

Relatedness and sex ratio in a primitively eusocial halictine bee

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Abstract. *Lasioglossum laevisimum* was studied in Calgary, Alberta, where it is eusocial with one worker brood. Estimates of relatedness were obtained among various categories of nestmate based upon four polymorphic enzyme loci, two of which exhibited significant levels of linkage disequilibrium. Relatedness estimates among workers and among reproductive brood females were very close to the expected 0.75 value that obtains when nests are headed by one, singly mated queen. However, relatedness between workers and the reproductive brood females they reared was significantly lower than 0.75. A low frequency of orphaning with subsequent monopolisation of oviposition by one worker brood female in orphaned nests may explain these results. Workers were significantly more and queens significantly less closely related to male reproductives than expected if all males were to have resulted from queen-laid eggs. Orphaning and worker-produced males contribute to this result. The sex investment ratio was 1:2.2 in favour of females, in excellent agreement with the predictions based upon relative relatednesses between workers and reproductive brood males and females. Adaptive intercolony variation in investment ratios was detected: the sex ratio was more heavily female-biased in nests in which the relative relatedness asymmetry between workers and reproductive brood was more female-biased. The study species is the most weakly eusocial hymenopteran for which relatedness estimates and sex ratio data are available. With high relatedness among nestmates and a strongly female-biased sex ratio, this study suggests the importance of indirect fitness contributions in the early stages of social evolution.

Key words: Relatedness – Sex ratio – Social evolution – Sweat bee

Introduction

Eusociality is characterised by societies in which individuals of the parental generation reproductively dominate

some members of the offspring generation. As this requires a reduction in the direct reproductive success of the worker offspring, some evolutionary mechanism other than simple individual selection is required to explain this phenomenon. The kin selection hypothesis remains the predominant paradigm in our thinking about the evolution of eusociality. One reason for this is its apparent success in explaining the multiple independent origins of eusociality among hymenopterous insects (Wilson 1971). Haplodiploidy results in a higher coefficient of relatedness between full sisters (0.75) than between a mother and her daughters (0.5), thus providing potential genetic benefits to a daughter that stays at home to raise sisters. However, haplodiploidy also results in a reduced relatedness of females to brothers (0.25) in comparison to sons (0.5) and, with an even sex ratio, this exactly cancels out the increased relatedness to sisters over daughters. Consequently, workers may only gain real genetic benefits if the colony sex ratio is female-biased and/or they produce some haploid eggs directly (Trivers and Hare 1976). Thus, major predictions of the haplodiploidy hypothesis are that (i) workers should have high relatednesses to the reproductive brood females that they help rear and (ii) colony sex ratios should be biased to promote the genetic interests of workers by being proportional to their relatednesses to male and female reproductives (Oster and Wilson 1978; Boomsma and Grafen 1991; Pamilo 1991).

A recent survey suggests that the first prediction is not supported: few social hymenopteran species have sufficiently high female nestmate relatedness values to suggest haplodiploidy as a major factor promoting eusociality (Gadagkar 1991). However, most of these estimates come from highly eusocial species, those in which the reproductive options for workers are limited by gross anatomical differences between the castes. Indeed the majority of estimates come from ants and polistine wasps – taxa which have been eusocial for over 100 million years as indicated by fossils from the Cretaceous (Brandao et al. 1989; Wenzel 1990). If a high coefficient of relatedness among nestmates is necessary for the ori-

gins of eusociality, these taxa are probably inappropriate test organisms: there have been over 100 million years for other factors (such as nutritional castration or behavioural manipulation of workers) to mask any initial importance of relatedness. To explain the origins of eusociality we need information from more primitively eusocial species, those in which morphological caste differences do not prevent workers from initiating nests alone. There is still a dearth of such information (Ross and Matthews 1989a, b).

The relative investment in male and female reproductive brood is frequently estimated and is of considerable interest in its own right (Charnov 1982). The well-known prediction is that under sterile worker control in a monogynous, monoandrous colony the investment ratio in reproductives should approximate 1:3 (males to females). Less female-biased to slightly male-biased ratios are expected with worker control, under conditions of polygyny, polyandry, male production by workers, some measure of queen control or combinations thereof (Trivers and Hare 1976).

During the earlier stages of social evolution phenomena such as orphaning and worker reproduction are expected to be comparatively common. Predicting the investment ratio of different classes of colony and the population as a whole is difficult under such circumstances. Building upon the work of Taylor (1988) and others, Pamilo (1991) has produced a model which incorporates orphaning and worker reproduction into sex allocation predictions (see below).

Recent advances stress the importance of intercolony variation in investment. The theory predicts that workers should bias the colony investment ratio in favour of the sex to which they are most highly related in comparison to the population average (Boomsma and Grafen 1991). For example, orphaning usually results in one "worker" becoming a replacement queen. The remaining workers thus raise a brood of nephews ($r=3/8$) and nieces ($r=3/8$) rather than brothers ($r=1/4$) and sisters ($r=3/4$) – "life for life" values are quoted here. The relative relatedness asymmetry between workers and the brood they rear is male-biased in orphaned colonies compared to the population average, unless all colonies are orphaned. Consequently, workers in orphaned nests should invest more in males than predicted by the Trivers and Hare (1976) model. If orphaning is reasonably common, the increased male production in such nests results in workers in queenright colonies favouring an even higher female bias to the sex ratio than 1:3. Empirical studies confirm the predicted patterns in both primitively eusocial (Boomsma 1991; Mueller 1991) and advanced eusocial species (Boomsma and Grafen 1990). Nonetheless, most studies of social hymenopteran investment ratios have involved highly eusocial taxa, among which ants predominate, and more data for primitively eusocial species are badly needed (Ross and Matthews 1989b).

In summary, relatedness values among hymenopteran nestmate females are often low, apparently contradicting the predictions of the haplodiploidy hypothesis. However, sex ratio variation among colonies often follows the pattern predicted under conditions of worker con-

trol. Unfortunately, sex ratio data have been presented along with relatedness estimates for only three species of primitively eusocial insect so far: two species of *Polistes* (Metcalf 1980) and the social sphecid *Microstigmus comes* (Ross and Matthews 1989a, b). Moderately high female-female relatedness values were found in each study but evidence for worker control over the investment ratio was found only for the sphecid wasp.

This study addresses the relationship between relatedness and investment ratio for a primitively eusocial halictine bee, *Lasioglossum (Dialictus) laevissimum*, studied in Calgary, Alberta (51°00'N, 114°10'W) throughout the summer of 1988. Halictine bees have often been considered to be among the best candidates for testing hypotheses of social evolution (Michener 1974, 1990; Sakagami 1974). The subgenus *Dialictus* in particular deserves attention because it has a large number of solitary as well as social species and sociality may have originated several times within the subgenus (Eickwort GC, personal communication).

Lasioglossum laevissimum seems particularly pertinent to studies of the origins of eusociality because it is such a weakly eusocial species (Packer 1992). A weakly eusocial species may be expected to have a high proportion of workers mated, a high proportion of workers with well-developed ovaries and a high proportion of males in the "worker" brood, a small degree of size dimorphism between the castes and comparatively few bees in the nest. Elsewhere (Packer 1992) I have shown that, of nine *Dialictus* species for which sufficient data are available, *L. laevissimum* received the lowest average rank for these five variables, which are generally considered important in determining the level of social evolution in halictines (Breed 1976; Packer and Knerer 1985). Thus, estimates of the crucial parameters of relatedness and sex ratio in this species should be of particular interest.

In this paper we provide relatedness estimates among various categories of nestmate based upon four polymorphic loci. Assumptions of at most weak selection and lack of linkage between loci are tested; the latter is unjustified for two of the loci. Lastly, patterns of variation in investment ratio and relatedness among colonies are analysed in the light of the recent theoretical advances made by Boomsma and Grafen (1991) and Pamilo (1991).

Methods

Sampling. Descriptions of the nest site and excavation techniques are provided by Packer (1992). Only perfectly excavated nests were included in the analyses presented here; whenever brood from adjacent nests may have become confused during digging, data from both nests were omitted from the analyses. All excavations were performed before 0900 hours or after 1800 hours, when no bees were out of the nest foraging. Individuals from imperfectly excavated nests were used to screen for variable enzyme loci or, for brood raised in the laboratory, to provide calibration plots of wet and dry weight against head width.

Adults found in nests were stored in one 1.5-ml Eppendorf tube per nest and frozen at -80°C until required for electrophoresis. Fully grown larvae and later developmental stages were placed

Table 1. Enzyme names, EC numbers, buffer systems, recipes and electromorphs

Enzyme	Symbol	EC # ^a	Buffer ^b	Electromorph mobilities (mm)
B-N-Acetylhexosaminidase 33	<i>Aha</i>	3.2.1.52	bI	<i>Aha</i> ₁ 18, <i>Aha</i> ₂ 24, <i>Aha</i> ₃ 28, <i>Aha</i> ₄ 33
Esterase	<i>Est</i>	—	RSL	<i>Est</i> ₁ 24, <i>Est</i> ₂ 28
D-2-Hydroxy-acid dehydrogenase	<i>Had</i>	1.1.99.6	bI	<i>Had</i> ₁ 7, <i>Had</i> ₂ 16, <i>Had</i> ₃ 26, <i>Had</i> ₄ 29
Peptidase phe-pro	<i>Pep</i>	3.4.13.8	RSL	<i>Pep</i> ₁ 18, <i>Pep</i> ₂ 23, <i>Pep</i> ₃ 31

^a Enzyme Commission numbers from Webb (1984)

^b Gel and electrode buffer systems were as follows: bI from Shaw and Prasad (1970) with 50 mg NAD added before degassing, RSL from Ridgway et al. (1970), all stain recipes from May et al. (1988) except *Est* from Shaw and Prasad (1970)

in individual depressions in wax filled petri dishes and reared to adulthood before being frozen individually. Contents of different nests were kept in separate dishes throughout rearing.

Electrophoretic techniques. Initial screening suggested that there were four usefully variable enzyme encoding loci – acetylhexosaminidase (*Aha*), esterase (*Est*), hydroxyacid dehydrogenase (*Had*) and peptidase with phenylalanine-proline as substrate (*Pep*) (see Table 1 for full enzyme names, electromorph mobilities and other details). Three loci (all but *Had*) stained best, or only, when extracts from gasters were used and these were ground in 25–40 µl (depending upon bee size) of a 1% solution of dithiothreitol in 0.1 M sodium phosphate buffer pH 7.0. This usually provided sufficient liquid for three wicks (4 × 14 mm Whatman #3). As only two buffer systems were required to resolve the four loci, an additional wick was left over for many individuals and this could be used to clarify some ambiguous results and to perform appropriate line-ups for rarer electromorphs. Single families containing reproductive brood and worker broods from multiple foundress associations were run with individuals from different nests on each of at least two gels to ensure homology of electromorphs across nests. Worker brood nests which had single foundresses were sufficiently small to permit direct comparison of mobilities across five or more families on a single gel.

Analyses of electrophoretic data. Linkage disequilibrium between loci was investigated directly from haplotype frequencies using the formulae of Hill (1974). For the analyses presented here, one male was chosen randomly from each nest and the coefficient of linkage disequilibrium (*D*) calculated for each pairwise comparison.

Selection on electromorphs was evaluated in two ways: (i) allele frequencies of parental and reproductive brood offspring generations were compared using chi squared tests, (ii) maternal genotype frequencies were compared to Hardy-Weinberg equilibrium expectation.

The method developed by Queller and Goodnight (1989) was used for estimating nestmate relatedness. This is an identity-by-descent measure based upon regression estimates of relatedness (Hamilton 1970) rather than “life-for-life” values (Hamilton 1972). The difference between the two approaches is that “life-for-life” values take asymmetries in the reproductive values of interactants into account. Thus, the expected values when female-to-male relatedness are being considered are double those that obtain with “life-for-life” values (Grafen 1986). Analyses were performed weighting nests equally. Single locus estimates were obtained with individuals not scored for a particular locus omitted from the estimate for that locus only. For analyses involving combinations of loci, all individuals with one or more missing values were excluded from the data set. As a result of the uneven distribution of missing data, sample sizes vary somewhat among analyses.

The method of Queller and Goodnight (1989) provides estimates of relatedness for each nest separately as well as global population estimates. These individual nest estimates have high standard errors associated with them but nonparametric statistical tests are appropriate to analyse patterns between relatedness and other vari-

ables of interest. Spearman’s rank correlation coefficient was used to analyse the relationship between relatedness and the investment ratio. Workers with higher relatedness to gynes are expected to bias the colony sex ratio towards females, consequently a one-tailed test is appropriate. Inspection of genotype arrays within nests permitted identification of some broods which could not have resulted from the monopolisation of oviposition by one singly mated queen. Sex ratios in nests with such complex genealogies were compared with those with genotype arrays consistent with monoandry and monogyny using the Mann-Whitney *U*-test, with the prediction of higher female bias in nests with apparently less complex genealogies and using one-tailed tests. Nonparametric statistical tests were taken from Siegel and Castellan (1988).

Sex investment ratio. Regression equations were obtained relating bee head width and body mass (both wet and dry) for males and females separately using recently enclosed individuals reared in the laboratory. This allowed estimation of the mass of individual brood members so that numerical sex ratio data could be transformed into accurate estimates of investment for each nest separately. Whenever the head width of an individual could not be measured due to damage in the field it was assumed to have had the average head width for nestmates of the same sex.

It was not possible to estimate investment ratios from pollen ball weights in this species as no clear distinction could be made between male and gyne destined provision masses. This is because of the broad overlap in mass distributions between the sexes. For a discussion of the effects of different mass estimators upon investment ratio calculations see Boomsma (1989) and Danforth (1990).

Sex and investment ratios were calculated for various time periods within the reproductive brood production phase. These data are cross-sectional as earlier excavated nests would have produced more late-emerging brood and early-emerging adults may have been missed in the last few days of excavation. Nonetheless, because of the short duration of reproductive brood production in this northern climate, all brood members could be sexed in 36 nests which were excavated over a 10-day period just before the beginning of reproductive brood eclosion. For these 36 nests, all brood members were weighed individually to estimate investment. The only exceptions were pupae which were damaged in the field, which were assumed to have weighed the same as their average nestmate of the same sex. The estimate obtained from this subsample of nests is considered to be the most accurate. Estimates of the investment ratio were obtained by weighting either nests or individuals equally.

Oviposition. Indirect estimates of relative queen and worker oviposition were obtained by dissection of bees excavated from nests in July at a time when reproductive brood eggs were being laid. Frozen bees were dissected and their head widths recorded. Estimates of ovarian development were made in two ways: first, by recording the length and breadth of the developing oocyte in each ovariole (these bees typically mature at most one oocyte per ovariole at a time). Total developing oocyte mass was estimated by summing the volumes of oocytes from each ovariole, these volumes

being estimated from the equation for the volume of a sausage

$$v = 2\pi r^2(l + 2r/3)$$

where r = the radius (half the oocyte width) and l = oocyte length. Secondly, the number of oocytes ready, or almost ready, to be laid, was counted for the two castes separately.

The possibility that oophagy had occurred was investigated by inspection of the gut contents of bees excavated from nests in July. Previous studies based upon dissections of thousands of bees have shown that three different categories of gut content can be identified: (i) a thick, translucent liquid which is presumably nectar, (ii) remains of pollen grains and (iii) a white substance with a consistency very similar to that of fully developed oocytes (Packer 1986). Discovery of the latter would be taken as evidence of oophagy.

Results

Overview of social biology

Nests were initiated in late May, mostly by solitary females, although six multiple foundress nests were found (less than 10% of all nests excavated during worker brood production and maturation), containing from two to five foundresses. Multiple foundress nests were omitted from the analyses of worker brood nests that follow. Accurate discrimination between multiple and solitary foundress colonies could not be ensured for reproductive brood nests and the absence of multiple foundress associations from this sample cannot be assumed.

A small worker brood was produced (averaging little more than 4 bees) of which 30% were males to give an average of less than 3 workers per solitarily founded nest. These workers averaged 7% smaller than their queen and, during the reproductive brood production phase in early July, 35% of them were mated and 63% had moderately well developed ovaries (containing at least one half-developed oocyte). Eclosion of reproductive brood commenced just before the middle of August.

Electrophoretic data

Four alleles were found at *Aha* and *Had*, two at *Est* and three at *Pep* (Table 1). *Est* was monomeric, all others dimeric.

Linkage disequilibrium was detected between *Had* and *Pep* ($D = -0.055$). This is a substantial amount of linkage disequilibrium as the observed value is fully 50% of the maximum possible given the allele frequencies in the sample analysed ($D_{\max} = -0.11$). Standard errors are greater than the corresponding point estimates of D for all other pairwise comparisons (Table 2). Similarly, the difference between observed and expected haplotype frequencies is significant only for the *Had-Pep* pair (Table 3). As a result, the data from these two loci cannot be considered as independent and estimates of relatedness probably should not be made by combining results from them.

Parental and reproductive brood offspring generation allele frequencies are not significantly different (Table 4)

Table 2. Linkage disequilibrium coefficients for all pairwise comparisons

Comparison	$D \pm SE$	G_{adj}	P
<i>Aha-Est</i>	-0.014 ± 0.023	0.32	> 0.5
<i>Aha-Had</i>	-0.011 ± 0.023	0.23	> 0.5
<i>Aha-Pep</i>	-0.016 ± 0.023	0.43	> 0.5
<i>Est-Had</i>	-0.012 ± 0.023	0.26	> 0.5
<i>Est-Pep</i>	0.006 ± 0.024	0.05	> 0.5
<i>Had-Pep</i>	-0.055 ± 0.02	4.98	< 0.05

Rare variants combined with common ones as follows: *Aha1* + *Aha2*, *Aha3* + *Aha4*, *Pep1* + *Pep2*

Table 3. Comparison of observed and expected haplotype frequencies for *Had* and *Pep*

Haplotype	Observed	Expected
<i>Had</i> ₁ <i>Pep</i> ₂	4	8.1
<i>Had</i> ₁ <i>Pep</i> ₃	16	11.9
<i>Had</i> ₂ <i>Pep</i> ₂	26	21.9
<i>Had</i> ₂ <i>Pep</i> ₃	28	32.1

$$\chi^2 = 4.8 \quad P < 0.05$$

Table 4. Comparison of parental and offspring generation allele frequencies taken from reproductive brood nests

Allele	Frequency		χ^2	P
	Parental	Offspring		
<i>Aha1</i>	0.256	0.263	0.70	> 0.5
<i>Aha2</i>	0.047	0.050		
<i>Aha3</i>	0.621	0.596		
<i>Aha4</i>	0.076	0.090		
Sample size	211	1809		
<i>Est1</i>	0.423	0.413	0.085	> 0.5
<i>Est2</i>	0.577	0.587		
Sample size	208	1813		
<i>Had1</i> + <i>Had3</i>	0.343	0.276	3.44	> 0.05
<i>Had2</i> + <i>Had4</i>	0.658	0.724		
Sample size	201	1764		
<i>Pep1</i>	0.035	0.056	1.87	> 0.1
<i>Pep2</i>	0.288	0.262		
<i>Pep3</i>	0.677	0.682		
Sample size	198	1695		

Rare alleles combined as in Table 2 unless otherwise stated

and observed maternal genotype frequencies do not depart significantly from Hardy-Weinberg expectation (Table 5). Thus, the assumption of at most weak selection acting on the loci appears to be met. Nonetheless, the highest χ^2 values were obtained for *Had* for both the above analyses and as this was one of the two loci for which linkage disequilibrium was detected: *Had* is probably the locus which can most profitably be excluded from analyses of relatedness based upon combinations of loci.

Table 5. Comparison of observed and Hardy-Weinberg expectation genotype frequencies

Genotype	Frequency		χ^2	<i>P</i>
	Observed	Expected		
<i>Aha11</i>	7	6.71	0.78	>0.5
<i>Aha12</i>	34	30.85		
<i>Aha22</i>	32	35.43		
<i>Est11</i>	13	13.30	0.03	>0.5
<i>Est12</i>	36	35.30		
<i>Est22</i>	23	23.40		
<i>Had11</i>	6	9.04	2.54	>0.1
<i>Had12</i>	38	31.87		
<i>Had22</i>	25	28.09		
<i>Pep22</i>	8	7.77	0.01	>0.9
<i>Pep23</i>	30	30.43		
<i>Pep33</i>	30	29.80		

Rare alleles combined as in Table 4

Table 6. Estimates of relatedness among various categories of nestmate presented for worker and reproductive broods separately

Comparison	Expected	Observed	SE	95% CL	<i>n</i>
Worker brood					
Queen to worker	0.50	0.42	0.10	0.22–0.62	18
Queen to male	1.00	0.92	0.08	0.76–1.08	8
Worker to worker	0.75	0.76	0.07	0.62–0.90	72
Worker to male	0.50	0.53	0.20	0.13–0.93	17
Reproductive brood					
Queen to worker	0.50	0.60	0.06	0.47–0.73	39
Queen to male	1.00	0.91	0.04	0.83–0.99*	37
Queen to gyne	0.50	0.57	0.06	0.46–0.68	41
Worker to worker	0.75	0.75	0.05	0.65–0.85	98
Worker to male	0.50	0.63	0.05	0.53–0.73*	108
Worker to gyne	0.75	0.64	0.04	0.55–0.73*	133
Gyne to gyne	0.75	0.74	0.03	0.68–0.80	701

The estimates are for three loci combined (*Had* excluded) with groups weighted equally. Multiple foundress nests are excluded from analyses of the worker brood. Standard errors were obtained by jackknifing across groups. Sample sizes are for bees in the first category mentioned in the comparison. Expected values are for nests headed by one, singly mated queen with no worker oviposition

* Significantly different from expected at $P \leq 0.05$

Relatedness

Estimates of relatedness between various categories of nestmate based upon three loci combined (*Had* excluded for reasons given above) are presented in Table 6. Similar data broken down on a locus-by-locus basis and also for all four loci combined are provided in the Appendix. Table 6 also shows the expected relatedness values assuming oviposition to be monopolised by one singly mated foundress. Most of the point estimates are in good agreement with the expected values: all but three of the latter are encompassed by the 95% confidence limits. The most marked exception is the relatedness between

workers and gynes which is significantly below the 0.75 expectation. However, relatedness values among workers and among gynes are both high (ranging from 0.71 to 0.73). Indeed, relatedness values among reproductive brood females are significantly higher than those between workers and gynes in the same nests ($U_s = 766$, $t_s = 1.80$, $n = 36$, $P < 0.05$, one-tailed test). These results imply that monogyny prevails during both worker and reproductive brood production but that there is lower relatedness between workers and female reproductives.

The confidence limits for two other relatedness estimates do not quite attain the expected value when based upon three loci: queens are significantly less and workers significantly more closely related to males than expected.

Investment ratios

The regression equations relating wet weight and bee head width were, for males: wet weight = $1.71 \times$ head width + 0.27, $r^2 = 0.69$; for females: wet weight = $1.54 \times$ head width + 1.51, $r^2 = 0.55$. These results provided slightly higher r^2 values than similar analyses based upon wet weight and the cube of head width and substantially better results than dry-weight regressions (perhaps because of the difficulty of obtaining accurate dry weight estimates for these very small bees). These equations were used to estimate investment in each reproductive brood individual in each nest. These values were then summed by sex to give investment ratios that either weighted individuals or nests equally. Table 7 summarises these data grouped into 5-day time periods for the entire data set and for the 10-day period for which the entire reproductive brood could be sexed in 36 nests. Not surprisingly for a protandrous species, the least female-biased estimate comes from the earliest time period and the most biased estimate from the last. The most accurate estimate gives a numerical sex ratio of 1:2.01 and an investment ratio of 1:2.19 when nests are weighted equally. These values become 1:2.26 and 1:2.52 respectively when individuals are weighted equally. The differences in investment ratios resulting from the different weighting schemes suggest that there is a higher female bias in the more productive nests (see below). Clearly both numerical and investment ratios are highly female-biased but the 95% confidence limits exclude both 1:3 and 1:1 investment ratios.

Inter-colony variation

Workers are expected to bias the investment ratio more heavily towards the sex to which they are more closely related in comparison to the population average. This was tested by comparing a nests' rank for proportionate investment in females to the rank of the ratio of worker relatedness to females over their relatedness to males. There was a significant positive relationship between the two ranks ($r_s = 0.30$, $z = 1.69$, $P < 0.05$, $n = 36$, one-tailed test) (Fig. 1) indicating that workers with higher average

Table 7. Sex and investment ratios by time period, for all nests combined and for the subsample of 36 nests for which all brood could be sexed

Time Period	No. of nests	Proportion of males in brood \pm SE			
		Numerical, weighted by:		Investment, weighted by:	
		Individuals	Nests	Individuals	Nests
03.8	3	0.359	0.324 ± 0.074	0.334	0.300 ± 0.040
04–08.8	36	0.289	0.306 ± 0.027	0.271	0.290 ± 0.028
10–14.8	30	0.327	0.362 ± 0.037	0.298	0.337 ± 0.041
15.8	14	0.170	0.192 ± 0.040	0.161	0.183 ± 0.039
Total	83	0.281	0.308 ± 0.020	0.261	0.290 ± 0.021
Best estimate	36	0.307	0.332 ± 0.023	0.284	0.312 ± 0.022

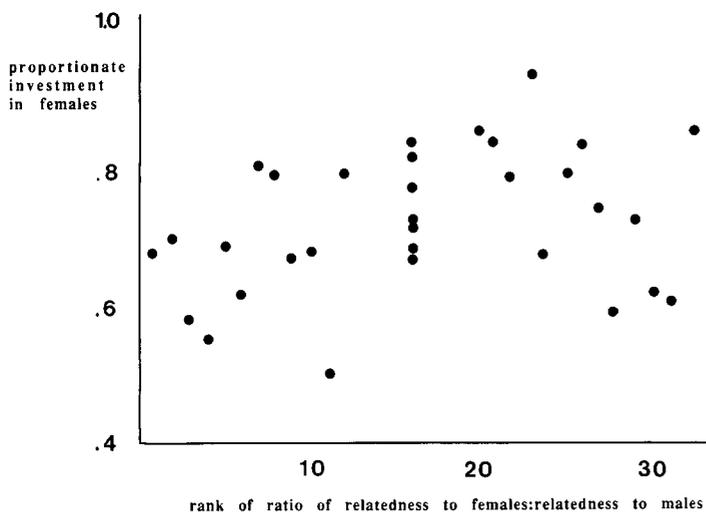


Fig. 1. Scatterplot of investment ratio against rank of relatedness ratio between workers and female and male reproductives

relatednesses to female in comparison to male brood invested more heavily in gynes.

During the excavation of reproductive brood nests it was not possible to identify those nests which had been initiated by multiple foundresses in spring. Consequently, some of the genetically more complex families could have resulted from nests which began as multiple foundress associations. Multiple foundress nests produced very large worker broods, averaging over 7 times the productivity of solitarily foundress nests (Packer 1992). These may be expected to produce very large reproductive broods. If multiple egg-layers occurred in multiple-foundress nests, we might expect lower estimates of relatedness between workers and reproductives in the most populous reproductive brood nests. The association between the ranks for worker to gyne relatedness and total nest reproductive brood productivity was almost significant ($r_s = 0.23$, $z = 1.55$, $P = 0.06$) but positive, indicating that the more productive nests were those with higher levels of relatedness between workers and gynes. The relationship between worker-to-male relatedness and nest productivity was negative but not significant, $r_s = -0.11$, $z = -0.72$, $P > 0.2$).

Table 8. Mean oocyte volumes, (SE) and number of eggs ready to be laid in queens and workers

Time	Oocyte volumes mm^3 per nest		Number of eggs ready to be laid	
	Queens	Workers	Queens	Workers
July 1–14 $n = 16$ nests	0.46 (0.06)	0.21 (0.07)	11	4
July 15–30 $n = 20$ nests	0.49 (0.06)	0.14 (0.04)	32	13
July 1–30 $n = 36$ nests	0.47 (0.04)	0.17 (0.04)	43	17

Ovarian development

Ovarian development data from nests excavated in July are presented in Table 8. Queens had more ovarian development than all of their workers combined in 33 of 36 nests ($P \leq 0.01$, sign test) and no one worker had oocyte volumes as large as their queen. Because the standard deviations were often larger than estimated sample mean oocyte volumes, Mann-Whitney U -tests were performed on these data. Individual worker oocyte volumes averaged larger in the first half of the reproductive brood production phase (i.e. when more haploid egg production is expected) than in the second half ($t_s = 1.86$, $P < 0.05$, one-tailed test). Conversely, queen oocyte volumes averaged larger in the second half of July (when more diploid egg production is expected) than in the first half, but not significantly so. However, there was no significant difference in the number of fully developed oocytes found in queens and workers between the two halves of the month ($G_{\text{adj}} = 0.03$, $P > 0.5$).

If either summed oocyte volumes or completely developed oocytes are compared between the castes, between one quarter and one third of all ovarian development was found in workers. Assuming oocyte development rates to be similar between the castes, this suggests that workers laid approximately 30% of the eggs. Based upon the numerical sex ratio data provided above, this could translate into workers producing almost all of the males

even in queenright nests. However, the relatedness estimates indicate that this is far from the case.

No evidence of oophagy was found by inspection of gut contents of bees.

Discussion

The kin selection hypothesis for the origin of a reproductive division of labour in eusocial Hymenoptera has been criticised on a variety of grounds (Evans 1977; Andersson 1984; Stubblefield and Charnov 1986). Perhaps most damaging is the observation that relatedness values among females in nests of eusocial species are usually low (Gadagkar 1991). However, very few estimates of relatedness are available for species for which eusociality is an evolutionarily recent phenomenon. The most pertinent studies are those of the eusocial sphecid *Microstigmus comes* (Ross and Matthews 1989a, b) and the halictine bee *Lasiglossum (Dialictus) zephyrum* (Crozier et al. 1987; Kukuk 1989), both of which have high relatedness values among females and *M. comes* also has a female-biased sex-investment ratio. *Microstigmus comes* is a member of a subtribe (the Spilomenina) for which sociality may be an ancestral condition (Matthews 1991). *Lasiglossum (Dialictus) zephyrum* is a member of a subgenus for which both solitary and social species are known (Weislo et al. 1993). The present paper provides data on an additional member of the subgenus *Dialictus* and, as outlined in the introduction, *L. (D.) laevissimum* would appear to be the most weakly eusocial insect for which relatedness and sex-ratio data have been obtained.

Before discussing the relatedness data in detail we have to assess the extent to which the assumptions of the models used to estimate such data are satisfied. Panmixis in the population analysed, unlinked loci and at most weak selection on the electromorphs are required if any confidence is to be placed in estimated relatedness values. No significant population structure was found at the level of small patches of nests within the aggregation although substructuring at a higher level was indicated (a detailed account of population structure in this species is in progress). We found no evidence of significant selection on any of the four loci analysed. Strong linkage disequilibrium was found between *Had* and *Pep* and as departures from Hardy-Weinberg equilibrium were highest for *Had* (although still not significant), relatedness estimates obtained from *Aha*, *Est* and *Pep* combined are considered the most accurate (Table 6). Estimates of relatedness are in reasonably good agreement across loci and most are very close to the theoretically expected values under conditions of monoandry and monogyny (Appendix).

Inspection of genotype arrays among nestmates suggest that a wide variety of causes resulted in departures from expected relatedness values. Some brood genotypes were suggestive of multiple mating, others of multiple egg-layers (including instances where orphaning seemed likely) and worker-laid males. Maximum likelihood analyses are required for accurate estimation of the frequencies of orphaning, worker oviposition, multiple

mating and so on, but considering the wide variety of potential causes of complex within-nest genealogies, such analyses will be difficult.

The complex range of genealogies also makes calculation of expected investment ratios difficult. Following Ross and Matthews (1989b), the simplest method is to estimate the worker-preferred sex-investment ratio as $1:r_f/r_m$ where r_f is the relatedness between workers and gynes and r_m the relatedness between workers and males (relatedness values here being the life-for-life estimates) (Oster and Wilson 1978; Crozier 1979). The mean relatedness values of workers to reproductive brood males and females are 0.29 and 0.64 respectively, giving a ratio of 1:2.21 (or 0.302), a value encompassed by the observed investment ratios (Table 7) and very close to the estimate obtained from 36 intact reproductive broods.

A more elaborate method can be derived from the results of Pamilo (1991) who provides equations predicting population-wide investment ratios under conditions of both queen and worker control and varying proportions of colony orphaning and worker-produced males in queenright colonies. The expected investment ratio in queenright nests from both queen and worker perspectives can be estimated from the equation

$$Y_q^* = \frac{g_m}{[1 + (1 - B)(1 - \psi)]g_f + g_m}$$

Where g_m and g_f are regression value relatednesses of the controlling caste to male and female reproductives respectively (these values being determined empirically), and B and ψ are the proportion of males in the population produced by orphaned colonies and by worker oviposition in queenright colonies respectively.

The population-wide sex investment ratio can similarly be calculated using the equation

$$Y^* = \frac{Y_q^*}{Y_q^* + (1 - B)(1 - Y_q^*)}$$

We cannot calculate the proportions B and ψ directly with our data. Nonetheless, substituting the relatedness

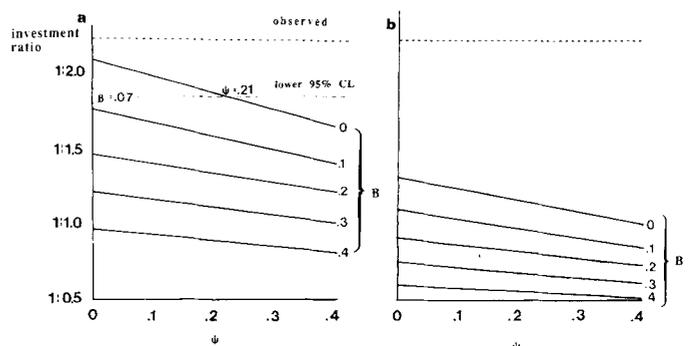


Fig. 2a, b. Plot of expected investment ratios against ψ , the proportion of males produced by workers in queenright colonies for a variety of values of B the proportion of males produced in orphaned colonies (values of B are given at the right hand ends of the lines). The dashed line shows the observed investment ratio, the dotted line shows the lower 95% confidence limit on this estimate. **a** The expected ratio for sterile workers, **b** the expected ratio for queens

values from Table 6 and various reasonable values for B and ψ , Fig. 2 was obtained. As can be seen, the observed sex ratio was more female biased than predicted with all of the combinations of B and ψ . Indeed, the lower 95% confidence interval for the investment ratio is surpassed only for worker control with values of B below 0.073 and ψ below 0.21. More striking is the discrepancy between the observed values and those expected under conditions of queen control of the sex investment ratio where the highest sex ratio predicted from theory comes nowhere near the lower 95% confidence interval. This indicates that it is the workers which are in control of the investment ratio in this species. The investment ratio favoured by replacement queens and ovipositing workers in queenright nests is expected to be intermediate between the values quoted for worker and queen control. As the observed value is even more female-biased than expected for sterile worker control for the majority of parameter combinations, it seems likely that it is the sterile workers which are in control of the investment ratio. The likely mechanism whereby control of the investment ratio is obtained would be manipulation of pollen ball size (Frank and Crespi 1989) which is known to serve as a proximal influence on the sex of the individual produced (Plateaux-Quénu 1983).

There was a significant positive association between the relative relatedness asymmetry between workers and females versus male reproductives and the proportional investment in female brood. This suggests that workers are capable of adjusting investment decisions according to their perception of family structure within the nest. At least two other primitively eusocial halictines produce different sex ratios in orphaned and queenright colonies (Yanega 1989; Boomsma 1991; Mueller 1991) and in at least one of them the increased male production in orphaned colonies is not merely a result of unmated females becoming replacement queens (Mueller 1991). Orphaning is one cause of reduced worker-gyne relatedness in *L. laevissimum*. It has been demonstrated for other halictine species that replacement queens mate after having become behaviourally dominant (Michener 1990). This is likely in *L. laevissimum* also as none of the reproductive brood nests lacked gyne brood. Hence, replacement queens laid both haploid and diploid eggs and the increased male bias in nests with low ratios of relatedness of workers to gynes in comparison to males cannot have resulted from replacement queens being unmated.

Whether summed fractions or only fully developed oocytes are considered, workers (as a group) seem to be about one-third as fecund as queens. However, the genetic data suggest the apparent near-monopolisation of oviposition by queens (and replacement queens) in this population. This discrepancy could be explained by (i) much faster rates of ovarian development in queens such that laid eggs are far more rapidly replaced by developing oocytes in queens than workers, (ii) higher rates of oocyte resorption in workers or (iii) differential oophagy such that queens consume most worker-laid eggs. No evidence for oophagy was found in dissected adult *L. laevissimum* and the third hypothesis can prob-

ably be discounted. Physiological studies are required to differentiate between the other two hypotheses.

This study of a primitively eusocial sweat bee provides the strongest support yet available for the importance of indirect fitness contributions and haplodiploidy for the evolution of eusociality. *Lasioglossum laevissimum* is the most weakly eusocial hymenopteran to receive studies of both relatedness and the sex ratio and predictions of the kin selection hypothesis appear to be met: (i) coefficients of relatedness among nestmates are close to the expected values, (ii) the sex ratio is female biased and in close agreement with the value expected based upon relatednesses of workers to reproductives of the two sexes, (iii) the population wide sex ratio is consistent with a model of sterile worker control, (iv) workers appear to have some ability to adjust the sex ratio appropriate to the relative relatedness asymmetries within colonies, (v) there is high variance in nest productivity (Packer 1992) and (vi) there is an almost significant tendency for the more productive nests to be those with higher relatednesses between workers and female brood indicating that one female largely monopolised reproductive brood oviposition even in multiple-foundress nests.

In conclusion, it seems premature to discount haplodiploidy as an important factor promoting eusociality in the Hymenoptera simply because most eusocial species have low relatedness values. All eusocial ants and vespids have been eusocial for over 100 million years and although the latter are mostly "primitively eusocial" their primitiveness does not indicate a recent evolution of eusociality (Carpenter 1989; Wenzel 1990). One would not attempt to find a reason for vertebrate colonisation of the land by studying the locomotory mechanisms of bats; similarly one should not assess hypotheses for the origins of eusociality by using the more advanced eusocial insects as test organisms. In contrast, the taxonomic distribution of eusociality in halictines suggests a much more recent date for social evolution in these bees with pemphredonine wasps probably being intermediate in this regard. Comparatively high estimates of nestmate relatedness have been obtained for both halictine species and the one pemphredonine studied to date (Crozier et al. 1987; Kukuk 1986; Ross and Matthews 1989a, b; this study) and female-biased sex ratios have been reported along with relatedness data for *L. (D.) laevissimum* and *M. comes* [a separate study indicated that *L. (D.) zephyrum* also produces female biased reproductive broods (Batra 1966)]. Clearly more data are required on this topic and phylogenetic studies of taxa which exhibit both solitary and eusocial behaviours will be crucial to permit the identification of those species most suitable for further investigations of the critical variables of relatedness and sex ratio.

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Appendix. Relatedness estimates among nestmates for worker brood and reproductive brood separately

Relatedness		<i>Aha</i>	<i>Est</i>	<i>Had</i>	<i>Pep</i>	Four loci (<i>Had</i> included)
of	to					
Worker brood						
Queens	workers					
Estimate		0.10	0.56	0.52	0.42	0.49
SE		0.15	0.13	0.15	0.15	0.08
95% CL		-0.20-0.40	0.30-0.82	0.22-0.82	0.12-0.72	0.3-0.65
<i>n</i>		20	27	27	27	18
Queens	males					
Estimate		1.00	1.00	0.84	0.75	0.88
SE		0.00	0.00	0.19	0.22	0.11
95% CL		1.00-1.00	1.00-1.00	0.46-1.22	0.31-1.19	0.66-1.10
<i>n</i>		8	9	9	9	8
Workers	workers					
Estimate		0.76	0.79	0.47	0.71	0.71
SE		0.08	0.10	0.16	0.11	0.06
95% CL		0.60-0.92	0.59-0.99	0.15-0.79	0.49-0.93	0.59-0.83
<i>n</i>		71	73	73	73	71
Workers	males					
Estimate		1.00	0.60	1.00	1.00	0.63
SE		0.00	0.27	0.00	0.00	0.16
95% CL		1.00-1.00	0.06-1.14	1.00-1.00	1.00-1.00	0.31-0.95
<i>n</i>		17	17	17	17	17
Reproductive brood						
Queens	workers					
Estimate		0.48	0.51	0.44	0.81	0.56
SE		0.12	0.11	0.13	0.09	0.06
95% CL		0.24-0.72	0.29-0.73	0.18-0.70	0.63-0.99	0.44-0.68
<i>n</i>		39	40	40	40	39
Queens	males					
Estimate		0.92	1.00	0.94	0.88	0.89
SE		0.06	0.00	0.06	0.07	0.06
95% CL		0.80-1.04	1.00-1.00	0.82-1.06	0.74-1.00	0.77-1.01
<i>n</i>		39	39	39	37	37
Queens	gynes					
Estimate		0.44	0.52	0.45	0.61	0.50
SE		0.10	0.10	0.14	0.10	0.06
95% CL		0.24-0.64	0.32-0.72	0.27-0.73	0.41-0.81	0.38-0.62
<i>n</i>		44	44	44	44	42
Workers	workers					
Estimate		0.77	0.68	0.59	0.80	0.72
SE		0.06	0.13	0.11	0.06	0.05
95% CL		0.65-0.89	0.56-0.81	0.37-0.81	0.68-0.92	0.62-0.82
<i>n</i>		101	101	101	104	98
Workers	males					
Estimate		0.56	0.58	0.44	0.80	0.58
SE		0.08	0.13	0.18	0.07	0.05
95% CL		0.41-0.71	0.32-0.84	0.08-0.80	0.66-0.94	0.48-0.68
<i>n</i>		108	111	108	108	108
Workers	gynes					
Estimate		0.58	0.70	0.67	0.65	0.64
SE		0.07	0.06	0.07	0.08	0.04
95% CL		0.45-0.71	0.64-0.76	0.53-0.81	0.49-0.81	0.56-0.72
<i>n</i>		122	126	122	120	113
Gynes	gynes					
Estimate		0.70	0.74	0.69	0.72	0.74
SE		0.04	0.04	0.05	0.06	0.03
95% CL		0.62-0.78	0.66-0.82	0.59-0.79	0.60-0.84	0.67-0.79
<i>n</i>		748	749	748	705	701

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