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The evolution of social behavior and nest architecture in sweat bees of the subgenus *Evylaeus* (Hymenoptera: Halictidae): a phylogenetic approach

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Summary. Phylogenetic studies are required to establish the direction of evolutionary change in behavioral characters. Here I produce a phylogeny for 8 Old World species of the subgenus *Evylaeus* based upon cladistic analysis of 26 informative allozyme loci. By mapping behavioral character states upon the resulting cladogram, the following major conclusions could be drawn: (1) Social *Evylaeus* species share sociality by descent from a eusocial common ancestor which might not have been an *Evylaeus* species; the solitary behavior of *Lasioglossum* (*E.*) *fulvicorne* may be a derived condition. (2) One reversal to solitary behavior within *Evylaeus* is proven for a Japanese montane population of *L.* (*E.*) *calceatum*. (3) The perennial societies of *L.* (*E.*) *marginatum* are derived from an annual social cycle and do not represent an independent evolution of sociality. (4) Multiple-foundress associations are a derived condition within *Evylaeus*, suggesting that **if** social behavior evolved within the group, then the semisocial route was not the one taken by these bees. (5) The nest architectural trait of excavating a cavity around clustered brood cells is a ground plan characteristic of *Evylaeus* but with a reversal in *L.* (*E.*) *marginatum*. (6) It is likely that extended opening of brood cells during juvenile development has originated independently twice among the species considered. Another benefit of phylogenetic studies is their use in predicting which taxa are most likely to exhibit particularly interesting behavioral states. In this regard, the phylogeny suggests that close relatives of *L.* (*E.*) *fulvicorne* and also most of the major species groups of *Evylaeus* which have not received any field study should be investigated both phylogenetically and behaviorally for a full evaluation of behavioral evolution in *Evylaeus*.

Introduction

Phylogenetics is clearly of importance to studies of behavioral evolution (Ridley 1983, 1986). Evolutionary studies of behavior involve comparisons between populations or species. Closely related species may share a behavioral pattern either by descent or by convergence. Phylogenetic studies involving the group(s) under consideration are required to differentiate these two hypotheses. When two taxa differ in behavior, it may be suggested that one pattern evolved "from" another. Again, phylogenetic studies are required to demonstrate the polarity of behavioral change (i.e., which state is primitive and which is derived) (Carpenter 1989; Coddington 1988). A third reason why behavioral biologists should understand the evolutionary history of their organisms relates to choice of taxa for further observation. Knowledge of the phylogenetic history of a group may suggest which taxa are likely to possess intermediate behavioral states. Such considerations can greatly reduce the amount of data required to test hypotheses of behavioral change.

In this paper, the organisms of study are bees of the subfamily Halictinae, the sweat bees, and the behavioral changes under consideration are transitions between solitary and various types of eusocial life cycles and among different nest architectural designs. Sociality appears to have arisen independently in many different sweat bee lineages (Sakagami 1974; Knerer 1980; Michener 1990). Consequently, comparisons of closely related species (or even conspecific populations) which exhibit both solitary and eusocial behaviors may be used to investigate the factors promoting the origin of sociality. However, in the absence of a phylogenetic hypothesis for the constituent taxa, it cannot be said whether it is the origin of sociality or a reversal to solitary behavior that is being investigated. Sweat bees also exhibit a wide range of nest architecture (Sakagami and Michener 1962), and, with one exception (Eickwort and Sakagami 1979), the evolutionary pathway among these various types remains unknown.

In this paper I reconstruct the evolutionary relationships among some European species of the subgenus *Evylaeus* (genus *Lasioglossum*) using allozyme loci as characters. Of all the Western Palaearctic *Evylaeus* whose social organization is known, only *L.* (*E.*) *nigripes* was unavailable for electrophoretic analysis. The phylogeny is used to investigate the evolution of the behavioral traits.

The subgenus *Evylaeus* contains both solitary and eusocial species (Packer and Knerer 1985). Temperate halictines typically overwinter as mated females which initiate nests the following spring. In univoltine solitary species, the brood produced will emerge and mate, and the females then enter diapause to start the cycle again the following year. Overwintered foundresses of eusocial species produce one or more worker broods during the summer and then reproductives later in the year; the colony then dies out. Thus, with one exception, eusocial taxa have an annual colony cycle. The exception is *L.* (*E.*) *marginatum*, the only halictine known to have perennial societies (Plateaux-Quénu 1959). Queens of this species produce one brood of workers each year for 4 or 5 years before producing reproductives, attaining colony sizes of over 500 adults and a foundress longevity approximately double that of a honeybee queen. The subgenus *Evylaeus* exhibits the widest range of social behaviors of any insect taxon of equivalent rank.

Evylaeus nest architecture is also highly variable (Sakagami and Michener 1962). Most species construct their brood cells in a tight cluster surrounded by a cavity in the soil, and this cavity arises directly from the main burrow. Others construct the cavity at the end of a short lateral branch or lateroid. Still others do not construct a cavity but have simple sessile brood cells along one or more main branch burrows.

In this paper, the taxon *Evylaeus* is understood as comprising those species possessing a carinate propodeum (the first abdominal segment which is fused to the thorax in apocritan Hymenoptera). Thus defined, it is equivalent to the "carinate" *Evylaeus* of earlier authors; the "carina-less" species should be transferred to the subgenus *Dialictus*, as recently suggested by Michener (1990).

Methods

Electrophoretic techniques. All species were sampled as adult females and [with the exception of *L.* (*E.*) *albipes*] were obtained from at least two localities (Table 1). Bees were killed in liquid nitrogen and stored in a $-80°$ C freezer until used for electrophoresis. Some enzyme staining systems produced poorer results after prolonged storage (a maximum of 22 months). This prevented comparison of out- and ingroup taxa for α-glucosidase which was otherwise well resolved among *Evylaeus* species. Before homogenisation, each bee was divided into two portions, the head plus thorax and the abdomen. Each portion was homogenised in distilled water or a 1% solution of dithiothreitol in 0.2 *M* phosphate buffer (pH 7.0), in an amount varying from 20 μl for *L.* (*D.*) *morio* to 120 μl for *L. xanthopus*. Filter paper wicks were impregnated with homogenate (1 wick for each 10 μl of liquid) and inserted into 11% starch gels. The buffer systems, staining recipes, and running conditions used for horizontal starch gel electrophoresis followed those of Packer and Owen (1989a, 1990) with slight modifications. A list of loci scored is provided in Table 2.

An extensive series of line-up gels ensured that electromorphs which appeared similar between species really did have the same mobility. The mean sample size (average number of diploid genomes per locus) exceeded 30 for all species except *L.* (*E.*) *albipes* and *L.* (*D.*) *morio*, for which an average of 7 and 8 individuals per locus was used, respectively. With the exception of *L.* (*E.*) *marginatum* and all samples from Greece, all collections were of overwintered bees from flowers, thus avoiding biases associated with sampling from nests. French *L.* (*E.*) *marginatum* were sampled by aspirating one female from each nest. Greek bees were mostly workers collected from flowers. This sampling protocol is adequate for the analyses performed, particularly considering the generally low level of intraspecific variation in these bees (Packer and Owen,

Table 1. List of species and collection localities

Species	Species group	Locality[a]	
		Prime	Subsidiary
Lasioglossum (*Dialictus*) *morio*	(*Dialictus*)	Les Eyzies	Paris
Lasioglossum (*Dialictus*) *politum*	(*Dialictus*)	Les Eyzies	Paris
Lasioglossum (*Evylaeus*) *fulvicorne*	*fulvicorne*	Les Eyzies	Paris
Lasioglossum (*Evylaeus*) *laticeps*	*laticeps*	Les Eyzies	Paris
Lasioglossum (*Evylaeus*) *calceatum*	*calceatum*	Les Eyzies	Paris
Lasioglossum (*Evylaeus*) *albipes*	*calceatum*	Les Eyzies	None
Lasioglossum (*Evylaeus*) *malachurum*	*malachurum*	Les Eyzies	Monemvasia
Lasioglossum (*Evylaeus*) *lineare*	*malachurum*	Monemvasia	Les Eyzies
Lasioglossum (*Evylaeus*) *pauxillum*	*pauxillum*	Les Eyzies	Paris
Lasioglossum (*Evylaeus*) *marginatum*	*marginatum*	Villajou	Monemvasia

[a] All localities are in France except Monemvasia, which is in southern Greece. Each sampling location covered an area of approximately 1 km^2

Table 2. List of enzyme loci used in phylogenetic analysis

Enzymes	EC no.	Symbol	Character number
Aconitate hydratase	4.2.1.3	*Ac-2*	1
Adenylate kinase	2.7.4.3	*Ak-1*	2
		Ak-2	3
		Ak-3	4
Alkaline phosphatase	3.1.3.1	*Akp*	5
Arginine kinase	2.7.3.3	*Ark*	6
Diaphorase (NADH)	1.8.1.*	*Dia-1*	7
Diaphorase (NADPH)	1.6.99.*	*Diap-1*	8
		Diap-2	9
Enolase	4.2.1.11	*Enol*	10
Fructose bisphosphate aldolase	4.1.2.13	*Ald*	11
Fumarate hydratase	4.2.1.2	*Fum*	12
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6pd*	13
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi*	14
α-Glucosidase	3.2.1.20	*Glu*	15
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*Gapd*	16
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3pd-1*	17
		G3pd-2	18
Guanine deaminase	3.5.4.3	*Gda*	19
Hexokinase	2.7.1.1	*Hk*	20
D-2-Hydroxy acid dehydrogenase	1.1.99.6	*Had*	21
Hydroxyacylglutathione hydrolase	3.1.2.6	*Hgh*	22
Isocitrate dehydrogenase	1.1.1.42	*Idh*	23
Malate dehydrogenase (NAD)	1.1.1.37	*Mdh-2*	24
Malate dehydrogenase (NADP)	1.1.1.40	*Me*	25
Peptidase (gly-leu)	3.4.11–13	*Pep(gl)*	26
Phosphoglucomutase	5.4.2.2	*Pgm*	27
Pyruvate kinase	2.7.1.40	*Pk*	28
Superoxide dismutase	1.5.1.1	*Sod*	29
Triosephosphate isomerase	5.3.1.1	*Tpi*	30

The following seven additional loci were monomorphic across all taxa: *Ac-1*, guanylate kinase (EC 2.7.4.8), *Mdh-1*, phosphoglycerate kinase (EC 2.7.2.3), *6Pgd* 6-phosphogluconate dehydrogenase (EC 1.1.1.43), phosphoglyceromutase (EC 5.4.2.1), and uridine kinase (EC 2.7.4.*). An additional enzyme, L-iditol dehydrogenase (EC 1.1.1.14), showed no overlap of electromorphs between species and so was omitted as being phylogenetically uninformative. Some other loci were highly variable, preventing sufficient comparisons among all electromorphs: These included acetylhexosaminidase (EC 3.2.15.2), alanine aminotransferase (EC 2.6.1.2), aldehyde dehydrogenase (EC 1.2.1.3), esterase (nonspecific), peptidase with leucine alanine as substrate (3.4.11–13*), and peptidase with phenylalanine proline as substrate (3.4.13.8)

unpublished data). Comparisons with conspecifics from the geographically distinct replicate samples confirmed that electromorphs were the same in both populations in all cases.

Voucher specimens are housed in the Royal Ontario Museum, Toronto, Ontario, and in my personal collection.

Cladistic analysis. Outgroup choice within the *Lasioglossum* group is problematic (McGinley 1986). Hypothetical ancestral character states were determined by comparisons of the ingroup with two members of *Dialictus: L.* (*D.*) *morio* and *L.* (*D.*) *politum*. Four members of the subgenus *Lasioglossum s. str.* (*laevigatum, xanthopus, pallens*, and *bimaculatum*) were also available for comparison but shared few electromorphs either among themselves or with the *Dialictus* and *Evylaeus* species. Only 7 loci that were phylogenetically informative for *Evylaeus* showed shared electromorphs between any of the four *Lasioglossum* and one or more in- or outgroup species. In each case, the allele shared was plesiomorphic within *Evylaeus* (i.e., was also shared with one or more *Dialictus* species). These analyses suggested three synapomorphic electromorphs for the taxon (*Dialictus*+*Evylaeus*) at the loci *Mdh-1, 6Pgd*, and *Sod*.

The reduced strength of the second intercubital vein and the comparatively thin apical regions of the metasomal terga also suggest monophyly for *Dialictus*+*Evylaeus* in relation to *Lasioglossum s. str.* The inner margin of the gonoforceps of *Evylaeus* has the apical half concave, often strongly so. This may be a synapomorphy for the subgenus as *Lasioglossum s. str.* and *Dialictus* have, at most, slightly sinuate inner margins to the gonoforceps (see figures in Do Pham et al. 1984). Rigorous morphological studies of these taxa remain to be done at the generic and subgeneric levels. Monophyly of *Evylaeus* is also suggested by one allozyme and one behavioral character (see below).

Phylogenetic analysis of electrophoretic data is highly controversial (Swofford and Berlocher 1987; Murphy et al. 1990). Swofford and Berlocher (1987) have recommended the use of gene frequency data. Their method has not been followed here because (a) few polymorphisms were found, (b) most polymorphisms consisted of an autapomorphic allele and a plesiomorphic one, thus providing no phylogenetically useful information, and (c) no two species shared more than one allele at any locus. Here I treat the locus as the character and the electromorph as the character state (Mickevich and Mitter 1981; Buth 1984; Murphy et al. 1990). Loci monomorphic within a species present no problems in such analysis. Polymorphisms were treated as follows. When a species exhibited a combination of an autapomorphic allele and one shared with other taxa, the character was assigned the plesiomorphic state. Polymorphisms involving two alleles which were both found in other species were treated as part of a transformation series. In those cases in which the polymorphism indicated a transformation from an outgroup electromorph to a derived state, the polarity of change was clear. This left only three polymorphisms (*Pgm* in *L.* (*E.*) *pauxillum* and *Gda* in *L.* (*E.*) *calceatum* and *L.* (*E.*) *marginatum*) for which polarity could not be determined until after initial cladogram construction. Once polarity was determined for informative polymorphisms, the taxon was coded for the derived character state. Thus, gains but not losses of electromorphs were included in the data to be analysed as in the shared alleles model (Mickevich and Mitter 1981).

Cladograms were generated using the i.e.* algorithm of HENNIG86, which finds all trees of minimal length (Farris 1988; Platnick 1989). Initial cladogram construction was based upon the raw data matrix (Table 3) with *Gda* and *Pgm* omitted for the reason given above. All characters exhibiting more than two states were coded as nonadditive, such that transition between any two states required only one step. The initial cladogram was used to polarize the polymorphisms of *Gda* and *Pgm* which were then coded as described above and the entire data set reanalysed. Consistency indices were calculated with autapomorphies removed following the method of Sanderson and Donoghue (1989).

Behavioral data. Behavioral data for *Evylaeus* species were taken from Packer and Knerer (1985, and references therein) with information on *L.* (*E.*) *albipes* from Plateaux-Quénu (1989). The following variables were considered: (1) sociality, whether a species is solitary or eusocial; (2) queen-worker morphological caste differentiation, i.e., overlapping or nonoverlapping size distributions; (3) worker mating, whether more or less than 1% of workers mate; (4) number of worker broods per year; (5) colony cycle, perennial or annual; (6) multiple-foundress or solitary nest initiation; (7) whether a cavity is constructed around clustered brood cells or not; (8) whether the cavity is constructed at the end of a short lateroid or attached to the main burrow directly; (9) whether brood cells remain open during larval development or not. The data matrix for behavioral characteristics is provided in Table 4, and an explanation of the character states for behavioral variables is given in the footnote.

Table 3. Raw allozyme data matrix, informative polymorphisms shown as both states with derived state underlined, coded according to relative mobility

Character Number	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
ancestral	0	1	1	1	0	1	1	2	0	01	0	2	2	2	?	0	0	1	2	0	2	0	0	2	5	0	1	0	0	1
fulvicorne	0	1	1	1	2	1	2	1	0	2	0	3	0	3	2	0	0	3	6	3	3	4	2	2	5	0	0	0	0	1
laticeps	0	2	0	2	3	1	1	3	2	0	0	0	3	1	2	0	2	4	3	1	2	3	2	5	4	0	2	3	0	1
calceatum	0	2	0	1	1	1	0	2	2	0	0	1	3	1	1	0	2	0	$0\underline{3}$	3	2	3	1	1	2	1	2	2	0	1
albipes	0	2	0	1	1	1	0	2	2	0	1	1	3	1	1	0	2	0	3	3	2	3	2	1	2	1	2	2	0	1
malachurum	0	2	0	1	3	1	1	3	2	0	0	3	3	1	2	1	2	1	4	1	2	1	2	4	0	1	2	1	0	1
lineare	0	2	0	1	3	1	1	3	2	0	0	3	3	4	2	$0\underline{1}$	2	1	5	1	2	3	2	4	4	1	2	3	0	1
pauxillum	0	$1\underline{2}$	1	1	2	1	1	2	2	2	0	3	1	5	2	0	3	1	1	3	1	2	2	0	3	2	$0\underline{2}$	4	0	1
marginatum	1	0	1	0	3	0	1	0	1	1	2	3	1	0	0	0	1	2	$\underline{0}1$	2	0	5	2	3	1	1	3	4	1	0

Table 4. Behavioral character state matrix

	Character number								
	1	2	3	4	5	6	7	8	9
ancestor	0/1	–	–	–	–	0/1	0	–	0
fulvicorne	0	–	–	–	–	0	1	0	0
laticeps	1	0	0	0	0	0	1	0	0
albipes	1	0	0	0	0	1	1	1	0
calceatum	1	0	0	0	0	1	1	1	0
lineare	1	1	1	0	0	1	1	0	1
malachurum	1	1	1	1	0	0	1	0	1
pauxillum	1	0	0	1	0	0	1	0	0
marginatum	1	0	1	–	1	0	0	–	1

Characters and character states: 1. solitary – 0, eusocial – 1; 2. overlapping caste size distributions – 0, nonoverlapping – 1; 3. proportion of workers mating >1% – 0, <1% – 1; 4. one worker brood per year – 0, more than one worker brood – 1; 5. annual societies – 0, perennial – 1; 6. solitary nest founding – 0, multiple-foundress nests – 1; 7. nest without cavity – 0, with – 1; 8. lateroid absent – 0; present – 1; 9. brood cells closed during juvenile development – 0, open – 1. In all cases a dash means not applicable

Results

Comparisons among electromorphs for all species were made for 37 loci. Of these, 7 were invariant across all *Evylaeus* and *Dialictus* species (footnote, Table 3). Four additional loci (*Ak-1, Ark, Sod, Tpi*) showed only autapomorphic variation among the 8 ingroup taxa. This left 26 loci that provided phylogenetically useful information for resolution of ingroup relationships. Table 3 shows the data from variable loci coded according to the relative mobility of electromorphs for each species.

A hypothetical ancestral character state could be assigned unambiguously for each character except one (*Enol*) for which the two outgroup species possessed different ingroup states. Preliminary cladograms were generated with two runs through the data, once for each potential ancestral condition for this locus. In each case the same most parsimonious tree was found (topologically the same as shown in Fig. 1). This permitted the polymorphisms for *Gda* and *Pgm* to be coded. The solution cladogram had a length of 84, consistency index of 94, and retention index (Farris 1989) of 92. Both potential ancestral conditions for *Enol* required one reversal, and another reversal is required in *Pep(gl)*; variation in all other characters is perfectly consistent with the tree.

Figure 1 shows that *Idh* (character 23) provides a synapomorphy for the included species of *Evylaeus. Enol* is shown on the figure with the slow allele (state 0) as plesiomorphic and with a reversal to the ancestral state in the *albipes* to *malachurum* lineage (transformation 0-2-0/1). The alternative interpretation of this locus is for the intermediate allele (1) to be plesiomorphic with a reversal to this state in *L.* (*E.*) *marginatum* (transformation 1-2-0/1). For most of the highly variable loci, *L.* (*E.*) *fulvicorne, L.* (*E.*) *pauxillum*, and *L.* (*E.*) *marginatum* possessed different character states not shared with any in- or outgroup taxon. Such character states are not included on the tree because it is not possible to determine which represent apomorphies. Thus, the number of autapomorphies for these three taxa is underestimated on the figure.

Figure 2 shows the cladogram with behavioral character state changes mapped onto it. The behavioral characters clearly exhibit more homoplasy (evolutionary reversal or convergence) than the electrophoretic ones.

Discussion

In this paper, I have constructed a phylogeny of some sweat bees whose social behavior is at least partly known. Even though many more species remain to be investigated phylogenetically and behaviorally, the results allow some conclusions to be made about behavioral evolution within *Evylaeus* and suggest suitable candidates for further field study.

The following discussion treats the characters of Table 4 and Fig. 2 in sequence and then suggests which additional species require further investigation.

1. *Origin of eusociality.* Social *Evylaeus* share sociality by descent from a eusocial common ancestor. However, it cannot be stated for certain whether this common ancestor was a member of *Evylaeus* or a lineage ancestral to *Dialictus*+*Evylaeus*. This is because the solitary *Evylaeus* included here (*fulvicorne*) is a sister taxon to the other species, and the outgroup (subgenus *Dialictus*)

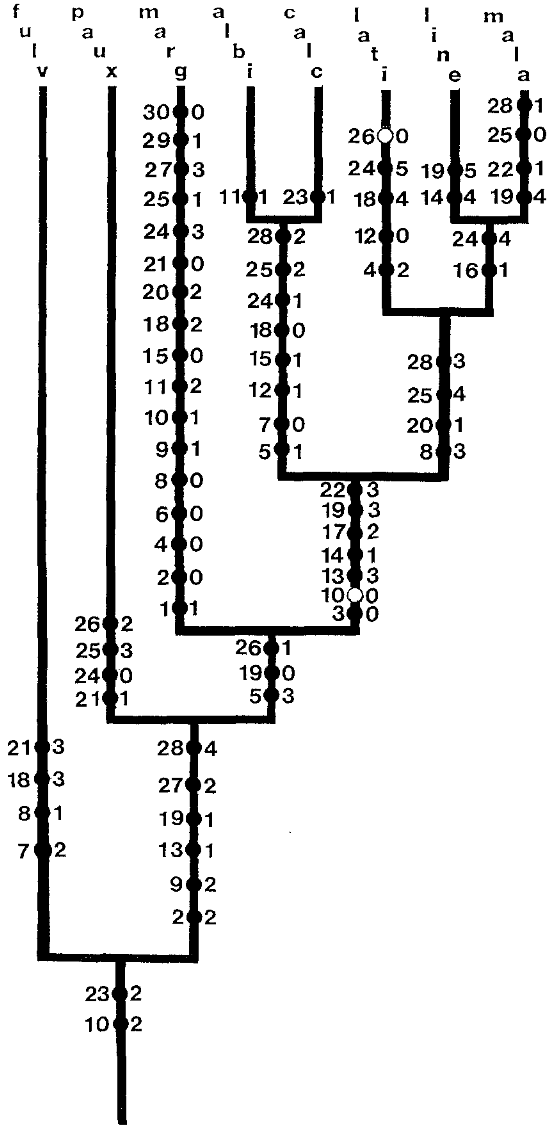

Fig. 1. Solution cladogram for *Evylaeus* based upon the data matrix of Table 3. Numbers on the *left* refer to the character number from Table 2, while numbers on the *right* indicate the derived character state. Origins of derived character states are shown as *solid circles*, reversals as *open circles*

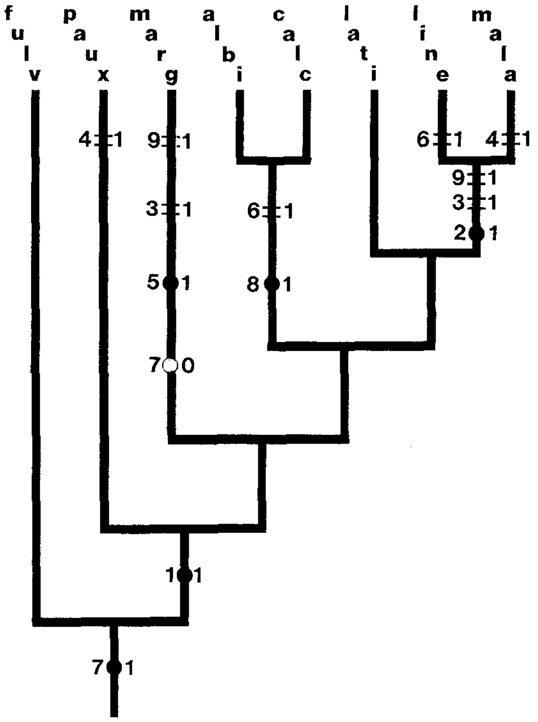

Fig. 2. Behavioral evolutionary changes in *Evylaeus* mapped upon the phylogeny derived from allozymes. *Solid circles* indicate changes from plesiomorphic to derived character states, *open circles* represent reversal to the plesiomorphic condition, and *double bars* indicate parallel evolution. Numbers on the *left* refer to character numbers, while those on the *right* represent character states, both taken from Table 4. The alternative pattern for character 1 (solitary and eusocial behavior) is for eusociality to be ancestral and solitary behavior to be derived in *L.* (*E.*) *fulvicorne* and at least one population of *L.* (*E.*) *calceatum* (see text)

contains both solitary and social species. Thus, solitary behavior may be plesiomorphic within *Evylaeus* (Fig. 2) or apomorphic in *L.* (*E.*) *fulvicorne*. However, reversal to solitary behavior is demonstrated by the phylogeny because a northern montane population of *L.* (*E.*) *calceatum* is solitary (Sakagami and Munakata 1972). Solitary behavior in *L. fulvicorne* may also be derived. That solitary behavior can be derived from eusociality is also suggested by mapping what is known of the social behavior of *Halictus* species onto Pesenko's (1985) phylogeny for the group (Packer 1986a; Michener 1990).

L. calceatum and most social *Halictus* species are weakly eusocial in terms of suppression of worker ovarian development and mating, colony size, morphological caste differentiation, etc. Reversal in the direction of social evolution may be easiest in such weakly social groups. Nonetheless, the assumption that social evolution does not reverse (except in the case of the origin of socially parasitic species) (Wilson 1971) is refuted by the data from sweat bees, as has long been suspected (Michener 1974, 1985; Sakagami and Munakata 1972).

2. *Queen-worker size dimorphism.* The degree of size dimorphism between the castes is highly variable in *Evylaeus*. Broad size overlap may indicate social flexibility in taxa with caste determination in the adult stage. Such flexibility may be important if foundress death before reproductive brood production occurs commonly. Production of totipotent females in the "worker" brood may be necessary in an environment in which the flight season is not always long enough to ensure two successful brood rearing phases – one for workers, one for reproductives (Packer et al. 1989a). Not surprisingly, the two species with the least differentiation between castes, *L.* (*E.*) *calceatum* and *L.* (*E.*) *albipes*, are also the two whose geographic range extends furthest north, and reversal to solitary behavior has been documented for one of them (see above).

Nonoverlapping size distributions suggest that caste determination is biased before the adult stage and indicate an obligate eusociality with small workers that are incapable of becoming foundresses (but see Knerer 1991). Among the included species, such extreme dimorphism is restricted to *L.* (*E.*) *lineare* and *L.* (*E.*) *malachurum* but is approached by *L.* (*E.*) *pauxillum*. None of these species reverts to solitary behavior at the northern extremes of their range. Thus, their comparatively well-developed sociality may constrain them to areas in which the flight season is always long enough to allow two discrete provisioning phases. It remains to be demonstrated that solitary behavior can evolve from such an advanced eusocial condition, and there is evidence that species with nonoverlapping size distributions between the castes may be incapable of facultative reversal to solitary behavior (Packer et al. 1989a). Only the more weakly eusocial species may be capable of facultative reversion to solitary behavior in short season climates or years with particularly bad weather. Brood divalent populations, in which foundresses produce a mixture of worker and reproductive females in the "worker" brood (see Yanega 1988), may be particularly amenable to such facultative reversal. As yet, there are no known instances of obligately solitary species being derived from ancestral eusocial ones in *Evylaeus*, although *Halictus quadricinctus* is probably an example of this in a related genus.

3. *Worker mating*. Mating by workers has been practically eliminated twice independently among the included species – once in *L.* (*E.*) *marginatum* and once in the *lineare-malachurum* clade. In *L.* (*E.*) *marginatum*, mating determines caste, and only nests containing reproductives are open during male flight, thereby permitting males to enter and mate with gynes – a mated worker is not possible in this species. Experimental manipulation is required to see whether males are comparatively unresponsive to the small worker females in *L.* (*E.*) *lineare* and *L.* (*E.*) *malachurum*, as they seem to be in *Halictus ligatus* (Packer 1986b), or whether workers repulse male advances as in *L.* (*D.*) *zephyrum* (Greenberg and Buckle 1981).

4. *Multiple worker broods*. Within the context of an annual colony cycle the character state "multiple worker broods" has originated twice – in lineages leading to *L.* (*E.*) *malachurum* and *L.* (*E.*) *pauxillum*. The number of worker broods produced per year is partly limited by the duration of weather suitable for brood production. Nonetheless, even sympatric species differ in the number of worker broods produced (Poursin and Plateaux-Quénu 1982). Both *L.* (*E.*) *malachurum* and *L.* (*E.*) *pauxillum* have only one worker brood at the northern edge of their ranges (Packer and Knerer 1985; Packer, unpublished data). Both of these species have well-defined caste differences which may be a prerequisite for the evolution of multiple worker broods in *Evylaeus*.

5. *Perenniality*. The unique social organization of the perennial *L.* (*E.*) *marginatum* was probably derived from an annual social cycle and does not represent an independent evolution of social behavior. The factors leading to this unique form of sociality remain obscure.

6. *Multiple-foundress associations*. The ancestral condition for social *Evylaeus* probably did not involve multiple-foundress associations in spring. The absence of multiple-foundress nests in both *L.* (*E.*) *pauxillum* (Smith and Weller 1989) and *L.* (*E.*) *marginatum* (Plateaux-Quénu 1959) indicates that solitarily founded nests are plesiomorphic among social *Evylaeus*.

L. (*E.*) *comagenense* is a member of the *fulvicorne* species group (Svensson et al. 1977) and is the only *Evylaeus* known to have multiple-foundress nests in spring without becoming eusocial in summer (Batra 1990; Packer et al. 1989a). What if such multiple-foundress associations without eusociality could be shown to be plesiomorphic in *Evylaeus*? The data suggest that the combination of multiple-foundress nests in spring with eusociality in summer is derived from a condition of solitary nest initiation in eusocial species. If eusociality did originate within *Evylaeus*, then the semisocial route (in which a reproductive division of labor among females of the same generation precedes the origin of mother-daughter castes) was probably not the one taken by these bees: The direct evolution of eusociality from a solitary ancestor may have occurred without such intermediate types of sociality intervening (Michener 1985). Further behavioral data and phylogenetic resolution of species in the *fulvicorne* group and several hitherto unstudied species groups are required to verify this (see below).

7. *Cavity construction*. The building of a cavity around a cluster of brood cells is a derived feature in the Halictidae as a whole (Eickwort and Sakagami 1979) but appears to be a ground plan characteristic in *Evylaeus*. It has been suggested that this architectural feature serves to prevent brood mortality from waterlogging (Packer and Knerer 1986; Packer et al. 1989b). Only partial cavities are excavated by *L.* (*E.*) *pauxillum*, possibly because the burrows of this species are too small for surface run-off water to enter them (Packer et al. 1989b).

Cavity loss in *L.* (*E.*) *marginatum* and in the later, large broods of *L.* (*E.*) *malachurum* requires explanation. It has been suggested that the number of brood cells produced by these species is too great for a cluster to be supported by thin earthen pillars (Sakagami and Hayashida 1960), but this does not explain in the lack of a cavity in the early broods of *L.* (*E.*) *marginatum* which may have as few as 2 cells. Young female *L.* (*E.*) *marginatum* overwinter in their natal brood cells. Consequently, these cells are constructed at comparatively great depth – below the frost line (hibernacula for overwintering halictids are always considerably deeper than their brood cells). If cavity formation is an adaptation to prevent waterlogging, then it may not occur at all in species with very deep brood cells, as these would be comparatively immune to percolating water after heavy rain. The overwintering behavior of this species seems to remove the advantage of cavity formation. Similarly, large numbers of brood cells in *L.* (*E.*) *malachurum* nests occur only in later brood nests in regions with long summers where the cells are situated deeply and in quite dry soil (Knerer personal communication) where the probability of waterlogging is very low.

Elsewhere in the Halictini, obligatory cavity formation is only known in the distantly related *Halictus quadricinctus* (Knerer 1980; Sitkidov 1988). Two Nearctic *Evylaeus* species, *cinctipes* (Packer et al. 1989b), and *cooleyi* (Packer and Owen 1989b), lack cavities around brood cells, but their taxonomic position remains unknown, and the evolution of this trait requires further investigation.

8. *Lateroid construction.* The construction of the cluster at the end of a lateroid is found only in *L.* (*E.*) *calceatum* and *L.* (*E.*) *albipes.* Two Asian species also exhibit this trait, *L.* (*E.*) *duplex* (Sakagami and Hayashida 1972) and *L.* (*E.*) *affine* (Sakagami et al. 1982). These four are extremely closely related based upon morphology, and the construction of a lateroid appears to be synapomorphic for them. The functional significance of this feature remains unknown.

9. *Open brood cells.* Some *Evylaeus* species keep the brood cells open during larval development, thereby facilitating interaction between adults and developing offspring. This is thought to be characteristic of a more advanced sociality (Knerer and Plateaux-Quénu 1966; Packer and Knerer 1985). The phylogeny suggests that the habit of opening brood cells during larval development has originated twice independently (Fig. 2). Parallel evolution of this trait is not unexpected considering the general sweat bee habit of occasional, temporary opening of brood cells to allow inspection of developing brood (Batra 1964, 1968; Plateaux-Quénu 1973, 1989).

Further studies

Old World *Evylaeus* species fall into about one dozen species groups based mostly upon morphology of the male genitalia, female propodeum, and female hind tibial spur (Ebmer, personal communication). Six groups are represented in the phylogenetic analysis, as indicated in Table 1. Behavioral studies of any member of the remaining groups have yet to be undertaken. The three Old World species not available for electrophoresis but for which behavioral data have been published are all members of the *calceatum* species group. These are *L.* (*E.*) *affine* (Sakagami et al. 1982), *L.* (*E.*) *duplex* (Sakagami and Fukuda 1989 and references therein), and *L.* (*E.*) *nigripes* (Knerer and Plateaux-Quénu 1970), all of which lack the extreme physiological and morphological caste differences found in *L.* (*E.*) *lineare* and *L.* (*E.*) *malachurum* (Packer and Knerer 1985). The behavior of some North American *Evylaeus* has been studied, but insufficient material was available for electrophoretic comparisons to be adequate, and their taxonomic affinities based upon morphology remain unclear.

Based upon morphological criteria, most of the species groups that remain to be studied behaviorally or electrophoretically appear to represent early branches from the tree (before the node leading to *L.* (*E.*) *marginatum* in Fig. 1). These groups are those of *L.* (*E.*) *leucopymatum*, *L.* (*E.*) *sibiriacum*, *L.* (*E.*) *tricinctum*, and *L.* (*E.*) *minutulum*. Further investigation of behavioral evolution in *Evylaeus* should involve field work on members of these taxa. Species closely related to *L.* (*E.*) *fulvicorne* should be studied (phylogenetically and behaviorally) to establish whether solitary behavior is the ancestral condition in *Evylaeus* or whether it is derived in *L.* (*E.*) *fulvicorne*. It appears particularly crucial to resolve the relationships among the various species of *Dialictus* (the sister group to *Evylaeus*) whose solitary or eusocial behavior has been established.

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