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Report

Phylogenomics Resolves Evolutionary Relationships among Ants, Bees, and Wasps

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Summary

Eusocial behavior has arisen in few animal groups, most notably in the aculeate Hymenoptera, a clade comprising ants, bees, and stinging wasps [1–4]. Phylogeny is crucial to understanding the evolution of the salient features of these insects, including eusociality [5]. Yet the phylogenetic relationships among the major lineages of aculeate Hymenoptera remain contentious [6–12]. We address this problem here by generating and analyzing genomic data for a representative series of taxa. We obtain a single well-resolved and strongly supported tree, robust to multiple methods of phylogenetic inference. Apoidea (spheciform wasps and bees) and ants are sister groups, a novel finding that contradicts earlier views that ants are closer to ectoparasitoid wasps. Vespid wasps (paper wasps, yellow jackets, and relatives) are sister to all other aculeates except chrysidoids. Thus, all eusocial species of Hymenoptera are contained within two major groups, characterized by transport of larval provisions and nest construction, likely prerequisites for the evolution of eusociality. These two lineages are interpolated among three other clades of wasps whose species are predominantly ectoparasitoids on concealed hosts, the inferred ancestral condition for aculeates [2]. This phylogeny provides a new framework for exploring the evolution of nesting, feeding, and social behavior within the stinging Hymenoptera.

Results and Discussion

Aculeate (stinging) Hymenoptera are behaviorally diverse, encompassing both solitary and eusocial species and exhibiting a variety of life history strategies including parasitoidism, predation, omnivory, and pollenivory [2, 13]. Multiple lines of evidence provide strong support for the monophyly of the Aculeata [9, 10], but relationships among the major lineages within this group have been a matter of continued uncertainty [7–9, 11, 12]. The position of ants, the most species-rich and ecologically dominant of all eusocial insects, has been particularly problematic [7–9, 11, 12] (Figure 1).

Advances in next-generation sequencing have unleashed the potential of genomic data to clarify many previously intractable parts of the Tree of Life [14–16]. Here we addressed the problem of aculeate Hymenoptera phylogeny by generating transcriptome data for ten representative species in nine families and genomic data for one key taxon (*Apterogyna*) for which RNA was unavailable (Table 1; see also Table S1 available online). We then combined these data with the published genome sequences of three bee species and three ant species, a transcriptome from one additional bee species, and genomic data from *Nasonia vitripennis*, a nonaculeate hymenopteran used as an outgroup. Orthology identification and matrix assembly was accomplished with the OrthologID pipeline [17]. This yielded multiple partitioned amino acid matrices, with different levels of gene representation across the 18 ingroup taxa, ranging from a 5,214-gene matrix (3,001,657 amino acid sites) to a stringently filtered matrix of 308 genes (175,404 sites) and only 14.98% missing data (see Experimental Procedures and Supplemental Experimental Procedures).

Table 1 shows the summary statistics for all the transcriptomes and for the genomic assembly. For the transcriptomes, we identified a range of protein sequence numbers, with the scoliid wasp *Crioscolia alcione* having the largest transcriptome size and the sweat bee *Lasioglossum albipes* the smallest. Whether variation in the transcriptome sizes represents actual variation in the number of genes present in these species or whether it represents variation in the quality of the assemblies is uncertain. The genomic library of *Apterogyna* AZ01 (family Bradynobaenidae) had only 1,717 genes, with a median amino acid length of 450. This reflects the relatively low coverage of sequencing (18×) and the apparent degradation of this older sample. Nevertheless, the *Apterogyna* sequence data were sufficient to reliably place this taxon within the hymenopteran tree.

Phylogenetic analyses produced a fully resolved tree of the aculeate Hymenoptera with robust support at all nodes (Figure 2). The same tree topology and relative branch lengths were obtained under a variety of analytical procedures, including partitioned maximum likelihood (ML) analyses and Bayesian analyses of concatenated data sets, as well as species tree estimates (Figures 2 and S1). All nodes in the topology have ML bootstrap support of 100% and Bayesian posterior probabilities of 1.0. Under species tree analyses, most nodes are also strongly supported, although support values drop for some of the deeper nodes in the tree (Figures 2 and S1). Most procedures employed the 308-gene matrix, but we also ran ML analyses of three other matrices of varying size and completeness (525, 3,018, and 5,214 genes, respectively), with the same results (Figures S1A–S1C).

As expected [6] we found that the cuckoo wasp (*Argochrysis*) is sister to all other aculeates, and that ants, bees, apoids, and vespid wasps are all monophyletic. We recovered the vespid wasps (represented by a nonsocial pollen wasp, *Pseudomasaris*, and a eusocial paper wasp, *Mischocyttarus*) as sister to all aculeates except the cuckoo wasp, a result that is in agreement with some other recent molecular studies [8, 11] although in strong conflict with morphology-based trees [7] in which vespids are nested well within the aculeate phylogeny, as sister to scoliid wasps (Figure 1A).

Of particular interest is the finding that ants are sister to Apoidea, a novel result that emphasizes a greater affinity of ants to the predatory wasps that characterize the earliest branching lineages of Apoidea than to scoliids, bradynobaenids, tiphiids, and other ectoparasitoid wasps with which they have been associated previously [1, 7, 9, 12]. This result

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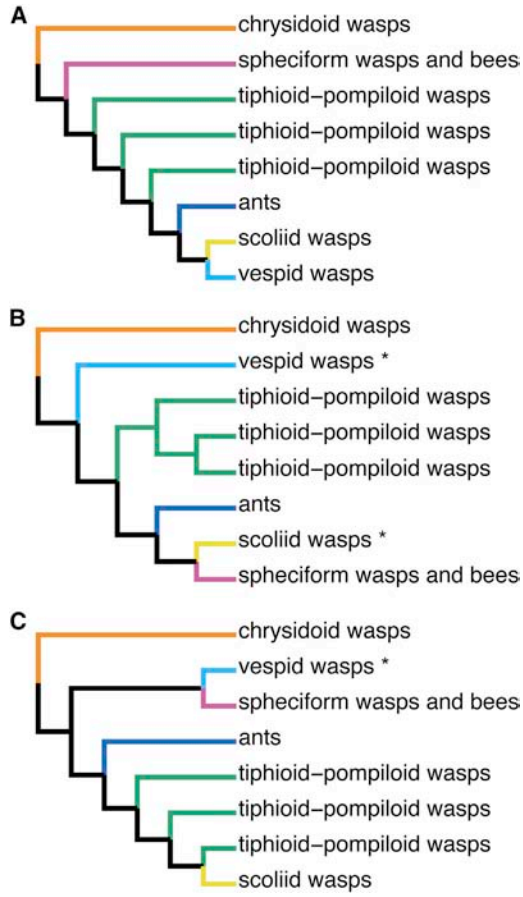

Figure 1. Previous Hypotheses of Phylogenetic Relationships among Ants, Bees, and Stinging Wasps

Morphological analysis of Brothers [7] (A) and molecular phylogenetic studies of Pilgrim et al. [8] (B) and Heraty et al. [9] (C). Major groups have differently colored branches. Vespid wasps * = Vespidae + Rhopalosomatidae; scoliid wasps * = Scoliidae + Bradynobaenidae.

receives significant statistical support ($p < 0.01$) under the Shimodaira-Hasegawa test [23] against five alternate phylogenetic placements, including ants as sister to scolioid wasps, and ants as sister to scolioid wasps plus apoids (Table S3). Thus, morphologically generalized apoid wasps such as Ampulicidae (cockroach wasps) and Sphecidae (digger wasps, mud dauber wasps, and relatives) may provide more insight into the early evolution of stem-group ants than the ectoparasitoid wasps that have previously served as models for the origin of ants.

Our results also provide new perspective on the lower Cretaceous fossil *Cariridris bipetiolata*, originally claimed to be the oldest fossil ant [24] but later reinterpreted to be a spheciform wasp, probably belonging to Ampulicidae [25]. Although this appeared to represent a major reassignment of the fossil, our discovery that ants and apoids are sister taxa helps to explain difficulty in the placement of *Cariridris* [26] and suggests that it is best treated as a lineage close to the root of the ant-apoid tree, perhaps not assignable with certainty to either branch.

The sister group of [ants + Apoidea] is [Scoliidae + Bradynobaenidae sensu stricto]. This more inclusive clade was also recovered in some other molecular studies [8, 11], but with a different branching order, such that the scolioid lineage was identified as sister to Apoidea. Our new results motivate a search for features in common between ants and apoids—not shared with scolioid wasps—that might predispose this group toward the evolution of sociality. The most obvious behavioral commonality is the collection and transport of resources (arthropod prey or pollen) to a constructed nest, a trait also shared with vespid wasps, the other group containing eusocial species. Scoliid wasps, in contrast, are ectoparasitoids on concealed hosts [2, 27]. (The life history of Bradynobaenidae remains to be elucidated.) It has long been argued that nest construction and provisioning are key prerequisites for the evolution of eusociality [1, 2]. The finding that ants and apoids are sister taxa suggests that this favorable combination of traits arose only once in their common ancestor, rather than separately from ectoparasitoid predecessors in the ant and apoid lineages, emphasizing that the preconditions for eusociality are rare and contingent.

Our phylogeny reveals a well-supported clade of tiphioid and pompiloid wasps, to the exclusion of the scolioids. This comports with an earlier molecular study [8], except that we find that the tiphiid wasp (*Brachycistis*) and the chyphotine wasp (*Chyphotes*) are sister taxa, to the exclusion of pompiloids (spider wasps and velvet ants), a result inconsistent with previous findings. Further taxon sampling is needed within this clade to test the monophyly and placement of tiphiid wasps (Tiphiidae) and velvet ants (Mutillidae), but our results confirm an earlier inference [8, 11] that the family Bradynobaenidae is not monophyletic, with true bradynobaenids (represented in our data set by *Apterogyna*) being sister to Scoliidae, whereas the subfamily Chyphotinae (represented here by *Chyphotes*) is part of the tiphioid complex. Bradynobaenid-like wasps share a number of morphological features [7], some unique, and these must be interpreted as examples of convergence between two distantly related clades, perhaps generated in part by the independent loss of wings in females of both groups. It should be noted that scoliid females are winged, as are some members of the tiphioid-pompiloid clade, so winged females are the ancestral condition for both clades.

The phylogenetic results presented here support the following scenario of behavioral evolution in aculeate Hymenoptera (Figure 3). The ancestral aculeate wasp was an ectoparasitoid, attacking and paralyzing concealed hosts and leaving its offspring in or near the host cavity [2]. In two major lineages (ants + Apoidea, and Vespidae), this behavior became modified as wasps adopted a more active predatory lifestyle, with increased importance of prey transport, nest construction, and parental care. More specialized feeding habits (pollenivory) were acquired later. Eusocial behavior evolved multiple times within both of these lineages [4, 28, 29]. The three remaining clades of aculeates (chrysidoids, scolioids, and the tiphioid-pompiloid clade) have largely retained ectoparasitoid habits, except for pompilids [30], and no examples of eusociality are known in these groups.

This is the first comprehensive phylogenomic analysis of aculeate Hymenoptera. It demonstrates the utility and feasibility of employing transcriptome data to resolve outstanding problems in insect phylogeny. The new tree provides a robust framework for investigating the evolution of nesting, feeding, and social behavior within the stinging Hymenoptera, and for exploring genomic signatures of changes in these characteristics.

Table 1. Species of Hymenoptera Sampled, and Summary Statistics for the Transcriptome Assemblies and the Genome Assembly

Species	Common Name	Assembly Size (MB)	Number of Sequences (>300 aa)	Mean Protein Length (aa)
Apterogyna ZA01 (Bradynobaenidae)	bradynobaenid wasp	284.2	1,717	450
Chyphotes mellipes (Bradynobaenidae)	bradynobaenid wasp	137.8	26,513	698
Argochrysis armilla (Chrysididae)	cuckoo wasp	91.6	18,777	690
Stigmatomma oregonense (Formicidae)	dracula ant	74.8	13,867	721
Sphaeropthalma orestes (Mutillidae)	velvet ant (wasp)	84.5	15,820	707
Pepsis grossa (Pompilidae)	spider wasp	150.3	27,549	703
Crioscolia alcione (Scoliidae)	scoliid wasp	219.2	45,155	689
Sceliphron caementarium (Sphecidae)	mud dauber wasp	161.3	33,499	659
Brachycistis timberlakei (Tiphiidae)	tiphiid wasp	84.3	15,036	627
Mischocyttarus flavitarsis (Vespidae)	paper wasp	76.8	15,907	676
Pseudomasaris vespoides (Vespidae)	pollen wasp	103.7	21,543	678
Lasioglossum albipes (Halictidae)	sweat bee	46.7	9,417	585

The assembly for *Apterogyna* is a partial genome assembly; all others are transcriptome assemblies. In addition to the assemblies derived from new sequence data generated in this study, we also assembled a transcriptome for the sweat bee *Lasioglossum albipes* based on raw paired-end short reads downloaded from the NCBI Sequence Read Archive (SRR578269). MB, megabases; aa, amino acids. See also Tables S1 and S2.

Experimental Procedures

Taxon Sampling

Eleven species from key families across the aculeate Hymenoptera were chosen for the generation of new phylogenomic data (Tables 1 and S1). These represent all the major lineages of stinging Hymenoptera that have been considered in previous hypotheses of phylogenetic relationships [6–12]. Ten species were collected in the field, while one rare species, *Apterogyna* ZA01, was available only as preserved specimens in ethanol. We included this second representative of Bradynobaenidae because of instability in the phylogenetic position of bradynobaenid wasps in previous studies [7, 8, 11, 12]. We supplemented our data on these eleven species with transcriptome data on one bee species (*Lasioglossum albipes*) from the NCBI Sequence Read Archive and published genome assemblies of three other bee species (*Megachile rotundata*, *Bombus terrestris*, and *Apis mellifera*) and three ant species (*Harpegnathos saltator*, *Linepithema humile*, and *Pogonomyrmex barbatus*).

Sequencing and Assembly

For the fresh collected samples, cDNA libraries were prepared, while for the ethanol-preserved sample, a DNA library was prepared (further details in Supplemental Information, including Table S2). Samples were pooled and sequenced on an Illumina HiSeq 2000 (100 bp paired-end). Transcriptomes were assembled using the Trinity software package [31], while ABySS was used for the genome assembly [32]. After translation of contigs into amino acid sequences, orthology was evaluated using a prerelease version 2.0 of the OrthologID pipeline [17]. OrthologID takes complete gene sets from all taxa and assigns them into gene clusters. It then generates a parsimony tree for each gene cluster and extracts one or more sets of orthologous genes. Orthologous sets of genes were then assembled into multiple partitioned matrices with different levels of taxon representation per gene, including (1) a 5,214-gene matrix with 3,001,657 amino acid sites and at least 9 ingroup taxa represented per gene partition, (2) a 3,018-gene matrix with 1,653,740 sites and at least 16 ingroup taxa represented per partition, and (3) a matrix that has every gene partition represented across all ingroup taxa with the additional requirement that they be single-copy in five publicly available ingroup genomes (525 partitions and 298,968 sites). For Bayesian inference and species tree estimation, a fourth, smaller 308-gene matrix with 175,404 sites was used. Of the 3,332,676 cells (19 taxa × 175,404 sites) in this 308-gene matrix, 73.42% are coded as amino acids, 11.60% are gaps, and 14.98% are missing.

Phylogenetic Analysis

For all four matrices, we performed partitioned (by gene) ML analyses with Γ-distributed rate heterogeneity over sites using RAxML v7.4.2 [18, 19]. The best protein substitution model for each gene partition was selected individually using the "ProteinModelSelection.pl" script [19] over 36 different models. For Bayesian inference, we used PhyloBayes MPI v1.3b [20, 21],

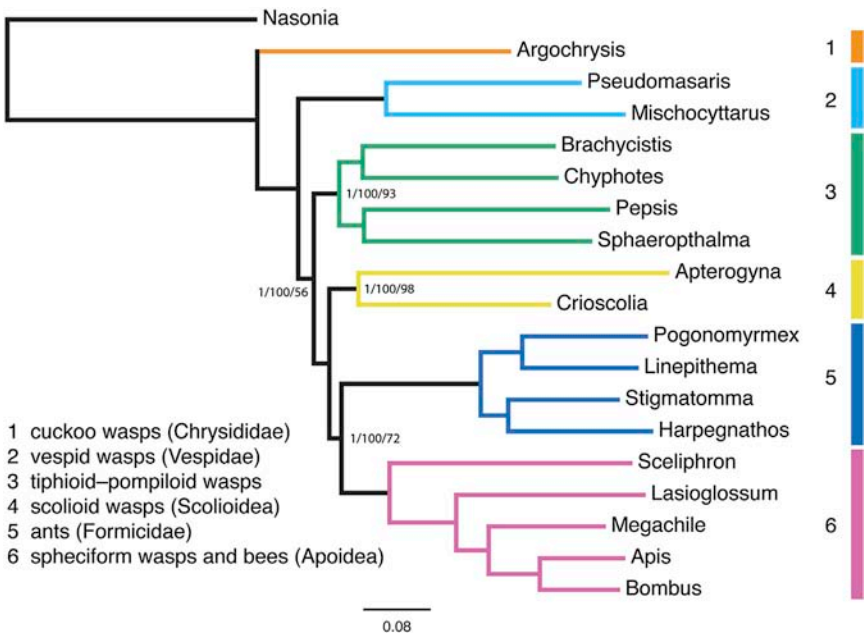

Figure 2. Maximum-Likelihood Tree of Aculeate Hymenoptera Derived from a 308-Gene Matrix

This tree was estimated with RAxML [18, 19] based on a 308-gene matrix with 175,404 amino acid sites. Support values are given in the following order: (1) posterior probabilities from a separate Bayesian analysis with PhyloBayes [20, 21], (2) RAxML bootstrap percentages based on 1,000 replicates, and (3) bootstrap percentages from a separate species tree analysis with STAR [22]. Unlabeled nodes have maximum support values (1/100/100). Scale bar indicates number of substitutions per site. From the top of the tree downward, species are as follows: *N. vitripennis* (Pteromalidae), *A. armilla* (Chrysididae), *P. vespoides* (Vespidae), *M. flavitarsus* (Vespidae), *B. timberlakei* (Tiphiidae), *C. mellipes* (Bradynobaenidae), *P. grossa* (Pompilidae), *S. orestes* (Mutillidae), *Apterogyna* ZA01 (Bradynobaenidae), *C. alcione* (Scoliidae), *P. barbatus* (Formicidae), *L. humile* (Formicidae), *S. oregonense* (Formicidae), *H. saltator* (Formicidae), *S. caementarium* (Sphecidae), *L. albipes* (Halictidae), *M. rotundata* (Megachilidae), *A. mellifera* (Apidae), and *B. terrestris* (Apidae). Major lineages are color coded using the same scheme as in Figure 1. See also Figure S1.

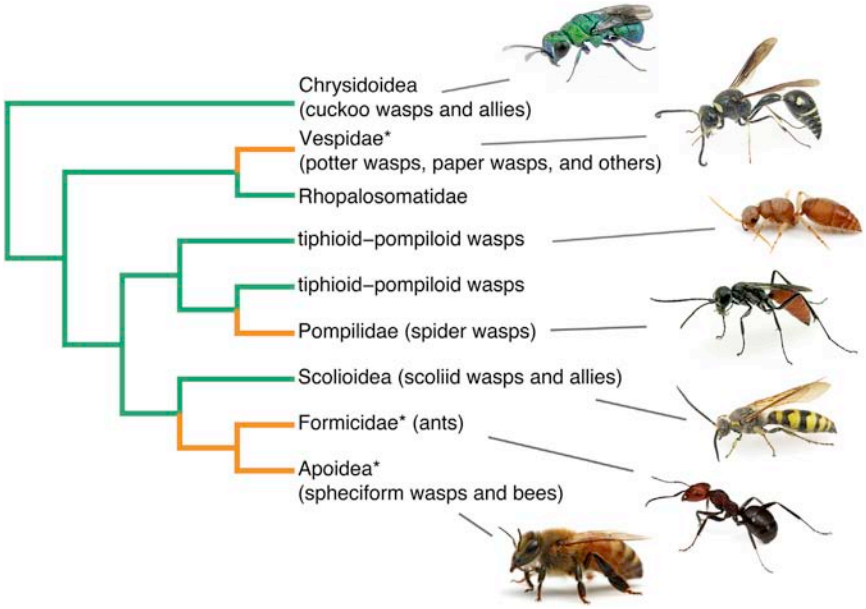

Figure 3. Evolution of the Aculeate Hymenoptera

Blue-green branches represent parasitoidism; orange branches represent nest construction and predation (with pollenivory and omnivory as derivative states thereof). Asterisks designate lineages containing eusocial species. Ants are entirely eusocial, but this is not true of all species of Vespidae and Apoidea. Biological information is from various sources, summarized in Gauld and Bolton [2] and Huber [13]. Names of superfamilies are modified from Pilgrim et al. [8]. Placement of Rhopalosomatidae is based on Pilgrim et al. [8] and Debevec et al. [11]. Images courtesy of Alexander Wild and Kurt Schaefer.

with CAT-GTR as the amino acid replacement model, in an unpartitioned analysis of the 308-gene matrix. A species tree was estimated on the basis of average ranks of gene coalescence events, as calculated in STAR [22]. The input for this analysis was 100 bootstrap replicate trees of each of 308 genes, built under maximum likelihood in RAxML. We also inferred a species tree with PhyloNet [33], which uses the parsimony-based criterion of minimizing deep coalescences [34]. We used 308 input trees with bootstrap support values generated in RAxML.

To evaluate alternate phylogenetic hypotheses against our best-scoring ML tree, we employed the Shimodaira-Hasegawa test [23]. Five constraints were considered (Table S3), and separate constrained partitioned analyses were conducted using RAxML on the same 308-gene matrix used to generate our ML tree (Figure 2). The five best trees satisfying the respective constraints were then subjected to the Shimodaira-Hasegawa test in RAxML ("-f h" option) to determine whether they were significantly worse than our best unconstrained ML tree.

Accession Numbers

Illumina reads have been deposited in the NCBI Sequence Read Archive with the accession number SRP020476. The matrices, partition files, and gene trees have been deposited in Dryad (http://doi.org/10.5061/dryad.jt440).

Supplemental Information

Supplemental Information includes one figure, three tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.08.050.

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