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Molecular tools in palaeobiology: divergence and mechanisms

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In 1987, Cambridge University Press published a volume entitled *Molecules and Morphology in Evolution: Conflict or Compromise?* edited by the esteemed British palaeobiologist Colin Patterson. Since the 1980s, we have witnessed a great deal of incorporation of the tools and data of molecular biology into palaeontological hypothesis building and testing. The degree of integration is substantial enough so as to rule out the rather pejorative subtitle of the 1987 volume, 'conflict or compromise'. We believe a new designation is appropriate: 'synergy'. Stated differently, our ability to address major questions in biological history requires the integration of molecular methods and data into the palaeobiologist's toolkit. The antagonism implicit in the notion of 'conflict or compromise' is more an artefact of disciplinary boundaries and analytical traditions, and is not firmly rooted in the data of biology. Palaeobiologists today routinely consider data from molecular biology in their research on the shape and antiquity of the tree of life ('divergence'), and in understanding the genetic and developmental mechanisms behind morphological change ('mechanisms').

This book documents aspects of this synergy, focusing on these two general categories: divergence and mechanisms. It derives from the symposium 'molecular tools in palaeobiology' that took place during the 2009 meetings of the Society of Vertebrate Paleontology in Bristol, UK. In retrospect, we realize that the 'vertebrate' orientation of that conference has resulted in a level of taxonomic focus in this book that excludes many important contributions regarding evolutionary divergence and mechanisms. Nevertheless, this is no small taxonomic category, and there has been much to say about it since 1987.

The unifying theme of that symposium, as in this edited volume, is general: how have molecular methods become critical in particular subfields of palaeobiology, and how does this constitute interdisciplinary synergy to understand life history?

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Divergence

Many aspects of the synergy we would like to emphasize in this book were evident to the authors of Patterson (1987). For example, on its cover, Patterson's volume depicted Ernst Haeckel's artistically rendered Tree of Life, published in German in 1874. From our perspective in 2011, careful inspection of that tree reveals the extraordinary extent to which the comparative anatomists of the nineteenth century 'got it right' in terms of the overall structure of animal, particularly vertebrate, phylogeny (Figure 1.1). Although biologists of the 1980s were unable to completely tease apart many issues in systematics – such as relations near the base of chordates, between birds and mammals, and among mammalian orders – for the species they had in common, Goodman *et al.* (1987) and Bishop and Friday (1987) proposed trees that were not far off from those outlined over a century before.

Concordance of vertebrate trees generated by molecular data today with those based on comparative anatomy of the nineteenth century is remarkable: not much has changed compared with the basic structure deciphered by naturalists two centuries ago. Haeckel (1874), Gill (1872) and others of their time considered dozens of major groups and hundreds of species, recognizing the nested relationships of vertebrates, gnathostomes, bony and cartilaginous fish, ray- and lobe-finned fish, tetrapods, amphibians and amniotes, diapsids and synapsids. Since then, there have been a few major questions regarding this understanding of vertebrate phylogeny, such as the possibility of a bird–mammal clade (Huxley 1868; Hedges *et al.* 1990; Gardiner 1993), the placement of coelacanths with ray-finned fish (Arnason *et al.* 2004), or the paraphyly of rodents (D'Erchia *et al.* 1996). However, subsequent analysis of more comprehensive data sets, often by the same investigators, has resolved these issues beyond any reasonable doubt (Hedges 1994; Murphy *et al.* 2001; Hallström and Janke 2009) and has left the Tree of Haeckel and Gill relatively unscathed. Changes within groups have occurred (particularly mammals), but perhaps the only substantial change among major vertebrate groups has been the recognition that tunicates are closer to vertebrates than *Branchiostoma* (Delsuc *et al.* 2008), switching two branches that Haeckel had already placed immediately adjacent to one another (Figure 1.1).

It's worth comparing, for example, the 88-taxa study by Hugall *et al.* (2007) with Haeckel's tree as displayed on the cover of Patterson (1987). The former (Hugall *et al.* 2007: figs. 1, 2) places hagfish and lamprey at the base of vertebrates, followed by a monophyletic frog–salamander–caecilian clade (they did not sample Chondrichthyes or Actinopterygia), followed by amniotes, which consist in turn of Mammalia as the sister taxon to an archosaur–squamate clade.

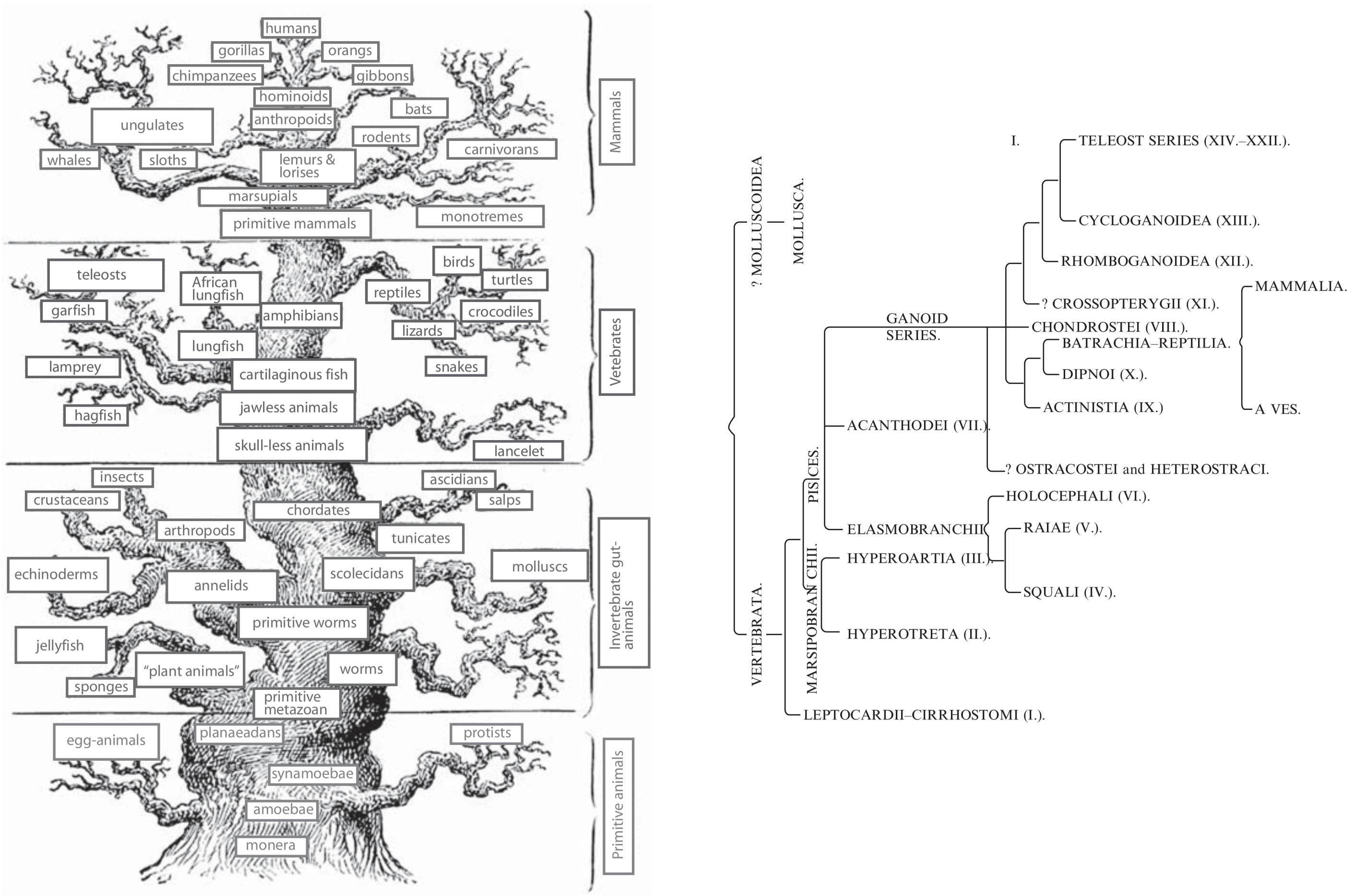

Figure 1.1 Nineteeth-century evolutionary trees of Ernst Haeckel (left) and Theodore Gill (right). Haeckel's image is from the 1897 English version of *Evolution of Man*, repeating his previously articulated views (e.g. Haeckel 1874, p. 513). Gill's phylogeny was published in 1872. Both show a remarkably high level of agreement with current ideas on vertebrate interrelationships, including the monophyly of craniate vertebrates, cyclostomes, gnathostomes, cartilaginous and bony fish, actinopterygians, sarcopterygians, tetrapods, amphibians and amniotes (Hugall *et al.* 2007; Delsuc *et al.* 2008). (See also colour plate.)

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The 179-gene data set of Delsuc *et al.* (2008: figs. 2 and 3) across deuterostomes similarly supported vertebrates, gnathostomes, cartilaginous and bony fish, actinopterygians, tetrapods, amphibians and amniotes. This basic structure, as inferred by both Hugall *et al.* (2007) and Delsuc *et al.* (2008), does not differ substantially from that proposed by Haeckel (1874) or Gill (1872), extending even to cyclostomes (i.e. hagfish and lamprey as sister taxa) and turtles within archosaurs (i.e. birds and crocodiles).

To put this in context, there are approximately 2×10^{157} ways in which the 88 taxa sampled by Hugall *et al.* (2007) could be interconnected as rooted, bifurcating trees (Felsenstein 1978). Why is it that out of this extraordinary number of possibilities Hugall *et al.* (2007) honed in on essentially the same pattern as that proposed by Haeckel and Gill in the nineteenth century? The answer of course speaks to the prediction made by Darwin in 1859, that there is a genuine Tree of Life subject to reconstruction using the evidence left behind by the mechanism of descent with modification, evidence that manifests itself on a developmental continuum between genotype and phenotype. Haeckel and Gill knew about the phenotypic side of this continuum. Hugall *et al.* (2007) focused on one small part of genotype: sequences of the nuclear *Rag-1* gene; Delsuc *et al.* (2008) had a much larger genetic data set with fewer sampled vertebrate taxa. That each source of data supports such a specific hypothesis out of such an astronomical number of possibilities is testament to the reality of Darwinian evolution. A largely consistent topological signal across independent data sets is what one would expect if animals actually share genealogical history with one another via the mechanism of descent with modification (Penny *et al.* 1982).

Phylogenetic consensus has been more elusive for parts of the Tree of Life other than vertebrates that (1) contain much more ancient branching points, (2) have not been as thoroughly documented genomically and anatomically, (3) are more prone to phenomena such as lateral gene transfer, and/or (4) have a much more limited fossil record (Woese 1987; Doolittle and Bapteste 2007). Nevertheless, evolutionary biologists who are focused on the most difficult branches on the Tree of Life should not understate the consensus elsewhere that has proven robust. Because it is natural for investigators to focus on areas of controversy and disagreement, uncertainty at one level (e.g. do metazoans share a single common ancestor?) has, on occasion, been inaccurately portrayed as an irreconcilable stumbling block for the entire phylogenetic enterprise. For example, Patterson (1987: p. 4) quoted a particularly pessimistic passage from Ernst Mayr's 1982 book *The Growth of Biological Thought* (p. 218): 'The futile attempts to establish the major phyla of animals induced at least one competent zoologist . . . [to call] common descent . . . a beautiful myth not established by any factual foundation. . . . Honesty compels us to admit that our ignorance

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concerning these relationships is still great, not to say overwhelming.' The zoologist to which Mayr is referring is Fleischmann (1901), hardly representative of biological thought from Mayr's perspective in 1982. Broadly speaking, this passage takes genuine uncertainty regarding pre-Cambrian divergences and overgeneralizes from this to common descent itself. On the contrary, given the agreement we now have about vertebrate interrelations (for example) such hyperbole is inaccurate and misleading for both students and the general public.

The scrutiny of protein sequences starting in the 1960s did indeed overturn several cherished ideas in systematics, such as an ancient human lineage to the exclusion of other great apes dating to the early Miocene (see Goodman *et al.* 1987; Andrews 1987). Zuckerkandl and Pauling's (1962: table 2) early application of the molecular clock to divergences within great apes yielded a surprisingly prescient result. For the differences they observed in human and gorilla alpha and beta haemoglobin molecules (one and two amino acid substitutions, respectively), they estimated a common ancestor between the two species to have existed approximately 11 million years ago. Such an interpretation is much closer to the view accepted today than to the previous theory that early Miocene apes, such as '*Ramapithecus*', shared ancestry with humans to the exclusion of other great apes (Simons 1972).

Interestingly, the calibration used for Zuckerkandl and Pauling's estimate was 'the common ancestor of man and horse [which] lived in the Cretaceous or possibly in the Jurassic period, say between 100 and 160 millions of years ago' (Zuckerkandl and Pauling 1962: p. 200). In hindsight, it is interesting to note that while some recent molecular clock studies support 'common ancestor of man and horse' (i.e. the divergence between Laurasiatheria and Euarchontoglires) just about 100 million years ago in the Cretaceous (Bininda-Emonds *et al.* 2007, but see Kitazoe *et al.* 2007, Hallström and Janke 2010, or Dos Reis *et al.* 2012), as a calibration this divergence has no palaeontological basis whatsoever (Wible *et al.* 2007; Benton *et al.* 2009). Nevertheless, it yielded a result for hominine divergence which is not far off from that accepted today based on our understanding of both the fossil record and a molecular clock (Lockwood 2007).

To some specialists of the mid/late twentieth century, even one with such a major influence and apparently broad perspective as Ernst Mayr, it seemed that after dethroning the idea of an independent human lineage dating to the early Miocene, all other such cherished ideas were soon to follow. They didn't. Again, and with the important qualification that many surprises have occurred within certain groups (e.g. mammals; see Asher *et al.* 2009), the genomic work of the last decade has confirmed the basic vertebrate topology first recognized in the nineteenth century, not scrambled it (Figure 1.1).

For mammals, at least, there remains today more uncertainty about divergence dates than topology among living clades. Bininda-Emonds *et al.* (this volume) have made substantial contributions towards understanding mammalian divergences. Building upon a supertree meta-analysis of mammals (Bininda-Emonds *et al.* 2007), one which has a far larger taxon sample of mammals than any previous study, they make the case that ordinal divergences within mammals substantially predate the Cretaceous–Tertiary (KT) boundary at 65 million years before present. Palaeontologists have known for some time that the earliest occurrences of undisputed crown-placental mammals do not predate the KT boundary (Kielan-Jaworowska 1978; Asher *et al.* 2005; Wible *et al.* 2009). Given the obvious presence of such clades as rodents and carnivorans in the early Palaeocene, it is reasonable to expect that at least some placental divergences predate the KT boundary. We know that first appearances in the fossil record are not synonymous with actual cladistic divergence (Benton *et al.* 2009). However, a missing record of crown placentals extending to the early Cretaceous, over 120 million years ago (Kumar and Hedges 1998), seems too much in conflict with our growing understanding of the Cretaceous fossil record (Foote *et al.* 1999; Reisz and Müller 2004; Hunter and Janis 2006), particularly given the limitations and pitfalls of the molecular clock (Graur and Martin 2004; Kitazoe *et al.* 2007). Bininda-Emonds *et al.* (this volume) offer perspective on this ongoing debate on the Mesozoic antiquity of crown-placental mammals.

Estimating divergence times from differences in molecular evolution is only one way of measuring rates of evolutionary change. Evolutionary rates can also be assessed in terms of morphological evolution, such as phenotypic or taxonomic change (Polly 2001). Simpson's (1944) classic work *Tempo and Mode in Evolution* indicates how palaeontologists have sought for many years to assess how evolutionary change can be measured and, ideally, quantified from a morphological perspective. The question remains, however, as to how morphological change through time can or should be measured, and if it is possible to use morphological data complementary to molecular methods for assessing rates of evolution. Larsson *et al.* (this volume) address this issue from a novel perspective and present a road map for using morphology not only as a tool to measure evolutionary rates through time, but also to estimate divergence times on a similar basis as molecular approaches.

Recent years have seen tremendous progress in the field of genomics as a result of major advances in DNA extraction and sequencing. At first glance this type of research appears unrelated to palaeobiology because of its strictly genetic nature and necessary focus on living forms. However, not only is it possible to reveal genomic data from extinct organisms (Paäbo 1989; Noonan *et al.* 2005), including some that are truly ancient (Organ *et al.* 2008), but in combination with methods from phylogenetic and comparative biology, fossilized structures can be used in conjunction with genomic data to gain insights into the biology of long extinct taxa (Organ *et al.* 2007; 2009). A review of this issue by Organ (this volume), demonstrates the great potential for 'palaeogenomics' in biology, and how it can reveal insights into both deep time and the recent past. He illustrates this potential with recent work on ancient genome size and sex chromosome reconstruction for species that have been extinct for tens of millions of years.

Estimating rates of speciation and extinction, and thus diversification, is a classical theme in macroevolution and for a long time could only be approached using fossil taxa (Raup 1978; Sepkoski *et al.* 1981). In recent years, major advances in bioinformatics and gene sequencing techniques enabled researchers to also use DNA-derived phylogenetic hypotheses and divergence estimates to calculate diversification rates (see Ricklefs *et al.* 2007). This novel approach did not make the palaeontological method obsolete, however, but instead stimulated and fostered new research on the underlying processes of diversification, resulting in a wealth of new studies from both neontology and palaeontology. The progress in this field also made it possible to reconsider a classical question of evolutionary theory: how does natural selection work, and on how many levels? Simpson and Müller (this volume) address the concept of species selection, an issue with a long tradition in palaeontology and often discussed as a possible evolutionary process. They describe how molecular phylogenies can be used to measure the extent and impact of species selection, and suggest that species selection is ubiquitous, providing deeper insights into the causes of diversification.

Mechanisms

All of the chapters in Patterson's 1987 book concerned the Tree of Life, methods for its reconstruction, and aspects of antiquity as inferred by the fossil record and molecular clocks. The focus of those authors was very much on phylogenetics, and virtually nothing was said regarding a field that is now of considerable importance to palaeontologists: evolutionary development. During the 1980s 'Evo-Devo' was of course a sophisticated discipline (Akam *et al.* 1988; Keynes and Stern 1988), but was generally the domain of those working on model organisms and beyond the practical remit of most palaeontologists.

This is quite different today, and the disciplinary scopes of palaeobiology and evolutionary development have become increasingly intertwined (e.g. Smith and Hall 1990; Hall 2002; Smith 2003; Donoghue and Purnell 2009; Sánchez-Villagra

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2010; Schmid and Sánchez-Villagra 2010; Takechi and Kuratani 2010). This coalescence is reflected in the majority of chapters in this book (summarized below) in which concepts of ontogeny and developmental genetics are applied to palaeontological questions, in stark contrast to the virtual absence of developmental subject matter in the chapters of Patterson (1987). While both Patterson's book and this one include too few chapters to comprise an infallible barometer of the course of palaeobiological thought over three decades, it is nevertheless tempting to view this difference as an indication of how the understanding of developmental genetics of model organisms has become a major springboard for hypothesis generation and testing in palaeobiology.

For example, Anthwal and Tucker (this volume) note the diversity of form among mammalian mandibles, in particular the variable presence and size of the condylar, coronoid and angular processes. Certain adult phenotypes such as the small, un-marsupial-like mandibular angle of the adult koala, are late occurrences during development (Sánchez-Villagra and Smith 1997); due to differential growth of other parts of the jaw during ontogeny, the mandibular angle may become more (or less) apparent. The coronoid process of the dentary is similarly variable; the mandibular condyle exhibits some variation across mammals but less than the angular and coronoid processes. Perhaps more importantly, specific phenotypes of the condylar, coronoid and angular processes observed in nature have also been observed within the phenotypic repertoire documented in past studies of transgenic mice and human genetic disorders. Anthwal and Tucker review these morphologies and note the complicated, but tractable, roles of several genetic loci among mouse knockout studies and human pathologies that are likely behind the variation observed in nature.

Buchholtz (this volume) discusses a long history of the study of the vertebrate axial skeleton, focusing on some of the mechanisms by which mammals in particular exhibit phenotypic diversity despite an extraordinary level of conservatism throughout the Order. Relative to other chordates, mammals show relatively little variation in vertebral counts, particularly among more cranial vertebral segments. The identification of skeletal modules, for example those patterned in somitic versus lateral plate mesoderm, has gained some support in the recent literature (Hautier *et al.* 2010). In the context of recent discoveries regarding the differential expression of *Hox* genes in specific mesodermal tissues (McIntyre *et al.* 2007), Buchholtz documents phenotypic modules across species and contributes to the understanding of the means by which mammals may deviate from a fundamentally conserved skeletal body plan.

One of the greatest enigmas in vertebrate evolution is the origin of turtles and, relatedly, the turtle shell (Rieppel 2009). Turtles are unique among chordates by possessing a shoulder girdle inside the ribcage. How this change

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occurred phylogenetically and developmentally has long been a perplexing question. Recent years have seen tremendous progress in both developmental studies on the evolution of the turtle shell (Nagashima *et al.* 2009) and the testudinate fossil record, for example the recent discovery of the Triassic turtle *Odontochelys semitestacea* (Li *et al.* 2008), a taxon with a complete plastron but rudimentary carapace. Kuratani and Nagashima (this volume) provide a thorough overview of the current state of the art and elegantly show how our current knowledge from the turtle fossil record can be combined with innovative studies from developmental biology, providing an explanatory framework for the origin and evolution of the turtle shell. According to Kuratani and Nagashima (this volume), *Odontochelys* resembles an early stage in modern turtle ontogeny, prior to the onset of carapace development, and it is the embryonic carapacial ridge that leads to the reversed positions of scapula and ribs through inward folding of the ventral body wall.

Palaeontology and developmental genetics have been successfully integrated in studies of tetrapod limb evolution, including the 'fin-limb transition', the increase or reduction of digits, and the overall loss of extremities (e.g. Shubin *et al.* 1997; Cohn and Tickle 1999; Shapiro *et al.* 2003). In many cases hypotheses generated from developmental genetics have been used to explain and understand the phenotypic diversity of limbs as revealed by the fossil record, and vice versa (Shubin *et al.* 2009). In the present volume, this area is tackled using two different examples, both focusing on the evolution of the autopodium. In the first one, Mitgutsch *et al.* (this volume) use the 'mole's thumb', a distinctive sesamoid bone found in talpid mammals, to address the long-standing issue of how to determine homology and understand morphological diversification. They emphasize how studies of developmental genetics require morphology to formulate appropriate research questions. In the second contribution, Richardson (this volume) reviews the recent advances in our understanding of the development of the bird wing, a hot topic in vertebrate palaeontology because of its relevance to the interrelationships between modern birds and Mesozoic theropod dinosaurs (e.g. Burke and Feduccia 1997; Wagner and Gauthier 1999; Larsson and Wagner 2002). Richardson shows that if only one single research discipline is considered, a proper understanding of the evolution of the avian wing is impossible, highlighting the need for integration.

Schmid (this volume) critically investigates the basis for inferring molecular mechanisms behind phenotype in long-extinct organisms. Applying basic principles of parsimony (simplicity is optimal), uniformitarianism (modern processes are relevant to past events) and actualism (the past is a manifestation of our own world), Schmid outlines possible genetic mechanisms behind several aspects of the peculiar phenotype of the Triassic actinopterygian fish

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Saurichthys, including body regionalization, fin position, jaw elongation, and scale phenotype. Specific mechanisms concerning somitogenesis and the expression of (for example) retinoic acid, *FGF*, *Eda*, and paralogues of *Hox* and *Tbx* are known to influence these phenotypes in modern organisms such as the zebrafish, cichlid fish, chicken and mouse. Close correspondence not only in detailed phenotype of the axial skeleton, fins, jaws and scales, but also correlations among these hard tissues, make a compelling case that the genetic basis of its phenotype is to a signficant extent tractable in a 240-million-year-old vertebrate.

Linking phenotype and genotype is also a focus of Sears *et al.* (this volume), who synthesize a large body of data concerning the embryology and genetics of marsupial forelimb development. They start with the observation that forelimb and hind limb development in marsupials, unlike that observed in other mammalian groups, is relatively uncoupled. At the time of birth, marsupials such as the short-tailed opossum (*Monodelphis domestica*) have no hind limb to speak of, but have just enough of a forelimb and rostrum to enable them to locate and climb from the reproductive tract to the mother's teat. Here, outside of the uterus, the majority of their embryonic and foetal growth occurs. Sears *et al.* (this volume) apply a microarray assay to survey how gene expression is different in forelimb and hind limb, and between mouse and *Monodelphis*. Both comparisons yield a number of interesting differences. Sears *et al.* (this volume) interpret their findings as supportive of a breakdown in the genetic modularity between forelimb and hind limb early in the course of marsupial evolution, enabling the high level of early specialization of the marsupial forelimb given its peculiar reproductive mode. This is potentially why we do not see, either in the fossil record or among living species, marsupials with divergent limb morphology such as hooves, flippers or wings.

Teeth are among the most common and important remains represented in the fossil record. Smith and Johanson (this volume) comprehensively summarize the debate regarding the origin of teeth in vertebrates. Embryologists and comparative anatomists have recognized for some time the common development that teeth share with other ectodermal structures, such as scales and hair. Yet there are important differences. For example, Smith and Johanson note that tooth addition in fossil taxa such as arthrodiran fish (Placodermi) occurs at the end of pre-existing dental rows, and is consistent with the tooth addition pattern observed in contemporary teleosts and lungfish. The capacity for continuous replacement of teeth differs from dermal structures such as scales. They argue that a co-option of genetic networks responsible for purely dermal structures is unlikely to be the primary mechanism by which teeth evolved among vertebrates. Rather, genetic networks regulating pattern of tooth

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