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# The evolution of insect biodiversity

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## SUMMARY

Insects comprise over half of all described animal species. Together with the Protura (coneheads), Collembola (springtails) and Diplura (two-pronged bristletails), insects form the Hexapoda, a terrestrial arthropod lineage characterised by possessing six legs. Exponential growth of genome-scale data for the hexapods has substantially altered our understanding of the origin and evolution of insect biodiversity. Phylogenomics has provided a new framework for reconstructing insect evolutionary history, resolving their position among the arthropods and some long-standing internal controversies such as the placement of the termites, twisted-winged insects, lice and fleas. However, despite the greatly increased size of phylogenomic datasets, contentious relationships among key insect clades remain unresolved. Further advances in insect phylogeny cannot rely on increased depth and breadth of genome and taxon sequencing. Improved modelling of the substitution process is fundamental to countering tree-reconstruction artefacts, while gene content, modelling of duplications and deletions, and comparative morphology all provide complementary lines of evidence to test hypotheses emerging from the analysis of sequence data. Finally, the integration of molecular and morphological data is key to the incorporation of fossil species within insect phylogeny. The emerging integrated framework of insect evolution will help explain the origins of insect megadiversity in terms of the evolution of their body plan, species diversity and ecology. Future studies of insect phylogeny should build upon an experimental, hypothesis-driven approach where the robustness of hypotheses generated is tested against increasingly realistic evolutionary models as well as complementary sources of phylogenetic evidence.

## Introduction

Together with Protura (coneheads), Collembola (springtails) and Diplura (two-pronged bristletails), the insects (Insecta) constitute Hexapoda, a clade of six-legged terrestrial arthropods. Insects comprise over half of all described species[1], and the ecological significance of six-legged life is thus hard to overstate. From the second half of the 19th century onwards, scholars have tried to make sense of insect biodiversity through the reconstruction of their phylogeny. Yet, exactly because of their unparalleled species diversity, their high morphological disparity[2] and ecological variety, insect phylogenetics has been plagued by controversy. It was the troubling complexity of reconstructing insect evolution that led the influential German entomologist Willi Hennig (1913–1976) to lay the foundation of modern phylogenetic systematics, igniting a revolution whose consequences reached far beyond the realm of insect science. Hennig emphasised distinguishing between ancestral and derived (apomorphic) characters, advocated a classification based strictly on monophyletic groups defined by the shared possession of derived characters (i.e. synapomorphies), and highlighted the importance of fossils in phylogenetic inference. His work culminated in the publication of his 1969 book *Die Stammesgeschichte der Insekten*[3] that set the scene for future extensive morphological treatments of insect phylogeny[4,5], and for the development of statistical phylogenetics[6].

A second revolution in our understanding of insect evolution was precipitated by the introduction of molecular phylogenetics. The first molecular phylogeny of insects was published in 1989 and relied on just a single gene marker for twelve species[7]. However, particularly as a result of the gargantuan effort of the 1KITE (1,000 Insect Transcriptome Evolution) consortium, the last decade has witnessed an explosion of genome-scale datasets. By 2021, over 600 insect genomes, 440 transcriptomes, 1,400 mitochondrial genomes and 69,000 DNA barcodes had been released[8,9]. The availability of hundreds or thousands of genes for representatives of all major living hexapod groups has facilitated large-scale insect phylogenomic[10] studies. This rich stream of genomic data will be mined for years to come,

elucidating the pattern, timescale and drivers of insect diversification and making insects a model system for studying macroevolutionary patterns and processes.

Although the growing size of genomic datasets has led to a new understanding of insect evolution, many controversies already recognised in Hennig's time remain unresolved, arising not only from incongruence between morphological and molecular phylogenies, but also between competing molecular studies[11–13]. Here, we review how genomics has revolutionised our view of the insect phylogeny and discuss the 'tricky nodes' that remain unresolved despite an unprecedented volume of genomic data. We discuss the computational challenges faced when addressing ancient radiations and outline possible approaches toward tackling tricky nodes in insect phylogeny. We argue that while genome scale data are key to resolving phylogenetic problems, data modelling[14–18], hypothesis testing[19] and the integration of morphological and molecular data[20] are crucial to achieving an integrated understanding of insect evolution and the origins of their megadiversity.

## Insect phylogeny in the molecular age

The advent of molecular phylogenetics uncovered novel relationships or verified controversial hypotheses previously proposed based on analysis of morphological data. Notably, phylogenomics fundamentally revised our understanding of the origins of hexapods. Myriapods (centipedes, millipedes and their kin) have traditionally been regarded as closely related to hexapods, as both groups share specialisations associated with life on land such as the structure of their tracheal respiratory systems, the presence of Malpighian tubules, unbranched legs and morphology of the head appendages[21]. However, since the late 1990s, analyses of molecular data have indicated that hexapods are terrestrial crustaceans, rather than close relatives of myriapods[22–25]. More subtle morphological evidence, such as the structure of the nervous system and ommatidia, as well as segmentation genes, confirmed that insects are 'crustaceans' that colonised land[26,27]. The now well-supported placement of hexapods within Pancrustacea (traditional 'Crustacea' plus Hexapoda)[28] provides a cautionary tale of how convergent morphological evolution can mislead phylogenetic inference.

Finding the sister group of the insects and their six-legged relatives (proturans, springtails and bristletails) is key to elucidate patterns and processes of terrestrialisation[28–31]. Early studies yielded conflicting results, some even controversially recovering hexapods as paraphyletic to crustaceans[32,33]. Eventually, expanded taxon sampling of crustaceans and mitigation of systematic errors converged on Remipedia as the closest living relatives of insects[23,28,34]. Remipedes are a cryptic group of small venomous and predatory crustaceans that exclusively inhabit coastal caves[35]. Exasperatingly, the extensive anatomical specialisations of remipedes to life in flooded caves, such as their biramous swimming appendages and lack of eyes or pigmentation, offer no clear insight into the nature of the earliest terrestrial pancrustaceans. However, remipedes are now increasingly employed as outgroups to study hexapod character evolution[36] and their inclusion in future studies of comparative development may help clarify the origin of key anatomical innovations that facilitated or where precipitated by their sea–land evolutionary transition, as well as the origin of hexapods.

Hexapod monophyly is well-supported[30,32,37], despite early mitogenomic studies failing to recover this clade[33,38]. Similarly, the monophyly of the insects (Insecta or Ectognatha) is also strongly supported[30]. However, the relationships among springtails, coneheads, and two-pronged bristletails, collectively known as 'Entognatha', remain contentious. Within insects, phylogenomic datasets based on whole genomes, transcriptomes, and mitochondrial genomes have converged to support several well-defined clades (Figure 1). Within insects, the ancestrally wingless, jumping bristletails (Archaeognatha) are the sister clade to a clade composed of the wingless silverfish (Zygentoma) and the Pterygota[30,39,40]. Pterygota is characterised by the possession of wings, although numerous pterygote lineages have lost them[30]. Several clades within Pterygota are consistently well-supported by genomic data. In particular, phylogenomics has largely clarified relationships within the most diverse insect group, the holometabolous insects (Endopterygota), which undergo complete metamorphosis including a distinct egg, larval, pupal and adult stage[41]. Hymenopterans (bees, wasps and ants) have been revealed as the sister group to the other holometabolous orders, which are then divided into two clades: Neuropteroidea (lacewings, dobsonflies and snakeflies, beetles and twisted-wing insects) and Mecopterida (caddisflies, butterflies, moths, scorpion flies, fleas and true flies). These results are largely congruent with the insect tree drafted by Hennig[3] and other entomologists in the 1960s and 1970s[5].

Besides corroborating the monophyly of the major hexapod clades, molecular phylogenetics has also settled several longstanding conundrums in insect phylogeny. These include the placement of highly modified insect groups, such as the eusocial termites[42] and parasitic lice[43–45], twisted-wing insects[46] and fleas[47,48], whose unusual morphology had puzzled generations of entomologists. Unlike other eusocial insects, such as bees and wasps, termites (Isoptera) stand out by being wood-feeding, diploid, and maintaining a prolonged reproductive bond between the male and female. Over a century ago, termites were proposed to be a specialised group of cockroaches[49]. Molecular and morphological studies have since firmly recovered termites as nested within the cockroaches (Blattodea)[30,42,50,51].

Lice (Psocodea) were traditionally divided into parasitic lice (Phthiraptera), free-living booklice and bark lice (Psocoptera). Molecular[43–45] and morphological[52] evidence has shown that psocopterans are a paraphyletic group, while parasitism probably originated only once in the ancestor of Phthiraptera[43]. Another group of specialised parasites, the twisted-winged insects (Strepsiptera) have been difficult to place on the insect tree due to their unique and unusual features. In molecular analyses, the phylogenetic position of the strepsipterans has been difficult to resolve due to tree-reconstruction artefacts[53,54]. The Strepsiptera problem was eventually resolved by harnessing phylogenomic datasets, which resolved them as the sister group to the beetles, together forming the clade Coleopterida[30,46,55].

Finally, the relationships of fleas (Siphonaptera), another group of specialised parasites, have also proved difficult to resolve. Early studies based on a small number of genes indicated that fleas may have been nested within the scorpionflies (Mecoptera)[56,57], while phylogenomic analyses often lent support to a sister-group relationship between fleas and scorpionflies[30,47]. When amino acid alignments were analysed[47] (better suited to

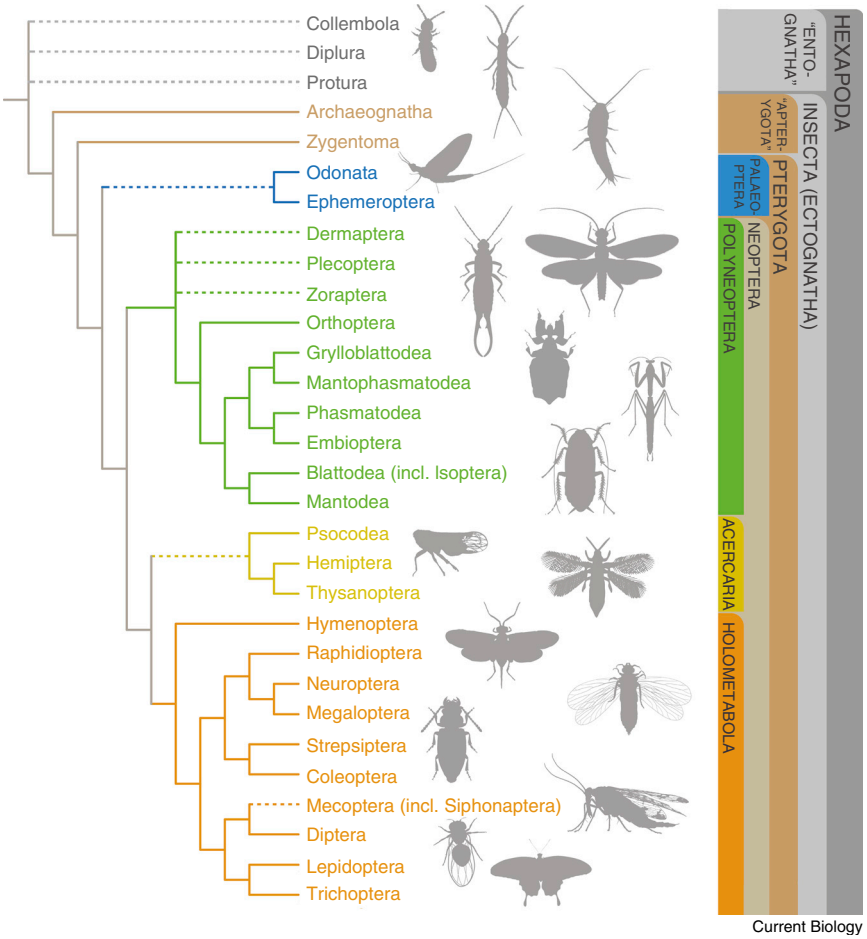


**Figure 1. Insect phylogeny.**
A best estimate of the relationships among the extant hexapod lineages based on current knowledge, with major clades indicated. Nodes that have traditionally been difficult to resolve with any dataset, molecular or morphological, or relationships incongruent between recent phylogenomic analyses, are indicated with dashed lines. Major contentious relationships include interrelationships of the early-diverging hexapod clades Collembola, Diplura, and Protura; monophyly of Palaeoptera; early branching events within Polyneoptera, particularly the position of Zoraptera; the monophyly of Acercaria with respect to Psocodea; and interrelationships within Mecoptera, specifically the placement of Siphonaptera. Silhouettes depict representatives of major insect orders (from top to bottom): Collembola, Diplura, Zygentoma, Ephemeroptera, Dermaptera, Plecoptera, Phasmatodea, Mantodea, Blattodea, Hemiptera, Thysanoptera, Hymenoptera, Neuroptera, Coleoptera, Mecoptera, Diptera, and Lepidoptera.

resolving relationships deep in the insect tree[58]), or when better-fitting evolutionary models were used[48], fleas were found to be nested within scorpionflies, as sister to the enigmatic Southern Hemisphere family Nannochoristidae, corroborating evidence from Mesozoic fossil species[59] and some morphological data[48]. With the knowledge that fleas represent the largest radiation of scorpionflies, they are now regarded as a member of Mecoptera[48].

## Tricky nodes in insect phylogeny

Although many traditional insect clades have been corroborated by phylogenomic analyses[30,43,55,60], there remain contentious nodes that are as difficult to resolve today as they were three decades ago (Figure 1). These long-lasting controversies, relating to deeply nested nodes in hexapod phylogeny, arguably hold the key to our understanding of the origin and early radiation of insects and their resistance to resolution reflects notorious challenges associated with resolving ancient radiations[61].

While the monophyly of hexapods is strongly supported, the earliest branching events within their phylogeny remain controversial[12]. The three non-insect hexapod groups, Collembola, Protura, and Diplura, were combined into the clade 'Entognatha' by Hennig[3] based on their shared possession of mouthparts deeply enclosed within the head (among other characters). However, morphological support for this grouping is equivocal[62,63], and results of molecular phylogenetic analyses vary considerably, with Diplura recovered either as a sister group to insects or to Protura[23,30,64,65]. These uncertainties obscure the origin of the hexapod body plan and our understanding of the morphological adaptations that underpinned their successful colonisation of land. Likewise, the origin of wings and flight in insects also remains elusive. The insect orders Odonata (dragonflies and damselflies) and Ephemeroptera (mayflies) were placed into the clade Palaeoptera, characterised by their inability to fold their wings over their abdomen, which distinguishes them from the remaining winged insects (Neoptera)[66]. However, the monophyly of Odonata and Ephemeroptera has been the subject of much debate, with the results of some morphological[4,67–69] and molecular analyses[11,30,70] suggesting that Palaeoptera may be paraphyletic with respect to Neoptera, and that Ephemeroptera may be representing the sister group of Neoptera[11]. Furthermore, the phylogeny of one of the three major radiations of neopterous insects, Polyneoptera (grasshoppers, roaches, mantises, stick insects, and their kin), has similarly remained difficult to resolve. While phylogenomic datasets now strongly support Zoraptera (a species-poor group of gregarious insects known mostly from the Tropics) as a member of Polyneoptera[30,60,71], its relationships to the early diverging polyneopteran orders Dermaptera (earwigs) and Plecoptera (stoneflies) have remained elusive[19,30,60].

Besides the refractory nature of many old controversies in insect phylogeny, phylogenomic studies have proposed unexpected relationships, such as the non-monophyly of hemipteroid insects (Acercaria or Paraneoptera, i.e., true bugs, thrips and their kin)[30,43]. These relationships require further testing, not least as they stand in stark contrast to available morphological[41] and mitogenomic evidence[72]. There are similar incongruences at

**Box 1. Phylogenomic datasets include many signals and are heterogeneous.**

Phylogenomic inference relies on detecting "phylogenetic signal", a measure of how much of the similarity between genetic sequences reflects relatedness of common ancestry[137]. Genomes also record phylogenetically non-informative signals that can overwrite genuine phylogenetic signal[138], and these can have different origins[139]. Standard phylogenomic methods rely on the concatenation of multiple sequence alignments of many orthologous genes (i.e. genes that are found in the genomes of the considered species because of inheritance, *via* speciation, from a common ancestor)[78]. These larger alignments are generally called 'superalignments'. The correct identification of orthologs to include in a superalignment is key to ensuring phylogenomic accuracy. Incorrect orthology assignment will result in datasets including a mixture of orthologs, paralogs and possibly xenologs, which will result in the inference of trees where nodes identify a mixture of speciation events (ortholog signal), gene duplication events (paralog signal) and possibly lateral gene transfers (xenolog signals). Such a tree is bound to disagree with the underpinning species phylogeny that is defined by the ortholog signal only[140], and a substantial amount of research is thus devoted to improve orthologue identification[141–143]. In addition, alignment of gene sequences might not be straightforward when site-specific substitution rates are high. Misalignments can lead to the recovery of incorrect trees and alignments are usually curated to trim ambiguously aligned positions, and sites with a high proportion of missing data[144,145].

The superalignments used in contemporary studies are composed of thousands of putative orthologous-gene alignments that, once curated, are routinely filtered to retain genes and species that, based on pre-specified criteria, can be expected to maximise the chances to infer a high-quality tree. Many such criteria exist, from occupancy (i.e., how many genes in the data set are scored by individual species), which dictates data decisiveness[146], to the saturation level of individual genes. As in the case of orthology assessment, how to select the orthologs to concatenate in a phylogenomic dataset is an active area of research, where a diversity of bespoke pipelines have been developed based on the diverse criteria briefly mentioned above (a non-exhaustive list of examples includes MARE[147], a well-established pipeline particularly in hexapod phylogenomics, ClanCheck[148], Agalma[149], geneSortR[150]). These pipelines do not use the same criteria to select genes and do not necessarily aim to achieve the same goals.

Superalignments are expected to show strong heterogeneity in the number and type of mutations observed across sites and lineages, due to gene-, site- and species-specific idiosyncrasies of the molecular evolutionary process[58,89,90]. Not accounting for the heterogeneity in phylogenomic datasets can lead to inaccurate phylogenetic reconstruction because of tree-reconstruction artefacts caused by systematic errors.

all levels of insect phylogeny, within orders and families, further confusing our understanding of critical events in insect evolution, such as the origins of flight or of eusociality[73–76].

## Tackling tricky nodes

Major questions in insect phylogeny have remained difficult to address despite the availability of unprecedented amounts of genomic data[3,5,77]. While it remains important to sequence new species to fill gaps in the insect phylogenetic tree, resolving these tricky nodes will require more than just sequencing. Rather, it will require a shift in focus, away from expanding taxon and locus sampling towards the testing of hypotheses through the application of ever improving strategies to mitigate dataset-assembly errors (Box 1) and systematic biases (Boxes 2 and 3), both of which can have detrimental effects on phylogenetic accuracy[78]. Of particular importance is the investigation of possible tree-reconstruction artefacts arising from systematic biases in molecular datasets, which can broadly be defined as errors that stem from incorrect analytical assumptions[79]. A key characteristic of tree-reconstruction artefacts is that the same error is repeatably and consistently recovered with high support from different datasets composed of the same type of data as long as the underlying biases remain unmitigated[80,81]. This has been cogently demonstrated for early animal evolution[82] where analyses performed using a model that could not accommodate site-specific compositional-heterogeneity (Box 3) systematically failed to reconstruct the target (true) tree when data were simulated using a site-specific compositional-heterogeneous model (Box 3), unless the target tree was isomorphic with the tree implied by the tree-reconstruction artefact imposed by the bias affecting the data. This study[82] also illustrates an important but counterintuitive problem with phylogenomic reconstruction: not all tree topologies are equally simple to find. Some target topologies are particularly difficult to recover. In such cases, it is to be expected that they will only be recovered under a very restricted set of models, and possibly with low support[82]. As such, consistent recovery of a highly supported tree by different datasets and studies using models that fit the data poorly or inadequately describe it is not a valid proxy for phylogenetic accuracy.

Tree-reconstruction artefacts lead to the systematic recovery of inaccurate tree topologies and are caused by the use of models that do not adequately describe the process that generated the data (unmitigated process heterogeneity). The most common sources of heterogeneity[83] are variation in the rate of sequence change between lineages or sites (rate heterogeneity), heterogeneity of nucleotide or amino acid composition across sites or species (compositional heterogeneity) and within-site rate variation (heterotachy; Box 2).

Statistical phylogenetic methods use an evolutionary model (Box 3) to account for amino acid (or nucleotide) substitutions. Different substitutions will be differently weighted when calculating the likelihood of trees, based on their rarity. If the model used in the analyses does not account for a type of heterogeneity that characterises the analysed data, amino acid substitutions will be incorrectly weighted, potentially leading to the identification of an incorrect best-fit tree. For example, two lineages with high GC content in a dataset where all the other lineages have low GC content will tend to be clustered together by models that do not account for lineage-specific compositional

**Box 2. Types of heterogeneity in the evolutionary process.**

### REPLACEMENT-RATE HETEROGENEITY

Substitution between amino acids (or nucleotides) is not homogenous. Replacement between different amino acids happens with different probability, depending on the physical and biochemical properties of the interchanging elements. For example, purines are more likely to interchange among each other than with pyrimidines because of molecular constraints. Similarly, the bulky amino acid tryptophan is more likely to interchange with the similarly sized phenylalanine than with the tiny glycine.

### ACROSS-SITE (AMONG-SITE) RATE HETEROGENEITY

Positions along a multiple sequence alignment accumulate mutations at different rates. This can be because of functional constraints or because of differences in selective pressures that are specific to different genomic regions[151]. For example, third codon positions mutate faster than first and second ones, leading to a greater accumulation of substitution and a faster deterioration of the phylogenetic signal at these sites[152]. On the other end of the mutational spectrum, functional sites in protein coding genes generally accumulate fewer mutations as changes happening at these sites can interfere with the functionality of the mutant protein.

### ACROSS-LINEAGE (AMONG-TAXA) RATE HETEROGENEITY

Different lineages might be differently prone to accept substitutions, for example because of linage-specific differences in the proofreading ability of their DNA polymerases[153]. As a consequence, different lineages may experience lineage-specific variation in evolutionary rates, and differential, lineage-specific accumulation of mutations.

### ACROSS-LINEAGE (AMONG-TAXA) COMPOSITIONAL HETEROGENEITY

Differences in the nucleotide/amino acid composition across taxa can be caused, for example, by adaptation to local habitats or lineage specific substitutional biases[58].

### ACROSS-SITE (AMONG-SITE) COMPOSITIONAL HETEROGENEITY

Different sites have different functional constraints and, despite proteins using 20 amino acids, a specific site might be able to accept only a few if the protein needs to have specific biochemical properties to function. An extreme example is represented by position 296 in the retinal binding domain of animal opsins. Across all opsins and all animals, this site invariably hosts a lysine. Other amino acids would cause a failure to bind retinal, making the protein dysfunctional and the organism unable to detect light[154].

### ACROSS-SITE HETEROGENEITY IN THE RATE OF SUBSTITUTION THROUGH TIME (HETEROTACHY)

The rate of substitution at a position may change over time, a process called heterotachy[155]. This process happens when a site had accumulated mutations at some point in the past (perhaps during an adaptive process) and then stopped accumulating mutations.

heterogeneity, as these models would conclude that the GC-rich sequences share many substitutions[79]. Long-branched taxa exacerbate problems of statistical inconsistency when the model used does not adequately describe the data[84]. Targeted taxon sampling, i.e., the inclusion of species that break long branches, can reduce attraction effects[85], but the addition of extra taxa in the absence of adequate modelling is generally insufficient to resolve tricky nodes. Furthermore, some long-branched clades have no close living relatives available for branch breaking.

Analyses of persistent problems in insect phylogeny have shown that systematic errors are indeed prevalent in trees inferred from large molecular datasets[14,70]. Adequate modelling is thus key, irrespective of taxon sampling density, to prevent the recovery of highly-supported but artefactual topologies and to this scope, a variety of models are continuously being developed (Box 3), implemented in software like IQ-TREE[86], PhyloBayes[87,88], and P4[89]. Indeed, considering process heterogeneity is so important to phylogenetic accuracy that modelling among-site rate heterogeneity using a Gamma distribution has long been the default approach of modern phylogenetics. However, other forms of heterogeneity, particularly across-site compositional heterogeneity, are much less frequently modelled, particularly in studies of insect evolution[30,43,55,60,76]. We contend that modelling across-site compositional heterogeneity should become the default approach in phylogenomics in general and in insect phylogenomics in particular, given that there is ample evidence that models that can accommodate this form of heterogeneity (e.g. CAT-based models[90]; Box 3) invariably provide a better fit to the data and lead to greater phylogenetic accuracy[82]. The effectiveness of CAT-based models has been demonstrated in various insect datasets[14,48,91] and analyses of insect phylogeny show strong sensitivity to model selection[14,48,92].

**Box 3. Mitigating tree-reconstruction artefacts by modelling heterogeneity.**

A common misconception is that heterogeneous substitution models represent a recent, perhaps unnecessary or even deleterious[156], development. This is incorrect, as heterogeneous models have a long history and their implementation is justified by biological and statistical (model fit and model adequacy) arguments. For example, general time reversible (GTR) matrices, including well-known precomputed GTR matrices for amino acid data (e.g. WAG or LG), assume at the least some heterogeneity of the amino acid (or nucleotide) replacement process.

The heterogeneity of the substitution process is not entirely described by a GTR matrix and an amino acid (or nucleotide) frequency vector, and to account for the observation that different sites accumulate substitutions at different rates (across-site rate heterogeneity; Box 2) models were developed that sample site-specific rates of evolution from a Gamma distribution fitted to the data[157]. Across-site rate heterogeneity is just the tip of a heterogeneity iceberg and models have been developed to account for other forms of heterogeneity. Across-site compositional heterogeneity has been abundantly shown to impact on phylogenetic accuracy[79,84,139]. It is caused by functional constraints that lead different sites to accept only a subset of the 20 amino acids and its modelling is therefore biologically justified (Box 2). Across-site compositional heterogeneity is modelled using category (CAT)-based models[90], which partition the sites of an alignment into categories of specific composition. CAT-based models (including the UMD and C10–60 models implemented in IQ-TREE as well as the CAT-F81 and CAT-GTR models implemented in Phylobayes) have been shown to invariably fit the data better than models that do not account for across-site compositional heterogeneity (e.g. LG+G), further justifying the implementation of these models that, in our opinion, should become the default choice in insect phylogenomics (Figure 2). While some studies have attempted to account for across-gene heterogeneity by partitioning individual genes into sets that were then assigned individual compositionally homogeneous models[158], modelling site-specific heterogeneity instead has been shown to achieve greater phylogenetic accuracy[159]. In agreement with this observation, the use of model adequacy tests has shown that CAT-based models more adequately describe superalignments than partitioned datasets and datasets that do not account for compositional heterogeneity[98].

Further forms of heterogeneity can affect phylogenomic datasets, from across-lineage compositional heterogeneity, which can be modelled by the Node Discrete Compositional Heterogeneity (NDCH) model[89], and the correspondence and likelihood Analysis (COaLA) model[160], to heterotachy[155] (Box 2), which can be modelled using the GHOST model in IQ-TREE[161]. It is to be expected that multiple forms of heterogeneity will affect the same dataset[162], with models such as CAT-GTR+G (for example) attempting to model at the same time replacement rate heterogeneity, across-site rate heterogeneity and across-site compositional heterogeneity.

Selection of an appropriate model should be based on a combination of model fit and model adequacy tests. While software packages such as ModelFinder in IQ-TREE automatically perform model fit[163] tests for a subset of known (generally pre-computed) amino acid models like LG+G or WAG+G, it is key to test also more complex models from GTR (i.e. a GTR matrix estimated from the data), to the CAT-based models (from C10–60 to the more complex CAT-F81 and CAT-GTR), as these models are expected to fit most datasets better than models that cannot account for compositional heterogeneity. However, even the best fit model might fail to describe specific aspects of the data adequately[98]. As this can affect phylogenetic accuracy[164], model adequacy should be more regularly tested. Model adequacy tests should be used to aid model selection evaluating whether the best fit (and other) models can also adequately describe key features of the data (e.g. its across-site compositional heterogeneity)[165]. However, model adequacy tests are not yet as broadly implemented as model fit tests and further software development in this area is urgently needed.

Complex models like CAT-GTR+G are not without limits. Their diffusion in the entomological research community may have been slow in part due to their higher computational cost and because for some datasets they can fail to reach convergence. In our view, these limitations are outweighed by their higher accuracy and applicability to the largest majority of phylogenomic datasets, or randomly subsampled versions of such datasets.

CAT-based models were initially designed to address recalcitrant nodes deep in the history of life. However, it has been shown that these models can be crucial also in the study of clades that have relatively short evolutionary histories[93,94]. Applied to insects, CAT-based models have the potential to help resolve both ancient relationships and recent radiations. Due to the short generation time of many insects, compositional biases are expected to emerge also in relatively recent radiations, and in radiations of fast-evolving lineages, such as parasitic groups[14,48,53]. For example, the model species *Drosophila melanogaster* has a generation time of 7–19 days depending on environmental conditions, and countless other insects reproduce even faster, with some aphids completing their reproductive cycle in a matter of days[95]. Unsurprisingly, model dependency can be observed when reconstructing *Drosophila*[96] phylogenies (Figure 2).

Among-site rate and compositional heterogeneity are not the only known form of heterogeneity affecting phylogenomic datasets (Box 2) and there is no universal remedy owing to the complex nature of the substitution process. Hence, model-fit and model-adequacy tests should be used to ensure analyses are performed under the best fitting model and that the best fitting model can adequately describe data heterogeneity[78]. Outside of insects, examples of the application of model adequacy tests can be found in analyses of mammal[93,94] and metazoan[97] relationships. Ultimately, no model can be expected to capture the

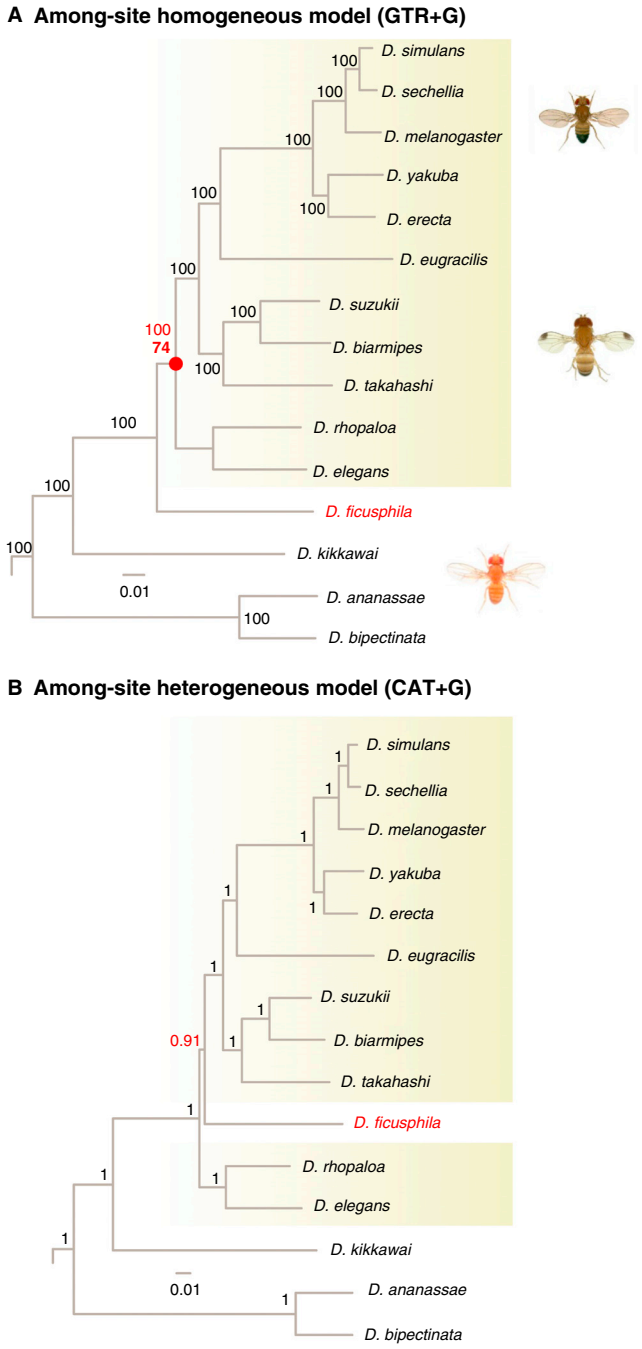


**Figure 2. Model dependency in insect phylogenomics.**
In this example, a compositionally heterogeneous, genome-scale nucleotide dataset of 182,271 nucleotides from 89 concatenated orthologous genes[96,166] recovers contrasting *Drosophila* trees. (A) When analysed using the GTR+G model (Box 3), implemented using RAxML[167], it recovers a tree different from the one inferred in (B) using Phylobayes[88] under CAT+G (Box 3). CAT-based models work by splitting a superalignment of multiple genes (Box 1) into its constituent sites, which are then reassigned to compositional categories. Accordingly, CAT+G can account for both among-site and among-gene compositional heterogeneity, differently from GTR+G, which does not account for these types of heterogeneity. An analysis of the same dataset performed using Astral, which can account for incongruences across gene trees, shows reduced support for the topology in (A). In this example, individual gene trees for the genes in the superalignment disagree among each other because of among-gene compositional heterogeneity and within-gene compositional heterogeneity. CAT+G can account for both forms of heterogeneity while Astral only for one (unless single gene analyses are performed using CAT-based models), and both analyses question the veracity of the GTR+G tree, which does not account for either. Support for the tree in (A) is maximal under GTR+G, significantly decreases under Astral, and disappears under CAT+G. That is, as modelling of heterogeneity improves, support for the tree in (A) disappears, demonstrating that, even within individual insect genera, phylogenetic reconstruction can be model-dependent and compositional heterogeneity can have a strong impact. Our comparative analyses suggest that the *Drosophila* GTR+G tree should be considered unreliable, despite its high support, and cautions against bold interpretations of evolutionary history solely based on analyses that use models that cannot accommodate the heterogeneity in the data. In (A; GTR+G and Astral analyses) numbers at nodes are bootstrap supports (BS). The bold number is the BS from an Astral analysis using 89 single-gene trees from RAxML as input and bootstrapping using gene-only resampling; all other Astral BS are 100. In (B; CAT+G analysis) supports at nodes are posterior probabilities. In all analyses, a discrete Gamma distribution with four rate categories was used to model among-site rate variation. Alignment and trees are available at Mendeley Data, V1, https://doi.org/10.17632/v3bt85fxch.1.

full complexity of the substitution process and, in some instances, none of the available models describe the data adequately[98]. Nevertheless, not all models are equal and model-fit and model-adequacy tests should be implemented to evaluate the trust we can place on alternative phylogenetic hypotheses and the efficacy of the evolutionary conclusions drawn from them.

Coalescence problems can also lead to the recovery of inaccurate trees[78,99]. This happens when gene trees disagree with the species tree because of incomplete lineage sorting and introgression[100]. Standard gene concatenation methods (like the one discussed above; Box 1) cannot account for such phenomena. Methods that relax the assumption that all genes follow the same history[101] have been implemented[102–104] and these methods can be employed to establish whether phylogenomic analyses are not mislead by population level phenomena and gene-level heterogeneity (Figure 2).

Application of some of these approaches to test controversial phylogenetic hypotheses has revealed that they are only recovered when suboptimal substitution models are employed[105]. This highlights the importance of hypothesis-testing in phylogenetics, particularly as new substitution models and phylogenetic approaches are developed (Boxes 1–3). As a group of highly specialised parasites, the phylogenetic position of fleas (Siphonaptera) has historically been difficult to resolve[30]. When methods addressing compositional heterogeneity are implemented, either by analysing less-saturated amino acid alignments[47] or using better-fitting site-heterogeneous models[48], fleas are robustly recovered within Mecoptera, reducing the number of extant holometabolan insect orders to ten[48].

Modelling of compositional heterogeneity and comparison of models using model fit and adequacy tests are already a standard component of studies that examine difficult phylogenomic problems outside of insects, which likewise defy resolution despite the accumulation of more sequence data. Recent examples include extremely ancient radiations, such as the relationships between prokaryotes, archaea, and eukaryotes, Metazoa, land plants, Lophotrochozoa and the monophyly of arachnids[97,106–110]. We anticipate that future advances in phylogenomic methods will result in the development of efficient

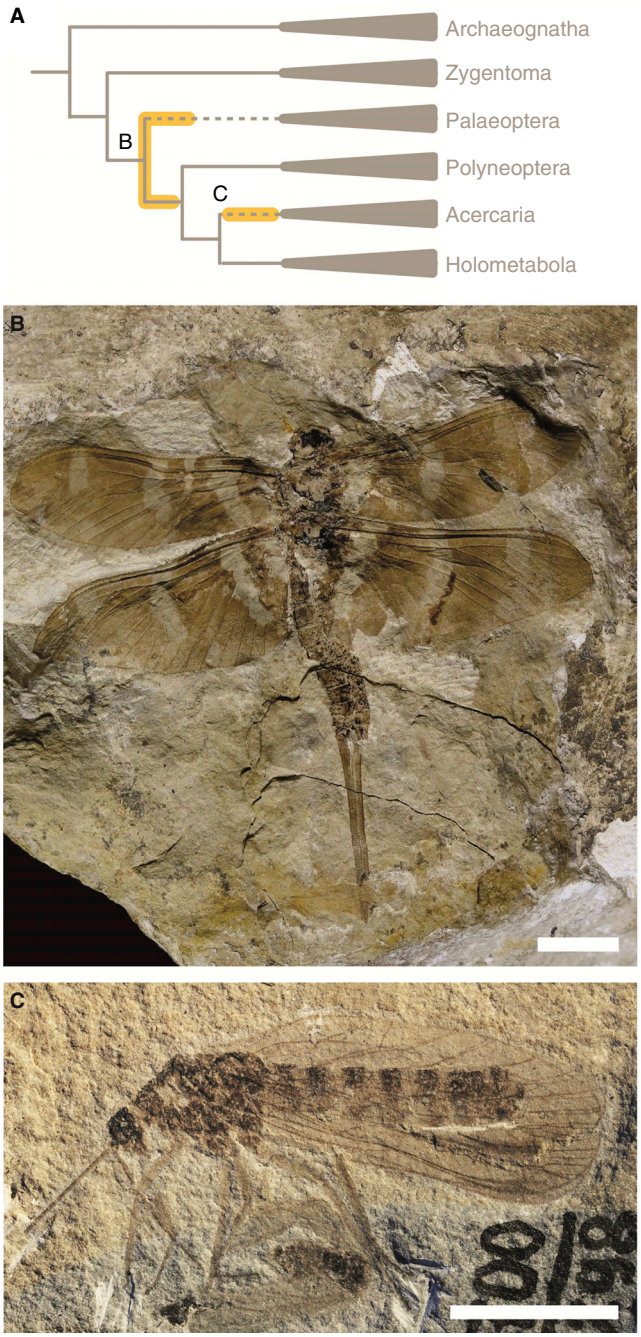


**Figure 3. Fossils and integrative insect phylogeny.**
Fossil insects belonging to the stem-group of major extant clades provide invaluable data for testing contentious phylogenetic hypotheses. (A) Insect phylogeny, highlighting the uncertain monophyly of Palaeoptera and Acercaria with dashed lines, and phylogenetic uncertainty regarding the placement of the extinct insect orders Palaeodictyoptera (B) and Hypoperlida (C) with orange bars (scale bars: 5 mm). Stem-groups are represented by lines, crown groups by triangles. (B) A palaeodictyopteran, *Dunbaria fascipennis*, from the Early Permian of Elmo, Kansas. Palaeodictyoptera were a Palaeozoic order of uncertain placement, representing either an early-diverging branch of Palaeoptera or stem-group to Neoptera[120]. (C) *Idelopsocus* sp. (†Hypoperlida) from the Early Permian Tshekarda locality, Russia. Hypoperlids were putative stem-group acercarians[168]. (Photo courtesy of Jakub Prokop.)

models accounting for further aspects of the substitution process[111]. Ultimately, however, only corroboration from multiple, independent lines of morphological and molecular evidence can discriminate between alternative phylogenetic hypotheses[112–114]. With the availability of gene-content datasets for almost all major insect orders[115], phylogenies inferred using this type of data[116], or based on pattern of gene deletions and duplications[117], can be expected to have an important role in testing hypotheses of hexapod relationships derived using amino acid data, within the philosophical framework provided by consilience analysis[112–114]. Finally, recent advances in microscopy and tomography offer unprecedented ways of studying insect morphology and will also contribute to testing phylogenetic hypotheses derived from molecular data[118].

## Restoring the dead to the insect tree of life

Phylogenomics captures only a snapshot of insect evolution, turning a blind eye to the immense diversity of extinct insect lineages. Numerous enigmatic insect groups that have been exceptionally diverse in the past, such as the Palaeodictyoptera and 'Protorthoptera' (or 'Grylloblattida'), have proven difficult to place on the insect phylogeny and in some cases represent heterogeneous paraphyletic assemblages rather than valid clades[119,120]. A robust backbone of the insect tree is required to firmly place extinct lineages of uncertain affinity. In a complementary fashion, fossil insects, particularly those belonging to the stems of major lineages, provide a powerful and independent source of evidence that can be used to test competing hypotheses of insect evolution (Figure 3). For example, Mesozoic stem-group fleas from the Jurassic share characters of scorpionflies and modern fleas[59], providing intriguing hints about flea ancestry and the evolution of the flea body plan.

Congruence between morphological and genomic data provides an important line of evidence for testing phylogenetic hypotheses. With large morphological datasets already available for major extant insect groups[121–123], phylogenetic software including BEAST[124], IQ-TREE[86], MrBayes[125], and RevBayes[126] facilitate integration of morphological and molecular data without having to compromise the quality of modelling of the molecular component. Following Hennig's lead, insect datasets have been the focus of these methodological developments[127,128], fulfilling his prophesy that, through careful analysis of the fossils, it would become possible to resolve phylogenetic structure within the taxonomic waste basket 'stem-groups' that he defined[3]. This is crucial not only to establishing evolutionary timescales and historical biogeography and ecology, but also patterns of character evolution in the assembly of insect body plans, crucial to informing models of developmental evolution[129–131]. Parallel to efforts in the field of phylogenomics, we expect the development of new models that more faithfully capture the complexities of morphological character evolution to become available[132,133].

Integrated into a rigorous phylogenetic framework, the vast fossil record of insects can provide invaluable insights into the history of terrestrial ecosystems over the last 400 million years. Although hexapods may not traditionally hold a prominent position in textbooks of invertebrate palaeontology, insects are not rare in the fossil record: they outnumber dinosaurs in terms of

fossil species richness 25 to one (~27,580 recognised fossil insect species in ~14,000 genera in the Palaeobiology Database as of 6 May 2021). This is especially the case for groups with sclerotised forewings, such as beetles and roaches. The vast fossil record of insects can be used to calibrate the timescale of insect evolution and estimate rates of diversification. Insects figure prominently as co-evolving pollinators of gymnosperms and angiosperms in understanding the establishment of modern terrestrial ecosystems during a Cretaceous Terrestrial Revolution[134]. Similarly, the apparently low extinction rates of insect clades at the Cretaceous–Palaeogene boundary stand in stark contrast to some other animal groups, most famously the non-avian dinosaurs, posing curious questions about extinction selectivity at the K–Pg boundary[135]. A dated integrative insect phylogeny, with its extinct branches restored among their living relatives, will improve our understanding of the macroevolutionary history of insects and the drivers of their unparalleled biodiversity among animals[136]. However, resolving the relationships of the dead may have to follow resolution of the relationships among the living for whom a comparatively infinite amount of data are available.

## The future of insect phylogeny

A well-resolved insect phylogeny is a pre-requisite for understanding the evolutionary success of the most diverse of all animal lineages. Debates in insect evolution have been dominated by discourse over several contentious relationships that have proven as difficult to resolve in the age of genomics as they were when systematics was based on comparative morphology alone. Conflicting results between genome-scale datasets confirm that tree-reconstruction artefacts are commonplace despite large amounts of data, and that systematic biases in phylogenomic datasets must be mitigated if we are to achieve an accurate phylogeny of insects. A powerful suite of computational tools exists for mitigating the effects of a highly heterogeneous molecular substitution process and resolving contentious nodes in insect phylogeny. Alternative types of genomic data (like gene-content data) and morphology can be used to help distinguish among competing hypotheses of insect relationships, not least through the integration of fossil species that can both reveal otherwise cryptic homologies and convergences. While targeted taxon sampling helps reduce some systematic errors by breaking down long branches[85], it is no panacea and to maximise our chances of resolving tricky nodes, judicious taxon and locus sampling has to be combined with substitution models that can adequately describe the data[93,94,98]. With an unprecedented resource of genomic datasets for insects, systematic entomologists should move away from inductivism towards a more deductive, experimental and hypothesis-driven approach to resolving insect phylogeny.

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## DECLARATION OF INTERESTS

The authors declare no competing interests.

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