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Evidence for AT-Transversion Bias in Wasp (Hymenoptera: Symphyta) Mitochondrial Genes and Its Implications for the Origin of Parasitism

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Abstract. We inferred the incidence of nucleotide conversions in the COI and 16S rRNA mitochondrial genes of members of the Symphyta and basal Apocrita (Hymenoptera). Character-state reconstructions in both genes suggested that conversions between A and T (AT transversions) occurred much more frequently than any other type of change, although we cannot wholly discount an underlying transition bias. Parsimony analysis of COI nucleotide characters did not recover phylogeny; e.g., neither the Tenthredinoidea nor Apocrita were recovered as monophyletic. However, analysis of COI amino acid characters did recover these relationships, as well as others based on fossil and morphological evidence. Analysis of 16S rRNA characters also recovered these relationships providing conversions between A and T were down-weighted. Analysis of the combined data sets gave relatively strong support for various relationships, suggesting that both data sets supported similar topographies. These data sets, both separately and combined, suggested that the phytophagous Siricidae were more closely related to the predominantly parasitic Apocrita than were the ectoparasitic Orussoidea. This suggests that the wasp parasitic lifestyle did not have a single origin, unless the Siricidae have more recently reverted to phytophagy. Alternatively, parasitism evolved twice independently, once in the Orussoidea and again in the Apocrita. The latter scenario is supported by the observation that the evolution of parasitism was accompanied by a tendency for the larvae to develop inside plant tissues. Adaptations that accompanied the movement of wasps into a confined, wood-boring habitat may have preadapted them to becoming ectoparasitic.

Key words: Transversion bias — Symphyta — Cytochrome oxidase I — 16S rRNA — Phylogeny — Parasitism

Introduction

Parasitism has evolved independently within a number of insect orders, primarily in the wasps (Hymenoptera) and flies (Diptera), but also in the beetles (Coleoptera) and several other orders. In the two main parasitic lineages (wasps and flies), there is strong phylogenetic evidence that endoparasitism evolved multiple times, probably from ectoparasitic ancestors (McAlpine 1989; Whitfield 1992; Dowton and Austin 1994a). However, the origin of ectoparasitism (and hence parasitism per se) is not well understood. In the present study, we investigated the origin of ectoparasitism in the wasps—one of the largest and most biologically diverse group of insects.

The wasps are traditionally divided into the paraphyletic Symphyta (sawflies and woodwasps) and the monophyletic Apocrita (flexible-waisted wasps) (Gauld and Bolton 1988). Our recent molecular systematic analysis of the Hymenoptera recovered many apocritan relationships but was unable to recover symphytan relationships

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Abbreviations: COI, cytochrome oxidase I; T-PTP, topology-dependent permutation tail probability

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Table 1. Biologies of taxa for which sequence data were generated or obtained for the present study

Family	Taxon	Gene sequenced	Biology[c]
Symphyta			
Xyeloidea			
Xyelidae	*Macroxyela ferruginea* (Say)	COI	Free-living, phytophagous
Tenthredinoidea			
Pergidae	*Perga condei* (Benson)	COI, 16S[a]	Free-living, phytophagous
	Phylacteophaga froggatti (Riek)	COI, 16S[a]	Leaf-mining, phytophagous
Tenthredinidae	Undetermined	16S[b]	Free-living, phytophagous
Cephoidea			
Cephidae	*Hartigia trimaculata* (Say)	COI, 16S[a]	Stem-boring, phytophagous
Siricoidea			
Xiphydriidae	*Xiphydria mellipes* (Harris)	COI	Wood-boring, phytophagous
Siricidae	*Sirex noctilio* (Fabricius)	COI	Wood-boring, phytophagous
	Tremex columba (L.)	16S[b]	Wood-boring, phytophagous
Orussoidea			
Orussidae	*Orussus terminalis* (Newman)	COI, 16S[a]	Ectoparasitoid
Apocrita			
Stephanoidea			
Stephanidae	*Schlettererius cinctipes* (Cresson)	COI, 16S[a]	Ectoparasitoid
Trigonalyoidea			
Trigonalidae	*Poecilogonalos costalis* (Cresson)	COI, 16S[a]	Endoparasitoid
	Orthogonalys pulchella (Cresson)	COI, 16S[a]	Endoparasitoid

[a] Data from Dowton and Austin (1994a)
[b] Data from Derr et al. (1992a,b)
[c] Biological information from Goule (1993).

(Dowton and Austin 1994a), presumably because the gene fragment used (16S rRNA) was too variable for this deeper level. The Symphyta are widely accepted as the group from which all other wasps were derived (Rasnitsyn 1980), while the Apocrita are more specialized and contain all the parasitic wasps apart from the ectoparasitic Orussoidea. The phylogenetic position of the Orussoidea relative to the Apocrita is therefore crucial in distinguishing between a single and multiple origin of ectoparasitism. A single origin for ectoparasitism has been implied by the placement of the Orussoidea as the sister group to the Apocrita (Gibson 1985; Johnson 1988; Whitfield et al. 1989), while multiple origins are implied by the placement of the Orussoidea within the Siricoidea *sensu lato* (Königsmann 1977; Smith 1979; Rasnitsyn 1980). Even if the Orussoidea are removed from the Siricoidea, there is conflicting evidence as to whether the remaining Siricoidea (*sensu stricto*) are a natural (monophyletic) group (Whitfield et al. 1989) or are paraphyletic (Gibson 1985; Heraty et al. 1994). The lack of consensus between these studies may be due to their reliance on relatively few anatomical structures and to the difficulty in distinguishing between informative and noninformative characters (Gibson 1985, 1993). We therefore examined this phylogeny using molecular sequence data from the highly conserved COI and 16S rRNA genes (Simon 1991; Simon et al. 1994). We amplified and sequenced a fragment of these genes from those wasps that previous studies have indicated are closely related to the Orussoidea (Königsmann 1977; Rasnitsyn 1980, 1988; Gibson 1985; Johnson 1988; Whitfield et al. 1989; Whitfield 1992; Dowton and Austin 1994a; Heraty et al. 1994).

Materials and Methods

Sequence Generation. Sequences were obtained either from the literature or generated from the superfamilies that appear in Table 1. For the COI gene fragment, genomic DNA was extracted and amplified (Dowton and Austin 1994a) with the primers COI-RLR (Roehrdanz 1993) and COI-MD (5′-ATTGCAAATACTGCACCTAT-3′). These primers anneal to nucleotides 2496–2515 (COI-RLR) and 2934–2953 (COI-MD) of the honeybee mitochondrial genome (Crozier and Crozier 1993). The PCR product was thus 458 base pairs. Double-stranded PCR products were directly sequenced (Dowton and Austin 1993, 1994b). Outgroup sequences were obtained from GenBank from the Diptera [*Drosophila melanogaster* Meigen (de Bruijn 1983), *Phormia regina* (Meigen) (accession no. L14946), *Lucilia illustris* (Meigen) (accession no. L14945)], as was the Lepidoptera [*Choristoneura rosaceana* (Harris) (accession no. L19099)]. Details of the 16S rRNA sequence generation appear in Dowton and Austin (1994a).

Analysis. The fragment of the COI gene from all species studied was of the same length; alignment was therefore straightforward. Nucleotides homologous to positions 2532–2915 of the honeybee mitochondrial genome (Crozier and Crozier 1993) were translated before phylogenetic analysis by maximum (protein) parsimony using PAUP version 3.1.1 (Swofford 1993). The number of informative characters was 44. Parsimony analysis employed a step-matrix that calculated the minimum number of substitutions needed to convert one amino acid to another, using the *Drosophila* mitochondrial code. Silent substitutions were thus ignored.

For the 16S rRNA gene fragment, sequences were aligned based on the secondary-structure model of the *Drosophila yakuba* 16S rRNA gene (Clary and Wolstenholme 1985), as outlined previously (Dowton

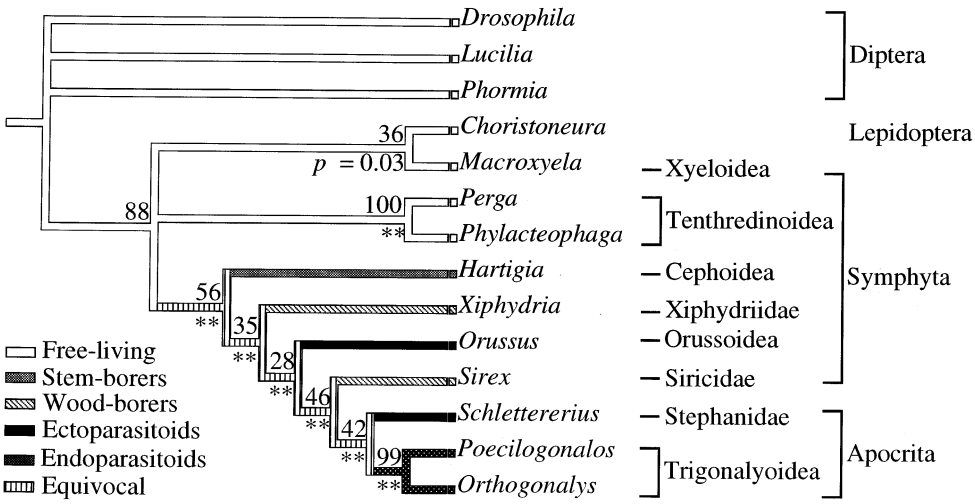

Fig. 1. Strict consensus of the four shortest trees after protein parsimony analysis of a fragment of the COI gene (128 amino acids) from various Hymenoptera. Four most parsimonious trees of 178 steps were found using the branch-and-bound algorithm. These differed only in the relationships between the dipteran outgroups and between the basal Hymenoptera (Xyeloidea and Tenthredinoidea). Taxa close to the Orussoidea were placed identically in each of the shortest trees. Boot strap values are indicated above the branch, while nodes supported by the T-PTP test at the $p < 0.01$ (**) level are indicated below the branch. The majority-rule tree of the most and near-most parsimonious trees [194 trees within 1% of the most parsimonious trees (Smith 1989)] also yielded a topology congruent with that shown. The shortest tree that placed the Orussoidea as the sister group to the Apocrita was five steps longer than the tree shown (see Table 2).

and Austin 1994a). One region, equivalent to nucleotides 910–965 of the fruitfly gene, was excluded due to its excessively high AT content (as much as 98% for some taxa). The number of informative characters was 215. Uninformative characters were not included in the analysis. A tree-length distribution of 100,000 randomly sampled trees was left skewed ($g_1 = -1.29$), indicating that the data contained significant phylogenetic information [$p < 0.01$ (Hillis and Huelsenbeck 1992)]. Parsimony analysis employed a step-matrix that weighed conversions between A and T as one step, with all other conversions given a weight of four (due to the approximately fourfold higher incidence of conversions between A and T). COI and 16S rRNA characters were analyzed both separately and combined. For the combined analysis, informative characters in both gene regions were placed into a single data matrix. Character-state changes were weighted as described above, according to the gene region from which the informative characters came. The degree of support for various nodes was assessed by the bootstrap (Felsenstein 1985) and T-PTP (Topology-dependent Permutation Tail Probability) (Faith 1991) tests. For these tests, 100 randomized or resampled data sets were assessed using the following search options: heuristic search, taxa added using the ''closest'' option, ten trees held at each step, and ''steepest descent'' option in effect. However, for the bootstrap analysis of the combined data set, the branch-and-bound algorithm was used to ensure that the shortest tree was found in each of the bootstrap replicates. A comparison of these two search strategies on the combined data set revealed that bootstrap proportions were slightly higher (1–2%) with the branch-and-bound strategy. For the T-PTP test, outgroup sequences were not randomized. In this test, the decay index (Bremer 1988) for each node is determined in the actual and each of the randomized data sets. For each node, p is the proportion of times a decay index is observed in the randomized data sets that is equal to or greater than the corresponding decay index in the actual data.

Results and Discussion

Analysis of the COI Gene Fragment

The COI gene is the most highly conserved mitochondrial protein-coding gene (Simon et al. 1994). It exhibits a level of identity of 70–91% between 280 and 90 Myr ago, respectively (Simon et al. 1994). As such, it should be appropriate for the present study, as the parasitic Hymenoptera first appeared in the fossil record during the early Jurassic (130–160 Myr ago: Rasnitsyn 1980). We found that the COI gene was highly conserved in the Hymenoptera; of the 128 amino acid positions sampled, only 56 were variable.

Parsimony analysis of COI nucleotide characters (first, second, and third positions analyzed separately, or all positions analyzed) did not recover phylogeny generally accepted from fossil and morphological evidence; e.g., the Xyeloidea and Tenthredinoidea: Pergidae should be among the most basal of the Symphyta (Rasnitsyn 1980, 1988). Recovery of relatively deep phylogenetic branches using nucleotide sequences may be confounded by the disparate evolutionary rates of synonymous and nonsynonymous sites, particularly in the presence of compositional bias (Loomis and Smith 1990), which is high in this gene fragment (Dowton and Austin 1995). Amino acid sequences are preferred in these conditions (Loomis and Smith 1990). Parsimony analysis of COI amino acid characters recovered the Xyeloidea and Pergidae:Tenthredinoidea as the most basal of the lineages included, as well as the nested pattern of relationships among the Symphyta (i.e., paraphyly; Fig. 1) that have been postulated from anatomical studies (Rasnitsyn 1980, 1988; Gibson 1985; Johnson 1988; Whitfield et al. 1989; Heraty et al. 1994). These results similarly suggested that COI amino acid sequences were most appropriate for parsimony analysis for the evolutionary timeframe assessed in the present study. Of the remaining sawflies, the Cephoidea were placed as least related to

Table 2. Bootstrap support and the shortest trees for various hypotheses concerning the closest relative to the Apocrita[a]

Closest relative to the Apocrita	Shortest tree			Bootstrap support		
	COI	16S	COI/16S	COI	16S	COI/16S
Siricidae	178	1,220	1,269	46	34	72
Orussoidea	183	1,251	1,306	1	0	0
Xiphydriidae	181	ND	ND	0	ND	ND
Cephoidea	179	1,238	1,288	9	0	5

[a] Informative characters were independently resampled, with replacement [bootstrapping (Felsenstein 1985)] prior to parsimony analysis (Swofford 1993). ND: not determined

the Apocrita, in agreement with certain anatomical studies (Gibson 1985; Johnson 1988; Rasnitsyn 1988; Whitfield et al. 1989; Heraty et al. 1994). The Siricoidea *sensu lato* (includes Siricidae, Xiphydriidae, Orussoidea) were found to be an unnatural group (i.e., paraphyletic), as has been previously suggested (Gibson 1985). This analysis placed Siricidae: *Sirex* as the most basal of the Symphyta, not the Orussoidea. Surprisingly, Lepidoptera: *Choristoneura* fell inside the Hymenoptera, whereas we expected it to fall outside the Hymenoptera with the Diptera. Although this disrupts the Panorpida, the result could reflect that this taxon is more closely related to the Hymenoptera than are the Diptera. Further, the sister relationship between Xyeloidea: *Macroxyela* and Lepidoptera: *Choristoneura* failed the T-PTP test. The observation that even randomized data tended to support this relationship indicates that this node should be collapsed. This leads to a polytomy between the most basal hymenopteran and a member of the Lepidoptera, with the relationships of the Panorpida and Hymenoptera unresolved.

Although the T-PTP test indicated that the various relationships were rarely recovered after randomization of the data, bootstrap proportions were generally low (Fig. 1), a likely result of the low number of informative characters used in the analysis (Hillis et al. 1994). Table 2 indicates the relative evidence for alternate hypotheses. Although the bootstrap supported Siricidae: *Sirex* as the sister to the Apocrita five times more than any other hypothesis, only one additional step was needed to collapse the Siricidae + Apocrita node to a polytomy including Cephoidea: *Hartigia*. Clearly, additional data were necessary to discriminate between these various hypotheses.

Evidence for AT-Transversion Bias

Our previous analysis of a broad range of hymenopteran 16S rRNA sequences did not resolve symphytan relationships (Dowton and Austin 1994a), presumably because the gene is too variable for this evolutionary time frame. However, differential weighting of character-state changes (according to their empirically determined frequency of occurrence) greatly increases the resolving power of such analyses (Hillis et al. 1994). For example, transversions are often given higher weight because they occur less frequently than transitions; analysis is thereby restricted to the more slowly evolving positions of the gene fragment. Measurement of the observed character-state changes in the hymenopteran 16S rRNA gene (Table 3), indicated that such weighting was inappropriate in this instance, as transitions occurred less frequently than transversions. Instead, conversions between A and T were observed much more frequently than any other character-state change (on average, four times more frequently than any other type of change). This result did not appear to be an artefact of assessing relatively remote taxa. Analysis of the most closely related clade of taxa (the Tenthredinoidea) indicated that AT transversions occurred six times more frequently than any other change. Similarly, AT transversions occurred five times more frequently than any other change in the COI gene. Further, a lack of transition bias was also observed in the protein-coding genes of the mitochondrial genome of *Apis* when compared with that of *Drosophila* (Crozier and Crozier 1993). These authors similarly suggested that changes between A and T are most frequent in the hymenopteran mitochondrial genome.

It has been argued that transitions universally outnumber transversions, even when transversion bias is indicated; assessment of more closely related species should reveal transition bias (Simon et al. 1994). Our evidence for AT-transversion bias, even within the Tenthredinoidea, cannot disregard this contention. Even if an underlying transition bias operates in these mitochondrial genes of the Hymenoptera: Symphyta, our evidence suggests that one particular type of transversion has predominated during the evolution of this group, a likely reflection of the high AT content of this genome. As such, down-weighting these mutations should have the same effect as down-weighting transitions when assessing more recently diverged lineages; i.e., the analysis will be restricted to the more slowly evolving positions of the gene fragment, extending the time frame for which the gene can recover phylogeny. Our finding that the down-weighted 16S rRNA parsimony analysis recovered a phylogeny very similar to a distinct data set in which AT-transversion bias was not presumed (see below) sup-

Table 3. Number of nucleotide substitutions observed after parsimony analysis (Swofford 1993) of a 16S rRNA gene fragment[a]

	To			
From	A	G	C	T
A	—	51.3	73.6	136.0
G	28.2	—	4.8	32.0
C	61.6	4.8	—	19.7
T	156.5	58.1	30.3	—

[a] Details of the analysis appear in Dowton and Austin (1994a). The present analysis was restricted to those wasps that appear in Fig. 2. Values were calculated from the average of all equally parsimonious character-state reconstructions (Maddison and Maddison 1992). Changes between A and T occurred, on average, four times more frequently than any other change

ports our contention that down-weighting transversions between A and T is appropriate in this instance.

Analysis of the 16S rRNA Gene Fragment

We subsequently analyzed symphytan/basal apocritan relationships using the 16S rRNA gene fragment, with changes between A and T weighted as a single step and all other changes weighted as four steps. Such a model of analysis, in the presence of compositional bias, has been recently suggested (Collins et al. 1994). Whereas unweighted parsimony did not resolve certain well-accepted relationships within the Symphyta (Fig. 2, left panel, e.g., the Apocrita should be monophyletic and more recently diverged than any of the Symphyta, and the Tenthredinoidea should be monophyletic and more basal than any of the other lineages surveyed), the down-weighted analysis placed these lineages appropriately (Fig. 2, right panel). Most importantly, Stephanidae: *Schlettererius* was resolved within the Apocrita (making the Apocrita monophyletic), while a member of the Tenthredinidae was resolved as the sister to the Pergidae, making the Tenthredinoidea monophyletic. We suggest that the down-weighting of conversions between A and T may generally increase resolution when analyzing high AT-content genes by parsimony.

The pattern of relationships recovered by the down-weighted 16S rRNA analysis was similar to the COI analysis and was identical to those recovered by the combined analysis (see below). The Tenthredinoidea were recovered as the most basal symphytan group included, while Siricidae: *Sirex* was recovered as the sister to the Apocrita. Further, the pattern of relationships recovered was identical when conversions between A and T were down-weighted across a broad range (four to ten times all other conversions).

Similar to the COI analysis, bootstrap proportions for the various relationships were generally low (Table 2), perhaps due to the relatively few informative characters. Evidence for alternate hypotheses appears in Table 2. Although bootstrap proportions were generally lower than for the COI analysis, no support was found for alternate hypotheses with this test, while 18 more steps were necessary to disrupt the sister relationship between Siricidae: *Tremex* and the Apocrita. Although evidence from this analysis was more discriminating than the COI analysis, the absolute bootstrap proportions were too low to constitute strong support. Evidence from simulation studies indicates that the bootstrap is a conservative estimate of phylogenetic confidence limits (Zharkikh and Li 1992; Hills and Bull 1993), with bootstrap proportions of $\geqslant 70\%$ corresponding to a probability that a particular clade is real of $\geqslant 95\%$ (Hillis and Bull 1993).

Combined Analysis of the 16S rRNA and COI Gene Fragments

We subsequently assessed phylogenetic relationships in the Symphyta/basal Apocrita after combining the COI and 16S rRNA data sets. We did not assess the relationships of Xyeloidea and Xiphydriidae in the combined analysis as sequence data were not available for both gene regions. The combined analysis was identical to the separate 16S rRNA gene analysis (Fig. 3). However, bootstrap proportions were much higher for the combined analysis (Table 2), probably due to the addition of informative characters that supported a similar topography. This analysis again suggested that the Siricidae, not the Orussoidea, were the sister group to the Apocrita, inferring multiple origins of parasitism in the wasps. Very little support was found for a monophyletic origin of parasitism; Orussoidea: *Orussus* was not recovered as the sister to the Apocrita in any of the bootstrap resampled data matrices, and 37 additional steps were required to make Orussoidea: *Orussus* the sister to the Apocrita.

Implications for the Evolution of Ectoparasitism

Within the Hymenoptera, the Apocrita contain all the parasitic wasps apart from the ectoparasitic Orussoidea. The phylogenetic placement of the Orussoidea therefore distinguishes between a single and multiple origins of parasitism in the wasps. In the present study, parsimony analyses of COI and 16S rRNA sequences, both separately and combined, suggested that the Siricidae were more closely related to the Apocrita than the Orussoidea. This phylogenetic arrangement allows one to infer that the ectoparasitic lifestyle did not have a single origin, in disagreement with certain other studies. The validity of the few anatomical characters that have been identified as synapomorphic for an Orussoidea + Apocrita relationship relies on the accurate deduction of the groundplan structure of those characters (Gibson 1993). This in turn relies on the identification of the sister group to the Hymenoptera, a relationship that is still extremely unclear given the isolated nature of the Hymenoptera relative to

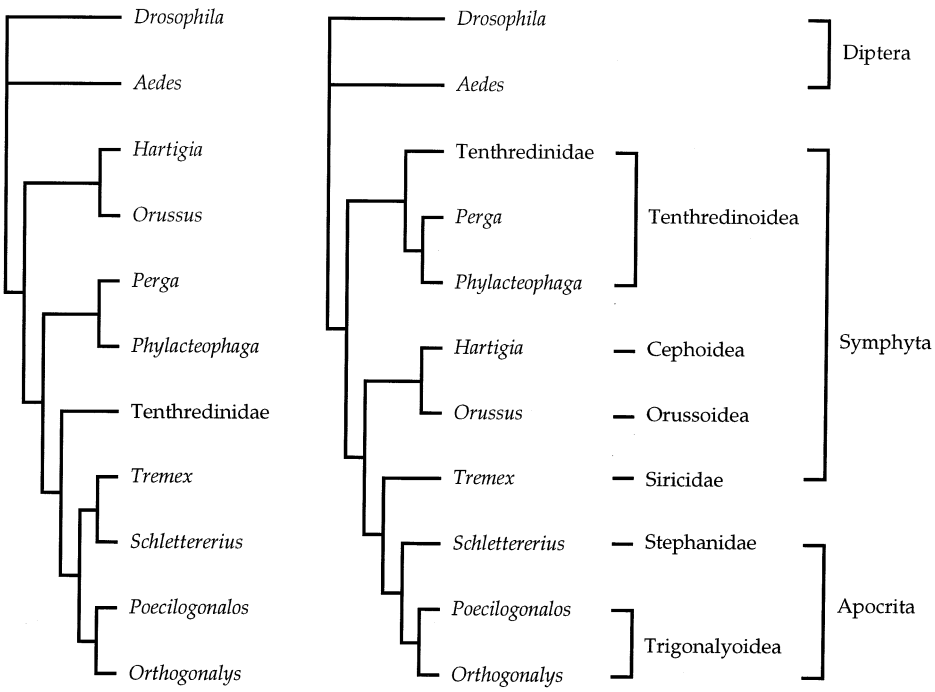

Fig. 2. Alternate phylogenies of the Symphyta/basal Apocrita based on analysis of a 16S rRNA gene fragment (Left) with all state changes weighted equally, and (Right) with changes between A and T down-weighted fourfold relative to all other changes. A single most parsimonious tree was found with both data sets using the branch-and-bound algorithm. In (the left panel) neither the Tenthredinoidea (*Perga, Phylacteophaga*, Tenthredinidae) nor the Apocrita (*Schlettererius, Poecilogonalos, Orthogonalys*) were recovered as monophyletic, whereas in (the right panel) both natural groups were recovered. Further, in (the right panel) the Tenthredinoidea fall out as the most basal of the included Symphyta, while the Apocrita were the most recently diverged lineage, in accord with morphological and fossil evidence.

other endopterygote and panorpid orders (Kristensen 1991). This limitation applies to both morphological and molecular studies and stresses the need for further investigation of higher phylogenetic relationships.

Our discrimination between these two alternate hypotheses relies on single exemplars from both the Siricidae and Orussoidea, and must be considered as preliminary evidence only. Since it is, we do not propose taxonomic revision of the Symphyta. Nevertheless, the only way to maintain the Orussoidea as sister group of the Apocrita in our analyses is to argue that the two gene fragments have convergently evolved in the Apocrita and two members of the Siricidae (*Sirex* and *Tremex*). Further, evidence from analysis of both COI and 16S rRNA genes separately and combined indicated that the models of analysis employed were appropriate; most well-accepted relationships were recovered. The only relationships that were questionable were the apparent sister relationships between Lepidoptera: *Choristoneura* and Hymenoptera: Xyeloidea: *Macroxyela* (COI analysis) and between Cephoidea:*Hartigia* and Orussoidea:*Orussus* (16S rRNA and combined gene analyses). However, bootstrap support for the latter relationship was clearly lower than for any other relationship, while both relationships failed the T-PTP test. The observation that randomized data tended to support these relationships indicates that these nodes are indeed questionable and should be collapsed.

Assuming the Siricidae are the sister group to the Apocrita, two equally parsimonious descriptions for the evolution of parasitism are possible. The first hypothesis maintains a single origin of parasitism (in the common ancestor of the Orussoidea + Siricidae + Apocrita), with a reversion to phytophagy in the Siricidae. Although it may seem implausible that a highly specialized parasitic lineage should revert to phytophagy, there are a number of lineages within the Apocrita that have discarded parasitism to adopt specialized forms of phytophagy (e.g., bees, seed wasps, gall wasps). The second hypothesis contends that parasitism had two independent origins, once in the Orussoidea and again in the Apocrita. Support for this hypothesis comes from the observation that the evolution of the Apocrita from their symphytan ancestors was accompanied by a tendency for the larvae to develop in relatively confined situations, such as within the stems of soft plant tissues and within woody tissues (Gibson 1985). The closest relatives (Xiphydriidae and Siricidae) of these two ectoparasitic lineages share a number of anatomical structures related to their wood-boring habitat (Benson 1955). Thus adaptations that accompanied the movement of wasps into a confined, wood-boring habitat may have preadapted them to becoming ectoparasitic. Given that anatomical (reviewed in Whitfield 1992), biological (Whitfield 1992), and molecular (Dowton and Austin 1994a) data confirm that endoparasitism in the wasps had multiple origins, it is

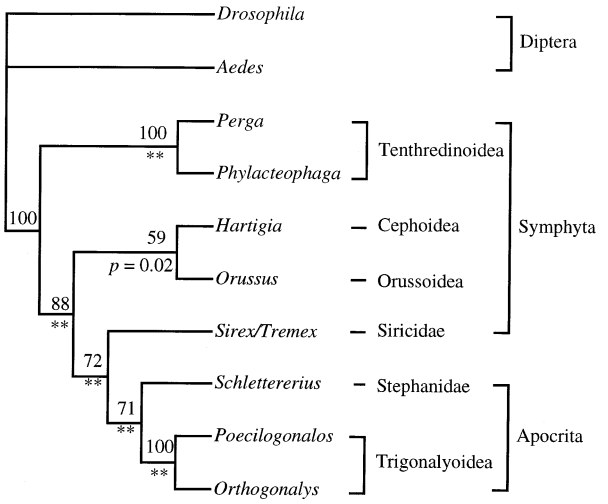

Fig. 3. Phylogeny of Hymenoptera closely related to the Orussoidea, based on combined COI and 16S rRNA data sets. A single most parsimonious tree was found, using the branch-and-bound algorithm (1,269 steps). Bootstrap values are indicated above the branch, while nodes supported by the T-PTP test at the $p < 0.01$ (**) level are indicated below the branch. Only one other tree was found within 1% of the most parsimonious tree. This tree (1,276 steps) had a topology identical to that depicted in Fig. 1 for the taxa included, i.e., *Hartigia* and *Orussus* were paraphyletic rather than sister groups. The shortest tree that placed the Orussoidea as the sister group to the Apocrita was 37 steps longer than the tree shown (see Table 2). *Phormia* indicates that this composite outgroup taxon consisted of *Phormia COI* with *Aedes* 16S rRNA sequence, due to the unavailability of a second dipteran outgroup in which both gene regions have been sequenced. Similarly, *Sirex/Tremex* consisted of *Sirex* COI with *Tremex* 16S rRNA sequence; both are from the family Siricidae (see Table 1).

perhaps less surprising that ectoparasitism might have multiple origins. The transition from an ectoparasitic to an endoparasitic lifestyle is an order of magnitude more complex, given that the endoparasitoid must not only deal with the behavioral defences of its host but also avoid the host's immune response (Whitfield 1992).

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