PHYLOGENOMICS AND THE RECONSTRUCTION OF THE TREE OF LIFE

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Abstract | As more complete genomes are sequenced, phylogenetic analysis is entering a new era — that of phylogenomics. One branch of this expanding field aims to reconstruct the evolutionary history of organisms on the basis of the analysis of their genomes. Recent studies have demonstrated the power of this approach, which has the potential to provide answers to several fundamental evolutionary questions. However, challenges for the future have also been revealed. The very nature of the evolutionary history of organisms and the limitations of current phylogenetic reconstruction methods mean that part of the tree of life might prove difficult, if not impossible, to resolve with confidence.

HOMOLOGOUS CHARACTERS
Homologous characters are those that are descended from a common ancestor.

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Understanding phylogenetic relationships between organisms is a prerequisite of almost any evolutionary study, as contemporary species all share a common history through their ancestry. The idea of phylogeny follows directly from the theory of evolution presented by Charles Darwin in *The Origin of Species*[1]: the only illustration in his famous book is the first representation of evolutionary relationships among species, in the form of a phylogenetic tree. The subsequent enthusiasm of biologists for the phylogenetic concept is illustrated by the publication of Ernst Haeckel's famous 'trees' as early as 1866 (REF. 2).

Today, phylogenetics — the reconstruction of evolutionary history — relies on using mathematical methods to infer the past from features of contemporary species, with only the fossil record to provide a window to the evolutionary history of life on our planet. This reconstruction involves the identification of HOMOLOGOUS CHARACTERS that are shared between different organisms, and the inference of phylogenetic trees from the comparison of these characters using reconstruction methods (BOX 1). The accuracy of the inference is therefore heavily dependent on the quality of models for the evolution of such characters. Because the underlying mechanisms are not yet well understood, reconstructing the evolutionary history of life on Earth solely on the basis of the information provided by living organisms has turned out to be difficult.

Until the 1970s, which brought the dawn of molecular techniques for sequencing proteins and DNA, phylogenetic reconstruction was essentially based on the analysis of morphological or ultrastructural characters. The comparative anatomy of fossils and extant species has proved powerful in some respects; for example, the main groups of animals and plants have been delineated fairly easily using these methods. However, this approach is hampered by the limited number of reliable homologous characters; these are almost non-existent in microorganisms[3] and are rare even in complex organisms.

The introduction of the use of molecular data in phylogenetics[4] led to a revolution. In the late 1980s, access to DNA sequences increased the number of homologous characters that could be compared from less than 100 to more than 1,000, greatly improving the resolving power of phylogenetic inference. A few genes became reference markers. In particular, owing to its considerable degree of conservation across all organisms, the gene that encodes small subunit ribosomal RNA (SSU rRNA) was extensively used for the classification of microorganisms and allowed the recognition of the Archaea as a third distinct domain of the tree of

Box 1 | Basic principles and methods of phylogenetic inference

Phylogenetic inference involves two crucial steps: first, homologous characters (those that are descended from a common ancestor) are identified among species; second, the evolutionary history of species is reconstructed from the comparison of these characters using tree-building methods for phylogenetic inference. Almost any type of character (for example, morphological structures, ultrastructural characteristics of cells, biochemical pathways, genes, amino-acids or nucleotides) can be used for inferring phylogenies, provided that they are homologous. In sequence data, homology is determined by similarity searching. Once homologous characters are identified, a character matrix is constructed, which scores the different character states (columns on the matrix) observed in each species (rows on the matrix).

Three main kinds of reconstruction method can then be used to infer phylogenetic trees from this character matrix as follows (see REF. 10 for an overview and REF. 34 for details).

Distance methods

These methods first convert the character matrix into a distance matrix that represents the evolutionary distances between all pairs of species. The phylogenetic tree is then inferred from this distance matrix using algorithms such as neighbour joining (NJ)[155] or minimum evolution (ME)[156].

Maximum parsimony

This method selects the tree that requires the minimum number of character changes to explain the observed data.

Likelihood methods

These methods are based on a function that calculates the probability that a given tree could have produced the observed data (that is, the likelihood). This function allows the explicit incorporation of the processes of character evolution into probabilistic models. Maximum likelihood (ML)[157] selects the tree that maximizes the probability of observing the data under a given model. Bayesian methods[106] derive the distribution of trees according to their posterior probability, using Bayes' mathematical formula to combine the likelihood function with PRIOR PROBABILITIES on trees. Unlike ML, which optimizes model parameters by finding the highest peak in the parameter space, Bayesian approaches integrate model parameters (see REF. 10 for a review).

life[5]. However, as more genes were analysed, topological conflicts between phylogenies based on individual genes were revealed. Moreover, information from a single gene is often insufficient to obtain firm statistical support for particular NODES of a phylogeny. As a consequence, numerous parts of the tree of life remained poorly resolved simply because of sampling effects that are due to the limited availability of data.

However, in the time it has taken you to read these lines, thousands more base pairs of sequence information will have been generated by large-scale genomic projects. This wealth of data, hardly imaginable only a decade ago, is giving birth to a new field of research, termed phylogenomics, which uses phylogenetic principles to make sense of genomic data[6]. One branch of phylogenomics involves the use of these data to reconstruct the evolutionary history of organisms. Access to genomic data could potentially alleviate previous problems of phylogenetics due to sampling effects by expanding the number of characters that can be used in phylogenetic analysis from a few thousand to tens of thousands. With this increase, the emphasis of phylogenetic inference is shifting from the search for informative characters to the development of better reconstruction methods for using genomic data. Existing models used in tree-building algorithms only partially take into account molecular evolutionary processes, and phylogenomic inference will benefit from an increased understanding of these mechanisms. Interestingly, phylogenomics is also providing the opportunity to use new 'morphological-like' characters that are based on genome structure, such as rare genomic changes (RGCs)[7,8].

In this review, we describe current methods for phylogenomic inference and discuss their merits and pitfalls in light of their recent application to diverse phylogenetic problems. The recent improvements in the resolution of the tree of life owing to large-scale studies in each of the three domains — Archaea, Bacteria and Eukaryota — are discussed. Despite holding considerable promise, the phylogenomic approach also has potential problems that stem from the limitations of current phylogenetic reconstruction methods. We present examples of method INCONSISTENCY that lead to tree-reconstruction artefacts, and tentatively propose solutions to these issues. Finally, we discuss the future potential of phylogenomics and specifically address the issue of how to corroborate results from phylogenomic analyses.

Current methods for phylogenomics – an overview

The two crucial steps of classical phylogenetic inference — the identification of homologous characters and tree reconstruction — are generally preserved in phylogenomics. Therefore, as for phylogenies that are based on morphological data or single genes, the reliability of a phylogenomic tree depends on the quality of the characters and the accuracy of the reconstruction methods. Theoretically, reliable characters can be considered as those that have undergone only a few changes over time (ideally, a single change). Multiple changes create HOMOPLASY (noise) in the form of CONVERGENCE and REVERSAL, masking the genuine phylogenetic signal[9].

The three main types of standard phylogenetic reconstruction method (distance, parsimony and likelihood methods[10]; BOX 1) have been adapted for use in

PRIOR PROBABILITY
The probability of a hypothesis (or parameter value) without reference to the available data. This can be derived from first principles, or based on general knowledge or previous experiments.

NODE
Nodes of phylogenetic trees represent taxonomic units. Internal nodes (or branches) refer to hypothetical ancestors, whereas terminal nodes (or leaves) generally correspond to extant species.

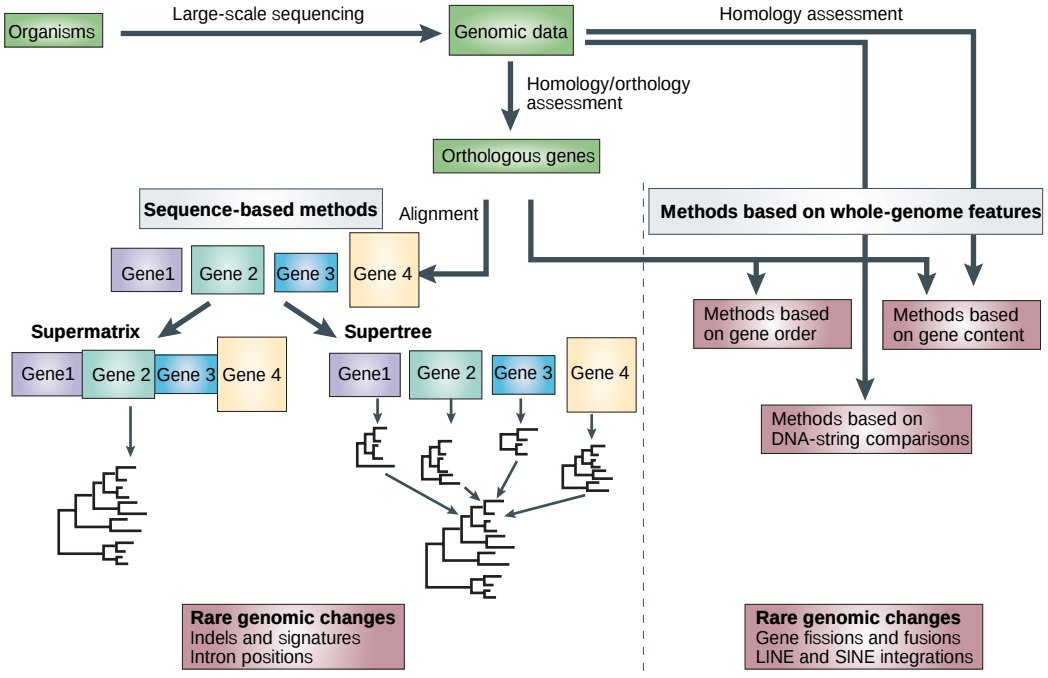
INCONSISTENCY
A phylogenetic reconstruction method is statistically inconsistent if it converges towards supporting an incorrect solution with increasing confidence as more data is analysed.

HOMOPLASY
Identical character states (for example, the same nucleotide base in a DNA sequence) that are not the result of common ancestry (not homologous), but that arose independently in different ancestors by convergent mutations.

CONVERGENCE
The independent evolution of similar character states in evolutionarily distinct lineages.

REVERSAL
The independent reacquisition of the ancestral character state in a given evolutionary lineage.

Box 2 | Methods of phylogenomic inference



The flowchart shows steps in the inference of evolutionary trees from genomic data. Genomic information is obtained by large-scale DNA sequencing. In general, sets of orthologous genes are then assembled from specific sets of species for phylogenetic analysis. This homology or orthology assessment is a crucial step that is almost always based on simple similarity comparisons (for example, BLAST[158] searches). Most methods used for the subsequent reconstruction of phylogenetic trees are either sequence-based or are based on whole-genome features.

Sequence-based methods

These methods require orthologous genes to be aligned using tools for multiple-sequence alignment (for example, the ClustalW program[159]) and the determination of unambiguously aligned positions (for example, using Gblocks[160]). Once this crucial step is achieved, two alternative approaches can be used to infer phylogenetic trees from the different gene alignments, which are usually of unequal lengths and contain different sets of species. The supermatrix approach involves analysing the concatenation of individual genes, and non-overlapping taxa are coded as missing data. Likelihood-based reconstruction methods (BOX 1) are particularly suited for the analysis of supermatrices. These methods take into account across-gene heterogeneity in evolutionary rates by using partitioned-likelihood models, which allow each gene to evolve under a different model[161]. Despite the increased number of extra parameters introduced by using an independent model for each gene, these partitioned models usually fit the data better than concatenated models[17,20,162]. Alternatively, the supertree approach[37] combines the optimal trees obtained from the analysis of individual genes, each of which contains data from only partially overlapping sets of taxa.

Methods based on whole-genome features

These methods infer phylogenetic trees from the comparison of gene content (also known as gene repertoire), gene order and 'DNA strings' (the distribution of oligonucleotides in genomes). Gene-content methods reconstruct phylogenetic trees from 'distances', which represent the proportion of shared orthologous genes between genomes using classical distance algorithms[50,51,55], or from matrices, which score the presence or absence of homologues or orthologues in genomes using maximum parsimony[56,57]. Gene-order methods construct phylogenetic trees by minimizing the number of breakpoints between genomes[65], or simply by scoring the presence or absence of pairs of orthologous genes[53,56]. Methods that are based on the distribution of DNA strings, which do not rely on homology assessment, can also be used[55,73–75]. These are based on oligonucleotide 'word usage' (the frequency of short-oligonucleotide combinations), which provides a characteristic signature of genome structure[72]. The few approaches that are currently implemented for this method calculate evolutionary distances among species from the difference in their oligonucleotide word usage, and reconstruct phylogenetic trees using standard distance-based algorithms[55,73–75].

Rare genomic changes

Rare genomic changes[7,8] — such as insertions and deletions (indels), intron positions, retroposon (SINE and LINE) integrations, and gene fusion and fission events — can be used as signatures that support particular nodes, and to reconstruct phylogenetic trees on the basis of their presence or absence.

phylogenomics. Phylogenomic reconstruction methods can be divided roughly into sequence-based methods and methods that are based on whole-genome features. The latter are becoming increasingly popular, but their relatively recent introduction limits their critical evaluation at this stage. As a consequence, methods based on multiple-sequence alignment, for which there is an extensive methodological background, currently remain the methods of choice. Sequence-based methods and methods based on whole-genome features, as well as the study of rare genomic changes, are discussed below.

Sequence-based methods

Number of characters versus number of species. Sequence-based phylogenomic methods are based on the comparison of primary sequences, and phylogenetic trees are inferred from multiple-sequence alignments. Around the year 2000, the move from single-gene to multiple-gene analyses, using sets of fewer than 20 genes (for examples see REFS 11–15), slightly preceded the phylogenomic era. Datasets that contained more than 100 genes were subsequently used[16–21], but at the cost of considering fewer species than in single-gene studies because of data availability and computational time constraints (for recent exceptions, see REFS 22,23).

A long-standing debate in phylogenetics is whether the greatest improvement in accuracy results from an increased number of characters (in this case, genes) or species[24–28]. Evidence from computer simulations has been equivocal[27,28], whereas empirical studies tend to support the importance of extensive species sampling[24,29,30]. In practice, increasing the number of genes is straightforward for species for which complete genomes have been sequenced (see REFS 16,18,19,21,31 for examples). However, the largest complete datasets (in which all genes are represented for all species) that can be mined from sequence databases are asymmetrical — that is, they include many species and few genes, or few species and many genes[22,32]. As it is likely to produce more accurate results[29,30], assembling phylogenomic datasets rich in both species and genes is necessary. However, this is invariably associated with missing data, as some genes are not represented for all species (see REFS 17,20,22 for examples), and the effects of these missing data are discussed below.

Supermatrices and supertrees. Once multiple-gene alignments have been assembled from the chosen dataset as described above, two alternative approaches can be used for phylogenomic reconstruction. Following from the total evidence principle of using all the relevant available data[33], the most popular strategy is to analyse the 'supermatrix' that is formed by the concatenation of individual genes (BOX 2) using standard sequence-based methods[34]. In this approach, the sequences of genes that are not represented for some species are coded as question marks. Several studies have implicitly made the assumption that a certain amount of missing data can be tolerated by tree-reconstruction methods (for example, 20% missing data in REF. 11, 12.5% in REF. 15, 25% in REF. 17). Recent empirical[20,22,35] and simulation[20,36] studies have shown that this proportion can be surprisingly high without losing too much accuracy. The impact of missing data is limited because species for which sequence information is incomplete are still represented by a large number of informative characters in phylogenomic studies[20]. In fact, having a species represented by only 10 genes out of 100 that are analysed in the whole study might generally be less problematic than not considering that species at all.

The robustness of the supermatrix approach to missing data makes it powerful for phylogenetic reconstruction, as phylogenomic datasets can be assembled at low cost by mining existing databases[22,32] or by the sequencing of multiple PCR-targeted loci[11,14,15], as well as cDNAs and ESTs[17,20,23]. This allows the incorporation of a large number of species, instead of being restricted to model organisms for which complete genome sequences are available.

The second sequence-based phylogenomic approach consists of analysing each data partition (such as genes) individually, and combining the resulting trees — which contain information from partially overlapping species — into a 'supertree' (BOX 2). Different methods for constructing supertrees have been proposed[37], but because of its intrinsic simplicity, the matrix representation using parsimony (MRP) method[38,39] is the most popular[40]. Supertree methods have mainly been used for combining trees that are obtained from disparate sources of data (such as morphological and molecular data) to provide an overview of the phylogeny of a given group. For example, this approach has been used in studies of placental mammals[41]. In phylogenomics, this approach has so far been restricted to a few studies that have attempted to reconstruct the phylogenies of Bacteria[42] and of model eukaryotic species for which complete genomes are available[31]. However, supertree reconstruction is currently an active area of research[40], and its use is likely to expand in the near future.

The relative merits of the two sequence-based approaches have not been thoroughly explored. Empirical comparisons indicate the superiority of the supermatrix approach over MRP in a study of crocodylians (crocodiles, caimans, alligators and gavials)[43], whereas the two approaches were found to be roughly equivalent in terms of the results produced when reconstructing the phylogeny of grasses[44]. However, the comparison between the two approaches is made difficult by the different types of character used in each method[45], and more work is needed to address these issues. Nevertheless, in phylogenomic studies of Bacteria, supermatrix[46,47] and supertree[42] analyses have produced relatively similar trees that are based on different datasets.

Methods based on whole-genome features

Gene content and gene order. Methods of phylogenetic reconstruction that are based on the comparison of whole-genome features beyond the sequence level — such as gene order and gene content (that is, the specific genes found in a genome) — have recently been developed (BOX 2). Unlike classical sequence-based

approaches, methods that are based on gene content and gene order do not rely on a multiple-sequence alignment step. However, they do still depend on HOMOLOGY and ORTHOLOGY assessment (see below). Changes in gene content and gene order within genomes result in characters with billions of possible states, as compared with only four states for nucleotide sequences. As a result, they are less prone to homoplasy by convergence or reversal, and might therefore potentially represent good phylogenetic markers[9], as long as they contain enough phylogenetic information[48]. Although they use different character types to those that are used in sequence-based approaches, these methods nevertheless use standard tree-reconstruction algorithms (see REF. 49 for a recent review).

Phylogenetic trees reconstructed from gene-content information have generally been reconstructed using distance[50–56] or parsimony[55–58] methods (BOX 2). One concern with gene-content analyses is the erroneous grouping of organisms with a similar number of genes[53,56,58,59]. This phenomenon, known as the 'big genome attraction' artefact[60], is thought to result from substantial convergent gene losses occurring in certain genomes; for example, those of intracellular parasites[58,59]. Progress in explicitly modelling the molecular details of genome evolution has recently been made with the development of probabilistic approaches, and this should ultimately lead to more accurate inferences that are based on gene content[60–62].

Gene order was recognized early on as a valuable phylogenetic character[63]. However, its use involves estimating evolutionary distance from the number of rearrangements necessary to transform one genome into another, which is a complex mathematical problem[64]. Even with the HEURISTIC approach of BREAKPOINT minimization, computational burden has significantly restricted the application of phylogenetic reconstruction that is based on gene order[65]. Only inversions are generally considered, but explicit models of gene-order evolution are being implemented to handle duplications, insertions and deletions[66]. More efficient algorithms still need to be devised before the full potential of this approach is realized. Whole-genome prokaryotic trees have nevertheless been constructed through this approach using parsimony and distance methods, under the drastically simplifying assumption that gene order can be described by the presence or absence of pairs of orthologous genes[53,56]. However, this approach suffers from the current lack of evaluation of its accuracy.

The issue of orthology assessment. Dependence on the assessment of orthology is an important issue in phylogenomic studies. This assessment is primarily based on sequence-similarity searches that can be misleading[67] owing to differences in evolutionary rates and/or base composition between species, and the occurrence of HORIZONTAL GENE TRANSFER (HGT)[68]. Orthology assessment ideally requires rigorous and time-consuming phylogenetic analyses of individual genes[47,69,70]. Although automation procedures have been proposed[71], this step is often overlooked in the reconstruction of phylogenies that are based on gene content, rendering their critical evaluation difficult. However, analyses of homologous[58] or orthologous[56] genes using the gene-content approach have yielded fairly congruent trees. Furthermore, recent gene-content analyses that have attempted to filter the noise introduced by HGT, and PARALLEL GENE LOSSES showed that these factors have only a limited effect on the results[52,54]. This indicates that gene-content methods might be more robust to the potential problems of orthology assessment than was first thought. The accurate modelling of HGT events and parallel gene losses will nevertheless be necessary to avoid the big genome attraction artefact[60].

The DNA string approach. Finally, another approach derived from whole-genome features, which is not dependent on homology or orthology assessment, is based on the distribution of oligonucleotides ('DNA strings') in genomes[55,72–75] (BOX 2). This approach is based on the observation that each genome has a characteristic 'signature' with regard to these strings; these are defined, for example, as the ratio between observed dinucleotide frequencies and those expected if neighbouring nucleotides were chosen at random[72]. The few methods currently used for this approach show that it is possible to extract phylogenetic signal using this oligonucleotide 'word usage'[55,73–75]. However, a comparison with SSU rRNA sequences in the context of the prokaryotic phylogeny shows that this usage seems to evolve much faster than SSU rRNA[74]. Therefore, SATURATION of the phylogenetic signal contained in oligonucleotides might limit the use of such approaches for inferring ancient divergence events.

The study of rare genomic changes

Genomes can also be studied using the traditional methodology used by comparative morphologists by looking for shared complex characters — known as rare genomic changes (RGCs) — that have a very low probability of being the result of convergence (BOX 2). As well as gene order, such RGCs include intron positions, insertions and deletions (indels), retroposon (SINE and LINE) integrations, and gene fusion and fission events[8,9].

Until now, only a few characters of this kind have been used to address specific phylogenetic questions, such as the phylogeny of placental mammals[76,77] and jawed vertebrates[78], or the position of the ROOT in the eukaryotic tree[79,80]. Although rare, homoplasy can also affect RGCs[81–84]; therefore inferences should not rely on only a few characters. With the sequencing of complete genomes, the statistical study of large numbers of RGCs certainly represents a promising avenue.

Recent achievements of phylogenomics

At the time of writing, 260 complete genomes have been sequenced (33 eukaryotes, 206 bacteria and 21 archaeans), and more than 1,000 genome projects are in progress. These figures illustrate the large datasets that are becoming available for phylogenomic studies and demonstrate the extraordinary potential of the

HOMOLOGY
Two sequences are homologous if they share a common ancestor.

ORTHOLOGY
Two sequences are orthologous if they share a common ancestor and originated by speciation.

HEURISTIC
A method of inference that relies on educated guesses or simplifications that limit the parameter space over which solutions are searched. This approach is not guaranteed to find the correct answer.

BREAKPOINT
In the context of phylogenetic methods that are based on gene-order comparison between genomes, a breakpoint is defined when a pair of genes are adjacent in one genome but not in the other.

HORIZONTAL GENE TRANSFER
The transfer of genetic material between the genomes of two organisms, which usually belong to different species, that does not occur through parent–progeny routes.

PARALLEL GENE LOSS
The independent loss of homologous genes in evolutionary distinct lineages.

SATURATION
Mutational saturation occurs when many changes at a given position have randomized the genuine phylogenetic signal.

ROOT
The root of a phylogenetic tree represents the common ancestor of all taxa that are represented in the tree. The position of the root is often determined using an outgroup taxon to determine the order of evolution in the group of taxa of interest.

MONOPHYLY
Monophyletic taxa include all the species that are derived from a single common ancestor.

phylogenomic approach to shed light on long-standing phylogenetic questions, spanning all levels of the tree of life (FIG. 1). In this section, we present recent advances for each of the three domains of life that have been enabled by phylogenomics.

Eukaryotes. The reconstructions of phylogenies of placental mammals and land plants represent the most spectacular examples of recent advances enabled by phylogenomics. The evolutionary history of placental mammals was traditionally considered to be irresolvable, owing to the explosive radiation of species that occurred in a short space of time. However, this has now been elucidated by the analysis of supermatrices that contain about 20 nuclear genes[14,15,85,86], and the resulting phylogeny has been confirmed by recent analyses of complete mitochondrial genomes[30,87], with only a few nodes left unresolved. All but 1 of the 18 morphologically defined extant mammalian orders have been confirmed by molecular studies. The notable exception to this is the insectivores, which have been split into two distinct orders by the recognition of an unexpected group of African origin named Afrotheria[14,15,77]. Four principal groups have been identified among the orders of placental mammals, and their origins might be explained by geographical isolation, resulting from plate tectonics, during the early stages of diversification among placental mammals[85]. Molecular studies have also revealed the prevalence of morphological convergence during the evolution of placental mammals, with the occurrence of parallel adaptive radiations[14]. This might partly explain why reconstructing the phylogeny of placental orders on the basis of morphological characters has been difficult.

Similarly, studies of multiple genes from all three compartments of the plant cell (the mitochondrion, chloroplast and nucleus) have helped to overcome long-standing uncertainties in land-plant phylogeny[11,88–92]. These advances have brought several changes to the traditional botanical classification of flowering plants, which were previously based on morphological features such as floral characteristics and leaf shape[93]. In this case, as with the placental mammals, molecular evidence revealed the plasticity of phenotypic characters, highlighting several examples of previously unrecognized convergent evolution. This is perhaps best illustrated by the case of the sacred lotus (*Nelumbo nucifera*), which was previously thought to be related to water lilies (Nymphaeaceae), whereas molecular studies unveiled a close phylogenetic affinity with plane trees (genus *Platanus*)[88].

At a larger scale, sequence-based phylogenomic studies of eukaryotic phylogeny confirmed the MONOPHYLY of most phyla, which were originally defined on the basis of ultrastructural or rRNA analyses. However, they also demonstrated the common origin of a group of morphologically diverse amoebae, which were previously thought to have evolved independently[17]. Phylogenomic analyses of nuclear[20,94] and mitochondrial genes[95] have also corroborated the long-suspected sister-group relationship between the unicellular choanoflagellates and multicellular animals. However, the lack of representatives from several principal lineages (that is, Rhizaria, Cryptophytes, Haptophytes and Jakobids) in phylogenomic studies currently prevents the study of most of the working hypotheses derived from decades of ultrastuctural and molecular studies. These hypotheses have proposed the division of the eukaryotic world into six main groups[80,96]: the Opisthokonta, Amoebozoa, Plantae, Chromalveolata, Rhizaria, and Excavata (FIG. 1). The validity of these proposed 'kingdoms' represents one of the most important outstanding questions that phylogenomics has the potential to answer.

Finally, the question of the origin of the eukaryotes has been recently addressed[97] using a gene-content method that allows the modelling of genome-fusion events[60]. The authors proposed that the eukaryotes originated from a fusion event between a bacterial species and an archaeal species, leading to a ring-like structure at the root of the tree of life[97]. This would account for the chimeric nature of the eukaryotes, which has been inferred from the observation that many eukaryotic metabolic genes have a greater number of similar counterparts in Bacteria, whereas most of those

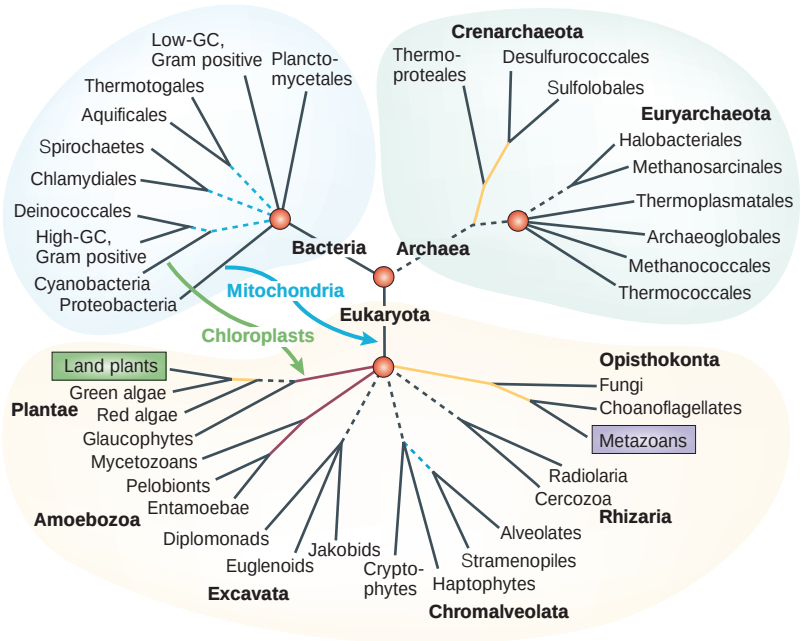


Figure 1 | **Phylogenomics and the tree of life.** A schematic representation showing recent advances and future challenges of the phylogenomic approach for resolving the main branches of the tree of life. This tree aims to represent a consensus view on evolutionary relationships within the three domains — Bacteria, Archaea, and Eukaryota — with hypothetical relationships indicated as dashed lines. The main branches that have been identified (purple) or confirmed (yellow) by phylogenomics are indicated. Blue dashed lines underline putative phylogenetic hypotheses that have been indicated by phylogenomic studies and need further investigation. The main uncertainties for which the phylogenomic approach might provide future answers are pinpointed by red circles. Note that most of the progress brought about by the phylogenomic approach has been realized at a smaller taxonomic scale for land plants, and for placental mammals within the metazoans (see main text). The two well-recognized endosymbiotic events involving bacteria that gave rise to eukaryotic organelles (mitochondria and chloroplasts) are indicated by arrows (blue and green, respectively). Note however, that other horizontal gene transfers and gene duplication events are not represented in this organismal tree, although they do constitute important aspects of genome evolution.

involved in information processing, such as transcription and translation, have more similar counterparts in Archaea. However, the mitochondrial endosymbiosis, a well-characterized fusion event, was followed by massive, lateral gene transfers to the eukaryotic nucleus and constitutes an important source of 'bacterial-like' genes in eukaryotes[98]. Distinguishing between this endosymbiosis and another earlier genome-fusion event is difficult, and the accuracy of the new gene-content method described above[60] has not yet been sufficiently evaluated to make a definitive statement on this fundamental evolutionary question.

Prokaryotes (Bacteria and Archaea). Despite the large number of complete prokaryotic genomes available, the picture of bacterial and archaeal evolution provided by SSU rRNA in the 1980s[99] remains surprisingly unchanged. The development of phylogenomic studies in prokaryotes have been largely held back by the supposedly predominant role of HGTs in shaping the evolutionary history of microorganisms, which is thought to have been so widespread that it might have blurred the phylogenetic signal for a prokaryotic phylogeny[100]. HGTs are undeniably an important source of genome evolution and innovation in prokaryotes[101]. Nevertheless, phylogenomic methods based on whole-genome features such as DNA strings[55,74,75], gene content[50,53,54], conservation of gene pairs[53,56] and protein domain structure[55,102], have all yielded phylogenetic trees that are similar to the corresponding SSU rRNA tree in the sense that they recovered the three domains of life and the main groups in both prokaryotic domains. Moreover, both supertree[42] and supermatrix[46,47,103] analysis identified a core of genes that rarely undergo HGT, from which it is possible to infer the phylogeny of prokaryotes. This indicates that HGTs do not prevent the recovery of a phylogenetic signal in prokaryotes, although they do constitute an extra source of noise[68]. For example, in Archaea, a major division between the Euryarchaeota and Crenarchaeota (FIG. 1) is supported by evidence from rRNA[99] and sequence-based phylogenomic studies[42,103]. However, it has been difficult to recover this division using gene-content methods[49], and this has been interpreted as being a consequence of HGT[53].

In the Bacteria, methods based on whole-genome features[50,53–56,58,74] and sequence-based phylogenomic inferences[42,46,47,103] have recovered the respective monophyly of all principal groups that were indicated by SSU rRNA (for example, Cyanobacteria, Spirochaetes, Chlamydiales and Proteobacteria; FIG. 1). However, the relationships between these groups, which are unresolved in the SSU rRNA tree[99], remain weakly supported. The only tentative groupings that might be proposed at this stage are Chlamydiales with Spirochaetes, Aquificales with Thermotogales, and high-GC Gram-positive bacteria (bacteria with a high GC content) with Deinococcales and Cyanobacteria, as they were recovered in independent analyses[42,46,47,56]. However, biases in amino-acid composition and differences in evolutionary rates, instead of a genuine phylogenetic signal (discussed below), might also explain these results. These potential new groupings can therefore only be considered as working hypotheses for future phylogenomic studies, which will be based on more species and will use methods that are specifically designed to tackle the issues raised above. The resolution of the bacterial radiation is perhaps the biggest challenge to phylogenomics at present.

Future challenges of phylogenomics

Because it uses many characters, phylogenomics leads to a drastic reduction in STOCHASTIC OR SAMPLING ERRORS associated with the finite length of single genes in traditional phylogenetic analyses. It is not, however, immune to SYSTEMATIC ERRORS, which are dependent on data quality and inference methods. The emergence of phylogenomics therefore brings the field full-circle to the roots of molecular phylogenetic analysis, with potential pitfalls in the form of tree-reconstruction artefacts, which were among the earliest issues faced by phylogenetics[104]. Here, we discuss systematic errors in the case of classical sequence-based methods (those based on supermatrices), as they are the best characterized. However, systematic error can also affect all other approaches, as exemplified by the occurrence of the big genome attraction artefact in gene-content methods. This artefact is analogous to the problem of compositional bias in sequence data (see below), and its impact can be reduced by the use of models of genome evolution inspired by sequence-based models[60].

Misleading effects of inconsistency. The use of large datasets generally results in a global increase in the resolution of phylogenetic trees, as measured by standard statistical indices such as bootstrap percentages — obtained using BOOTSTRAP ANALYSIS[105] — and BAYESIAN POSTERIOR PROBABILITIES[106]. However, obtaining a strongly supported tree does not necessarily mean that it is correct. These statistical indices only assess sampling effects, and give an indication of tree reliability that is conditional on the data and the method. So, if the method does not correctly handle properties of the data, an incorrect tree can receive strong statistical support (BOX 3).

A phylogenetic reconstruction method is statistically consistent if it converges towards the true tree as more data are analysed. All phylogenetic reconstruction methods make assumptions about the process of sequence evolution either implicitly (in the case of parsimony methods) or explicitly (in the case of distance and probabilistic methods). In theory, these tree-building methods are statistically consistent as long as their assumptions are met. However, every method is known to be inconsistent under some conditions[34]. When their assumptions are violated, current methods are prone to converge towards an incorrect solution, as shown by simulation studies[107–109]. In practice, method assumptions are always violated to some extent, as current models fail to capture the full complexity of sequence evolution[110]. These model violations generate an erroneous signal (noise) that will compete with the genuine phylogenetic (historical) signal. In general, noise is randomly distributed in sequences, and tree-reconstruction

STOCHASTIC OR SAMPLING ERROR
The error in phylogenetic estimates caused by the finite length of the sequences used in the inference. As the size of the sequences increases, the magnitude of the stochastic error decreases.

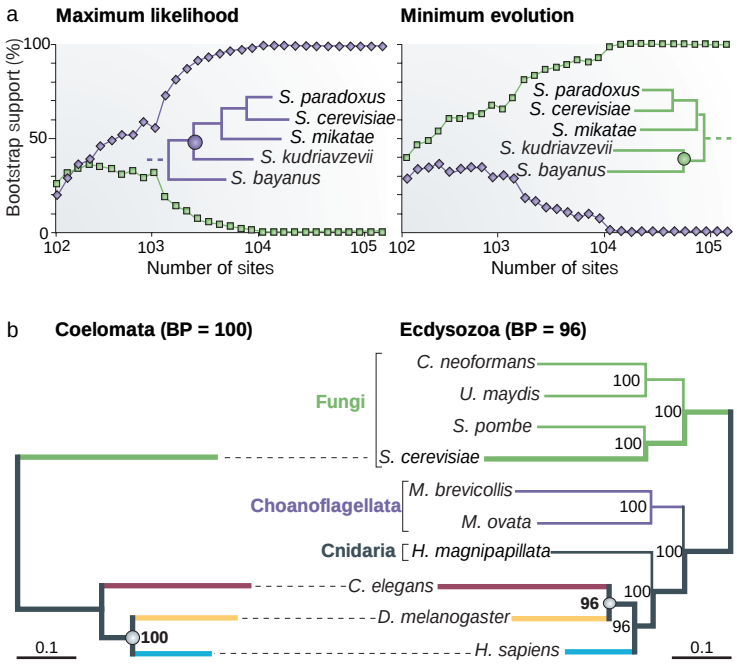
SYSTEMATIC ERROR
The error in phylogenetic estimates that is due to the failure of the reconstruction method to fully account for the properties of the data.

BOOTSTRAP ANALYSIS
A type of statistical analysis used to test the reliability of specific branches in an evolutionary tree. The non-parametric bootstrap proceeds by re-sampling the original data, with replacement, to create a series of bootstrap samples of the same size as the original data. The bootstrap percentage of a node is the proportion of times that node is present in the set of trees that is constructed from the new data sets.

BAYESIAN POSTERIOR PROBABILITY
In Bayesian phylogenetics, the posterior probability of a particular node of a tree is the probability that the node is correct, which is conditional on the data and the model used in the analysis both being correct.

Box 3 | Inconsistency and its causes

A phylogenetic reconstruction method is statistically inconsistent if it converges towards supporting an incorrect solution as more data are analysed. This happens when the assumptions made by the methods about the sequence evolutionary process are violated by the data properties. The three main kinds of bias that are not efficiently handled by most current reconstruction methods are known to be responsible for inconsistency.



Compositional bias

Similar nucleotide composition can lead phylogenetic methods to artefactually group unrelated species together. As an illustration, part **a** shows a phylogenomic dataset of 127,026 nucleotide sites (106 genes) for 8 yeast species[19] that was analysed using variable-length bootstrap analysis[163]. This method allows visualization of the change in statistical support (expressed as bootstrap percentages; BP) for a particular phylogenetic hypothesis as the number of sites increases. In the left panel, maximum likelihood (ML) using a parameter-rich model (GTR+Γ+I) that accounts for substitution-rate heterogeneity among sites converges towards supporting a tree (purple curve) that groups *Saccharomyces kudriavzevii* with *Saccharomyces mikatae*, *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* to the exclusion of *Saccharomyces bayanus*. The statistical support for this tree reaches 100% when more than 10,000 sites are analysed (purple diamonds). By contrast, on the right panel, the distance-based minimum evolution (ME) method, using the same model, converges towards supporting an alternative tree (green curve) where *S. kudriavzevii* and *S. bayanus* are grouped together, also with 100% BP support for more than 10,000 sites (green squares). Note that these two phylogenetic hypotheses are mutually exclusive because the support for the alternative solution converges towards 0 in both panels. As the two trees cannot both be correct, one of the methods must be inconsistent. In this case, ME has been shown to be misled by the fact that *S. kudriavzevii* and *S. bayanus* share similar base compositions[144]. This shows that the increase in statistical support provided by phylogenomics does not always guarantee convergence on the correct tree. All calculations were done using the PAUP* program[164].

Long-branch attraction

Unrelated species sharing high evolutionary rates can be artefactually grouped together because most phylogenetic methods become inconsistent under these conditions[104]. As an example, part **b** shows a phylogenomic dataset, which encompasses 146 nuclear proteins (35,346 amino-acid positions), that was assembled to study the relationships between *Homo sapiens*, *Drosophila melanogaster*, *Caenorhabditis elegans* and *S. cerevisiae*[23]. The ML tree obtained using a JTT+Γ model (which accounts for substitution-rate heterogeneity among sites for these four species) strongly groups *H. sapiens* and *D. melanogaster* together (BP = 100). This arrangement corresponds to the classical Coelomata hypothesis (in which arthropods are grouped with vertebrates). The same analysis including six more outgroups (three fungi, two choanoflagellates and a cnidarian) results in a highly supported tree in which *D. melanogaster* and *C. elegans* are grouped together (BP = 96). This corresponds to the Ecdysozoa hypothesis (in which arthropods are grouped with nematodes). In this case, ML is probably inconsistent for the 4-taxa dataset, as the 'long branch' of the *C. elegans* is attracted by the long branch of *S. cerevisiae*, which is broken by the use of more outgroup species in the 10-taxa dataset[23,112]. All calculations were done using the PHYML program[165]. Numbers indicated above nodes correspond to bootstrap percentages and the scale bars represent the number of estimated substitutions per site. *C. neoformans*, *Cryptococcus neoformans*; *H. magnipapillata*, *Hydra magnipapillata*; *M. brevicollis*, *Monosiga brevicollis*; *M. ovata*, *Monosiga ovata*; *S.pombe*, *Schizosaccharomyces pombe*; *U. maydis*, *Ustilago maydis*.

Heterotachy

Heterotachy[166] refers to the variation in the evolutionary rate of a given position of a gene or protein through time. This phenomenon has been recently confirmed as an important process of sequence evolution[113], and can lead to phylogenetic reconstruction artefacts[109] in cases where the proportions of invariable sites of unrelated taxa have converged[167–169]. Unlike other types of bias, heterotachy does not leave any evident footprints in sequences[109], and therefore leads to insidious artefacts that are particularly difficult to detect[168,169].

methods are able to extract the more structured historical signal. However, when the historical signal is weak, such as for ancient phylogenetic relationships, and/or the noise is predominant, because the same biases are shared by phylogenetically unrelated organisms (see below), the phylogenetic inference can be misled.

Sources of inconsistency. There are several causes of model inadequacy, as several simplifying assumptions are generally made. These include the independence of evolutionary changes at different sites and the homogeneity of the nucleotide-substitution process. For example, compositional biases can result in the artefactual grouping of species with similar nucleotide or amino-acid compositions[111], because most methods assume the homogeneity of the substitution process and the constancy of sequence composition (stationarity) through time (BOX 3A). Moreover, variations in the evolutionary rate among species can cause the well-known and widespread long-branch attraction (LBA) artefact[104,112]. Here, high evolutionary rates increase the chance of convergence and reversal, leading to the artefactual grouping together of fast-evolving species[104] (BOX 3B). These biases are the best-characterized sources of inconsistency in sequence data. In addition, the confounding effects on phylogenetic inference of HETEROTACHY[113] are only now beginning to be better understood (BOX 3).

Examples of inconsistency. Two recent examples highlight the problem of inconsistency in phylogenomic studies. The first concerns the controversy surrounding the phylogenetic position of *Amborella trichopoda* in the flowering plants (angiosperms). In contrast to the classical two-way division of flowering plants into monocotyledons and dicotyledons, this dicotyledonous plant has been removed from dicotyledons (now called the eudicotyledons) and is now thought to have been one of the earliest angiosperms to evolve, on the basis of several lines of molecular evidence[11,114]. However, recent phylogenomic analyses of complete chloroplast genomes argued for a return to the traditional phylogeny that separates monocotyledons and dicotyledons, with *A. trichopoda* being part of the dicotyledons[115,116]. The limited taxon sampling used in these studies, combined with high levels of heterogeneity in evolutionary rates among species (the grasses, which were used as representatives of monocotyledons, are particularly fast evolving), have been identified as possible sources of this discrepancy[117,118]. More specifically, maximum likelihood (ML) phylogenetic analyses have been carried out using models that take into account among-site rate variation and use a dataset that includes a slowly evolving monocotyledon (*Acorus gramineus*)[118]. These analyses found strong support for the early emergence of *A. trichopoda*, and this indicates that an LBA artefact was responsible for the apparent early emergence of the fast-evolving grasses that were included in previous analyses. However, as *A. trichopoda* is the only extant representative of its family, its basal position in the angiosperms might prove difficult to resolve conclusively[118].

The second controversial example relates to the phylogeny of bilaterian animals. The classical gradualist view that divides bilaterians into Acoelomata, Pseudocoelomata and Coelomata on the basis of the nature of their body cavity (coelom) has been overturned owing to accumulating molecular evidence[119,120]. The starting point of this revolution was the recognition, on the basis of analyses of SSU rRNA, of a monophyletic group of moulting animals — named the Ecdysozoa — that includes arthropods and nematodes[121]. The new animal phylogeny consists of three main bilaterian groups: the Deuterostomia (including vertebrates), the Lophotrochozoa (including brachiopods, annelids and molluscs) and the Ecdysozoa. However, more recent phylogenomic studies of model animals have resurrected the original view that supports the grouping of arthropods and vertebrates (Coelomata), to the exclusion of the nematodes[16,21,31,122]. Although these studies consider a very large number of genes, they nevertheless suffer from poor species sampling as they include a maximum of 10 species, a configuration that is potentially prone to phylogenetic artefacts (BOX 3B). In fact, the analysis of a much larger species sample has revealed the extent of the effect of the LBA artefact on animal phylogenomic analyses, leading to the artificial grouping of fast-evolving nematodes and platyhelminthes[23]. When different methods were used to avoid this artefact, strong support was obtained in favour of the new animal phylogeny that includes the Ecdysozoa[23].

Reducing the perils of inconsistency. In the pre-genomic era, the most straightforward way of detecting an erroneous phylogenetic result was the observation that incongruent trees are obtained from different genes. For example, the misplacement of microsporidia at the base of the eukaryotic tree, instead of as a sister-group of fungi, was revealed by the comparison of several single-gene phylogenies[123]. However, when whole-genome information is used, an erroneous result that is due to inconsistency is difficult to ascribe as only one tree is produced.

As increasingly large datasets are analysed, the probability increases that strongly supported but erroneous groupings remain undetected. Therefore, using the most accurate tree-reconstruction methods available is of the utmost importance. Arguably, probabilistic methods of phylogenetic inference, such as ML and Bayesian methods should be preferred, as they explicitly incorporate the processes of sequence evolution in the models that they use[110]. The use of the most complex models will reduce the probability of becoming inconsistent, as they will fit the data better. However, despite the fact that simulation studies indicate that probabilistic methods are relatively robust to violations of the model's primary assumptions[124–126], this might not hold in extreme cases[109].

More realistic models of sequence evolution are therefore needed. Research in this area is continuing, with the most recently developed models relaxing the assumption of independence among sites by taking into account the

HETEROTACHY
The variation of evolutionary rate of a given position of a molecule through time.

Box 4 | Phylogenomics and computational burden

The problem of tree space

Phylogenetic inference is a computationally demanding task, given the large number of trees that are possible — the 'tree space' — even when a relatively small number of species is considered. Indeed, the number of rooted trees (that is, those for which the common ancestor of all the species included is known) for 10 species is 34,459,425, and for 50 species this number increases to more than 10^{75}. It is therefore impossible to look at all of these trees and assess which best explains the data. As a consequence, phylogenetic inference relies on various heuristic optimization algorithms that explore only a subset of the possible trees[34], but this should not be done at the expense of the accuracy of tree reconstruction. Likelihood-based methods are computationally very demanding because they incorporate numerous parameters in their underlying models. However, by using numerical procedures, such as MARKOV CHAIN MONTE CARLO (MCMC) methods, the Bayesian approach allows the implementation of complex models while remaining computationally tractable[106].

Assessing confidence

Assessing the statistical confidence of phylogenetic trees adds another dimension to the computational burden. Using resampling procedures such as the non-parametric bootstrap[105] is very time-consuming because they involve repeating the initial phylogenetic analysis many times. The computational burden is particularly high with maximum likelihood (ML) methods, but it can be considerably reduced by resampling the sitewise log-likelihoods (RELL bootstrap) instead of the original characters[170]. With Bayesian methods, the measure of confidence is less computationally demanding because it is directly computed from the original data in the form of Bayesian posterior probabilities (PPs)[106]. Originally thought to be roughly equivalent to non-parametric bootstrap percentages (BPs)[106], PPs have been shown to appear consistently higher than BPs in a wide range of empirical studies (see REF. 171 and references therein) and to provide an overestimate of accuracy in a phylogenomic dataset[172]. In fact, the PP of a tree represents the probability that the tree is correct, assuming that the model is correct[173]. However, Bayesian methods can be sensitive to model misspecification[173,174], whereas the ML non-parametric bootstrap method seems to be more robust[173]. As a consequence, the non-parametric bootstrap remains the method of choice for assessing confidence, particularly as its computation can easily be distributed on parallel processors.

Divide and conquer

Given the rate of sequencing, the size of sequence-based phylogenomic datasets will soon make phylogenetic analysis computationally problematic when using classical methods. The resolution of large phylogenetic problems can be tackled by using 'divide-and-conquer' strategies. These methods break the dataset down into smaller subsets (that is, a fraction of the species), infer optimal trees for these subsets, and finally combine these trees into a larger tree. The first implementations of this strategy have been based on quartets including four species[175] and DISK-COVERING METHODS using larger species subsets are currently being developed[176]. The combination of the supermatrix and supertree approaches (BOX 2) might therefore represent a solution for reconstructing phylogenomic trees with thousands of species to eventually obtain a full picture of the tree of life[40,177].

occurrence of context-dependent[127], multiple-nucleotide[128] and structurally constrained[129,130] substitutions. Likelihood models that relax the assumptions of homogeneity and stationarity have also been designed to handle sequences with heterogeneous composition[131,132]. Methods of tree reconstruction that are based on the COVARION MODEL OF MOLECULAR EVOLUTION (first proposed by Fitch[133]) have been proposed[134,135] and implemented within an ML[136] and Bayesian[137] framework to handle heterotachy. Recently, these efforts have been followed by the development of mixture models, allowing distinct models for different classes of site, to accommodate among-site heterogeneities in evolutionary dynamics[138,139]. In general, mixture models seem to represent a promising avenue to correctly handle sequences that evolved through heterogeneous processes[109]. Ultimately, evolutionary models should integrate all these improvements simultaneously. However, ML methods could produce incorrect results because of the increased variance associated with the estimates of large numbers of parameters. The development of complex models should therefore avoid falling into the 'infinite parameter' trap[34].

However, improved probabilistic methods, as described above, might not hold all the answers to the inconsistency problem. Fast-evolving characters are particularly challenging for phylogenetic inference because they are likely to have experienced many changes, eroding the phylogenetic signal[9]. At these sites, using the correct model of sequence evolution is of particular importance for inferring hidden changes in order to separate signal from noise. However, as the perfect model does not exist, various strategies have been developed to reduce the impact of systematic errors. One efficient approach is to improve species sampling (BOX 3B), as multiple changes are most easily detected when many species are analysed. Moreover, different species violate model assumptions to variable degrees, and inconsistency might only occur for particular combinations of species. For example, datasets that consist of species with heterogeneous evolutionary rates are more likely to show inconsistency than those with more homogeneous rates. With single-gene datasets, focusing on the most slowly evolving taxa has been shown to counteract the LBA artefact[121]. In phylogenomics, although increasing the number of species is important (BOX 3B), it also significantly raises the computational burden, which can become a serious issue (BOX 4).

Focusing on the rarest substitution events is another way of improving phylogenetic inference. Because nucleotide substitutions between bases belonging to the

MARKOV CHAIN MONTE CARLO
A computational technique for the efficient numerical calculation of likelihoods.

DISK-COVERING METHODS
A family of 'divide-and-conquer' algorithmic methods for large-scale tree reconstruction. They use graph theory to optimally partition the input dataset into small overlapping sets of closely related species, reconstruct phylogenetic trees from these subsets, and combine the subtrees into one tree for the entire set of species.

COVARION MODEL OF MOLECULAR EVOLUTION
In this model, although some sites in a macromolecule are vital to function and can never change through time, most switch between being free to evolve in some species and being invariable in others.

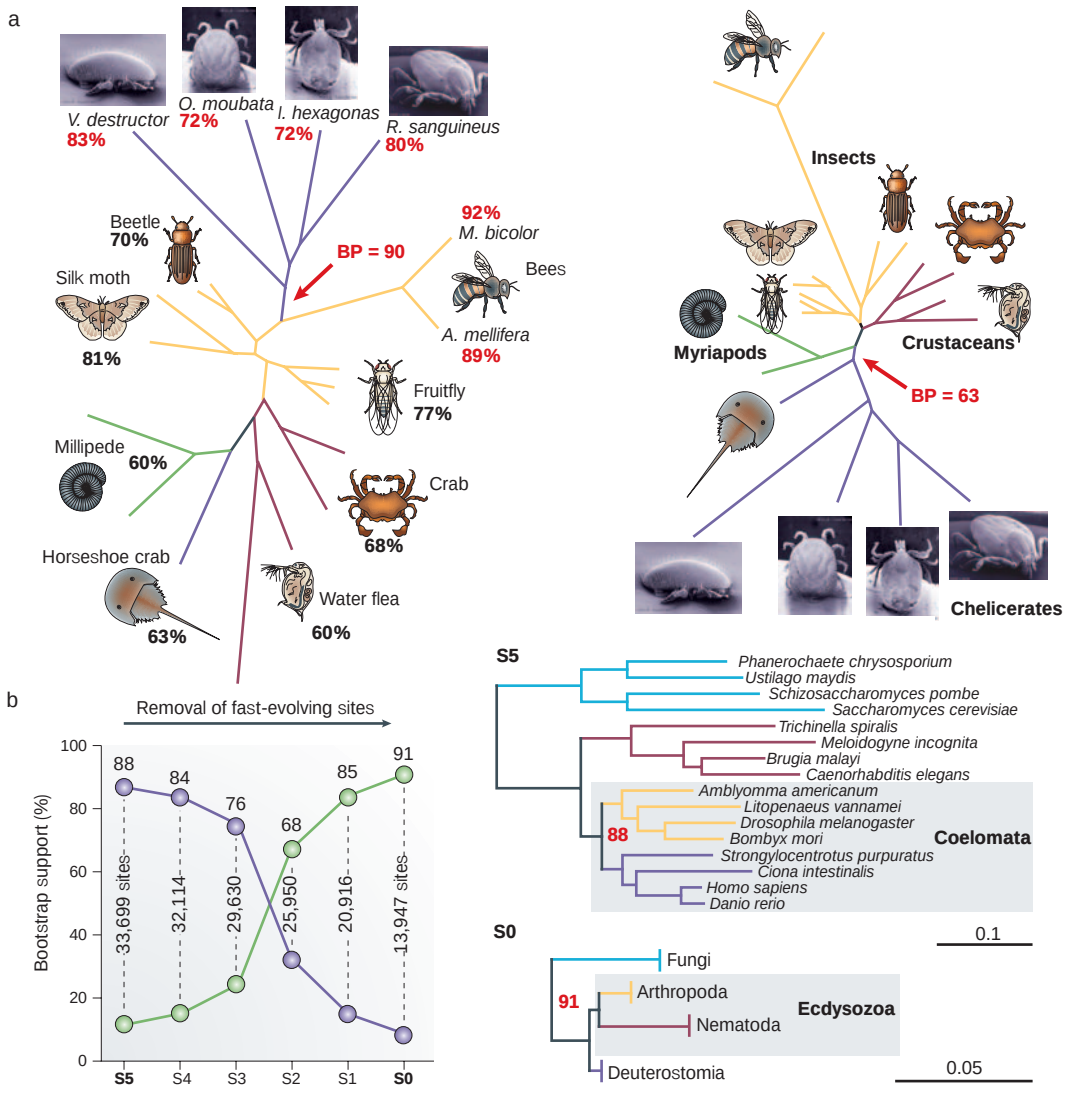


Figure 2 | **'Garbage in, garbage out': inconsistency and the use of reliable characters.** If the characters used in phylogenomics are unreliable, even the most accurate tree-reconstruction method can fail. Therefore, methods that focus on the most reliable characters have been developed to reduce the impact of inconsistency. **a** | Purine–pyrimidine coding (RY coding). This strategy considers only transversion events between the two families of bases in DNA (purines and pyrimidines). In the case of arthropod phylogeny that is based on the four most conserved mitochondrial genes (3,729 nucleotides)[141], the maximum likelihood (ML) criterion using the GTR+Γ+I model recovers an incorrect but strongly supported result (BP (bootstrap percentage) = 90; left panel). The four ticks (*Varroa destructor*, *Ornithodoros moubata*, *Ixodes hexagonus* and *Rhipicephalus sanguineus*) are nested in the insects as a sister-group of bees (*Melipona bicolor* and *Apis mellifera*), instead of clustering with the other chelicerates that are represented by the horseshoe crab (*Limulus polyfemus*). Mitochondrial genomes of some ticks and bees have converged towards high proportions of AT residues (in red), and this is not accounted for by the model, which assumes base-composition homogeneity. RY coding (right panel) allows the recovery of the correct phylogeny, supporting the monophyly of chelicerates (BP = 63) when analysed under ML using the 2-state CF+Γ+I model[178]. Therefore the removal of half the original information by RY coding (the number of parsimony steps is roughly halved) has removed the inconsistency of ML. All calculations used the PAUP* program[164]. Photographs are reproduced, with permission, from the Biodic web site © (2003) Free University of Brussels. **b** | The slow–fast (SF) method. The misleading effect of the long-branch attraction artefact[104] is tackled here using the SF method[147] for a phylogenomic dataset from animals and fungi[23]. Using this method, different subsets of the data (S0, S1,…, Sn) are constructed containing sites that have experienced a total number of substitutions that is equal to or less than 0, 1,…, n in predefined monophyletic groups. The 'evolution' of the phylogenetic signal for a particular hypothesis is then monitored as fast-evolving sites are progressively removed from the original dataset. The ML analysis under the JTT+Γ model of the S5 matrix yields a tree that supports the Coelomata hypothesis (arthropods + deuterostomes) with a bootstrap support of BP = 88. However, the progressive removal of fast-evolving positions results in a decrease of the support for the Coelomata hypothesis (green curve) and a concomitant increase (green curve) in the support for the Ecdysozoa hypothesis (arthropods + nematodes). The support in favour of the new animal phylogeny (Ecdysozoa) is reaching BP = 91 with the 13,947 slowest-evolving sites for which no substitutions occurred in each of the four groups (S0). The initial support observed in favour of Coelomata is attributable to the fastest-evolving sites, probably causing a long-branch attraction artefact, with fast-evolving nematodes being attracted by the distant fungal outgroup[23]. All calculations used the PHYML program[165].

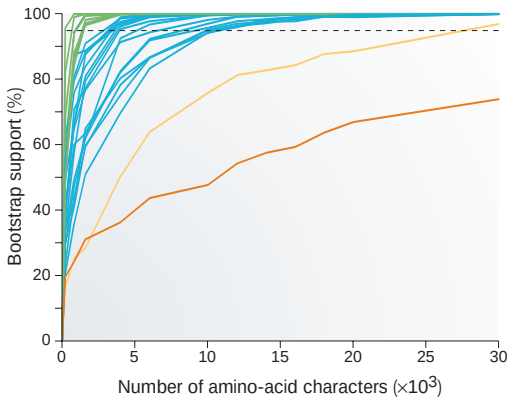


Figure 3 | **Phylogenomics and the resolution of phylogenetic trees.** This figure illustrates the differential increase in resolution provided by phylogenomics for different internal nodes of a given phylogenetic tree. A maximum likelihood (ML) tree (not shown) was reconstructed for a phylogenomic dataset of 141 genes representing a total of 31,731 amino-acid sites for 35 eukaryotes (N. Rodríguez-Ezpeleta, personal communication). The level of statistical support expressed as bootstrap percentages (BP) for internal nodes observed in the ML tree was plotted as a function of the number of jack-knife resampled sites[179]. As expected, the resolution increased with the use of more characters, and four types of profile can be defined with respect to the amount of data that is needed to define different nodes. First, numerous nodes (green) can be resolved with a small number of genes and often a single one (BP > 95 reached for less than 2,500 sites). Second, most nodes (blue) are resolved with multigene datasets including less than 10,000 sites as recently achieved in plants[11] or mammals[85]. Third, few nodes (yellow) can only be resolved by a phylogenomic approach that considers a large amount of characters (that is, more than 100 genes[17,23]). Fourth, very rare nodes (orange) are virtually irresolvable because the extrapolated number of sites required to reach BP = 95 exceeds the number of homologous sites that can be extracted from complete genomes. In this last case, only the improvement of the species sampling or of the tree-reconstruction method could possibly change the situation.

same family (transitions) occur more frequently than between bases from different families (transversions), reducing the data to purines (A,G = R) and pyrimidines (C,T = Y) efficiently reduces saturation (FIG. 2a). Compositional bias is also decreased by this so-called RY-coding strategy, as base-composition differences are often most pronounced between bases of the same family[140]. RY coding has proved helpful in studies that have used mitochondrial genomes[141–143], and has allowed the inconsistency of distance methods in yeast phylogenomics to be avoided[144].

Other approaches have been proposed for identifying and removing the fastest-evolving sites [145–149]. For example, the slow–fast (SF) method has been developed primarily for studying ancient divergence events[147] and has been helpful in tackling the LBA artefact when reconstructing the phylogeny of eukaryotes[79]. For example, FIG. 2b illustrates the usefulness of this approach in the phylogenomics of bilaterian animals. When using a complete phylogenomic dataset of almost 150 genes, the fast-evolving nematodes tend to be artefactually 'attracted' by the distant fungal outgroup[23] (see BOX 3B for an explanation). This shows that even a sophisticated phylogenetic method such as ML can be misled by the bias introduced through differences in evolutionary rates among species. The objective exclusion of the noisiest characters using the SF method led to an alternative topology, in which the long branch of nematodes is no longer grouped with the long branch of fungi.

Phylogenomic datasets offer the luxury of focusing solely on the more reliable characters using the methods described above. When 100,000 characters are available, removing the 20–30% fastest-evolving characters is unlikely to alter the statistical significance of the results, as would be the case using single-gene datasets (see REF. 79 for examples). At present, these types of approach[52,54,142,145,147,149] might be the only way to handle cases where the presence of several confounding factors misleads even the most accurate methods of phylogenetic inference.

Phylogenomics and corroboration. The congruence of results obtained from various datasets and/or various methods is the key validation of evolutionary inferences[150]. To corroborate results, single-gene phylogenies have been compared with classical morphological and ultrastructural studies, and subsequent multigene phylogenies were generally contrasted with previously obtained molecular trees. However, whole genomes represent the ultimate source of characters from which the evolutionary history of organisms can be reconstructed; therefore, how can we corroborate phylogenomic results?

The similarity between phylogenomic results and the SSU rRNA tree of prokaryotes has been viewed as a first validation of the new large-scale approaches. Eventually, corroborating the results of different large-scale approaches should become a standard method of validation (see REFS 56,151 for examples). It is therefore desirable that methods that are based on whole-genome features should become as sophisticated and accurate as sequence-based methods. This requires a better understanding of the processes driving genome evolution, which could be achieved by comparing genomes from closely related taxa. With better methods and the use of the more reliable characters, rendering inconsistency less likely, the definitive proof for corroboration of phylogenomic results will certainly be their robustness to varying the species that are sampled. This will allow verification that the same results are obtained with different subsets of species. Corroboration in phylogenomics is a necessary prerequisite for tackling the large-scale resolution of the tree of life.

Perspectives — a fully resolved tree of life?

Recently, concerted efforts have been made towards realizing Darwin's dream of having "…fairly true genealogical trees for each great kingdom of Nature…" in the form of collaborative network initiatives for assembling the tree of life. Several phylogenomic research programmes (see Online links box) that are targeting various groups — such as eukaryotes, fungi,

METAGENOMICS
The functional and sequence-based analysis of the collective microbial genomes contained in an environmental sample of uncultured organisms.

arthropods, nematodes, dipterans or birds — will soon lead to important improvements through the consideration of a dense taxon sampling for these groups. Moreover, continuous progress from METAGENOMICS will continue to reveal the extent of microbial diversity[152].

At first sight, these efforts should ultimately lead to a fully resolved tree of life. However, as shown in FIG. 3, not all nodes of a phylogenetic tree are equal with respect to the increase in resolving power provided by the phylogenomic approach. This is expected, as the resolution of phylogenetic trees ultimately depends on the evolutionary pattern by which organisms diversified. If time intervals between speciations were particularly short, it is likely that even complete genome data might not provide enough characters to accurately resolve certain nodes, for which almost no phylogenetic signal will be recovered[153]. Furthermore, given the importance of taxon sampling for phylogenetic inference, it is possible that isolated species that are the sole living member of a particular group (for example, the coelacanth, the tuatara and *A. trichopoda*), or groups for which sampling is naturally scarce with only a few representative extant species (for example, monotremes), will prove difficult to position with confidence. Therefore, there will be nodes that are likely to be left unresolved because of the nature of the evolutionary process, and this will in itself tell us a lot about the evolution of organisms. However, despite the power of phylogenomics to reveal evolutionary relationships, we might have to accept the idea of a partially resolved tree of life.

Finally, the reconstruction of the topology of the organismal phylogeny is not in itself the ultimate goal. The challenge is to understand the evolutionary history of organisms and their genomes, the functions of their genes, and how this relates to their interactions with the environment. Assembling the tree of life represents the first step towards achieving the big picture of phylogenomics where, to paraphrase Theodosius Dobzhansky[154], nothing in genomics makes sense except in the light of evolution.

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Competing interests statement

The authors declare no competing financial interests.

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