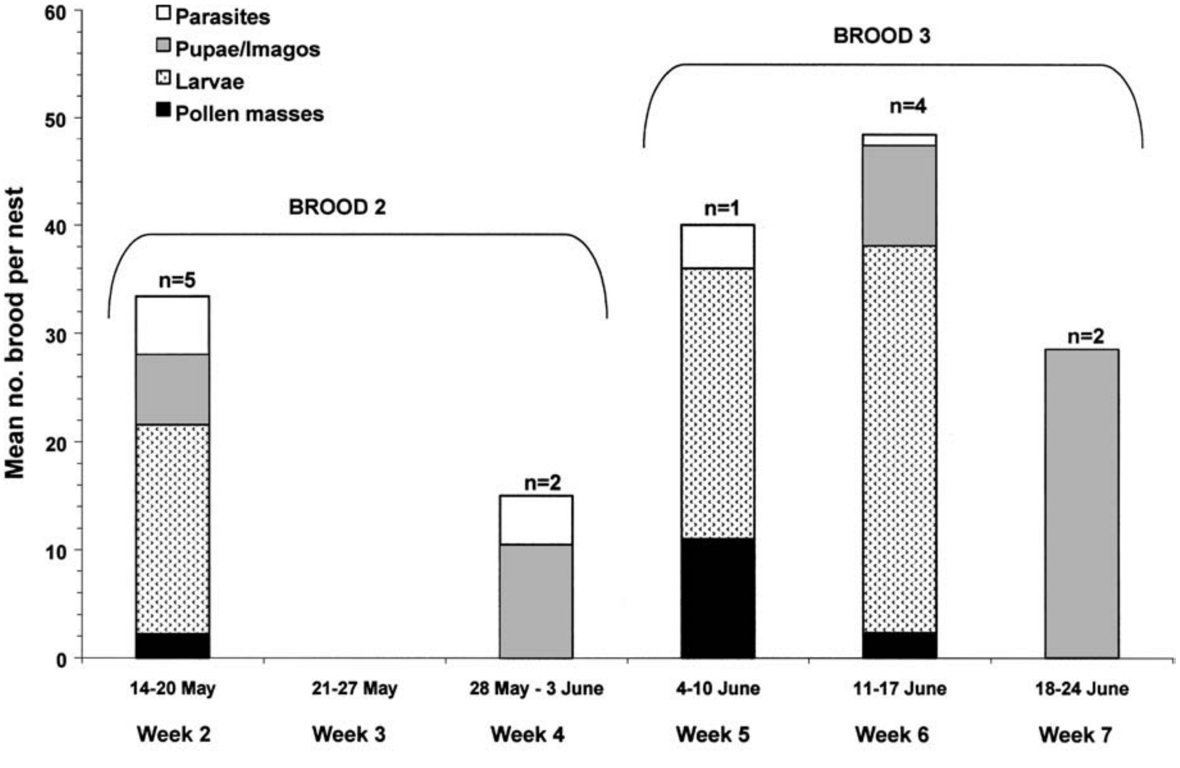

Figure 1. Brood development at the ANM and MALA1 aggregations from weeks 2 to 7, in terms of average number of brood per nest, including parasite larvae (mainly bombyliids). Numbers above the bars represent the number of nests excavated each week. Data from week 7 are from partially excavated nests.

newly emerged adults still in their brood cells, and adults, as well as unidentified bombyliid larvae that had consumed or were consuming *L. malachurum* larvae and their provisions. Only two nests contained no living *L. malachurum*. The first, excavated in week 5, contained only bombyliid larvae and dead workers, while the second, excavated in week 6, contained only dead workers.

Brood 1

Brood 1 probably emerged in early to mid-April, before the onset of observations in 2000. There were no male pupae or adults in nests excavated in week 2, so Brood 1 was probably composed solely of workers. The average number of workers produced in Brood 1, estimated as the number of adult workers in fully excavated nests from both aggregations in weeks 2 and 4, was 7.0 ± 4.2 (n = 7, see below). Since the second brood was already being produced and some Brood 1 workers might already have succumbed, this number probably underestimates the size of Brood 1.

Brood 2

Brood 2 consisted solely of workers. By week 4, excavated nests contained only pupae, indicating that brood production had ceased, so the size of Brood 2 was estimated from nests excavated during week 2. The mean size of Brood 2 at MALA1 was 32.5 ± 17.7 (n = 2) and at ANM was 34.0 ± 2.6 (n = 3), with an overall mean of 33.4 ± 9.1 (n = 5, Fig. 1).

Brood 3

Brood 3 egg-laying began during week 5, as we found only pollen masses and larvae that week. In week 7 we found only pupae and imagos, but brood production may have continued in other nests. Brood 3 was a mixed brood of workers, gynes, and males. The mean size of Brood 3, based on ANM nests excavated in week 6, was 48.3 ± 13.3 (n = 4, Fig. 1). Brood 3 was almost significantly larger than Brood 2 (Kruskal-Wallis F = 3.99, df = 1,8, $p < 0.0860$).

Worker and gyne larvae and pupae were distinguished based on size (see below). Of 154 sexable, Brood 3 larvae and pupae, 77 (50.0%) were males, 8 (5.2%) were workers, 41 (26.6%) were gynes, and 28 (18.2%) were females of unknown caste. Assuming that the proportions of workers and gynes were the same in unknown as known-caste females (and thus were produced in a ratio of about 1 : 4), on average each nest produced about 24.1 males, 20.2 gynes, and 3.9 workers in Brood 3. Brood 3 was slightly protandrous, male brood being slightly older on average than females (Table 1).

Queens and workers

Of 12 fully excavated nests from weeks 2–6, 10 contained living queens and two (one in week 2 and one in week 5) did not (Table 2), so it is likely that most queens survive to produce gyne brood. Nine nests were haplometrotic, but one nest had two live queens.

The average number of workers per nest, estimated from nest excavations, rose from 3.6 ± 1.5 (n = 5 nests) in week 2, to 15.5 ± 4.9 (n = 2) in week 4. In week 6, there were 10.0 ± 1.0 workers per nest. The average number of foragers per nest was assessed by collecting foragers as they departed from nest entrances. In week 2, the average number of foragers per nest per day at MALA1 was 4.2 ± 2.1 (n = 9 nests), in week 5 was 4.5 ± 2.1 (2 nests), and in week 7 was 13 (1 nest). These numbers suggest that most, but not all, workers forage when nests are active.

Table 1. Protandry of Brood 3 suggested by differences in the age structure of sexable brood, particularly during week 6. Pupae include newly emerged adults still in their brood cells

Week (no. nests)	Stage	Males	Workers	Gynes	Females of unknown caste
5 (n = 1)	Larvae	5	1	2	0
6 (n = 4)	Larvae	16	5	16	15
	Pupae	27	3	5	5
7 (n = 2)	Pupae	29	1	17	7
Total		77	10	40	27

The proportion of nests with guards was estimated by nest observations during forager collections and using foot surveys of the entire MALA1 aggregation. In 9 surveys carried out during foraging periods between 11 May and 24 June, on average about 35% of nests were active (nests entrances were open and we could see a guard or foragers), and 96% of open nests were guarded (had an adult female sitting in the entrance).

Caste characteristics

Body size

With few exceptions, queens and gynes were discernibly larger than workers (Fig. 2). Queens (mean head width 2.46 ± 0.09 mm, n = 14) and gynes (2.48 ± 0.04 mm, n = 40) were very similar in size but significantly larger than workers (2.20 ± 0.07 mm, n = 381) (ANOVA: $F_{2,435} = 360.10$, $p < 0.0001$, Bonferroni (Dunn), MSE = 0.00512, df = 433; non-parametric tests yield the same results). Based on head widths, queens were 10.3% larger than their own workers; size differences ranged from 5.4% to 14% in excavated nests. Based on subcostal vein lengths, queens (n = 14) were 12.9% larger than workers (n = 322); size differences ranged from 6.0% to 17.6% in excavated nests.

Female brood collected during weeks 5–7 (Brood 3) fell into two distinct size classes (Figs. 2 and 3), indicating that both workers and gynes are produced in the third brood. Gynes (inferred dry weight 9.18 ± 0.62 mg, n = 40) were about 51% larger than Brood 3 workers (6.05 ± 1.34 mg, n = 10) and twice the size of males (4.54 ± 0.81 mg, n = 59). Workers in their turn were about 33% larger than males. All differences are statistically significant (ANOVA: $F_{2,106} = 390.04$, $p < 0.0001$, Bonferroni (Dunn) MSE = 0.657038, df = 106). Accordingly, we assigned caste to female pupae excavated in weeks 6 and 7, based on size.

Newly emerged gynes and worker pupae exhibited non-overlapping head width distributions, although in week 6, the

Table 2. Social characteristics of *L. (Evylaeus)* in 15 populations and study years. When data were reported from both nest excavations and flower-caught workers, we used the former for principal components analyses. When ranges were given, the midpoint was used. Where caste size differences are reported based on both head widths and wing lengths, we used the latter. See methods for explanations of coding

Populations and reference	Locality	Type of nest founding	No. worker broods	Mean no. first brood workers %	Males in first brodd(s) %	Workers with developed ovaries	Workers mated %	Q-W size difference %	Gyne wintering location
L. albipes (Plateaux-Quénu, 1992)	Dordogne, France	1	1	3.0[a]	20–50	0.0	100.00[a]	5.6[d]	0
L. apristum (Miyanaga et al., 1999)	Japan	0	1	6.9[b]	5.5[b]	39.0 8.7[c]	0.0 4.4[c]	7.7[d]	1 (13/16 dug new burrows)
L. baleicum (Cronin and Hirata, unpubl.)	Nishioka, Japan	1	1	2.8	12–16	26.0	50.0	6.0[d]	1
L. duplex (ref. in Packer and Knerer, 1985)	Hokkaido,	1	1	4.6	10.0	20.2	8.5	9.6[d]	0
L. laticeps (Packer, 1983; Packer and Knerer, 1985)	Dorset England	0	1	4.1	23.8	53.0	18.0	7.3[e] 4.5[d]	1
L. lineare (Knerer, 1983; Packer and Knerer, 1985)	Paris, France	2	1	4.1	1.0	13.6	0.5	21.0[e]	0
L. malachurum (Knerer, 1992; Packer and Knerer, 1985)	Isle of Wight, England	0	1	2.3	2.3	3.4	4–20[c]	15.0[e]	1
L. malachurum (Knerer, 1992; Packer and Knerer, 1985)	Dordogne, France	0	2	2.0	2.0	8.0	8.0	15.8[e]	1
L. malachurum (Knerer, 1992; Packer and Knerer, 1985)	Estepona,	0	2	1.5	0	12–61	0.0	16.6[e]	1
L. malachurum (Knerer, 1992; Packer and Knerer, 1985)	Marseilles France	0	3	6.8	0.0–1.3	16.3–34.6	5.8	15.9[e]	1
L. malachurum (Richards, 2000)	Agios Nikolaos Monomvasias, Greece 1998	0	3	4.0	1.0	67.0[c]	50–70[c]	14.3[d]	1
L. malachurum (this study)	Agios Nikolaos Monomvasias, Greece 2000	1	3	7.0	0.0	58.0	0.7	10.3[d]	1
L. nigripes (ref. in Packer and Knerer, 1985)	Dordogne, France	2	1	7.2	4.0	59.0	1.0	10.3[d]	0
L. pauxillum (ref. in Packer and Knerer, 1985)	Tulln, Austria	0	2	4,0	5,0	15.0	1,0	14.5[e]	1

[a] Data from lab-reared nests.
[b] Data from greenhouse-raised nests.
[c] Data based on flower-caught females.
[d] Size difference based on head width.
[e] Size difference based on wing length.

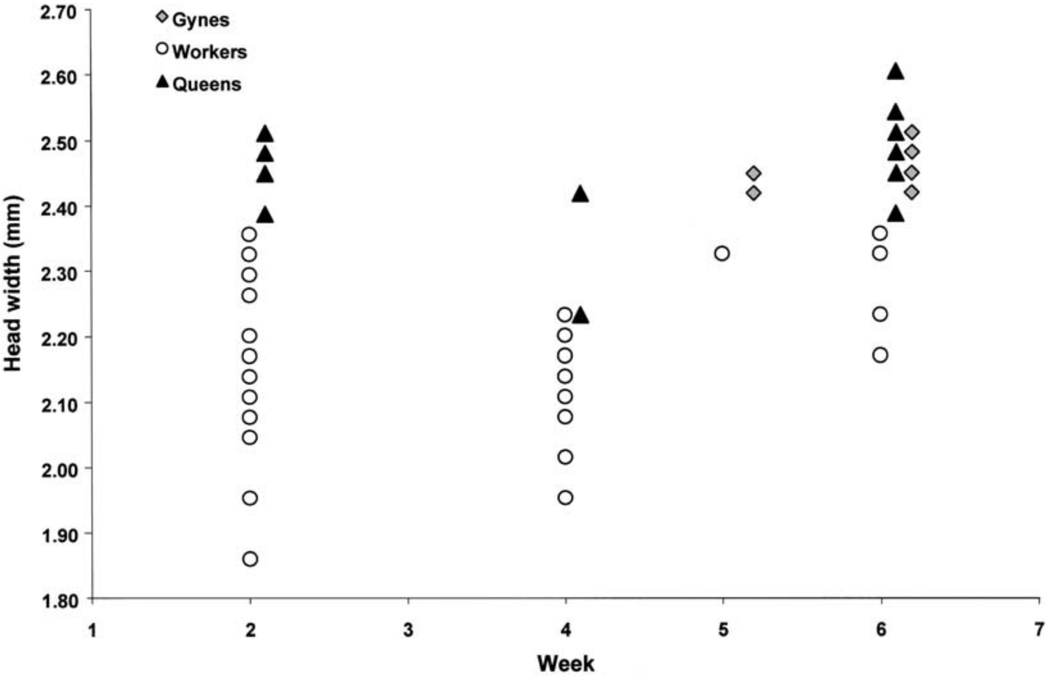

Figure 2. Weekly distributions of female head width. Young, queen-sized individuals (gynes) first appeared in week 5, when worker brood were also still being produced. Body size distributions of gynes and workers are non-overlapping but those of queens and workers are not.

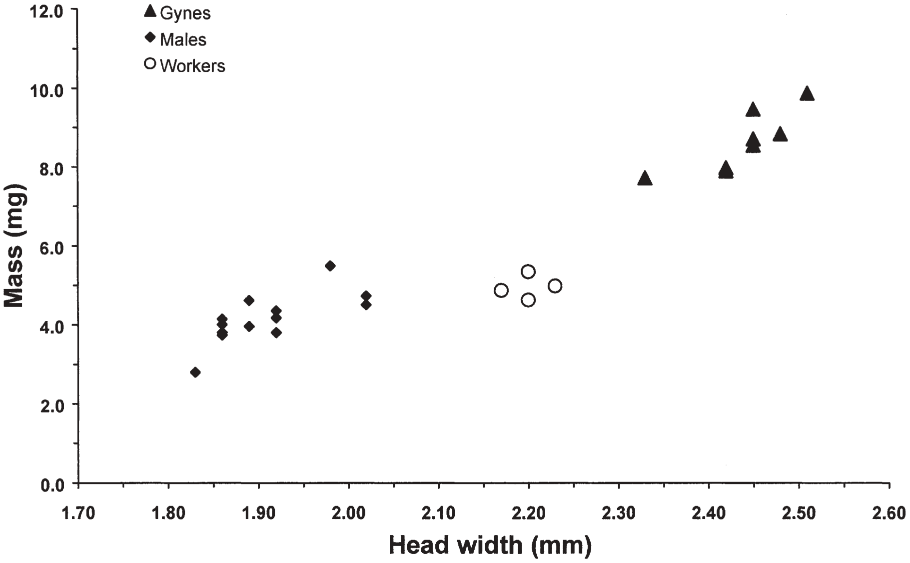

Figure 3. Dry weights of newly emerged workers, gynes, and males, in relation to head width. Note the non-overlapping size distributions of gynes and workers.

smallest gynes were only slightly larger than the largest workers (Fig. 2). However, in week 4, we found a worker-sized queen with a head width of only 2.23 mm, well within the range for workers. That she was a queen, not a worker or replacement queen, was indicated by the fact that she was mated at a time when there were no adult males in the population, so she must have mated the previous season. Moreover, compared to the other adult females in her nest, she was the largest, as well as having the highest ovarian development, mandibular wear and wing wear scores (which were 2.5, 4, and 4, respectively). Note that gynes and queens are not the same individuals and may not be members of the same physical caste (see Methods for definitions).

Reproductive characteristics

The reproductive status of queens and workers is compared in Figure 4. All queens (n = 13) were mated (although one queen was damaged and could not be assessed). The ovaries

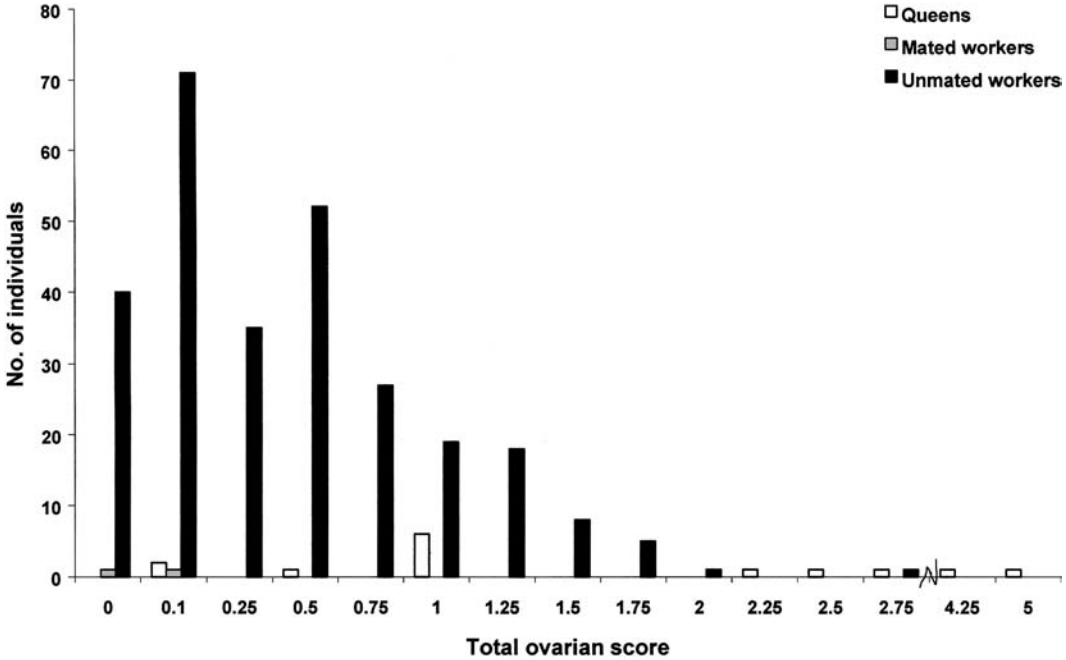

Figure 4. Total ovarian scores and frequency of mating for queens and workers.

of two queens found in week 6 were degrading, but the remaining 12 queens had at least one $^1/_2$-developed oocyte, and 3 of these (25%) had at least one fully mature egg ready to lay. In the single pleometrotic nest, both queens had total ovarian scores of 1.0.

Queens had high wear scores, the minimum being a score of 7, and most having wear scores of 9 or 10. The two queens from the same nest both had total wear scores of 10. Queen wing and mandibular wear were not correlated (Pearson ϱ = 0.09, n = 15, p > 0.05), but this statistical test has very little power since almost all queens were very worn. Queen total wear and ovarian development were not correlated (Pearson ϱ = –0.021, n = 14, p > 0.05), but this analysis also has very little power.

Whether or not workers had mated could be determined for 278 individuals; only two (0.7%) had spermathecae full of sperm (Fig. 4). These two workers were from a nest excavated during week 7, and both had worn mandibles. About 42% (142/297) of dissected workers exhibited little or no ovarian development (scores ≤ 0.1), whereas 28 % (84/297) had at least one $^1/_2$-developed oocyte and 8.4% (25/297) had at least one fully developed oocyte. The highest ovarian development score for a worker was 2.0, and was observed in a worker caught during week 4.

Since only two workers were mated, and these had undeveloped ovaries, almost all egg-laying workers must have laid only male eggs. Oviposition of male eggs probably began in week 5 (4–10 June, Fig. 1). The proportion of workers with at least one oocyte developed to $^1/_4$ final size, increased from 27% (n = 51) in week 2 to 78% (n = 90 workers) in week 4 and 72% (n = 89) in week 5. This was followed by a decline in weeks 6 and 7 (week 6: 34%, n = 32; week 7: 0%, n = 6), and another increase in week 8 (100%, n = 12). The increases coincide with the estimated peak oviposition periods for Brood 3, during week 5 (Fig. 1), suggesting workers may often lay male eggs.

Many workers were collected soon after emergence and would not yet have had much opportunity to engage in brood maintenance or reproductive activities, so the following analyses exclude workers with no sign of wear (total wear score = 0) and no clear sign of developing ovaries (ovarian development scores of 0 or 0.1). Mandibular and wing wear were correlated although mandibular wear tended to be greater (Pearson ϱ = 0.24, p = 0.0011, n = 290). Workers were considerably less worn (mean total wear score 3.2) than queens (mean score = 9.1, Kruskal-Wallis F = 162.2, df = 1, 302, p < 0.0001). Worker ovarian development was negatively correlated with total wear, but the correlation was not significant (Pearson ϱ = –0.07, n = 286, p > 0.05).

Fat stores

Dissections of newly emerged workers (n = 6), gynes (n = 3), and males (n = 2) found in their brood cells revealed that all imagos eclose with abdomens full of fat bodies. In addition, 16 foragers (15 in week 4 and 1 in week 7) caught at nest entrances, also had abdomens full of fat bodies. All had ovarian development scores of 0 or 0.1 and 88% (14/16) had mandibular wear scores of 0 or 1, suggesting that these individuals were newly emerged or young, but two of them had mandibular wear scores of 2 (week 4) and 4 (week 7).

Interspecific comparisons

Social characteristics of the *L. malachurum* populations were compared to other eusocial *Evylaeus* (Table 2) using principal components analysis (Table 3). The first three principal components (PC) had eigenvalues greater than 1 and explain 80% of the total variance in these variables (Table 3). Loading scores greater than 0.70 were deemed to be significant in explaining variation among the 15 populations. Characteristics loading most strongly on PC1, which accounted for 34% of the total variance, were the proportion of males in the early brood, and the proportion of mated workers. PC2 explained 24% of the total variance; the two traits with significant loading scores were mode of colony founding and the overwintering locale of gynes. PC3 explained 20% of the total variance; the only significant trait was the proportion of workers with developed ovaries.

The relative positions of the 15 *Evylaeus* populations and species with respect to the first three components are shown in Figure 5. In general, the *L. malachurum* populations cluster together (Fig. 5a), because compared to other eusocial *Evylaeus*, they have lower rates of worker mating, fewer males in early broods (high values of PC1), and are usually haplometrotic (low values of PC2). The *L. malachurum* populations also show a nearly perfect north-south gradient of increasing worker ovarian development (increasing values of PC3, Fig. 5b).

Discussion

Local variation in colony social organization

Although *L. malachurum* is obligately eusocial, the strength of eusociality varies considerably across its geographic range (Richards, 2000; Knerer, 1992). Southern colonies are larger, experience longer breeding seasons and colony cycles, and have workers with higher rates of ovarian development. We have also found evidence for marked social variation among aggregations and years in the same region of southern Greece, despite the fact that the three aggregations studied probably represent one large panmictic population, with no evidence for weather or microclimatic differences among aggregations and years.

Richards (2000) suggested that geographic patterns of colony social organization might be explained in terms of queen control of worker behaviour, suggesting that early queen mortality in southern areas might lead to increased worker reproductivity. However, this explanation now seems more appropriate to explain the peculiar circumstances of 1998 at ANM, rather than explaining geographically based social variation across Europe. At ANM, the differences between 1998 (Richards, 2000) and 2000 are particularly dramatic. In 1998, the few nests excavated were all queenless and contained only about 4 brood. Among workers, 50–70% were mated and 67% had developing ovaries. At the same point in the colony cycle in 2000, most nests had queens, average brood size was about 48, only 0.7% of workers were mated, and 58% had developing ovaries. What could cause such a disparity? The aggregation studied in 1998 (which did not exist in 2000) was in the midst of olive groves that were regularly sprayed with insecticides, which would probably have caused high adult and brood mortality, explaining the small colony sizes and early queen mortality that year. Behaviourally, insecticide spraying seems to have set up an inadvertent experiment on the effects of early queen removal. Early removal of queens in 1998 was associated with a high frequency of worker mating, whereas non-removal of queens in 2000 was associated with a very low frequency of worker mating. However, rates of worker ovarian development were about the same, suggesting that queens may influence worker behaviour, and thus colony social organization, by inhibiting mating and thus preventing worker production of diploid eggs.

Geographic variation in colony social organization

It is likely that the factors promoting social variation between aggregations and years within a single population, will also explain much of the variation between populations. In this section, we compare colony social organization in southern Greece to other parts of Europe (as summarized in Table 2).

Table 3. Results of the principal components analysis based on the data in Table 3. Factor loading scores ≥ 0.70 (after rounding) were considered to be significant (indicated in boldface)

Variable	Factor loading scores		
	PC 1	PC 2	PC 3
Type of colony founding	–0.39125	**0.77398**	0.16631
Number of first-brood workers	0.20250	0.64804	0.46664
Workers with developed ovaries	0.22831	0.10344	**0.87902**
Workers mated	**–0.69813**	–0.39184	0.18859
Queen-worker size difference	0.67569	0.20842	–0.57802
Males in early brood	**–0.87411**	–0.33560	0.12496
Gyne overwintering location	0.55439	**–0.73686**	0.27288
Number of worker broods	0.66833	–0.22630	0.37414
Eigenvalue	2.7082	1.9335	1.6178
Percent Explained	33.5	24.2	20.2
Cumulative Percent	33.5	58.0	78.2

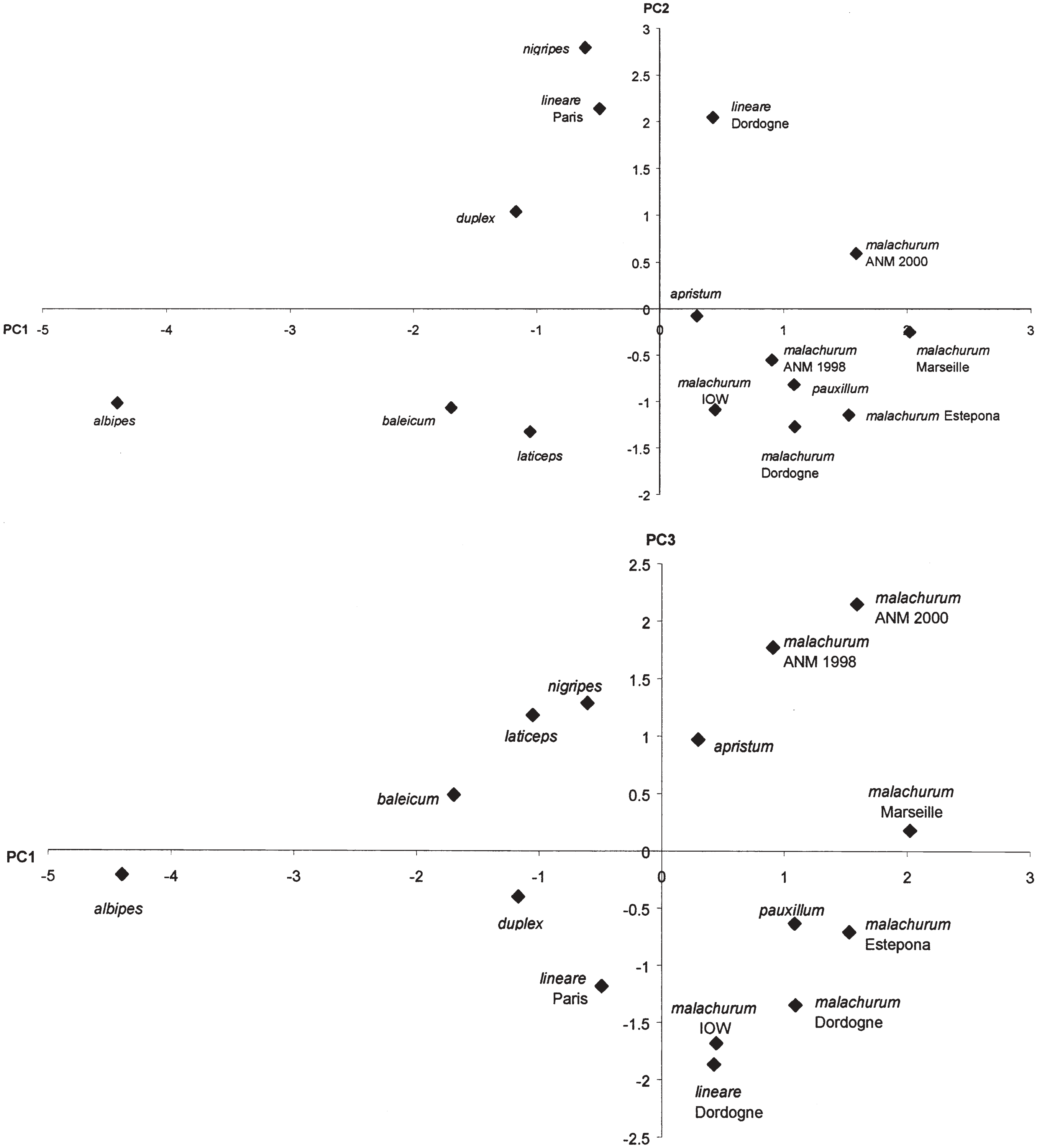

Figure 5. Principal components analysis of the 15 *Evylaeus* populations described in Table 3. PC1 represents decreases in the proportions of mated workers and males in the first brood. PC2 represents increased numbers of pleometrotic nests and overwintering away from the nest. PC3 represents increase proportions of workers with ovarian development.

Pleometrosis has never before been observed in any population of *L. malachurum* (Smith and Weller, 1989; Knerer, 1992) but in southern Greece as many as 20% of nests may have more than one foundress [microsatellite analyses support the hypothesis of occasional pleometrosis (M.H. Richards and D. French, unpubl. data)]. *L. malachurum* gynes overwinter away from their natal nests (Knerer, 1992), so accidental encounters in spring resulting in multi-foundress associations are unlikely. More likely, foundresses fighting for the same nest in spring occasionally become tolerant of each other and end up cohabiting the same nest.

Colony sizes are larger in more southerly populations, and this is due to the interpolation of successive worker broods before production of the reproductive (final) brood. In northern populations (such as the Isle of Wight) queens produce one worker brood (including a few males), and these workers provision the reproductive brood. In more southerly populations, early worker broods provision later worker broods which provision the reproductive brood. Since the size of the first brood is generally about the same in all populations (Table 2), this suggests that for about the same amount of effort (in terms of provisioning and brood cell excavation), queens in southern colonies may eventually produce many more gynes and males than queens in northern colonies.

Across Europe, *L. malachurum* populations display north-south clines of increasing worker ovarian development and decreasing worker mating (Table 2, Fig. 5). Based on the increased rates of worker ovarian development that we observed just prior to the onset of male production at ANM, it seems likely that workers often lay male eggs. A similar temporal pattern of worker ovarian development was observed in Tulln, Austria (Knerer, 1992). Halictine eggs are relatively large (J. Mou and M.H. Richards, unpubl. data), and must be metabolically expensive to produce, so workers should not exhibit relatively high levels of ovarian development unless they have real opportunities for successful oviposition. However, workers might have developed ovaries but not actually lay eggs, if they hold themselves in readiness to quickly produce eggs should the death of the foundress-queen create the opportunity to become a replacement queen. It seems likely that in a competition to become the replacement queen, a worker with more highly developed ovaries is likely to win (Strassmann and Meyer, 1983). If so, the proportion of workers with developing ovaries will tend to strongly overestimate the actual number of reproductive workers.

The strong association between frequency of worker mating and proportion of males produced in the early worker broods suggests that male availability influences whether or not, and how many, workers can mate (Yanega, 1993; Yanega, 1996). In northern populations where more males are produced in the early brood, workers are more likely to mate but have lower rates of ovarian development. In southern populations where few or no males are produced in the early broods, workers are less likely to mate but have higher rates of ovarian development (Table 2). This suggests that diploid egg production by workers is more likely in northern colonies, and that haploid egg production might be more common in southern colonies. Genetic evidence for worker maternity of gynes has been detected in a population of *L. malachurum* from Tubingen, Germany (Paxton et al., 2002), where there is only one worker brood per year.

In halictine bees, queen control of worker behaviour is through physical domination, which is more effective when queens are substantially larger than their daughters (Crozier and Pamilo, 1996; Michener, 1974; Kukuk and May, 1991; Smith and Weller, 1989). Different studies have defined the degree of queen-worker size dimorphism based on different characteristics (for example, Knerer 1992 used wing length and abdominal width). The degree of caste dimorphism at ANM is somewhat less than in other populations, but presents no obvious geographic pattern. Queen-worker size difference also was not a significant trait in the principal components analysis. This may be because other traits are more important or may reflect the different measurements used in different studies. We note that an average queen-worker size difference of 10% in terms of head width, does not seem that dramatic, but at ANM this represented about a 50% size difference in terms of body mass, which must be important in agonistic interactions.

At ANM as in other populations (Stöckhert, 1923; Knerer, 1992), the size distributions of newly emerged gynes and workers, whether measured in terms of head width, wing length, or body mass, are basically non-overlapping. However, body size alone (or the amount of food consumed by female larvae) is not sufficient to determine caste in *L. malachurum*, since queens are occasionally worker-sized. Knerer (1992) suggested that worker-sized queens might have resulted from gyne eggs mistakenly laid on male-destined pollen masses, implying a mechanism of caste determination distinct from body size, such as the presence of fat bodies in newly emerged females that allow females to diapause (Knerer, 1992; Richards and Packer, 1994). However, these explanations are unlikely for two reasons. First, if females accidentally lay female eggs on male pollen balls, they should also sometimes lay male eggs on gyne balls, producing males that are twice their normal size. We have no evidence for this. Second, we found that at Agios Nikolaos, all newly emerged workers, males and gynes had numerous fat bodies, so this cannot be the determinant of diapause.

An alternative explanation for the existence of occasional worker-sized queens, is that they are workers that seize the opportunity to mate and enter diapause, effectively switching caste by overwintering. In southern Greece, the most likely candidates for such behaviour are the estimated 10% of the final brood which are workers. Simultaneous production of workers and gynes has recently been observed in another population of *L. malachurum* in Germany (Strohm and Bordon-Hauser, 2003). There may be some advantage to producing a small number of workers in the gyne brood. Under the right conditions, they can continue to provision and raise brood, either extending the production of Brood 3 or producing a separate Brood 4. In other eusocial halictines, such as *Halictus rubicundus* (Yanega, 1988; Yanega, 1989), simultaneous production of workers and gynes occurs in the early ('worker' broods), but the worker and gyne castes exhibit broadly overlapping body size distributions in comparison with *L. malachurum*.

Intraspecific vs. interspecific social variation in L. (Evylaeus)

Within *L. malachurum*, clinal social variation is most striking with respect to the number of worker broods produced in each colony cycle, the proportion of mated workers, and the degree of worker ovarian development (Table 2). In contrast, interspecific variation in social *Evylaeus* is defined by the proportion of males in the early brood, the proportion of workers mated, mode of colony founding, gyne overwintering locale, and the proportion of workers with developed ovaries, approximately in that order. Packer and Knerer (1985) gave a similar list of important traits in an earlier study of social *Evylaeus*. The differences in the two lists of characteristics hint that there may be different constraints on intra- and interspecific social evolution, because not all species show the same plasticity in their social traits. For example, *L. malachurum* can produce 1–3 worker broods in response to local environmental variation, but cannot produce zero worker broods as can facultatively solitary species like *L. albipes* or *L. calceatum* (Plateaux-Quénu, 1992, 1993; Plateaux-Quénu et al., 2000; Sakagami and Munakata, 1972; Packer, 1991). Thus differences in social plasticity may lead to different patterns of social variation within and between species.

Insofar as stronger eusociality is defined by greater queen monopolization of oviposition (high reproductive skew), and weaker eusociality by an increasing worker contribution to brood production (low reproductive skew), then we can compare the strength of eusocial colony organization among populations and species. Taking rates of worker ovarian development as an indication of worker oviposition, southern *L. malachurum* colonies must have lower skew than northern colonies and so are more weakly eusocial. This creates a definitional contradiction, because the weakly eusocial colonies in the south are larger, have fewer males produced in the early broods, and fewer mated workers, characteristics that are usually associated with stronger eusociality (Michener, 1974, Packer and Knerer, 1985). Moreover, compared to the other eusocial *Evylaeus* populations, *L. malachurum* populations have lower rates of worker mating, produce fewer males in early broods, and are more often haplometrotic, again traits traditionally associated with stronger eusociality. However, Figure 5b suggests that *L. malachurum* populations exhibit as wide a range of reproductive skew patterns as all other *Evylaeus* combined. Perhaps ovarian development is not an appropriate tool for comparing worker reproductivity among populations, traditional notions about how social traits are correlated are not be correct, or possibly trait correlations within species are not necessarily the same as those between species. Certainly, our principal components analysis suggests that within *L. malachurum* and eusocial *Evylaeus* as a whole, worker ovarian development is either a poor indicator of skew or that this trait is not correlated with the other social traits in the expected manner. If so, then we cannot label southern *L. malachurum* colonies as less eusocial than northern ones, although the fact of clinal social organization remains true. One reason why intra- and interspecific patterns of skew might be differently associated with other eusocial traits, is that halictine queens have limited egg-laying ability. When colonies reach large sizes, workers can gather provisions at a rate outstripping the ability of the queen to lay eggs: *L. malachurum* queens can lay no more than 6 eggs a day (Knerer, 1992), so worker oviposition in large colonies may be a necessity.

Conclusion

There is no doubt that social variation in *L. malachurum* is clinal and associated with local ecological conditions, but we now need to identify the specific mechanisms by which environmental variation is translated into social variation, since differences in queen longevity no longer seem a likely explanation. In only two halictine bees, *L. albipes* (Plateaux-Quénu, 1993; Plateaux-Quénu et al., 2000) and *H. rubicundus* (Soucy and Danforth, 2002; Soucy, 2002) have there been studies explicitly designed to dissect apart the relative contributions of intrinsic (genetic) and extrinsic (environmental) sources (Wcislo, 1997) of social variation within and among species. *Lasioglossum malachurum* would be a good candidate for more of these studies.

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