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Deep homology and the origins of evolutionary novelty

Neil Shubin[1,2], Cliff Tabin[3] & Sean Carroll[4]

Do new anatomical structures arise *de novo*, or do they evolve from pre-existing structures? Advances in developmental genetics, palaeontology and evolutionary developmental biology have recently shed light on the origins of some of the structures that most intrigued Charles Darwin, including animal eyes, tetrapod limbs and giant beetle horns. In each case, structures arose by the modification of pre-existing genetic regulatory circuits established in early metazoans. The deep homology of generative processes and cell-type specification mechanisms in animal development has provided the foundation for the independent evolution of a great variety of structures.

It is not possible to identify what is new in evolution without understanding the old. This is a reflection of the way evolution works, with some novelties being traceable as modifications of primitive conditions and others having origins that are much less obvious. As a result, the problems of novelty and homology have been deeply intertwined for the past century and a half. It is here, at the interface between these two great concepts of evolutionary biology, where fresh data from developmental biology have had an extraordinary impact. One of the most important, and entirely unanticipated, insights of the past 15 years was the recognition of an ancient similarity of patterning mechanisms in diverse organisms, often among structures not thought to be homologous on morphological or phylogenetic grounds. In 1997, prompted by the remarkable extent of similarities in genetic regulation between organs as different as fly wings and tetrapod limbs, we suggested the term 'deep homology'[1] to describe the sharing of the genetic regulatory apparatus that is used to build morphologically and phylogenetically disparate animal features[1,2].

Homology, as classically defined, refers to a historical continuity in which morphological features in related species are similar in pattern or form because they evolved from a corresponding structure in a common ancestor. Deep homology also implies a historical continuity, but in this case the continuity may not be so evident in particular morphologies; it lies in the complex regulatory circuitry inherited from a common ancestor. In some instances, recognition of deep homologies can help in the identification of cryptic classical homologies, when morphological data alone are inadequate to make the case for homology. For example, the photoreceptors present in various extant clades would not be recognized as homologous without the observation of common underlying genetic cassettes (discussed in the next section).

Deep homology, however, can also be found in contexts in which structures are not homologous in the classical sense. As we explored in 1997, appendages in vertebrates, arthropods and other bilaterians evolved independently, but their derivation was dependent on regulatory networks present in a common 'urbilaterian' ancestor. Most strikingly, the genetic regulatory cascade comprising a key transcription factor and downstream effector genes eliciting outgrowth (such as the *Drosophila melanogaster* gene *Distal-less* or its mouse homologue *Dlx*) seems to have been present in such a common ancestor and has been repeatedly used to control outgrowth formation in the protostome and deuterostome lineages[3]. Moreover, a series of deep homologies exist in the genetic systems used to pattern the appendages of vertebrates and arthropods, many of which have come to light since our original paper was written (for example, proximal-appendage specification by *homothorax* in *D. melanogaster* or its homologue *Meis1* in mice[4,5]). The similarities are much more than the use of a common genetic tool kit of genes: they involve the use of genes and regulatory circuits that have previously evolved complex roles in an ancestral organism.

Deep homology is important for the generation of novelties because ancient regulatory circuits provide a substrate from which novel structures can develop. In this Review, we explore three of Charles Darwin's exemplars of evolution: animal eyes, tetrapod limbs and the giant horns of beetles. New data from studies of these features are offering surprising twists on classic examples of evolution. And, together, these examples illustrate how deep homology enables researchers to understand the generation of novelty in cases in which fossils are not informative; to make predictions about morphological transformation that can be tested by experimental and expeditionary work; and to see the extent to which common genetic mechanisms are used to generate diverse adaptations and can lead to the parallel evolution of novelties.

What eyes tell us

Darwin noted in *On the Origin of Species*[6] that "Amongst existing Vertebrata, we find but a small amount of gradation in the structure of the eye, and from fossil species we can learn nothing on this head. In this great class we should probably have to descend far beneath the lowest known fossiliferous stratum to discover the earlier stages, by which the eye has been perfected."

Unless an animal lives in complete darkness, there are tremendous advantages to being able to sense light and have high-acuity vision. Accordingly, complex eyes are present in a wide variety of taxa, from jellyfish and molluscs to insects and vertebrates. But how and when did they develop?

The eyes in widely divergent organisms are of such different structures, and develop in such distinct embryological contexts, that biologists

[1]Department of Organismal Biology and Anatomy, University of Chicago, 1027 East 57th Street, Chicago, Illinois 60637, USA. [2]The Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, Illinois 60605, USA. [3]Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, NRB 360k, Boston, Massachusetts 02115, USA. [4]Howard Hughes Medical Institute, University of Wisconsin-Madison, 1525 Linden Drive, Madison, Wisconsin 53706, USA.

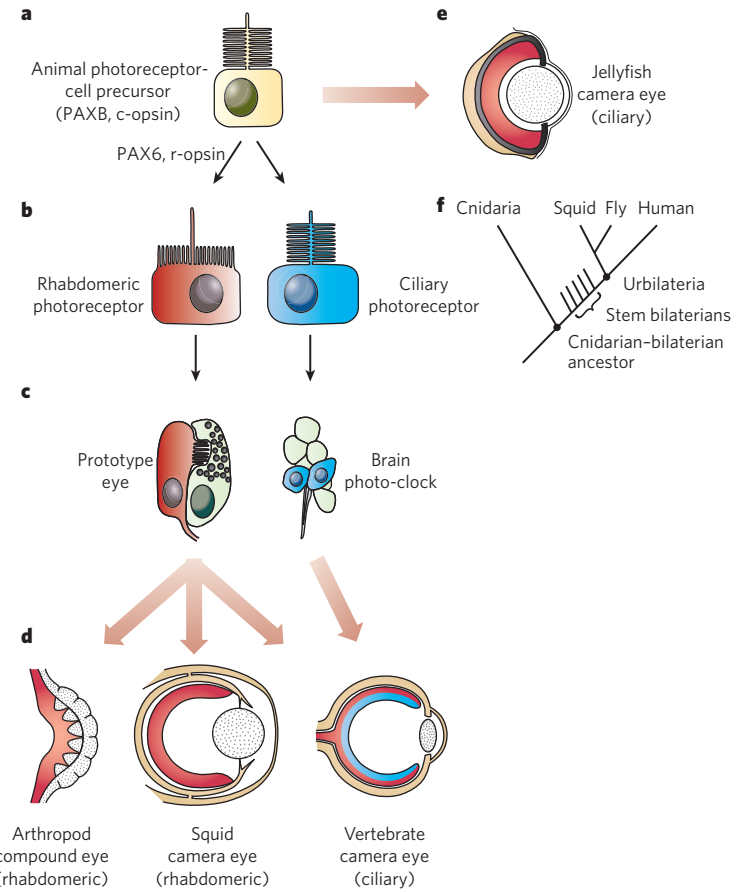

Figure 1 | Deep homology of eye development and the parallel evolution of animal eyes. A route for the evolution of photoreceptor cell types and different forms of eyes. **a**, The cnidarian–bilaterian ancestor had photoreceptors that expressed c-opsin and PAXB. **b**, Rhabdomeric photoreceptors, r-opsins and PAX6 evolved in ancestral-stem bilaterians, after the split between the cnidarian and bilaterian lineages. **c**, The last common ancestor of all bilaterians (Urbilateria) probably had two types of light-sensing organ: a prototypical eye and a brain photo-clock, which are both found in the annelid *Platynereis dumerilii*. **d**, The photoreceptor types established in the Urbilateria were then incorporated in different ways in the parallel evolution of different eyes in various phyla. Rhabdomeric photoreceptors were the foundation for the evolution of compound and camera-type eyes in arthropods and molluscs, respectively. Both types of photoreceptor were incorporated into the vertebrate eye, with ciliary receptors carrying out phototransduction and rhabdomeric receptors being transformed into ganglion cells and functioning in image processing. Pigment cells are not shown. **e**, The ciliary camera-type eyes of box jellyfish are also proposed to have evolved in parallel in the cnidarian lineage. **f**, Cladogram depicting the evolutionary relationships of the taxa shown in **a**–**e**. (Panels **a**–**e** courtesy of L. Olds (University of Wisconsin, Madison); panel **f** courtesy of K. Monoyios (University of Chicago, Illinois).)

have historically inferred that eyes had evolved independently dozens of times[7]. But deciding where homology ends and novelty begins is not always straightforward, particularly for structures that lack a fossil record, such as eyes. Over the past 15 years, many insights into the evolution of eyes have come from descending beneath the visible diversity of animal eyes into the genetic machinery that controls their development.

The unexpected finding that the homologous transcription factors Eyeless and PAX6 have crucial roles in the formation of the eyes of *D. melanogaster* and vertebrates was the first indication that the markedly different eyes of long-diverged phyla had more in common than was previously thought[4]. This discovery spurred comparisons of the detailed genetic circuitry underlying eye formation in diverse animals. It is now known that a small set of transcription factors, including those encoded by members of the *eyeless, atonal* and *eyes absent* gene families in *D. melanogaster* and their homologues in vertebrates, are widely used in the specification and formation of various types of animal eye (see refs 8 and 9 for reviews). Moreover, in all light-sensing organs in animals, the ability to detect light depends on a cascade involving opsin proteins. This observation led to the view that all modern variations of light sensing in bilaterians can be traced to the existence of photosensitive cells in a common ancestor with PAX6 and other transcription factors at the top of a genetic regulatory pathway leading to opsin production. This is a textbook example of deep homology[1,2]: morphologically disparate organs whose formation (and evolution) depends on homologous genetic regulatory circuits.

The deep homology of regulatory mechanisms suggests a number of different hypotheses for evolutionary transformation[10,11]. Are all eyes homologous in the classical sense of being diverse forms of the same structure? Have different eyes instead evolved entirely independently and just happened to use similar genetic components? Or have different eyes evolved in parallel as elaborations of structures and genetic regulatory mechanisms present in common ancestors? Weighing up these possibilities requires a deeper examination of eye development and a broader survey of taxa. It turns out that far more striking developmental similarities exist between diverse taxa than would ever have been expected. However, there are also many cases in which, on close inspection of the details, the similarities break down.

One well-established argument for the independent evolution of eyes in arthropods, squid and vertebrates concerned their photoreceptor composition and light-transduction mechanisms. All animal eyes are composed of photoreceptors located adjacent to cells that produce light-shielding dark pigment. The photoreceptors of different taxa are of two main types, rhabdomeric and ciliary (Fig. 1b), which have distinct phototransduction signalling cascades. The eyes of insects and other invertebrates rely on rhabdomeric photoreceptors and a phospholipase-C-based cascade, whereas vertebrates use ciliary photoreceptors and a phosphodiesterase-based cascade. How can the fact that similar camera-type eyes of vertebrates and squids rely on different kinds of photoreceptor and transduction process be explained, other than by asserting completely separate origins?

It is clear that these photoreceptors were not separate inventions in each respective lineage. In polychaete annelids, both photoreceptor types are found, with the ciliary-type opsin (c-opsin) expressed in the brain and rhabdomeric opsin (r-opsin) expressed in the eyes of the same animal[12]. The best explanation for the phylogenetic distribution of photoreceptor types is that both cell types coexisted in the common bilaterian ancestor of vertebrates and invertebrates (Fig. 1c), and each lineage then used different cell types for light detection in their visual systems. Both types of photoreceptor seem to have been incorporated into the evolving vertebrate eye, with the rhabdomeric photoreceptor cells being transformed into ganglion cells and having a key role in image processing[12] (Fig. 1d). So one fundamental element of the deep homology of eye development lies in the specification of cell types.

The deep homologies are not limited to the photoreceptors. The target interneurons specifically involved in processing the visual signal in the vertebrate retina and the *D. melanogaster* optic lobe are not only functionally analogous but are specified by homologous transcription factors (CHX10 and VSX2). Moreover, both cell types project to visual centres of the brain, and both sequentially express genes encoding the

same two transcription factors: first, MATH5 or its homologue Atonal, and then BRN3B or ACJ6 (in mice and *D. melanogaster*, respectively)[13]. These findings imply that these different bilaterian eyes are related, not just in the homology of cell types but in the deep homology of cellular circuitry for interpreting visual input, which predates the independent evolution of the modern eyes in the two lineages.

How deep do the cell-type homologies extend in the phylogenetic tree of metazoans? The cnidarians, the likely sister group to the bilaterians, include the box jellyfish, which has a camera-type eye with a cornea, a lens and a retina. These animals not only have retinas with c-opsin, typical of vertebrate-type eyes[14,15], but also express the full set of orthologous genes used in vertebrate phototransduction[16]. Furthermore, the pigment function is combined in jellyfish photoreceptors that use the same melanogenic pathway seen in vertebrates, involving, for example, *mitf* and a homologue of *Oca2* (ref. 16).

The cnidarian photoreceptors reveal that ciliary photoreceptors, c-opsin and the phosphodiesterase signal-transduction cascade predate the divergence between cnidarians and bilaterians and, because r-opsins have not been detected in cnidarians, it is probable that r-opsins are a bilaterian innovation[17,18]. The anatomical resemblances between cnidarian and vertebrate camera-type eyes perhaps reflect conservation of a camera-type eye 'program' and consequent parallel evolution of eyes in the two phyla (Fig. 1a). If a ciliary-type camera-style eye was assembled in a cnidarian–bilaterian ancestor, it must have been lost in other bilaterians except for a vertebrate ancestor. Given the advantages of vision, it is difficult to imagine why such structure would be lost before being reinvented using rhabdomeric photoreceptors.

Taken together, the available data suggest that the eyes of jellyfish, squid, arthropods and vertebrates are not the product of rampant convergent evolution, as once thought, but of parallel evolution that is based on a shared history of generative mechanisms and cell types — deep homologies — established early in animal evolution.

Tetrapod limbs and fish fins

In *On the Origin of Species*, Darwin wrote of the similarities of tetrapod limbs: "What can be more curious than that the hand of a man, formed for grasping, that of a mole for digging, the leg of the horse, the paddle of the porpoise, and the wing of the bat, should all be constructed on the same pattern, and should include similar bones, in the same relative positions?"

The origin of limbs in the Devonian period allowed the invasion of land and the later evolution of vertebrates that could fly, dig, run, hop and climb. Consequently, tetrapod limbs are classic examples of evolutionary novelties[19,20]. But they are also a prime example of homology: all tetrapod limbs have similar bone morphology and development, and this can be traced back to the limbs of Devonian vertebrates. As was clear to Darwin, this homology is immediately apparent in the detailed similarities of morphology and development of the bones in the limbs of all tetrapods. More controversial, however, have been attempts to compare these bones with those in the paired fins of fish. It is here where deep genetic homologies and the discovery of fossils conspire to offer fresh insights.

The most striking differences between limbs and fins lie in the distal region of the appendage, as the pattern of the proximal bones is essentially identical among lobe-finned fish and tetrapods[19,20]. Much of the surface area of fins is supported by dermal rays — bones that are completely absent in limbs. In place of these rays, limbs have a set of endochondral elements — the digits and wrist or ankle bones — that look unlike, and function differently from, fin radials. Importantly, these bones contain a series of characteristic joints that allow flexion and extension, particularly in those that enable a 'palm' area to lie flush with the ground[21]. Tetrapods, then, have traded a dermal skeleton in the distal appendage for a complex endochondral one, and their evolved joints and bones allow the distal appendage to support the weight of the body.

Genetic discoveries in the 1990s reinforced a classical view that digits, wrists and ankles have no direct correlate in fins[1,22–25]. Developmental studies of *Hoxd9*, *Hoxd10*, *Hoxd11*, *Hoxd12* and *Hoxd13* gene expression in tetrapod limbs revealed a discrete phase of expression directly associated with digital specification[22,23]. There are four important features of this late-phase expression: first, expression occurs in a distal segment of the limb, the 'paddle', that does not overlap with more proximal zones; second, this expression occurs while the digits and mesopodial bones are being specified; third, the domains of expression of the 5′ *Hoxd* genes display 'reverse colinearity', such that *Hoxd13* expression (for example) extends more anteriorly than the expression domains of more 3′ genes; and fourth, late-phase expression is regulated independently from early-phase expression by a separate enhancer that drives distal expression[26]. This distinct expression pattern of the *Hoxd* genes has been referred to as 'phase 2 expression'. Phase 2 expression, with all the characteristics described above, was unknown in fish fins in the late 1990s, as the zebrafish (*Danio rerio*) was reported to have only a single phase of expression[23]. The fossil record also seemed to support this conclusion, as the sister group to limbed vertebrates (panderichthyids) seemed to lack any bones comparable to digits[1,19,20]. Data from both types of study supported the idea that the origin of novelty at the morphological and functional levels would have happened in parallel to that at the genetic level.

Just as for eyes, comparative data refined the hypothesis of novelty (Fig. 2). Work on a variety of non-model vertebrates has revealed that a late phase of *Hoxd* expression exists in the distal fin bud. Indeed, it is now known to be a general feature of gnathostomes, having been discovered in basal actinopterygians[27], lungfish[28], zebrafish[29] and a chondrichthyan[30]. Although the details of late-phase patterns of expression vary between these taxa, some but not all aspects of the tetrapod phase 2 *Hox* pattern are present in fins. The most notable difference is that late-phase expression in the fins of osteichthyans (paddlefish, zebrafish and lungfish) spatially overlaps with earlier phases of expression, whereas in tetrapods the phases of expression are segregated proximodistally such that phase 2, but not phase 1, expression is found in the autopod. Despite these differences, basal actinopterygians and lungfish have broad zones of expression that, like tetrapods, exhibit reverse colinearity.

The key question is whether these late phases of expression in tetrapod limbs and fish fins reflect the same process. There is, as yet, no evidence of independent regulation of the form described in tetrapods. Lacking this evidence, there are two tenable hypotheses: either fish have phase 2 expression homologous to that which specifies the digits in tetrapods, or the late-phase expression observed in fins is a temporal and spatial extension of a highly conserved early phase with unique dynamic properties in the fish. The two cases suggest different molecular scenarios for the origin of the autopod. If fish have true phase 2 *Hox* gene expression, the main difference between tetrapod limbs and fish fins lies not in the origin of late-phase *Hox* gene expression but in changes in the timing of this expression and/or in changes in genes acting downstream of the *Hox* genes that must have shaped the tetrapod form. Alternatively, if fish lack true phase 2 *Hox* gene expression, the evolution of the phase 2 regulatory module would be a unique and perhaps defining autopod invention of the tetrapod lineage. In either case, the evolutionary process reveals the impact of deep homology: different kinds of appendage arose by modifications to an ancient and conserved developmental system. Even if the morphological structures (fin rays and digits) are not homologous, there is deeper homology in the network of *Hox* genes and their targets within the limb or fin field, as well as in phase 2 regulation — if indeed it predated the evolution of the autopod.

From experiments to expeditions

If components of the genetic machinery that builds the skeleton of limbs were present in fins, the closest fossil relatives of Devonian tetrapods may indeed have had equivalents of wrist and ankle bones or digits. In developmental genetics, the database is expanded by carrying out experiments on different taxa. In palaeontology, knowledge is expanded by discovering new fossil forms. The origin of limbs is a problem that is ripe for analysis. When cladograms of basal limbed vertebrates and their finned relatives are placed on a stratigraphic column, it becomes apparent that new information on the fin-to-limb transformation is most likely to come from expeditionary work in the

Frasnian and Givetian stages (385 million to 375 million years ago) of the Devonian. For more than 50 years, it has been known that some lobe-finned fish have homologues of two wrist bones, the intermedium and ulnare. But what was not known until recently is what the endoskeleton distal to these bones looked like in the closest relatives of tetrapods and how it functioned.

Targeting the Frasnian rocks of Arctic Canada for fossil vertebrates ultimately led to the discovery of *Tiktaalik roseae,* the closest finned sarcopterygian to limbed forms[21,31]. *T. roseae* is highly informative because most of the skeleton is exceptionally well preserved. In addition, because its appendages are known from multiple specimens, pectoral fins have been prepared both in articulation and as isolated elements preserved in three dimensions, with bony articular surfaces preserved in several specimens. *T. roseae* retains a dermal skeleton, with large unjointed dermal rays, the lepidotrichia and a scaly cover for the humerus. However, like tetrapods, the lepidotrichia are reduced, and the distal endoskeleton is expanded, having several transverse joints at both the level of the proximal carpus and the distal carpus of tetrapods. What is most interesting is the parallel between the loss of the lepidotrichia and the enhanced mobility of the fin joints; the greatest reduction of the fin rays occurs proximally in the regions over the elbow and presumptive wrist joints, not distally as previously supposed. The origin of joints capable of extensive flexion and extension involved not only the origin of facets on the endochondral bones but also the loss of the rays that would have covered them. The phylogenetic position of *T. roseae* reinforced the idea that the reconstruction of the pectoral fin of *Panderichthys rhombolepis* lacking radials was incorrect; indeed, recent analyses have shown that it has a complement of radials that articulate with the ulnare and intermedium[31].

Fossil and genetic data lead to the following picture of the sequence of changes associated with the origin of limbs. The diverse range of fin skeletons seen in fish is generated by a conserved set of genetic tools that are common to all fish that have paired appendages, in particular the early phases of *Hox* gene expression. The adaptive radiation of fish in Devonian aquatic ecosystems, including both near-shore habitats and shallow freshwater streams, was composed of a variety of appendage designs (Fig. 2). One lineage in this radiation acquired fins with an appendage composed of humerus, radius, ulna, ulnare and intermedium and distal bones. One of these lineages, exemplified by *T. roseae*, reduced the dermal rays and expanded the distal joints during the evolution of appendage-based support for the body. Complete loss of the dermal skeleton is associated with a true digital array first seen in Devonian taxa such as *Acanthostega gunnari*. The open question is the extent to which these last steps may be associated with changes to the enhancers that drive phase 2 *Hox* expression in limbs.

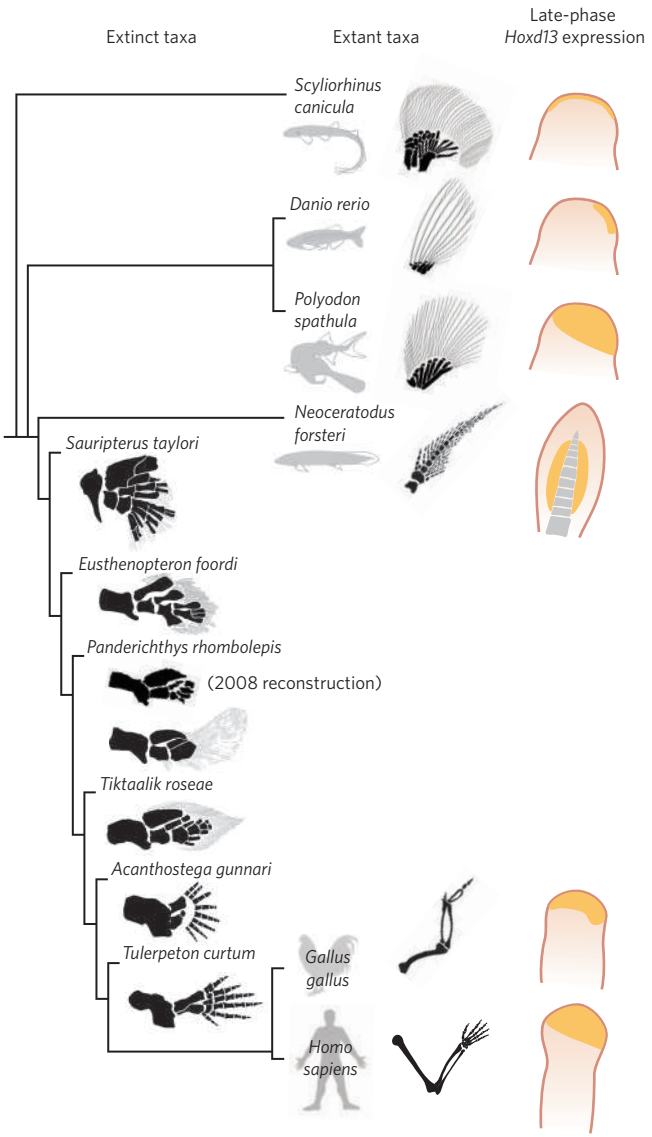

Figure 2 | Deep homology of late-phase *Hox* expression. A phylogenetic tree of gnathostomes, showing the pectoral fins of relevant extant and fossil taxa with known examples of late-phase expression of *Hoxd13* (yellow). Late-phase expression is a primitive feature to sarcopterygians, but its details differ among finned taxa. Basal actinopterygians and lungfish have broad expression domains that resemble the autopodial expression in tetrapods. (Fin illustrations courtesy of K. Monoyios.)

Deep homology and the origin of fins

These advances in understanding the assembly of the tetrapod limb push the issue of limb origins further back in time. How did fins, the foundation for the tetrapod limb, originate? Here, too, deep homology allows predictions to be tested. Both arthropod appendages and tetrapod limbs develop as outgrowths of the body wall that are patterned along three axes — the proximodistal, anteroposterior and dorsoventral axes — often using homologous genes to establish the ordinate axes. If the extraordinary similarities between arthropod and vertebrate appendages reflect the homology of patterning mechanisms, those mechanisms should be present in basal deuterostomes. But limbs are not present in the taxa intermediate between arthropods and vertebrates in deuterostome phylogeny. The clear prediction is that continuity should be found in the outgrowth-patterning mechanisms in arthropods and vertebrates in some other vertebrate structure that predates the origin of fins. Branchial arches and nervous systems are likely candidates, as both are patterned along three major axes, with the former developing as small outgrowths in which many components of limb-patterning circuits are deployed. If this scenario is true, then fins would have arisen by the co-option and ectopic deployment of outgrowth-promoting circuits at novel anatomical sites.

The ideal way to explore this hypothesis would be to examine the state of the limb-development program in finned taxa and in closely related primitively finless taxa. Such comparisons are not possible, however, as the latter are long extinct. But there is now an emerging model within a group of insects that offers the opportunity to reconstruct the more recent origin of a novel outgrowth that seems to have arisen by the co-option of a limb-outgrowth program.

The origin of beetle horns

The scarab beetles (superfamily Scarabaeoidea), which encompass stag beetles (Lucanidae), dung beetles (Scarabaeinae), rhinoceros beetles (Dynastinae) and several other families, include thousands of species with horns of different sizes and shapes that extend from dorsal segments of the head or from the pronotum. In some species, the horns are larger than any of the other appendages, such as the antennae, mouthparts and legs, and can account for up to 30% of the body weight. These extravagant beetles have attracted collectors for centuries, notably Darwin and Alfred Russel Wallace, who each brought new species back from their travels.

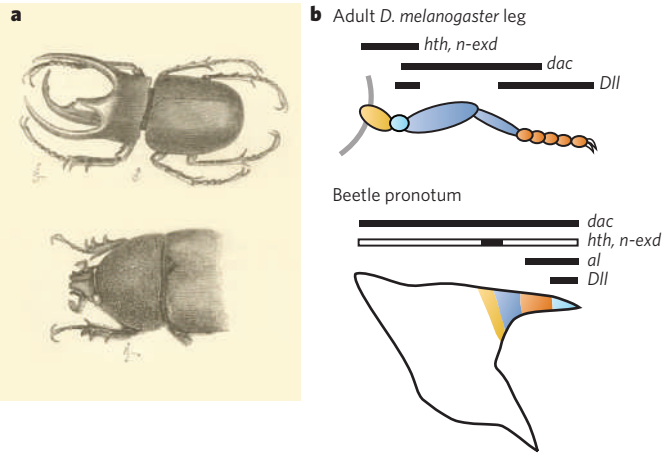

Figure 3 | The evolution of beetle horns by co-option of a limb-outgrowth program. a, Illustration from Darwin's *The Descent of Man* showing the male (top) and female (bottom) forms of the Atlas beetle, *Chalcosoma atlas*. Note the large horns extending from the head. **b**, Similarities in the expression of limb proximodistal axis-patterning gene expression in the adult leg of *Drosophila melanogaster* (top) and the developing horns of *Onthophagus* dung beetles (bottom). Coloured regions denote segments of the leg and regions of the developing horn. Black bars denote gene-expression patterns, indicating the relationship between the expression domains of individual genes in the developing outgrowths. The white bar shows where the listed proteins are co-expressed at only low levels. The expression of outgrowth-promoting genes in beetle horns indicates that these structures evolved by the co-option of an ancient outgrowth program and its deployment at novel anatomical sites. (Panel **a** reproduced from ref. 32; panel **b** modified, with permission, from ref. 35.)

The aptly named Atlas beetle (Fig. 3a) prompted Darwin to remark in *The Descent of Man*[32]: "if we could imagine a male … with its polished bronze coat of mail, and its vast complex horns, magnified to the size of a horse, or even of a dog, it would be one of the most imposing animals in the world."

The Atlas beetle and other extreme species featured prominently in Darwin's conception of sexual selection. Horn formation is typically sexually dimorphic, with males producing large horns and females producing much smaller horns or none at all. Among males of a given species, horn size often varies considerably relative to body size such that small males may not produce horns at all. In Darwin's time, no one had seen horns being used in male combat, so he concluded that they were ornaments used to attract females. But beetle horns are weapons, and there is now extensive evidence that beetles with longer horns prevail in combat with rival males and have a fitness advantage[33].

Co-option of a limb program

Horns are tubular, cuticular projections from the body wall, rather like insect appendages such as antennae, legs and mouthparts. However, unlike typical appendages, horns are unjointed, lack muscles and nerves, and arise from parts of the body where insects do not generally develop outgrowths, so they lack obvious homology to appendages. Most beetle species are hornless, including the vast majority of scarab species; therefore, horns are clearly evolutionary novelties. So how did they arise? The key to answering this question is understanding the developmental basis of horn formation.

Recent studies of horn development in the dung beetle *Onthophagus* have shown that they form from compact discs of epidermal cells that proliferate during the late larval period and then evert to their full length during the pupal moult, just as typical body appendages do[30]. Most strikingly, developing horns express a suite of genes that subdivide the proximodistal axis of typical insect limbs, including expression of the *Distal-less* (*Dll*) gene in the distal tip of the developing horn and expression of the *homothorax* (*hth*) gene and the nuclear form of the Extradenticle protein (encoded by *exd*) in the most proximal base of the developing horn[34–36] (Fig. 3b). These results suggest that beetle horns are the product of the co-option and deployment of an outgrowth program at novel anatomical sites.

An inordinate fondness for beetle horns?

The widespread distribution and great diversity of horns has prompted many naturalists to consider the evolution of horns in scarabs[37]. This raises the question of whether horns have arisen independently numerous times and, if so, whether this has occurred by the same mechanism. Although analyses of the distribution of horns in adults have suggested numerous independent gains, even within a single genus, recent studies of development in larvae have shown that the potential to make horns is widespread, even among hornless species. Armin Moczek and colleagues have shown that although certain members of the dung beetle genus *Onthophagus* develop pronotal horns during the late larval period and retain them to adulthood[38], many other species transiently develop horns as larvae and then resorb them in the pupal period. Furthermore, these authors uncovered evidence that the larval horns have an important role in the splitting of the head capsule during the larval-to-pupal moult.

These findings explain why the capacity to make adult horns has been widely maintained and could therefore account for the multiple independent gains (and losses) of adult horns in this genus. Furthermore, because the adult pronotal horns are clearly derived from larval horns, the findings suggest that adult *Onthophagus* horns did not evolve from scratch for male combat but are exaptations (structures that first originated to serve another, unrelated, function).

Horn development has been studied less in other clades, so it is not clear how often horns have arisen. However, it has been suggested that their presence at distinct anatomical sites on the head and the pronotum, which are each associated with deployment of proximodistal axis-patterning genes[36], results from at least two independent co-options of an outgrowth-promoting program[39].

Deep homology and parallel evolution

Darwin closed *The Descent of Man*, his second greatest work, with the line: "Man still bears in his bodily frame the indelible stamp of his lowly origin." These words have never rung more true. Researchers are now starting to appreciate the presence of far more indelible stamps of humanity's lowly metazoan origins than Darwin could ever have imagined. The detection of deep homologies offers more than new glimpses of evolutionary history, however. Such homologies provide a profound insight into the evolutionary process. Studies of deep homology are showing that new structures need not arise from scratch, genetically speaking, but can evolve by deploying regulatory circuits that were first established in early animals. But herein lies a challenge for the next generation of biologists: if the mechanisms behind the formation of diverse organs are ancient and highly conserved, then parallel evolution must be considered a fact of life in the phylogenetic history of animals[2].

With the growth of developmental genetics, it is possible to see beyond the view of homologies working at the level of whole organs. The mechanisms that define the ordinate axes of structures, the genetic circuits that pattern them, and the cell types with which organs are formed can be considered. The more that researchers look, the more they will find that the same tools have been used to build a great variety of structures long thought to have independent histories[40–42]. Discerning what has been conserved and what is novel in the origins of organs and body plans will be possible only with more comparative data, experiments on non-model animals, and targeted fossil discoveries from crucial nodes in the tree of life. ■

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