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Phylogeny of the Bee Genus *Halictus* (Hymenoptera: Halictidae) Based on Parsimony and Likelihood Analyses of Nuclear EF-1α Sequence Data

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We investigated higher-level phylogenetic relationships within the genus *Halictus* based on parsimony and maximum likelihood (ML) analysis of elongation factor-1α DNA sequence data. Our data set includes 41 OTUs representing 35 species of halictine bees from a diverse sample of outgroup genera and from the three widely recognized subgenera of *Halictus* (*Halictus* s.s., *Seladonia*, and *Vestitohalictus*). We analyzed 1513 total aligned nucleotide sites spanning three exons and two introns. Equal-weights parsimony analysis of the overall data set yielded 144 equally parsimonious trees. Major conclusions supported in this analysis (and in all subsequent analyses) included the following: (1) *Thrincohalictus* is the sister group to *Halictus* s.l., (2) *Halictus* s.l. is monophyletic, (3) *Vestitohalictus* renders *Seladonia* paraphyletic but together *Seladonia* + *Vestitohalictus* is monophyletic, (4) Michener's Groups 1 and 3 are monophyletic, and (5) Michener's Group 1 renders Group 2 paraphyletic. In order to resolve basal relationships within *Halictus* we applied various weighting schemes under parsimony (successive approximations character weighting and implied weights) and employed ML under 17 models of sequence evolution. Weighted parsimony yielded conflicting results but, in general, supported the hypothesis that *Seladonia* + *Vestitohalictus* is sister to Michener's Group 3 and renders *Halictus* s.s. paraphyletic. ML analyses using the GTR model with site-specific rates supported an alternative hypothesis: *Seladonia* + *Vestitohalictus* is sister to *Halictus* s.s. We mapped social behavior onto trees obtained under ML and parsimony in order to reconstruct the likely historical pattern of social evolution. Our results are unambiguous: the ancestral state for the genus *Halictus* is eusociality. Reversal to solitary behavior has occurred at least four times among the species included in our analysis.

INTRODUCTION

Halictid bees (Halictidae) are model organisms for studying the evolution of social behavior in insects. Of all the bees, this family includes the greatest number of eusocial species and shows the greatest intra- and interspecific variability in social behavior (Michener, 1974). Within the halictid subfamily Halictinae there are seven genera and subgenera that include all or some eusocial species: *Halictus* (*Halictus*), *Halictus* (*Seladonia*), *Lasioglossum* (*Evylaeus*), *Lasioglossum* (*Dialictus*), *Augochlora*, *Augochlorella*, and *Pereirapis;* and in most cases, even closely related species show widely differing levels of social organization (Michener, 1990a; Packer, 1993; Wcislo, 1997; and Yanega, 1997 all provide reviews of halictid social diversity). However, despite their importance, research on halictid sociality has been hindered by our poor understanding of phylogenetic relationships at the generic and species levels.

Phylogenetic analyses of social organisms have made important contributions to our understanding of social evolution. Within social insects, phylogenetic approaches have been used to analyze the origins of eusociality in the vespid wasps (Carpenter, 1989, 1991), carinate *Lasioglossum* (subgenus *Evylaeus*) (Packer, 1991), *Halictus* (Richards, 1994), Augochlorini (Danforth and Eickwort, 1997), and corbiculate bees (formerly the Apidae; cf. Roig-Alsina and Michener, 1993) (Michener, 1990b; Cameron, 1991, 1993; Prentice, 1991; Sheppard and McPheron, 1991; Chavarria and Carpenter, 1994; Engel and Schultz, 1997). Recent phylogenetic evidence from bees indicates that evolutionary reversals from eusociality to solitary nesting may occur more frequently than independent origins of eusociality (Wcislo and Danforth, 1997).

The genus *Halictus* was described by Latreille in 1804 and includes over 300 species of small to large, primarily eusocial bees. We chose to study this genus for several reasons. First, there is no previous phyloge-

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netic analysis of the higher-level relationships within the genus (Richards (1994) included only two of the three widely recognized subgenera, and Pesenko (1984) provided a morphological analysis of only one of the three subgenera). Like many genera of halictine bees, morphological variation within *Halictus* is limited and traits are often continuously distributed. With few discrete morphological characters for phylogeny reconstruction, higher-level relationships remain obscure. Second, *Halictus* presents some interesting biogeographic patterns that could be interpreted in light of a phylogeny. *Halictus* is found in all continents of the world except Australia (Moure and Hurd, 1987) but is most diverse and speciose in the Palearctic region (Michener, 1978b). Members of two of the three subgenera apparently colonized the New World via the Bering land bridge during the Pleistocene (Michener, 1979). Finally, within this genus are solitary species (e.g., *Halictus* (*Halictus*) *quadricinctus* (Sakagami, 1974; Knerer, 1980)), socially polymorphic species (e.g., *Halictus* (*H.*) *rubicundus* (Bonelli, 1967; Knerer, 1980; Yanega, 1988, 1989, 1990, 1992, 1993)), and eusocial species (e.g., *Halictus* (*H.*) *farinosus* (Eickwort, 1985)). By mapping social behavior onto a well-resolved phylogeny for *Halictus*, we can infer the most likely scenario for social evolution.

For this study we chose a nuclear protein-coding gene, elongation factor-1α (EF-1α). This gene encodes an enzyme involved in the GTP-dependent binding of charged tRNAs to the acceptor site of the ribosome during translation (Maroni, 1993). Recent work by Jerome Regier and colleagues (Friedlander *et al.*, 1992, 1994, 1997) has shown that EF-1α (as well as other nuclear protein-coding genes) can provide an extremely good source of data for higher-level studies of insect phylogenetics. The advantages of nuclear protein-coding genes include their slow rate of substitution, unbiased nucleotide composition, and ease of sequence alignment (especially when introns are excluded). Previous cladistic analyses of EF-1α sequence data have found that this gene provides useful phylogenetic information across a wide range of divergence times. Regier and Shultz (1997) used EF-1α amino acid data to reconstruct arthropod relationships, and McHugh (1997) and Kojima (1998) found that EF-1α provided evidence of polychaete paraphyly. Within insects, EF-1α has been shown to recover higher-level relationships in the moth subfamily Heliothinae (Cho *et al.*, 1995) and the moth superfamily Noctuoidea (Mitchell *et al.*, 1997), and Belshaw and Quicke (1997) used EF-1α to reconstruct relationships within the braconid subfamily Aphidiinae.

We investigated the utility of EF-1α for resolving relationships within and among genera of halictine bees. While only the protein-coding regions (exons) are generally used for higher-level studies (e.g., Cho *et al.*, 1995; Mitchell *et al.*, 1997), we reasoned that for more closely related taxa introns might provide sufficient variation to resolve species-level relationships whereas exons could provide resolution at deeper levels. Indeed, in initial analyses of EF-1α sequence variation among halictine genera this appears to be the case.

Danforth and Ji (1998) showed that EF-1α occurs as two paralogous copies (F1 and F2) in Hymenoptera, as in Diptera (Hovemann *et al.*, 1988). In our analysis, we sequenced most of the F2 copy, which is roughly 1600 bp in length and contains three introns (Danforth and Ji, 1998).

MATERIALS AND METHODS

Bees for this study were collected by the authors in Europe and North America. Specimens used for sequencing were primarily preserved in 95% EtOH but recently collected pinned specimens and frozen specimens were also used. Pinned specimens older than 3–5 years were not suitable for DNA extractions but those collected more recently provided good quality, high-molecular-weight DNA for PCR. Outgroup and ingroup taxa included in this study, locality data, specimen voucher numbers, and GenBank accession numbers are listed in Table 1. Voucher specimens are housed in the Cornell University Insect Collection.

DNA extractions followed standard protocols detailed in Danforth (1999). Two sets of PCR products were used to generate the data set (Table 2). Initially, primers were designed based on a comparison of published *Drosophila* (Hovemann *et al.*, 1988), *Apis* (Walldorf and Hovemann, 1990), and moth (Cho *et al.*, 1995) sequences. Primers that initially amplified at least some halictid species included For1-deg, For3, and Cho10 (Table 2). Based on initial comparisons of the F1 and F2 copies of EF-1α in halictid bees, we developed a new, F2-specific, reverse primer (F2-Rev1). For the downstream (3′) end of EF-1α we used primers For3/Cho10 (Table 2). These primers amplify both EF-1α copies; however, the presence of a roughly 200- to 250-bp intron in the F2 copy allows these PCR products to be separated on low-melting-point agarose gels. Only the F2 copy was included in the present analysis.

PCR amplifications were carried out following standard protocols (Palumbi, 1996), with the following cycle conditions: 94°C, 1 min denaturation; 50–56°C, 1 min annealing; 72°C, 1 min to 1 min 20 s extension. Prior to sequencing, PCR products were either gel-purified in low-melting-point agarose gels (FMC, Rockland, ME) overnight at 4°C or purified directly using the Promega (Madison, WI) Wizard PCR Preps DNA Purification kit. PCR products purified in this way provided consistently good sequencing results.

For manual sequencing we used ^{33}P-labeled dideoxy chain termination reactions (Thermo Sequenase radiolabeled terminator cycle sequencing kit; Amersham

TABLE 1

Ingroup and Outgroup Taxa Included in This Study

Voucher code	Species	Locality	Classification: Michener (1978)	Classification: Ebmer (1987)	Classification: Pesenko (1984)	GenBank Accession Nos.
Ingroups						
Subgenus *Halictus*						
Hacr361	*H. crenicornis* Blüthgen, 1923	Carataunas, Spain	Group 1		Monilapis	AF140296
Hasi86	*H. simplex* Blüthgen, 1923	Dordogne, France	Group 1		Monilapis	AF140297
Hafu363	*H. fulvipes* (Klug, 1817)	Trevelez, Spain	Group 2		Hexataenites	AF140298
Hali62	*H. ligatus* Say, 1837	Ithaca, NY, USA	Group 2		Odontalictus	AF140299
Hali-c	*H. ligatus* Say, 1837	Rock Hill, SC, USA	Group 2		Odontalictus	AF140300
Hamc408	*H. maculatus* Smith, 1848	Nancy, France	Group 2		Tytthalictus	AF140301
Hapo1	*H. poeyi* Lepeletier, 1841	Marathon, FL, USA	Group 2		Odontalictus	AF140302
Hapo-d	*H. poeyi* Lepeletier, 1841	Rock Hill, SC, USA	Group 2		Odontalictus	AF140303
Hapo435	*H. poeyi* Lepeletier, 1841	Hoke Co., NC, USA	Group 2		Odontalictus	AF140327
Haps430	*H. pseudomaculatus* Blüthgen, 1936	Mount Hermon, Israel	Group 2		Tytthalictus	AF140328
Hasc87	*H. scabiosae* (Rossi, 1790)	Dordogne, France	Group 2		Hexataenites	AF140329
Hasc365	*H. scabiosae* (Rossi, 1790)	Carataunas, Spain	Group 2		Hexataenites	AF140330
Hasx83	*H. sexcinctus* (Fabricius, 1775)	Mont Serein, France	Group 2		Hexataenites	AF140331
Hafa25	*H. farinosus* Smith, 1853	Locality unknown	Group 3		Nealictus	AF140332
Hapa438	*H. parallelus* Say, 1837	Chatham Co., NC, USA	Group 3		Nealictus	AF140333
Haqu89	*H. quadricinctus* (Fabricius, 1776)	Dordogne, France	Group 3		Halictus	AF140334
Haru32	*H. rubicundus* (Christ, 1791)	Missoula, MT, USA	Group 3		Protohalictus	AF140335
Haru431	*H. rubicundus* (Christ, 1791)	Moore Co., NC, USA	Group 3		Protohalictus	AF140336
Subgenus *Seladonia*						
Haco301	*H. confusus* Smith, 1853	Junius Ponds, NY, USA		*tumulorum*		AF140304
Hage366	*H. gemmeus* Dours, 1872	Laujar de Andarax, Spain		*gemmeus*		AF140305
Hake239	*H. kessleri* Bramson, 1879	Vienna, Austria		*kessleri*		AF140306
Hasm376	*H. smaragdulus* Vachal, 1895	Carataunas, Spain		*seladonius*		AF140307
Hasb95	*H. subauratus* (Rossi, 1792)	Les Eyzies, France		*seladonius*		AF140308
Hasb374	*H. subauratus* (Rossi, 1792)	Trevelez, Spain		*seladonius*		AF140309
Hatr93	*H. tripartitus* Cockerell, 1895	Chiricahua Mtns., AZ, USA		Not treated		AF140310
Hatu91	*H. tumulorum* (Linnaeus, 1758)	Paris, France		*tumulorum*		AF140311
Subgenus *Vestitohalictus*						
Hapl369	*H. pollinosus* Sichel, 1860	Trevelez, Spain				AF140312
Havs373	*H. vestitus* Lepeletier, 1841	Trevelez, Spain				AF140313
Outgroups						
Aupu333	*Augochlora pura* (Say, 1837)	Ithaca, NY, USA				AF140314
Aume334	*Augochloropsis metallica* (Fabricius, 1793)	Ithaca, NY, USA				AF140315
Mgge247	*Megalopta genalis* Meade-Waldo, 1916	Republic of Panamá				AF140316
Ncdi249	*Neocorynura discolor* (Smith, 1879)	Colombia				AF140317
Agko12	*Agapostemon kohliellus* (Vachal, 1903)	Dominican Republic				AF140318
Agse162	*Agapostemon sericeus* (Forster, 1771)	Ithaca vicinity, NY, USA				AF140319
Agty230	*Agapostemon tyleri* Cockerell, 1917	Portal, AZ, USA				AF140320
Agvi161	*Agapostemon virescens* (Fabricius, 1775)	Ithaca vicinity, NY, USA				AF140321
Mxaz97	*Mexalictus arizonensis* Eickwort, 1978	Miller's Canyon, AZ, USA				AF140322
Psbr347	*Pseudagapostemon brasiliensis* Cure, 1989	Brazil				AF140323
Spmi21	*Sphecodes minor* Robertson, 1898	Nova Scotia, Canada				AF140324
Spra337	*Sphecodes ranunculi* Robertson, 1897	Ithaca, NY, USA				AF140325
Thpr434	*Thrincohalictus prognathus* (Perez, 1912)	Golan Heights, Israel				AF140326

Inc., Cleveland, OH) and standard 8% polyacrylamide gel electrophoresis, as indicated in the Amersham product manual.

Automated sequencing of PCR products was performed on an ABI 377 automated sequencer available through the Cornell Automated Sequencing Facility. Overall, we sequenced EF-1α F2 in 35 species, 5 of which were represented by more than one locality (giving a total of 41 OTUs). The region analyzed below corresponds with positions 196 to 1266 in the *coding* region of the insect EF-1α gene (Danforth and Ji, 1998); thus, our data set spans 77% of the 1386-bp coding region (Walldorf and Hovemann, 1990). As in the previous report (Danforth and Ji, 1997), we found two

TABLE 2

Primers Used in PCR Amplification and Sequencing

Primer name	Sequence	Position[a]
Forward primers		
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
Reverse primers		
Cho10	5′-AC RGC VAC KGT YTG HCK CAT GTC-3′	1887
F2-Rev1	5′-A ATC AGC AGC ACC TTT AGG TGG-3′	1600

[a] Positions based on the 5′ end of the primer in the honey bee, *Apis mellifera* (Walldorf and Hovemann, 1990).

introns within the region analyzed (at locations 753/754 and 1029/1030).

Taxon Sampling

Taxon sampling has been shown to be very important, and to greatly influence phylogenetic conclusions (Lecointre *et al.*, 1993; Graybeal, 1998; Hillis, 1998; Poe, 1998; Robinson *et al.*, 1998; Soltis *et al.*, 1998). We sampled 13 outgroup species within the subfamily Halictinae, representing nine genera and the two tribes (Augochlorini and Halictini) (Table 1). The five included genera from Halictini represent a good sample of the diversity within the strong-veined members of this tribe (Michener, 1978a,b).

In the present study we intended to test both the monophyly of the 3 subgenera of *Halictus* and their phylogenetic relationships. We thus included representatives of all 3 subgenera recognized by Blüthgen (1961; see Table 1), *Halictus* s.s., *Seladonia*, and *Vestitohalictus*. For the largest subgenus, *Halictus* s.s., we were able to include three of Michener's (1978b) four informal groups (the fourth being monotypic) and 7 of Pesenko's (1984) 12 subgenera.

Parsimony Analysis

Phylogenetic analyses of nucleotide and amino acid sequences were performed using a test version of PAUP*4 (PAUP v. 4.0d64; Swofford, 1998; see Swofford and Begle, 1993 for details on earlier versions of the program). For parsimony analyses we used heuristic search with TBR branch swapping, random addition sequence for taxa, and 50 replicates per search. We applied various weighting procedures, including successive-approximations character weighting (Farris, 1969; Carpenter, 1988) and implied weights (Goloboff, 1993). We also analyzed the data set in two different ways: (1) exons only and (2) introns + exons (the total data set). The reason for using the two different data sets was to evaluate how introns contribute to tree resolution. Bootstrap analysis (Felsenstein, 1985) and Bremer support (decay indices; Bremer, 1994) were used to evaluate branch support on parsimony trees. Bootstrap values were calculated based on 100 replicates with 10 random sequence additions per replicate.

Because our data set includes noncoding intron sequences, we observed insertion/deletion mutations in the two included introns. In preliminary analyses of the data set this indel variation appeared to contain phylogenetically informative characters, and so we attempted to code indels in a way that would give individual indel mutations (of whatever length) a weight equal to a single base substitution while at the same time retaining information on sequence variation within indels. Several indel coding methods have been proposed: (a) exclude gap regions, (b) code gaps as missing data, (c) code gaps as a fifth state, (d) exclude gap regions and code indels as new binary characters, and (e) keep gap regions, code gaps as missing data, and code indels as new binary characters. After criticizing the five common solutions listed above, we propose a new solution, which can be regarded as a mix of (c) and (e) (depending on the length of the gap).

Solutions (a) and (b) ignore indel variation completely. The problem with solution (c) is that when several unit gaps are contiguous each unit gap will result in one state change (one step), thus weighting what is likely to be a single indel event more than a single base change (*n* times as much, if *n* is the length of the total gap). Indels are taken into account in solution (d) with reasonable weight but variation among bases within the indel is ignored (see DeSalle and Brower, 1997). A sophisticated sixth method, very close to solution (d), has been proposed by Barriel (1994) and used by Bourgoin *et al.* (1997). It involves recoding regions containing gaps into a series of binary characters. Barriel's method is quite confusing and (in our opinion) more complicated than necessary.

Our method of gap coding differs slightly from all those outlined above. We propose that gaps be coded in the following way: when gap length (in all taxa in which it occurs) is one, code the gap as a fifth state; when gap length exceeds one, code it as missing data and add a new character to code the indel information. When gaps vary in length, the new character can be multistate, with each character state referring to a different gap length. Table 3 provides a simple example of our gap-coding method, as applied to a sample data set. Gaps coded in this way were treated as unordered in all our analyses but could potentially be ordered (if one wished to impose a "model" of indel mutation). Our method (and any method that incorporates missing data) could potentially suffer from a problem outlined by Maddison (1993) in which missing data lead to a spurious, long-distance interaction effect. However, we believe that this effect is unlikely to arise in most real data sets.

We applied the method described above to our alignment using MacClade (version 3.07; Maddison and Maddison, 1997) with the data matrix in standard

format (10 states, which we symbolized A, C, G, T, 0, 1, 2, . . .). For parsimony analysis we selected the default option for handling gaps (gaps are treated as missing data). By this method, we introduced a total of 16 new multistate characters: 6 in intron 1 and 10 in intron 2. Alignments for both the original and the recoded data sets are available from the senior author.

Maximum Likelihood Analysis

For the maximum likelihood (ML) analyses we initially used the equal-weights parsimony trees to estimate the log likelihood of each tree under 17 possible models of sequence evolution (Sullivan and Swofford, 1997; Frati *et al.*, 1997; Huelsenbeck and Crandall, 1997), from the simplest (Jukes–Cantor with equal base frequencies) to the most parameter-rich (GTR with empirical base frequencies, gamma distributed rates [with four rate categories], and six substitution types). We estimated the log likelihood of all models for two different data sets (1) exons only and (2) introns + exons (the total data set), as in the parsimony analysis.

Because the GTR model with empirical base frequencies and site-specific rates (GTR + SSR) had the highest log-likelihood (see Results), we selected this model for additional branch swapping. We performed an iterative analysis with a series of increasingly exhaustive branch-swapping algorithms in the following order: NNI, SPR(1), SPR(2), TBR(1), and TBR(2) (T. Schultz, pers. comm.). At each iteration the ML parameters were reestimated based on the trees currently in memory and applied to the next round of branch swapping. The parameter estimates resulting from this search algorithm are discussed below.

MacClade v. 3.07 (Maddison and Maddison, 1992, 1997) was used to map behavioral characters onto trees and to investigate alternative tree topologies.

RESULTS

Alignment

The 41 sequences were aligned using MegAlign in the Lasergene software package (DNASTAR Inc., Madison, WI) using the clustal alignment method and then adjusted by eye. The coding region of *Apis mellifera* (Walldorf and Hovemann, 1990) was included as a reference to determine the reading frame of the sequences and the limits of exons and introns. Our sequence consists of two introns and three exons.

Together, the three exons represent 1071 bp of aligned sequence with no insertion/deletion (indel) mutations observed. Intron 1 includes 270 aligned nucleotide sites (with 6 gap-coded characters), and intron 2 includes 228 total aligned nucleotides (with 10 gap-coded characters). The entire alignment includes 1513 total aligned nucleotide sites plus 16 characters representing gap-coded variation.

TABLE 3

Gap-Coding Methods Illustrated

Character	00000000011111
Number	12345678901234
Taxon 1	ATT----3AATGAT
Taxon 2	ATT----3CATGAT
Taxon 3	ATTAC--2CATG0T
Taxon 4	ATTGC--2CATGTT
Taxon 5	ATTGCA-1GATGTT
Taxon 6	ATTGCAA0GATGTT

Note. Character 8 is the gap-coding character that provides information on gap length. Character 13 represents a single base deletion (in Taxon 3) coded as "0." In both cases characters are treated as unordered. Dashes (-) indicate missing data.

TABLE 4

Base Composition of EF-1α Sequence Data

	A	C	G	T	*P* value[a]
Exon	26.5	25.2	24.1	24.2	0.999
nt1	28.6	18.1	38.5	14.8	1.0
nt2	30.2	26.3	16.0	27.5	1.0
nt3	20.6	31.2	17.9	30.3	0.049
Intron	28.5	16.7	20.2	34.6	0.999
Overall	27.0	22.8	23.0	27.2	0.914

[a] *P* values refer to the probability of rejecting the null hypothesis of homogeneity among taxa in base composition.

Base Composition

The overall base composition and the base composition broken down by character partition is shown in Table 4. Overall, the base composition was only slightly A/T biased. The A/T bias was most significant in introns, in which As and Ts accounted for over 63% of the nucleotides.

Phylogenetic Analysis

Unweighted parsimony analysis of nucleotides and amino acids. We initially analyzed the data set with and without introns using equal-weights parsimony. With introns excluded, we obtained 586 trees of length 586. The overall resolution was poor, presumably because only one-half of the total parsimony-informative sites occur in the exons (Table 5) despite the fact that exons include more than twice as many nucleotide positions. With the inclusion of the intron sequence, we obtained improved resolution. Figure 1a shows a consensus tree of the 144 total trees obtained with introns plus exons included. This tree topology recovers all nodes obtained in the analysis of exons only and provides considerably better resolution within certain groups. Overall, this tree is highly congruent with subgeneric limits and with Michener's groups (although Group 2 appears to be paraphyletic with respect to Group 1 in some resolutions). Interestingly, *Vestitohalictus* arises well within *Seladonia*, thus rendering *Seladonia* para-

TABLE 5

Composition of Introns and Exons

	Total	Const.	Parsimony uninformative	Parsimony informative
Exons	1071	798	59	214
nt1	357	331	7	19
nt2	357	340	11	6
nt3	357	127	41	189
Introns	442	188	76	178
Amino acids	357	325	16	16

phyletic, and bootstrap support for this group is high (99%). (This same pattern is obtained with all subsequent analyses and appears to be well supported by the data.) Additional aspects of the tree that are well supported include *Halictus* monophyly (bootstrap support = 99%) and sister group relationship between *Thrincohalictus* and *Halictus* (bootstrap support = 100%).

Whereas the equal-weights analysis recovers the main lineages within *Halictus* s.l., relationships *among* these lineages are not clear. For example, *Seladonia* + *Vestitohalictus* is placed as sister to *Halictus* s.s. in the majority (58%) of trees, while in others *Seladonia* + *Vestitohalictus* forms the sister group to Michener's Group 3, thus rendering *Halictus* s.s. paraphyletic.

When nucleotide sequences are translated to amino acid sequences, much of the parsimony-informative variation (third position and intron variation) is lost. Not surprisingly, the translated sequence yielded only 16 parsimony-informative sites with virtually no resolution within the ingroup (tree not shown). Tree statistics for all parsimony analyses are given in Table 6.

Successive approximations and implied weights parsimony. In order to obtain improved tree resolution and to apply weights that are determined by character congruence, we applied two different weighting methods: successive approximations (using the rescaled consistency index) and implied weights (with K = 2). These analyses support many conclusions obtained in the equal-weights analysis: *Thrincohalictus* is the sister group to *Halictus*, *Halictus* s.l. is monophyletic, Michener's three groups are distinct (but his Group 1 makes Group 2 paraphyletic), *Vestitohalictus* arises from within *Seladonia*, and *Seladonia* + *Vestitohalictus* are monophyletic. Overall, the weighted analyses gave greater resolution than the unweighted analyses and improved branch support. In three of the four alternative weighted analyses we obtained support for the sister group relationship between *Seladonia* + *Vestitohalictus* and Group 3 (although bootstrap support was only slightly greater than 50%; Fig. 1b), while in the fourth (implied weights on the full data set) we were again unable to resolve the basal relationships within *Halictus*. Overall, based on the two weighting methods used, we are unable to determine conclusively whether *Seladonia* + *Vestitohalictus* forms the sister group to *Halictus* s.s. or whether *Seladonia* + *Vestitohalictus* arises from within *Halictus* s.s., as sister to Michener's Group 3. It should be noted that this is ultimately a question of where to place the root of *Halictus* s.l.: (a) between *Seladonia* + *Vestitohalictus* and *Halictus* s.s. or (b) within *Halictus* s.s. (between Groups 1 + 2 and Group 3 + *Seladonia*).

Maximum likelihood analysis of nucleotides. We applied ML to our data for several reasons. First, since we are analyzing protein-coding sequences there is substantial rate heterogeneity among sites. For EF-1α the vast majority of parsimony-informative sites are in third positions (Table 5), and these show the greatest frequency of change on the parsimony trees. Second, with a data set consisting of both rapidly evolving introns and slowly evolving exons, we wanted to accomodate the rate heterogeneity between these two data partitions. Finally, there is evidence of transition/transversion bias. Depending on the model of sequence evolution selected, transitions occur at a rate 2.60 to 2.89 times that of transversions, indicating that character state transformations within positions are not all equal.

As expected, the log likelihoods increased with increasingly complex models of sequence evolution (Fig. 2). The model based on site-specific rate variation (GTR + SSR) had a considerably higher likelihood score than any other model for both the complete data set (introns + exons) and for the exon-only data set, and we selected this model as the most appropriate for the current data set.

Branch swapping led to only slight increases in likelihood (for the total data set branch swapping improved the likelihood score by 4 units: from −7903.95 to −7899.82), indicating that the parsimony trees come very close to the tree topologies estimated under ML. With introns excluded we obtained nine trees of equal likelihood and with introns included we obtained 3. Figure 3a shows the consensus trees of the three trees obtained under the GTR + SSR model. The trees obtained with and without introns are perfectly congruent within the ingroup; however, the consensus tree obtained when introns were excluded is slightly less well resolved. That ML obtains identical tree topologies whether introns are included or excluded is remarkable. Figure 3b shows one of the three trees obtained under the ML analysis with all positions included to illustrate branch length variation. Estimates of the relative rate of substitution indicated that third positions evolve at roughly the same rate as introns, and both introns and third positions evolve roughly an order of magnitude faster than either first or second positions: introns, 1.72; nt1, 0.15; nt2, 0.06; nt3, 1.90.

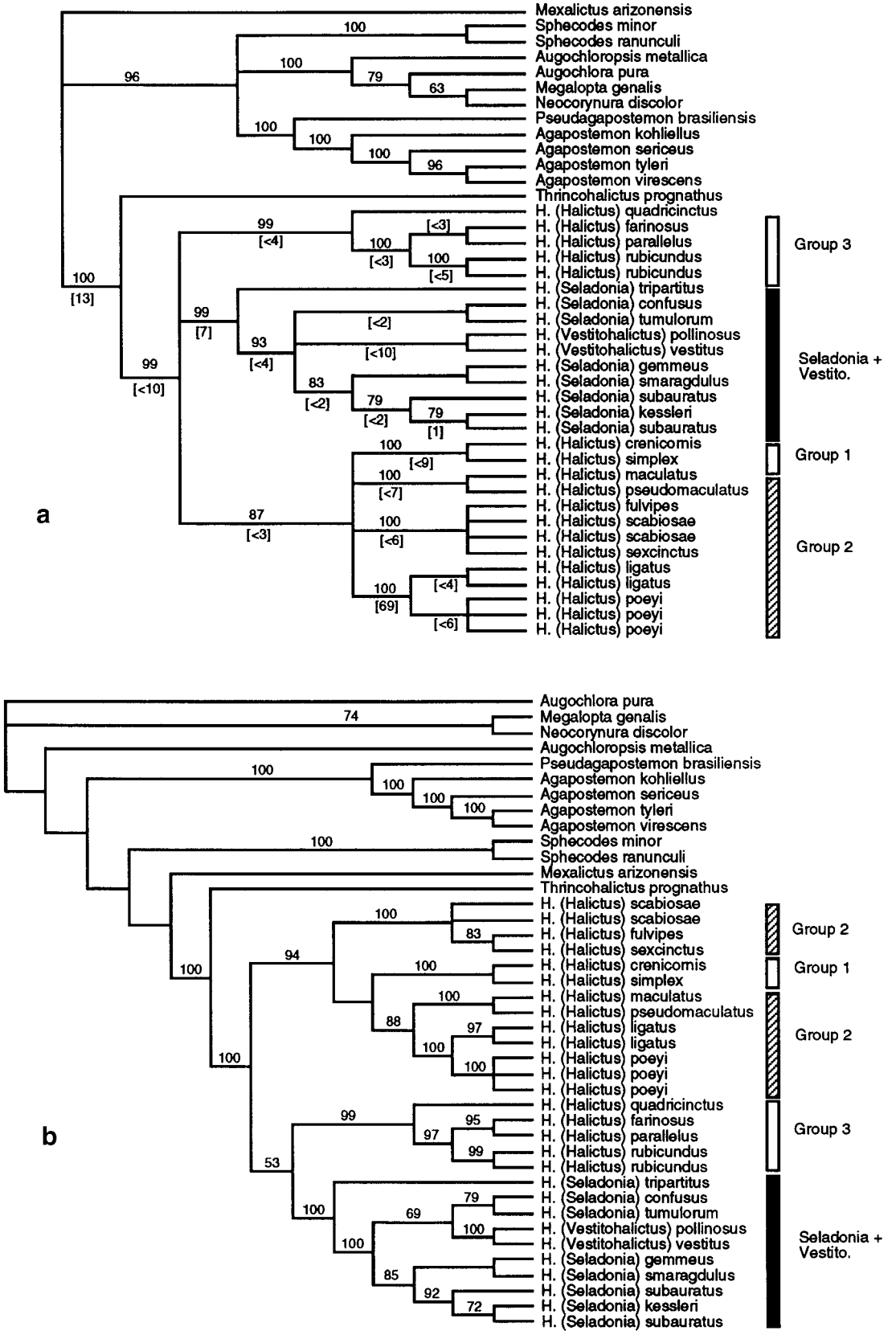

FIG. 1. Parsimony analysis of EF-1α data. (a) Consensus of 144 equally parsimonious trees obtained in an unweighted analysis of introns + exons. Length = 1186, CI = 0.5637, RI = 0.8061. (b) Consensus of 4 equally parsimonious trees obtained after successive-approximations character weighting. Length = 594.16, CI = 0.7484, RI = 0.9082. Numbers above branches indicate bootstrap support values and numbers below branches indicate decay indices.

DISCUSSION

Conclusions on the Phylogeny of Halictus

Overall, we prefer the tree shown in Fig. 3a because in using the GTR + SSR model we allow for transition/transversion differences as well as rate heterogeneity among sites and among coding and noncoding regions. The estimates of rate heterogeneity and the R matrix (representing the frequencies of transformations from one base to another) are estimated directly from the data beginning with equal-weights parsimony trees. We view ML as a method for simultaneously applying weights appropriate for DNA sequence data. The approach used here, in which model parameters are iteratively reestimated, can be viewed as a form of reciprocal illumination (sensu Hennig, 1966), since the parameters of the ML model are determined based on previous tree topologies (Swofford *et al.*, 1996). For the data set that we analyzed, the ML analysis appears to have made more efficient use of the nucleotide variation for resolving basal branches of the *Halictus* s.l. cladogram.

Comparison of Our Results with those of Previous Studies

Michener (1978b) and Pesenko (1984) provide alternative (although mutually congruent) higher-level classifications for the species of *Halictus* s.s. based on morphology. Michener (1978b) defined four groups within *Halictus* s.s., and Pesenko (1984) considered the 3 subgenera as different genera and proposed 12 subgenera for his genus *Halictus* (*Halictus* s.s. in our sense). Richards (1994) performed a cladistic analysis using allozyme data, and Packer (1997) performed a combined analysis of Pesenko's morphological data with Richards' allozyme data. Below, we discuss how our results compare to the classifications of Michener (1978b) and Pesenko (1984) for *Halictus* and Ebmer (1987) for *Seladonia* and to the higher-level phylogenetic results of Richards (1994) and Packer (1997).

TABLE 6

Tree Statistics for Parsimony Analyses

Method	CI	RI	No. steps	No. trees
Amino acids				
Equal weights	0.7353	0.8816	49	1
Exons only				
Equal weights	0.5114	0.7956	586	586
Successive approx.	0.7184	0.9044	262.05	12
Implied weights	0.5114	0.7956	586	6
Exons + introns				
Equal weights	0.5637	0.8061	1186	144
Successive approx.	0.7484	0.9082	594.16	4
Implied weights	0.5637	0.8061	1186	4

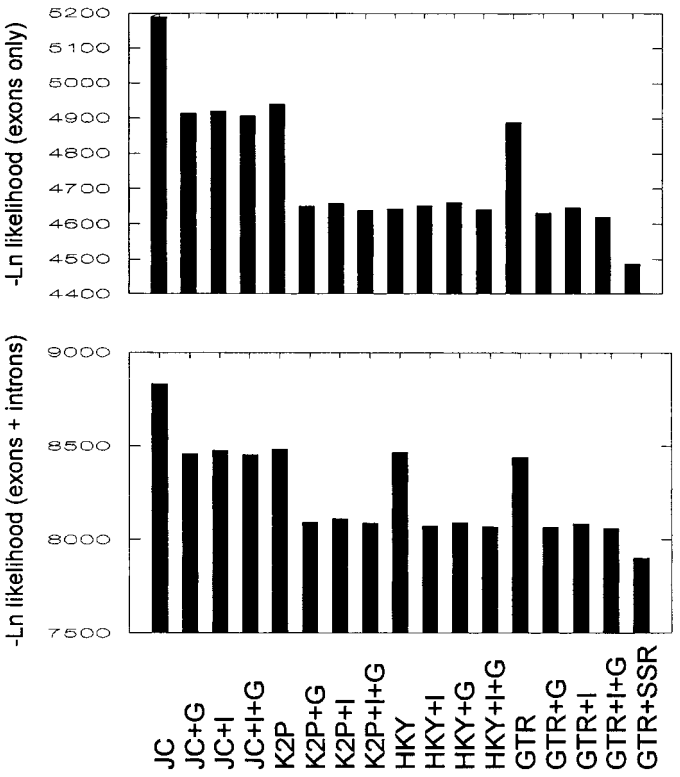

FIG. 2. −Ln likelihoods based on the equal-weights parsimony trees for 17 models of sequence evolution, with introns excluded (top) and included (bottom). Likelihoods improved slightly with branch swapping, as described under Materials and Methods. GTR + SSR refers to the GTR model with site-specific rates for introns, first, second, and third positions.

Congruence with Michener's and Pesenko's groupings. Our results are highly congruent with the classifications of *Halictus* s.s. proposed by Michener (1978b) and Pesenko (1984). We recovered all three of Michener's informal groups; however, we found evidence that Group 1 arises from within Group 2, thus rendering Group 2 paraphyletic. While Michener (1978b) recognized *Vestitohalictus* as a distinct subgenus of *Halictus* s.l., our trees provide convincing evidence that *Vestitohalictus* renders *Seladonia* paraphyletic. Interestingly, Michener stated that "it may well be that some species [of *Vestitohalictus*] intergrade with *Seladonia*" (Michener, 1978b, p. 530), indicating that the distinction between these two subgenera is weak. With regard to the relationship between *Seladonia* and *Halictus* s.s., Michener (1978b) also speculated that Group 3 shared a morphological character with *Seladonia*, the presence of a clump of specialized setae on the inner surface of the gonostylus (p. 533; illustrated in Figs. 88–99), although he did not state whether this was a synapomorphy or a symplesiomorphy. If the presence of these hairs is a derived state, this character would provide additional support for the placement of Group 3 and *Seladonia* + *Vestitohalictus* as sister groups (Fig.

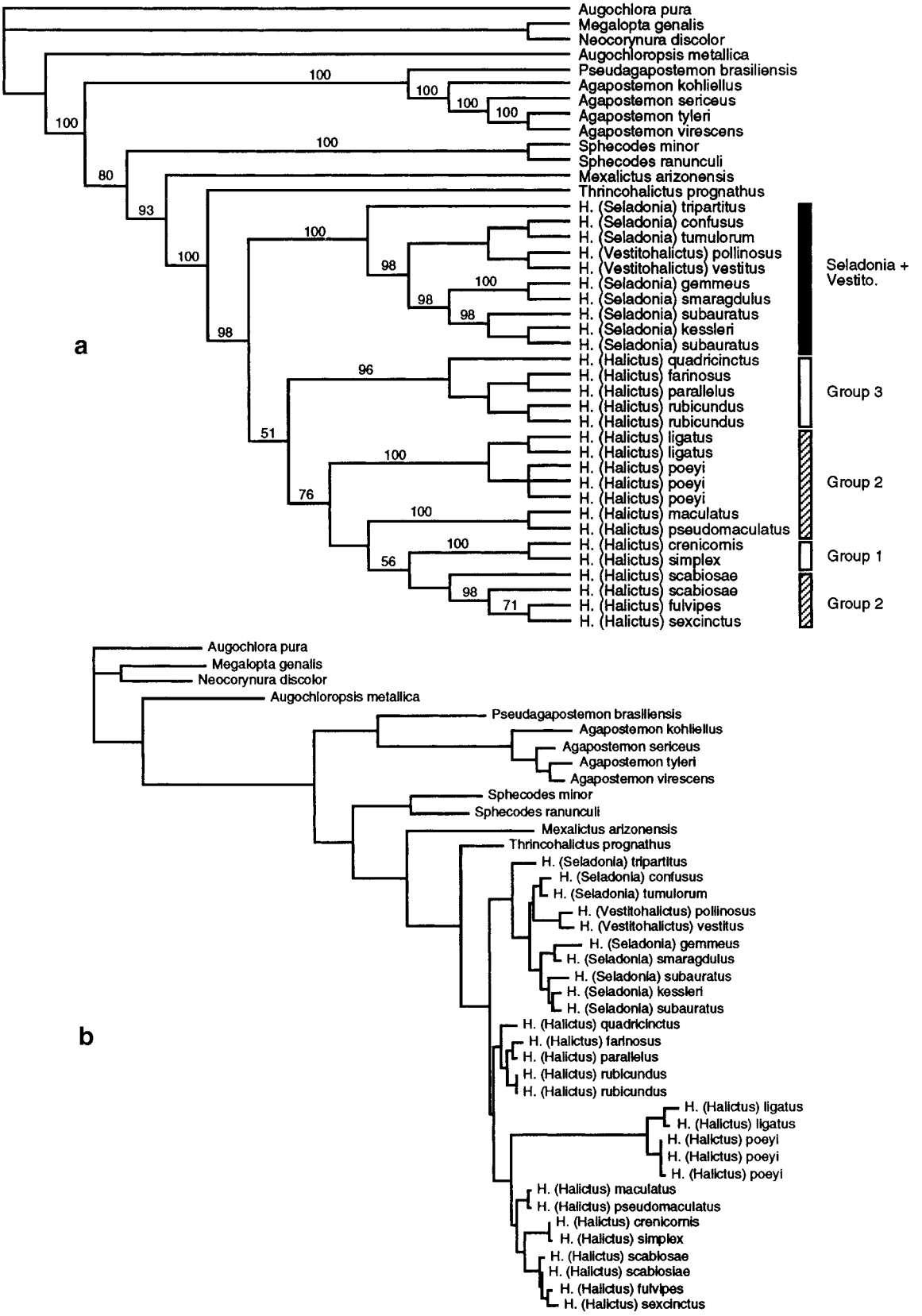

FIG. 3. Maximum likelihood analysis of EF-1α data. (a) Consensus of 3 trees obtained using the GTR + SSR model. −Ln likelihood = 7899.82. (b) One of three trees obtained using the GTR + SSR model. −Ln likelihood = 7899.82. Branch lengths are shown as proportional to character changes.

1b). However, since this character is also present in *Thrincohalictus* (Michener's Figs. 70 and 71) and the agapostemonine genera (Roberts and Brooks 1987), we interpret the *absence* of the tuft of hairs as the derived state, thus supporting the monophyly of [Group 1 + Group 2] (Fig. 3a).

For Pesenko's (1984) subgenera, at least those included in our analysis, our results suggest that all are monophyletic (with a test provided only for *Nealictus, Hexataenites, Tytthalictus,* and *Monilapis*). Our results are congruent with Pesenko's in that we recovered two main branches within *Halictus* s.s.: (1) a monophyletic group including *Nealictus, Protohalictus,* and *Halictus* (which together comprise Michener's Group 3) that is sister to (2) a monophyletic group including *Monilapis* (= Michener's Group 1), *Odontalictus, Tyttalictus,* and *Hexataenites* (which together comprise Michener's Group 2) (Fig. 52 in Pesenko, 1984).

Congruence with Ebmer's (1987) groupings within Seladonia. Ebmer (1987) recognized six species groups for European species of the subgenus *Seladonia.* We included representatives of four of these groups (species groups included were *seladonius, smaragdulus, gemmeus,* and *tumulorum*). While we found support for the monophyly of the *tumulorum* group (*H.* (*Seladonia*) *confusus* + *H.* (*Seladonia*) *tumulorum*) in all analyses, Ebmer's *seladonius* group (including *H.* (*Seladonia*) *subauratus* + *H.* (*Seladonia*) *smaragdulus*) appears paraphyletic with respect to both the *gemmeus* and the *kessleri* groups (Fig. 3b). *H.* (*Seladonia*) *tripartitus* was not included in Ebmer's classification, since this species occurs only in North America. Our results indicate that *Vestitohalictus* forms the sister group to Ebmer's *tumulorum* species group in both the parsimony and the ML analyses (Figs. 1b and 3a).

Comparison with Richards' allozyme results. Richards (1994) performed a phylogenetic analysis (by equal-weights parsimony) of 15 species of *Halictus* plus *Agapostemon virescens* (as an outgroup) using 17 variable allozyme loci. Her study included fewer ingroup and outgroup species than ours, fewer parsimony-informative characters, and representatives of only two of the three recognized subgenera (*Vestitohalictus* was not available). One of the strongest conclusions of her study was that *Seladonia* arises well within *Halictus* s.s. (as sister group to a paraphyletic Group 3; her Fig. 1a), thus rendering *Halictus* s.s. paraphyletic. We found this same pattern in some analyses (e.g., when successive-approximations character weighting is applied to the total data set, Fig. 1b) but this result was not supported by bootstrap values much greater than 50% and may simply be an artifact of the extremely short branch supporting *Halictus* s.s. monophyly (Fig. 3b). It is interesting to note that when we used only *Agapostemon virescens* to root our EF-1α trees we obtained a tree topology similar to that obtained by Richards (with *Seladonia* arising from within *Halictus* s.s.). This shows that the two data sets are reasonably congruent but that outgroup sampling has a profound effect on ingroup relationships.

Comparison with Packer's combined analysis of morphological and allozyme data. Packer (1997) presented a combined analysis of Pesenko's (1984) and Richards' (1994) data sets. For this analysis Packer coded the species of *Seladonia* included in Richards' data set for the morphological characters listed in Pesenko (1984) and rooted the tree with *Agapostemon virescens.* Packer obtained 12 equally parsimonious trees in the equal-weights parsimony analysis and a single tree after successive-approximations character weighting (his Fig. 4). Remarkably, this tree is fully congruent with our ML trees in placing *Seladonia* as sister to *Halictus* s.s. and Michener's Group 3 as a basal branch of *Halictus* s.s. and in Michener's Group 1 arising from within Group 2 (with Group 1 + 2 forming a monophyletic group) (as in Fig. 3a).

Biogeographic Considerations

Our results shed light on the biogeography of the genus *Halictus.* The vast majority of *Halictus* species are in the Old World. *Halictus* s.s. and *Seladonia* are Holarctic in distribution but both are most abundant and species rich in the Palearctic. Of the 250 or so species of *Halictus* s.s. and *Seladonia* only 12 are found in the Western Hemisphere: 7 in *Seladonia* and 5 in *Halictus* s.s. (Moure and Hurd, 1987; Carman and Packer, 1997). *Vestitohalictus* is found exclusively in the Palearctic (from the Canary Islands to China through the Mediterranean basin) and tends to be found in arid regions (Michener, 1979). Our phylogenetic results confirm an observation made by Michener (1979) that there appears to have been two independent invasions of *Halictus* s.s. into North America from the Old World. Older invasions (as judged by the topology in Fig. 3) involved members of Michener's Group 3: *Halictus* (*H.*) *farinosus, H.* (*H.*) *parallelus,* and *H.* (*H.*) *rubicundus* (which also occurs in the Old World). A more recent invasion occurred in the common ancestor of *Halictus* (*H.*) *ligatus* and *H.* (*H.*) *poeyi.* In both cases, these invasions presumably occurred via the Bering land bridge during the Pleistocene, when sea levels would have been low (Eickwort *et al.,* 1996). The climate at that time was essentially boreal, with short summers (Lozhkin *et al.,* 1993), although the land bridge itself was a steppe prairie that supported flowering plants (Matthews, 1980). Eickwort *et al.* (1996) hypothesized that populations of *H. rubicundus* that crossed the Bering land bridge would have been solitary and later reverted to eusociality at lower latitudes in North America.

The extremely long branch leading to *Halictus ligatus* + *H. poeyi* (Fig. 3b) suggests that dispersal from the Old World to the New World may have resulted in a

substantial founder effect, such that these two species are highly divergent from all other *Halictus* s.s. The species-level distinction between *H. ligatus* and *H. poeyi* has only recently been made based on allozyme markers (Carman and Packer, 1997) and mitochondrial DNA sequence data (Danforth *et al.*, 1998). *H. poeyi* is confined to the southeastern United States, whereas *H. ligatus* is distributed from Quebec southward to Alabama and westward to Texas, Arizona, and New Mexico. We included two individuals of *H. ligatus* (from different localities) and three of *H. poeyi* in our analysis and confirmed, based on a nuclear gene, that these two species are distinct.

Implications for Social Evolution in the Genus Halictus

By mapping social behavior onto our cladogram (Fig. 4) we can infer the historical pattern of social evolution within the genus *Halictus*. We would infer from Fig. 4 that the ancestor of *Halictus* s.s. was eusocial but that there have been multiple independent losses of eusociality within the genus. This inference is true whether we select the trees obtained under ML (Fig. 3) or under parsimony (Fig. 1) and is largely in agreement with the conclusions of Richards (1994) and Packer (1997).

Halictus quadricinctus is the only species of the genus included in our study for which eusociality has never been recorded (Knerer, 1980; Sitdikov, 1988), although nest sharing has been documented (Vasić, 1967). Four additional species included herein have been recorded as both solitary and eusocial (as well as quasisocial in some instances). These are *H.* (*H.*) *rubicundus* (Knerer, 1980; Yanega, 1988), *H.* (*H.*) *sexcinctus* (Bonelli, 1965; Knerer, 1980), and the sibling pair *H.* (*S.*) *tumulorum* (Sakagami and Fukushima, 1969; Sakagami and Ebmer, 1979) and *H.* (*S.*) *confusus* (Dolphin, 1966; Tuckermann, pers. comm.). It thus seems probable that eusociality has been lost, at least facultatively, a total of four times among the taxa included here: three times in *Halictus* s.s. and at least once in *Halictus* (*Seladonia*). There are two additional species of *Halictus* known to lack eusociality: *H.* (*H.*) *tsingtsouensis* (Sakagami, 1980) and *H.* (*S.*) *virgatellus* (Eickwort, pers. comm.). The former is a member of Michener's Group 1, with which it shares, among other derived features, a concave gena in the male. It is thus closely related to two species included in our study: *H.* (*H.*) *crenicornis* and *H.* (*H.*) *simplex*, both of which are behaviorally unknown. Nonetheless, the solitary behavior of *H.* (*H.*) *tsingtsouensis* probably represents another independent loss of eusociality. *Halictus* (*S.*) *virgatellus* also probably represents another independent loss of eusociality (Janjic and Packer, unpublished data). It seems probable that eusociality has been lost as many as six times within the genus. Our results thus confirm a speculation made by Eickwort (1985) that "multiple reversion to solitary existence from eusocial ancestry is a more parsimonious hypothesis than the reverse for *Halictus*."

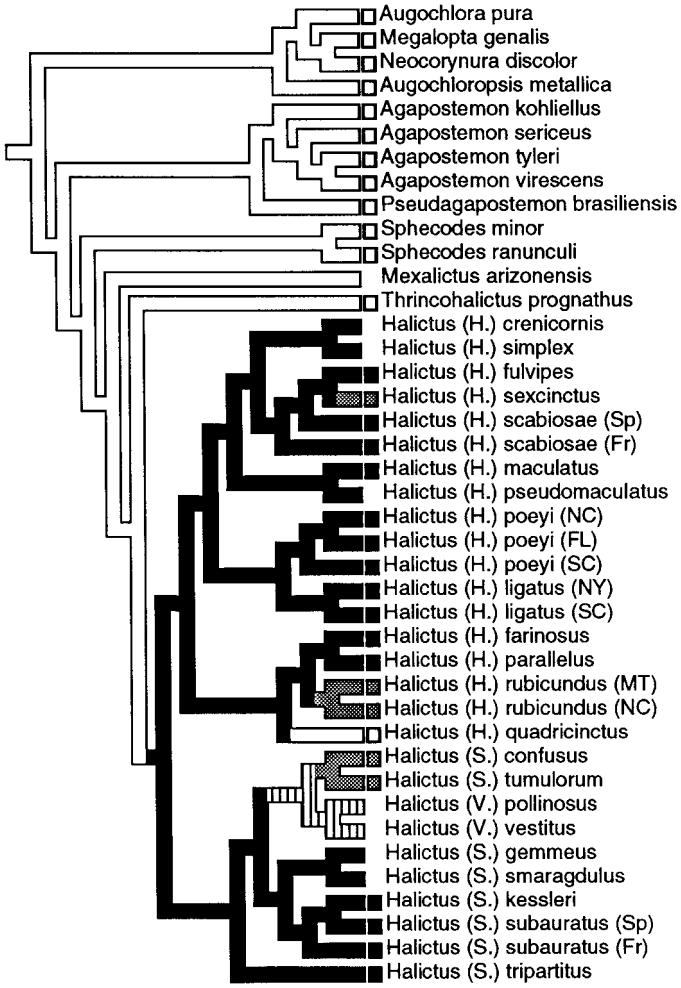

FIG. 4. Social behaviors mapped onto the maximum likelihood tree obtained under the GTR + SSR model (Fig. 3b). Black indicates eusocial taxa, shaded bars indicate socially polymorphic taxa, and white bars indicate communal, semisocial, or solitary taxa (i.e., noneusocial taxa). Behavioral data come primarily from Richards (1994) and additional references cited in the text.

Packer (1997) surveyed social variation within and among halictine taxa and concluded that, for examples in which reversals to solitary behavior had occurred, most involved species or populations at high altitude or latitude. This is certainly not the case for most of the taxa under consideration here as, with few exceptions, all climates experienced by populations of solitary taxa are as warm, or warmer, than temperate habitats in which many halictine bees are known to be eusocial (e.g., Ithaca, NY). All social *Halictus* species which have been studied in detail possess high levels of ovarian development among workers (Knerer, 1980) in comparison to those levels found in many other eusocial taxa, such as species in the *Lasioglossum* subgenus *Evylaeus* (see Packer, 1991). The only genetic data

available for any *Halictus* species are those of Richards *et al.* (1995) for *Halictus* (*H.*) *ligatus*. These authors found evidence for direct reproduction by workers in this species. Yanega's (1988) observations on *Halictus rubicundus* suggest that the first ("worker") brood contains a high proportion of individuals that overwinter and initiate nests the following spring. Similarly, the observations of Dolphin (1966) and Tuckermann (pers. comm.) suggest a wider range of possible reproductive outcomes for worker brood individuals than typically expected for halictine workers. All of these observations indicate substantial flexibility in social roles for "worker" brood females in *Halictus*, suggesting that social evolution has not led to groups of supraspecific taxa in which complete loss of the worker caste has become impossible. This suggests the possibility that factors intrinsic to the social biology of the genus may be at least partially responsible for some of the losses of eusociality.

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