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Genome duplication, extinction and vertebrate evolution

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Vertebrate evolution has been punctuated by three episodes of widespread gene or genome duplication, which have been linked with the origin of vertebrates, gnathostomes and teleosts, respectively. These three events coincide with bursts of character acquisition and increases in phenotypic complexity, and many researchers have suggested a causal relationship between the two. However, this pattern is derived from data for living taxa only; we argue here that, when fossils are taken into account, bursts of character acquisition disappear and gen(om)e duplication in vertebrate phylogeny can no longer be correlated with the origin of body plans. If patterns of character acquisition or morphological gaps between higher taxa are a reflection of phenotypic complexity, then more inclusive data sets incorporating fossil taxa provide no support for hypotheses linking gen(om)e duplications and the evolution of complexity in vertebrates.

Duplication, duplication, duplication

The distinction between invertebrates and vertebrates has long been considered fundamental, and it pervades all discussions of animal biology. With the recognition that invertebrates are paraphyletic (see Glossary) and do not constitute a natural group, the distinction became little more than a convenient shorthand in communication, but discoveries in molecular biology over the past decade have revealed that the division has real genetic significance. Comparative analysis of invertebrate and vertebrate genes has borne out earlier suggestions [1] that vertebrate genomes are more complex than are those of their invertebrate relatives. *Hox* genes, a family of homeobox-containing transcription factors that lie in adjacent positions along a chromosome, have been particularly significant in these explorations of molecular complexity. Adjacent *Hox* genes form a 'cluster', and all invertebrates, including amphioxus (the closest living invertebrate relative of vertebrates), have only one *Hox* cluster. All vertebrates, however, have more than one [2]. A similar pattern has been found in many other gene families [3–5], and it now appears that the origin of vertebrates coincided with a widespread (possibly even genome-wide) gene duplication event [3]. Indeed, continued surveying has revealed that vertebrate evolution has been punctuated by at least two other duplication events, one at the origin of the gnathostomes [6] and the other somewhere along the lineage leading to teleost fishes, after their divergence from sturgeons [7] or gars [8].

All three duplication events are associated either with dramatic jumps in morphological complexity or with adaptive radiations and innovations of body design. This has led several authors to suggest a relationship between gene duplication and complexity [5–7,9–23], with some arguing that increases in complexity were caused by gene duplications [11,13,24]. We contend, however, that the apparent correlation between the phylogenetic timing of duplication events and increases in phenotypic complexity and diversity is an artefact of incomplete taxonomic sampling.

Evolutionary jumps, fossils and extinction

A fundamental problem with the hypothesis linking gen(om)e duplication events in vertebrates with evolutionary jumps is that extinct lineages are ignored. This is unfortunate because, in all three instances, the gulf between the living branches of the vertebrate tree is bridged by a series of extinct clades that are taxonomically and anatomically intermediate. These fossils can provide insight into the nature of morphological evolution through the period in which gen(om)e duplication is implicated,

Glossary

Adaptive radiation: rapid diversification of a lineage, linked with adaptation to a range of different ecological niches.
Apomorphy: an advanced group-diagnostic character.
Basal clade: a monophyletic group that occurs low within the topology of a phylogenetic tree.
Clade: monophyletic group.
Crown group: least inclusive clade encompassing the living members of a group and the extinct taxa that nest among them.
Monophyly: the condition where a group contains the common ancestor of all its members and all the descendents of that common ancestor.
Paralogue: used to describe two or more genes that occur within a genome that are not homologous but are derived through duplication from a single ancestral gene.
Paraphyly: the condition where a group contains the common ancestor of all its members, but not all of the descendents of that common ancestor.
Plesiomorphy: a primitive trait, not diagnostic within the phylogenetic context considered; the quality of primitiveness.
Symplesiomorphy: a shared, primitive trait that diagnoses a more inclusive clade.
Stem lineage: a paraphyletic assemblage of extinct taxa that are not members of a given crown group, but are more closely related to it than they are to any other crown group. For example, stem vertebrates lie outside the vertebrate crown group, but are more closely related to crown vertebrates than they are to their nearest living relative, amphioxus (a crown cephalochordate).

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constraining models of developmental evolution, rates of character acquisition and hypotheses of adaptive radiation.

In the case of comparative genomics, omission of fossils is a matter of necessity, given that no genes are preserved. However, when attempts are made to compare or integrate analyses of genomic and morphological evolution, there is no logical reason for excluding extinct taxa. One consequence of their exclusion is that attempts at integrated analysis are based on an incomplete morphological data set. More significantly, analyses based on extant diversity alone reveal an apparent pattern of bursts in character evolution and morphological complexity that is misleading. We argue here that this is not merely a scientific inconvenience: the pattern is a consequence of the incomplete sampling of taxonomic and morphological diversity.

The best understood of these three important episodes of vertebrate phylogeny is the origin of Gnathostomata, where accelerated rates of increasing morphological complexity have been identified as coincident with an inferred genomic doubling [4–7,11,17,22,25]. All living vertebrates, except the hagfishes and lampreys, are members of Gnathostomata, but what are gnathostomes? Etymologically, and in common parlance, gnathostomes are widely considered to be vertebrates with jaws. Curiously, however, they are both more and less than this.

Gnathostomes: more than just a pretty smile

The jaw is just one of a long inventory of characters that separate extant jawed vertebrates from lampreys, their nearest living relatives (Mazan *et al*. [26], for example, record 56 characters, and this is a considerable underestimate). Indeed, most of the typical features of what is generally thought of as the vertebrate body plan (e.g. a phosphatic internal skeleton, vertebrae and paired appendages) are gnathostome characters, and some authors recognize a distinct gnathostome body plan [27]. By any measure, there is an abrupt step-change in the rate of phenotypic character acquisition in the portion of the phylogenetic tree between living jawless and jawed vertebrates and this is congruent with patterns of genetic complexity.

Does this abrupt change constitute evidence of correlated increases in molecular and morphological complexity? Possibly, but incorporation of palaeontological data reveals a much less dramatic pattern of morphological and physiological change. This is because a spectrum of extinct lineages lies between the extant jawless and jawed vertebrates (Figure 1). We can resolve their intermediate position, and their degrees of relationship to living jawed vertebrates because, in addition to the plesiomorphic vertebrate characters that they all share, they also have subsets of that inventory of characters that distinguishes living jawed vertebrates from lampreys. For instance, conodonts, which were otherwise anatomically comparable to lampreys, have a single gnathostome apomorphy (a mineralized skeleton), indicating that they are more closely related to living jawed vertebrates than they are to lampreys [28]. Pteraspidomorphs have a mineralized dermoskeleton, as do anaspids, which also have an anal fin and a distinct stomach, betraying still closer affinity to sharks and bony fishes. Galeaspids have these characters and more, including a mineralized endoskeleton, which indicates that they are even more closely related to living jawed vertebrates [29,30]. And osteostracans are more closely related still, having paired pectoral appendages, slit-shaped gill openings, a dorsally elongated tail fin, cellular bone, sclerotic ring and ossified sclera; all characters that are absent from lampreys but present in living jawed vertebrates [29,31]. Finally, placoderms, the extinct group that is most closely related to living jawed vertebrates, diminish the inventory of characters that distinguish extant jawed vertebrates still further, having mineralized neural and haemal vertebral elements, a horizontal semi-circular canal, paired pelvic fins and, among other characters, jaws. Few characters remain that are exclusive to living jawed vertebrates, of which the anterodorsal attachment of the superior oblique eye muscle [31] is perhaps the most convincing. Is this sufficient justification for recognizing a distinct gnathostome body plan?

One might argue that placoderms are more appropriately considered members of Gnathostomata because, after all, they have jaws. But why should this single character be considered more ‘significant’ or ‘essential’ than any of the many other characters that distinguish sharks and bony fishes from lampreys? As with the other extinct lineages considered above, placoderms do not exhibit the full complement of characters shared by living jawed vertebrates. If placoderms are to be incorporated into Gnathostomata, then so should all the others, from osteostracans to conodonts, which on the basis of gnathostome synapomorphies are demonstrably more closely related to sharks and bony fishes than they are to lampreys. Regardless of whether these extinct clades are formally considered members of Gnathostomata, their affinity to extant jawed vertebrates is conventionally recognized through their inclusion in what is known as the gnathostome total group, within which the extant lineages are distinguished as crown Gnathostomata, and the remaining paraphyletic ensemble of extinct lineages as stem Gnathostomata [28].

Stem lineages and evolutionary patterns

Whichever way one classifies this parade of dead fishes, stem gnathostomes are fundamental to our discussion because their subsets of gnathostome characters reveal that the construction of the gnathostome body plan occurred piecemeal and, if the dates of appearance of these groups in the fossil record are anything to go by [32], this assembly of characters occurred over a protracted period of time between the Middle Cambrian and the Late Ordovician, or possibly even later, a matter of 70 million years (myr) or more (molecular clock dates suggest an even more protracted interval [32]). Stem gnathostomes also demonstrate that the pattern of character acquisition was not smooth, with more characters acquired at some nodes than at others (Figure 1). This apparent variation in rate might be real, but, given the nature of the fossil record, it is possible that it is itself an artefact of incomplete taxon sampling in that we might have omitted some extinct lineages that are as yet unknown or incompletely understood from the fossil record [32]. After

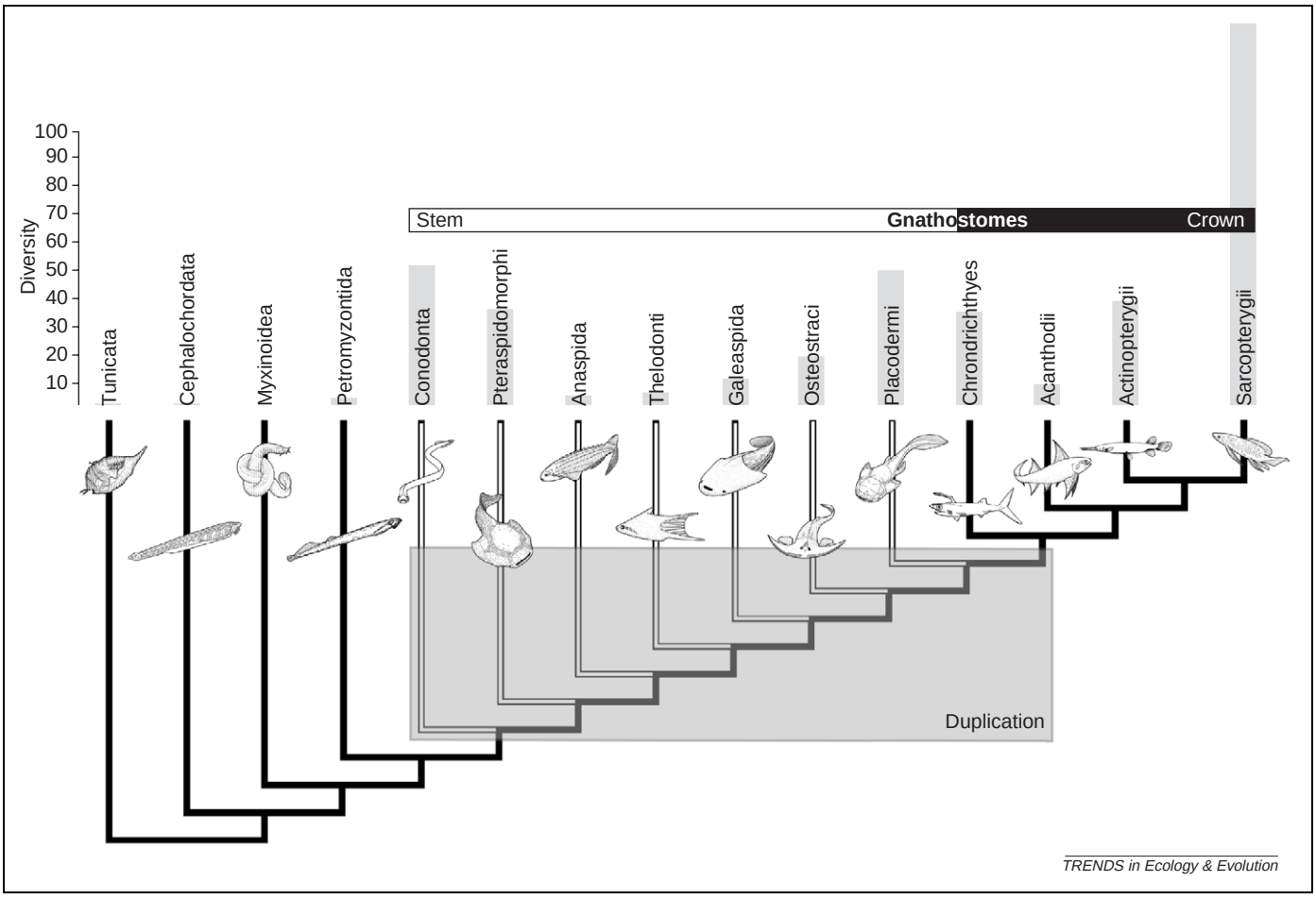

Figure 1. Gnathostome origins and the timing of gen(om)e duplication. The cladogram shows the hypothesis of relationships among living (black lines) and extinct (white lines) clades of lower vertebrates. The grey box shows the lack of precision in placing the gnathostome duplication event after the origin of Petromyzontida (lampreys) but before the origin of Chondrichthyes (sharks, skates and rays), and the range of fossil clades that fall within this interval. The vertical bars and scale above the cladogram show diversity (number of families, total for each clade). Diversity is based on data for the Palaeozoic because much of the increase in chondrichthian, actinopterygian and sarcopterygian diversity occurred during the Mesozoic and Cenozoic, hundreds of millions of years after the genomic doubling event implicated in gnathostome origin. Relationships based on [28,31]; familial diversity from [50].

all, although most of the groups that we now place among stem gnathostomes have been known since the 19th century [33], others have been discovered or resolved as being vertebrates only relatively recently (galeaspids in 1965 [34], pituriaspids in 1991 [35] and conodonts in 1993 [36]).

The significance of stem relatives of extant higher taxa also extends to inferred patterns of diversity change, and the adaptive radiations that are identified as a result. For instance, many of the clades of stem gnathostomes are extremely speciose and would do nothing to diminish the inferred burst of diversity in the crown were they extant (and, therefore, members of the crown) (Figure 1). Indeed, until their demise (most became extinct during the Late Devonian, ~375 million years ago), they were numerically superior to contemporary lineages that now lie within the crown group. It is not until well after the decline of the major groups of stem gnathostomes (i.e. pteraspidomorphs, galeaspids, osteostracans and placoderms) that crown gnathostomes became more diverse [41].

The same pattern can also be demonstrated for the pre-teleost portion of actinopterygian fish phylogeny in which a further episode of gen(om)e duplication has been implicated (Box 1). Proponents of this event make the evolutionary consequences clear. With almost 24 000 living species [37] teleosts are the most diverse and successful group of vertebrates, and their phenotypic diversity, number of species [8,15,38], and even increased phenotypic complexity [8] have all been linked to gen(om)e duplication. Closer scrutiny of the apparent congruence between duplication and increases in diversity and complexity, however, reveals a different picture. The timings and topology advocated by Hoegg *et al*. [8], for example, appear to resolve the position of the duplication to a point intermediate between teleosts and their nearest living relatives, but the authors make no mention of the 11 extinct clades [39] that intercalate between these two lineages. These stem teleosts fill not only the phylogenetic, but also the morphological chasm separating living teleosts from other living actinopterygians [39,40], smoothing patterns of character acquisition previously taken to suggest an evolutionary burst or sudden increase in phenotypic complexity at the origin of teleosts. Furthermore, the phylogenetic positioning of the gen(om)e duplication event (Box 1) does not even coincide with the major teleost radiation, which occurred within the derived acanthomorph sub-clade (Polymixiiformes+Paracanthopterygi+Atherinomorpha+Percomorpha).

Box 1. The 'fish-specific' duplication and teleost evolution

Of the three vertebrate ge(nom)e duplications, the event inferred to have occurred within ray-finned fishes (actinopterygians) is the least well understood and the most open to question. It is clear that many teleosts have more paralogues of *Hox* and other genes than do other gnathostomes [8,15], but the precise pattern and phylogenetic point of duplication is less well understood than other events because the number of *Hox* clusters in living basal ray-finned fishes is unknown [7]. Recent papers narrow the timing down to somewhere after the common ancestor of teleosts and sturgeons (acispenseriforms) [7], or after the origins of the clade [sturgeons + gars (lepisosteids) + bowfin (*Amia*)] [8] (if they form a clade [51]). All agree that it predates the origins of living teleosts.

The pre-teleost duplication has been linked to the well known diversity and phenotypic complexity of living teleosts. However, when their stem is taken into account, patterns of character acquisition no longer fit the model (see main text for details). The protracted teleost stem, spanning the interval of inferred genome duplication, also makes it impossible to determine the phylogenetic point at which duplication occurred. It might be possible to infer duplication, based on the 'predicted' pattern of increased diversity, but the inclusion of palaeontological data also changes relative taxon richness significantly: Chondrostei (*sensu* [52]), for example, includes only six living genera, but a further 11 extinct genera are known; the bowfin, *Amia calva* is the sole Halecomorphi survivor, but a total of seven families are known from the fossil record [53]; some classifications recognize up to 42 families of extinct Palaeonisciformes [37]. These clades unequivocally pre-date the fish-specific duplication but are more taxon rich than are most stem-teleost clades (Figure I). Family-level data might be expected to be the most robust for an investigation of this type, yet the most basal clade of crown teleosts, Osteoglossomorpha, contains only seven families [54,55], and other crown clades contain even fewer families. Thus, when fossil data are taken into account, there is no close correlation between genome duplication and the appearance of clades exhibiting greater taxon richness. This is all the more surprising given the 'pull of the recent' in teleost diversity data; 43% of extant teleost families have no fossil record [54], suggesting that, if we had a full picture, extinct actinopterygian clades and stem teleosts would be even more taxon rich than current data suggest.

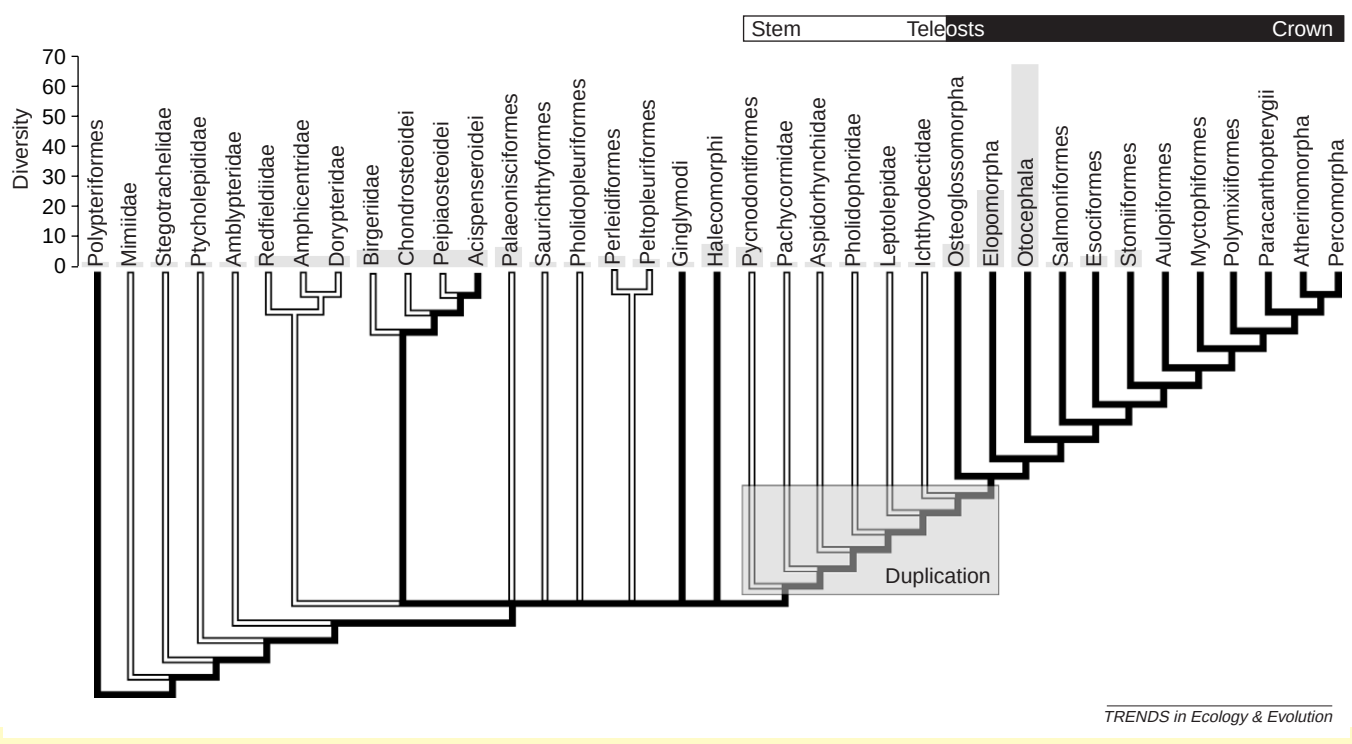

Figure I. Hypothesis of relationships among living (black lines) and extinct (white lines) clades of ray-finned fishes. The vertical bars and scale above the cladogram show diversity (number of families, total for each clade; values for most derived teleosts not shown). The grey box shows the degree of uncertainty in placing the fish-specific duplication event within the teleost stem. The relationships among the most derived non-teleost fishes are shown as unresolved because of uncertainties concerning whether gars (Ginglymodi) or bowfin (Halecomorphi) are more closely related to teleosts [40], and because if they form a clade with sturgeons (Acispenseroidei) [51] the positions of the intervening extinct clades are currently unknown. This lack of resolution in this part of the tree does not affect the fact that an extensive teleost stem exists, and that there is no correlation between taxon diversity and the phylogenetic timing of the duplication. The position of Osteoglossomorpha as the most basal crown teleosts follows [37,51,56,57]. Relationships and taxon counts based on [37,52–55,58], and the summary cladograms from [42].

The situation regarding the gen(om)e duplication at the origin of vertebrates is more problematic. It appears to be associated with an evolutionary jump from amphioxus to hagfish but determining whether this is real or an artefact of extinction is difficult. Only the pattern of character evolution through the vertebrate stem can reveal the answer. However, this is confused by preservational biases and difficulties inherent in assigning fossils to the stems of clades with deep divergence times (Box 2). Even among crown vertebrates, the most basal clades, hagfish and lampreys, lack mineralized tissues, and the preservation potential of chordates with no hard parts is low, even in instances of exceptional preservation. This is further compounded by the fact that most crown-vertebrate apomorphies are soft tissue, ultrastructural and especially embryological characters. Therefore, the current inventory of exceptionally preserved soft-bodied animals could include the remains of stem vertebrates that have not

Box 2. Recognizing stem vertebrates

Unravelling the pattern of character acquisition through the origin of vertebrates is complicated by the low preservation potential of chordates lacking mineralized skeletal components, and the absence of any unequivocal stem vertebrates in the known fossil record. Conodonts provide an interesting illustration of this preservational bias. Their fossil record extends from the late Cambrian to the end of the Triassic (some 300 myr), and comprises millions of specimens of thousands of species. However, if it were not for the phosphatic elements of the conodont feeding apparatus (their sole gnathostome apomorphy), the known fossil record would comprise 12 specimens assigned to three species, the number that preserve traces of the body. Furthermore, some of these specimens preserve few vertebrate characters, and it is doubtful whether one would be recognized as a chordate if it were not clear from its phosphatic skeletal apparatus that it is a conodont.

Most crown-vertebrate apomorphies are soft tissue, ultrastructural and embryological characters and, for this reason, phylogenetic placement of the few fossils currently identified as hagfishes and lampreys is open to question (Figure I). Although they have features that are interpreted as hagfish and lamprey apomorphies, they lack key vertebrate characters [59–62], and some could be stem vertebrates. Similarly, *Pikaia*, *Yunnanozoon*, *Haikouella* and *Myllokunmingia* from the Lower Cambrian Chengjiang Fauna, as well as a spectrum of mitrate carpoids, are candidate stem vertebrates. With the recognition that echinoderms and hemichordates are each other's nearest relatives, however, the many putative homologies uniting carpoids and vertebrates have been resolved as deuterostome symplesiomorphies, and carpoids are most readily interpreted as stem echinoderms [63]. Of the remaining taxa, it is currently unclear whether the chordate characters that they have are unique to the crown group, or appeared somewhere along the chordate stem. Some, such as a perforated pharynx and pharyngeal arches, are evidently deuterostome symplesiomorphies [63]. If these fossils do not unequivocally qualify as members of the chordate crown group, interpretations placing them as stem cephalochordates or stem vertebrates are even more doubtful.

From what we know of other major clades, it seems most unlikely that there was not an extensive vertebrate stem. Unfortunately, however, we lack the evidence needed even to begin an assessment of the taxon richness or character evolution, and their possible congruence with gen(om)e duplication, through this crucial phase of vertebrate evolution.

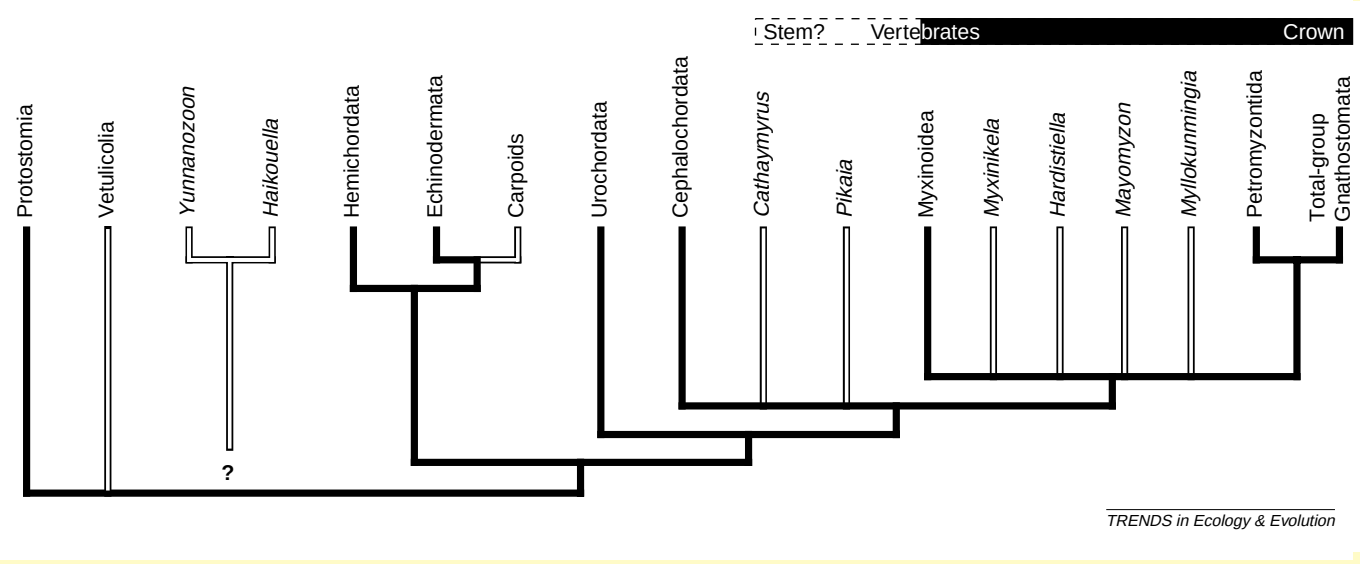

Figure I. Hypothesis of relationships among living (black lines) and extinct (white lines) clades of deuterostomes. Relationships of extant groups are based on [63] and references therein. The affinities of *Vetulicolia* are extremely uncertain. *Yunnanozoon* and the suspiciously similar *Haikouella* have been variously interpreted as stem deuterostomes, hemichordates, cephalochordates, craniates or vertebrates owing to equivocation over interpretation and character polarity. *Pikaia* and *Cathaymyrus* have hitherto been recognized as fossil cephalochordates but they fail to exhibit any unequivocal cephalochordate apomorphies.

been recognized as such. Candidates include *Pikaia* from the Middle Cambrian Burgess Shale, and *Yunnanozoon*, *Haikouella* and *Myllokunmingia* from the Early Cambrian Chengjiang Fauna, but none of these can be placed unequivocally within the vertebrate stem (Box 2).

What a more inclusive and integrated perspective on vertebrate phylogeny reveals, therefore, is that the purported jumps in character acquisition and diversity in vertebrates coincident with the gen(om)e duplication events are artefacts of higher than average pruning of lineages from the Tree of Life by extinction. Neither is there anything particularly special about the extent of these stem lineages that could be linked to inferred gen(om)e duplication events. Tetrapoda, Mammalia and Aves also have extensive stem lineages [42], and we should not be seduced into thinking that there is anything special about the nature of the origin of vertebrate higher taxa just because they have long stem lineages. Rather, the anatomical distinctiveness of these groups of organisms is an artefact of extinction of phylogenetic intermediates.

Constraining the timing of gen(om)e duplication events

Although the elucidation of stem vertebrates, stem gnathostomes and stem teleosts refutes the hypothesis that dramatic jumps in character acquisition are associated with the origin of their respective crown groups, some authors have been careful to couch their hypotheses of causality between gen(om)e duplication, molecular and morphological evolution in terms that allow for these events to have occurred somewhere between extant lineages [6,18,43]. Yet the precise timing of the inferred

gen(om)e duplications relative to morphological character evolution is crucial to the entire thesis of causality (or permissiveness [6]). If the gen(om)e doubling were found to postdate the acquisition of most of the characters within the stem, hypotheses of causality and permissiveness would be rejected; gen(om)e duplication would be consigned to an interesting but insignificant phenomenon of vertebrate evolution.

Clearly, only data from stem taxa can resolve this issue. We are never going to be able to sequence any of their genes but it might be possible to constrain the timing of gen(om)e duplication by inferring the existence of new paralogues from evidence of their first phenotypic expression in fossils from within the stem (phenotypic expression being understood from studies of knockout, mis-expression and *in situ* hybridization in extant crown group representatives). Such fossil evidence would postdate true paralogue origin (and even origin of function) but might provide some insight into the relative timing of gen(om)e duplication events (i.e. early or late within the stem).

For example, *Dlx* genes occur as six paralogues in gnathostomes (*Dlx1–6*), and four in lampreys (*DlxA–D*). Two of the lamprey paralogues (*DlxA* and *DlxC*) appear to be unique to lampreys, whereas *DlxD* and *DlxB* appear to be orthologous to gnathostome *Dlx1*, *Dlx6*, *Dlx7*, and *Dlx2*, *Dlx3*, *Dlx5*, respectively [4]. In gnathostomes, all six genes coordinately regulate jaw [17] and tooth development [44]; *Dlx3*, *Dlx5* and *Dlx6* are integral to cartilage, bone and tooth development in the pharyngeal, axial and appendicular skeletons [45]. Given that placoderms are the most plesiomorphic vertebrates in which these skeletal systems occur in mineralized form [46], it would not be unreasonable to conclude that manifest function of gnathostome *Dlx* paralogues was late in the gnathostome stem, coincident with the acquisition of jaws. However, all six paralogues are also expressed in fin and/or limb development [45], and the presence of pectoral fins in osteostracans and fin-like structures in even more plesiomorphic members of the gnathostome stem (e.g. anaspids and thelodonts) could, therefore, indicate much earlier manifestation of *Dlx* function.

However, deletion experiments show that, with respect to the skeleton, there is considerable redundancy between paralogues [45], perhaps because a similar range of functions was once performed by a smaller repertoire of ancestral genes. In other words, function and phenotype were acquired before the origin of gnathostome paralogues, among which function was subsequently partitioned. This lies at the heart of the duplication–degeneration–complementation model of paralogue fate following gene duplication [47]. Under this model, paralogues, which initially are functionally redundant, can be either silenced or retained, with retention favoured where paralogues acquire novel functions (neofunctionalization), or because they accumulate complementary degenerative mutations such that both are required for combined maintenance of ancestral functions (subfunctionalization). Subfunctionalization in particular provides an explanation for the prevalence of paralogues with a partitioning of ancestral expression patterns and protein functional domains. One corollary of the subfunctionalization model is that complexity, in terms of the body plan, regulatory gene networks and/or gene expression patterns, is established before gen(om)e duplication, and genomic complexity is thus an effect, rather than the cause of developmental and/or phenotypic evolution (although neither model precludes further increases in developmental complexity and, potentially, phenotypic complexity, after duplication). With respect to *Dlx* and gnathostomes, however, under either model of paralogue retention, the phenotypic effect of *Dlx* duplication occurred late within the gnathostome stem, after many gnathostome characters had already been acquired.

Testing the congruence of genetic and phenotypic complexity

The hypothesis that gen(om)e duplications have been significant in facilitating or driving vertebrate evolution requires duplications to be congruent with increases in phenotypic complexity and/or morphological innovation and/or taxon richness. If only extant taxa are considered, the coincidence of character acquisition and taxon richness with duplication events in the lineages leading to living jawed vertebrates and to teleosts, for example, seems obvious, but it is an artefact of incomplete taxon sampling, resulting entirely from the unjustified exclusion of extinct lineages. The pattern disappears when the fossil record of extinct members of those lineages is taken into account. However, it could be argued that the hypothesis is not falsified by this result because phenotypic complexity is something more than character acquisition or taxon richness.

So is there a correlation between genomic and phenotypic complexity? Unfortunately, finding an answer is problematic. First, research effort is strongly biased towards the molecular side of the hypothesis and, in contrast to the hundreds of papers investigating genome complexity, very few have attempted to analyse phenotypic complexity, innovation or taxon richness through the phylogenetic intervals in question (see Box 3 for a discussion of the interrelationships between complexity, innovations, taxon richness and body plans). This might be because, when extant taxa only are considered, the apparent evolutionary bursts within vertebrate phylogeny appear too obvious to be worth further enquiry, but extinct taxa cannot simply be ignored.

A further problem arises from the meaning of complexity. In the context of duplication events, 'genomic complexity' is unambiguous. However, with very few exceptions [48], the meaning of 'phenotypic complexity' or how it might be measured has not been addressed (Box 3). We are aware of only two attempts to measure the evolution of complexity that have included the interval of vertebrate evolution of interest here: one counted cell morphotypes [48,49], the other used an index based on character counts [5]. Unfortunately, these analyses were based on extant vertebrates only (cell morphotype number cannot be determined directly in extinct vertebrates); thus, in both cases, the magnitude of the increase in complexity measured between living jawless and jawed vertebrates is, similar to the pattern of character acquisition, a

Box 3. Complexity, characters and body plans

Suggested correlates of vertebrate genome duplications include increases in phenotypic complexity, morphological innovation, taxon richness (i.e. diversification) and the origin of body plans. All these are linked.

A detailed discussion of complexity in this context is beyond the scope of this article. 'Complexity' is considered by some authorities to have lost any precision or general meaning [64,65], but there is a degree of general agreement that the morphological complexity of an organism is some function of its component parts, with more complex organisms have more parts or more interactions between parts. The difficulties of actually measuring organismal complexity, however, are not insignificant. Some progress has been made in formulating narrower definitions of complexity [65,66] and these at least provide a context within which comparisons can be made and scientific questions framed, but research into patterns of phenotypic complexity has not progressed much beyond this.

With the single exception noted in the main text [48], these problems remain unacknowledged in hypotheses of genome duplication and complexity increase in vertebrates. Used without qualification or explicit explanation, 'phenotypic complexity' has no meaning or value in scientific investigation.

Body plans are linked to complexity through the hypothesis that the origin of body plans and establishment of body-plan characters are emergent properties of increasing phenotypic complexity [67]. Characters that define the body plan are those that attained greater importance and became evolutionarily crystallized because other characters are functionally or developmentally predicated on them. This idea applies to both phenotypic and genomic complexity, and has some theoretical support [68], but one of the difficulties in this area of research is determining whether body plans are real or merely perceptual artefact [67]. Consideration of the stems of extant clades points strongly toward perceptual artefact.

Some authors have linked genomic duplication to innovation, rather than phenotypic complexity *per se*, but this is really just character acquisition by another name in that:

- Innovations (or 'key innovations') are causally linked to intervals of accelerated phenotypic evolution (reviewed in [69]);
- Such intervals are usually identified by increases in taxon richness;
- From a morphological perspective, an interval of increased taxon richness is the same thing as an increase in rate of character acquisition.

A further difficulty with key innovations comes in establishing the causal relationship with accelerated evolution. However, multiple comparisons of the evolutionary history of clades with and without a putative innovation, and some level of correspondence between the appearance and diversification of the clade are generally taken to be valid tests [70]. It is difficult to see how this could be applied in the context of vertebrate and gnathostome origin, or teleost diversification, however, as they are, by their nature, one-off events.

The relationship between key innovations and body-plan characters is more subtle, but becomes clearer when extinction is taken into account. Both refer to characters shared by a clade that are interpreted as having special significance and are correlated with diversification. The only difference is that, in the case of body-plan characters, diversification occurred deep within phylogeny and the subsequent extinctions of basal taxa have left the clade in question as a terminal survivor, morphologically isolated from its nearest living relative.

consequence of missing all the taxa in intermediate phylogenetic positions.

The upshot is that we cannot currently test for congruence between genomic duplications and increases in phenotypic complexity (or morphological innovation; Box 3) because these concepts are defined too loosely for falsification of the hypothesis. If we are to incorporate the fossil record into the hypothesis (and to do otherwise renders analysis futile) then the taxon richness of clades is directly correlated with rates of character acquisition and this, as we have shown, provides no support for congruence. This discussion also serves to emphasize a broader point: to understand fully the nature of developmental evolution it is necessary to include extinct, not just extant, organisms. The fossil record might be famed for its incompleteness but it is far more complete than a phylogeny that includes only those organisms that, by chance, are alive today.

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