

**DATA FROM THE ELONGATION FACTOR-1 $\alpha$  GENE CORROBORATES THE PHYLOGENETIC PATTERN FROM OTHER GENES REVEALING COMMON ANCESTRY OF BUMBLE BEES AND STINGLESS BEES (HYMENOPTERA: APINAE)**

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**SUMMARY**

A phylogenetic analysis of the tribes of corbiculate tribes (honey bees, stingless bees, bumble bees and orchid bees) was conducted using DNA sequence of the nuclear gene elongation factor-1 $\alpha$ . Results based on equal weights parsimony analysis and maximum likelihood showed strong support for the sister group relationship between stingless bees and bumble bees, consistent with all other genes analyzed thus far. A combined parsimony analysis of EF-1 $\alpha$  with four genes used in previously published studies (nuclear long-wavelength rhodopsin and 28S rDNA and mitochondrial cytochrome b and 16S rDNA) gave the same strong support for Meliponini+Bombini. There was no significant incongruence among the gene partitions but strong incongruence between the DNA sequences and the morphology data. In spite of that incongruence, a combined analysis of genes (3,513 characters) plus morphology (95 characters) gave the same strong support for Meliponini+Bombini. Relationships of Apini and Euglossini were ambiguous. The phylogeny can be used as a framework for interpreting variation in behavior among the bees. For example, the evolution of different recruitment mechanisms in stingless bees and honey bees suggests that the waggle dance evolved only in Apini and the more erratic movements seen in Bombini and Meliponini evolved in a separate lineage.

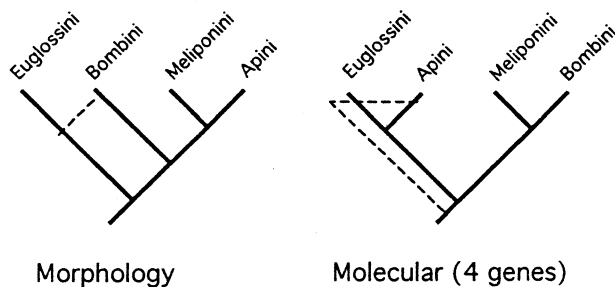
**Keywords.** Apini, Bombini, Euglossini, Meliponini, EF-1 $\alpha$ , molecular phylogeny

**INTRODUCTION**

The importance of stingless bees (tribe Meliponini) is given special recognition by this III Mesoamerican Seminar. While most participants will be interested in economic applications or in various aspects of the behavior of these tropical bees, it is nonetheless important to have an evolutionary perspective of how this tribe fits into the overall scheme of relationships with its social relatives. Knowledge of these phylogenetic relationships gives power to understanding and predicting features of meliponine biology.

Meliponini comprises a tribe within the subfamily Apinae, currently known as the corbiculate clade (Schultz et al. 1999), named for the putative synapomorphy (the specialized pollen-carrying structure on the hindleg known as the corbicula) that distinguishes the clade from all other bees. The other three tribes in the corbiculate clade are the Euglossini (orchid bees), Bombini (bumble bees) and Apini (honey bees), which, with the exception of the solitary or communal Euglossini, constitute the major groups of eusocial bees. Extensive reviews of the behavior and ecology of all four tribes have been compiled by Michener (1974, 2000) and Roubik (1989). Examinations of comparative morphology, most recently that of Roig-Alsina and Michener (1993), reviewed in Michener (2000), have shown the corbiculate bees to be monophyletic. Simultaneous analysis of DNA sequences from multiple genes (16S rDNA, 28S rDNA, Cytochrome b and Long-Wavelength *Rhodopsin*— major opsin) and outgroups from closely related tribes (Cameron and Mardulyn 2001) also supports monophyly of the clade.

To a group of stingless bee experts, the important element of the phylogenetic research on corbiculate bees is that the relationship of Meliponini to the other corbiculate tribes is controversial. Recent consensus from morphological analyses of extant taxa (Roig-Alsina and Michener 1993; Schultz et al. 1999) and incorporation of fossil information (Engel 2001) have shown meliponines as sister group to the highly eusocial honey bees (Fig. 1a). An analysis by Lockhart and Cameron (2001) of the relevant morphological characters extracted from Roig-Alsina and Michener's character matrix for the long-tongue bees (Roig-Alsina and Michener 1993) showed equal support for Michener's (1974) hypothesis (Fig 1a, dashed line): (Euglossini+Bombini)+(Apini+Meliponini), an indication that there is uncertainty in the position of the root of the tree.



**Figure 1.** Two phylogenetic hypotheses of corbiculate bee tribal relationships based on (a) Morphology and (b) Molecular data from 16S, cyt b, opsin and 28S genes.

In contrast to the morphological data, DNA sequences have indicated that stingless bees are more closely related to bumble bees than to honey bees (Fig. 1b). Results from the study by Cameron and Mardulyn (2001) of four genes (16S, 28S, cytochrome b, opsin) are consistent with all previous DNA studies, showing strong support for a (Meliponini+Bombini) clade, with uncertainty concerning the position of Apini and Euglossini—as either sister groups (shown above) or with Apini as a separate lineage (Fig. 1b, dashed line): (Apini+(Euglossini+(Bombini+Meliponini))). Although strongly incongruent with morphology, the DNA data sets are congruent with one another using standard statistical tests of congruence (Cameron and Mardulyn 2001, 2003).

Nonetheless, the burden of proof is on the DNA data because those results challenge the traditional view of the stingless bee-honey bee relationship. Therefore, a fifth gene, Elongation Factor-1 alpha (EF-1 $\alpha$ ) has been newly added to the analysis, which is the subject of this report. This particular gene was selected because it has proven useful in numerous studies of relationships among other groups of bees (Danforth et al. 2003; Schwarz et al. 2003; Michel-Salzat and Cameron 2004). Results from analysis of EF-1 $\alpha$  (reported here) are congruent with those of all other molecular analyses, showing the same strongly supported Bombini+Meliponini relationship.

## MATERIALS AND METHODS

*Elongation factor-1 alpha* (EF-1 $\alpha$ ) has two divergent copies in bees (Danforth and Ji 1998), which are easily separated using specific PCR primers. The F2 copy was PCR-amplified using one of the following forward primers (HaF2for1: ggg yaa agg wtc ctt caa rta tgc or EmphF2for: gcc tgg gta ttg gat aag ctg aa) and one of the reverse primers (F2 rev: aat cag cag cac ctt tag gtg g or EmphF2rev: tgg att gtt ytt rga gtc acc ag), provided by S. Sipes and B. Danforth. Most of the corbiculate taxa amplified with HaF2for1 + F2rev. DNA extraction, amplification and sequencing followed established protocols. EF-1 $\alpha$  PCR conditions (Danforth and Ji 2001; Sipes personal communication) included denaturation 1 min. at 94°C, annealing 1 min. at 50°C, elongation 1.5 min. at 72°C, 35 cycles. Tribal taxa used in the analysis are those given in Cameron and Mardulyn (2001); outgroup taxa were *Anthophora pacifica* and *Xylocopa virginica*. GenBank Accession numbers for EF-1a are AY208277-AY208290; accession numbers for the other genes are given in Cameron and Mardulyn (2001). Aligned sequences (Clustal X; parameters as in Cameron and Mardulyn 2001) are available from the author, and vouchers are with the author until deposition at the Natural History Survey at the University of Illinois at Urbana-Champaign. Morphological characters are given in Cameron and Mardulyn (2001).

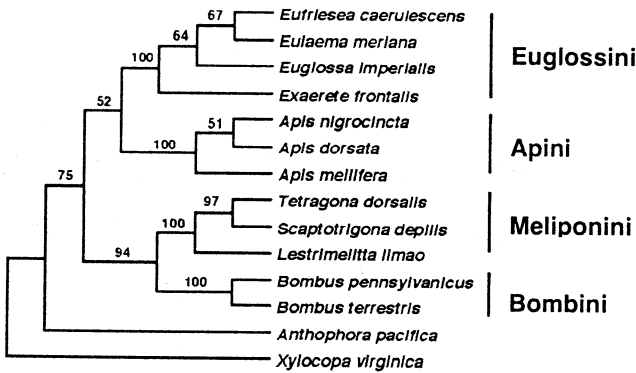
Maximum parsimony (branch and bound, equal weights assigned to all characters or nt3 transitions for opsin given 4x less weight) and maximum likelihood (input parsimony tree, HKY85+I+G model, parameters estimated from the data) were implemented in PAUP\* (version 4.10b) (Swofford 2001). Consistency indices (CI) are given with uninformative characters excluded. Bootstrap analyses (branch and bound 400-1000 replicates) for branch support estimates were implemented in PAUP\*. Tests of data partition congruence are as given in Cameron and Mardulyn (2001, 2003). All analyses were run with and without the two intron sequences. Because in all cases the results were unchanged except for a lack of infra-tribal resolution with introns excluded, trees and statistics are reported for analyses that include the introns.

**RESULTS**

Maximum parsimony (MP) analysis of the EF-1 $\alpha$  sequences resulted in strong support for the Bombini+Meliponini sister group relationship (Fig. 2), with high bootstrap support. Maximum likelihood (ML) results gave identical branching patterns (-Ln likelihood = 4801.04, ti/tv ratio = 3.15, invariable sites = 0.61; shape parameter = 6.78).

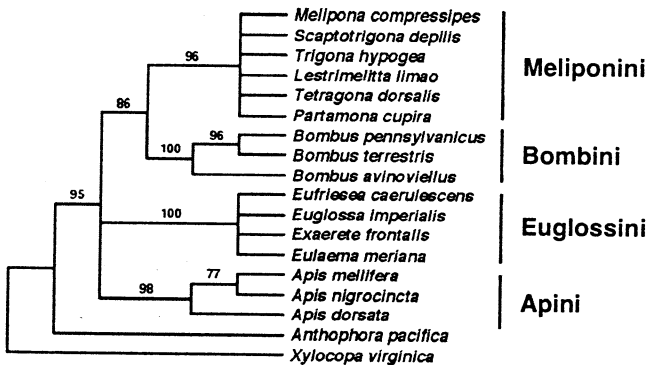
Combined MP analysis of 5 gene partitions (no incongruence among the gene partitions; p>0.05) resulted in the same Bombini+Meliponini clade (Fig. 3), but showed less intratribal resolution, due in part to missing taxa among data sets.

**EF-1 $\alpha$  bootstrap consensus**



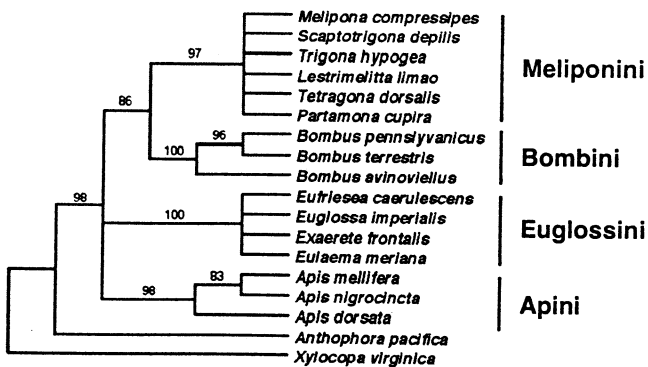
**Fig. 2. Maximum parsimony (MP) bootstrap tree (400 branch and bound replicates). Numbers along branches are bootstrap values. Separate MP (branch and bound) gave 2 trees (1071 characters, 252 parsimony informative; tree length = 718; CI = 0.588; RI = 0.666)**

**5 genes bootstrap consensus**



**Fig. 3. MP all-genes bootstrap tree (1000 replicates with 10 random addition sequences per replicate). Separate MP analysis (branch and bound) gave 9 trees (3513 characters, 978 parsimony informative; tree length = 3401; CI = 0.480; RI = 0.533).**

**5 genes plus morphology bootstrap**



**Fig. 4. MP all-genes + morphology bootstrap tree (1000 replicates with 10 random addition sequences per replicate). Separate MP analysis (branch and bound) gave 9 trees (3608 characters, 996 parsimony informative; tree length = 3130.25; CI = 0.499; RI = 0.560).**

Lastly, MP analysis of the complete data set containing all gene partitions and morphology resulted in the same tree topology (Fig. 4) as the 5-genes tree (Fig. 3), again with strong support for the Bombini+Meliponini clade.

## DISCUSSION AND CONCLUSIONS

The results of the EF-1 $\alpha$  analyses further advance the view that the stingless bees are not the sister group to the honey bees, but instead share common ancestry with the bumble bees. This is the fifth gene that gives support to this pattern. It seems reasonable to conclude that the weight of evidence falls on the side of the DNA data and that the pattern suggested by the morphology data alone (Fig. 1a) is not accurate. Additional investigation of morphology is warranted. Future molecular research on this clade should include a larger representation of taxa from each tribe. This is currently being addressed by several ongoing studies in the author's laboratory, including a generic level analysis of the Euglossini (Michel-Salzat and Cameron, 2004), a worldwide molecular phylogeny of the Meliponini (Rasmussen and Cameron in progress) and a worldwide molecular phylogeny of Bombini (Cameron and Hines in progress).

Meanwhile, the origin of Meliponini is strongly and consistently indicated via DNA data to share common ancestry with Bombini. These results open to question the traditional view (Roig-Alsina and Michener 1993; Schultz et al. 1999; Engel 2001) that highly eusocial behavior evolved once through a derived common ancestor of honey bees and stingless bees, passing first through a primitively eusocial (bumble bee-like) phase. Thus, highly eusocial behavior, thought to be homologous in honey bees and stingless bees, may indeed have evolved twice independently, suggesting parallel pathways and adaptive influences in the evolution of complex social systems as well as shared homologies between bumble bees and stingless bees. One potentially exciting homology relevant to communication appears to be the zigzag run or "excited run" (Dornhaus and Chittka 2001) found in all stingless bees studied thus far (Dyer 2002), and in *Bombus terrestris* (Dornhaus and Chittka 2001) and possibly in *B. transversalis* (Dornhaus and Cameron 2003). Once thought to be either convergent in bumble bees and stingless bees or primitively retained in stingless bees and lost in honey bees (Fig. 1, Chittka and Dornhaus 1999), this simple form of excitatory running over the brood comb to increase foraging activity may in fact be homologous in bumble bees and stingless bees.

## Acknowledgments

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