Syst. Biol. 52(1):23–36, 2003
DOI: 10.1080/10635150390132687

Phylogeny of Eusocial *Lasioglossum* Reveals Multiple Losses of Eusociality within a Primitively Eusocial Clade of Bees (Hymenoptera: Halictidae)

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Abstract.— We performed a phylogenetic analysis of the species, species groups, and subgenera within the predominantly eusocial lineage of *Lasioglossum* (the *Hemihalictus* series) based on three protein coding genes: mitochondrial cytochrome oxidase I, nuclear elongation factor 1α and long-wavelength rhodopsin. The entire data set consisted of 3,421 aligned nucleotide sites, 854 of which were parsimony informative. Analyses by equal weights parsimony, maximum likelihood, and Bayesian methods yielded good resolution among the 53 taxa/populations, with strong bootstrap support and high posterior probabilities for most nodes. There was no significant incongruence among genes, and parsimony, maximum likelihood, and Bayesian methods yielded congruent results. We mapped social behavior onto the resulting tree for 42 of the taxa/populations to infer the likely history of social evolution within *Lasioglossum*. Our results indicate that eusociality had a single origin within *Lasioglossum*. Within the predominantly eusocial clade, however, there have been multiple (six) reversals from eusociality to solitary nesting, social polymorphism, or social parasitism, suggesting that these reversals may be more common in primitively eusocial Hymenoptera than previously anticipated. Our results support the view that eusociality is hard to evolve but easily lost. This conclusion is potentially important for understanding the early evolution of the advanced eusocial insects, such as ants, termites, and corbiculate bees. [Comparative methods; eusociality; phylogeny; social behavior; social evolution; systematics.]

Eusocial organisms are characterized by reproductive division of labor, overlap of generations, and cooperative brood care (Wilson, 1971; Michener, 1974). The insect order Hymenoptera exibits more independent origins of eusociality and more eusocial species than any other animal group, including mammals (Jarvis, 1981; Sherman, et al., 1991), shrimp (Duffy, 1996, 2000), thrips (Crespi, 1992; Crespi and Mound, 1997; Crespi et al., 1998), aphids (Aoki, 1977; Stern, 1994; Stern and Foster, 1996, 1997), and platypodid beetles (Kent and Simpson, 1992). As a result, the Hymenoptera provide an ideal group in which to investigate the evolutionary origins and maintenance of eusociality. However, although many groups of eusocial Hymenoptera can provide insights into the organization of eusocial colonies and the maintenance of eusociality, few lineages are recent enough in origin to provide insights into the evolutionary origins of eusociality. Some of the best known and most well-understood eusocial insects, such as ants (Grimaldi and Agosti, 2000), termites (Emerson, 1968), and corbiculate bees (Michener and Grimaldi, 1988a, 1988b; Engel, 2001a, 2001b), evolved eusociality in the Cretaceous (146–65 million years ago), and closely related solitary species are no longer extant. Such advanced eusocial taxa cannot provide insights into the earliest stages of eusocial evolution because the transition from solitary living to eusociality occurred long ago (Bourke and Franks, 1995). In contrast, primitively eusocial insects, such as halictid bees and vespid wasps (Hunt, 1999), can provide insights into the evolution of eusociality in its earliest stages. In halictid bees, eusociality is of recent origin (at least in *Halictus* and *Lasioglossum* and possibly also in the Augochlorini), eusociality has arisen repeatedly, and there is great variation in social behavior within clades of closely related species (Michener, 1974; Brockmann, 1984; Andersson, 1984; Seger, 1991; Crozier and Pamilo, 1996; Bourke, 1997).

In spite of their potential importance for understanding social evolution, reconstructing the patterns of social evolution in halictid bees has been hindered by our poor understanding of both higher level (generic and tribal) and lower level (subgeneric and species) phylogenetic relationships. In the absence of well-corroborated phylogenies for the halictid bees, it has been impossible to reconstruct social trajectories. Recent phylogenetic studies of halictine bees based on morphology (Danforth and Eickwort, 1997; Engel, 2000), allozyme data (Packer, 1991; Richards, 1994), and DNA sequence data (Danforth, 1999, 2002; Danforth et al., 1999; Danforth and Ji, 2001) have shed some light on social evolution in selected groups of halictid bees. Danforth and Eickwort (1997) determined that eusociality had a single origin in the halictine tribe Augochlorini with one reversal to solitary nesting within the eusocial lineage. Engel (2000) confirmed these results based on a reanalysis of the same data set with additional characters and taxa. Danforth et al. (1999) determined that the genus *Halictus* had a eusocial common ancestor, with between four and six reversals to either solitary nesting or facultative eusociality within the predominantly eusocial clade, a result consistent with earlier allozyme studies (Richards, 1994) and combined analyses based on allozymes and morphology (Packer, 1997). Overall, the previous studies on the genus *Halictus* and the tribe Augochlorini have supported a pattern of few origins and multiple reversals from eusociality to solitary nesting (Wcislo and Danforth, 1997).

One lineage of primitively eusocial halictine bees, the genus *Lasioglossum*, is an important group for understanding the early transitions in social behavior. *Lasioglossum* is a large genus with a cosmopolitan distribution and diversity in social behavior (Table 1). The genus is presumed to be of recent origin because there are no known fossils (Engel, 2001a), and the group is nested

TABLE 1. Classification of the subgenera of *Lasioglossum* (modified slightly from Michener, 2000). Total numbers of species from Michener (2000).

Subgenus (no. species)	Social behavior
Lasioglossum series (716 total)	
Australictus Michener (11)	Solitary, communal
Callalictus Michener (8)	?
Chilalictus Michener (134)	Solitary, communal
Ctenonomia Cameron (196)	Solitary
Echthralictus Perkins and Cheesman (2)	Cleptoparasitic (presumed)
Glossalictus Michener (1)	?
Homalictus Cockerell (101)	Solitary, communal
Lasioglossum Curtis (162)	Solitary
Parasphecodes Smith (99)	Solitary, communal
Pseudochilalictus Michener (2)	?
Hemihalictus series (552 total)	
Acanthalictus Cockerell (1)	?
Austrevylaeus Michener (19)	?
Dialictus Robertson (285)	Solitary, eusocial
Evylaeus Robertson (215)	Solitary, eusocial
Hemihalictus Cockerell (1)	Solitary
Paradialictus Pauly (1)	?
Paralictus Robertson (5)	Social parasite
Sellalictus Pauly (11)	?
Sphecodogastra Ashmead (8)	Solitary
Sudila Cameron (6)	Solitary, communal

well within the halictid tribe Halictini, sister to *Thrincohalictus* + *Halictus* (Danforth, 2002). Approximately 40% of its 1,268 described species are eusocial or within predominantly eusocial subgenera (Michener, 2000).

Lasioglossum is commonly divided into numerous subgenera. Michener (2000) recognized 18 subgenera and divided the subgenera into two higher categories: the *Lasioglossum* series (with a strong first r-m crossvein in females; Danforth, 1999: Fig. 1a) and the *Hemihalictus* series (with a weak first r-m crossvein in females; Danforth, 1999: Fig. 1d). The predominantly eusocial subgenera (*Dialictus* and *Evylaeus*) are in the *Hemihalictus* series (Table 1). Recent molecular studies (Danforth, 1999; Danforth and Ji, 2001) have supported monophyly of both the *Lasioglossum* and *Hemihalictus* series, and for the purposes of this article we treat the predominantly eusocial *Hemihalictus* series as a monophyletic sister group to the communal and solitary *Lasioglossum* series. Table 1 lists the *Lasioglossum* subgenera as they are recognized herein.

Social behavior among species of *Lasioglossum* is extraordinarily variable. There is more variation in social behavior among species of *Lasioglossum* than among all other groups of eusocial halictid bees combined. Species are known to exibit solitary nesting (*Lasioglossum* (*L.*) *leucozonium* [Stöckhert, 1933] and *Lasioglossum* (*Hemihalictus*) *lustrans* [Daly, 1961]), primitive eusociality (*Lasioglossum* (*Dialictus*) *zephyrum* [Michener, 1990]), and social parasitism (*Lasioglossum* subgenus *Paralictus* [Wcislo, 1997b]). Colony sizes vary widely, from small colonies of a single queen four or fewer workers (in *L.* (*Evylaeus*) *laticeps* [Packer, 1983]) to huge colonies of >400 workers and perennial life cycles (in *L.* (*Evylaeus*) *marginatum* [Plateaux-Quénu, 1959, 1960, 1962, 1972]). In addition, there is substantial variation among populations in some species, such as *L.* (*Evylaeus*) *calceatum* (Sakagami and Munakata, 1972), in which low-elevation populations exibit eusociality and high-elevation populations exibit solitary nesting.

The typical halictine life cycle, whether in a solitary or a eusocial species, begins with nest founding by an overwintering gyne who mated the previous year and carries sperm in her spermatheca. Females generally construct nests in soil or in wood, and nest founding may be performed alone or in groups (Eickwort, 1986; Packer, 1993). This early stage of nest founding is referred to as the *foundress phase* (Mueller, 1996). In solitary species of *Lasioglossum*, as in most solitary bees, females rear one or more broods of approximately equal numbers of male and female offspring, and all female offspring mate and enter diapause until the following spring. In eusocial colonies, females rear multiple broods, with the first brood typically exhibiting a female-biased sex ratio. These midsummer or early season females are typically smaller than the foundress generation and generally become workers (Soucy, 2001), although in some species first-brood females may mate and immediately enter diapause, essentially behaving as an overwintering gyne (Yanega, 1988, 1989). The emergence of the first brood marks the beginning of the *worker phase* in eusocial colonies. Following the emergence of the first brood, queens generally cease foraging and do not leave the nest. During the matrifilial or worker phase, the workers provision cells with pollen and nectar and queens lay eggs in the provisioned cells. Depending on the length of the growing season, colonies may produce from one to three worker broods. The reproductive brood is produced toward the end of the active growing season and typically exhibits a slightly male-biased sex ratio. The emergence of the reproductive brood marks the beginning of the *reproductive phase*. Most females produced late in the season mate and enter diapause until the following spring. Much variation exists among species and populations in aspects of the life cycle, including variation in sex ratio in the early broods, the number of worker broods, and the total size of the worker population (Michener, 1974; Yanega, 1997). Additional factors that vary among species and populations include the degree of queen–worker dimorphism, the proportion of workers mated, and the proportion of workers who have developed ovaries (Breed, 1976; Packer and Knerer, 1985). Caste differentiation in *Lasioglossum*, as in most halictine bees, is subtle, and queens and workers are generally broadly overlapping morphologically. In most eusocial halictine bees, both female body size and offspring sex ratio change gradually over the course of the nesting season, with colonies initially producing small female-biased offspring early in the season and larger male-biased offspring later in the season (Mueller, 1996; Soucy, 2001). The gradual changes in body size and sex ratio have been related to temperature and day length (Yanega, 1997).

Here, we analyze phylogenetic relationships among species of *Lasioglossum* in the predominantly eusocial group, the *Hemihalictus* series (Table 1). We combined

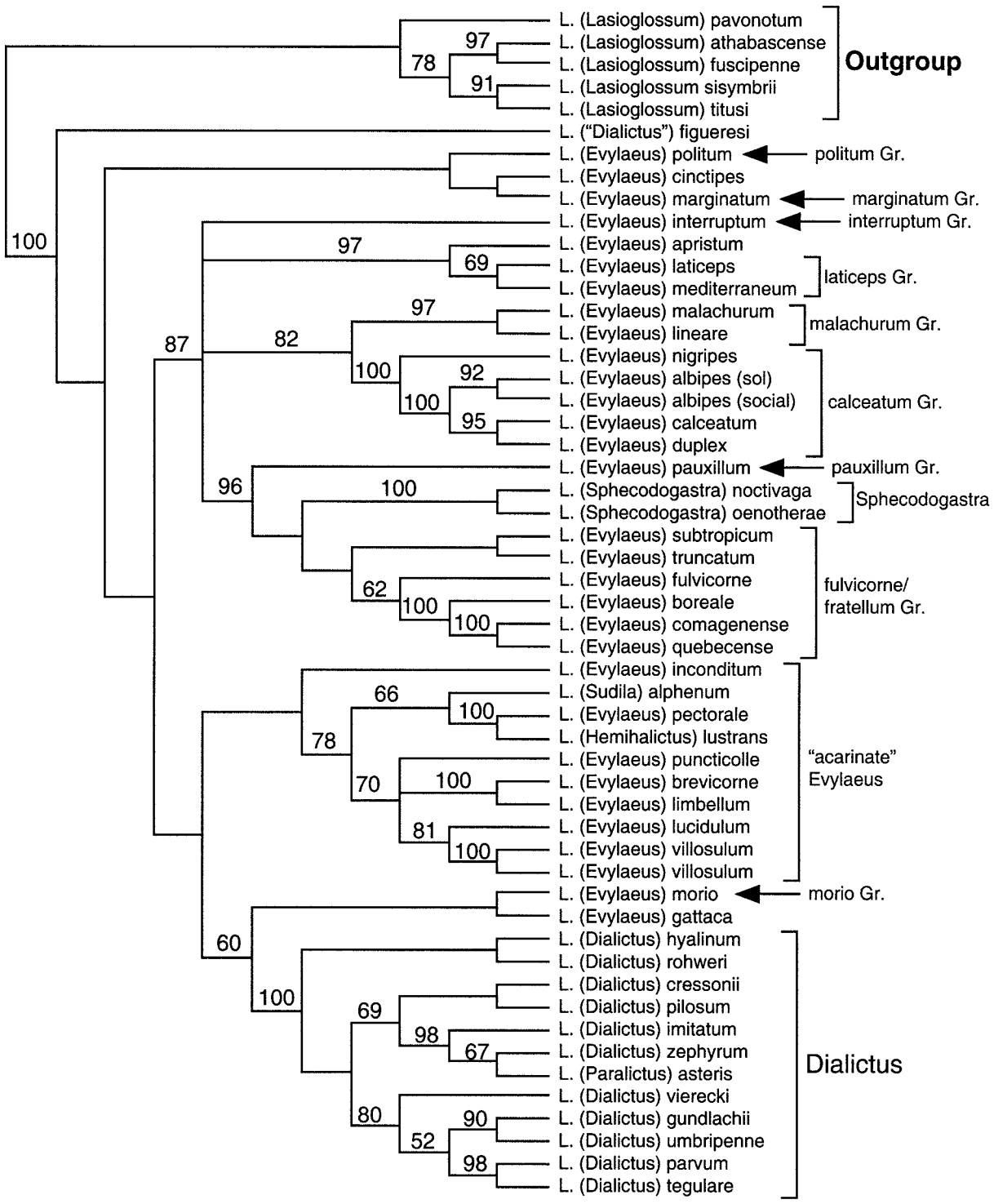

FIGURE 1. Consensus of five trees based on equal weights parsimony analysis with COI nt3 included (length = 5,115; CI = 0.2689; RI = 0.5132). Numbers above nodes indicate bootstrap values (Felsenstein, 1985) after 500 replicates with 10 random addition sequences per replicate.

these phylogenetic results with data available in the extensive literature on social behavior in *Lasioglossum* (reviewed by Michener, 1974, 1990; Packer, 1993; Yanega, 1997) to reconstruct the likely evolutionary transitions that characterize this primitively eusocial lineage of bees. For the purposes of this study, we have defined the social states following the terminology of Wilson (1971) and Michener (1974). *Solitary* species are those in which all females mate and construct and provision cells and in which there is no reproductive division of labor when females share nests. *Eusocial* species are those in which there is overlap of generations, cooperative brood care,

and reproductive division of labor during some part of the colony life cycle at most localities where the species has been studied. *Socially polymorphic* species are those in which the majority of colonies are eusocial at some localities, whereas at other localities the majority of colonies are solitary. *Social parasites* are those species in which females enter the nests of closely related species and reside there as inquiline queens, laying eggs and utilizing the worker population of the host nest (see Wcislo, 1997b, for a detailed account of one social parasite and its host). Few of the >1,000 species of *Lasioglossum* have been studied in detail, and our information on social behavior is necessarily fragmentary. Wcislo and Danforth (1997) provided additional comments on social flexibility in *Lasioglossum* and other bees.

Materials and Methods

To generate a robust phylogeny for the subgenera of *Lasioglossum* within the predominantly eusocial group (the *Hemihalictus* series), we combined data from three genes: elongation factor 1α (EF-1α, F2 copy; Danforth and Ji, 1998), long-wavelength rhodopsin (LW opsin; Chang et al., 1996, Mardulyn and Cameron, 1999; Ascher et al., 2001), and mitochondrial cytochrome oxidase I (COI; Simon et al., 1994; Danforth, 1999). Primers used are listed in Table 2. DNA extractions, PCR, and sequencing protocols followed standard methods detailed by Danforth (1999), Ascher et al. (2001), and Danforth and Ji (2001). Alignments for all genes were generated in the Lasergene DNA Star software package using Clustal W. For noncoding regions (e.g., two introns in opsin and two introns in EF-1α), alignments were improved by eye and unalignable regions were excluded from the analysis. Reading frames and intron/exon boundaries were determined by comparison with published sequences for the honey bee, *Apis mellifera* (COI: Crozier and Crozier, 1993; opsin: Chang et al., 1996; EF-1α: Danforth and Ji, 1998).

TABLE 2. Primers used in PCR and sequencing of *Lasioglossum* subgenera.

Primer	Sequence	Position[a]
COI		
Ron	5′-GGA TCA CCT GAT ATA GCA TTC CC-3′	2049
HemiFor	5′-CGA ATA AAY AAT ATA AGA TTT TG-3′	2073
Jerry	5′-CAA CAT TTA TTT TGA TTT TTT GG-3′	2481
Madeline	5′-TTC TTT TTT HCC WCT TTC RTT RAA-3′	2585
Pat	5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′	3380
EF-1α		
For1-deg	5′-GY ATC GAC AAR CGT ACS ATY G-3′	462
For3	5′-GGN GAC AAY GTT GGY TTC AAC G-3′	1496
F2-Rev1	5′-A ATC AGC AGC ACC TTT AGG TGG-3′	1600
Cho10	5′-AC RGC VAC KGT YTG HCK CAT GTC-3′	1887
LW opsin		
LWRhF	5′-AAT TGC TAT TAY GAR ACN TGG GT-3′	398
LWRhF3	5′-AGA TAC AAC GTR ATC GTS AAR GGT-3′	512
LWRhR	5′-ATA TGG AGT CCA NGC CAT RAA CCA-3′	946

[a] Based on the 5′ end of the primer in the honey bee, *Apis mellifera* (COI: Crozier and Crozier, 1993; EF-1α: Walldorf and Hovemann, 1990; LW opsin: Chang et al., 1996).

We included a total of 48 ingroup sequences (with two species, *L.* (*Evylaeus*) *villosulum* and *L.* (*Evylaeus*) *albipes*, represented by more than one population) representing 6 of the 10 currently recognized subgenera within the *Hemihalictus* series (Table 3) plus five outgroups from the *Lasioglossum coriaceum* group, a basal branch of the *Lasioglossum* series of subgenera (Danforth and Ji, 2001). The four subgenera excluded from the analysis (*Acanthalictus, Austrevylaeus, Paradialictus*, and *Sellalictus*) represent just 32 of the 552 total species, and social behavior is unknown for all four. All voucher specimens are deposited in the Cornell University Insect Collection. GenBank accession numbers and specimen voucher codes are listed in Table 3. Our combined data set is available from the *Systematic Biology* web site (http://systematicbiology.org/).

We performed most phylogenetic analyses using PAUP* v.4.0b8 (Swofford, 1999). Initially we performed equal weights parsimony analyses on each of the three data sets and then combined the data sets into a single analysis. We tested for data set congruence using the incongruence length difference test (ILD test; Farris et al., 1995) implemented in PAUP*. In spite of the problems that this test may have (Dowton and Austin, 2002), it provides a useful measure of incongruence when data sets are roughly equal in size. For the four intron regions, gaps were coded as a fifth state because gaps were relatively short ($\leq$4 bp in length) and indel mutations were mostly autapomorphic. We also coded gaps as missing data and according to methods described by Danforth et al. (1999), which treats gaps of varying lengths as alternative states of a multistate character. Alternative methods of gap coding yielded virtually identical tree topologies. Branch support for the individual data sets and the combined data set was estimated using bootstrap analysis (Felsenstein, 1985). For parsimony searches, we performed 50 random sequence additions. For calculating bootstrap proportions, we performed 500 replicates with 10 random sequence additions per replicate.

We also performed maximum likelihood (ML; using PAUP* v.4.0b8) and Bayesian (using MrBayes; Huelsenbeck and Ronquist, 2001) analyses in which separate rate categories were assigned to 1 of 11 discrete data partitions: COI nucleotide (nt) 1, COI nt2, COI nt3, EF-1α nt1, EF-1α nt2, EF-1α nt3, EF-1α introns, opsin nt1, opsin nt2, opsin nt3, and opsin introns. We used a general time-reversible (GTR) + site-specific rate (SSR) model for tree searching, with one or more of the equal weights parsimony trees as starting trees for branch swapping. Separate ML analyses were performed on each data set individually by calculating the model parameters for each data set on the equal weights parsimony trees. We use a GTR+I+G model for this analysis to infer the substitution patterns characteristic of each gene and each data partition within genes. ML provides an explicit method for comparing substitution patterns among genes in a combined analysis. ML has rarely been used in this context (see Reed and Sperling, 1999, for one such example), but an understanding of how subsitution patterns vary among data sets can provide important insights into the

TABLE 3. *Lasioglossum* species included in the present study, with GenBank accession numbers and locality data.

Taxon	Author	Abbrev.	No.	GenBank no. EF-1α	GenBank no. LW opsin	GenBank no. mtCOI	Locality
Outgroups							
L. (*Lasioglossum*) *athabascense*	(Sandhouse)	Laat	556	AF435390	AF448867	AF104645	USA: New York, Tompkins Co.
L. (*L.*) *fuscipenne*	(Smith)	Lafu	65	AF264844	AF448868	AF104648	USA: Michigan
L. (*L.*) *pavonotum*	(Cockerell)	Lapa	339	AF264851	AF448869	AF104654	USA: California, Marin Co.
L. (*L.*) *sisymbrii*	(Cockerell)	Lasi	253	AF264852	AF448870	AF104656	USA: Arizona, Cochise Co.
L. (*L.*) *titusi*	(Crawford)	Lati	167	AF264854	AF448871	AF104657	USA: California, San Bernardino Co.
Ingroups							
L. ("*Dialictus*") *figueresi*	Wcislo	Difi	341	AF264802	AF448903	AF435357	Costa Rica: San José Province
L. (*Dialictus*) *cressonii*	(Robertson)	Dicr	66	AF264801	AF448908	AF103963	Canada: Ontario
L. (*D.*) *gundlachii*	(Baker)	Digr	48	AF264803	AF448909	AF103965	Puerto Rico
L. (*D.*) *hyalinum*	(Crawford)	Diha	277	AF264804	AF448910	AF103966	USA: Arizona, Pima Co.
L. (*D.*) *imitatum*	(Smith)	Diim	27	AF264805	AF448911	AF103967	USA: New York, Tompkins Co.
L. (*D.*) *parvum*	(Cresson)	Dipa	7	AF264806	AF448912	AF103968	Puerto Rico
L. (*D.*) *pilosum*	(Smith)	Dipi	71	AF264807	AF448913	AF103969	USA: New York, Seneca Co.
L. (*D.*) *rohweri*	(Ellis)	Dirh	79	AF264808	AF448914	AF103970	USA: New York, Seneca Co.
L. (*D.*) *tegulare*	(Robertson)	Ditg	81	AF264809	AF448915	AF103971	USA: New York, Seneca Co.
L. (*D.*) *umbripenne*	(Ellis)	Dium	322	AF264810	AF448916	AF103975	Republic of Panama: Panama Province
L. (*D.*) *vierecki*	(Crawford)	Divi	67	AF264811	AF448917	AF103972	USA: New York, Seneca Co.
L. (*D.*) *zephyrum*	(Smith)	Dizp	74	AF264812	AF448918	AF103973	USA: New York, Seneca Co.
L. (*Evylaeus*) *albipes* (soc)	(Fabricius)	Eval	99	AF264814	AF448873	AF103976	France: Dordogne
L. (*E.*) *albipes* (sol)	(Fabricius)	Eval	104	AF264813	AF448872	AF103977	France: Vosges
L. (*E.*) *apristum*	(Vachal)	Evap	145	AF264815	AF448874	AF103978	Japan: Shimane Prefecture
L. (*E.*) *boreale*	Svensson	Evbo	262	AF264816	AF448875	AF103979	Canada: Northwest Territories
L. (*E.*) *brevicorne*	(Schenck)	Evbr	389	AF435365	AF448876	AF435358	Spain: Almeria Province
L. (*E.*) *calceatum*	(Scopoli)	Evca	105	AF264817	AF448877	AF103980	France: Dordogne
L. (*E.*) *cinctipes*	(Provancher)	Evci	311	AF264818	AF448878	AF103981	USA: New York, Tompkins Co.
L. (*E.*) *comagenense*	(Knerer and Atwood)	Evco	255	AF264819	AF448879	AF103982	Canada: Nova Scotia
L. (*E.*) *duplex*	(Dalla Torre)	Evdu	142	AF264820	AF448880	AF103983	Japan: Miyagi Prefecture
L. (*E.*) *fulvicorne*	(Kirby)	Evfu	310	AF264821	AF448881	AF103984	France: Vaucluse
L. (*E.*) *gattaca*	Danforth and Wcislo	Evga	324	AF264834	AF448898	AF104639	Republic of Panama: Chiriqui Province
L. (*E.*) *inconditum*	(Cockerell)	Evin	407	AF435366	AF448883	AF435359	USA: Arizona, Santa Cruz Co.
L. (*E.*) *interruptum*	(Panzer)	Evin	385	AF435367	AF448882	AF435360	Spain: Almeria Province
L. (*E.*) *laticeps*	(Schenck)	Evla	117	AF264822	AF448884	AF103985	France: Dordogne
L. (*E.*) *limbellum*	(Morawitz)	Evlm	388	AF435368	AF448886	AF435361	Spain: Almeria Province
L. (*E.*) *lineare*	(Schenck)	Evli	137	AF264823	AF448893	AF103986	France: Meurthe et Moselle
L. (*E.*) *lucidulum*	(Schenck)	Evlu	383	AF435369	AF448887	AF435362	Spain: Almeria Province
L. (*E.*) *malachurum*	(Kirby)	Evml	111	AF264826	AF448890	AF103988	France: Dordogne
L. (*E.*) *marginatum*	(Brulle)	Evmg	108	AF264825	AF448889	AF103987	France: Dordogne
L. (*E.*) *mediterraneum*	(Bluthgen)	Evme	289	AF264824	AF448888	AF435363	France: Dordogne
L. (*E.*) *morio*	(Fabricius)	Evmo	148	AF264827	AF448891	AF103989	France: Dordogne
L. (*E.*) *nigripes*	(Lepeletier)	Evng	129	AF264828	AF448892	AF103990	France: Vaucluse
L. (*E.*) *pauxillum*	(Schenck)	Evpa	131	AF264829	AF448885	AF104634	Austria: Vienna
L. (*E.*) *pectorale*	(Smith)	Evpe	10	AF264830	AF448894	AF104635	USA: Florida, Polk Co.
L. (*E.*) *politum*	(Schenck)	Evpo	122	AF264831	AF448895	AF103636	France: Dordogne
L. (*E.*) *puncticolle*	(Morawitz)	Evpu	128	AF264832	AF448896	AF104637	France: Dordogne
L. (*E.*) *quebecense*	(Crawford)	Evqu	325	AF264833	AF448897	AF104638	no locality data
L. (*E.*) *subtropicum*	Sakagami	Evsu	139	AF264835	AF448899	AF104640	Japan: Okinawa Prefecture
L. (*E.*) *truncatum*	(Robertson)	Evtr	312	AF264836	AF448900	AF104641	USA: New York, Tompkins Co.
L. (*E.*) *villosulum*	(Kirby)	Evvi	125	AF264837	AF448901	AF104642	France: Dordogne
L. (*E.*) *villosulum*	(Kirby)	Evvi	382	AF435370	AF448902	AF435364	Spain: Almeria Province
L. (*Hemihalictus*) *lustrans*	(Cockerell)	Helu	186	AF264838	AF448904	AF104643	USA: Texas, Bastrop Co.
L. (*Paralictus*) *asteris*	(Mitchell)	Paas	30	AF264856	AF448919	AF104659	USA: New York, Tompkins Co.
L. (*Sphecodogastra*) *noctivaga*	Linsley and MacSwain	Stno	258	AF264859	AF448905	AF104661	USA: Texas, Ward Co.
L. (*S.*) *oenotherae*	(Stevens)	Stoe	54	AF264860	AF448906	AF104662	USA: New York, Tompkins Co.
L. (*Sudila*) *alphenum*	(Cameron)	Sual	390	AF264861	AF448907	AF104663	Sri Lanka: NE District

phylogenetic utility of different genes. Site-specific rate models have been criticized by Buckley et al. (2001) and Buckley and Cunningham (2002), who recommend using a variety of models when performing ML analyses. Our results are robust irrespective of method of analysis or model choice.

To reconstruct the likely history of social evolution in *Lasioglossum*, we mapped social behavior on the trees using MacClade 3.07 (Maddison and Maddison, 1992). We were able to assign social behavior to 44 of 54 taxa/populations based on previously published reports (reviewed by Michener, 1974, 1990; Packer, 1993; Yanega, 1997).

To test the hypothesis that the pattern of social evolution observed based on our character mapping was significantly different from a pattern that could be obtained by randomly associating a social behavior with each species on the tree (i.e., no phylogenetic or historical

constraint to social evolution), we mapped our social behavior data onto 1,000 random tree topologies and counted the number of steps that occurred in the evolution of social behavior. We applied a one-tailed test of significance and asked whether our observed number of steps deviated significantly from those of the 1,000 randomly generated topologies. If social behavior is constrained at all by phylogeny, the observed number of steps should be significantly lower than the number of steps observed on 1,000 random tree topologies. This analysis was performed with MacClade 3.07.

RESULTS

The entire data set consisted of 3,421 aligned nucleotide sites for the three genes. All taxa were sequenced for all three genes, so little data were missing from the analysis. The EF-1α data set consisted of 1,524 aligned sites (29 of which were excluded), the COI data set consisted of 1,239 aligned sites, and the opsin data set consisted of 658 aligned sites (63 of which were excluded). Alignments were unambiguous except within those regions excluded from the analysis. The data set could be subdivided into 11 data partitions corresponding to noncoding positions and codon positions for each gene. Introns were located in the EF-1α data set at positions 733/754 and 1029/1030 (relative to the coding region of the *A. mellifera* EF-1α F2 copy; Danforth and Ji, 1998) and in the opsin data set at positions 639/640 and 898/899 (relative to the *A. mellifera* LW opsin coding sequence; Chang et al., 1996). Our two opsin introns match introns 4 and 6 reported for nymphalid butterflies (Hsu et al., 2001), suggesting that opsin introns are conserved across insect orders. Table 4 shows the total number of sites, the number of parsimony informative sites, the consistency index (CI) for each data partition, and the estimated rates of substitution.

Base composition among genes and among data partitions is shown in Table 5. Base composition overall was slightly A/T biased (61.1% A/T) primarily because of a huge A/T bias in COI nt3 (90.7%). Base composition among taxa did not differ significantly for the overall data set or any data partition except for COI nt3 ($P < 0.0001$). Exclusion of COI nt3 resulted in an overall A/T bias of 57%.

TABLE 4. Data partitions for *Lasioglossum* analyses.

Gene	Total sites	No. parsimony-informative sites	CI[a]	Relative rate[b]
COI				
nt1	413	93	0.2332	45.7
nt2	413	27	0.4048	7.03
nt3	413	333	0.1954	234.4
EF-1α				
nt1	359	8	0.6923	2.36
nt2	359	2	1.0	1.0
nt3	358	129	0.4332	31.9
int	419	135	0.5570	30.7
LW opsin				
nt1	163	26	0.4921	16.63
nt2	163	7	0.6667	5.46
nt3	163	53	0.5820	26.5
int	106	41	0.6512	26.6
Overall	3,329 (92 sites excluded)	854	0.2689	

[a]Excluding uninformative sites.

[b]Based on GTR+SSR model. The slowest rate (EF-1α nt2) is standardized at 1.0.

TABLE 5. Base composition by gene and data partition within gene. The *P* value refers to the probability of rejecting the null hypothesis of homogeneity among taxa in base composition.

Gene	A	C	G	T	*P*
COI					
nt1	33.2	13.9	19.1	33.8	1.0
nt2	20.6	22.4	14.0	43.0	1.0
nt3	47.8	8.7	0.60	42.9	<0.001
EF-1α					
nt1	28.5	18.2	38.2	15.1	1.0
nt2	30.1	26.1	16.2	27.6	1.0
nt3	20.6	29.6	18.4	31.3	1.0
int	27.3	16.9	19.7	36.1	1.0
LW opsin					
nt1	31.3	13.6	26.7	28.4	1.0
nt2	22.3	23.1	18.8	35.8	1.0
nt3	16.6	39.3	25.7	18.5	1.0
int	27.5	24.9	18.9	28.7	1.0
Overall	28.9	20.2	18.6	32.3	1.0
Overall, excluding COI nt3	26.2	21.9	21.2	30.8	1.0

We performed equal weights parsimony analyses with COI nt3 included and excluded. Exclusion of COI nt3 was justified based on the extraordinarily high base compositional bias, the significant heterogeneity in base composition among taxa (Table 5), and the extremely high rate of COI nt3 evolution (roughly 5 times higher than that of any other data partition and 234 times higher than that of the slowest data partition, EF-1α nt2; Table 4). Inclusion of COI nt3 yielded 5 trees (Fig. 1; length = 5,115; CI = 0.2689; retention index [RI] = 0.5132), and exclusion of COI nt3 yielded 24 trees (Fig. 2; length = 2,196; CI = 0.3895; RI = 0.6757). Overall, the tree topologies were very similar, but inclusion of COI nt3 provided additional resolution within the subgenus *Dialictus* while decreasing bootstrap support for the deeper nodes (Fig. 2). The biased base composition in COI nt3 justified exclusion of this data partition. Using the ILD test, we were not able to detect any significant incongruence among genes even with COI nt3 included ($P = 0.75$).

Next, we employed ML to investigate substitution patterns among the three genes and rates of substitution among the 11 data partitions. Using one of the equally parsimonious trees obtained from the equal weights analysis (with COI nt3 excluded), we calculated the relative rates of the 11 data partitions (under the GTR+SSR model) and the transformation matrices for the coding region of each gene plus the noncoding regions (introns) of EF-1α and LW opsin. Rates varied substantially among data partitions (Table 4) with EF-1α nt2 showing the lowest (standardized at 1.0) and COI nt3 showing the highest (234.4). For both opsin and EF-1α, the rate of nt3 substitution was virtually identical to the rate of intron substitution (Table 4). Comparing the two nuclear genes, EF-1α and opsin, opsin had substantially more nt1 and nt2 variation than EF-1α, suggesting more

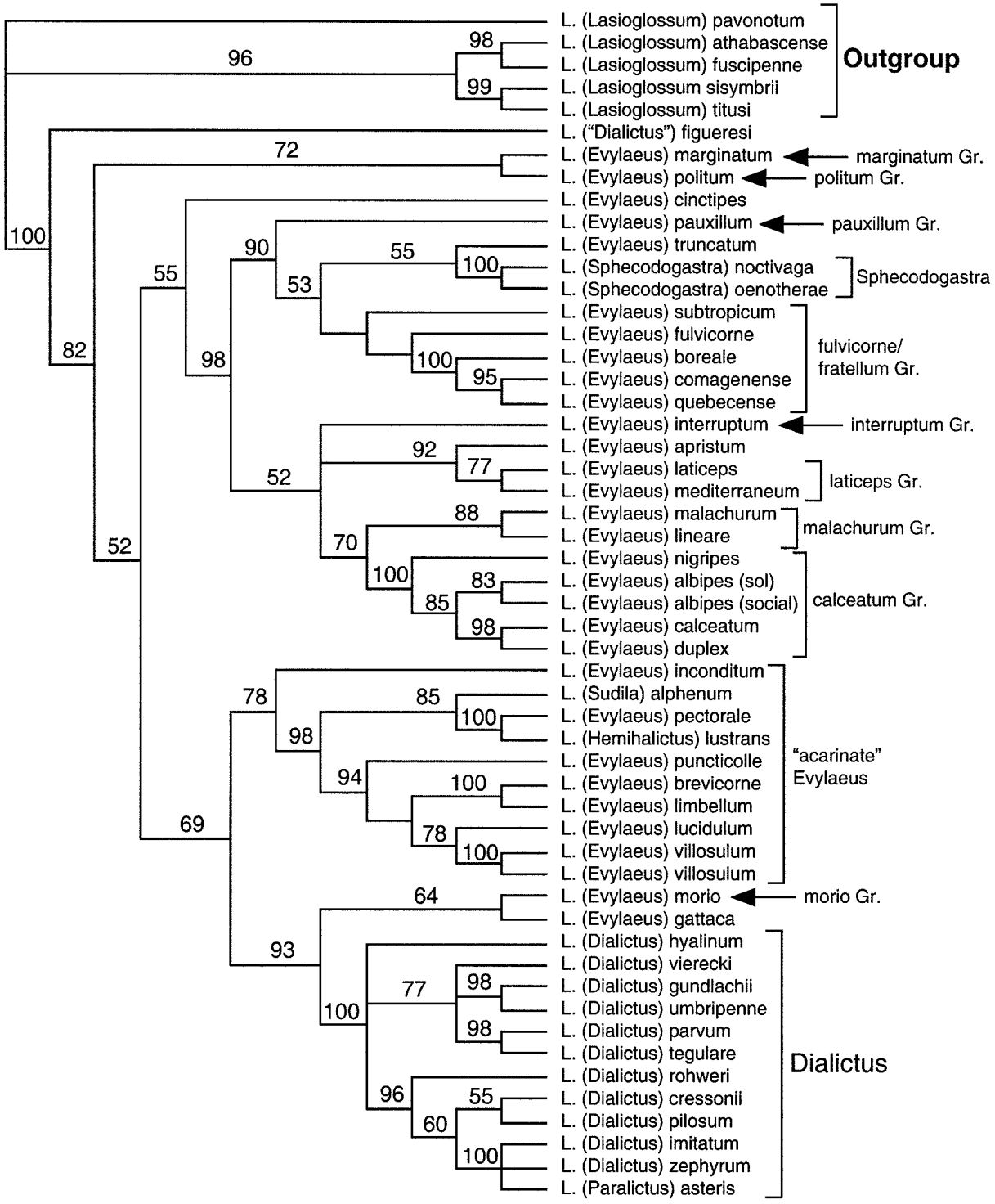

FIGURE 2. Consensus of 24 trees based on equal weights parsimony analysis with COI nt3 excluded (length = 2,196; CI = 0.3895; RI = 0.6757). Numbers above nodes indicate bootstrap values (Felsenstein, 1985) after 500 replicates with 10 random addition sequences per replicate.

nonsynonymous changes for opsin than for EF-1α. For nt1 and nt2, opsin had an intermediate rate of substitution in comparison with COI (fast) and EF-1α (slow). For nt3, the two nuclear genes had comparable rates that were roughly eightfold slower than that of COI nt3 substitutions.

Transformation rate matrices (under the GTR + I + G model) for each gene and for the noncoding regions are shown in Figure 3. The nuclear genes together had more symmetrical transformation matrices (with a slight bias toward transitions) than did the mitochondrial gene. Whereas T-C transitions dominated in the COI data set,

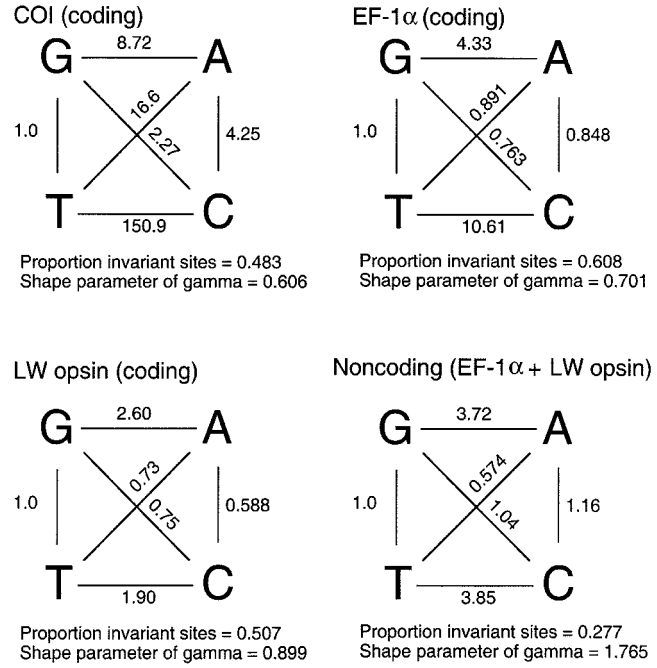

FIGURE 3. ML parameter estimates from separate analyses of four data partitions using the GTR + I + G model and the equal weights parsimony trees obtained with exclusion of COI nt3. The proportion of invariant sites and the shape parameter of the gamma distribution are shown for each data partition.

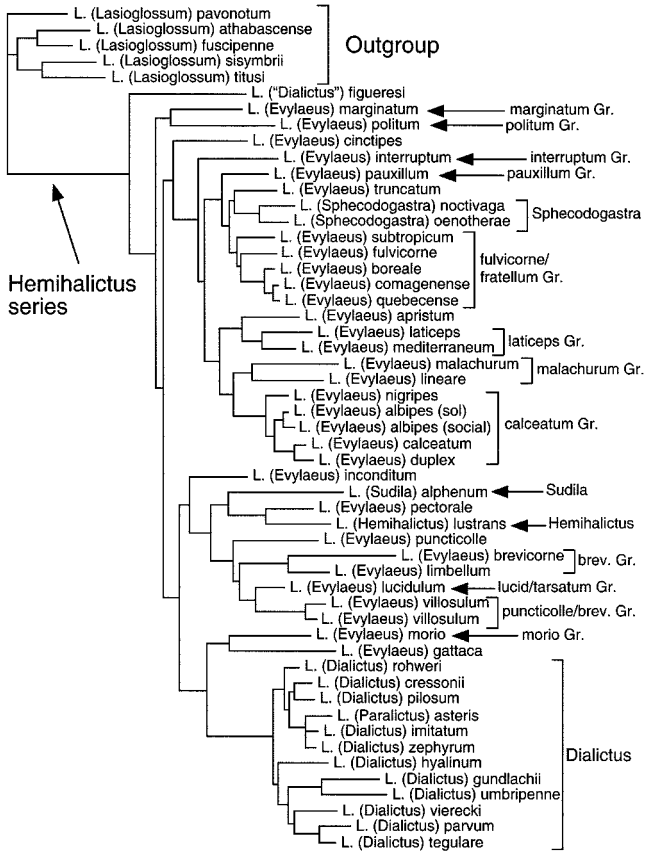

FIGURE 4. Tree resulting from an ML analysis using the GTR + SSR model with separate rates for each of the 11 data partitions. The same tree topology is obtained whether COI nt3 are included or excluded. Starting trees for the ML analysis were based on the equal weights parsimony trees. Subgenera and species groups of *Evylaeus* are indicated.

EF-1α and opsin showed no such highly skewed transformation. The skewed transformation rate matrix is a potential problem for phylogenetic analysis because a biased transformation rate matrix will most likely yield higher levels of homoplasy than an unbiased one.

We also compared the three genes in terms of their phylogenetic utility by calculating the 50% bootstrap consensus of the combined data set and then comparing the number of nodes recovered in the 50% bootstrap consensus of each of the three genes analyzed separately. For the total ("combined") data set, we recovered 44 nodes with >50% bootstrap support. For the individual data sets, we recovered 17 "combined" nodes for opsin and COI (each analyzed alone) and 30 "combined" nodes for EF-1α (analyzed alone). Overall, EF-1α recovered more nodes with high bootstrap support than did either of the other two data sets and yet included fewer parsimony-informative sites than did the COI data set.

We performed an ML analysis on the combined data set using the GTR+SSR model and equal weights parsimony trees as starting trees for branch swapping. We obtained the same tree topology (but different branch lengths) whether we included or excluded COI nt3 (Fig. 4). This tree differs little from the consensus of most-parsimonious trees obtained by equal weights parsimony but provides slightly greater resolution. Bayesian analysis using the same model yielded the same tree topology. We used the Bayesian posterior probabilities as estimates of branch support (shown on Fig. 5). Most branches show posterior probabilities close to or equal to 100. Overall, our results were highly stable to alternative methods of analysis (whether we used parsimony, ML, or Bayesian analyses) and whether we included or excluded COI nt3.

We used the ML/Bayesian tree to infer the likely history of social behavior within the genus *Lasioglossum*, but our conclusions would have been the same if we had used the parsimony tree topologies. Mapping social behavior onto the tree yielded a single origin of eusociality and multiple reversals from eusociality to solitary nesting, social polymorphism, or social parasitism (Fig. 5). We did not detect any secondary acquisitions of eusociality within the secondarily solitary clades. Overall, we detected nine transitions in social behavior on the tree.

Using 1,000 random tree topologies for these 53 species/populations, we estimated that on average 16.18 ± 1.57 ($n = 1{,}000$) transitions in social behavior would be expected (Fig. 6). Our observed number (nine) is significantly less ($P < 0.0001$) than the null distribution obtained from 1,000 random trees (it lies outside of the distribution of steps for the 1,000 trees; Fig. 6). This result supports the view that sociality is correlated with the phylogeny, either because sociality is a heritable trait with a significant historical component or because closely related taxa inhabit similar environments that shape their social behavior in similar ways.

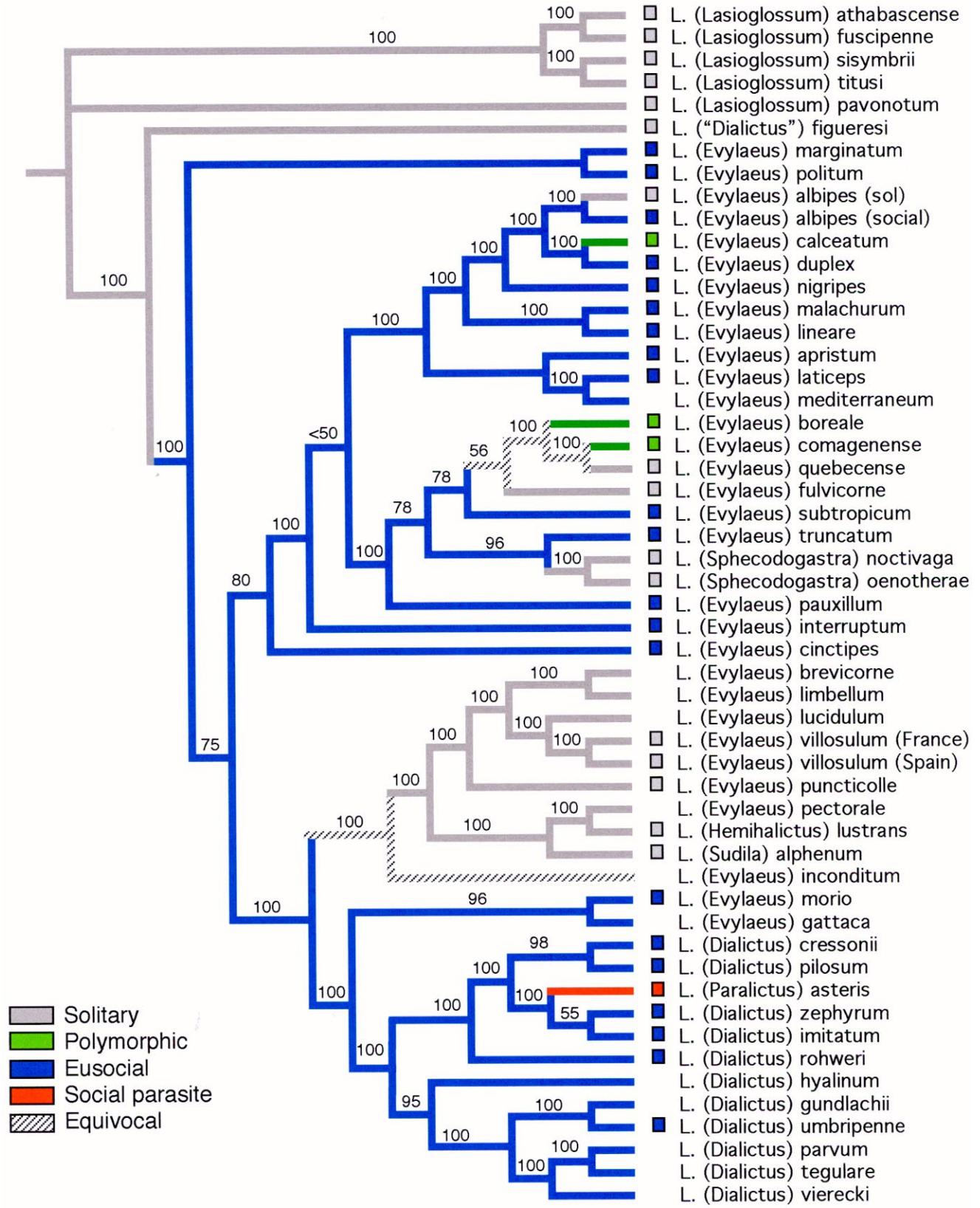

FIGURE 5. Mapping of social characters onto the ML tree topology (Fig. 4). Data on social behavior were obtained from review papers and the primary literature. Taxa for which social behavior is not known were coded as missing (no box). Characters were mapped using MacClade 3.07. Values on the branches indicate Bayesian posterior probabilities.

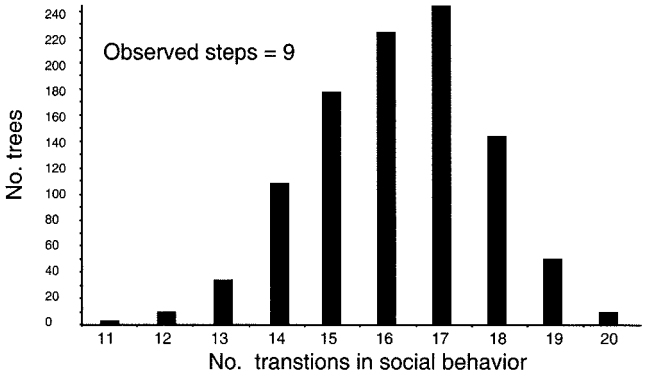

FIGURE 6. Distribution of the number of transitions in social behavior based on reconstructions for 1,000 random tree topologies for the same taxa included in Figure 5. The observed number of transitions in social behavior is 9, whereas the number of steps on the 1,000 random trees ranges from 11 to 20.

DISCUSSION

Phylogeny of Lasioglossum

Our results provide the best estimate of the phylogenetic relationships among the subgenera and species groups within the predominantly eusocial lineage of *Lasioglossum*. No previous phylogenetic study (e.g., Packer, 1991) has included as many species, species groups, and subgenera. Nevertheless, we are far from a complete understanding of *Lasioglossum* phylogeny because we were unable to include several smaller subgenera (e.g., *Acathalictus, Austrevyleus*, and *Sellalictus*, none of which have been assayed for social behavior) and we did not include all the species groups of *Evylaeus* (Ebmer, 1987, 1995, 1997). Nevertheless, *Evylaeus* is paraphyletic with respect to several other currently recognized subgenera, including *Dialictus, Paralictus* (treated as a part of *Dialictus* by Michener, 2000), *Sphecodogastra, Hemihalictus*, and *Sudila*.

Phylogenetic Utility of Nuclear versus Mitochondrial Genes

Our comparison of substitution patterns among genes (Fig. 3) and among data partitions within genes reveals some striking differences that may be related to phylogenetic utility. The mitochondrial gene (COI) showed several undesirable attributes for phylogenetic analysis, including a highly skewed nt3 base composition and substantial heterogeneity in base composition among taxa (Table 5), asymmetry in the transformation rate matrix (Fig. 3), and an extraordinarily high rate of nt3 substitution (mirrored by a low CI when the data partition was analyzed alone; Table 4). In spite of this bias, the COI data clearly contributed to the overall resolution of the tree. The COI data set is not incongruent with the other data sets and, based on a 50% bootstrap consensus, resolved approximately half of the nodes recovered in the total evidence analysis. Based on a comparison of three mitochondrial and three nuclear genes in stalk-eyed flies, Baker et al. (2001), concluded that mitochondrial genes performed less well than nuclear genes overall, an observation congruent with our results for *Lasioglossum*.

EF-1α appears to be the most effective of the three genes at recovering relationships among species of *Lasioglossum*. It recovered more nodes than the other two genes in a 50% bootstrap consensus tree, had a symmetrical transformation rate matrix (Fig. 3), and had unbiased base composition (Table 5). EF-1α has been used widely in insect molecular systematics studies (reviewed by Caterino et al., 2000), apparently with good success. Caution should be exercised, however, because the gene occurs in two copies in several insect orders, including Diptera (Hovemann et al., 1988), Hymenoptera (Walldorf and Hovemann, 1990; Danforth and Ji, 1998), and Coleoptera (Kelly Miller, pers. comm.).

Opsin appears to be a promising gene for phylogenetic analysis at the level used here. The gene had unbiased based composition (Table 5), a symmetrical transformation rate matrix (Fig. 3), and intermediate rates of nucleotide substitution (Table 4). Unlike EF-1α, which had almost no nt1 and nt2 variation, opsin had substantial nt1 and nt2 variation, suggesting that it should be less prone to saturation at deeper levels. Rates of intron and nt3 substitution are comparable to those of EF-1α. Furthermore, opsin recovered roughly as many nodes in a 50% bootstrap consensus as did COI, in spite of the fact that the opsin data set is approximately half the size of the COI data set and includes one quarter of the parsimony-informative sites. Opsin was of questionable utility at higher (tribal, subfamilial, and familial) levels in bees (Ascher et al., 2001) and strongly supported incorrect nodes in an analysis of vertebrate higher level phylogeny (Chang and Campbell, 2000).

Social Evolution in Lasioglossum

Our results with regard to social evolution are clear and unambiguous. We infer a single origin of eusociality and several reversals from eusociality to solitary nesting, suggesting that losses of eusociality are far more common than origins. This pattern is congruent with previous results obtained for *Lasioglossum* based on a data set for COI + EF-1α (Danforth, 2002), for a closely related (predominantly eusocial) genus, *Halictus* (Danforth et al., 1999), and for distantly related allodapine bees (Reyes et al., 1999). Danforth et al. (1999) hypothesized a single origin of eusociality in the common ancestor of *Halictus* and up to six independent transitions from eusociality to solitary nesting or social polymorphism. Likewise, Reyes et al. (1999), based on a combined analysis of morphology and molecular data, inferred that eusociality was the plesiomorphic state for the Australian allodapine genus *Exoneurella* and that two derived members (*E. eremophila* and *E. setosa*) had secondarily evolved solitary life histories. Wcislo and Danforth (1997) reviewed other cases in which transitions from eusociality to solitary nesting were documented.

Furthermore, the observed pattern of social evolution in *Lasioglossum* deviates significantly from a random distribution of social states on the tree. We infer

from this finding that sociality in the genus has a historical/phylogenetic component, in spite of the fact that there is substantial evidence of environmental effects on sociality in halictine bees (Crespi, 1996; Wcislo, 1997a). Soucy and Danforth (2002) provided a similar analysis of phylogeographic relationships among populations of a socially polymorphic halictine bee, *Halictus rubicundus*. Their results support those we obtained here. Social behavior among populations of *H. rubicundus* was constrained by phylogenetic affinities among populations, as determined by an analysis of over 2,000 bp of COI and COII sequence (Soucy and Danforth, 2002). Likewise, Plateaux-Quénu et al. (2000) recently conducted a "common garden" experiment in which they tested the hypothesis that social behavior in *L.* (*Evylaeus*) *albipes* has a genetic component. Two social forms of this species are known. One form is typically solitary and occurs in eastern France, and the other form is typically eusocial and occurs in western France. Foundresses from both forms, when reared under similar conditions, expressed the social behavior typical of their population of origin. The authors, however, could not rule out the possibility that the two "forms" represent two cryptic species with different social behaviors.

Examination of the cladogram in Figure 5 in detail reveals some interesting patterns. First, the basal branch of the *Hemihalictus* series (given the taxa that we have included) is *L.* ("*Dialictus*") *figueresi*, a solitary Neotropical species with an extraordinarily long development time (from egg to pupa; Wcislo et al., 1993; Wcislo, 1997c). The life history of *L.* ("*D.*") *figueresi* has features found in temperate eusocial species. For example, following cell provisioning, females enter an inactive period during which they no longer leave the nest, similar to the inactive period toward the end of the foundress phase of colony development. However, unlike a eusocial halictine bee, the foundresses die within their nests prior to the emergence of the first brood, precluding any interactions between foundresses and their offspring (Wcislo et al., 1993). This species, which was originally described as a *Dialictus*, is morphologically apomorphic and clearly not a member of *Dialictus* based on our data.

Second, among the species of *Evylaeu*s, there is one, *L.* (*Evylaeus*) *marginatum*, that has a particularly unusual form of sociality (Plateaux-Quénu, 1959, 1960, 1962, 1972; Michener, 1974; M. Richards, pers. comm.). This species is the only halictine bee with perennial colonies. Colonies are started by a single foundress and build up over 5–6 years until the worker population exceeds 400 individuals. In the final (5th or 6th) year, colonies produce male and female offspring. Mating takes place within the nests, which are opened in September, and females enter diapause as fertilized gynes who become new foundresses the next year. In spite of the huge colony size and apparently obligate workerlike behavior, this species exhibits little queen/worker dimorphism. Richards (pers. comm.), who has studied this species in Greece, considers *L.* (*E.*) *marginatum* to be an advanced eusocial halictine bee because workers and queens appear to be distinct behavioral castes. According to our results, this species is relatively basal within the tree as sister to *L.* (*Evylaeus*) *politum*, another eusocial species. Both *L.* ("*Dialictus*") *figueresi* and *L.* (*E.*) *marginatum* have extraordinarily long larval development times (2 months from egg to adult in *L.* (*E.*) *marginatum*).

Our results indicate that eusociality in halictine bees is relatively difficult to evolve but is easily lost. This makes sense in light of the life histories of solitary and eusocial halictid bees. The evolution of eusociality from solitary behavior requires the addition of several novel behavioral attributes. Putative queens must evolve the ability to vary the sex ratio over the course of the season (producing female-biased worker broods and male-biased reproductive broods). Queens must also vary their daughters' body sizes over the season (possibly through the amount of pollen provisioned per cell), because workers are generally smaller than foundresses. Queens also must evolve behaviors that enforce workerlike behavior in their first brood daughters, such as "backing" and "nudging" observed in artificial nests of *L.* (*Dialictus*) *zephyrum* (Michener, 1990). At the same time, eusocial species retain all the attributes of a solitary nesting halictid bee. Nest founding, burrow excavation, cell construction, foraging for pollen and nectar, and nest guarding are all behaviors retained (and performed) by foundress females in eusocial species. Solitary nesting could be viewed as a bet-hedging strategy in socially polymorphic species such as *H. rubicundus* (Eickwort et al., 1996; Soucy, 2001) and *L.* (*E.*) *calceatum* (Sakagami and Munakata, 1972). Reversions to solitary nesting may be facultative in many (but probably not all) eusocial halictid bees, and this behavioral flexibility may be what explains the repeated losses of eusociality observed in *Lasioglossum*.

Some groups of secondarily solitary species are almost certainly obligately solitary. Such groups include the subgenera *Sphecodogastra* and *Hemihalictus*. Both subgenera include oligolectic (floral specialist) species. The eight species within the subgenus *Sphecodogastra* are oligolectic on plants in the genus *Oenothera* (Onagraceae; McGinley, in press), and species are matinal (Knerer and MacKay, 1969), crepuscular, or even nocturnal (during the full moon; Kerfoot, 1967a, 1967b). The monotypic subgenus *Hemihalictus* (incuding *L.* (*Hemihalictus*) *lustrans*) is oligolectic on *Aplopappus* (Compositae; Daly, 1961). Oligolecty is generally presumed to preclude the development of eusocial colonies because the brief period of pollen availability makes it impossible to rear more than one brood of offspring.

The pattern of social evolution observed in *Lasioglossum* may shed some light on the earliest stages of social evolution in the advanced eusocial lineages of insects, such as ants, termites, and corbiculate bees. For these lineages, it is impossible to reconstruct the earliest stages of social evolution because eusociality arose so long ago (in the Cretaceous for all three; Emerson, 1968; Michener and Grimaldi, 1988a, 1988b; Grimaldi and Agosti, 2000) and because closely related solitary species are no longer extant. The earliest stages of social evolution in these advanced eusocial lineages may have resembled the pattern observed for *Lasioglossum*. Eusociality may have had few

origins and may have been be characterized by multiple reversals back to solitary living (or possibly the retention of substantial social flexibility). Such secondarily solitary species may have gone extinct, leaving what appears to be an exclusively eusocial clade of advanced eusocial insects.

ACKNOWLEDGMENTS

We are grateful to the following collaborators for providing specimens for this and future studies of halictid relationships: John Ascher, Manfred Ayasse, Terry Griswold, Penelope F. Kukuk, Yasuo Maeta, Robert Minckley, Ryouichi Miyanaga, John L. Neff, Beth Norden, Alain Pauly, Robert Paxton, Tai Roulston, Jerome G. Rozen, Jr., Roy R. Snelling, and Douglas Yanega. We are particularly grateful to Laurence Packer, Cecile Plateaux-Quénu, Kenneth Walker, William T. Wcislo, and Andreas W. Ebmer for providing crucial taxa and help with identifications. Belinda Chang provided suggestions on using ML to investigate substitution patterns among genes and data set quality. The following people commented on early drafts of this report: Sheryl Soucy, Sedonia Sipes, Cecile Plateaux-Quénu, Miriam Richards, John Ascher, Chung-Ping Lin, William T. Wcislo, and one anonymous reviewer. Improvements were made to the manuscript as a result of comments provided by the associate editor (Ted Schultz) and editor (Chris Simon). This project was supported by National Science Foundation Research Grants in Systematic Biology (DEB-9508647, DEB-9815236), and travel funds were provided by the Cornell International Agriculture Program and the National Geographic Society (grant 6946-01).

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First submitted 13 December 2001; reviews returned 8 May 2002; final acceptance 22 September 2002
Associate Editor: Ted Schultz