

Research article

Variable worker behaviour in the weakly eusocial sweat bee, *Halictus sexcinctus* Fabricius

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Summary. Studies of eusocial halictines suggest that workers have many reproductive options, including sterile altruism in the maternal nest, combined helping and personal reproduction, and diapause and spring nest founding. How and when workers exercise these various options influences the strength of colony social organization. *Halictus sexcinctus* exhibits highly polymorphic social behaviour, with solitary colonies in central Europe and both eusocial and communal colonies in southern Greece. Indirect evidence suggests that some worker-brood females are actually gynes. A distinctly bimodal size distribution among foundresses in 1998, the lower size peak being close to the modal body size of workers from 1997, suggests that large worker-brood females overwinter and return to the aggregation as eusocial foundresses. Other first-brood females remain in the maternal nest as workers, although few can be classified as classical, sterile altruists. Only 17% of older, healthy workers are sterile (i.e. had ovarian development scores ≤ 0.1), whereas about 83% are reproductive, exhibiting at least one 1/4-developed oocyte. About 57% of older workers have at least one fully or 3/4 developed oocyte, signifying that they are ready or almost ready to lay. Sterile workers exhibit greater total wear (combined mandibular and wing wear) scores than reproductive workers, suggesting that they are older, have higher activity rates, or both.

Key words: Halictidae, worker reproduction, worker subfertility, social evolution.

Introduction

Halictine sweat bees exhibit considerable variation in colony social behaviour, both within and among species. Much, although not all, of this variation is due to interactions between bees and their local environmental conditions. The behaviour of workers is particularly critical in determining

the strength of eusociality in particular colonies and populations. When workers lay few or no eggs (i.e. when they behave altruistically), then colonies are more strongly eusocial, whereas when workers lay many or all of the eggs (i.e. when they behave selfishly) colonies are more weakly eusocial (Richards et al., 1995). Thus variability in worker reproductive behaviour is the key determinant of the strength of eusocial colony organization. A critical element in understanding this variability lies in identifying the various reproductive options that workers exercise in species with different types of colony social organization. Studies of eusocial halictines have suggested that halictine workers (or perhaps more precisely, worker-brood females) have many reproductive options, including sterile altruism in the maternal nest, as in *Lasioglossum marginatum* (Plateaux-Quénu, 1962), combined helping and personal reproduction in the maternal nest as in *Halictus ligatus* (Richards et al., 1995), and diapause and spring nest founding as in *H. rubicundus* Christ (Yanega, 1988).

In terms of its social behaviour, *Halictus sexcinctus* is the most strongly polymorphic sweat bee yet discovered. It exhibits solitary behaviour in central Europe (Noll, 1931; Knerer, 1968), but eusocial and communal behaviour in southern Greece (Richards et al., 2001, 2003). Its phylogenetic position (Danforth, 2002) implies that the solitary populations represent an evolutionary reversal from eusociality, suggesting that communal social organization might be an intermediate step in this transition (Richards et al., 2003). Two major changes in the reversion would be the loss of caste-based colony social organization and probably a switch from two broods per year to only one. Both types of changes are likely to be reflected in the behaviour of females produced in the first brood of eusocial nests. In this paper, I present indirect evidence for partial bivoltinism and for high rates of reproduction by first-brood females that remain in their maternal nests as workers.

Methods

This study is part of a larger investigation into the social biology of a population of *Halictus sexcinctus* nesting at Pyla-Daimonia, Demos Asopos (previously referred to as Demos Molai in Richards, 2001) in the southeastern Peloponnesos, Greece. Field studies were carried out from May to July in 1997 and 1998, using methods previously described (Richards, 2001). Nests and adult female bees were individually marked. Adult female bees caught during nest excavations or during behavioural observations, were stored immediately in 70–95% ethanol and dissected to determine their reproductive status (whether or not they had mated and the extent of ovarian development). Assessment of matedness (presence or absence of sperm in the spermatheca) was straightforward. 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 which had no oocytes that had reached 1/4 final size, were assigned a score of 0.1. Females with at least one 1/4-developed oocyte were assigned a total ovarian development score representing the summed fractions (1/4, 1/2, 3/4, or 1) of all developing oocytes. Females were classified as sterile if they had no ovarian development or only slightly thickened ovaries (ovarian development scores of 0 or 0.1).

Age 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, with 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). A total wear score was obtained by adding the mandibular and wing wear scores.

Body size was measured in terms of head width and wing length at 12.5–20× magnification on a dissecting microscope equipped with an eyepiece micrometer, 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 of the costal cell, from the beginning of the subcostal vein to the stigma, along the inside of the costal cell.

Caste was identified based on behaviour and relative size, but not reproductive status (Richards, 2001). Foundresses were identified when nests were established or after worker emergence, as the largest, most worn females in the nest. Workers were identified as females that emerged in the first brood and which were smaller than foundresses. 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.

Since wing wear, mandibular wear, and ovarian development scores were non-normally distributed, statistical analyses were based on non-parametric tests.

Results and discussion

Daughters that leave the nest: indirect evidence for partial bivoltinism

A large discrepancy between the size of the first brood and the actual number of workers observed suggests that some worker-brood females leave the maternal nest to overwinter and become foundresses the following spring (Yanega, 1988, 1997). Further indirect evidence supporting this hypothesis is found in the size distributions of workers and gynes collected in 1997 and foundresses collected in 1998 (Fig. 1). The 1997 worker distribution was unimodal, skewed to the left ($g_1 = -0.401$, $n = 154$, $p < 0.05$), and leptokurtotic (excess

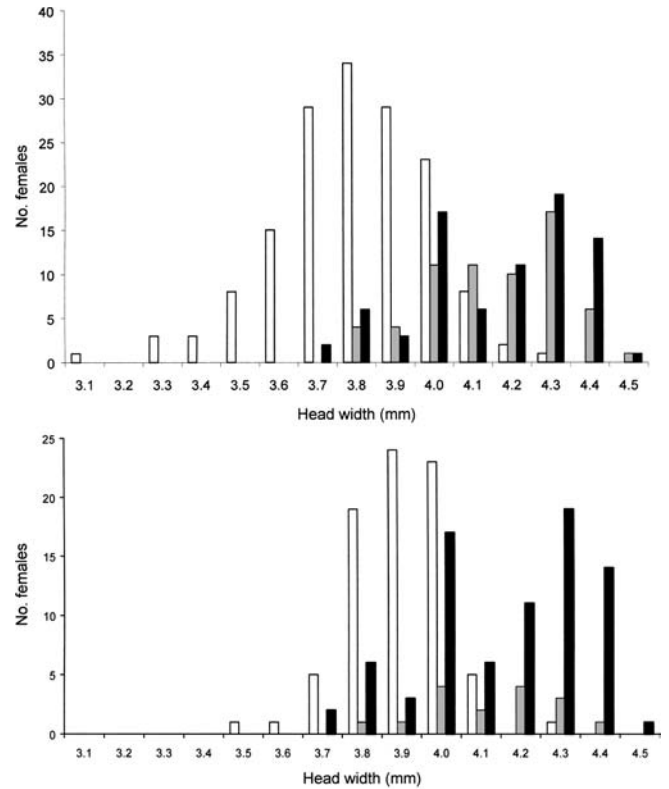


Figure 1. Head width distributions of *H. sexcinctus* females. Top: Females born in 1997 include workers (white bars) and gynes (grey bars) collected in 1997, and foundresses (black bars) collected in 1998. Bottom: Females collected in 1998 are shown for comparison

numbers of mean sizes, $g_2 = -2.199$, $p < 0.05$). Note that these statistics are mainly descriptive as their associated probabilities are difficult to assess (Sokal and Rohlf, 1981). The 1997 and 1998 gyne distributions did not depart significantly from normality, although sample sizes were small (Richards, 2001). In contrast, the 1998 foundress distribution was bimodal, causing skew to the left ($g_1 = -0.54586$, $n = 77$, $p < 0.05$), with one peak at about 4.0 mm and a second peak at about 4.3 mm. The bimodal distribution of foundress size could be explained if some of the missing workers actually disappeared into overwintering diapause, reappearing as spring nest foundresses the following spring. If large, first-brood females were more likely to overwinter, then this would explain why the lower foundress size peak corresponds closely with the upper size range of workers from 1997.

In the Daimonia population of *H. sexcinctus*, as many as 3/4 of females raised in the first brood disappear from their colonies (Richards, 2001). While some of them probably succumb to predators or parasites, it seems likely that some are actually gynes that become eusocial foundresses the following spring. It is unlikely that the missing first-brood females become foundresses in communal nests, as communal foundresses have relatively short wings and workers do not (Richards et al., 2003). Observational evidence that worker-brood females become overwintering gynes and then euso-

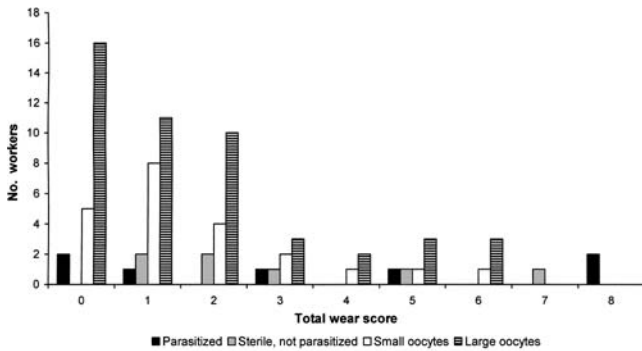


Figure 2. Total wear patterns for older workers, comparing sterile (parasitized versus unparasitized) and reproductive (with only small oocytes versus with at least one 3/4 or fully developed oocyte) individuals

cial nest foundresses has been found in *H. rubicundus* (Yanega, 1988) and *H. ligatus* (Packer, 1986) (Richards and Packer, 1994), and the phenomenon may be widespread in eusocial halictines (Yanega, 1997). Unfortunately, statistical methods for detecting platykurtosis are not especially reliable (Sokal and Rohlf, 1981), and this may lessen the chances that bimodal foundress size distributions will be noticed. As an example, *H. ligatus* foundresses nesting in southern Ontario also exhibited a previously unremarked bimodal size distribution (Richards and Packer, 1998), although the distribution did not deviate strongly from normality.

Daughters that stay home: Altruism vs. reproduction

Workers collected while foraging or from excavated nests were divided into three classes on the basis of ovarian development. Of 115 workers dissected, 22 (19.1%) had developing ovaries but no large (at least 3/4-developed) oocytes, 48 (41.7%) had large oocytes, and 45 (39.1%) were sterile. The sterile workers included 7 females parasitized by conopid larvae.

Mandibular, wing, and total wear scores were used to estimate age and activity rates of workers. Females with no ovarian development and total wear scores of 0 (n = 31) were deemed to be newly emerged adults and were excluded from further analyses, as they were not old enough to assess their reproductive activity. The ovarian status and total wear scores of the remaining 84 ‘old’ individuals are shown in Fig. 2. Most old workers (n = 70, 83.3%) had at least one 1/4-developed oocyte, and 48 (57.1%) had at least one 3/4 or fully developed oocyte. As many nests are queenless by the time the second brood is produced (Richards, 2001), workers with well developed oocytes are probably egg-layers. Only 14 (16.7%) old workers were sterile, including the seven that were parasitized.

Sterile and reproductive workers did not differ in size (in terms of head width: Wilcoxon two-sample test, z = -0.118, n.s.; wing length z = -0.118, n.s.). However, sterile workers were more likely than reproductive workers to have high total wear scores (Fig. 2; z = 2.053, p < 0.05). This difference was due mainly to the higher mandibular wear scores of sterile

workers (mandibular wear: z = 2.395, p < 0.05; wing wear: z = 1.314, n.s.). Among sterile workers, parasitized and unparasitized individuals did not differ in size (head width: z = 0.291, n.s.; wing length: z = -0.868, n.s.) or wear (total wear: z = 0.000, n.s.; mandibular wear: z = 0.473, n.s.; wing wear: z = -0.461, n.s.). Among reproductive workers there was no relationship between head width and ovarian development score (Pearson correlation coefficient, $\rho = 0.132$, n = 55, n.s.; not all individuals could be measured) or between ovarian and total wear scores (Pearson correlation coefficient, $\rho = 0.124$, n = 70, n.s.).

In addition to the seven parasitized workers, three parasitized queens were found, as well as one adult female of undetermined caste. Rates of parasitism were not perceptibly higher among workers than among foundresses (7/84 older workers vs. 3/86 foundresses, likelihood ratio $X^2 = 1.647$, d.f. = 1, n.s.).

That workers with developing ovaries were on average less worn than sterile workers can be explained in several ways. First, sterile workers may be older than non-sterile workers as mandibular and wing wear, as well as rates of parasitism, are expected to increase with age. Second, sterile workers may work harder and thus wear out faster. Third, average differences between sterile and reproductive workers might be due mainly to the effects of parasite infestation, as 7 of the 14 sterile workers were parasitized. The third possibility is unlikely. The higher average wear scores of sterile workers are not simply due to the effects of parasitism, since the wear patterns of parasitized and non-parasitized sterile workers are similar (see above) and 3 of 7 parasitized workers had very low wear scores (Fig. 2). Nor is there any reason to believe that conopid infestation increases rates of wing or mandibular wear. Moreover, although some parasites may increase the lifespan of their hosts, those infesting *H. sexcinctus* females eventually consume the entire contents of their hosts’ abdomens, which is more likely to shorten the hosts’ lifespans. The following discussion therefore pertains to the first two possibilities.

It is difficult to distinguish between the alternative hypotheses that sterile workers are older or sterile workers work harder, because wing and mandibular wear are correlated with both age and activity. The maximal age difference between the oldest and youngest workers in a nest based on the length of the foundress provisioning period, is about 3–4 weeks (Richards, 2001), which is probably sufficient time for older workers to accumulate significant wear. If sterile workers are older than reproductive workers, this could be because they live longer, emerged earlier, or because workers that do lay eggs, do so as soon as possible after emergence, before accumulating much evidence of wear. The latter explanation is supported by the observation that many workers with high ovarian scores or with oocytes ready to lay, exhibit little or no wear (Fig. 2). On the other hand, if sterile workers do more work than reproductive workers, this would suggest that they are more altruistic. The high rate of parasitization among the sterile workers suggests that subfertile individuals are more likely to be altruistic (Craig, 1983). In this population of *H. sexcinctus*, only about 50% of foundresses survive to work-

er-brood emergence, so it is likely (and in fact, necessary) that many second-brood eggs are worker-laid. Thus subfertile workers can help to raise siblings or nieces and nephews, and the associated inclusive fitness payoff may be enough to make up for the loss of personal reproductive opportunities.

The influence of parasites on worker reproduction has been noted in two other eusocial hymenopterans, which like *H. sexcinctus*, have egg-laying workers. In the ant *Leptothorax nylanderi* (Forster), cestode infections render individual workers sterile (Salzemann and Plateaux, 1987). In bumblebees (*Bombus terrestris* L.) (Shykoff and Schmid-Hempel, 1991; Schmid-Hempel and Schmid-Hempel, 1993), intestinal infestation by trypanosome gut parasites (*Crithidia bombi*) significantly lowers the rate of worker ovarian development, but apparently does not result in complete sterility. Parasitized workers tend to be older than healthy workers. Infestation has consequences for colony social organization, because in infested nests, worker-laid eggs (which would be male) appear about 5 days later than in uninfested nests.

Conclusion

In the *Daimonia* population of *Halictus sexcinctus*, there seem to be at least two kinds of worker-brood females, those that remain in the maternal nest and those that disappear. Indirect evidence suggests that many of the latter are gynes that will return as eusocial foundresses the following spring. First-brood females that remain in the natal nest become workers and may be either sterile or reproductive workers. Sterile workers that help to raise the offspring of nest-mates are by definition altruistic, but reproductive workers may also be somewhat altruistic if they assist their mothers or sisters to raise brood, in addition to raising their own. However, some reproductive workers remain unworn and may do no work at all, and so may be categorized as wholly 'selfish' individuals that parasitize the parental investments of their nestmates.

In weakly eusocial insects such as *H. sexcinctus* in southern Greece, it is much more difficult to define the level of worker altruism than it is in strongly eusocial species. When all workers are sterile, there is clearly only one set of reproductive tactics. If the majority are sterile, but a few lay most of the male eggs, as seems to be true of strongly eusocial species like *Lasioglossum marginatum* (Plateaux-Quénu, 1962), then there is a clear behavioural demarcation between the many altruistic workers and the few reproductive ones. In weakly eusocial colonies, the differences among workers represent the extremes of a behavioural continuum that seems also to include queens.

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