Online Material

SUPPLEMENTARY METHODS

1. Taxonomic sampling

The corbiculate bees are a monophyletic group of long-tongued bees within Apinae, defined most notably by the specialized pollen transporting structures on the hind legs known as pollen baskets or corbicula (Sakagami and Michener, 1987; Roig-Alsina and Michener, 1993; Michener, 2000). The corbiculate bees include four tribes: the solitary or communal Euglossini (orchid bees; 210 species in five genera; Roubik and Hanson, 2004; Ascher, 2007), the primitively eusocial Bombini (bumble bees; 246 species in the genus *Bombus*; Williams, 1998; Ascher, 2007), and the advanced eusocial Meliponini (stingless bees; 471 species in 20–40 or more genera depending on the classification; Michener, 2000; Camargo and Roubik, 2005; Ascher, 2007) and Apini (honey bees; six to 11 species in the genus *Apis*; Engel, 1999; Michener, 2000).

A list of taxa examined in the present study is given in Supplementary Table I. The monophyly of each extant corbiculate tribe is well established both by morphology (Roig-Alsina and Michener, 1993; Michener, 2000) and molecular data (Cameron and Mardulyn, 2001; Michel-Salzat et al., 2004; Arias and Sheppard, 2005; Cameron et al., 2007; Rasmussen and Cameron, 2007), so use of exemplars is appropriate. Because our focus was to sequence more genes rather than to sample more taxa, sampling was limited to two or three exemplars per tribe. However, an effort was made to sample across a greater diversity of genera and species, as corroborated by recent molecular phylogenetic analyses of each tribe (Kawakita et al., 2004; Michel-Salzat et al., 2004; Arias and Sheppard, 2005; Cameron et al., 2007; Rasmussen and Cameron, 2007). Outgroup taxa were sampled from four tribes that are suggested as closely related to the corbiculate bees by morphology (Michener, 1990; Roig-Alsina and Michener, 1993) and a recent molecular phylogenetic analysis (Danforth et al., 2006a).

2. Genes analyzed and primers for the polymerase chain reaction (PCR)

We assembled sequences of 12 protein-coding nuclear genes for this study (Supplementary Tab. II). The following seven genes have been previously utilized in bee phylogenetics: arginine kinase (Argk; Kawakita et al., 2003), CAD (Danforth et al., 2006b), elongation factor 1-alpha F2 copy (EF-1α; Danforth and Ji, 1998), sodium-potassium ATPase (NaK; Danforth et al., unpublished), RNA polymerase II (Pol II; Danforth et al., 2006a,b), long-wavelength rhodopsin (Rh; Mardulyn and Cameron, 1999) and wingless (Wg; Danforth et al., 2004a). The primer sequences have been previously published and those used in the present study are listed in Supplementary Table II. The white (W) gene has been used in insect systematics, e.g., mosquitoes (Besansky and Fahey, 1997); Danforth et al. (2004b) designed the primer for bees. The remaining four genes have not been explored previously for phylogenetic analysis and were newly developed for this study: mitotic checkpoint control protein (Bub3), Ca^{2+}/calmodulin-dependent protein kinase II (CamkII), deoxyribonucleoside kinase (Dnk), and glycerol kinase (Gyk). For these four loci, we compared the sequences derived from the genomic databases of *Apis mellifera* and *Drosophila melanogaster* and those contained in the ETS database of *Heliconius* butterflies (Papanicolaou et al., 2005) to identify conserved regions within the exons for designing degenerate primers. Because our aim was to sample more genes but not longer sequences from individual loci, care was taken to keep the length of a PCR fragment, including introns, to about 1000 bp in order to facilitate direct sequencing. Primer sequences and maps showing primer and intron positions for these four genes are provided as Supplementary Figure.

The Argk, EF-1α, and Rh sequences of the following species have been previously published and were retrieved from the GenBank database: *Euglossa imperialis* (AY267176/ AY267144/ AY267160), *Exaerete frontalis* (AY267175/ AY267143/ AY267159), *Bombus ardens* (AF492897/ AF492964/ AF493031), *Bombus diversus* (AF492894/ AF492961/ AF493028), *Meliponula bocandei* (AY267177/ AY267145/ AY267161), *Apis dorsata* (AY267178/ AY267146/ AY267162), and *Centris cockerelli* (AY267180/ AY267148/ AY267164). The remaining sequences were newly obtained in this study and deposited in the GenBank database under accession numbers EU184704–EU184857.

3. Molecular methods

Genomic DNA was extracted from thoracic tissue of ethanol-preserved or pinned specimens with

Supplementary Table I. Taxa examined.

Tribe	Species	Locality
Ingroup		
Euglossini	*Euglossa imperialis* Cockerell, 1922	Panama
	Exaerete frontalis (Guérin-Méneville, 1845)	Panama
	Eufriesea pulchra (Smith, 1854)	Panama
Bombini	*Bombus ardens* Smith, 1879	Kyoto, Japan
	Bombus diversus Smith, 1869	Kyoto, Japan
Meliponini	*Trigona fuscipennis* Friese, 1900	Costa Rica
	Cephalotrigona capitata (Smith, 1854)	Costa Rica
	Meliponula bocandei (Spinola, 1853)	Gabon
Apini	*Apis florea* Fabricius, 1787	Mahaxai, Laos
	Apis dorsata Fabricius, 1793	Laksao, Laos
	Apis cerana Fabricius, 1793	Kyoto, Japan
Outgroup		
Centridini	*Centris cockerelli* Fox, 1899	New York, USA
Ericrocidini	*Epiclopus gayi* Spinola, 1851	Chile
Melectini	*Thyreus takaonis* (Cockerell, 1911)	Okinawa, Japan
Anthophorini	*Anthophora plumipes* (Pallas, 1772)	Kyoto, Japan

standard phenol/chloroform methods. PCR amplifications were carried out with the following reaction conditions: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 sec, annealing for 30 sec, and extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. Annealing temperatures depended on primers used and are provided in Supplementary Table II. All PCR amplifications were aided by Ex Taq polymerase (TaKaRa, Otsu, Japan) and carried out using a GeneAmp PCR System 9700 (Perkin-Elmer, Foster City, CA). Amplified PCR products were purified using a NucleoSpin Extract II Kit (Macherey-Nagel, Düren, Germany). Sequencing reactions were performed using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer), and eloctrophoresis was conducted on an ABI 3100 sequencer (Perkin-Elmer). The CamkII gene displayed extensive length variation in the first intron; full-length sequences could not be obtained by using only the PCR primers in some taxa. We therefore designed internal sequencing primers located at the boundaries between the first intron and the flanking exons, and used them to obtain sequences of only exons and the second intron.

4. Phylogenetic analysis

Because the intron sequences varied greatly in length among taxa, only exon sequences were used for analyses. Alignment of the exon sequences was straightforward. Only a single, three-base insertion in the CAD gene of *Thyreus takaonis* was necessary. The number of nucleotide sites for each gene partition varied between 166 and 942 (Supplementary Tab. III), and the combined data matrix consisted of 6018 aligned nucleotide sites. The number of parsimony-informative characters for the corbiculate ingroup ranged between 16 and 196 for each partition, and in total, 1387 sites were informative (Supplementary Tab. III). Within the ingroup, sequences could not be obtained in *Trigona fuscipennis* and *Meliponula bocandei* for CAD, *Trigona fuscipennis* and *Euglossa imperialis* for CamkII, and *Apis cerana* and *Apis florea* for Dnk. In the outgroup, *Epiclopus gayi* could not be sequenced for Dnk, EF-1α, and Gyk, *Thyreus takaonis* for Argk and EF-1α, and *Anthophora plumipes* for Argk and Dnk.

All phylogenetic analyses were done using PAUP* version 4.0b10 (Swofford, 2002), unless otherwise stated. Parsimony, likelihood, and Bayesian analyses for the nucleotide characters were performed with the following procedures. We constructed the most parsimonious trees by heuristic searches with 100 random addition analyses and tree bisection-reconnection (TBR) branch-swapping. Robustness of the trees was validated with non-parametric bootstrap analysis with 1000 replications. For the maximum likelihood analysis, we used the program Modeltest 3.0 (Posada and

Supplementary Table II. Primers used for this study. Superscript letters after primer name indicate annealing temperatures; a, 40 °C; b, 45 °C; c, 50 °C; d, 55 °C; e, used for sequencing only. When using primers with different superscript letters, the lower annealing temperature was used.

Gene	Primer name	Strand	Primer sequence (5′ to 3′)	Reference
Argk	ArgKfor2[c]	F	GAC AGC AAR TCT CTG CTG AAG AA	Kawakita et al. (2003)
	ArgKrev2[c]	R	GGT YTT GGC ATC GTT GTG GTA GAT AC	
Bub3	Bub3for1[a]	F	GTC AAT GCH ATM TTV ACK GGH GGH TG	this study
	Bub3f2[c]	F	GAT CCY AGA ACA CCY ACY TGT GTW GG	
	Bub3rev1[a]	R	CG YTT YTT RTT RAA RCC ATC CC	
	Bub3r2[c]	R	GC AAA YGT RTT ATA MGT WGA ATG AA	
CAD	apCADfor1[c]	F	GGW TAT CCC GTD ATG GCB MGW GC	Danforth et al. (2006b)
	apCADrev1mod[c]	R	GC CAT YRC YTC BCC YAC RCT YTT CAT	
CamkII	CamkIIF[b]	F	TTY GCY GGG ACR CCM GGT TAY CT	this study
	CamK_int_F[e]	F	G CAG TAY CCR AGY CCR GAA TG	
	CamkIIR[b]	R	TT GCG CCT YGC RTT RAA TTT CT	
	CamK_int_R[e]	R	CT TAC GTC GTA YGW TCC	
Dnk	dNKf1[c]	F	GAR GGY AAY ATM GGH AGC GGK AAR AC	this study
	dNKf2[c]	F	TAS AAG AAC CAR TAG AAC TTT GGC G	
	dNKr1[c]	R	AG CCA KTC MTC RTG MAT MTT RTG MAG YT	
	dNKr2[c]	R	T TTC TTC TTT YCT YGC TCT KGY TT	
EF-1α	ef1aF2f[d]	F	GGA CAC AGA GAT TTC ATC AAR AA	Kawakita et al. (2003)
	ef1aF2r[d]	R	TTG CAA AGC TTC RTG RTG CAT TT	
Gyk	GlyK-F[d]	F	ACG GGY GGS AAR GAC GGT GG	this study
	GlyKf2[c]	F	TGG GAT CCC ACV TTR TGC AGA TAT TT	
	GlyK-R[d]	R	AA AGC TGG YAC RAA RTA TAC GTC	
	GlyKr2[c]	R	AG TTT CAT ATT ATC TCG YAR CCA	
NaK	NaKfor2[d]	F	GCS TTC TTC TCB ACS AAC GCC GTY GAR GG	Danforth et al. (2004b)
	NaKrev2[d]	R	AC CTT GAT RCC GGC YGA WCG GCA CTT GGC	
Pol II	polfor2a[c]	F	AAY AAR CCV GTY ATG GGT ATT GTR CA	Danforth et al. (2006b)
	polrev2a[c]	R	AG RTA NGA RTT CTC RAC GAA TCC TCT	
Rh	LWRhF[c]	F	AAT TGC TAT TAY GAR ACN TGG GT	Mardulyn and Cameron (1999)
	LWRhR[c]	R	ATA TGG AGT CCA NGC CAT RAA CCA	
	LwRhFor3[c]	F	AGA TAC AAC GTR ATC GTS AAR GGT	Danforth et al. (2004a)
W	whitefor2[c]	F	GCT GAR GGW AGA GTA GCY TTC ATG GG	Danforth et al. (2004b)
	whiterev2[c]	R	C SGC RAA RAC GTT CTG GAA RGT CAT ATT	
Wg	LepWG1a[b]	F	GAR TGY AAR TGY CAY GGY ATG TCT GG	Brower and DeSalle (1998)
	LepWG2a[b]	R	AC TIC GCA RCA CCA RTG GAA TGT RCA	
	beewgFor[b]	F	TGC ACN GTS AAG ACC TGY TGG ATG AG	Danforth et al. (2004a)

Supplementary Table III. Summary of basic sequence statistics and phylogenetic results (analyses were done without the outgroups). Likelihood bootstrap values and partitioned Bremer support (PBS) are provided for the node supporting Bombini + Meliponini.

Parition	Total sites	Informative sites	A + T (%)[a]	CI[b]	Likelihood bootstrap	PBS	PBS/ min steps[c]	Relative rate[d]	Alpha[d]	PI[d]
Argk	530	119	52.6	0.641	32	2	0.012	1.122	0.337	0.583
Bub3	413	38	64.2	0.754	30	1	0.016	0.453	0.096	0.746
CAD	594	136	54.0	0.719	96	9	0.037	1.455	0.272	0.605
CamkII	332	16	44.2	0.696	19	–1	–0.024	0.368	1.492	0.743
Dnk	428	94	69.8	0.781	69	6	0.035	1.258	0.703	0.435
EF-1α	529	81	56.6	0.660	53	5	0.037	0.806	0.218	0.657
Gyk	422	79	62.2	0.767	11	–1	–0.009	0.747	0.193	0.663
NaK	942	196	44.8	0.649	98	9	0.027	1.417	0.109	0.720
Pol II	766	148	62.8	0.696	98	9	0.048	0.822	0.168	0.689
Rh	502	117	56.5	0.714	63	–3	–0.016	1.128	0.349	0.570
W	166	45	50.9	0.662	27	0	0.000	1.179	0.335	0.591
Wg	394	45	38.9	0.740	28	–5	0.068	0.618	0.062	0.765

[a]Average proportion of A's and T's across sequences of the ingroup taxa.

[b] Consistency index excluding uninformative characters.

[c] Partitioned Bremer support divided by minimum number of steps for each partition.

[d] Relative substitution rate, shape parameter of the gamma distribution, and proportion of invariable sites as estimated from combined Bayesian analysis.

Supplementary Table IV. Comparison of base compositions among the ingroup taxa for the combined 12-gene data set. There is no significant heterogeneity in base composition as judged by the chi-square test ($\chi^2 = 25.0096$, df = 30, $P = 0.7246$).

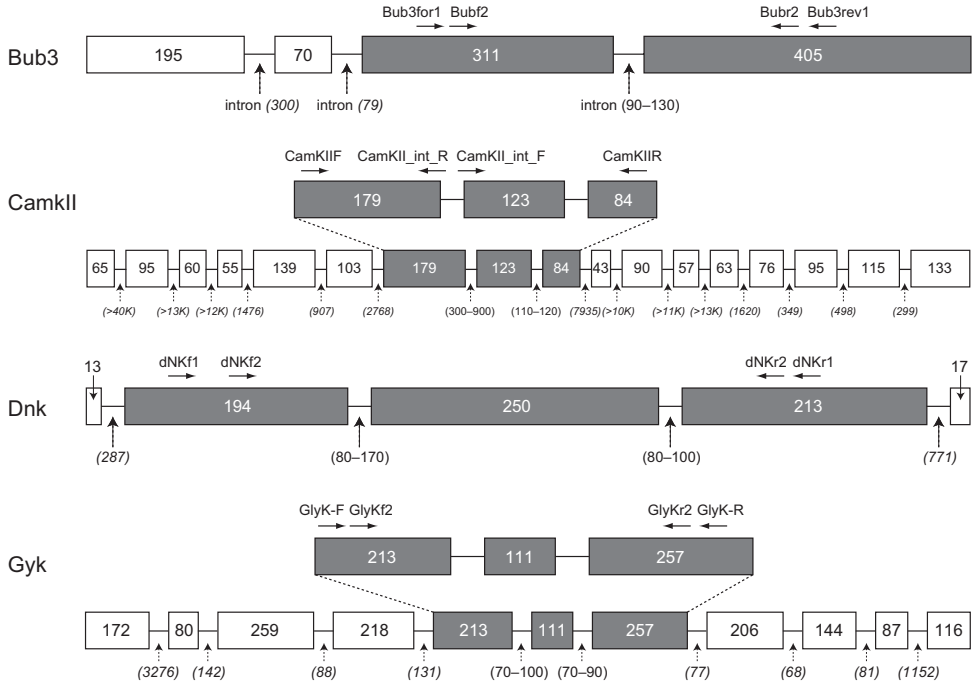
Taxon	Sites	A	C	G	T
Euglossa imperialis	5620	0.2886	0.2155	0.2354	0.2605
Exaerete frontalis	5786	0.2814	0.2157	0.2421	0.2608
Eufriesea pulchra	6003	0.2909	0.2096	0.2351	0.2645
Bombus ardens	5896	0.2808	0.2144	0.2388	0.2660
Bombus diversus	5841	0.2851	0.2111	0.2371	0.2668
Trigona fuscipennis	5084	0.2797	0.2182	0.2469	0.2552
Cephalotrigona capitata	5944	0.2784	0.2221	0.2470	0.2525
Meliponula bocandei	5379	0.2814	0.2172	0.2460	0.2554
Apis florea	5420	0.2832	0.2137	0.2391	0.2639
Apis dorsata	5820	0.2944	0.2049	0.2298	0.2709
Apis cerana	5568	0.2827	0.2132	0.2378	0.2664

Crandall, 1998) to select an appropriate model of base substitution and to estimate model parameters, which were used in maximum likelihood heuristic searches with 10 random addition analyses and TBR branch-swapping. Likelihood node support was assessed by performing 100 non-parametric bootstrap replications. The Bayesian analysis was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) and appropriate substitution models selected by MrModeltest 2.2 (Nylander, 2004). In the simultaneous analysis of the 12 genes, we partitioned the data set by genes, and substitution models were selected separately by using MrModeltest. The unlink command was used to obtain parameter estimates separately for each partition. In the Bayesian tree searches, we ran four simultaneous chains for 5×10^5 generations, saving trees every 100 generations for a total of 5001 trees. We plotted ln-likelihood of the sampled trees against generation time to identify the region of the analysis in which the parameter estimates were stable. We discarded the burn-in region (trees and parameter estimates obtained before equilibrium; the initial 1001 tree), and the remaining 4000 samples were used to obtain the posterior distribution of tree topology, branch lengths, and substitution parameters. To ensure that analyses were not trapped on local optima, we carried out two independent runs and compared the results for consistency.

Parsimony analysis of amino acid sequences was performed using PAUP* as described for nucleotide sequences. The best-fit model of amino acid sequence for the likelihood analysis was selected using Modelgenerator (Keane et al., 2006), and the likelihood search and 100 replications of bootstrap analysis were performed with MultiPhyl (Keane et al., 2007) using the nearest neighbor interchange (NNI) swapping algorithm. The Bayesian search was performed using MrBayes and by setting the prior for the amino acid model to "mixed" under which the program estimates the relative contribution of different amino acid models according to their posterior probabilities. The shape parameter of the gamma distribution and proportion of invariable sites were also estimated as suggested by Modelgenerator.

4.3. Analysis of correlation between nodal support values and statistical properties of individual gene partitions

Because individual gene partitions supported variable tribal relationships, we compared nodal support values provided by each gene for the Bombini + Meliponini group, and tested whether the strength of support for this topology was correlated with the following six parameters: number of informative sites, base composition (proportion of A + T), consistency index (CI; excluding uninformative characters), relative substitution rate, shape parameter of the gamma distribution



Supplementary Figure. Gene map showing positions of exons (boxes) and introns (lines), and the primers newly designed in this study. Exons are proportional to their lengths, and the number of nucleotide sites is given for each exon. Regions sequenced in this study are indicated in gray. Approximate lengths of introns in species examined in this study are given for the regions sequenced. Lengths for other introns are those of *Apis mellifera*.

(α), and the proportion of invariable sites (PI). The first three parameters were calculated using PAUP*. The remaining three parameters were estimated within the Bayesian framework. The advantage of the Bayesian approach is that various substitution parameters can be estimated (without assuming a particular tree topology a priori) and compared across different partitions when analyzed simultaneously using the same model (Lin and Danforth, 2003; Danforth et al., 2005). We first estimated relative substitution rate and α by employing the GTR+G model to all gene partitions and performing Bayesian searches. Parameter estimates were obtained separately for each gene by using the unlink command. We then obtained estimates of PI using the GTR+I model across all genes. We did not estimate α and PI simultaneously (GTR+G+I model) because the two parameters are dependent on each other and thus would more appropriately be compared across partitions when estimated separately.

The following four support indices were used to assess the correlation between the above parameters and phylogenetic performance: parsimony and likelihood bootstrap support, Bayesian posterior probability, and partitioned Bremer support (PBS; Bremer, 1994). The command file for calculating PBS was produced using TreeRot version 2 (Sorenson, 1999). Because PBS is dependent on the amount of signal content of a given partition, we divided PBS by the minimum number of steps for that gene (Baker et al., 2001) to obtain a standardized comparative measure of relative contribution of each gene to the node of interest.

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