Nesting biology and social organization of *Halictus sexcinctus* (Fabricius) in southern Greece

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Abstract: *Halictus sexcinctus* is a large halictine bee species widely distributed across central Europe and into the Middle East. Although its behaviour had not previously been studied in detail, it is known to exhibit solitary behaviour in central Europe. An aggregation nesting beside the seashore at Pyla-Daimonia, Demos Molai, in the southeastern Peloponnesos, Greece, was studied during the summers of 1997 and 1998. In southern Greece, *H. sexcinctus* exhibits weakly eusocial colonies, based on a partially bivoltine colony cycle, so across its range it is socially polymorphic. Weak eusociality in this Mediterranean population is characterized by haplometrotic nest-founding, a relatively high degree of queen–worker size dimorphism, high rates of worker mating and ovarian development, relatively short queen life-spans, and relatively low second-brood productivity. Overall, the sex ratio of the first brood is highly female-biased, while that of the second brood is variable, the proportions of males and females varying significantly in the 2 years. An unusual characteristic of nest foundresses in this population is that some are un-inseminated and produce all-male first broods rather than workers.

Résumé : *Halictus sexcinctus* est une espèce d'abeille halictinée commune dans tout le centre de l'Europe, jusqu'au Moyen-Orient. Bien que son comportement n'ait pas été étudié en détails, elle est reconnue comme une abeille solitaire dans le centre de l'Europe. Un regroupement de ces abeilles nichant sur la grève à Pyla-Daimonia, Demos Molai, dans le sud-est du Péloponnèse, en Grèce, a été étudié durant les étés de 1997 et 1998. Dans le sud de la Grèce, on re-trouve des colonies d'*H. sexcinctus* légèrement eusociales d'après le cycle partiellement bivoltin de la colonie, ce qui fait que, dans l'ensemble de sa répartition, l'espèce est socialement polymorphe. L'eusocialité faible chez cette population méditerranéenne est caractérisée par le fait que la colonie a été fondée par une seule reine, que le dimorphisme de la taille entre les reines et les ouvrières est important, que les taux d'accouplement et de développement ovarien de ouvrières sont élevés, que les reines vivent relativement peu de temps et que la productivité du deuxième couvain est faible. Dans l'ensemble, le rapport mâles : femelles du premier couvain favorise beaucoup les femelles, alors que celui du second est variable; le pourcentage de mâles et de femelles a été significativement différent les 2 années. Une caractéristique particulière des femelles fondatrices de cette population est que certaines ne sont pas inséminées et leur premier couvain est constitué exclusivement de mâles.

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Introduction

Halictine sweat bees are justifiably famous for the extreme range and variability of social systems that they exhibit. Because closely related species may exhibit quite divergent levels of social behaviour (Packer and Taylor 1997; Packer 1998), sweat bees are ideal subjects for elucidating the ecological, behavioural, and evolutionary factors that influence and regulate social behaviour. In addition, it is clear that halictine bees exhibit a great deal of social variation within as well as among species, phenotypic variation that has a genetic basis, an environmental basis, or both (Wcislo 1997; Plateaux-Quénu et al. 2000). For example, populations of *Lasioglossum* (*Evylaeus*) calceatum (Scopoli) living at low altitude exhibited eusocial colony organization, whereas a mountain-top population exhibited solitary behaviour (Sakagami and Munakata 1972). *Halictus rubicundus* (Christ) also exhibits

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this extreme social variability, with populations at high altitude (Eickwort et al. 1996) and in areas with short growing seasons (Hogendoorn and Leys 1997) exhibiting solitary behaviour. The social behaviour of *Lasioglossum (Evylaeus) albipes* (Fabricius) may also be a response to the length of the breeding season, since this bee is eusocial in southwestern France but solitary in inland France, where the climate is cooler (Plateaux-Quénu 1989, 1993). *Augochlorella striata* Provancher, which is further discussed below, may be a fourth example of a socially polymorphic species, since some foundresses appear to omit worker-brood production in response to harsh environmental conditions (Packer et al. 1989; Packer 1990).

Even obligately social sweat bees, which are unable to revert to completely solitary behaviour, may exhibit substantial plasticity in their social behaviour. In *Halictus ligatus* Say and *Lasioglossum (Evylaeus) malachurum* (Kirby), widespread species in North America and Europe, respectively, there is a pattern of increased queen monopolization of oviposition under harsher environmental conditions and an increasing worker share of oviposition in milder environmental conditions (Michener and Bennett 1977; Packer 1986; Knerer 1992; Richards 2000). If the degree to which reproductive skew favours queens is taken as a measure of the strength of eusocial colony organization, there may be a general tendency for obligately social species to display stronger eusocial colony organization in harsher environments, the opposite tendency to that displayed by the socially polymorphic species.

A third type of intraspecific social variability in halictine bees is demographically, rather than behaviourally, based. In virtually all populations of social sweat bees, some nests remain technically solitary for demographic reasons; for instance, the queen or the entire worker brood may perish before emergence. In contrast, in solitary nests of socially polymorphic species, the worker brood is actually omitted, because the foundress makes no attempt to produce workers (Eickwort et al. 1996). This type of social polymorphism should be particularly frequent in populations existing in difficult ecological conditions, such as at the edge of their geographic ranges, but may be difficult to distinguish from true social polymorphism. Both types occur in a population of A. striata nesting at the northern end of its range, in a marginal environment in Cape Breton, Nova Scotia. Although A. striata is considered to be eusocial elsewhere (Ordway 1965; Ordway 1966a; Ordway 1966b), Packer et al. (1989) observed that in Cape Breton fewer than half the nests became eusocial. Part of the reason was that in nests whose foundress died before worker emergence, the "workers" excavated overwintering burrows (thereby becoming gynes) instead of reactivating the nests (Packer 1990), so their nests remained solitary for demographic reasons. On the other hand, some A. striata foundresses apparently omitted worker production: only 70% of nests with surviving foundresses were reactivated in the summer, and nests were more likely to become eusocial if the first offspring produced was a female (and a small one at that) (Packer et al. 1989; Packer 1990). This implies a difference between solitary and eusocial foundresses in this A. striata population. At the same site, nests of Lasioglossum (Evylaeus) cinctipes (Provancher), which elsewhere is eusocial (Knerer and Atwood 1966), also failed to produce workers, thus remaining technically solitary, and they became extirpated from that site.

In this study I add another species to the list of socially polymorphic halictine bees that exhibit both solitary and eusocial colony organization in different parts of their range. *Halictus sexcinctus* is a relatively large sweat bee species with a geographic range across Europe and into the Middle East. It is considered to be solitary in central Europe, where it lives in very large aggregations (Knerer 1968), but as shown here, it is weakly eusocial in southern Greece.

Methods

Study site

The study site was located on the beach road running through Pyla-Daimonia, Demos Molai, in the southeastern Peloponnesos, Greece. The climate and general geography of southeastern Greece are described in Richards (2000). Generally, this is a typical Mediterranean landscape dominated by scrub and olive groves, with cool, rainy winters and very hot, very dry summers.

Field studies were carried out from May to July in 1997 and 1998. Observations are reported on a weekly basis starting with the first Sunday in May (week 1). At site 1, about 500 *H. sexcinctus* nests, as well as nests of several other species of halictid and megachilid bees, were dug into the vertical sandy retaining wall of a taverna that was probably created when the beach road was cut

out of the sandy cliffs by the seaside. These 500 nests were ideally suited for behavioural observations but could not be excavated, owing to the damage that would have been sustained by the taverna's patio. Site 2 was located about 10 m away and comprised 50–60 nests in another vertical sandy wall created by cutting a private driveway through a small cliff overlooking the beach. These nests could be excavated without causing undue damage. The identity of specimens from both field sites was confirmed by P. Andreas W. Ebmer (personal communication). Site 1, which was destroyed in spring 2000 when the retaining wall was replaced with a decorative stone wall, may have served as a source of bees for site 2, since this area of the aggregation also contained no *H. sexcinctus* nests in spring 2000.

Field methods

Field methods were generally as previously described (Richards 2000). Behavioural observations took place from early morning when bee activity began until mid to late morning at site 1. Nests were individually marked with coloured flags made of tape and souvlaki sticks on which individual nest numbers were written in indelible ink. Adult female bees were marked on the thorax with Testor's enamel so that they could be individually identified when departing from and arriving at the nest. These marks could usually be observed on guards at nest entrances as well. Although no site 1 nests could be excavated, adult bees associated with particular nests, as well as other adults flying around the nest aggregation, were caught and stored in 70–95% ethanol.

Nests at site 2 were excavated in their entirety to obtain "snapshots" of nest and brood development from spring until summer. Nests were excavated after white talcum powder was blown into the entrances and tunnels, making them easier to follow, especially when the sand occasionally collapsed. Nest excavations took place before foraging activity began in the morning or after it had ceased in the afternoon, to make sure that all nest occupants were in the nest. All nest occupants, including brood, adults, and nest parasites, were collected and stored in 70–95% ethanol.

Dissections and measurements

Female bees caught as adults were dissected to determine their reproductive status and age. Since these are relatively large sweat bees (as large as Mediterranean honey bees), assessment of matedness (presence or absence of sperm in the spermatheca) was straightforward, and particularly easy for specimens dissected within a few days of capture. Ovarian development was also assessed. Females with no ovarian development (ovaries were threadlike or had degenerated and disappeared) were assigned an ovarian development score of 0. Females whose ovaries were thickening but contained no oocytes that had reached 1/4 of their final size were assigned a score of 0.1. Females with at least one large (1/4 size) oocyte were assigned a total ovarian development score representing the summed fractions (1/4, 1/2, 3/4, or 1) of all developing, large oocytes.

Ages and activity rates of adult females are reflected in the degree of wear they sustain. Mandibular wear, which should reflect nest-digging activity, was ranked on a scale of 0 (no wear, mandibles shiny and with a sharp subapical tooth) to 5 (very worn, no separate tooth remaining). Wing wear, which is expected to reflect flight activity, and possibly aggressive interactions among adult females, was also assessed on a scale of 0 (wings undamaged, no nicks or tears) to 5 (original wing margin obliterated by damage).

Body size was measured in terms of head width and wing length at $12.5-20\times$ magnification on a dissecting microscope equipped with an eyepiece reticle marked in divisions of 1/10 mm. Head width was measured as the widest distance across the head, including the compound eyes. Wing length was measured as the length along the inside of the costal cell from the beginning of the subcostal vein to the stigma.

Fig. 1. Brood development in colonies of *Halictus sexcinctus* at Pyla-Daimonia, Greece, in 1997 (A) and 1998 (B). The stages of brood shown are provision masses with or without eggs (pollen balls), larvae, pupae, and newly emerged adults (imagos).



In most studies of social sweat bees, caste is assigned on the basis of a combination of characteristics: foundresses are large, are always mated, and have highly developed ovaries, whereas workers are small, may or may not have mated, and may or may not exhibit ovarian development. One problem with this scheme is that if caste is assessed on the basis of reproductive characteristics, then comparing these characteristics in foundresses and workers can be problematic. For instance, if all foundresses are assumed to be mated, then discovery of unmated foundresses leads to the conclusion that either they are not foundresses or that a mistake was made in assessing matedness. In this study, most foundresses were identified early in the season as the females that actually founded the nests, or if they were not found until after the emergence of workers, they were defined as the largest, most worn bees in the nest. Similarly, workers were identified as females that emerged in summer or were smaller and noticeably less worn than the foundress. In a few cases where these criteria were not sufficient to designate a female's caste with a high degree of confidence, caste was left as undetermined.

Results

Colony phenology and brood development

First brood

In both 1997 and 1998, observations began in early May. Foraging activity by foundresses probably began in week 1 or 2, since foundresses captured in the first 2 weeks of May had low wear scores (combined wing and mandibular wear score < 3). The oldest brood in nests excavated during week 3 in 1997 and 1998 were larvae (Fig. 1). Foundresses continued to forage until the third or fourth week in May. No nests contained more than one foundress, except during episodes

Brood	Year	No. of nests	Average no. of brood per nest				
			Females	Males	Ratio (females:males)	1997 vs. 1998	
First brood	1997 (week 7)	10	5.2 (81)	1.2 (19)	4.3		
	1998 (week 6)	25	3.8 (74)	1.3 (26)	2.9	ns	
Second brood	1997 (week 11)	17	4.1 (34)	7.8 (66)	0.52	0.0004	
	1998 (week 10)	20	3.0 (69)	1.4 (31)	2.2	p < 0.0001	

Table 1. Sex ratios for first and second broods of *Halictus sexcinctus* nesting at Pyla-Daimonia in 1997–1998, based on pupal counts.

Note: Differences between 1997 and 1998 were compared using Fisher's exact test. Values in parentheses are percentages.

of attempted nest usurpation, which usually lasted for only a few minutes (M.H. Richards, unpublished data).

Nests excavated in weeks 3–7 contained provision masses, eggs, larvae, pupae, newly emerged adults, previously used brood cells, cells containing dead brood, and various parasites, including bombyliids. In both years, first-brood adults emerged around week 6 (Fig. 1), so egg-to-adult development time is probably about 4.5–5 weeks.

Based on excavations in week 7 of 1997, and assuming that all brood cells were provisioned and an egg was laid in each, foundresses laid an average of 9.6 first-brood eggs (range 1–17) of which 8.5 were alive at the time of excavation, a survival rate of about 89%. Based on excavations in week 6 of 1998, foundresses laid about 10.2 eggs (range 1– 27), which produced 7.0 live brood, a survival rate of only about 68%. The difference in survival rates in the 2 years was highly significant (likelihood-ratio χ^2 test, $G_{[1]} = 46.386$, p < 0.001). However, no statistical differences in first-brood size distributions were detected between 1997 and 1998 (even using non-parametric tests), because too few nests were sampled, given the wide range of brood sizes.

Counts of pupae and newly emerged adults found in their brood cells were used to determine numerical sex ratios (Table 1). In 1997, 81% of the first brood were females, while in 1998, 74% were female. This difference is not significant (Fisher's exact test, p > 0.05). For the 2 years pooled, females composed 77% of the first brood. Hereinafter, females of the first brood are referred to as workers, while those of the second brood are referred to as gynes.

Second brood

In both 1997 and 1998, field observations were terminated before all bee foraging activity completely ceased, so the size of the second brood could have been slightly larger than that given here. Provisioning of the second brood began immediately after emergence of workers around weeks 6 and 7 and continued until at least mid-July. The first second-brood pupae were found in week 10, about 3 weeks after the beginning of the worker-foraging period, confirming the estimate of egg-to-adult development time given above.

In 1997, no provision masses were found in 10 nests excavated during week 11, implying that by then most foraging had ceased and broods were essentially complete. At that time, nests contained an average of 14.1 brood cells, of which 4.9 contained live brood. Since it was not possible to determine with absolute certainty whether diseased or previously used brood cells belonged to the first or second brood, the survival rate of the second brood could not be estimated. In 1998, bees were still provisioning during week 10. At that time, nests contained an average of 12.8 cells, of which 5.8 contained live brood. Since observations were terminated before foraging ceased in 1998, this is a minimum estimate of second-brood size. No statistical differences in secondbrood size distributions between 1997 and 1998 could be detected.

As with the first brood, counts of pupae and newly emerged adults were used to determine numerical sex ratios of the second brood (Table 1). In week 11 of 1997, females constituted 34% of the brood, while in week 10 of 1998, females constituted 69% of the brood. These two sex ratios are significantly different (Fisher's exact test, p < 0.0001). Based on proportions of males in nests excavated in 1997, the second brood is protandrous (week 9: 92% males, n = 4 nests; week 10: 80% males, n = 8 nests; week 11: 66% males, n = 17 nests).

Foundress and worker survival

All nests in the aggregation contained only a single foundress, so foundress survival (Fig. 2) was estimated as the proportion of nests with surviving queens at the time of nest excavation. All the nests excavated in week 3 of 1997 (n =2) and 1998 (n = 2) had live foundresses in them. By week 7 of 1997 (n = 10 nests) and week 6 of 1998 (n = 25 nests), at least 50% of foundresses had died. In 1997, no live foundresses were found in 29 nests excavated in weeks 9-11. In 1998, about 15% of queens were still alive in week 10 (n = 20nests). In other words, about 50% of foundresses survive to the onset of second-brood production and perhaps 10% survive to the end of second-brood production. It cannot be stated with certainty what proportion of these surviving foundresses were the original foundresses in each nest, although the rate of successful nest usurpation appeared to be low (M.H. Richards, unpublished data).

Workers began foraging in about week 6. In 1997, the average number of adult workers found in excavated nests was only about 0.4, while the number was 1.3 during the same week in 1998. These numbers are considerably lower than predicted: according to the sex ratios and productivities observed for brood 1, the average number of workers per nest would have been 6.9 (81% of 8.5 brood) in 1997 and 5.2 (74% of 7.0 brood) in 1998. Judging mainly from nests excavated in 1997, worker numbers stayed relatively constant from week 6 to week 8, but declined to almost zero by week 10. In 1997, worker foraging had ceased by week 11, although in 1998 a few nests still contained foragers at about this time.

Fig. 2. Survival rates of *H. sexcinctus* foundresses (\bullet , \bigcirc) and workers (\blacktriangle , \triangle) in 1997 (\bigcirc , \triangle) and 1998 (\bullet , \bigstar).



Reproductive activity

Foundresses

Reproductive activity (or more properly, potential reproductive activity) of adult females was assessed by dissecting them to determine whether they were mated and their degree of ovarian development. Very surprisingly, 4 of 14 foundresses from 1997 and 5 of 69 foundresses from 1998 were found to have empty spermathecae, indicating that they had not mated. These proportions are roughly similar (Fisher's exact test, p = 0.110, ns), so about 11% of foundresses had not mated.

The observation that there were unmated foundresses suggests that visual inspection of spermathecae is not a completely reliable technique for determining whether adult females have mated, but the existence of all-male first broods supports the observation that some foundresses do remain uninseminated. Four of the unmated foundresses were found in excavated nests, with the following brood counts: 3 males and no females, 8 males and no females, 8 males and 1 female, and 2 males and 4 females. In the two nests that contained only male brood, these could have been the brood of the resident foundress, and it should be noted that several other nests with all-male broods (but without foundresses) were also found. The two broods that included females could have been produced at least partially by other foundresses, since nest usurpation is frequently attempted in this population.

The ovarian development scores of foundresses are indicated in Fig. 3A. Of 83 foundresses, 78 (94%) had at least one 1/4-developed oocyte and 45 (54%) had at least one fully developed oocyte ready to lay. All the unmated foundresses had at least one 1/4-developed oocyte (Table 2). The levels of ovarian development in mated and unmated foundresses were not different, although this may simply reflect the small number of unmated foundresses (ANOVA for the effects of matedness and year on ovarian development, $F_{[2.82]} = 0.31$, ns).

Table 2. Frequency of mating and ovarian development in *H. sexcinctus* foundresses and workers for 1997 and 1998 combined.

		Ovarian development?				
Caste	Mated?	Yes	No	Subtotal		
Foundresses	Yes	69 (83.1)	5 (6.0)	74 (89.1)		
	No	9 (10.8)	0 (0)	9 (10.8)		
	Subtotal	78 (94.0)	5 (6.0)	83		
Workers	Yes	45 (47.4)	8 (8.4)	53 (55.8)		
	No	25 (26.3)	17 (17.9)	42 (44.2)		
	Subtotal	70 (73.7)	25 (26.3)	95		

Note: In this analysis, ovarian development was scored as positive if females contained at least one 1/4-developed oocyte. Values in parentheses are percentages.

Workers

Dissections of workers revealed significantly different reproductive patterns than those observed in foundresses (Table 2 and Fig. 3). About half (56%) of dissected workers had mated (6/20 in 1997 and 47/75 in 1998; Fisher's exact test, p = 0.178) and about 74% had developing ovaries (defined as containing at least one 1/4-developed oocyte; 13/20 in 1997 and 57/75 in 1998; Fisher's exact test, p = 0.844). Mated workers exhibited a significantly higher degree of ovarian development than unmated workers (two-way ANOVA for the effects of matedness and year on ovarian development: $F_{[2,92]} = 4.36$, p = 0.0155; for matedness, partial F = 8.60, p = 0.0042; for year, partial F = 0.12, ns).

A comparison of the reproductive activity of foundresses and workers (Table 2 and Fig. 3) indicates that foundresses were significantly more likely than workers to have mated (log-likelihood χ^2 test, $G_{[1]} = 27.88$, p < 0.001). Overall, foundresses also had significantly higher levels of ovarian development than workers (statistics are given in Table 3), **Fig. 3.** Ovarian development (OD) scores for foundresses, or queens (A), and workers (B) collected in both 1997 and 1998. OD scores represent summed totals of developing oocytes that had reached at least 1/4 of their final size. Open bars represent mated females and solid bars represent unmated females.



Table 3. ANOVA table comparing ovarian development in foundresses and workers from 1997 and 1998.

Source of variation in	Sum of	Mean			
ovarian development	df	squares	square	F	р
Complete model	3	39.19	13.06	11.62	< 0.0001
Error	174	195.61	1.12		
Caste (foundress vs. worker)	1	18.08	18.08	16.08	< 0.0001
Mated (yes vs. no)	1	7.12	7.12	6.32	0.0129
Year (1997 vs. 1998)	1	0.11	0.11	0.10	ns

Note: Partial effects for the three predictor variables (caste, whether or not mated, and year) are given in terms of Type III sums of squares.

	1997		1998		
Caste or sex	Head width	Wing length	Head width	Wing length	ANOVA for differences in head width (1997 vs. 1998)
Males	3.02 ± 0.16 (76) S	5.60 ± 0.33 (14)	3.07 ± 0.16 (62)	5.47 ± 0.44 (34)	$F_{[1,136]} = 1.21$, ns
Foundresses	$4.13 \pm 0.22 \ (18)$	6.17 ± 0.47 (19) S	4.20 ± 0.19 (77)	5.95 ± 0.30 (55)	$F_{[1,93]} = 1.85$, ns
Workers	3.93 ± 0.12 (78)	5.89 ± 0.34 (32) S	3.85 ± 0.19 (154)	5.79 ± 0.38 (57)	$F_{[1,230]} = 14.52, p = 0.0002$
Gynes	$4.15 \pm 0.15 \ (17)$	6.04 ± 0.34 (8)	$4.20\pm0.17(64)$	5.90 ± 0.22 (8)	$F_{[1,79]} = 1.67$, ns

Table 4. Head widths and wing lengths of different classes of *H. sexcinctus* collected in 1997 and 1998.

Note: Measurements are given as the mean ± 1 standard deviation, with sample sizes in parentheses. Annual variation in head width (i.e., 1997 vs. 1998) was compared using one-way ANOVA on the rank of head width. Samples designated "S" exhibited significant skewness (p < 0.05) toward smaller sizes (no samples exhibited significant skewness toward larger sizes).

Fig. 4. Total wear (mandibular wear + wing wear) scores (scale 0-10) for workers and foundresses captured in 1997 and 1998.



but in comparison with their own workers, foundresses sometimes had lower levels of ovarian development. In June 1998, a comparison of foundresses and workers collected from 11 nests indicated that foundresses had the highest degree of ovarian development in 8 nests, whereas in 3 nests a worker (a small female with a low wear score) had the highest degree of ovarian development. In one of these three nests, foundress infertility was due to endoparasitism, probably by conopids, but in the other two nests the foundresses appeared to be healthy.

Wear and tear as a measure of activity

In adult females, greater digging activity is expected to result in increased mandibular wear, while increased foraging activity should lead to increased wing wear. Mandibular and wing wear are correlated in both foundresses (Pearson's correlation coefficient, $\rho = 0.505$, n = 82, p < 0.0001) and workers ($\rho = 0.497$, n = 108, p < 0.0001), so total wear (mandibular wear + wing wear) is probably a better overall measure of parental investment activities such as brood-cell construction, nest maintenance, and brood provisioning. Wear patterns of foundresses and workers are compared in Fig. 4. The degree of wear exhibited by foundresses was significantly higher than that exhibited by workers, even when newly emerged workers (those with no wear and an ovarian development score of 0) were excluded from the comparison: the mean wear score for foundresses was 5.5 (n = 81), whereas the mean score for workers was only 1.7 (n = 78; $F_{1,157} = 104.91$, p < 0.0001).

In foundresses, total wear was not correlated with total ovarian development score ($\rho = 0.092$, n = 82, p > 0.05). In contrast, in workers, total wear was positively correlated with the degree of ovarian development (r = 0.345, n = 108, p = 0.0003), but the correlation may be inflated by the inclusion of newly emerged workers. When these were excluded (see above), the correlation between total wear and ovarian development disappeared (r = 0.157, n = 56, p > 0.05).

Body-size patterns

In general, females were larger than males, as is usual among sweat bees, and except for workers, body sizes were similar in 1997 and 1998 (Table 4). Only workers exhibited any change in body size, being slightly smaller in 1998 than in 1997 (Table 4). Since head-width distributions showed evidence of non-normality, leptokurtosis, and skew toward small sizes, the following statistical analyses are based on head-width rank rather than on head width per se. Foundresses and gynes did not differ in size $(F_{[3,172]} = 1.18, \text{ ns})$, but foundresses were clearly larger than workers $(F_{[3,323]} =$ 64.96, p < 0.0001). In terms of overall averages, in 1997 workers were about 4.8% smaller than foundresses, but in 1998 they were 8.3% smaller, a significant increase in the size differential (partial $F_{[1]} = 7.43$, p = 0.0068); statistically, this change can be ascribed to the fact that workers were smaller in 1998 than in 1997. Another way to look at queenworker size differences is to compare nestmates. Foundresses were always larger than the workers in their own nests, the size difference being 6.9% in 1997 (n = 4 nests) and 11.8% in 1998 (n = 11 nests).

Discussion

Social behaviour of H. sexcinctus in southern Greece

In southern Greece, *H. sexcinctus* exhibits a colony cycle and social behaviour typical of many social halictines that produce two or more broods per year. Key social characteristics exhibited by *H. sexcinctus* in southern Greece include haplometrotic (single foundress) nest-founding, overlap between foundress and daughter generations at the time when the second brood is produced, substantial queen–worker size differences, and reproductive skew favouring queens but allow-

Table 5. Comparison of important colony social parameters among eusocial populations of *H. sexcinctus* at Pyla-Daimonia, Greece (this study), *H. rubicundus* in New York, 1982–1987 (Yanega 1988, 1989), and *H. ligatus* in southern Ontario in 1984 and 1990–1991 (Packer 1986; Richards et al. 1995; Richards and Packer 1995, 1998).

	H. sexcino	ctus		
	1997	1998	H. rubicundus	H. ligatus
Method of nest-founding	Haplometrotic		Haplometrotic	~90% haplometrotic, ~10% pleometrotic
Foundress survival to worker-brood emergence (%)	50		69	45
Average no. of surviving first brood	8.5	7	5.8	5.8-9.0
Sex ratio of first brood (% males)	19	26	16–31	5-15
Average no. of first-brood females ^a	6.9	5.2	4.0-4.8	5.2-8.5
Average no. of adult workers in summer	0.4	1.3	3.8	≤4.5
Average no. of second brood	4.9	5.8	5.5-6.2	12-15
Sex ratio of second brood (% males)	66	29	58-85	50-60
Queen-worker size difference (%)	4.8-6.9	8.3-11.8	1.2	11.4-15.8
Proportion of workers mated (%)	26	63	69	42-52
Proportion of workers with developing ovaries	65	76	12–33	60
Proportion of sterile workers ^{b} (%)	25	16	na	26

Note: All averages are the number of individuals per nest.

^{*a*}Average number of surviving first brood \times (1 – proportion of males).

^bWorkers that were not mated and exhibited no ovarian development.

ing workers frequent opportunities for oviposition of males and gynes.

The following is a synopsis of the colony cycle in southern Greece. In spring, i.e., late April or early May, foundresses emerge from hibernation and establish nests. Each nest is established by a single foundress who provisions and raises the first brood. Most foundresses are mated and produce both male and female offspring in the first brood, but a very small number are unmated, produce only males, and do not produce a second brood. The sex ratio of the first brood is heavily female-biased. After the emergence of the first brood by mid-June, some first-brood daughters become foragers that provision and raise the second brood, more or less cooperatively. Probably fewer than half of the nests become matrifilial at this point, owing to high mortality of foundresses. The second brood, which begins to emerge in late June to early July, is protandrous. In 1997, the second brood had a male-biased sex ratio and in 1998 it had a female-biased sex ratio. If observations ceased too early, the proportion of females in the second brood would be underestimated because the youngest larvae in the nest would have been mostly female. The whole colony cycle takes about 10-12 weeks. Since both foundresses and their daughters evidently lay eggs in the second brood, the colony cycle is properly characterized as "partially bivoltine". Differences between 1997 and 1998 are summarized in Table 5.

Temperate-zone sweat bees are likely to experience substantial and unpredictable variation in annual or seasonal weather patterns, and such variation can strongly affect colony social organization (Wcislo 1997; Richards and Packer 1998). In southern Greece the weather is predictably hot and sunny by the end of May each year, so it seems unlikely that in this region local weather variation would lead to annual variation in sweat bee behaviour or demography. Yet even in this apparently predictable environment, there was evidence for annual variation in demographic parameters that could have important consequences for colony social organization. First-brood productivity was slightly, but not statistically significantly, lower in 1998 (7.0 brood per nest) than in 1997 (8.5 brood per nest), despite a significantly lower brood survival rate in 1998. Workers produced in 1998 were also significantly smaller than workers produced in 1997. Although second-brood productivities were similar, significantly more males were produced in this brood in 1997 than in 1998. The more male-biased sex ratio in 1997 was associated with a lower queen survival rate, a pattern similar to that observed in some other eusocial insects including H. rubicundus and A. striata in which a more male-biased sex ratio is observed in queenless nests (Yanega 1989; Mueller 1996). Perhaps foragers had more difficulty provisioning brood in 1997 than in 1998. Much less pollen is required to produce males, which are much smaller than gynes (Table 4), so a more male-biased sex ratio in 1997 might reflect a scarcity of pollen (Frank and Crespi 1989). However, I have no observations that indicate any differences in floral resources between 1997 and 1998.

One of the most curious observations in this study is that some H. sexcinctus foundresses remained un-inseminated. Although it has been hypothesized that in social halictines mating is a major determinant of whether a female subsequently acts as a worker or as a gyne (Yanega 1997), observations from several species militate against this hypothesis. In this study, I observed both un-inseminated foundresses and many inseminated workers. Un-inseminated foundresses have also been observed in Lasioglossum apristum (Vachal) (Miyanaga et al. 1999), and their existence is implied by occasional all-male first broods in Halictus scabiosae (Rossi) (Knerer and Plateaux-Quénu 1967). Mating seems to be unassociated with the subsequent reproductive behaviour of worker-brood females in L. (E.) albipes (Plateaux-Quénu and Packer 1998). Lasioglossum marginatum Brullé remains the only known halictine in which the relationship between mating and caste is unequivocal; in this bee, experimental evidence indicates that mated females become foundresses and unmated females become workers (Plateaux-Quénu 1962). The fact that in some social halictines, some foundresses remain un-inseminated, suggests that the same is true of some solitary halictines, which might be one reason for the repeated evolution of sociality in this group (Michener 1974). In a solitary species, if a predictable proportion of solitary foundresses produce all-male first broods, then other foundresses may be selected to produce female-biased first broods, which in turn favours eusociality should it arise (Seger 1983).

Comparative social level of *H. sexcinctus* and other *Halictus* species

In both social behaviour and colony cycle, H. sexcinctus in southern Greece resembles other eusocial halictines with annual colony cycles. In general, more strongly eusocial sweat bees are expected to exhibit more female-biased sex ratios in the worker brood, lower rates of worker reproductivity, a greater degree of queen-worker size dimorphism, and larger brood sizes, while more weakly eusocial species should have less female-biased worker broods, higher rates of worker reproductivity, a lesser degree of queen-worker size dimorphism, and relatively small brood sizes (Michener 1974; Breed 1976; Packer and Knerer 1985). Two other eusocial Halictus species that have been studied extensively are H. ligatus in Victoria, Ontario, Canada (Packer 1986; Richards and Packer 1995; Richards et al. 1995), and H. rubicundus in New York (Yanega 1988, 1989, 1993). Table 5 shows that, based on many important demographic and social parameters, the three species are roughly similar. However, H. sexcinctus has low queen survival to worker emergence, substantially fewer workers, apparently high rates of disappearance of first-brood females, and the highest rate of worker ovarian development, and produces the fewest second brood per nest. Halictus ligatus has similar levels of queen survival and worker ovarian development but a greater queen-worker size difference and much higher second-brood productivity. Halictus rubicundus exhibits the highest rate of queen survival, the lowest queen-worker size difference, the lowest rates of worker ovarian development, and intermediate brood productivity per nest. These patterns suggest that increased queen longevity is associated with decreased worker reproductivity (as indicated by ovarian development). This pattern is expected, because halictine queens prevent worker egg laying through physical intimidation (Kukuk and May 1991), so the level of worker reproduction is expected to rise after the death of the queen. However, a higher degree of queenworker dimorphism should also be associated with lower worker reproductivity, and yet worker ovarian development is lowest in H. rubicundus, which also has only 1.2% queenworker size dimorphism (this value is probably an underestimate: I based the calculation on a population sample of female wing lengths presented in Fig. 2 of Yanega (1989), but size dimorphism should really be calculated using queens and workers from the same nests).

An important characteristic of all three species is the discrepancy between the number of workers per nest and the higher number of first-brood females actually produced (Table 5). Yanega (1997) proposed that the discrepancy reveals that some first-brood females do not become workers in the maternal nest, but instead disappear and return as foundresses the following year (Yanega 1988, 1997). Table 5 shows that the discrepancy between the number of females produced and the number that become workers is largest for H. sexcinctus, followed by H. rubicundus, and lowest for *H. ligatus.* Two mechanisms could explain the variation among species. First, there may be variation in the timing of caste determination and the strength of caste differentiation. If caste-determination processes begin earlier in development (Yanega 1997), then proportionally more first-brood females should become workers. Second, there may be variation in the fitness payoffs due to worker versus foundress behaviour, so that in some circumstances, acting as a worker is better, whereas in other circumstances it might be better to become a foundress. Per-capita second-brood productivity (including workers and queens) can be estimated from the data given in Table 5: about 2.9 brood per bee for *H. sexcinctus*, 1.1-1.3 for H. rubicundus, and 2.2-2.7 for H. ligatus, which suggests that in all three species, the fitness payoff due to worker behaviour is probably lower than that due to foundress behaviour. Thus, differences in rates of worker disappearance among the three species could reflect the strength of caste differentiation, which is strongest in *H. rubicundus* and H. ligatus and weakest in H. sexcinctus. Strongly determined workers are less likely to leave the maternal nest. In H. ligatus, caste determination evidently occurs during the larval stages (Richards and Packer 1994), perhaps in response to the type of larval food provided by the queen. On the other hand, increased worker activity may not always enhance foundress fitness. Perhaps foundresses actively adjust the proportion of worker-brood daughters that actually become workers versus foundresses in such a way as to enhance their own fitness. This would imply that H. sexcinctus foundresses have less to gain from worker help than do H. rubicundus and H. ligatus foundresses.

Geographic variation in social behaviour

Halictus sexcinctus has a wide geographic range, from central Europe to the eastern Mediterranean and into Turkey and Iraq (P.A.W. Ebmer, personal communication). Although its colony organization has not been studied in detail in northern parts of its range, it is known to be solitary in central Europe (Knerer 1968). Thus, the species as a whole is socially polymorphic, being solitary in the northern part of its range and social in the southern part. Social polymorphism could represent either behavioural plasticity in response to environmental variation or a genetically fixed, and presumably adaptive, difference in behaviour among populations (Wcislo 1997). In fact, populations of H. sexcinctus in southern Greece and central Europe belong to two distinct subspecies (based on morphological criteria; P.A.W. Ebmer, personal communication), so behaviourally distinct populations may also be genetically distinct.

Several other sweat bee species also exhibit such extreme social polymorphism across their ranges. Two strongly socially polymorphic species, *H. rubicundus* (Yanega 1989, 1993; Eickwort et al. 1996; Hogendoorn and Leys 1997) and *L. (E.) calceatum* (Sakagami and Munakata 1972), revert to solitary nesting behaviour at high altitude, where breeding seasons are too short to allow the production of two broods. In solitary populations of *H. rubicundus*, it appears that foundresses omit the production of a worker brood, so the switch from eusocial to solitary nesting is accompanied by a switch from production of two broods to only one. The association between climate and behaviour within spe-

cies can be interpreted as evidence for behavioural plasticity. However, the fact that solitary populations of *H. rubicundus* and *L.* (*E.*) calceatum are isolated and clearly represent evolutionary reversions to the solitary mode (Packer 1991; Richards et al. 1995; Wcislo and Danforth 1997), and the observation of allozyme differences between social and solitary populations of *L.* (*E.*) albipes (Packer and Taylor 1997) provide a tantalizing suggestion of genetically based social polymorphism in these species.

Conclusion

A recurring theme in studies of sweat bee social behaviour is the reproductive opportunism of workers. In weakly eusocial species such as H. sexcinctus, females of the socalled worker brood may behave as wholly altruistic workers that never lay their own eggs, as partially altruistic workers that combine investment in their own offspring with cooperative investment in their relatives, or as nest foundresses the following spring. They may even attempt to usurp neighbouring nests (M.H. Richards, unpublished observation). In more strongly eusocial sweat bees, workers' reproductive options are narrower, because they are constrained by the social context of colonies and probably also by an inherent lack of behavioural flexibility. But even in obligately and strongly eusocial species such as L. (E.) malachurum, workers can often avail themselves of both cooperative and selfish reproductive opportunities (Richards 2000). Now that the extent of behavioural plasticity of workers is becoming well understood, the next scientific step will be to determine how the behaviour of individuals in colonies is transmuted into population-wide patterns of social variability.

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