Report

Current Biology

Phylogenomic Insights into the Evolution of Stinging Wasps and the Origins of Ants and Bees

Highlights

- UCE phylogenomics provides a highly resolved phylogeny of the stinging wasps
- Ants are the sister group to bees and apoid wasps
- Bees are nested inside crabronid wasps and sister to Pemphredoninae+Philanthinae
- Outgroup choice and taxon sampling can strongly impact phylogenomic inference

Authors

Michael G. Branstetter, Bryan N. Danforth, James P. Pitts, ..., Michael W. Gates, Robert R. Kula, Seán G. Brady

Correspondence

mgbranstetter@gmail.com

In Brief

Branstetter et al. present a densely sampled phylogeny of the stinging wasps, inferred using UCE phylogenomic data. They confirm that ants are sister to bees and apoid wasps and that bees are specialized crabronid wasps. They also demonstrate that taxon sampling can have a strong impact on phylogenetic results even when using genome-scale data.

Branstetter et al., 2017, Current Biology *27*, 1019–1025
April 3, 2017 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.cub.2017.03.027

Current Biology
Report

Phylogenomic Insights into the Evolution of Stinging Wasps and the Origins of Ants and Bees

Michael G. Branstetter,[1,2,8,9,*] Bryan N. Danforth,[3] James P. Pitts,[4] Brant C. Faircloth,[5] Philip S. Ward,[6] Matthew L. Buffington,[7] Michael W. Gates,[7] Robert R. Kula,[7] and Seán G. Brady[2]
[1]Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA
[2]Department of Entomology, National Museum of Natural History, Smithsonian Institution, PO Box 37012, 10th Street and Constitution Avenue NW, Washington, DC 20560, USA
[3]Department of Entomology, 3119 Comstock Hall, Cornell University, Ithaca, NY 14853, USA
[4]Department of Biology, Utah State University, 5305 Old Main Hill, Logan, UT 84322-5305, USA
[5]Department of Biological Sciences and Museum of Natural Science, Louisiana State University, Baton Rouge, LA 70803, USA
[6]Department of Entomology and Nematology, University of California, Davis, 1 Shields Avenue, Davis, CA 95616, USA
[7]Systematic Entomology Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, US Department of Agriculture, c/o Department of Entomology, National Museum of Natural History, Smithsonian Institution, PO Box 37012, 10th Street and Constitution Avenue NW, Washington, DC 20560, USA
[8]Lead Contact
[9]Twitter: @bramic21
*Correspondence: mgbranstetter@gmail.com
http://dx.doi.org/10.1016/j.cub.2017.03.027

SUMMARY

The stinging wasps (Hymenoptera: Aculeata) are an extremely diverse lineage of hymenopteran insects, encompassing over 70,000 described species and a diversity of life history traits, including ectoparasitism, cleptoparasitism, predation, pollen feeding (bees [Anthophila] and Masarinae), and eusociality (social vespid wasps, ants, and some bees) [1]. The most well-studied lineages of Aculeata are the ants, which are ecologically dominant in most terrestrial ecosystems [2], and the bees, the most important lineage of angiosperm-pollinating insects [3]. Establishing the phylogenetic affinities of ants and bees helps us understand and reconstruct patterns of social evolution as well as fully appreciate the biological implications of the switch from carnivory to pollen feeding (pollenivory). Despite recent advancements in aculeate phylogeny [4–11], considerable uncertainty remains regarding higher-level relationships within Aculeata, including the phylogenetic affinities of ants and bees [5–7]. We used ultraconserved element (UCE) phylogenomics [7, 12] to resolve relationships among stinging-wasp families, gathering sequence data from >800 UCE loci and 187 samples, including 30 out of 31 aculeate families. We analyzed the 187-taxon dataset using multiple analytical approaches, and we evaluated several alternative taxon sets. We also tested alternative hypotheses for the phylogenetic positions of ants and bees. Our results present a highly supported phylogeny of the stinging wasps. Most importantly, we find unequivocal evidence that ants are the sister group to bees+apoid wasps (Apoidea) and that bees are nested within a paraphyletic Crabronidae. We also demonstrate that taxon choice can fundamentally impact tree topology and clade support in phylogenomic inference.

RESULTS AND DISCUSSION

Phylogenomic Analysis

To resolve relationships among major stinging wasp lineages (superfamilies and families), we employed a phylogenomic approach that combines the targeted enrichment of ultraconserved elements (UCEs) with multiplexed next-generation sequencing (NGS) [12]. The UCE approach relies on DNA and allows for the efficient sequencing of hundreds of loci from both fresh and museum-preserved specimens. We followed published lab protocols [7, 12] (see also the Supplemental Experimental Procedures) and used a Hymenoptera-specific bait set designed to enrich 1,510 UCE loci [7]. We sequenced new molecular data from 139 taxa and combined these with data from 16 taxa previously sequenced [7] and UCE loci harvested from 32 genomes, resulting in a final dataset containing 187 taxa (see Data S1 for sample information).

We included 136 samples of stinging wasps, representing 30 out of 31 recognized families, missing only Scolebythidae. We sampled densely within the bees and apoid wasps (Apoidea), including 53 species from 23 out of 25 recognized bee subfamilies, and 16 species from outside bees, including the phylogenetically enigmatic families Ampulicidae and Heterogynaidae. We also sampled 14 species from four out of eight subfamilies within Crabronidae, including two subfamilies hypothesized to be closely related to bees (Pemphredoninae+Philanthinae) [5]. For outgroups, we sampled all superfamilies within the sawfly grade (Symphyta), and eight out of 12 non-aculeate superfamilies from the Apocrita (Parasitica), including Trigonaloidea, Evanioidea, Ichneumonoidea, and Ceraphronoidea, which previous analyses suggested are closely related to Aculeata [8, 10, 13–15].

Among the taxa from which we sequenced enriched UCE loci, we captured an average of 966 UCE contigs per sample, with a mean contig length of 801 bp and an average coverage per UCE contig of 80× (see Data S1 for assembly information). We evaluated the effects of filtering alignments for various levels of taxon occupancy (percentage of taxa required to be present in a given locus) and selected the 75% filtered locus set ("Hym-187T-F75") as the primary locus set for analysis. This dataset included 854 loci and 203,095 bp of sequence data, of which 143,608 sites were informative (see Data S1 for alignment matrix information).

We analyzed the Hym-187T-F75 dataset using maximum-likelihood (ML; RAxML v8 [16]), Bayesian (BI; ExaBayes v1.4 [17]), and species-tree (ST; ASTRAL-II [18]) approaches. For ML analyses, we compared several different data-partitioning schemes (see Data S1 for more information) and two approaches designed to mitigate phylogenetic error caused by base composition heterogeneity and/or substitution saturation. For the latter approaches, we created one dataset in which we converted the entire matrix to RY coding and one in which we removed loci exhibiting signs of base composition heterogeneity among taxa (47 loci removed). For ST analysis, we employed weighted statistical binning to reduce error from loci with low information content [19].

We recovered a robust phylogeny of the Aculeata, with topologies being nearly identical across all analyses (Figures 1, S1, and S2). We observed topological conflict at eight nodes, with the most important difference concerning relationships among families of the Chrysidoidea (cuckoo wasps and relatives). We recovered the Trigonaloidea as sister to stinging wasps (Aculeata) with maximum support in all analyses. Although we lacked several parasitoid superfamilies in our dataset, this result is congruent with most recent molecular analyses [8, 10, 15]. Importantly, we did not recover the Ichneumonoidea, a long-standing candidate as the sister group to Aculeata [13], to be closely related to the stinging wasps in any analysis. Within Aculeata, we found Chrysidoidea to form a paraphyletic grade, with the clade containing Sclerogibbidae+[Embolemidae+Dryinidae] recovered as the sister group to remaining non-chrysidoid lineages in most analyses. The rest of the aculeate superfamilies divided into two major clades that were each highly supported in all analyses. Overall, relationships among superfamilies largely agree with a recent transcriptome-based study [6], except for the placement of Vespoidea. Ants (Formicidae) were inferred to be the sister group to bees and apoid wasps with maximum support in all analyses, except for the RY-coded ML analysis (96%) and the ST analysis (90%).

Within the clade containing bees and apoid wasps (Apoidea), our results for relationships among families and higher-level clades are identical across analyses and largely agree with those of Debevec et al. [5]. Most significantly, we found the bees (Anthophila) to be nested inside of a paraphyletic Crabronidae and sister to Pemphredoninae+Philanthinae, confirming the finding first reported in [5], which was based on only four molecular markers and received only moderate support. Within Apoidea, we also found Ampulicidae to be the sister group to all other apoid families and Heterogynaidae, a phylogenetically enigmatic family, to be the sister group to Crabroninae+Sphecidae. Among bees (Anthophila), our results are largely congruent with previous studies of higher-level relationships [20]. Most notably, we found Melittidae to be the sister group to the remaining bee families, and we recovered monophyly of the eusocial corbiculate tribes (Apini, Bombini, and Meliponini) in all concatenated analyses (not recovered in the ST analysis).

Testing the Phylogenetic Position of Ants and Bees

Identifying the phylogenetic positions of ants and bees within the stinging wasps is of critical importance. Ants are ecologically dominant social insects in virtually all terrestrial ecosystems, and bees are among the most important pollinators of the largest lineage of vascular plants on earth—the angiosperms. To evaluate the robustness of our phylogenetic results, we performed two types of analyses. First, we evaluated a range of previous phylogenetic hypotheses for both ants and bees using the Shimodaira-Hasegawa (SH) test [21]. For the SH tests, we analyzed nine alternate positions for ants and 14 alternate positions for bees (Table 1), taking into account previous phylogenetic hypotheses for both taxa (ants [4, 6, 8, 9]; bees [5, 22–25]). We performed the tests with the most taxon-rich 187-taxon dataset and with a taxonomically balanced 100-taxon dataset. In all cases, the alternative topologies were rejected ($p < 0.01$), providing unequivocal support for the preferred topology presented here. Based on these analyses and our current level of taxon sampling, ants are the sister group to Apoidea, and bees are clearly highly derived crabronid wasps.

Second, we analyzed the impact of taxon sampling on our phylogenetic results. Previous phylogenomic studies based on far fewer taxa than we included here have obtained conflicting results regarding the placement of ants vis-à-vis Apoidea. The transcriptome-based study of Johnson et al. [6] found ants to be sister to Apoidea (the result here), whereas the UCE-based study of Faircloth et al. [7] found ants to be sister to all other aculeates, with the exception of Chrysidoidea, which was not included in their analyses. We divided the taxon-sampling experiments into the following categories (Figure 2): (1) variations of Johnson et al. [6], (2) variations of Faircloth et al. [7], and (3) variations of the current taxon set. In the first category, we generated two datasets, one with exactly the same taxon sampling as [6] ("Johnson-19T") and one with the chrysidoid *Argochrysis armilla* removed ("Johnson-18T"). We included this particular manipulation because the major difference between [6] and [7] was the presence or absence of Chrysidoidea, which is the sister taxon to all other aculeate groups. For the Faircloth et al. [7] manipulations, we recreated the original 45-taxon matrix ("Faircloth-45T") and several alternative taxon sets. First, we added a single chrysidoid ("Faircloth-46T"), and then we continued to add additional aculeates to balance taxa across major lineages ("Faircloth-52T," "Faircloth-56T," and "Faircloth-61T"). We also balanced the dataset by removing excessive ant taxa from the original dataset ("Faircloth-26T") and then adding in a chrysidoid ("Faircloth-27T"). For the third category, we generated a dataset with most outgroups removed ("Hym-147T"), leaving *Nasonia* as the earliest diverging outgroup and *Megaspilus* (Ceraphronoidea), Evanioidea, and Trigonaloidea as more recently diverging outgroups. From this taxon set, we removed chrysidoids ("Hym-133T") and chrysidoids plus trigonaloids ("Hym-131T"). We also created what we considered to be the most balanced dataset by removing excessive ant, bee, and wasp taxa ("Hym-100T").

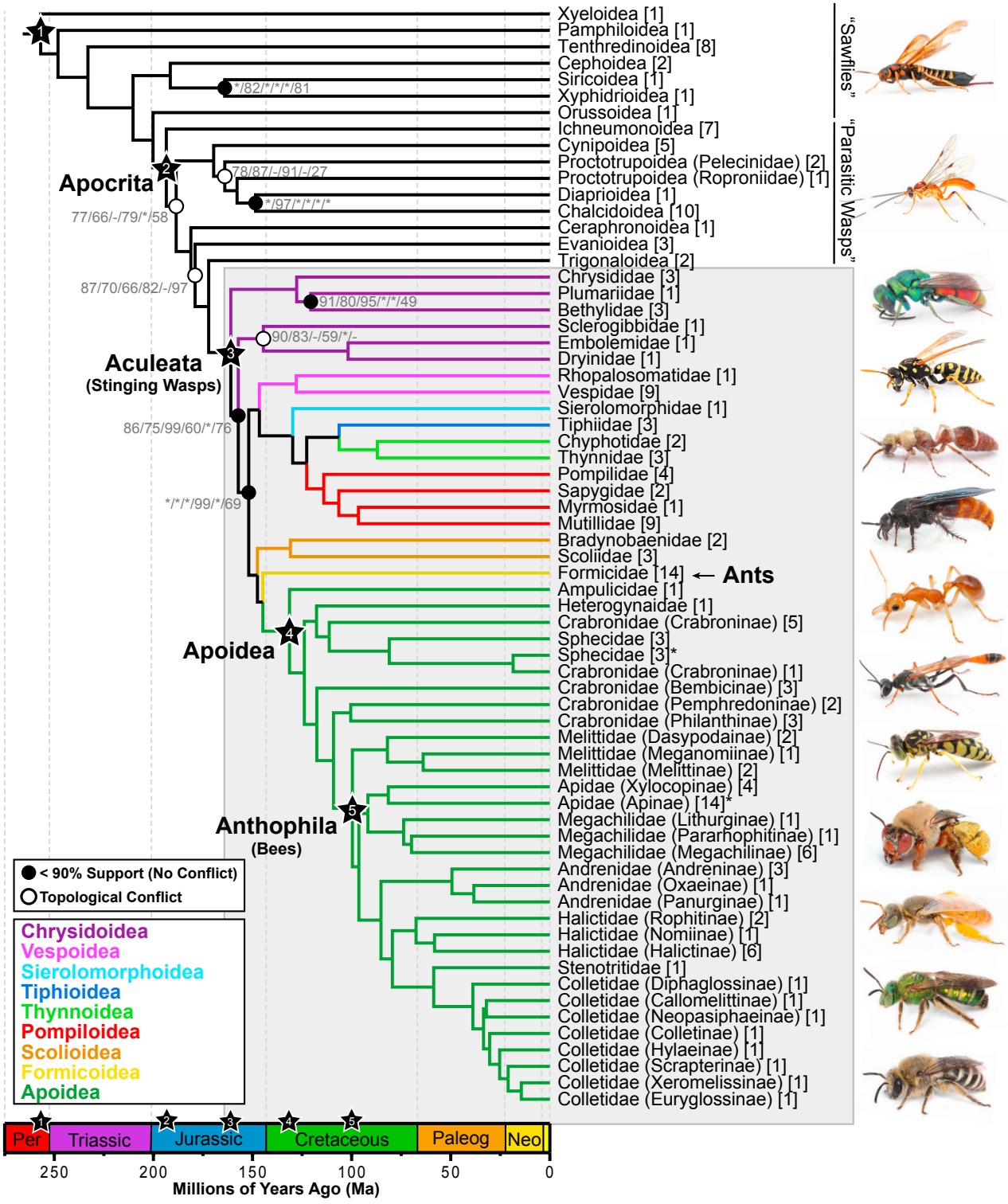

Figure 1. Dated Phylogeny of Aculeate Wasps and Outgroups

We inferred the topology by analyzing the Hym-187T-F75 matrix in RAxML (partitioned by k-means algorithm; 854 loci; 203,095 bp of sequence data) and estimated the dates in BEAST (50 random loci; fixed topology; 38 calibration points). Black dots indicate nodes that were recovered in all analyses but that received <90% support in at least one analysis. White dots indicate nodes with some topological conflict among analyses. Support values are provided for six analyses and are given in the following order: raxml-rcluster/raxml-kmeans/raxml-ry-coding/raxml-bcomp/exabayes-kmeans/astral. The asterisk and dash indicate 100% and 0% support, respectively. An asterisk by a terminal taxon name indicates paraphyly, and bracketed numbers indicate the number of samples. Sawfly, parasitoid wasp, and ant images are ©Alex Wild, used with permission. All other images are ©Joseph S. Wilson, used with permission. See also Figures S1 and S2 and Data S1.

Table 1. Results from SH Tests Comparing Our Favored Placement of Bees and Ants with 14 Alternative Positions for Bees and Nine Alternative Positions for Ants

		100-Taxon Matrix				187-Taxon Matrix			
Tree	Position of Bees	Likelihood	D(LH)	SD	Significance	Likelihood	D(LH)	SD	Significance
Best tree	bees + [Pemphredoninae+Philanthinae]	−8,889,479	NA	NA	NA	−10,006,566	NA	NA	NA
Alt. tree 1	bees + Philanthinae	−8,889,755	−276	40	<0.01	−10,006,870	−304	38	<0.01
Alt. tree 2	bees + Pemphredoninae	−8,889,790	−311	38	<0.01	−10,006,904	−338	36	<0.01
Alt. tree 3	bees + Crabronidae (excl. Crabroninae)	−8,890,408	−928	74	<0.01	−10,007,072	−506	61	<0.01
Alt. tree 4	bees + Bembicinae	−8,890,609	−1,130	66	<0.01	−10,007,264	−698	53	<0.01
Alt. tree 5	bees + apoid wasps (excl. Ampulicidae)	−8,891,776	−2,297	117	<0.01	−10,007,885	−1,319	96	<0.01
Alt. tree 6	bees + [Heterogynaidae+[Crabroninae+ Sphecidae]]	−8,891,895	−2,416	113	<0.01	−10,008,008	−1,442	92	<0.01
Alt. tree 7	bees + Crabronidae (incl. Sphecidae)	−8,892,128	−2,649	121	<0.01	−10,008,220	−1,654	101	<0.01
Alt. tree 8	bees + Heterogynaidae	−8,892,298	−2,819	122	<0.01	−10,008,354	−1,788	101	<0.01
Alt. tree 9	bees + [Crabroninae+Sphecidae]	−8,892,303	−2,824	123	<0.01	−10,008,352	−1,786	102	<0.01
Alt. tree 10	bees + Crabronidae (excl. Sphecidae)	−8,892,355	−2,876	129	<0.01	−10,008,517	−1,951	110	<0.01
Alt. tree 11	bees + Crabroninae	−8,892,953	−3,474	140	<0.01	−10,008,952	−2,386	120	<0.01
Alt. tree 12	bees + Sphecidae	−8,892,966	−3,487	140	<0.01	−10,008,974	−2,408	119	<0.01
Alt. tree 13	bees + all apoid wasps	−8,893,198	−3,719	140	<0.01	−10,008,819	−2,253	115	<0.01
Alt. tree 14	bees + Ampulicidae	−8,893,210	−3,731	139	<0.01	−10,008,838	−2,272	114	<0.01
Tree	Position of Ants	Likelihood	D(LH)	SD	Significance	Likelihood	D(LH)	SD	Significance
Best tree	ants + Apoidea	−8,889,479	NA	NA	NA	−10,006,566	NA	NA	NA
Alt. tree 1	ants + [Scolioidea+Apoidea]	−8,889,710	−231	39	<0.01	−10,006,715	−149	32	<0.01
Alt. tree 2	ants + Scolioidea	−8,889,720	−241	37	<0.01	−10,006,677	−111	33	<0.01
Alt. tree 3	ants + Aculeata (excl. Chrysidoidea)	−8,890,755	−1,276	86	<0.01	−10,007,370	−804	73	<0.01
Alt. tree 4	ants + [Vespoidea+Tiphioidea]	−8,890,868	−1,389	83	<0.01	−10,007,479	−913	69	<0.01
Alt. tree 5	ants + Vespoidea	−8,891,195	−1,716	94	<0.01	−10,007,756	−1,191	81	<0.01
Alt. tree 6	ants + Tiphioidea	−8,891,245	−1,766	93	<0.01	−10,007,781	−1,215	81	<0.01
Alt. tree 7	ants + [Vespoidea+Scolioidea]	−8,891,453	−1,974	108	<0.01	−10,007,901	−1,335	90	<0.01
Alt. tree 8	ants + Aculeata	−8,892,268	−2,789	126	<0.01	−10,008,670	−2,104	113	<0.01
Alt. tree 9	ants + [Tiphioidea+Scolioidea]	−8,897,047	−7,568	205	<0.01	−10,011,698	−5,132	178	<0.01

We performed the analyses unpartitioned using the complete 187-taxon matrix and a taxonomically balanced 100-taxon matrix. Our favored topology was significantly better than the alternatives in all cases. D(LH), difference in likelihood scores; alt., alternative; excl., excluding; incl., including; NA, not applicable.

The results of the taxon-sampling experiments (Table 2) support the conclusion that, even with genome-scale data, both outgroup choice and taxonomic balance impact phylogenetic results. The Faircloth et al. [7] study suffered from both of these issues, and we suspect that the trees obtained in that study are incorrect with regard to the position of ants. Focusing on the placement of ants (Formicoidea: Formicidae), we recovered three alternative topologies (Figure 2; Table 2): ants sister to Apoidea (topology A); ants sister to all other groups, minus Chrysidoidea (topology B); and ants sister to Apoidea plus Scolioidea (topology C). In both of the Johnson et al. [6] matrices, we recovered topology A. Analysis of the original Faircloth et al. [7] taxon set (Faircloth-45T) produced topology B, as in the original study. For Faircloth-46T, Faircloth-52T, and Faircloth-56T, we also recovered topology B. However, in the Faircloth-61T analyses, the topology shifted to C, placing ants as sister to Scolioidea plus Apoidea. The difference between Faircloth-56T and Faircloth-61T was the addition of several chrysidoids (Embolemidae and Dryinidae), Rhopalosomatidae (Vespoidea), and Ampulicidae (Apoidea), with the latter two taxa breaking long branches. Reducing and balancing the taxa of Faircloth-45T also altered the resulting topology. Reducing the number of ant taxa from 22 in Faircloth-45T to three taxa in Faircloth-26T changed the topology to A. The Hym-147T matrix and variants (Hym-133T, Hym-131T, and Hym-100T) also produced topology A. For the Hym-100T matrix, in which we reduced the number of ant and bee taxa to balance the larger taxon set, all relationships, in addition to the placement of ants, were the same as those in the ML analysis of the Hym-187T matrix. In addition to the topological differences just described, removing outgroups from matrices (chrysidoids or trigonaloids) usually resulted in decreased bootstrap scores for the position of ants (Table 2).

Biological Implications

Our results resolve long-standing debates in aculeate phylogeny and provide a solid framework for understanding both the importance of pollenivory as a driver of bee diversification and the importance of eusociality as a driver of ant diversification and ecological dominance. Ants and bees are surprisingly closely related, which impacts how we view the evolution of important

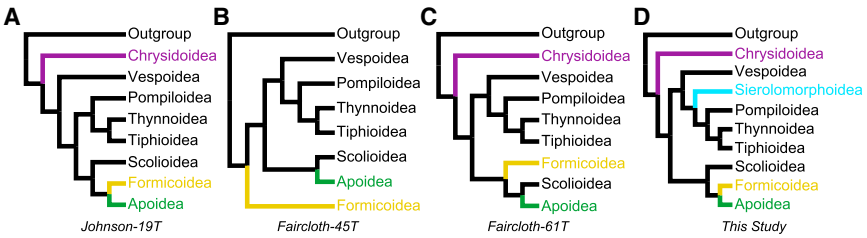

Figure 2. Alternative Hypotheses for Relationships among Aculeate Superfamilies

Topology from Johnson et al. [6] (A), topology from Faircloth et al. [7] (B), topology from analysis of the Faircloth-*61T* alignment supermatrix (C), and preferred topology inferred in this study (includes Sierolomorphoidea) (D). Topologies correspond to those reported in Table 2, except that topologies A and D are equivalent in terms of ants being sister to Apoidea.

behaviors, such as nest building, central place foraging, and eusociality in Aculeata [6]. It is important to highlight the fact that eusociality has evolved once at the origin of ants and at least six to eight times within bees [20], which means that the clade containing ants and bees may be particularly pre-disposed to becoming social. As discussed in [6], understanding the biology of all of the lineages within the Apoidea (bees and apoid wasps) will provide new insights into the biological factors that promote the evolution of social behavior.

Our results largely corroborate previous findings regarding relationships within Apoidea [5] and bees [20]. We confirm the placement of Ampulicidae as sister to the remaining Apoidea and the placement of bees as sister to the crabronid subfamilies Philanthinae+Pemphredoninae. The close affinities of bees to the crabronid subfamilies Philanthinae and Pemphredoninae have been suggested previously in studies based both on morphological and molecular data (reviewed in [5]). Philanthinae include ground-nesting wasps that hunt a variety of prey, including beetles, ants, and, ironically, bees. Pemphredoninae include small, mostly cavity-nesting wasps that hunt diverse prey, including Collembola (springtails), Thysanoptera (thrips), and an array of plant-feeding Hemiptera (aphids, scales, psyllids, cicadellids, cercopids, and membracids). Together, Pemphredoninae and Philanthinae comprise just over 2,200 described species [26]. That the bees, with over 20,000 described species, are sister to a group of just 2,200 hunting wasp species would suggest that the switch from predation to pollenivory was a significant driver of diversification in bees. Future studies should include an even broader sampling of Pemphredoninae and Philanthinae to test this hypothesis.

Within bees, our results provide further confirmation that Melittidae, previously thought to be sister to long-tongued bees (Apidae+Megachilidae) based on morphology [27], is monophyletic and sister to the remaining bee families. Family-level relationships in bees are fully congruent with previous studies [20]. It is notable that most of our analyses recovered the eusocial corbiculate bees (honeybees, bumblebees, and stingless bees) as monophyletic and sister to the weakly social Euglossini (orchid bees), thus favoring a single origin of eusociality within the group. Relationships among these taxa have been controversial, but our result agrees with a recent phylogenomic study that found that controlling for base-compositional heterogeneity favored monophyly of eusocial corbiculates [28].

Conclusions

The coupling of NGS with reduced representation phylogenomics has driven a revolution in molecular systematics, making it possible to generate large datasets at a fraction of the cost of traditional methods [29, 30]. Here, we further applied one promising approach, the targeted enrichment of UCEs [12], to the megadiverse insect order Hymenoptera, greatly extending a previous study that first employed the UCE method in arthropods [7]. We focused on family-level relationships of the stinging wasps (Aculeata) and produced a robust backbone phylogeny that provides many insights into the evolutionary history of this group. In addition, by carrying out a series of taxon-sampling experiments, we have demonstrated that even in the era of phylogenomics, careful taxon sampling can be of critical importance, with both outgroup choice and taxon evenness having a significant impact on topology and bootstrap support.

EXPERIMENTAL PROCEDURES

UCE Sequencing Workflow and Bioinformatics

The protocols for generating UCE data followed those reported in Faircloth et al. [7] and are described in detail in the Supplemental Experimental Procedures. For newly sampled taxa, we performed the following steps: DNA extraction, library preparation, sample pooling, UCE enrichment, enrichment verification, final pooling, and Illumina sequencing. For all bioinformatics steps, from read cleaning to alignment, we used the PHYLUCE v1.5 software package [31].

Phylogenomic Analyses of the Complete Taxon Set

Using the Hym-187T-F75 locus set, we carried out ML and BI analyses on the concatenated matrix with the programs RAxML v8 [16] and ExaBayes v1.4 [17], respectively (additional analytical details are in the Supplemental Experimental Procedures). For ML searches, we compared the following partitioning schemes: (1) unpartitioned, (2) partitioned by locus, (3) partitioned with the hcluster algorithm in PartitionFinder v1 [32] (data pre-partitioned by locus), (4) partitioned with the rcluster algorithm in PartitionFinder v2 [33], and (5) partitioned with the k-means algorithm [34] in PartitionFinder v2. For the BI analysis, we used the k-means partitioning scheme because this scheme resulted in the highest log likelihood in ML analyses (see Data S1 for partitioning results). For ST analysis, we used the summary method implemented in ASTRAL-II v4.8.0 [18] and employed weighted statistical binning [19], which was developed to reduce ST inference error caused by the inclusion of loci that have few informative sites.

To further test our results and to remove potential data biases, we carried out two additional analyses on the Hym-187T-75T locus set using RAxML. For the first analysis, we converted the concatenated matrix to RY coding, and for the second analysis, we used the program BaCoCa v1.1 [35] to identify and remove loci deviating significantly ($p < 0.01$) from base composition homogeneity among taxa. The latter analysis identified 47 offending loci, leaving 807 loci for concatenation and analysis ("Hym-187T-F75-BComp").

Divergence Dating

We employed node dating and used the program BEAST v1.8.2 [36] to estimate divergence dates on the complete 187-taxon tree. To calibrate the analysis, we used 37 fossils representing taxa from across Hymenoptera and one secondary calibration (252 Ma; taken from [37]) for the root node (see Data S1 for calibration information). For Aculeata, we selected a subset of the fossils used in two recent molecular studies [38, 39]. Importantly, we included the oldest known fossils for bees (*Melittosphex burmensis*), ants (*Haidomyrmex*

Table 2. Results of the Taxon Inclusion/Exclusion Experiments as Evidenced by Topological and Bootstrap Support Differences

Taxon Set	Topology	BS (Ants+Sister Group)	Outgroup	Notes
Johnson-18T	A	89	no chrysidoid	–
Johnson-19T	A	100	–	same taxon set as in [6]
Faircloth-26T	A	88	no chrysidoid	–
Faircloth-27T	A	97	–	–
Faircloth-45T	B	100	no chrysidoid	same taxon set as in [7]
Faircloth-46T	B	99	–	–
Faircloth-52T	B	98	–	–
Faircloth-56T	B	100	–	–
Faircloth-61T	C	100	–	–
Hym-100T	A	100	–	most balanced taxon set
Hym-131T	A	90	no chrysidoid or trigonaloid	–
Hym-133T	A	100	no chrysidoid	–
Hym-147T	A	100	–	–
Hym-187T-F75	A	100	–	this study

The results suggest that both outgroup choice (chrysidoid presence/absence) and taxon evenness can affect outcomes. The matrix name indicates whether the taxon set is a version of Johnson et al. [6], Faircloth et al. [7], or this study (Hym-). Three different topologies were recovered: ants sister to Apoidea (A); ants sister to all other aculeate superfamilies, except Chrysidoidea (B); and ants sister to Apoidea+Scoliodea (C). Bootstrap support indicates support for the clade that includes ants plus its sister group. Topologies correspond to those shown in Figures 1A–1C, in relation to the position of ants only. BS, bootstrap support.

cerberus and *Kyromyrma neffi*), Apoidea (Angarosphecidae), and Aculeata (*Sclerogibbodes embioleia*). We used the Fossilworks database [40] (http://www.fossilworks.org) to date fossils, and we followed best practices for node dating [41]. To decrease computation time in BEAST, we used a constraint topology, and we tested three different locus sets: (1) 25 best loci (where "best" indicates the highest mean gene-tree bootstrap score), (2) 50 best loci, and (3) 50 randomly selected loci. For additional details, see the Supplemental Experimental Procedures.

ACCESSION NUMBERS

For newly sequenced samples, raw sequence reads and contigs representing UCE loci are available from the NCBI Sequence Read Archive (SRA) and GenBank, respectively (NCBI BioProject: PRJNA379583). Additional data and results, including Trinity assemblies, alignments, alignment supermatrices, and all phylogenetic trees, are available from the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.r8d4q).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and one data file and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.03.027.

AUTHOR CONTRIBUTIONS

All authors conceived the ideas and designed methodology, M.G.B. collected and analyzed the data, and M.G.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGMENTS

We would like to thank Dave Smith and Crystal Cooke-McEwen for donating specimens. We thank Jeffrey Sosa-Calvo, Ana Ješovnik, and Mike Lloyd for assistance with lab work. For sequencing, we thank Joe DeYoung at the University of California, Los Angeles Neurosciences Genomics Core and Peter Schweitzer at the Cornell Genomics Facility. Lab work for this study was conducted at the Smithsonian National Museum of Natural History Laboratory of Analytical Biology (LAB), and phylogenetic analyses were performed using the Smithsonian's High-Performance Computer Cluster (Hydra) and the CIPRES Science Gateway [42]. We thank two anonymous reviewers for helpful suggestions. This work was supported, in part, by a Smithsonian Institution Peter Buck Postdoctoral Fellowship (M.G.B.) and several US National Science Foundation grants (DEB-1354996, DEB-0814544, DEB-0742998, and DEB-1555905). Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture (USDA). The USDA is an equal opportunity provider and employer.

Received: October 7, 2016
Revised: February 10, 2017
Accepted: March 13, 2017
Published: April 3, 2017

REFERENCES

1. Grimaldi, D., and Engel, M.S. (2005). Evolution of the Insects (Cambridge University Press).
2. Hölldobler, B., and Wilson, E.O. (1990). The Ants (Belknap Press).
3. Michener, C.D. (2007). The Bees of the World, Second Edition (The Johns Hopkins University Press).
4. Pilgrim, E.M., von Dohlen, C.D., and Pitts, J.P. (2008). Molecular phylogenetics of Vespoidea indicate paraphyly of the superfamily and novel relationships of its component families and subfamilies. Zool. Scr. *37*, 539–560.
5. Debevec, A.H., Cardinal, S., and Danforth, B.N. (2012). Identifying the sister group to the bees: a molecular phylogeny of Aculeata with an emphasis on the superfamily Apoidea. Zool. Scr. *41*, 527–535.
6. Johnson, B.R., Borowiec, M.L., Chiu, J.C., Lee, E.K., Atallah, J., and Ward, P.S. (2013). Phylogenomics resolves evolutionary relationships among ants, bees, and wasps. Curr. Biol. *23*, 2058–2062.
7. Faircloth, B.C., Branstetter, M.G., White, N.D., and Brady, S.G. (2015). Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. Mol. Ecol. Resour. *15*, 489–501.
8. Heraty, J., Ronquist, F., Carpenter, J.M., Hawks, D., Schulmeister, S., Dowling, A.P., Murray, D., Munro, J., Wheeler, W.C., Schiff, N., and

Sharkey, M. (2011). Evolution of the hymenopteran megaradiation. Mol. Phylogenet. Evol. *60*, 73–88.

9. Brothers, D.J. (1975). Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. Univ. Kans. Sci. Bull. *50*, 483–648.
10. Klopfstein, S., Vilhelmsen, L., Heraty, J.M., Sharkey, M., and Ronquist, F. (2013). The hymenopteran tree of life: evidence from protein-coding genes and objectively aligned ribosomal data. PLoS ONE *8*, e69344.
11. Brothers, D.J., and Carpenter, J.M. (1993). Phylogeny of Aculeata: Chrysidoidea and Vespoidea (Hymenoptera). J. Hymenopt. Res. *2*, 227–304.
12. Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T., and Glenn, T.C. (2012). Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. Syst. Biol. *61*, 717–726.
13. Sharkey, M.J. (2007). Phylogeny and classification of Hymenoptera. Zootaxa *548*, 521–548.
14. Sharkey, M.J., Carpenter, J.M., Vilhelmsen, L., Heraty, J., Liljeblad, J., Dowling, A.P.G., Schulmeister, S., Murray, D., Deans, A.R., Ronquist, F., et al. (2012). Phylogenetic relationships among superfamilies of Hymenoptera. Cladistics *28*, 80–112.
15. Castro, L.R., and Dowton, M. (2006). Molecular analyses of the Apocrita (Insecta: Hymenoptera) suggest that the Chalcidoidea are sister to the diaprioid complex. Invertebr. Syst. *20*, 603–614.
16. Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics *30*, 1312–1313.
17. Aberer, A.J., Kobert, K., and Stamatakis, A. (2014). ExaBayes: massively parallel bayesian tree inference for the whole-genome era. Mol. Biol. Evol. *31*, 2553–2556.
18. Mirarab, S., and Warnow, T. (2015). ASTRAL-II: coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. Bioinformatics *31*, i44–i52.
19. Bayzid, M.S., Mirarab, S., Boussau, B., and Warnow, T. (2015). Weighted statistical binning: enabling statistically consistent genome-scale phylogenetic analyses. PLoS ONE *10*, e0129183.
20. Danforth, B.N., Cardinal, S., Praz, C., Almeida, E.A.B., and Michez, D. (2013). The impact of molecular data on our understanding of bee phylogeny and evolution. Annu. Rev. Entomol. *58*, 57–78.
21. Shimodaira, H., and Hasegawa, M. (1999). Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. *16*, 1114–1116.
22. Ohl, M., and Bleidorn, C. (2005). The phylogenetic position of the enigmatic wasp family Heterogynaidae based on molecular data, with description of a new, nocturnal species (Hymenoptera: Apoidea). Syst. Entomol. *31*, 321–337.
23. Alexander, B.A. (1992). An exploratory analysis of cladistic relationships within the superfamily Apoidea, with special reference to sphecid wasps (Hymenoptera). J. Hymenopt. Res. *1*, 25–61.
24. Prentice, M.A. (1998). The comparative morphology and phylogeny of apoid wasps (Hymenoptera: Apoidea). PhD thesis (University of California, Berkeley).
25. Melo, G.A.R. (1999). Phylogenetic relationships and classification of the major lineages of Apoidea (Hymenoptera), with emphasis on the crabronid wasps. Sci. Pap. Univ. Kansas Nat. Hist. Mus. *14*, 1–55.
26. Pulawski, W.J. (2017). Catalog of Sphecidae *sensu lato* (=Apoidea excluding Apidae). https://www.calacademy.org/scientists/projects/catalog-of-sphecidae.
27. Roig-Alsina, A., and Michener, C.D. (1993). Studies of the phylogeny and classification of long-tounged bees (Hymenoptera: Apoidea). Univ. Kans. Sci. Bull. *55*, 124–162.
28. Romiguier, J., Cameron, S.A., Woodard, S.H., Fischman, B.J., Keller, L., and Praz, C.J. (2016). Phylogenomics controlling for base compositional bias reveals a single origin of eusociality in corbiculate bees. Mol. Biol. Evol. *33*, 670–678.
29. Lemmon, E.M., and Lemmon, A.R. (2013). High-throughput genomic data in systematics and phylogenetics. Annu. Rev. Ecol. Evol. Syst. *44*, 19.1–19.23.
30. McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C., and Brumfield, R.T. (2013). Applications of next-generation sequencing to phylogeography and phylogenetics. Mol. Phylogenet. Evol. *66*, 526–538.
31. Faircloth, B.C. (2016). PHYLUCE is a software package for the analysis of conserved genomic loci. Bioinformatics *32*, 786–788.
32. Lanfear, R., Calcott, B., Ho, S.Y.W., and Guindon, S. (2012). Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. *29*, 1695–1701.
33. Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., and Calcott, B. (2016). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. Published online December 23, 2016. http://dx.doi.org/10.1093/molbev/msw260.
34. Frandsen, P.B., Calcott, B., Mayer, C., and Lanfear, R. (2015). Automatic selection of partitioning schemes for phylogenetic analyses using iterative k-means clustering of site rates. BMC Evol. Biol. *15*, 13.
35. Kück, P., and Struck, T.H. (2014). BaCoCa--a heuristic software tool for the parallel assessment of sequence biases in hundreds of gene and taxon partitions. Mol. Phylogenet. Evol. *70*, 94–98.
36. Drummond, A.J., Suchard, M.A., Xie, D., and Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. *29*, 1969–1973.
37. Zhang, C., Stadler, T., Klopfstein, S., Heath, T.A., and Ronquist, F. (2016). Total-evidence dating under the fossilized birth-death process. Syst. Biol. *65*, 228–249.
38. Wilson, J.S., Dohlen, C.D., Forister, M.L., and Pitts, J.P. (2012). Family-level divergences in the stinging wasps (Hymenoptera: Aculeata), with correlations to angiosperm diversification. Evol. Biol. *40*, 101–107.
39. Cardinal, S., and Danforth, B.N. (2013). Bees diversified in the age of eudicots. Proc. Biol. Sci. *280*, 20122686.
40. Alroy, J. (2016). Fossilworks: gateway to the paleobiology database. http://www.fossilworks.org.
41. Magallon, S.A. (2004). Dating lineages: molecular and paleontological approaches to the temporal framework of clades. Int. J. Plant Sci. *165*, S7–S21.
42. Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Proceedings of the Gateway Computing Environments Workshop (GCE) (IEEE), pp. 1–8.