Ancient Rapid Radiations of Insects: Challenges for Phylogenetic Analysis

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Abstract

Phylogenies of major groups of insects based on both morphological and molecular data have sometimes been contentious, often lacking the data to distinguish between alternative views of relationships. This paucity of data is often due to real biological and historical causes, such as shortness of time spans between divergences for evolution to occur and long time spans after divergences for subsequent evolutionary changes to obscure the earlier ones. Another reason for difficulty in resolving some of the relationships using molecular data is the limited spectrum of genes so far developed for phylogeny estimation. For this latter issue, there is cause for current optimism owing to rapid increases in our knowledge of comparative genomics. At least some historical patterns of divergence may, however, continue to defy our attempts to completely reconstruct them with confidence, at least using current strategies.

WHAT IS AN ANCIENT RAPID RADIATION?

In this review, we focus on cases of diversification in which lineages of insects have diverged in rapid succession within a relatively short time span in the ancient past, generating patterns of molecular and morphological change that are difficult to discern phylogenetically. We refer to these patterns as ancient rapid radiations, with no implication that evolutionary change has accelerated in these cases; instead, it is lineage splitting or diversification that has happened rapidly. While insects diverged spectacularly in the Permian, and again in the Jurassic, and have been diverging ever since, "ancient" in this context refers not necessarily to a specific age, but to a high ratio between the amount of time that has elapsed since divergences occurred and the time span in which they occurred. These high ratios are of course more characteristic of divergences that are many millions of years old.

THE PHYLOGENETIC SIGNATURE OF A RAPID RADIATION

Rokas et al. (103) have referred to the typical molecular phylogenetic pattern that characterizes an ancient rapid radiation as its signature. The signature can be described as the significantly closer temporal spacing (compression) of a number of cladogenetic or lineage-splitting events in a phylogeny than would be expected by either stochastic or relatively constant diversification. **Figure 1** depicts a pattern of this sort. The only evidence of some relationships among the taxa we will ever see must have accumulated on these short horizontal branches (internodes) that link the taxa together. All subsequent changes along the branches may have an impact on how similar taxa appear, but will not bear directly on their relationship.

Although such a phylogenetic pattern is to be expected from an ancient rapid radiation, its signature can be obscured by other

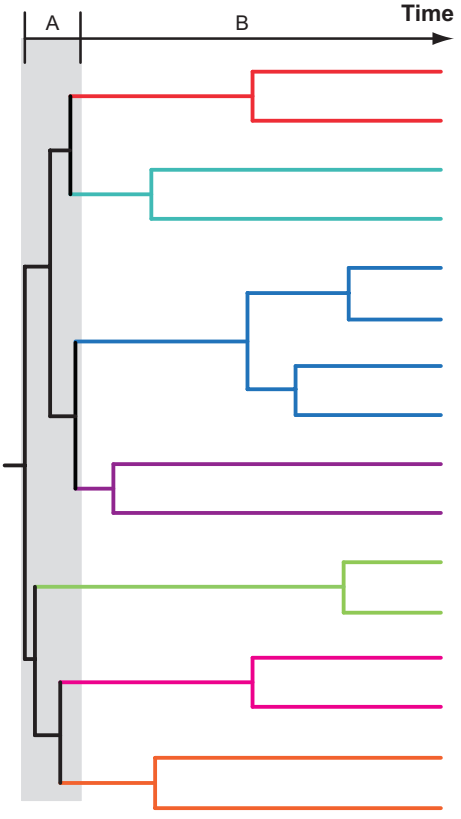


Figure 1

Diagrammatic representation of an ancient rapid radiation, with the crucial short internal branches highlighted with a gray bar.

factors such as inadequate data, conflict within or among datasets, or loss of phylogenetic signal over time. Unfortunately, many insect datasets are plagued by short, ancient internodes, lineage-specific substitution rate biases (substitutions accumulate at different rates among lineages), and lineage-specific base compositional biases (the nucleotides are sometimes found in different proportions among lineages, and among regions of the genes). These problems frequently combine with the inclusion of data whose substitution rates are wildly inappropriate for the questions they are intended to address, to make insect phylogenetics a particularly challenging enterprise. We briefly review some methods for diagnosing confounding factors with

ancient radiations—as more extensive recent reviews are available (101, 129).

COMPLICATIONS WITH ANALYZING ANCIENT RAPID RADIATIONS

Phylogenetic studies of insects, especially studies that address relationships among higher taxa, often exhibit a portion of the phylogeny with low support or resolution. When a large amount of data has been analyzed, a possible cause for the poor support is a rapid radiation, because it would result in truly short interdivergence times for characters to accumulate within. Unfortunately, such a signature of a rapid radiation can also result from, or alternatively be obscured by, a variety of other causes related to data quality. It is thus important to test whether the available data are appropriate for resolving relationships at the hierarchical level being analyzed, and to determine whether confounding biases in the data are interfering with signal extraction.

Are the Genes Appropriate?

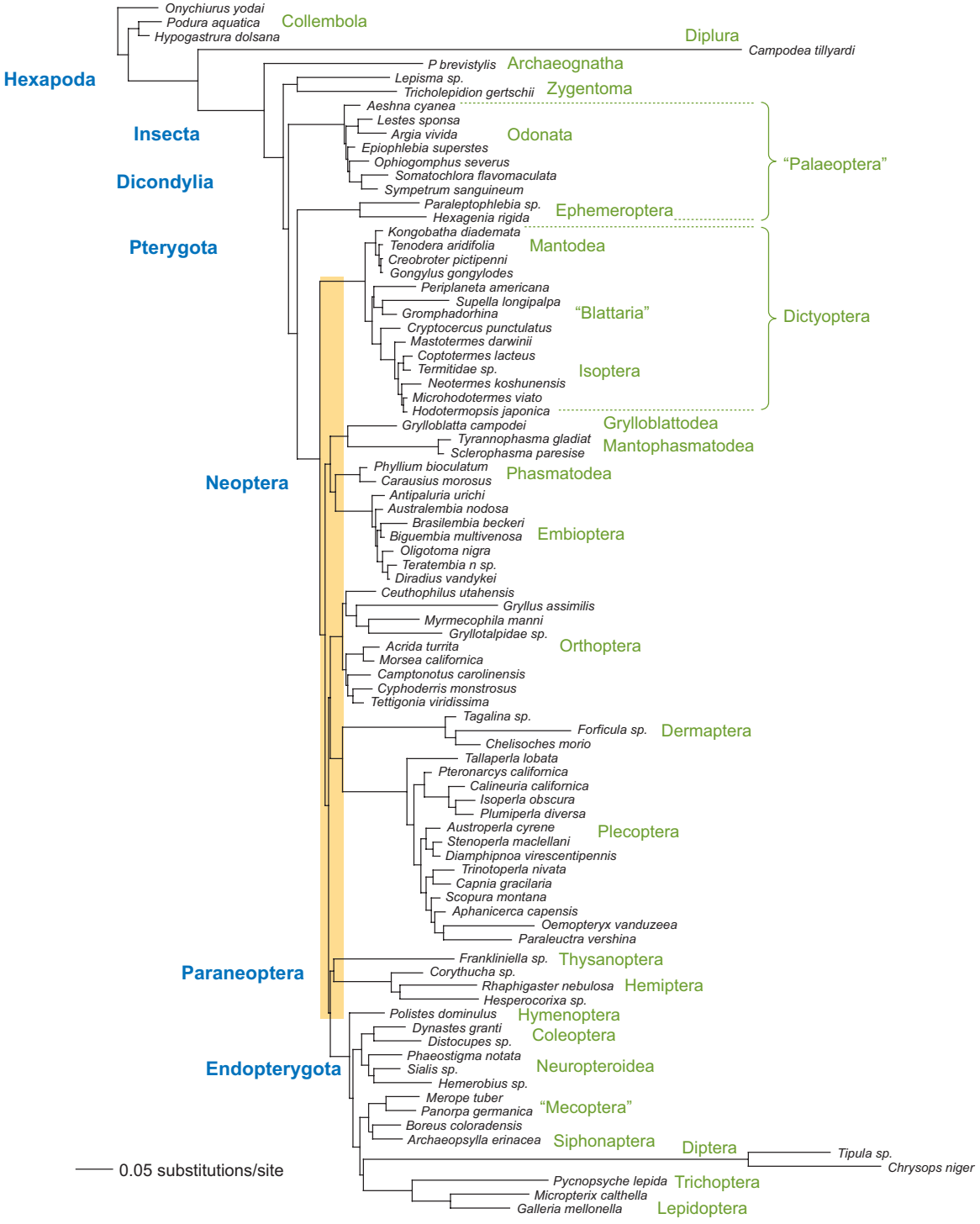
The rates at which sites in a gene change should be coordinated to the phylogenetic question at hand. Just as one would not measure continental drift with a stopwatch, some genes evolve too quickly to be useful for some deep phylogenetic questions. In general different genes are appropriate for estimating different divergence times. The time span appropriate to some genes may be broad, as is the case for nuclear ribosomal RNAs, which possess such a wide array of regionally variable substitution rates that portions of the data can be used to estimate relationships from within recently diverged genera (1, 138) to relationships among arthropod classes and beyond (81, 119). Protein-coding genes may have regional rate variations that broaden their window of utility as well. Substitutions accumulate in different codon positions at different rates, and even if the synonymous nucleotide substitutions are essentially randomized, there may still be useful phylogenetic signals in second codon sites or amino acids.

Selection of genes is not always made according to predictions of phylogenetic utility, but it is frequently based on ease of PCR, economics, and tradition. There are several dominant data sources in the insect systematics toolbox. For inferring the deepest splits in the insect tree, nuclear rRNA, histone H3, elongation factor-1α (EF-1α), and mitochondrial genes have been the most common sources of phylogenetic data. Each of these sources is different in substitution rates, nucleotide composition, and other analytical considerations, and can also be evaluated according to predicted phylogenetic utility when ancient internodes are short. For example, the most obvious difference among genes apparent in our neopteran example presented below is the extreme variation in tree lengths, resulting from the differences in substitution rates among genes.

One approach to solving a difficult phylogenetic problem is to collect a lot of data. However, the effectiveness of this approach is linked directly to the branch lengths of the internodes (which represent the time span upon which all evidence for relationship must accumulate) relative to the branch lengths of terminal taxa. Fast-evolving sites are more likely to change on an ancient short internode, but these changes are also more likely to be subsequently overwritten, especially if there is a lag in time between the first appearance of a lineage and the origin of extant subgroups from which we can sample today. **Figures 2** and **3** illustrate this pattern. Slowly evolving sites are unlikely to change on short internodes, but when they do, they are less likely to be overwritten. Extremes of slowly and fast-evolving sites, at least with parsimony, would be expected to perform in opposite ways. Although it is widely understood that the phenomenon of long-branch attraction (30) can lead to phylogenetic error, what is often overlooked is the prediction that, when terminal branches (undivided branches that start at the most recent node and lead to an extant species)

Split: a partition of the taxa in a phylogenetic tree into two groups, supported by a character or data pattern

Long-branch attraction: the tendency of phylogenetic methods to group long but (in reality) nonadjacent branches



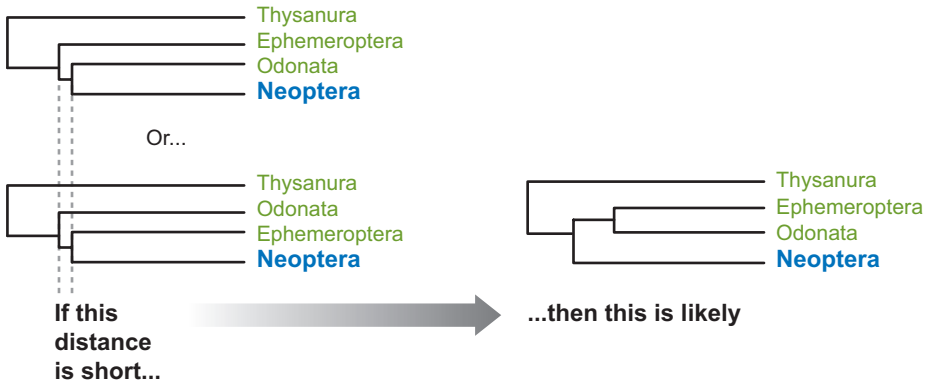


Figure 3

An illustration of the Palaeoptera problem. If the left panel is true, with arrows representing that either Odonata or Ephemeroptera could be the sister taxon to Neoptera, then parsimony will likely support a monophyletic Palaeoptera, shown in the right panel. This is because both possibilities would include a short time span (represented by the internal branch) upon which evidence of relationships could accumulate, followed by a long period, during which this evidence could have been overwritten.

are much longer than short internodes, adding more data to the problem actually decreases the probability that the truth will be recovered (30). Even the accumulation of slow data can lead to phylogenetic inconsistency when internodes are so short that homoplasy is more probable than synapomorphy. Likelihood-based methods have a chance at reconstructing these divergences correctly because a certain amount of homoplasy (parallel-evolving or convergent changes) is expected under likelihood and likelihood-based Bayesian methods. However, even with these tree shapes, it is better to have data that are slower and therefore less homoplastic. Modeling data that are evolving at rates appropriate to the problem can still be a challenge as well, if different lineages evolve at different rates, with different compositional biases, and unaccommodated covariation. Problems with long branches and homoplasy, as well as how "fast" is misleading and how "slow" is desirable, have yet to be thoroughly explored in higher-level insect phylogenetics.

Early molecular phylogenetic studies of insects often employed mitochondrial genes for a broad spectrum of applications, in part because universal primers for PCR were available and amplification was easy. Ballard et al. (2) explored deep arthropod history with 12S sequences, and Liu & Beckenbach (73) explored insect phylogenetics at the ordinal level with COII. These early works using mitochondrial data have not held up well when judged by several criteria including phylogenetic expectations and stability to analytical assumptions. For example, Liu & Beckenbach (73) show six different trees resulting from

Figure 2

Likelihood branch length estimate from the rRNA (SSU and LSU) data presented by Kjer et al. (58). Constraints were constructed for Dicondylia, Pterygota, Neoptera, Dictyoptera, Holometabola, and their orders, and a parsimony analysis was performed under these constraints. These relationships were recovered in the unconstrained analysis of a larger dataset by Kjer et al. (58). Branch lengths of this tree were then estimated with maximum likelihood, under a GTR+I+G model. This tree is therefore not an estimate of phylogeny, but rather an illustration of branch lengths for this tree. The orange bar highlights the problem area for the orthopteroid radiation; its left edge encompasses Neoptera, and its right edge includes most of the extant orders.

different analytical assumptions and conclude that both additional data and taxa would be needed to resolve ordinal relationships. However, the increased taxon sampling strategy requires that additional taxa can subdivide longer branches (which is nearly impossible when internodes are short, or when extinctions have resulted in a distribution of extant taxa that share a common ancestor relatively much more recent than the lineage as a whole).

Individual mitochondrial gene datasets are approaching (or exceeding) the limits of their phylogenetic utility within several orders, including Odonata (86), Orthoptera (31), Trichoptera (57), and Hymenoptera (128). Extant representatives of all these orders are considerably younger than the ancient internodes that separate the orthopteroids, or palaeopterans (39), so it can be inferred from these studies that if the phylogenetic utility clock is ticking too fast for these orders, it is also too fast for the deeper nodes.

Cameron et al. (15, 16) have examined over 100 mitochondrial genomes in order to infer phylogeny among insect orders. Their analyses show that increasing the number of taxa and the length of the sequences does result in an improvement over earlier relatively much smaller studies. However, some of their results are hard to accept, such as the Odonata appearing as sister to the Orthoptera. While their final studies are yet to be published, Cameron et al. (16) mention problems with nucleotide compositional biases, extreme rate variation among lineages, and long-branch attraction. Their data support the monophyly of many insect orders but provide little corroboration of relationships among orthopteroid or palaeopteran orders. These results are consistent with what we would expect if the short internodes shown in **Figure 2** reflect reality.

In recent years, an increasing number of nuclear protein-coding genes (**see Supplemental Table 1** and references therein; follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org**) have been explored for use in phylogeny estimation, especially at moderately shallow taxonomic levels (e.g., genera, tribes, subfamilies, and families). The utility of these genes for phylogenetics has varied widely, especially when employed for even deeper hierarchical levels such as relationships among orders in insects or among arthropod classes. More recently it has become customary to explore the quality of the phylogenetic signals of individual genes before investing in them for larger analyses. Unfortunately, cases of inappropriate phylogenetic use of genes still persist, perhaps because it is often easier to use familiar genes than to explore new or less broadly tested ones.

For shallower phylogenetic depths, such as species within genera and recently diverged genera within families, gene selection can be relatively straightforward, focusing on having enough alignable variation present in the gene to provide some signal for analysis. In many such cases, data from as few as three to four genes spanning the mitochondrial and nuclear genomes (or two to three genes plus morphology) may suffice for good phylogenetic resolution and support [although of course difficult exceptions exist, e.g., the human-chimp-gorilla divergences (101)]. Choice of phylogenetic methodology tends to be also less crucial for shallow phylogenetic depths, as homoplasy is lower and the effects of compensating for systematic bias are small.

The Need to Account for Systematic Biases in Older Divergences

At deeper phylogenetic levels, analysis becomes more complex, and the use of substitution models that take into account major nonphylogenetic patterns or tendencies in the data is recommended for extracting the full phylogenetic signal (93, 94). Multiple substitutions at the same sites, taken to an extreme, cause problems for deeper-level phylogeny, if not actually make phylogeny estimation impossible for deep nodes (44, 87, 88, 92, 93). A number of other biases in data can exacerbate the loss of signal due to homoplasy in more

directly misleading ways for phylogeny estimation. Although even a small amount of signal can overcome a large amount of random noise, it is when biases are not random that they have the potential to mislead. Among the nonrandom sources of noise are variations in the nucleotide (or amino acid) composition among taxa, differences in rate of change among sites within genes, differences in the distribution of variable sites among taxa, and differences in the rate of sequence evolution among lineages. We briefly review these factors and their effects below; the reader is referred to more extensive reviews of data analysis considerations (107, 111).

Nucleotide base compositional heterogeneity has been implicated in causing systematic errors in phylogeny estimation in a number of studies (24, 40, 53, 78, 94). The difficulty here is the tendency for lineages that share similarities in composition to group together even when they are distantly related. The main challenge in accommodating compositional heterogeneity (78) has been to compensate for it alongside other parameters of commonly used substitution models.

Differences in the rate of substitution among sites in a gene [among-site rate variation (ASRV), alternatively called rates across sites] are also important to take into account in phylogenetic analysis (37, 54, 67, 115, 135, 136). The most broadly employed methods for taking ASRV into account in evolutionary models include incorporating an estimate of the proportion of invariable sites (77, 114) and/or using a gamma distribution to model the distribution of rate variation (135, 136). Simplified, the gamma distribution can fit the data to a curve in which many sites are expected to change slowly, and a few are expected to change many times. Another approach is to use site-specific rate models (59). Whichever the approach, most of the common computer-implemented methods currently have the disadvantage of assuming that all the lineages analyzed show the same patterns of variation among sites, which may not be true.

If the lineages one is analyzing differ in their rates of change, and even in the patterns of nucleotide sites that are changing, the effect on analysis can be profound (52, 75). The erroneous grouping of more distantly related lineages is often caused by these rate and pattern differences (76), especially when some lineages are characterized by long branches (the long-branch attraction problem). Unfortunately it is precisely these lineage-specific effects that have been most difficult to incorporate into evolutionary models for likelihood or Bayesian analysis.

It is still not entirely clear exactly how best to analyze cases in which molecular evolution is strongly heterogeneous among lineages. Chang (23) and Kolaczkowski & Thornton (60) presented some simulated cases in which parsimony analysis outperformed maximum-likelihood methods in recovering the correct phylogeny, and the latter authors suggested that this might generally be the case with heterogenous (covarion-like) evolution. Subsequent work (34, 36, 75, 112, 113) showed that the range of examples presented by Kolaczkowski & Thornton (60) was only a tiny part of the spectrum of possible scenarios. In addition, all studies showed that likelihood or Bayesian methods generally performed worse than parsimony only when the evolutionary model incorrectly assumed homogeneous evolution. Thus, it is evident that more realistic models of heterogeneous evolution incorporating lineage-specific rate and site pattern differences will be important to employ and develop further. Some progress in developing such models is evident (35, 41, 49, 55, 118) and most of these recent approaches are available in computer implementations.

The above complications apply to a particular gene individually and can be expected to come into play more significantly with older divergences than with younger divergences, and with faster-evolving (possibly substitutionally saturated) than with slowly evolving genes. It is best to use the slowest-evolving genes to analyze the oldest divergences; the problem for ancient divergences (especially

Compositional heterogeneity: significant differences among lineages in the proportions of the nucleotides present in homologous sequences

Among-site rate variation (ASRV): variation among nucleotide sites (loci) within a gene in the rate of substitutional change

Covarion-like evolution: differences among lineages in the actual distribution of sites within a gene that tend to vary

Consensus network: a phylogenetic network that displays the splits found in source trees that have the same (completely overlapping) set of taxa

Supernetwork: a phylogenetic network incorporating the splits found in a series of source trees that contain overlapping, but not identical, sets of taxa

200–300 mya and older) is that no known genes may actually possess the desired rate of variation. A common mistake is to equate stability of amino acids among taxa with slow change among nucleotides. For instance, both histone H3 and EF-1α are exceedingly conservative in their amino acids, but most of their parsimony informative nucleotide sites are silent third-codon positions, inappropriate for long-diverged taxa. For the most ancient divergences, we may well need to employ rare genomic features such as gene order and short- and long-interspersed nuclear elements (SINEs and LINEs), which have little likelihood of homoplasy (9, 102), rather than comparative nucleotide sequence data. Unfortunately, observing these kinds of rare changes on short internodes would be hit or miss, with "miss" increasingly likely, proportional to how short the internode was. What you would expect from the patterns we see in **Figure 1** would be mostly uninformative changes that define individual lineages without linking them to others (e.g., supporting the monophyly of the orders in **Figure 2** but not providing evidence of relationships among them), and nothing supporting the short internodes. For example, mitochondrial genome rearrangement was initially considered promising in uncovering relationships among insect orders, because the first genomes sequenced were, by chance, highly rearranged. However, as genomes accumulated, it became clear that little evidence will emerge from mitochondrial genome rearrangements, except for within Psocodea and Neuropterida, because most insect orders share a plesiomorphic genome arrangement (16), and those orders that differ are autapomorphic.

Gene Trees May Conflict with Each Other

When multiple genes are sequenced and analyzed to estimate the same phylogeny, the hope is that all, or most, of the genes will converge on the same phylogeny. Other than investigator error (such as PCR contamination or mislabeling), there are a number of reasons why the individual gene trees might not all be congruent, especially if the divergences estimated were closely spaced in time or if the divergences are ancient. First, if the divergences estimated are closely spaced, there may be ancestral polymorphisms of alleles that have not been sorted out between divergences, such that the gene trees and species trees (even when correctly estimated) are not congruent (27, 80, 89, 106). In this case it may be that the most common (or well-supported) topology recovered among the genes corresponds to the underlying species phylogeny, but this is not always the case (27). Second, the systematic biases discussed above may be present in one or more of the genes analyzed, such that some deviations from the correct underlying phylogeny may occur even when best efforts are made to accommodate the biases. This effect is minimized if care is taken to fit substitution models to the data that are as accurate as possible for each gene, as is done with programs that permit different genes or sites to be modeled independently in a combined, mixed-model analysis (104). In some cases (especially rRNA genes with hypervariable regions) it may be necessary to exclude some hypervariable regions from analysis in order to maximize the phylogenetic signal-to-noise ratio. An alternative approach to mixed-model analysis is to estimate each gene tree separately and examine conflict and congruence among the gene trees using consensus networks or supernetworks of the gene trees (45, 46, 51). Using filtered supernetworks, researchers may examine how many genes are contributing to each uncertainty in the combined analysis and filter out those rogue relationships that are only sporadically recovered (51).

EXAMPLES OF INSECT RADIATIONS

Insects in Terrestrial Radiations

As the most diverse group of terrestrial animals, insects should supply many examples

of ancient rapid radiations. Many of the most spectacular radiations involve familiar interactions with angiosperm plants, which also radiated extensively during the Cretaceous and Tertiary. However, insects as a whole were already highly diverse by the Permian, long before the origin of angiosperms. In some cases we can only speculate why the insect groups diversified as rapidly as they did. We critically discuss a variety of prominent cases below that exemplify not only the phylogenetic challenges posed by rapid radiations, but also the wide variety of ecological contexts in which they developed.

The Paleozoic Diversification of the Insect Orders

Exploring the relationships among insect orders offers a classic example of how some parts of the tree are well corroborated and other parts are best described as a polytomy. Before the advent of molecular systematics, Kristensen (61–65) proposed a tree derived from morphological characters that contained Hexapoda, Insecta, Dicondylia, Neoptera, Dictyoptera, Paraneoptera, and Holometabola. One major part of the tree was left unresolved: the relationships among the orthopteroid orders (or Polyneoptera). Since then, molecular data have done little to resolve the consensus view of insect ordinal relationships, with each study contradicting previous work (even by the same authors with much of the same data, or in the same paper with different analytical assumptions: 22, 73, 116, 123, 131, 132) and virtually no corroboration among independent datasets, except those nodes that have been firmly established by morphology (58). Although analytical differences may account for some of the disagreements, this lack of agreement may be rooted in phylogenetic reality. One of the hallmarks of short ancient internodes is their instability to slight variations in analytical assumptions and changes in taxon sampling or outgroup choice. Here we discuss two problem areas of Kristensen's (61) tree that are still unresolved: Palaeoptera and the orthopteroids.

The Palaeoptera problem. Odonata, Ephemeroptera, and their extinct stem lineages, along with the extinct Palaeodictyoptera, are winged insects that lack the ability to fold their wings over their abdomens as neopterans can. Neopterans possess a coordinated series of morphological features including wing sclerites, muscle attachments, wing veins, and flexion points that make Neoptera almost universally accepted as a monophyletic group (although the morphological evidence for whether neoptery is apomorphic or plesiomorphic is still open to debate; 47). Similarly, Pterygota, including Odonata and Ephemeroptera, is also nearly universally accepted, supported by both morphological (7, 8, 61) and molecular datasets (48, 58, 81, 116, 123), with Thysanura as its sister taxon. This arrangement, in which there is a polytomy at one point in the tree (at the "Palaeoptera") but strongly corroborated resolution both above and below this polytomy, is unusual and indicative of the possibility of a short ancient internode. If we accept Pterygota, Neoptera, and the monophyly of both Odonata and Ephemeroptera, then there are only three possibilities: (*a*) Palaeoptera (Odonata plus Ephemeroptera), (*b*) Metapterygota (Odonata plus Neoptera), or (*c*) Chiastomyaria (Ephemeroptera plus Neoptera). One serious problem is that if either Metapterygota or Chiastomyaria is a true grouping, then parsimony analyses will still tend to favor Palaeoptera (**Figure 3**) because these relationships dictate a short, ancient internode followed by long terminal branches that lead to the extant representatives of Odonata and Ephemeroptera. Morphological data favor Metapterygota (7, 8, 63), but all possibilities have some morphological support (Palaeoptera: 4, 42, 69; Chiastomyaria: 10, 18). Molecular datasets support all three possibilities as well, even with the same data, depending on analytical methods (48, 58, 81, 91).

Polytomy: a node in a phylogenetic tree that subtends more than two descendant branches

Another problem with the Palaeoptera is in reference to the radical changes associated with flight. Determining which characters are plesiomorphic or ancestral, and which are apomorphic or new, for structures that did not previously exist poses another problem (47). Many of the morphological characters that support one relationship over another are dependent on interpretation of relative apomorphy without a relevant outgroup. Yet another problem is the excessive rate variation among lineages in the vicinity of Palaeoptera. Dipluran rRNA is excessively autapomorphic, whereas Odonata is excessively slow. This pattern can be seen in the phylograms presented by Yoshizawa & Johnson (137) and Kjer et al. (58).

Finally, the age of extant palaeopteran taxa is deceptively young. We tend to associate Odonata with "ancient" because one of the most familiar of all extinct insects is the giant Carboniferous odonatoid, *Meganeura*, with its meter-long wingspan flying among the cycads as the first fish-like amphibians crawl onto the land over 350 mya. However, fossils for extant odonate families are only as old as the Jurassic (39); epiophlebiid fossils have not been discovered, but as the sister taxon to Anisoptera, they may be as old as Triassic (39). One can see this gap in **Figure 2**, in which we have sampled all three extant suborders. The time span between the emergence of *Meganeura* and its extant descendents is surely 100 million years long, and possibly as long as 200 million years, and this branch cannot be subdivided with extant taxa. During this huge span of time, the few synapomorphies that had accumulated on any of the potential internodes may have been overwritten by parallel changes on the branches leading to the extant orders (**Figure 1**). This problem can be seen in many of the long branches leading to extant orders (**Figure 2**).

The Neoptera explosion. The Palaeoptera problem is dwarfed by the problems of estimating relationships among the early orthopteroids. There are 10 neopteran lineages in a virtual polytomy: Plecoptera, Dermaptera, Embioptera, Phasmatodea, Mantophasmatodea, Grylloblattodea, Dictyoptera, Zoraptera, Orthoptera, and Paraneoptera plus Holometabola. Terry & Whiting (116) show that different rRNA alignment gap costs result in different relationships among orthopteroids. There is some molecular support for Plecoptera plus Dermaptera, Embioptera plus Phasmatodea, and Grylloblattodea plus Mantophasmatodea (58, 116). Two of these relationships are contradicted by the mitochondrial data (15, 58), which place Mantophasmatodea with Phasmatodea. Morphological analyses do not support the molecular hypotheses (7, 8, 39).

The upper and lower time frame limits from which these 10 lineages diverged is circumscribed by the first emergence of land plants 475–425 mya (121) and by the emergence of the extant fossil orders 280 mya. For dates near the upper limit, it is hard to imagine terrestrial animal life of any kind before there were plants to feed upon and offer shelter. At the lower limit, fossil Plecoptera, Orthoptera, and Dictyoptera have been found from the Permian. Thus, all these lineages must have emerged at least within 200 million years of one another, although more likely these divergences are bounded by the emergence of the putative stem neopterans (Paoliidae) in the mid-Carboniferous (39), which would mean that the orthopteroids diversified within a span of less than 50 million years. Even if each bifurcation was regular, that leaves a series of short internodes between 5 and 20 million years in duration but that are over 300 million years old. **Figure 2** indicates that this ancient diversification was not regular, but appears nearly instantaneously, favoring the shorter estimate of internode lengths. The branches following this diversification, up to the first emergence of extant families, are unusually long. The Permian mass extinction appears to have had a major impact on insect diversity and may be responsible for the pattern of long internodes leading to extant orders; these lineages were present before the

Permian mass extinction and then were almost (but not entirely) eliminated, only to rebound in the Mesozoic (71).

If these estimations of branch lengths shown in **Figures 2** and **4** are even close to accurate, then we have a serious problem with phylogenetic estimation among orthopteroid orders, especially for parsimony analysis. We provide a **test dataset** (and references therein) that examines character evidence with MacClade, which compares the nuclear rRNAs with the histone H3 data on a consensus tree of insect ordinal relationships. We invite the reader to explore the individual characters in the data directly to understand more intimately some of the issues we have discussed.

Insect Diversifications Associated with the Cretaceous/Tertiary Angiosperm Radiation

The four examples presented below include prominent diversifications of insects that are generally associated with plants. Not surprisingly their diversifications correspond well in time with major diversification of plants as well.

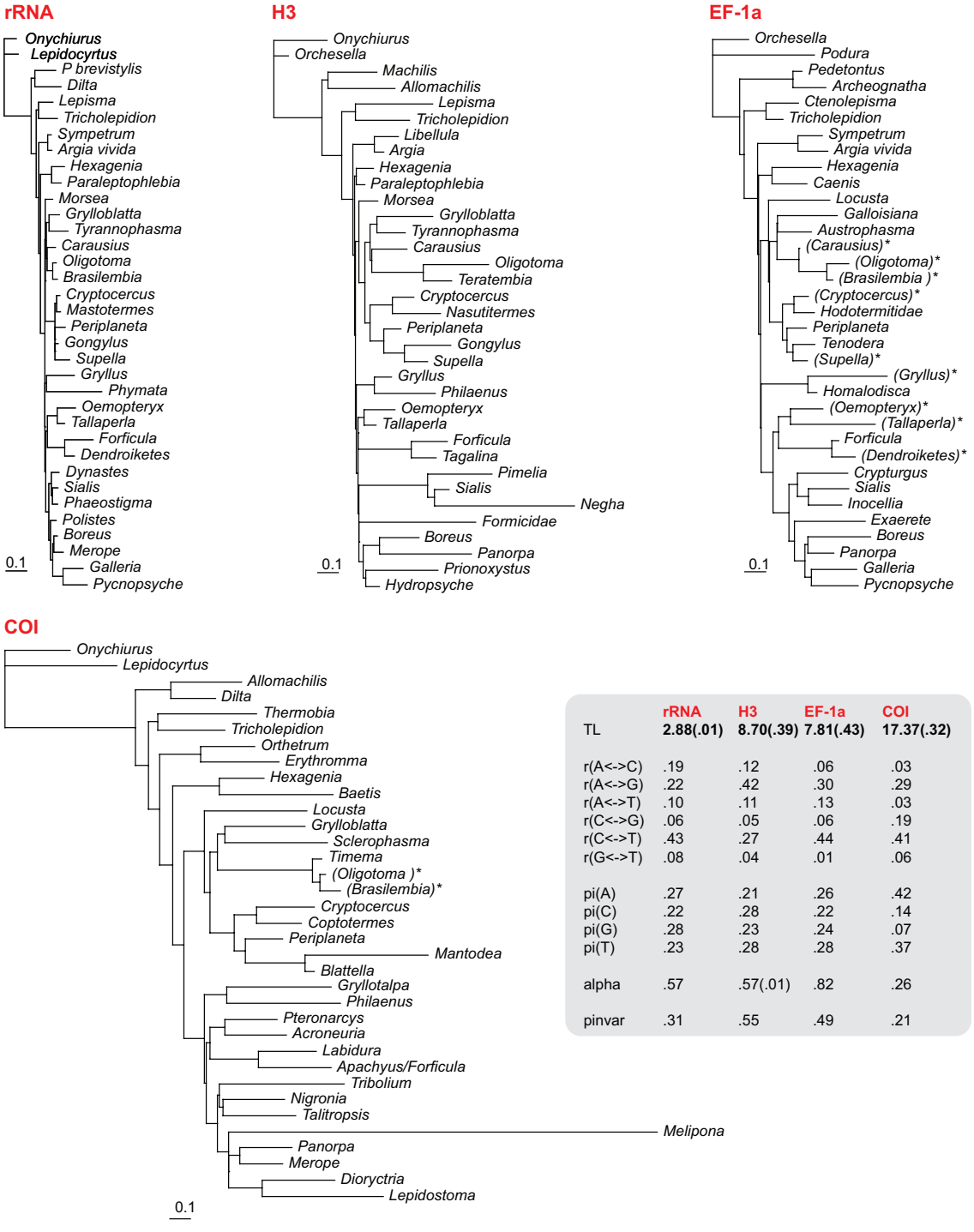
Corbiculate bees. The corbiculate bees are a clade composed of four tribes of Apidae: Apini (honey bees), Bombini (bumble bees), Euglossini (orchid bees), and Meliponini (stingless bees). These bees, dating as a lineage to the late Cretaceous but likely diversifying in the early Tertiary (39), include familiar, abundant, and economically important pollinators of many plants and represent a broad range of nesting biology, from solitary to highly eusocial. As a result they have been intensively studied for many years. Although the monophyly of the corbiculate clade as a whole is generally well supported by analyses and broadly accepted by bee systematists, the relationships among the four tribes have been extraordinarily controversial.

From morphological characters, a variety of different hypotheses (14, 17) have been put forward for how these four tribes are related. The generally preferred morphological tree groups the highly eusocial Apini and Meliponini as sister taxa, with the primitively eusocial Bombini sister to those two and the solitary Euglossini sister to the other three, although there is some support also for grouping the Bombini and Euglossini.

None of these morphology-based hypotheses has been best supported by the available molecular data. Instead, analysis of an increasing number of genes (16S, cytochrome *b*, 28S, longwave opsin, EF-1α) converged on two other alternative trees, both of which group Bombini and Meliponini as sister taxa but disagree as to the placement of Apini and Euglossini. Recent, taxonomically more thorough analyses of individual tribes that include the other tribes as outgroups have continued to support the Bombini-Meliponini relationship (12, 13, 43, 83, 97), as have the overwhelming majority of analyses of 8–10 additional genes (56). The latter analyses have perhaps identified the Apini-Euglossini alternative grouping as an artifact of similarity in base composition in some genes.

Robust resolution of the relationships among these four tribes may have profound implications for the evolution of highly social behavior in bees, yet it does not appear to be coming easily (74). At this point, the molecular and the more recent combined analyses overwhelmingly favor the Bombini-Meliponini relationship (**Figure 5** shows a supernetwork of the five gene trees), but acceptance of this result is not universal in part because additional individual morphological (11, 109) and behavioral (90) studies have appeared to support the traditional morphological hypothesis. A recent paper (117) concluded that the study of the evolution of sociality in this group currently needs to consider both phylogenies as possible frameworks for interpretation.

Both morphological and molecular data do support one common pattern, however. The monophyly of each of the four tribes is strongly supported by the data, whereas the amount of data relevant to grouping any



	rRNA	H3	EF-1a	COI
TL	**2.88(.01)**	**8.70(.39)**	**7.81(.43)**	**17.37(.32)**
r(A<->C)	.19	.12	.06	.03
r(A<->G)	.22	.42	.30	.29
r(A<->T)	.10	.11	.13	.03
r(C<->G)	.06	.05	.06	.19
r(C<->T)	.43	.27	.44	.41
r(G<->T)	.08	.04	.01	.06
pi(A)	.27	.21	.26	.42
pi(C)	.22	.28	.22	.14
pi(G)	.28	.23	.24	.07
pi(T)	.23	.28	.28	.37
alpha	.57	.57(.01)	.82	.26
pinvar	.31	.55	.49	.21

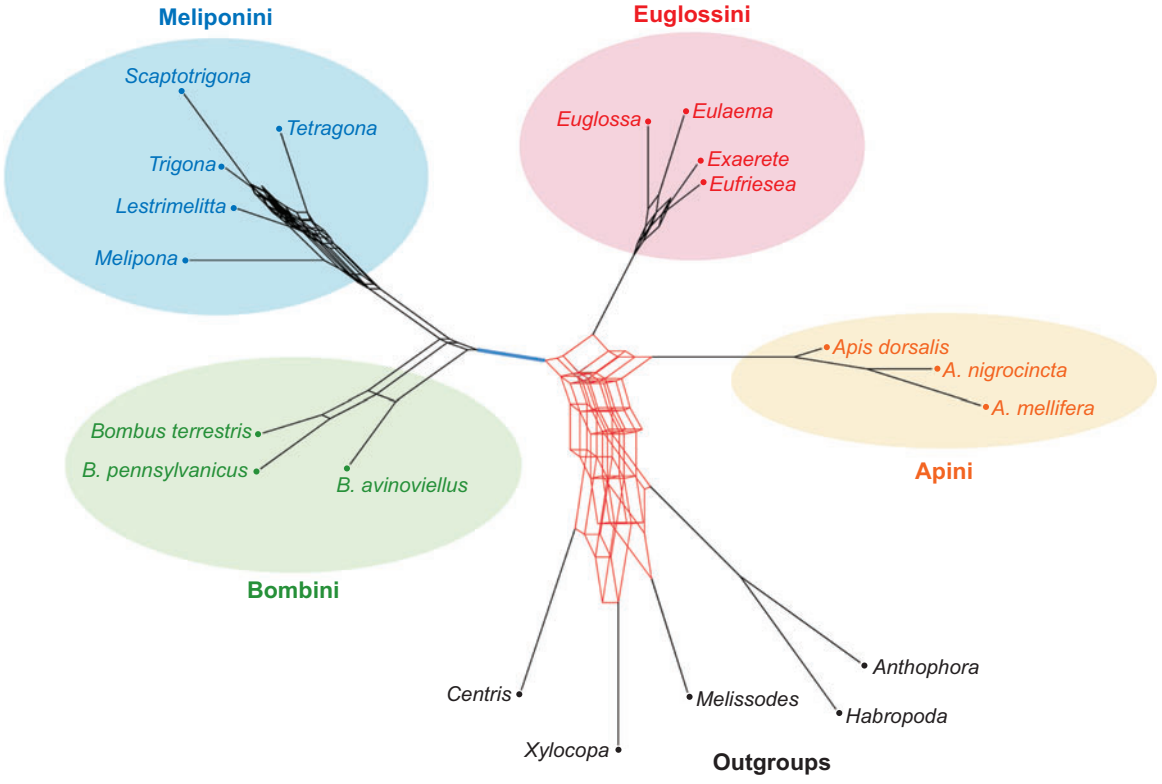


Figure 5

Filtered z-closure supernetwork (51) of corbiculate bee relationships, in which only splits represented in three or more of the five available gene trees are represented. The blue branch indicates the principal well-supported ingroup tribal relationship; the others are essentially unresolved.

subsets of the four tribes is comparatively tiny (74). In this respect, all data sources so far suggest the possibility that the time between the divergences among the four tribes in question may have been small compared with the time these tribes have continued to evolve. Thus it is not surprising that the relationships have been difficult to support with convincing data. This may also mean that if or when we do resolve the relationships, they might not tell us

Figure 4

A combined, unconstrained Bayesian likelihood analysis of nuclear rRNA, histone H3, EF-1α, and mitochondrial cytochrome oxidase I (COI) data constructed to compare branch lengths among the genes, as well as model parameters. A GTR+I+G model was used, with parameters and branch lengths unlinked. Each tree has the same topology (estimated from the combined analysis), with different representatives of higher taxa (chimeras in the combined analysis), as indicated in the different panels. Branch lengths were then estimated separately, from each partition with likelihood in PAUP, using the gene-specific parameters and the input tree from the combined Bayesian analysis. Missing taxa are marked with an asterisk and placed in parentheses. The model parameters are shown in the gray box (r, relative proportion of changes; pi, percent of each nucleotide base; alpha, gamma shape parameter; and pinvar, estimated proportion of invariant sites). This should not be taken as an estimate of phylogeny (it is a contrived dataset with poor taxon sampling).

as much as about the evolution of sociality as was hoped, because almost none of the evolution of the tribes was shared by any two of them!

Ditrysian Lepidoptera. Despite their possible early origin as detritivores, most extant Lepidoptera are herbivores (96) and as such are expected to have diversified extensively during periods of plant, especially angiosperm, diversification. The fossil record of Lepidoptera extends at least back to the lower Jurassic (122), with a few earlier fossils somewhat questionably placed in the order (39, 66). This predates all but the earliest angiosperms. Most of the lepidopteran diversification of suborders appears to belong to the Cretaceous, however, and the huge radiation of the Ditrysia (approximately 95% of extant lepidopteran species) occurred in the mid to late Cretaceous and into the early Tertiary, coinciding with major angiosperm radiations (38, 39, 66).

Extensive earlier morphological and more recent molecular phylogenetic results have converged strongly on the same clear progressive pattern of early lepidopteran suborder diversification (32, 33, 133, 134). Within the Ditrysia, however, patterns of relationship have been elusive beyond monophyly of some superfamilies and a few other groupings based on morphological data (66, 85). The Apoditrysia in particular appear to have diversified largely during the time (late Cretaceous–early Tertiary) that many angiosperms and associated holometabolous insects were also diversifying (39, 66). So far, molecular studies on ditrysians have focused largely on relationships within families or superfamilies rather than more broadly. An ongoing NSF-funded Assembling the Tree of Life (AToL) project on Lepidoptera promises to apply considerably more data across the span of taxa than has previously been brought to bear for ditrysian phylogeny.

Parasitoids of herbivorous lepidopteran larvae. Lepidopteran larvae, especially ditrysians, are attacked by a variety of hymenopteran parasitoids from various superfamilies. The order Hymenoptera as a whole dates back to the Triassic as phytophages (98–100, 108), yet it is the parasitoid habit that enabled the order to diversify massively when angiosperms and ditrysian lepidopterans did (ignoring the comparatively modest radiation of the Tenthredinoidea in the Cretaceous) (126).

As with the Lepidoptera, the older, early-diverging superfamily relationships have been relatively well resolved and supported by both morphological and molecular data (28, 120). The Cretaceous diversification of many parasitoid lineages has led to poorly resolved relationships among most hymenopteran superfamilies, however (19, 28, 105, 110, 124, 126), and much disagreement remains even about the monophyly of several superfamilies.

At a lower taxonomic scale, several groups have received more intensive study, including the braconid wasps of the microgastroid assemblage of subfamilies. This assemblage has been studied morphologically and molecularly to estimate relationships both for developing a robust classification (130) and for investigating relationships with symbiotic polydnaviruses (125, 127). While there continues to be some uncertainty about relationships among subfamilies, the generic relationships within the largest subfamily, Microgastrinae, have been difficult to estimate using either molecular or morphological data (or both combined). Mardulyn & Whitfield (82) initially suggested, after several statistical analyses of the phylogenetic signal in a three-gene dataset and morphological data, that the difficulty was likely due to an underlying rapid radiation, which is now dated to approximately 50 mya (125). Broader taxon sampling (130) and the addition of four more nuclear protein-coding genes (longwave opsin, wingless, arginine kinase, and EF-1α) to the analysis (3) increased resolution among and support for some major lineages, but only partially so. Relative to the outgroup relationships, the generic relationships resemble a star

phylogeny, with only a few genera resolved (45, 51). A set of approximately 10 genes might largely resolve the major patterns, but extrapolations from current data suggest that even 10 times this much data might not suffice to resolve all relationships. In the same group only four genes (16S, ND1, 28S, and long-wave opsin) sufficed to resolve species-level relationships within the genus *Cotesia* (84).

Phytophagous Coleoptera (Chrysomeloidea and Curculionoidea). The diversification of beetles is truly impressive, and extant Coleoptera live practically everywhere on earth except the open ocean. The origin of Coleoptera as a group is difficult to pinpoint from fossils. The earliest Protocoleoptera date from the early Permian (68). These Protocoleoptera are coincident with early non-holometabolous insects and have less than fully sclerotized elytra (39, 68) but seem to be transitional toward modern Coleoptera. By the late Permian, more fully cupedid-like fossils called Archecoleoptera had appeared (26). By the late Triassic (240–220 mya), species possessing elytra-like veinless hardenings of the forewings appeared (95). By the Jurassic, beetle diversity had exploded in terms of family diversity if not species richness.

Studies of extant species have solidified around the recognition of four coleopteran suborders: Archostemata (four small extant families), Adephaga (nine extant families including 10% of current coleopteran diversity), Myxophaga (five families including only 65 species), and Polyphaga (a morphologically and trophically diverse assemblage of approximately 90% of all current coleopteran species).

Monophyly of each of the four suborders has been supported by nearly all studies. Relationships among these four suborders have been generally considered to follow the pattern (Archostemata + (Adephaga + (Myxophaga + Polyphaga))) (5, 6, 25). However, some morphological studies (70, 72, 79) and recent molecular studies (21) have challenged this view in different ways. The first (morphological) challenge considers the Polyphaga as actually sister to the other extant Coleoptera (on the basis of wing venation, articulation, and pattern of loss of cervical sclerites), whereas the second (molecular, 18S rRNA data) challenge switched the position of Adephaga and Myxophaga relative to the traditional pattern. Later, combined studies (20) are somewhat ambiguous with respect to these complications. Given the small amount of molecular data so far applied to the overall pattern, it seems likely that the subordinal relationships will be resolved within the near future.

The enormous species-level diversity within the Coleoptera, however, is the result of high levels of diversification primarily within some polyphagan groups. Farrell (29) examined the phylogeny and diversity of Phytophaga using a combined 18S rRNA and morphology dataset and came to the conclusion that while the origins of many phytophagan families predate the angiosperm radiations, the high species-level diversification of many of the chrysomeloid and curculionoid clades does in fact coincide with the radiations of their host plants, most spectacularly during the Cretaceous and early Tertiary. Whether this pattern will be corroborated by analyses of other polyphagan groups remains to be seen. As with the other megadiverse insect orders, Coleoptera is the focus of a current NSF AToL project and we should know much more in the near future.

PROSPECTS AND RECOMMENDATIONS FOR THE FUTURE

We have several recommendations for recognizing short, ancient internodes. First, researchers should examine phylograms for individual partitioned datasets, using model-based methods that are likely to more accurately account for superimposed substitutions and systematic biases. Do the contentious issues in insect phylogenetics correspond to internodes that appear short on phylograms?

Multiple independent datasets should also be explored. Are there corroborated nodes among independent datasets? Short, ancient internodes will typically not be reconstructed similarly by multiple datasets. They are also difficult to recover under different analytical assumptions. The recovery of short internodes is likely to vary even with small perturbations of analytical assumptions, taxon sampling, or outgroup selection. The bounds of divergence times can sometimes be inferred from fossils (another sort of independent dataset). Do these bounds dictate short internodes? We also recommend that investigators look at the individual characters mapped on the tree and ask whether any relatively consistent characters support short internodes, or do the characters homoplastically toggle back and forth among states, with no observable phylogenetic pattern?

Data Exploration/Visualization is a Good Thing!

It seems axiomatic in biology that the more data one has that bears on a problem, the greater the chance of resolving that problem. It does not follow, however, that mere compilation of data will result in a more accurate analysis, especially when the phylogenetic signal is small relative to other variation that is present. As outlined above, it may be essential to rule out systematic bias (differences in base composition, sites free to vary, and rates among lineages) before a clear phylogenetic pattern can emerge. It also may be true that lack of resolution in a combined-data phylogeny is the result of conflict among datasets. In this case it is useful to examine what those conflicts are and the impacts they are having on analyses. It is an advantage if multiple data sources are employed (e.g., a variety of independent genes), but because rapid radiations often result in low amounts of phylogenetic signal for some nodes in the phylogeny, it is still possible that only one or a few data sources with strong, but misleading, signal can confound analysis. Using all apparently relevant data in a "total evidence" approach may sometimes be worse than taking a serious critical look at data quality.

SUMMARY POINTS

1. Ancient rapid radiations cause problems for phylogenetic analysis because they take place over short periods of time, allowing few phylogenetic markers to accumulate for the relationships among the lineage of interest, and because the time since these lineages arose is long, allowing much opportunity for the historical signal to be erased by subsequent changes.
2. A number of biases in sequence data can confuse estimation of phylogeny, including base compositional bias, differences in ASRV, variation among lineages in both base composition and evolutionary rate, and differences among lineages in the sites that actually change.
3. Parsimony methods are efficient and effective at low to moderate levels of homoplasy, but less so with highly homoplastic data. Homoplasy is expected (and corrected for) under model-based methods, which are generally superior both at estimating the correct topology and at estimating reasonable branch lengths. This advantage of model-based methods, however, depends on whether the substitution model is appropriate (i.e., accurate) for the data.

4. Orthopteroid insect orders emerged almost simultaneously, around 300 mya, but the most recent common ancestor to all extant descendents in each order is much younger, resulting in a difficult tree shape to recover: short internodes followed by long branches.
5. The Odonata, Ephemeroptera, and Neoptera present a challenging phylogenetic tree shape, regardless of their true relationships, because the first pterygotes may have emerged up to 400 mya, but the earliest representatives of their extant descendents are Mesozoic.
6. The corbiculate bees, ditrysian Lepidoptera, parasitoids of ditrysian Lepidoptera, and phytophagous Coleoptera all represent likely rapid radiations during the Late Cretaceous and early Tertiary, when the angiosperm plants upon which they depend were also rapidly diversifying.
7. Taxon sampling can salvage some difficult phylogenetic problems, but there are some cases in which no extant taxa can possibly subdivide a long branch, because the common ancestor of the extant representatives is much younger than the first emergence of the lineage whose relationships are in question.
8. Adding some kinds of data that vary among short, ancient internodes actually increases the probability of phylogenetic error.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Contents