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Phylogenomic Insights into Animal Evolution

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Animals make up only a small fraction of the eukaryotic tree of life, yet, from our vantage point as members of the animal kingdom, the evolution of the bewildering diversity of animal forms is endlessly fascinating. In the century following the publication of Darwin's *Origin of Species*, hypotheses regarding the evolution of the major branches of the animal kingdom — their relationships to each other and the evolution of their body plans — was based on a consideration of the morphological and developmental characteristics of the different animal groups. This morphology-based approach had many successes but important aspects of the evolutionary tree remained disputed. In the past three decades, molecular data, most obviously primary sequences of DNA and proteins, have provided an estimate of animal phylogeny largely independent of the morphological evolution we would ultimately like to understand. The molecular tree that has evolved over the past three decades has drastically altered our view of animal phylogeny and many aspects of the tree are no longer contentious. The focus of molecular studies on relationships between animal groups means, however, that the discipline has become somewhat divorced from the underlying biology and from the morphological characteristics whose evolution we aim to understand. Here, we consider what we currently know of animal phylogeny; what aspects we are still uncertain about and what our improved understanding of animal phylogeny can tell us about the evolution of the great diversity of animal life.

Introduction

Our understanding of the evolutionary relationships between major animal (metazoan) groups has matured to an extraordinary degree in the past quarter of a century [1]. This is very largely due to the widespread use of computational analysis of molecular data. Genomes contain a vast quantity of informative data, and molecules have various desirable qualities as phylogenetic markers. Not least of these qualities is that most of the informative heritable change molecules contain is independent of the aspects of phenotype whose evolutionary paths might interest us. Perhaps as a result of this relative independence from phenotype, research publications using molecular data tend to be rather removed from morphology — the most common finished product of such a study ideally being a more accurate evolutionary tree. Here, we aim to begin to bridge this gap by considering what our improved knowledge of animal phylogeny might tell us about the patterns and processes of animal evolution.

Post-Darwinian Phylogenies

The separate treatment of morphology and phylogeny is in striking contrast to the standards prevailing for most of the first hundred or so years of animal phylogenetics, starting with Ernst Haeckel [2], in which phylogeny generally emerged directly from a consideration of morphological evolution — of the likely homology and transformations of characters and the scientist's conception of how that morphology evolved. This process was one of reciprocal illumination in which the evolution of phenotypic characters suggested the topology of the tree at the same time as the tree implied a certain evolutionary history of those characters. The intellectual basis for this process has been summarised as "the criterion for a reliable scenario [being] its inner coherence" [3].

There were many different morphology-based schemes for relating the animals (and for explaining the origins of their morphology and embryology) but a few significant themes can be recognised [4]. One major theme was the generally sensible idea of a progression from simple to more complex, with a special focus on the evolution of the number of developmental tissue layers (diploblast to triploblast), planes of body symmetry (radial to bilateral), organ systems (organized as tissues or as more specialized organs) and guts (blind-ended guts to through guts with mouth and anus). A second major theme concerned the likely characteristics of the common ancestor of the bilaterally symmetrical animals ('Urbilateria'): solid like a sponge planula larva or an acoelomate platyhelminth (acoeloid/planuloid), schizocoelomate (forming coelomic body spaces by splitting the mesoderm, supposed to be typical of protostomes) or enterocoelomate (coelom formation by budding from the gut, supposed to be typical of deuterostomes). Equally important were different interpretations of primitive and derived characteristics of other aspects of embryology, most notably the pattern of cleavage (primitively radial or spiral) and the fate of the blastopore becoming either mouth (protostome) or anus (deuterostome). Each of these aspects of hypothetical ancestors has a strong effect on the topology of the tree and hence on the implied direction of character evolution, because each manoeuvres a different modern group, with its particular body plan and mode of development, towards the base of the

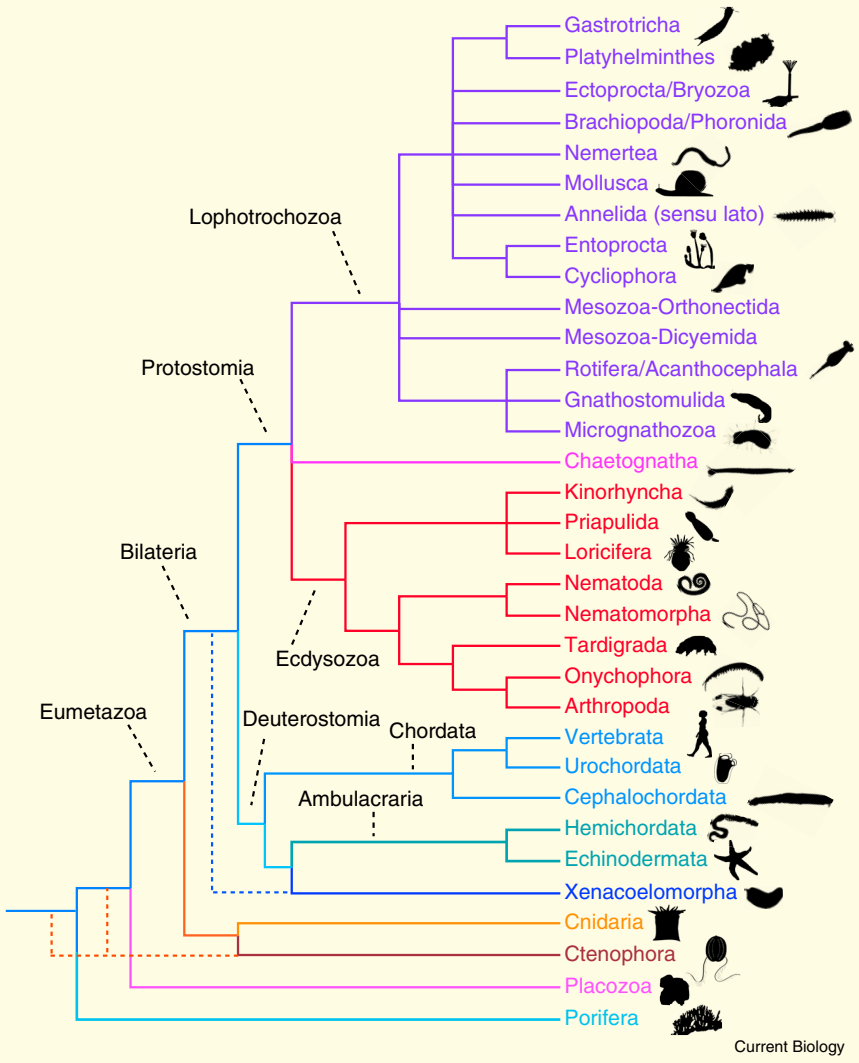

Figure 1. Current consensus phylogeny.
Our current best estimate of the phylogenetic relationships of major animal phyla. Major clade names are indicated. Alternative possible positions for groups with the most contentious positions are indicated by dashed lines. In the case of Ctenophora and Xenacoelomorpha systematic biases in evolutionary patterns, in particular rapid evolution, seem a likely cause of the difficulty in positioning the clades. The lack of resolution within the Lophotrochozoa seems to be plausibly explained by a rapid radiation. While Deuterostomia is a very long accepted clade, support for this grouping is weak compared to the support for Protostomia, suggesting the branch leading to Deuterostomia is (at best) short. A short branch leading to Deuterostomia has the corollary that there is a shorter evolutionary path from Urbilateria to Urdeuterostomia than from Urbilateria to Urprotostomia and that Urbilateria might be predicted to be more deuterostome-like than protostome-like. In any case, the overall phylogenetic distribution of the characters 'deuterostomy' and 'radial cleavage' implies Urbilateria shared both these canonical deuterostomian characters. Animal silhouettes from Phylopic (www.phylopic.org), credits from top: Michelle Site, Mali'o Kodis, Mali'o Kodis, uncredited, Scott Hartman, Scott Hartman, Scott Hartman, Mali'o Kodis, Michelle Site, (Rotifera: Diego Fontaneto, Elisabeth A. Herniou, Chiara Boschetti, Manuela Caprioli, Giulio Melone, Claudia Ricci, and Timothy G. Barraclough, vectorized by T. Michael Keesey), Matthew Hooge (vectorized by T. Michael Keesey), Вальдимар (vectorized by T. Michael Keesey), Scott Hartman, Michelle Site, Michelle Site, Mali'o Kodis, Frank Förster, Eduard Solà Vázquez (vectorized by Yan Wong), Dinah Challen, Michelle Site, Nicolas Gompel, T. Michael Keesey, Yan Wong, Mali'o Kodis, Mali'o Kodis, (drawing by Manvir Singh), Hans Hillewaert (photo, T. Michael Keesey, vectorization), Noah Schlottman, uncredited, Noah Schlottman, Michelle Site, uncredited.

bilaterian animals and derives other phyla from this early body plan and ontogeny.

While this multitude of ideas shows a profound lack of consensus, some aspects of phylogeny and character evolution seem, nevertheless, to have been accepted. Most significant is the accurate and, for the most part, unchallenged grouping of species into phyla, such as chordates, molluscs, annelid worms or arthropods, whatever the subsequent discussion of between-phylum and within-phylum relationships. A phylum is the most inclusive classificatory subdivision within the animal kingdom. Phyla (like all clades) are characterised by a set of diagnostic characters unique to the group — notochord and dorsal nerve chord for chordates, shell, radula and muscular foot in molluscs. To an extent, the designation of a phylum is an admission of ignorance regarding these higher-level relationships: the body plan defining a phylum is well defined but relationships to groups with other body plans are less clear.

In truth, of course, some phyla are more closely related to each other than others, so a higher level of classification linking subsets of phyla should be possible. Indeed, further to the largely accurate assigning of species to phyla, phylogenies from the late 19th century onwards reveal various super-phyletic groups also seen in the most credible of today's molecular phylogenies (Figure 1). The most obvious grouping of phyla is the bilaterians (all bilaterally symmetrical animals); other currently accepted groups, such as Ambulacraria (echinoderms and hemichordate worms), Deuterostomia (Ambulacraria and chordates) and Protostomia (all bilaterally symmetrical animals excluding deuterostomes) have also all been initially proposed over 100 years ago. However, other groups that in the past provoked little dispute we now know are not monophyletic: Articulata incorrectly linked annelids and arthropods and implied that the latter is derived from something resembling the former, perhaps by way of an onychophoran worm [5–7]. And many authors linked both the chaetognaths and the lophophorates (brachiopods and phoronids) to the deuterostomes (chordates, echinoderms and hemichordates) because of their shared embryological characters such as enterocoely and a pattern of 'radial' cleavage in the early embryo [8] as well as the ciliated feeding structure (lophophore) common to the lophophorates and the deuterostome hemichordates [9]. It is important to emphasise the success of morphology-based estimates of

animal relationships — almost all species are assigned to the correct phylum based purely on morphology — but establishing the relationships between the phyla using morphology and embryology has had a more mixed success.

In comparison to morphological and embryological features, characters encoded in the genome, in addition to being largely independent from phenotypic evolution, have three major advantages for reconstructing accurate phylogenetic trees. First is the ease with which one can detect primary homology — the likelihood of convergent evolution of specific sequences of hundreds or thousands of nucleotides or amino acids is negligible [10]. Second, genes are ~100 times more numerous than codable morphological characters, and each gene contains hundreds or thousands of nucleotide characters. Third, compared to the enormous diversity of morphological characters, it is relatively straightforward to mathematically model the evolution of the large sample sizes of genetic characters (4 nucleotides, 20 amino acids, 64 codons) [11]. These enormous advantages allow us to address the two limitations of any statistical inference very efficiently: stochastic error arising from a limited amount of data, and systematic errors resulting from incorrect modelling of the underlying process of change [12]. All these advantages of molecular data explain why it is preferable to map morphological characters onto a molecular tree rather than *vice versa* (see review by Lee and Palci in this issue). Reconstructing a tree from molecular data is not quite as straightforward as this discussion implies, however.

Stochastic and Systematic Errors in Phylogenetic Reconstruction

The first comprehensive efforts to reconstruct the relationships between animal phyla using molecular data used 5S ribosomal RNA sequences, but the topologies these analyses produced based on just 120 nucleotides [13,14] suffered from major stochastic or sampling errors, ameliorated somewhat by the subsequent use of the ~1800 nucleotides of 18S rRNA [1,15–17]. Stochastic error derives from the use of small samples of molecular data that contain randomly distributed homoplasy (the presence of the same character in distantly related animals due to convergent evolution). With few nucleotides or amino acids in a data set, the likelihood of random homoplasies predominating over informative sites at certain branches of the tree is high. Larger samples will allow the informative sites to predominate.

Sequencing hundreds of nucleotides from many thousands of genes in a transcriptome or even an entire genome is now straightforward and, except for very ancient comparisons, there are numerous conserved regions that can be unambiguously aligned, typically yielding >100,000 homologous positions [18,19]. Such large data sets drastically reduce most effects of stochastic error and contrast with morphological data sets, where the biggest matrices contain a few hundred characters.

While there is a strong psychological bias towards preferring phylogenetic trees with the greatest number of characters used, the impact of careful taxon sampling makes it clear that the remaining problems of tree reconstruction cannot necessarily be solved simply by using more characters [20–22]. Systematic error, where the mode of sequence evolution differs from our simplified models, was previously generally masked by stochastic error but turns out to be the main limitation when larger datasets are available. The most famous (and frequently encountered) systematic error in phylogenetics is the long-branch attraction artefact, whereby fast-evolving branches in the tree are artefactually clustered. A fast evolving lineage (long branch) may either be grouped with a second fast-evolving lineage or by the long branch (not necessarily fast-evolving) leading to a distant outgroup [23]. The problem derives from the fact that, while long-branch taxa evolve away from their true sibling species, the large number of changes along independent long branches results in occasional convergent changes in non-sibling taxa which artificially link them. The artefact is not in fact due to the accelerated rate *per se*, since branch length is always modelled in probabilistic methods, but to the fact that fast lineages often evolve differently, violating the homogeneity hypotheses of the model. A second well-known systematic error results from cases of heterogeneity of nucleotide or amino acid composition across lineages and leads to the incorrect grouping of taxa sharing the same bias.

Despite many years of progress in improving tree reconstruction methods (Box 1), artefacts remain and, once stochastic error has been eliminated, violations of the assumptions underlying the models used to infer the tree explain much of the incongruence observed between (and within) studies. Models generally assume, for example, that all characters in the data set evolve according to the same rules and that the process of evolution is homogenous across different branches of the tree; these assumptions are likely to be frequently violated. The rest of the incongruence between studies comes from data errors (e.g. undetected contaminations, use of non-orthologous genes or frameshifts affecting predicted amino acids) and high amounts of missing data (Figure 2). In theory, systematic error should be solvable by improving the model of evolution. However, the evolutionary process is highly complex, both at the level of the gene (duplication, horizontal transfer, incomplete lineage sorting or conversion, all of which conspire to make gene trees different from the species tree), and at the level of primary sequences (e.g. heterogeneity across positions and over time, mutation/selection balance, interdependence of sites within and between genes).

The gene-level problems (apart from the incomplete lineage sorting) can be dealt with through the careful identification and use of orthologous genes (i.e. genes whose relationships reflect speciation), a feasible task for animals. For the characters within sequences, the problem of correctly modelling character evolution depends on the handling of the heterogeneity of the substitution process across sites and over time. In this context, CAT-like models which, in addition to the well known site-specific rates, have site-specific equilibrium frequency profiles (categories hence 'CAT'). The CAT model allows different characters within an alignment to be modelled using different parameters, meaning, for example, that an amino acid found buried in a cell membrane (typically hydrophobic) will not be assumed to evolve according to the same rules as one sticking out into the cytoplasm (hydrophilic). Such non-homogenous models constitute the most significant recent trend towards improving the accuracy of phylogenomics [24]. An alternative, simpler approach to avoiding such errors is the careful selection of taxa, genes and positions, ultimately identifying and discarding the data that

Box 1. Sources of phylogenetic error.

Phylogenetic reconstruction is susceptible to systematic error whatever the type of character used, but the cause of error has been best characterised in the use of classical methods based on primary sequences of orthologous genes, typically on a concatenation of multiple gene alignments (i.e. a super matrix). The evolutionary process is extremely complex, meaning that dealing with systematic error via model improvement is challenging. The heterogeneity of mutational and selective pressures across time and across genomes, for example, due to epistasis and heterogeneity of environment, makes the evolution of a given nucleotide or amino acid position highly site-specific. This makes perfect modelling of sequence evolution an impossible task and forces researchers to focus on modelling heterogeneity that is the most mathematically and computationally accessible. The handling of the heterogeneity of the substitution process across sites through the CAT-like models, which drastically improve the fit of the models to data and hence the accuracy of phylogenomics, is the most significant area of recent progress. In contrast, heterogeneity of processes over time has been addressed for rate (heterotachy) and global amino acid composition [103] but improvements have been less marked. The heterogeneity of the substitution process over time (heteropecily) has been shown to bias animal phylogeny based on mitochondrial genomes [104] but has not yet been modelled. Each of these diverse model improvements have generally, for mathematical and computational tractability, been made separately but in the same way that the joint estimate of alignment and phylogeny is desirable, joint modelling of these factors is ultimately required [105].

Alternative approaches to avoiding tree reconstruction errors that are less technical but perhaps easier to implement are the careful selection of taxa, genes and positions with genes. The principle here is that, by preferentially discarding the data that most significantly violate model assumptions (e.g. a fast evolving species with an aberrant nucleotide composition), one can minimise errors [25].

An important question that deserves further research is how to find the best compromise between increasing the number of species and increasing model complexity; both these approaches are known to improve accuracy at the cost of increased computational time. Finally, it should be noted that the incompleteness of numerous phylogenomic matrices (often >50% missing data) decreases accuracy by reducing the effective number of species [106]. Such a consideration may explain some previously observed incongruence between studies (Figure 2).

violate model assumptions most significantly, such as a fast evolving species with an aberrant nucleotide composition [25].

Molecular Phylogeny of Animals and Implications for Character Evolution

Despite problems due to incomplete taxon sampling for some elusive animals, systematic biases and some nodes remaining difficult to resolve, molecular phylogenetics of animals has made a great deal of progress [26]. Our current best estimate of the tree relating the animal phyla is shown in Figure 1; see [26] for a recent alternative view. As discussed, some important aspects of the tree, such as the grouping of Bilateria, Deuterostomia, Ambulacraria and Protostomia, support conclusions from the study of morphology. A number of other features, however, diverge from previous ideas of animal relationships. The old consensus linked annelids and arthropods (Articulata); in contrast, molecular trees show that these phyla are found on opposite sides of the most basic division of Protostomia, arthropods are members of the Ecdysozoa alongside nematodes and priapulids, while annelids are in the Lophotrochozoa with molluscs and others [20]. The acoelomate Platyhelminthes — once thought to be one of the earliest branches amongst the Bilateria — are also in the Lophotrochozoa [27]. The lophophore-bearing taxa and chaetognaths, previously linked by many to the deuterostomes, are in fact closer to these protostomian taxa [8,28–33]. Each of these phylogenetic discoveries has had a significant influence on our interpretation of the evolution of morphological characters and on the reconstruction of animal ancestors and these new interpretations will be returned to later in this discussion

In morphology-based analyses, trees are based on hypotheses of so-called ‘primary’ homology between character states in different taxa, which are then tested by their distribution in the most parsimonious tree(s). Synapomorphies — shared novel characters that define monophyletic groups — are in this view hypotheses of ‘secondary’ homology posterior to the tree analysis. However, with accurate molecular trees, another approach is to map morphological states onto the morphology-independent tree (Box 2). The distribution on such a tree of putatively (i.e. primarily) homologous morphological characters can also establish (secondary) homology, and provide an answer as to whether primary estimates of morphological homologies are supported by their distribution on a molecular tree. In both cases, the relationship between trees and an understanding of phenotypic evolution, intimately linked in the pre-molecular era, remains essential if we are interested in understanding how the diversity of the animals arose. In parallel with the increasing confidence in phylogenetic trees, there is a renaissance in morphology, with many new techniques becoming available that have led to new insights from the morphology side of the equation [34–36]. With this in mind, it is interesting to consider a number of important conclusions derived from the modern phylogenetic consensus regarding character evolution deep within animal evolution and the likely make up of metazoan ancestors.

A Moulting Clade of Arthropods, Nematodes and Priapulids

The existence of a clade of moulting animals, the Ecdysozoa, comprising arthropods, priapulids, nematodes and relatives, and that moulting (ecdysis) is thus a homologous character defining this group are by now well established [20,37,38]. The homology of this character in animals as morphologically distinct as nematodes and arthropods was by no means obvious. Previously, pseudocoelomate nematodes and related

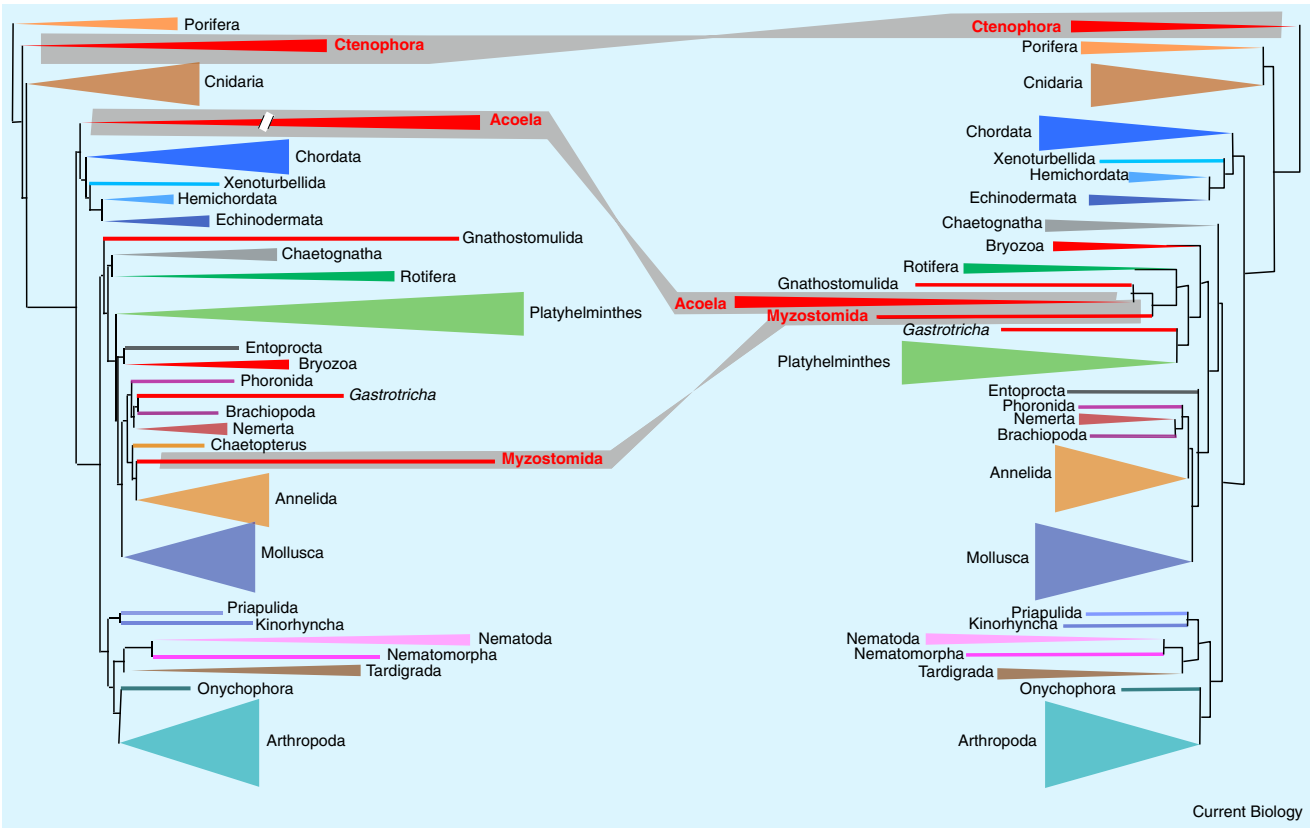

Figure 2. Effects of data quality and optimal models on the prevalence of systematic error.
The original dataset is Figure 1 from Dunn *et al*. [65]. These authors used relatively relaxed parameters for selection of reliably aligned positions from their super matrix of concatenated genes, which have 21,152 positions and 55.5% of missing data. The resulting data set was analysed using the WAG +G model. Updated data set: The same data set updated by Philippe *et al*. [21]. These authors identified some instances of frameshift and some contaminating sequences. They used more stringent parameters when discarding unreliably aligned positions (producing a super-matrix of 18,463 positions) and used the site heterogenous CAT model to reconstruct the tree on the data set, which had fewer instances of missing data (35.6%). The effects of these procedures aimed at minimizing the systematic bias of long-branch attraction can be seen in the different positions for three fast evolving (long branched) taxa highlighted in red. Ctenophores move from the earliest branching position within Metazoa to a position closer to Cnidaria and Bilateria. Acoela and Myzostomida move from being grouped with other long-branch taxa to positions with Deuterostomia (Acoela) or Annelida (Myzostomida).

introvert-bearing worms were considered an early branch within the Bilateria, distant from the coelomate arthropods. Ecdysis of an external cuticle and lack of locomotory cilia were thought of as convergently evolved. Moulting, however, turns out to be a better indication of relationships than a seemingly complex character such as segmentation. That this result is not obvious even when knowing the corresponding genetic basis of the character illustrates the limitations of phylogeny based only on morphological characters; it is worth noting here that some aspects of the new phylogeny, such as a Lophotrochozoa-like clade, were proposed as long ago as 1899, and that palaeontologists also suggested this by the end of the 1980s [5,39].

Evolution of Early Embryonic Cleavage Patterns

The spiral arrangement of blastomeres seen when an early cleavage stage embryo is viewed from the animal pole is found in several phyla, most notably annelids, molluscs, nemerteans and platyhelminths [40,41]. This pattern is strongly associated with a conserved set of defined blastomere fates. The recognition that the Lophotrochozoa are monophyletic — a clade that includes all the phyla with classic spiral cleavage — strongly supports the homology of this mode of early development. Recent reports of spiral cleavage in the lophophorate phoronids [42] and in entoprocts [43] make sense in the light of their inclusion in the Lophotrochozoa alongside the canonical spiral cleavers.

This single origin of spiral cleavage in a sub-group of protostomes is in contrast to the interpretation of this character in phylogenetic schemes placing the platyhelminths and nemerteans at the base of the Bilateria. These acoeloid/planuloid theories must interpret spiral cleavage either as a primitive bilaterian character lost in taxa with radial cleavage or as convergently evolved. Of related significance is the recognition that the arthropods are not close relatives of the spirally cleaving annelids. The previously widespread acceptance of the Articulata (annelids plus arthropods) had led to the interpretation of arthropod early cleavage stages as being a derived form of spiral cleavage [6,7]. While taxa without spiral cleavage show various modes of development, the most common alternative to spiral cleavage is radial cleavage, most firmly associated with the deuterostomes. According to its distribution on the new molecular tree, radial cleavage seems certain to be the primitive character state of metazoans; in addition to deuterostomes radial cleavage is found in diploblasts (poriferans, cnidarians and ctenophores),

Box 2. Mapping morphological data onto a phylogeny.

Mapping morphological characters onto a phylogenetic tree is a complex task. Unfortunately, by far the mot common method used is maximum parsimony. While parsimony constituted a major advance in evolutionary biology by formalizing hypothesis comparisons, it is now well established that probabilistic methods, even using the simple Mk or threshold models [107,108], outperform parsimony [109]. The obvious advantages of probabilistic methods are that they take into account rate heterogeneity across sites and branches (e.g. they can account for different branch lengths) and allow multiple changes on a single branch. Few efforts have been made in developing an accurate model of morphological character evolution (but see [110]), but this is a crucial step to take full advantage of the now well-established animal phylogeny. In the long run one can envision uniting trait evolution models with more mechanistic insights/knowledge from developmental genetics and in the short run phenomenological models using the existing mathematical tools, allowing great flexibility [111].

ecdysozoans (priapulids, as well as in some arthropods with primitive non-yolky eggs and holoblastic cleavage) and chaetognaths [9,44–46].

Ancestry of Through Guts

Cnidarians and ctenophores both have blind-ended guts, in which the single opening functions both as mouth and anus. It is reasonable to suppose (but see [47]) that this is the primitive condition, although if coelenterates (Cnidaria and Ctenophora) turn out to be monophyletic [48], this character would be unpolarised — i.e. coelenterates could as easily have lost a through gut as bilaterians gained one. The widespread presence of a through gut with separate mouth and anus in deuterostomes and in both major clades of protostomes suggests that the through gut is an innovation that was present in the common ancestor of both groups — an ancestor that is synonymous with 'Urbilateria' unless Xenacoelomorpha branch before this point (see below). Platyhelminthes, which have a blind gut, are nested within the Lophotrochozoa and so seem most likely to have lost a through gut (all their closest relatives have one); the same can be said of the Xenacoelomorpha if they are indeed members of the Deuterostomia rather than an early branch of Bilateria [49]. This inference, along with the observation that acoelomate, pseudocoelomate and coelomate taxa are intermingled in the Deuterostomia, Ecdysozoa and Lophotrochozoa shows that the traditional picture of a steady increase in complexity in these characters (from blind gut to through gut or from acoelomate via pseudocoelomate to coelomate) is untenable.

Primitive Deuterostomy?

The primitive gut or archenteron archetypically forms by an ingression of cells when the spherical blastula undergoes gastrulation; the opening of the tube that forms during gastrulation is called the blastopore. The fate of the blastopore has a clear significance in the name of the two major clades of Bilateria — the protostomes, in which the blastopore forms the mouth and the deuterostomes in which the blastopore forms the anus and the mouth is secondary. Amphistomy refers to an intermediate condition in which the blastopore is slit-like and closes in the middle to form both mouth and anus. The recognition that some phyla once classed as deuterostomes, specifically the chaetognaths and at least some lophophorates, group with the protostomes may suggest that deuterostomy was the primitive condition. The recent discovery of deuterostomy in the ecdysozoan priapulids [50] and possibly in onychophorans [51] reinforces this view. In reality, there is no clear pattern as in the protostomes everything from protostomy, through amphistomy to deuterostomy can be found in different taxa. It thus seems that at least the Ecdysozoa are likely to be primitively deuterostomous, radial cleavers, and that this may extend to all of the Protostomia (and thus Bilateria).

Major Areas of Phylogenetic Controversy

Xenacoelomorpha

While the precise position of Platyhelminthes ('flatworms') within the Lophotrochozoa is still uncertain, one aspect of platyhelminth evolution that has been resolved is the removal from this phylum of three groups of marine worms: the single species *Xenoturbella bocki* and the two related acoelomorph flatworm groups the Acoela and the Nemertodermatida. That none of these three groups is a flatworm is where the current consensus ends. After having been briefly assigned to the molluscs due to DNA contamination with their principal food (bivalve molluscs [52]), *Xenoturbella* has since been linked to the Ambulacraria (echinoderms and hemichordates) [53]. Several studies, by contrast, place the acoelomorphs as the earliest branch of bilaterians, branching off from the main lineage of before the protostome–deuterostome split [54–56]. More recent work groups *Xenoturbella* with the acoelomorphs (Xenacoelomorpha) [57,58]; the remaining tension is between those who put xenacoelomorphs as a branch outside the Protostomia and Deuterostomia and those who place them as the sister group of the Ambulacraria [49].

If xenacoelomorphs are an early branching clade intermediate between diploblasts (sponges, cnidarians and ctenophores) and all other bilaterians, their morphological simplicity [47] as well as the lack in Xenacoelomorphs of some Hox genes [54,59] and microRNAs [60] present in other Bilateria would result from their having branched off from the main bilateral lineage before the evolution of these characters [57]. A deuterostome affinity, on the other hand, would imply that the Xenacoelomorpha are simple and lack the characters mentioned not because they retain primitive features, but because they have lost these characters, as well as characters typical of deuterostomes such as gill slits and a through gut through a process of simplification and loss [18,61].

Chaetognatha, Ecdysozoa and Lophotrochozoa

While lophophorates and chaetognaths, previously believed to be associated with deuterostomes, have been shown unambiguously to belong to the protostomes, only the lophophorate phyla have been positioned more precisely, within the Lophotrochozoa. The chaetognaths, or 'arrow worms', in contrast have been linked by different studies to Ecdysozoa, Lophotrochozoa or as a sister group to both [29–33]. The likely early branching position of chaetognaths relative to other protostomes means their deuterostome-like early developmental features (radial cleavage, deuterostomy and enterocoely) may indicate that such

traits are primitive within the protostomes and hence primitive within the Bilateria as a whole. The composition of the Ecdysozoa and Lophotrochozoa is now well established but relationships between their constituent phyla are much less clear [18,62]. The lack of resolution amongst ecdysozoan phyla is simpler as there are fewer clades and the Priapulida (and possibly their likely sister taxa the Kinorhyncha and Loricifera) seem likely to be the earliest branch [58]. This would mean the 'cycloneuralian' worm-like phyla of the Ecdysozoa (Priapulida, Kinorhyncha. Loricifera, Nematoda and Nematomorpha) do not form a clade and would imply that the ecdysozoan ancestor possessed the features (most notably a worm-like body and proboscis) shared by these phyla. The phylum-rich Lophotrochozoa are more complex: certain former phyla (Sipunculida, Echiura, the ectoparasitic Myzostomida and the beard worms Pogonophora/Vestimentifera) have been subsumed into the phylum Annelida, and the Sipunculida and Echiura, unsegmented as adults, have been shown to go through a segmented phase as embryos [63,64].

The Lophotrochozoa uncontroversially contain the phyla Annelida (sensu lato), Mollusca, Nemertea, Platyhelminthes, Brachiopoda and Phoronida, as well as several phyla of small animals, such as Gastrotricha, Rotifera, Entoprocta, Cycliophora, Gnathostomulida and Bryozoa/Ectoprocta. However, there is no obvious consensus for the relationships between them. Brachiopods and phoronids are very likely linked — phoronids may even be shell-less brachiopods — and molecular phylogenies and recent reports of spiral cleavage link these two phyla to the spirally cleaving annelids, molluscs and nemerteans [42]. The spiral cleavage and trochophora-like Müller's larva of some platyhelminths also suggests a link to these phyla. A number of molecular studies, however, group platyhelminths with the diminutive phyla mentioned above in a cluster that has been called Platyzoa [58,62,65,66]. Most studied members of these phyla, including platyhelminths, are fast evolving (long-branched), however, and a systematic error is the most plausible explanation for this grouping (Figure 2) [21,67,68]. One interesting interpretation of the difficulty in resolving the relationships between the phyla of the Lophotrochozoa is that the lack of resolution is caused by the phyla having diverged from one another in a rapid radiation of new body plans. Lack of resolution here argues for the use of the adaptive radiation framework, well developed for recent cases (e.g. cichlid fishes [69]).

Non-Bilaterian Animals

Outside the bilaterally symmetric animals, the ctenophores (sea gooseberries/comb jellies) share characteristics with the cnidarians and bilaterians including nervous system, true muscle cells and aspects of their early development [9,70]. These characters are absent in sponges (Porifera) and the single placozoan species *Trichoplax adhaerens*. Ctenophores were linked in many morphological phylogenies to the cnidarians in a group called the 'Coelenterata' ('sack-guts', reflecting their blind guts). This phylogenetic position of ctenophores would suggest that the shared characters just mentioned appeared after the branching off of the sponges. The placement of the ctenophores using molecular phylogenetic data is another simmering controversy involving another fast-evolving clade: some recent phylogenomic studies place ctenophores not with bilaterians and cnidarians (collectively Eumetazoa) but as the earliest diverging metazoan group more distant than sponges to the Eumetazoa [65,71–73]. This early divergence of ctenophores has been supported by analyses of the content of ctenophore genomes that lack many characters present in sponges, cnidarians and bilaterians [74,75]. Such an early branching placement of ctenophores would suggest that the characters they have in common with cnidarians and bilaterians appeared convergently in both clades. The alternative explanation of independent losses of these morphological characters in both sponges and *Trichoplax* is less parsimonious [76].

While an early branching position of ctenophores has repeatedly been recovered in molecular phylogenetic studies [77], the fast evolutionary rate and the low extant taxonomic diversity of this clade make long-branch attraction artefacts possible (Figure 2). Phylogenetic studies addressing the perceived problem of long branches leading to the ctenophores have supported instead an unresolved multifurcation of cnidarians, ctenophores, placozoans and bilaterians excluding the poriferans [21,48]. Further studies are required, and the question of the characteristics of the common ancestor of all animals remains open [76,77].

Interpreting Patchy Characters

Characters formerly widely considered homologous and used to define clades include the body segments of the (widely accepted) Articulata and the coelomic cavities of the (less broadly accepted) Coelomata. In the modern molecular tree, these characters show a patchy phylogenetic distribution, i.e. they are shared by various distantly related taxa. This patchy distribution immediately calls into question the idea that these characters are homologous. If segmentation in annelids and arthropods is homologous and thus derived from their common ancestor, then it has been lost in multiple other protostome phyla that also descend from the common ancestor of both ecdysozoans and lophotrochozoans.

A second character with a patchy distribution is the biphasic life cycle involving a larval stage followed by a metamorphosis into a very different looking adult. Restricting ourselves to the ciliated larvae of aquatic taxa (the larva of holometabolous insects is clearly derived, for example, as are life stages of parasites such as digenean Platyhelminthes), these seem at least superficially similar — from the dipleurula of echinoderms and hemichordates in the deuterostomes to the trochophore of annelids and molluscs, the pilidium of nemerteans and the Müller's larva of polyclad Platyhelminthes.

Whether all or even some of these larvae are indeed homologous and so derived from a common ancestor is, however, far from clear [78]. On the one hand, the morphological similarities are obvious, with all the larval types mentioned using bands of cilia to swim and having a similar ciliated apical organ [79]. On the other hand, many phyla have no ciliated larva and the potential for convergent evolution of such a useful life-history character has been emphasized [80]. The potential to re-use adult developmental circuits for patterning larval equivalents might even produce similar expression patterns of homologous genes in non-homologous larval organs.

There are two related ways in which to think about patchy characters. First, the more complex the similarity (and more importantly, the more complex the evolutionary path), the more

reasonable it becomes to accept homology, despite multiple losses in taxa that lack the character [81]. Second, likelihood of convergence is higher where there is an obvious external reason for the similarities we find, other than shared ancestry. For example, the fact that the same adult anterior patterning genes (e.g. *Six3*, *rx*, *FoxQ2*) might pattern (anterior) apical organs in ambulacrarian and trochophora larvae can be explained as a convergent re-use of a conserved adult patterning mechanism [82]. In contrast, detailed similarities in the patterning of something that cannot have come from an adult (for example, the same neuropeptide provoking the onset of larval metamorphosis) are less likely to have evolved through convergence [83].

A Complex 'Urbilateria' — Integrating Molecular Trees and Fossils

A major difference between pre-molecular and molecular trees is that numerous simple organisms had been misplaced and, owing to their lack of 'advanced' characters, incorrectly located deeper in the tree, as also observed for unicellular eukaryotes [84]. For instance, acoelomates or pseudocoelomates were thought to branch closer to the root than more sophisticated bilaterians, and the simple urochordates (e.g. sea squirts) rather than more fish-like cephalochordates (amphioxus) were incorrectly assumed to be the earliest diverging chordate lineage [85]. Molecular phylogenies have corrected these misconceptions and show that many seemingly simple animals are deeply nested within clades of complex ones (e.g. Myzostomida within annelids [86,87], Myxozoa within Cnidaria [88] and Platyhelminthes within Lophotrochozoa). An analogous process happens with incomplete or poorly preserved fossils which effectively preferentially 'lose' advanced characters during fossilization, causing them to be placed lower down in a tree. This systematic error of pre-molecular phylogenetics is likely due to the strong prejudice, inherited from Aristotle and his 'great chain of being', that simple organisms are ancestral to more complex forms. The number of simple organisms incorrectly placed as early branches in the molecular trees may still be underestimated [89].

One implication of this observation is that simplification is a major driving force in evolution and that the ancestors of extant phyla are likely to have been more complex than previously thought. The eukaryotic ancestor, for example, appears to have had a genome rich in genes and introns to an extent that would have been unthinkable 20 years ago [90]. Numerous complex characters are likely to have been present in Urmetazoa and Urbilateria, the ancestors of metazoans and bilaterians, respectively. In particular, numerous genetic networks involved in cell–cell interactions and in multicellular development are shared by all animals (even the simple *Trichoplax* [91]) and even by the close relatives of animals (choanoflagellates [92] or ichthyosporeans). Given the prevalence of simplification during evolution and given its clearly rich gene repertoire, it is reasonable to assume a morphologically complex Urbilateria.

Another finding of molecular phylogenetics — the shorter branch leading from Urbilateria to the deuterostomes compared to protostomes, or even the possibility of paraphyletic deuterostomes [93] — leads to the intriguing possibility that Urbilateria was more deuterostome-like than protostome-like. We have seen already that deuterostomian developmental characters, such as radial cleavage and deuterostomy, are likely to be primitive within the Bilateria. Perhaps the adult form of Urbilateria was most similar to a deuterostome as well. Evolution from this complex, possibly deuterostome-like ancestor by simplification provides a straightforward explanation to the conundrum of the multitude of patchy characters discussed above. However, a complex Urbilateria does not mean that simpler ancestors did not exist and that complexity appeared instantaneously. Instead, complexity appeared more or less gradually and, at a given stage, it would have provided a sufficient selective advantage to outcompete the closely related lineages that, had they survived, could have revealed all the intermediate steps. The only access we now have to these intermediates is through fossils.

Given that all evolution leading to the animal phyla took place in now extinct stem groups [94], the fossil record might provide help to interpret the trees generated by molecular phylogeny (and *vice versa*). Animals with hard parts start to appear in the fossil record just before the beginning of the Cambrian period some 542 million years ago (see primer by Briggs in this issue). While the Cambrian yields a continuous and diverse fossil record, the fossil record before is discontinuous (i.e. fossil finds rely on rare instances of exceptional preservation) and few taxa can be seen to cross into the Cambrian. The fossils from the Precambrian are generally problematic and no clear consensus exists about their status, even though some have been claimed to be animals or even bilaterians and members of crown-group phyla. These problems of interpreting the Precambrian record have led to wide variations in charts that attempt to plot animal phylogeny against the fossil record [95].

One of the principal problems of interpreting the early fossil record has been that our search image for ancestors of various clades has been so poorly constrained. If the stem eumetazoans and stem bilaterians evolved from either benthic or planktonic larvae, either from adult ancestors actually resembling modern larvae (a classic Haeckelian view) or via heterochronic modes of evolution such as progenesis (the 'planula hypothesis'), it is obvious that predicting the forms of stem lineage fossils (or even finding them) will be challenging. This problem is confounded by the fact that fossils on the stem leading to modern clades will lack a subset of the characters that define that clade as these characters accumulated gradually along this stem. Some control exists though. For example, despite the general patchiness of the fossil record, some remarkable discoveries of microscopic fossils have been made in the Precambrian (e.g. Doushantou Formation from South China), yet none of these can be confidently assigned to bilaterians [96]. In contrast, the emerging field of studies of microscopic organic fragments in the Cambrian reveals many such fragments that are clearly bilaterian in origin [97]. Even if bilaterians were tiny in the Precambrian, they would be capable of being preserved in the microfossil record, suggesting that their absence is real.

Uncertainties about the timing of animal origins are exacerbated by the continuing problems in both molecular clock methodology and how to calibrate them [98,99]. For example, dating of the split of crown-group bilaterians to about 675 Ma [95] implies a gap of something like 140 million years between their appearance and their first definitive traces in the fossil record.

These early dates for Urbilateria seem, however, to be incompatible with the view that Urbilateria was complex. One argument along these lines is that if Urbilateria were complex, then

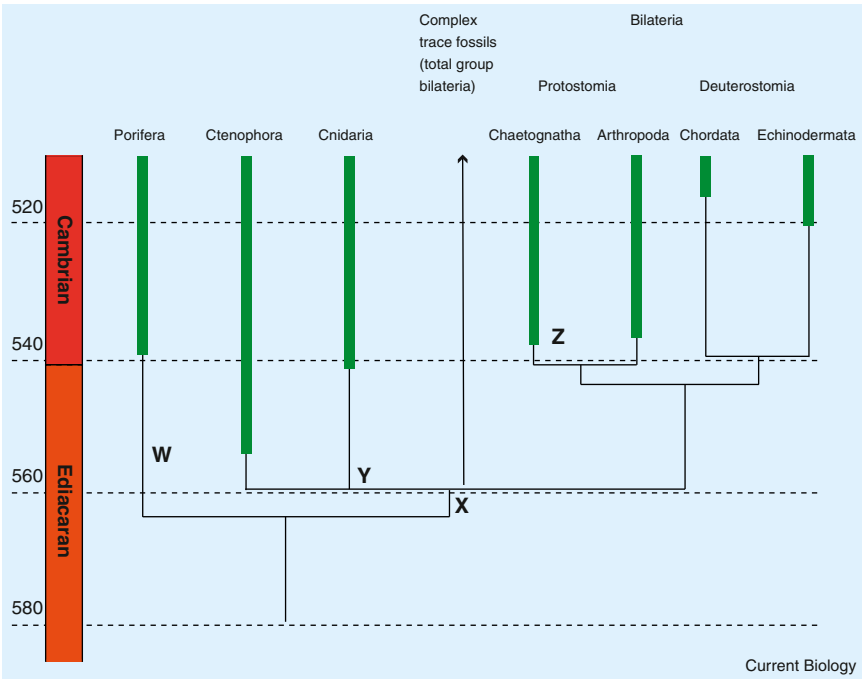

Figure 3. A simplified order of appearance of major taxa in the fossil record.
Green bars represent (relatively) reliable occurrences of the clade, thin lines represent implied 'ghost lineages (no distinction between stem and crown-group members). Note that the fossil record in itself does not necessitate an appearance of crown group metazoans before about 565 million years ago (Ma). Sponges: hexactinellid spicules from China. Ctenophores: we accept *Eoandromeda* as a probable stem-group ctenophore, otherwise early Cambrian examples from China. Cnidarians: probably *Corumbella* and allied forms from the latest Ediacaran of Brazil. Total group bilaterians: complex trace fossils from later than 560 Ma. Protostomia: total-group chaetognaths (e.g. from China) from close to the base of the Cambrian and arthropod trace fossils from a similar time. Deuterostomia: total-group echinoderms from around 521 Ma (data from [112]); chordates from the Chengjiang biota (e.g. [113]; a tunicate is also described [114]). Several earlier fossils imply earlier occurrences of some groups but their affinities are unclear. A recently published Doushantuo fossil that may date as early as 600 Ma was described as a sponge [115]. (Key to possible earlier fossils: W, various forms from the Ediacaran assemblages from c. 565–550 Ma have been suggested to be sponges; X, trace fossils from the about 565 Ma Mistaken Point assemblage may reflect a "coelenterate" grade; Y, *Haootia* from Mistaken Point has been suggested to be a cnidarian [116]; Z, a clear protostome diversification takes place as reflected in the "small shelly fossil" record [112].

it is likely to have been relatively large as well [94] and thus to have been capable of leaving either body fossils or, at the very least, trace fossils. Despite various controversial earlier findings, the oldest widely accepted trace fossils that are complex enough necessarily to have been made by bilaterians (either stem or crown group) are younger than about 560 million years [100]. This is an important time datum that allows us to state quite clearly that by this time at least stem-group sponges, ctenophores, cnidarians, placozoans and bilaterians must have diversified. A further corollary of the view that Urbilateria was complex, however, is that this time interval also represents the period when bilaterians were diversifying — if complex bilaterians existed much earlier, they should surely have been able to leave some sort of evidence, at least as trace fossils. From very low diversity in the late Ediacaran (from around 560 million years ago onwards) to the very high diversity of exceptional preservation in the later Cambrian, such as the Sirius Passet and Chengjiang biota, it is clear that a very rapid expansion of bilaterian, and incidentally, cnidarian and poriferan, clades took place. This sense of rapidity is reinforced by emphasizing that there are no even vaguely plausible candidates for early animal fossils before 600 million years ago. A straightforward reading of the early fossil record combined with the view above that the Urbilateria was complex suggests that the earliest stages of animal evolution took place perhaps around 590–580 million years ago, and that it was not until around 560 million years ago that bilaterians began to emerge (Figure 3). If the 'mainline' of animal evolution did in fact take place in large complex adult benthic forms [94,101,102], then this means that there is a much better chance of tracing the earliest phylogenetic stages of the animals in the fossil record. From this discussion, the possibility of reciprocal illumination between the fossil record and molecular phylogenies becomes clear: phylogenies can help direct us to what sort of organism we should be looking for, whereas the fossil record can help test such theories.

Conclusion

Many aspects of the animal phylogeny have been established for some time and backed up with corroborating evidence from sources such as unique genomic features. Nevertheless, we have highlighted a number of issues that remain unresolved. Improvements in the accuracy of animal phylogeny will, we suggest, require a 'more and less' approach. More genomic data are required from a broader diversity of species. The aim for now, however, should not be to build complete trees of tens of thousands of species using thousands of genes, but rather to concentrate on ensuring the accuracy of the principal features of the tree. To achieve this, we should set out to select the least problematic taxa (i.e. slow evolving with minimal systematic biases in substitution patterns); to sample taxa with the aim of breaking long branches; and to select amongst all genes the most relevant data (e.g. genes for which we are best able to model their evolutionary properties). In parallel, it is essential that more sophisticated evolutionary models be developed to approximate more closely the inherently complex reality of genomic evolution.

Our central theme emphasises the use of the increasingly stable phylogenetic framework of the animal kingdom as the basis for understanding the pattern and process of morphological evolution. Trees contain information concerning both relationships

between species and, in the lengths of internal and terminal branches, on the timing of divergences as well as the non-uniformity of genetic evolution. Mapping characters onto accurate trees is the next step in this process. It should be clear from our discussion that the character-mapping step is liable to be as fraught with problems as has been the construction of the underlying framework. While determining character state homology may be helped to an extent by mapping onto an accurate tree, this is still a far from trivial problem in practice. More sophisticated methods for mapping characters are necessary (Box 2) but the potential for convergent evolution on the one hand and for character loss or character state reversion on the other is not easily overcome (especially given that, as we have underlined, simplification is an evolutionary driving force). The next step of mapping homologous characters onto trees will require the collaboration between morphologists, developmental biologists, comparative genomicists, palaeontologists and phylogeneticists.

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