

# The origin and evolution of appendages

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**ABSTRACT** Current awareness of gene expression patterns and developmental mechanisms involved in the outgrowth and patterning of animal appendages contributes to our understanding of the origin and evolution of these body parts. Nevertheless, this vision needs to be complemented by a new adequate comparative framework, in the context of a factorial notion of homology. It may even be profitable to categorize as appendages also gut diverticula, body ingrowths and 'virtual appendages' such as the eye spots on butterfly wings. Another unwarranted framework is the Cartesian co-ordinate system onto which the appendages are currently described and where it is supposed that one patterning system exists for each separate Cartesian axis. It may be justified, instead, to look for correspondences between the appendages and the main body axis of the same animal, as the latter might be the source of the growth and patterning mechanisms which gave rise to the former. This hypothesis of axis paramorphisms is contrasted with the current hypothesis of gene co-option. Recapitulationism is a common fault in current Evo-Devo perspectives concerning the origin of the appendages, in that the evolutionary origin of appendages is often expected to be the same as one of the key mechanisms involved in the ontogenetic inception of appendage formation. This unwarranted perspective is also evident in the current debate on the nature of the default arthropod appendage. Most likely, a default arthropod appendage never did exist, as the first appendages probably developed along the trunk of an animal already patterned extensively along the antero-posterior body axis.

**KEY WORDS:** *homology, axis paramorphism, default appendages, recapitulationism, gene co-option*

## Evolutionary developmental morphology

To a very large extent, the categories we use to describe animal structure in the context of evolutionary developmental biology are still those of comparative morphology and embryology, but this is more an effect of conceptual and lexical inertia than the product of deliberate and firmly grounded choice.

Appendage, for example, is a concept of morphology, defined in terms of topography, and possibly also of function. Function, however, is basically extraneous to evo-devo biology, and topography is only relevant in so far as position is determined by the developmental mechanism of which we want to investigate the nature, origin and evolution.

Position, in any case, is not a specific attribute of appendages. What is specific to them, in an evo-devo perspective, is the fact that appendages are new body axes – that is, additional or secondary to the main body axis – with a more or less conspicuous proximo-distal patterning.

But even in this respect appendages are far from unique. For example, Podlasek *et al.* (2002) have convincingly argued that the genito-urinary tract of vertebrates can be considered a

developmental axis comparable to that of a limb. In particular, studies of the expression of genes such as *Hoxd-13*, *Hoxa-13*, *Hoxa-10*, *Shh*, *Bmp-4* have shown that this axis undergoes segmental patterning followed by region-specific morphogenetic growth in a manner similar to what we observe in the limb.

Increasing knowledge of genetically controlled developmental mechanics will inevitably require a new categorization of body features such as to allow the identification of more sensible units of which to investigate origin and evolution. Present knowledge is perhaps too incomplete as to serve as the basis for a new full-fledged evolutionary developmental morphology. It may be profitable, however, to start looking at the available data from less conventional points of view. Animal appendages may offer good opportunities for such an exercise.

## A bestiary of appendages

Examples of appendages are the oral tentacles of hydra and sea anemones, as are echinoderm tube feet, or the retractile stalks supporting the eyes of most terrestrial slugs and snails. But virtually all research on appendages carried out so far from

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the viewpoint of developmental biology deals only with the paired appendages of vertebrates and arthropods. This is also basically true for developmental genetics, despite the precious comparative insights we have got from a limited exploration of other kinds of appendages, such as the tube feet of the echinoderms, or the parapodia of polychaete annelids. The limits of present knowledge about the developmental origin of most kinds of appendages has important consequences on our ability to reconstruct the origin of animal appendages from an evolutionary point of view. Efforts in this direction are in fact all too often limited to extrapolations from a straight comparison of two systems only, the arthropod and the vertebrate, out of the context of a more articulated comparative framework.

In this paper I will not attempt to review the bulky, and precious, recent literature on the developmental genetics of the few model systems investigated thus far. I will outline, instead, a few points I regard as critical for an understanding of the origin of appendages, in both developmental and evolutionary terms.

I will start with a few remarks on the diversity of metazoan body appendages. I will then discuss a few questions of ontogeny; in particular, how appendages are developmentally related to the main body axis, how the different germ layers contribute to the production of the appendages, and whether the notion of gene co-option may be relevant to an understanding of the origin of appendages. The latter point will lead us straight to the evolutionary aspects of our enquiry. Here, two main points will be discussed: whether we can legitimately imagine that the common ancestor of all triploblastic animals, the Urbilateria, was already provided with appendages from which those of all modern animals should have been derived, and whether we can legitimately search for a 'default' ancestral kind of appendage in those animals, like insects, which are endowed with several different kinds of specialized appendages such as antennae, mandibles, and legs.

We cannot seriously start enquiring into the origin of animal appendages, however, unless a few lexical (but also conceptual) questions are addressed.

In principle, 'appendage' is a largely neutral term. It refers to body outgrowths irrespective of mechanism of origin, position, or patterning.

Position, however, is usually regarded as a fundamental argument in evaluating homology of appendages. Thus, its consolidated knowledge that tetrapod limbs are homologous to the paired fins of bony fishes, in the same way as beetle elytra correspond to the membranous fore wings of most other insects, or fly halteres to the membranous hind wings of most of the remaining insects, beetles included. Within one animal, position is again important in determining what is usually called serial homology. In this way, a beetle's elytra are serially homologous to its membranous hind wings, while each of its three pairs of legs is serially homologous to its antennae, mandibles, and maxillae, or to the labium. This kind of equivalence was perfectly well established one century before the first homeotic mutants showed that two extra legs may replace the antennae of a fruitfly, and two extra wings its halteres.

Patterning is also important. Several body appendages, such as the oral tentacles of many polypoid animals, are little more than cylindrical outgrowths, more or less distinctly tapering towards their nondescript distal end. Others are a little more

complex, e.g. the capitate tentacles of some cnidarians, or the segmented parapodia of many syllid polychaetes. Sheer complex patterning, however, is the privilege of arthropod and vertebrate appendages, although not one shared by all body outgrowths of these animals. For example, a vertebrate tail (reasons for regarding it as an appendage rather than as a part of the main body axis are given below) is less complex than a typical fish fin or a typical tetrapod leg. Same for arthropod appendages such as the posterior paired cerci of many insects, or the unpaired and equally posterior filum terminale of the silverfish, in comparison with a maxilla or a thoracic leg.

The most interesting aspect of this diversity of appendages, however, is not so much the disparity of models evolved by representatives of the same phylum, e.g. the Arthropoda or the Chordata, as is instead the broad correspondence between the nature (or complexity) of body patterning along the main body axis and the nature (or complexity) of the most extensively patterned appendages developed by the same animal. It is possibly not by chance that segmented appendages are only present in animals whose main body axis is also segmented, a point to which I will return later in this article.

### Developmental complexity and the complexity of the appendages

It is worth noticing, additionally, that the degree of morphological complexity in the proximo-distal pattern of an adult appendage is often matched by the process complexity of the (post-embryonic) development of the same animal (Minelli 1996, 2003). There are many examples in arachnids, where adult legs are often more complex than usual in the two orders where the post-embryonic development is not direct as in spiders and scorpions, but includes the metamorphosis of a 6-legged larva into an 8-legged adult. This happens in mites as well as in the little and obscure order of the Ricinulei. Mite larvae are often quite different from their adults and one to three morphologically distinct nymphal stages do usually separate the larva from the adult. It is also possibly not by chance that ricinuleans have more richly patterned legs than the other arachnids have. As for the mites, the extremely small size of these arthropods does not seem to allow for the expression of extra leg joints, nevertheless their legs are often provided with complex and widely diversified apical structures.

The best example of a correspondence between the complexity of an animal's developmental schedule and the complexity of the same animal's appendages is provided, however, by the blister beetles. These insects have a hypermetabolous life cycle. That is, their post-embryonic development is much more complex than the cycle of a ladybird (or, by the way, of a fruit fly). In particular, the sequence of their active larval stages is punctuated by a resting stage that is not equivalent to the pupa of holometabolous insects. The true pupa, and finally the adult, close the cycle only after the blister beetle has gone through not less than four different juvenile stages. The morphological counterpart to this unusual developmental complexity is found in the antennae of several representatives of this family. In particular, the antennae of the males of several *Meloe* species have a couple of strongly modified articles at mid-length, whereas both the proximal and the distal ones are of

simple, normal shape. Similar interruptions of an otherwise uniform series of antennal segments are very rare in arthropods. In these appendages, more or less complex patterns are very common at either the proximal or the distal end of the appendage, or both. Something comparable to these beetle antennae is basically limited to copepods, where the male antennule is generally provided with a mid-length singularity which has some 'equivalent' along the main body axis and, possibly, in the complexity of the post-embryonic development (cf. Minelli, 1996; Boxshall and Huys, 1998).

### Vertebrate tail and echinoderm radii

Despite the apparently easy application of the morphological term 'appendage', there are at least two interesting cases whether a distinction between main body axis and axis of appendage is controversial. One of these cases is the vertebrate tail, the other is the echinoderm radii.

In vertebrate zoology, the tail is usually described as the posterior section of the main body axis, but this interpretation is open to question. In terms of developmental mechanisms, it is still uncertain (Kanki and Ho, 1997) whether the tail is actually a product of gastrulation, as is the animal's trunk. To be sure, no endodermal derivative is involved in forming the tail, a circumstance that puts the tail on the same side as limbs, rather than on the same side as the trunk. A further argument in favour of the appendicular nature of the tail is provided by those experiments where a frog tail blastema treated with retinoic acid failed to regenerate a tail but gave rise instead to supernumerary limbs (Mohanty-Hejmadi *et al.*, 1992; Maden, 1993; Brockes, 1997). If the traditional comparison between the vertebrate tail and the tail of the ascidian tadpole larva is to be taken seriously, that is, if the two tails are really homologous, we must contemplate some degree of morphological assimilation between vertebrate trunk and vertebrate tail. Indeed, there is an indisputable morphological continuity between the skeletal and neural axis of the two regions. A possibly final argument in favour of the interpretation of the vertebrate tail as a body appendage derives from developmental genetics, as no *Hox* gene expression is known to have its anterior boundary in the tail (Prince *et al.*, 1998).

The other critical case is echinoderms. In this phylum, a distinction between appendages and main body axis is an all but easy question (e.g., Hotchkiss, 1998; Popodi and Raff, 2001). To be sure, these animals *have* obvious appendages, such as their tube feet (or podia) or the tentacles surrounding the mouth opening of many holothurians. But what about the five (or more) arms of a sea star? Are all these arms equivalent, in the sense that all five can be described as multiple main body axes, or are they the five appendages of a virtually non-existent trunk? The pattern of expression of a posterior class *Hox* gene in the adult primordium of the sea urchin larva, together with arguments from palaeontology and comparative morphology, suggests the existence of one main body axis only (Peterson *et al.*, 2000). If this interpretation is correct, it would imply that four out of the five axes originated as appendages, but a subsequent process of assimilation led to the virtual identity of all body axes, irrespective of their primary or secondary origin. This is in essence the same interpretation as the one just proposed for the vertebrate tail.

### Limbs and genitalia

Among the new vistas on homology opened by developmental genetics there is the notion that many genital appendages (but not all of them, see Minelli, 2002, 2003) are serially homologous with limbs. One of the first pieces of evidence pointing in this direction was Shearn *et al.*'s (1987) report on two *Drosophila* loci where mutations cause different homeotic transformations of the appendages, e.g., antenna-to-leg, proboscis-to-leg, and haltere-to-wing, but also genitalia-to-leg and genitalia-to-antenna. Developmental equivalence of limbs and genital appendages has been also reported for vertebrates, humans included (e.g., Del Campo *et al.*, 1999). In the mouse, the morphogenesis of the penial bone (baculum) is controlled by group 11, 12 and 13 *Hox* genes in the same fashion as are the size and the number of the digits (Dollé *et al.*, 1993).

In the context of the homologization of limbs and genitalia it is also worth mentioning that a pair of appendages may well have its homologue in an unpaired appendage. Even if the example does not refer to appendages in the proper sense of the word, this equivalence is convincingly shown by cyclopia, the developmental defect by which the distance between the two eyes of a vertebrate is reduced to zero and only one median, bilaterally symmetrical eye is eventually formed.

To be sure, all these appendages are homologous under certain criteria only, but not under others. This circumstance requires abandoning the traditional all-or-nothing notion of homology in favour of a more articulated, factorial or combinatorial concept (Minelli, 1998, 2002).

### 'Internal' and 'virtual' appendages

From an evo-devo point of view it may be sensible to include, in a study of body appendages, three more kinds of structures that a morphologist would not obviously put into the same category. Those of the first two kinds have, indeed, their own morphological axis; and those of the third kind share with 'true' appendages at least one key gene expression pattern.

A first kind is internal appendages, such the paired diverticula of a leech gut or our own caecal appendage. What is most peculiar, in this case, is not so much the fact that these structures do not project externally as outgrowths of the main body axis, as is the fact that they involve endodermal derivatives. Indeed, a basic feature of nearly all 'true' appendages is the fact they are made of ectodermal and mesodermal material only. In this respect, ecto+mesoderm and endoderm show a large degree of independence. But this is not an absolute one. In the case of leeches, the production of segmentally arranged gut diverticula is induced by mesodermal influences on a 'segmentally naive' endoderm (Wedeen, 1995; Wedeen and Shankland, 1997).

Second, one might grant the status of 'negative appendages' to ingrowths such as insect tracheae, where a proximodistal axis is indeed formed and patterned (cf. Arthur *et al.*, 1999).

Finally, modern developmental genetics invites to grant the status of 'virtual appendages' to some body features which are neither outgrowths nor ingrowths but nevertheless share with conventional appendages one or more critical gene expression patterns. Typical in this respect are the eyespots on butterfly wings, whose position on the wing disc is first manifested by a

localized expression of the *Distal-less* gene, quite similar to the *Distal-less* expression that marks the position and eventually the distal end of future paired appendages such as legs or antennae (Carroll *et al.*, 1994).

### Cartesian co-ordinates?

A system of co-ordinates is obviously useful in describing an appendage or its developing anlage, but the choice of a suitable reference system is a far from trivial problem, if we just move from the level of plain morphological description to the investigation of the developmental processes responsible for the growth and patterning of the appendage. In the latter case, to expect that there should be one distinct patterning system for each Cartesian axis (proximo-distal, antero-posterior, and dorso-ventral) is simply unwarranted. There is no a priori reason why the animal's own description of the appendage should actually use our own preferred spatial reference system. More specifically, there is no reason to expect that every spatial axis should have its own independent patterning system. Indeed, a multi-axial patterning system is actually involved in the development of the appendages of *Drosophila* (Lecuit and Cohen, 1997; Niswander 2003).

Another point is that it is perhaps unwarranted to equate the dorso-ventral axes of the vertebrate and arthropod appendages, which are patterned by completely different systems, whereas some similarity exists in the genes involved in patterning the antero-posterior and proximo-distal axes (Lawrence and Struhl, 1996; Shubin *et al.*, 1997). In vertebrates, however, the actual axis patterned by the *Hox* code does not correspond with either the proximo-distal or the antero-posterior geometrical axis of the appendage, but with a proximal/anterior to distal/posterior one (Gardiner *et al.*, 1998).

Last point, one might contend that an unambiguous proximo-distal axis of the appendage does indeed exist as the spatial counterpart of time dimension of growth and differentiation, but this is again unwarranted, in arthropods especially. In fact, while vertebrate appendages do usually (but not always) grow and differentiate in a proximo-distal sequence, in arthropods these processes go on otherwise. In insects, the boundaries separating leg segments are formed in a sequence which is neither proximal-to-distal nor distal-to-proximal. In a cockroach leg, for example, the boundary between femur and tibia forms before the one between the trochanter and the femur, followed in turn by the boundary between tibia and tarsus (Norbeck and Denburg, 1991). Quite similar is the segmentation sequence of the *Drosophila* leg, despite its much delayed development from an imaginal disc (Rauskolb, 2001). The segmentation schedules of arthropod antennae are quite diverse, and this diversity is not fully covered by the contrast (Imms, 1940) between 'truly segmented antennae', with intrinsic musculature articulating every antennal article with those preceding and following it, and 'flagellar antennae', formed by a sequence of flagellomeres whose articulation is not served by musculature.

### The first appendage and the pitfalls of recapitulationism

One might speculate that the first appendages evolved by animals were simple unpatterned outgrowths, such as the

simplest of oral tentacles or cirri. Highly complex appendages such as insect legs or human limbs would be the result of a long evolutionary history. A history made easy, perhaps, by the successive co-option, into the limb-patterning system, of individual genes or gene cassettes previously evolved to the service of other morphogenetic processes.

Reasonable as it may seem to be, this scenario is indeed quite nebulous as to the first origin of the appendages. Moreover, it fails to offer a simple explanation to the fact that an animal's appendage tends to share many nontrivial structural and developmental traits with the main body axis of the same animal. Indeed, if the appendage is a secondary body axis, why should it originate by a completely different process than the main one?

One of the most widespread but theoretically less justified attitudes in evolutionary developmental biology is equating developmental mechanisms of organ induction with the evolutionary origin of the same organ. This is a blatant but seldom acknowledged example of recapitulationism, an attitude in singular contrast with the widespread disrepute into which Haeckel's principle is apparently held. An example of such an equation of developmental and evolutionary origin is the hypothesis that the first limb outgrowth was induced by localized expression of fibroblast growth factor (FGF) molecules (Wilkins, 2002). This hypothesis rests on the results of experiments in which implantation of beads soaked in a FGF into the flanking mesoderm of chick embryos prior to limb emergence induced supernumerary limbs, whose wing vs. leg identity depended on the precise site of application of the FGF (Cohn *et al.*, 1995, 1997).

Indeed, vertebrate limb evolution is often described in overt recapitulatory terms: the proximal-to-distal addition of elements during ontogeny would roughly parallel the repeated evolutionary addition of new terminal (distal) elements (Shubin *et al.*, 1997). In Wilkins's (2002) words, "the contemporary molecular genetic foundations of tetrapod limb development largely reflect a series of recruitments of successively more downstream elements, whose expression occurs in successively more distally developing regions of the limb" (p. 293).

But there are clear instances of intercalary evolution, for instance, the evolution of the phalanges preceded the evolution of carpals and tarsals, which are proximal to them (Lebedev and Coates, 1995; Shubin, 1995).

According to a recent scenario for vertebrate fin and limb evolution, the first step was the outgrowth of the limb buds under the stimuli provided by the apical ectodermal ridge and by diffusing fibroblast growth factors. Subsequent recruitment of *Hox9a* and *Hox9d* genes (phase I *Hox* gene expression) would have permitted the development of the future proximal segment of the limb (the stylopod) in the sarcopterygian grade of vertebrate organization. Further recruitment of more 5' *Abd-B*-like *Hox* genes (paralogy groups 11 through 13; phase II and phase III *Hox* gene expression) would have permitted the evolution of a more distal segment (the zeugopod) and, finally, of the terminal limb segment (the autopod). In this scenario, the differentiation of fore- and hindlimbs is seen as an additional step, involving modulation of *Hox* gene activities (Wilkins, 2002).

In other terms, three successive waves of *Hox* genes expression would have progressively caused the differentiation of

stylopod, zeugopod, and autopod, in the order. But there are problems with this idea. According to the phylogenetic scenario reconstructed by Mabee (2000), sharks and rays are expected to have a phase III expression of *Hox* genes, but they lack an autopod; conversely, ray-finned fishes have phase I and phase II *Hox* genes expression, but lack both stylopod and zeugopod.

A major problem with modern recapitulationist interpretations is their gene-reductionist nature. This is indeed in line with the widespread belief in the existence of master control genes whose expression single-handedly 'decides' that an eye or a heart are to be made. The question is not to deny the evidence for the developmental role of these genes. The problem is that no mutation in any single gene, no change in its promoter sequences, no novelty in its temporal and spatial patterns of expression could give rise by itself to the first eye, or heart, or appendage.

### Problems of categorization and Urbilateria's appendages

In my opinion, the major problem here is one of categorization, as anticipated in the introduction. Tradition in comparative anatomy invites to look for less controversial homologies, thus suggesting that the main body axis of animal A shall be compared to the main body axis of animal B, while an appendage, homoplasy permitting, might be compared to another appendage: one of the same animal, in terms of serial homology, or one of another animal, in terms of special homology. Comparing an appendage to a main body axis would be outrageous except perhaps in the case of the echinoderms. But this is only true in terms of 'finite products'. In developmental terms, very different comparative scenarios may be legitimate. An Urbilateria provided with appendages (perhaps also with heart, eyes, body segmentation, and so on; e.g., Kimmel, 1996; De Robertis, 1997; Holland *et al.*, 1997; Holland and Holland, 1998; Palmeirim *et al.*, 1997; Christ *et al.*, 1998; Gehring and Ikeo, 1999; Dewel, 2000) is an expression of the traditional comparative blueprint, whereas alternative scenarios may lead to very different *evodevo* perspectives.

For a while, a main argument in favour of an Urbilateria provided with some kind of appendages rested on the discovery of the virtually universal involvement of a *Distal-less* expression in marking the site where appendages are about to develop. This was not limited to the appendages of vertebrates (Beauchemin and Savard, 1992; Dollé *et al.*, 1992) and insects (Cohen *et al.*, 1989; Cohen, 1990), as *Distal-less* expression was found to mark the prospective tip of the appendages, segmented and unsegmented alike, of animals belonging to the most diverse phyla (Popadic *et al.*, 1996).

A more specific claim seemed to be warranted by Rodriguez-Esteban *et al.*'s (1997, 1998) reports on the similar expression patterns, during the early outgrowth and proximo-distal patterning of *Drosophila* and vertebrate limbs, of genes such as *hedgehog*, *patched* and *decapentaplegic* in the insect and their homologues *Sonic hedgehog*, *patched* and *Bone morphogenetic proteins* in the vertebrates. These correspondences were seen as a solid proof that these appendages should derive from those of a common ancestor. De Robertis (1997) hypothesizes the presence in Urbilateria of a 'humble appendage or antenna-

like outgrowth', based on the presence of *fringe*, *serrate* and other genes; a view shared by others (e.g. Morata and Sánchez-Herrero, 1999; Panganiban *et al.*, 1997, with doubt; Dewel, 2000; Dong *et al.*, 2001), but rejected by others (e.g., Mittman and Scholtz, 2001).

Comparative evidence on insect and vertebrate limb development, however, is compatible with different evolutionary scenarios. Tabin *et al.* (1999) summarized arguments in favour and against the four following views: (1) arthropods and vertebrates derive from a primitive bilaterian (Urbilateria) already provided with some kind of appendages, produced and patterned by essentially the same genes as those which produce appendages in the modern representatives of the two phyla; (2) Urbilateria possessed some kind of body outgrowths not homologous to modern appendages but growing under the control of genes that were later co-opted to provide patterning of both arthropod and vertebrate modern appendages; (3) arthropod and vertebrates recruited, independently, a basically identical "cassette" of genes whose internal networking and patterns of expression were already integrated, but used to purposes other than to form appendages; (4) limb-patterning genes shared by arthropods and vertebrates were individually recruited in this role and their common presence in the two phyla is just coincidental.

On the balance, Tabin *et al.* (1999) came to the safe conclusion that arthropod and vertebrate appendages are *nothistorical* homologues, but this left unexplained the expression of several identical genes in the outgrowth and patterning of appendages in the two phyla.

This may be explained, however, if we adopt a suitable comparative perspective. That is, if we look into the growth and patterning of the main body axis as to a source of possible mechanisms for the origination and patterning of the appendages. This means that we should regard the appendages as a kind of evolutionarily divergent duplicates of the main body axis. More specifically, according to this hypothesis appendages such as arthropod and vertebrate limbs *originated by duplicate expression of genes already involved in the growth and patterning of the main body axis*. This is the notion I proposed under the name of *axis paramorphism* (Minelli, 2000b; see also Arthur, 2002; Held, 2002).

### Axis paramorphism

Let's thus look for pattern features common to the appendages and the main body axis (Minelli, 1996, 2000b, 2002, 2003).

A first conspicuous feature is the occurrence of segmented appendages in those animal groups where the main body axis is also segmented, and in these only, whereas non-segmented appendages are unusual in both vertebrates and arthropods (although common, but not universal, in annelids). It is worth noting that segmentation is not limited to the paired fins and legs of gnathostome vertebrates and to the paired appendages of arthropods (antennae, mouth-parts and legs). It extends indeed to unpaired appendages such as the tail of the vertebrates and the posterior dorsal and anal fin of *Latimeria*, and to the caudal appendages of the silverfish and of several arachnids among the arthropods.

But this is just a first gross level of segmentation. In many segmented appendages it seems to be justified to distinguish at least two different levels of segmentation. In the case of arthropod antennae, for example, morphologists distinguish between 'true' segments, as are those of the centipede and millipede antenna, from flagellar annuli as those forming most of the antenna of a silverfish or a winged insect. In a more or less comparable way, the subdivision of the insect tarsus into individual tarsomeres may be regarded as secondary in respect to the subdivisions of the leg into 'primary segments' such as femur, tibia and tarsus. Same for the phalanges of the tetrapod digits, in comparison to the articulation of the limb into stylopod, zeugopod and autopod. Interestingly, primary and secondary levels of segmentation may be also recognized along the main body axis, at least in some body districts, in all major groups of segmented animals (Minelli, 2000a, 2001, 2003).

At still smaller scale there are additional items which have hitherto escaped consideration in this comparative framework, where they should be placed indeed. One at least of these items is worth mentioning here, the lepidotrichia, i.e. the multiple segmented and typically branched bony axes which support the fin membrane in ray-finned fishes. Individual lepidotrichia behave as developmentally independent segmented axes. If wounded, each one of these rays will regenerate, following the formation of its own blastema (Akimenko *et al.*, 2003). In a sense, lepidotrichia are integrated into the fin in the same way as multiple morphologically and morphogenetically independent cilia combine to form a ctenophoran's comb-like plate.

The presence or absence of segmentation is just one gross criterion of morphological equivalence between an animal's main body axis and the axes of its appendages. Many additional comparisons have been suggested elsewhere (Minelli, 1996, 2000b, 2003). These examples involve a diversity of appendages such as the antennae of dipterans and centipedes, the antennules of copepods, the spinnerets of spiders, as well as the paired appendages of the vertebrates. In every case, morphological trends in complexity (or specialization) of the appendages mirror parallel trends in the structure of the main body axis.

The relevance of all these arguments, however, may be questioned as simply derived from comparative morphology. But there is a not less impressive array of developmental genetic evidence, which may be brought in support to the paramorphism hypothesis. In his recent book on the developmental genetics of the imaginal discs, Held (2002) articulates arguments in favour and against the notion of paramorphism, as applied to the leg-to-trunk relationships in *Drosophila*. Genes expressed in the leg disc include two classes, the "wide zone" class and the "periodic zone" class, which might correspond to the gap genes and the segment-polarity genes, respectively. The similarity is not limited to gene structure, but extends to some aspects of their expression and putative developmental role. To begin with, "wide-zone" genes are generally expressed earlier than periodic-zone genes, paralleling the earlier expression of gap over segment-polarity genes in the segmenting trunk. Two pair-rule genes (*hairy* and *odd-skipped*), better known for their role in trunk segmentation, also belong in the leg's periodic class. Moreover, loss-of-function mutants of *dishevelled* cause similar defects on both axes (double-joint

syndrome along the appendage and extra segment boundaries along the main body axis (Held *et al.*, 1986; Bishop *et al.*, 1999). All these arguments are clearly in favour of the paramorphism hypothesis. Held (2002) remarks that in *Drosophila* the Notch pathway is involved in leg, but not in body segmentation (Rusconi and Corbin, 1999; Wesley, 1999); however, an ancestral involvement in the latter role has been hypothesized, so the present condition in *Drosophila* may well be a derived one (Dearden and Akam, 2000; Rauskolb and Irvine, 1999).

Vertebrates offer additional molecular genetic arguments supporting the paramorphism hypothesis. There are mice *HoxD* mutants, for instance, where both the appendages and the main body axis are simultaneously affected by skeletal defects, thus suggesting the existence of a common multiaxial patterning system (Dollé *et al.*, 1993). Growth and patterning of the main body axis and the corresponding events along a limb axis share several components, including WNT signalling (Moon *et al.*, 1997; Shubin *et al.*, 1997; Tabin *et al.*, 1999): in comparable terms, in *Drosophila* *wingless* has a role in patterning both the antero-posterior body axis and the proximo-distal axis of the leg (Campbell and Tomlinson, 1995; Nagy and Williams, 2001).

On the other hand (Held, 2002) one must admit that insect leg segmentation does not involve the cell-lineage restrictions that characterize compartments along the main body axis (Bryant and Schneidermann, 1969). Moreover, as to the pair-rule genes belonging to the periodic zone class, there is no evidence of two-segment periodicity in their expression in the leg disc (Cohen, 1993; Godt *et al.*, 1993; Kojima *et al.*, 2000).

These differences, however, are not enough to invalidate the hypothesis of axis paramorphism. To be sure, we shall expect increasing evolutionary divergence of the paramorphic axes (trunk ad appendages) through of new regulatory interactions under changing selective regimes over a few hundred million years of evolution (Shubin and Marshall, 2000).

We can thus articulate an evolutionary scenario where the developmental similarities between arthropods and vertebrates, as to the way their appendages are originated and patterned, does not require the hypothesis of an Urbilateria already provided with body appendages. Vertebrate and arthropod limbs are not true historical homologues. Behind them, however, there is the historical homology between the main body axis of the vertebrates and the main body axis of the arthropods, including but not limited to the expression of the zootype genes. This is the common ancestral background that has been independently re-expressed, with modification, in their paramorphic secondary axes, that is, in their appendages. These are, in other terms, the homoplastic paramorphs of true historical homologues (Minelli 2000b).

The hypothesis of axis paramorphism (a view somehow anticipated by speculations of Dollé *et al.* (1993) and Held (1995)) is quite different from the hypothesis of gene co-option (e.g., Tabin *et al.*, 1999; Arthur *et al.*, 1999), according to which individual genes or batteries of genes, originally involved in developmental process other than limb production, became progressively involved in the growth and patterning of the appendages. In this alternative scenario, the animal would have 'invented' its appendages independent of the genes now involved in its growth and patterning. Prima facie, this hypothesis may seem more likely than the paramorphism scenario, but

it leaves several questions unresolved. How did the animal actually originate its appendages? From which reserve of genetic tools could it derive the mechanisms by which it could produce its secondary axes, if not from those already available and actually used in producing and patterning the main body axis? The hypothesis of axis paramorphism suggests an answer to these questions.

From this point of view, the production of the first unspecialized appendages would have been a process similar to lateral budding. But with a proviso: that these 'buds' were devoid of an endodermal component. The presence of caecal gut outgrowths in the legs of the sea spiders and a few other animals must be quite confidently regarded as a later innovation. As to the cnidarian tentacles, which contain an either solid or hollow core of gastrodermal cells, one might even question whether the cnidarian epidermis and gastrodermis are actually homologous to the ectoderm and endoderm of the Bilateria.

Cnidarians, however, offer an excellent example of the broad equivalence of body appendages and reproductive buds. In several species of boloceroidid sea anemones, tentacles may actually give rise to new polyps. In two species studied by Pearse (2002), single tentacles were pinched off at the sphincter and shed into the coelenteron, where they regenerated into minute new polyps; in a third species, new polyps originated from fan-like clusters of short tentacles, distinct from the longer tentacles involved in feeding and locomotion.

### The arthropod default appendage

Despite the current rapid growth of the literature aiming at interpreting developmental mechanisms in evolutionary perspective, explicit phylogenetic frameworks are all too rarely adopted. As a consequence, hot dispute may ensue between alternative views both of which are hardly interesting, or even meaningful, if placed in the right historical context. One of these bones of contention is the nature of the insect default appendage.

That insect antennae, mouth-parts and legs are serially homologous appendages has never been disputed, but different opinions have been manifested as to the evolutionary relationships among them. A lively debate has recently contrasted two alternative views of the putative insect default appendages, but both of them are actually unreliable in the light of the above arguments.

In the past, the prevailing notion was that head appendages are a kind of modified legs. This notion fitted well into the traditional view of arthropods as modified annelids. A basically homonomous worm-like body would have evolved an homonomous series of paired appendages. A regionalization of the main body axis would have ensued, thus causing the paired appendages to specialize, starting with those at the anterior body end.

Experimental evidence has been used to back this view. For example, the distal part of a *Drosophila* antenna is transformed into leg in the absence of *spineless* activity (Duncan *et al.*, 1998) and an antenna-to-leg transformation is observed if the function of *extradenticle* or *homothorax* is removed (e.g., Dong *et al.*, 2000). This has been interpreted as a proof that the default state of the insect appendage is leg-like rather than

antenna-like (Casares and Mann, 1998), in accordance with the traditional views.

Other experimental evidence, however, would point in the opposite direction. Appendages other than the antenna are changed into something similar to an antenna if the specific input required for their determination is lacking (Stuart *et al.*, 1991; Percival-Smith *et al.*, 1997; Hughes and Kaufman, 2000). This has been regarded as a proof that the antennal segment and its appendage actually represent, in phylogenetic terms, the ground plan from which all other segments and their appendages have evolved (Rogers and Kaufman, 1997). Another argument advocated in support of the idea that the antenna is more primitive is the higher complexity of the leg, due to the presence of a distinct intermediate domain between the proximal and the distal one (Dong *et al.*, 2001). Casares and Mann (2001) have clearly perceived that this contrasting evidence asks for a less extreme view than to regard either the leg or the antenna as the ground state of the arthropod antenna. Accordingly, these authors suggest that the ground state common to antennae and legs, as well as to genitalia and analia, is a leg-like appendage consisting of two main sections: a proximal segment and a distal tarsus.

In my view, however, all these views are just one more example of an evolutionary origin directly inferred from ontogenetic control mechanism of organ origination. A similar recapitulationist view underpins the view that an altered expression of the 5' *HoxD* genes may have been crucial to the 'invention' of vertebrate digits (Sordino *et al.*, 1995; Zeller and Deschamps, 2002).

Two lines of evidence, however, have undermined these scenarios definitely. First, there is good evidence that extensive body patterning, as provided by the 'zootype genes' (Slack *et al.*, 1993, and subsequent literature), evolved prior to the origin of the arthropods. Second, new vistas on animal phylogeny are increasingly against the notion of a superphylum Articulata (with Arthropoda closely related to the Annelida), thus suggesting a separate origin of body segmentation in the annelid ancestor (from within the Lophotrochozoa) and in the arthropod ancestor (from within the Ecdysozoa) (e.g., Minelli and Bortoletto, 1988; Eernisse *et al.*, 1992; Aguinaldo *et al.*, 1998).

What all these reconstructions lack is an adequate historical, i.e. phylogenetic, framework. If a degree of regionalization was already present in the arthropod ancestor where paired appendages were first developed, then there are good reasons to imagine that a degree of specialization between, say, head and trunk appendages was already present, despite the basically similar processes underlying the outgrowth and the patterning of all these serially homologous parts. That is, the most likely evolutionary scenario is not one of specialization of originally identical pairs of appendages, but one of production, along a series of already differentiated (sets of) segments, of appendages diverging in morphology since their very first appearance (Minelli, 1992). A strictly homonomous body on whose segments identical serially homologous appendages were borne has probably never existed except in the mind of typologically thinking biologists.

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