The emerging conceptual framework of evolutionary developmental biology

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Over the last twenty years, there has been rapid growth of a new approach to understanding the evolution of organismic form. This evolutionary developmental biology, or 'evo-devo', is focused on the developmental genetic machinery that lies behind embryological phenotypes, which were all that could be studied in the past. Are there any general concepts emerging from this new approach, and if so, how do they impact on the conceptual structure of traditional evolutionary biology? In providing answers to these questions, this review assesses whether evo-devo is merely filling in some missing details, or whether it will cause a large-scale change in our thinking about the evolutionary process.

Evolutionary developmental biology has its origins in the comparative embryology of the nineteenth century, and in particular in the work of von Baer[1] and Haeckel[2,3], whose 'laws' were put forward as being generally applicable to the way in which development evolves, regardless of the taxon concerned. Following a quiescent period of almost a century, present-day evo-devo erupted out of the discovery of the homeobox in the early 1980s (refs 4, 5). One principal focus over the last 20 years has been the comparative study of the spatiotemporal expression patterns of developmental genes, both homeobox-containing[6] (hence coding for transcription factors) and others. Although there are many cases of conservation of expression patterns, there are also cases where these patterns differ markedly between closely related species, even when the gene concerned acts at a very early developmental stage[7].

We have thus moved from one extreme to the other: from laws that turn out to be, at best, overgeneralizations, to a situation where it almost seems that anything goes, that is, any developmental gene, its expression pattern and the resultant ontogenetic trajectory can evolve in any way. If this were true, no generalizations would be possible, let alone universally applicable laws. However, the search for general patterns is fundamental to science and is not easily suppressed, even when the relevant data set looks very complex, as it currently does in evolutionary developmental biology. There are signs, particularly over the last few years that new general concepts are emerging. I discuss these below, after a brief look at two of evo-devo's foundations.

Comparative embryology and phylogeny

What is the current status of the laws of von Baer and Haeckel? One view is that Haeckel's recapitulation was wrong but von Baer's embryonic divergence essentially right[8]; however, it can be argued[9] that the pattern that is observed depends on the type of comparison being made. For comparisons among animals of the same level of phenotypic complexity, such as different vertebrate classes, von baerian divergence may be the norm (but see below). However, when comparisons are made between very different levels of complexity, the pattern that emerges is broadly recapitulatory, although only in a very imprecise way, in the sense of recapitulating levels of complexity rather than precise morphological details (Fig. 1). So, taking a broad view, both von Baer and Haeckel captured elements of the truth: evolution leads both to embryonic divergence and, in some lineages, to a lengthening of the ontogenetic trajectory leading to more complex adult phenotypes with greater numbers of cells, their embryos passing through simpler, quasi-ancestral forms. There is, however, an important restriction to this general model. Von Baer's divergence applies only after the 'phylotypic' stage[10]. Earlier development is interspecifically variable, converging to this point of maximum similarity, and only after that diverging again. This 'egg-timer' or 'hourglass' model of development[11] renders the situation messier, but should not lead us to abandon the idea of von baerian divergence altogether, especially as the hourglass is a very asymmetric one, with the point of constriction close to the beginning.

Studies of the proposed phylotypic stage in vertebrates reveal more variation than was recognized by either von Baer or Haeckel, partly as a result of screening a wider range of vertebrate species[12]. The result is recognition of a phylotypic period, rather than a precise phylotypic stage, and that this period is one of relative, but by no means absolute, conservation. This can be regarded as a specific example of a general phenomenon: the more taxa that are examined, the more any developmental process that initially seemed conserved may be found to vary. This is a strong argument for broad comparative studies.

Such studies rest on another foundation, namely, the explicitly phylogenetic approach to systematics developed by Hennig[13] between the 1950s and 1970s, plus later refinements of his methodology. This body of work is particularly important, given that the influence of the 'modern synthesis' of the 1930s and 1940s (see below), with its primary focus on evolutionary mechanisms at the population level, resulted in the near-eclipse of phylogenetic considerations from mainstream evolutionary thinking for many years. Most work inspired by the synthesis had no particular antagonism towards phylogeny; its main focus of interest merely lay elsewhere. However, remarks made by a few pro-synthesis biologists reveal their active dismissal of the importance of phylogenetic considerations rather than merely neglect, as, for example, in the Medawars' statement[14] that "nothing of any importance turns on the allocation of one ancestry rather than another".

The importance of the work of Hennig is twofold. First, phylogeny was moved back to a centre-stage position, but without displacing mechanistic approaches from that position, thus setting the scene for a truly combined pattern-and-process approach, even though Hennig focused entirely on pattern. Second, Hennig achieved this by developing the cladistic method for the reconstruction of phylogenetic pattern that is based on a rigorous treatment of data—in terms of shared derived characters—rather than intuitive feelings about systematic relationships arising from accumulated personal experience of particular taxa. Furthermore, his methodology, although initially developed to deal with morphological data, is of very broad applicability and is now in widespread use in the analysis of molecular sequence data, including the sequences of many developmental genes.

Advocates of evolutionary developmental biology are of course interested in the ways in which developmental characters map to phylogenies, as exemplified by studies of comparative echinoderm[7] and anuran[15] development, and the phylogenetic scope of various characters in the zebrafish model system[16]. However, that focus of interest should not be taken to imply that they favour the ontogenetic method of polarizing characters in phylogeny reconstruction[17]. In fact, this method has not been widely adopted; rather, the outgroup method has retained favour.

The mapping of developmental processes onto a phylogeny reveals the kinds of alteration in development that we need to explain. Equally, such mapping enables us to exclude from consideration illusory evolutionary changes that never took place, such as the supposed annelid–arthropod transition (see below).

From the homeobox to expression patterns

Evolutionary and developmental biology uncoupled at around 1900, partly due to the latter becoming more experimental and less comparative[18]. They stayed largely separate, with some notable exceptions[19,20], until about 1980. Their re-integration was prompted by several factors, including the widespread acceptance of phylogenetic systematics (see above), the growth of a more genetic approach to development, especially in *Drosophila*[21], and the appearance, over a short period around 1980, of a cluster of books on the interface between evolution and development[8,22,23]. But the single most important factor was the discovery of the homeobox and its widespread phylogenetic conservation[4,5].

The importance of homeobox-containing genes (including the *Hox* genes), from an evolutionary perspective, is that they reveal the existence of a general mechanism underlying the development of morphologically diverse organisms. This constitutes something of a paradox—where does the diversity come from if the genes are highly conserved?—the resolution of which may lie in lower levels of conservation of downstream genes, although this is still largely a presumption. Not only are homeobox genes highly conserved in sequence (especially within the 180-base-pair homeobox region itself), but in many cases their expression patterns are highly conserved also, implying a conservation of function. One of the most enduring visual images of the comparative study of expression patterns of homeobox-containing genes carried out in the 1990s is the picture of 'stripey' embryos of various arthropods[24,25]. These all show the segmentally reiterated transverse bands of expression of the homeobox-containing gene *engrailed*, which reflect its role in determining segment polarity (Fig. 2).

Conservation is, of course, a relative thing. Over long enough periods of evolutionary time, even highly conserved developmental genes evolve, and when they do, important morphological consequences follow. Sometimes altered expression patterns are such as to give more or less of the same thing within a still-conserved overall body plan. An example is divergence in the anteroposterior expression patterns of *Hox* genes in insects and crustaceans, and, associated with this, morphological divergence, for example in the number of leg-bearing segments[6]. Here, the basic design is still the same at a more fundamental level—a segmented arthropod. In other cases, altered expression patterns lead to more fundamental shifts in morphology, and give rise to an entirely distinct body plan. For example, the expression patterns of some homeobox-containing genes in echinoderms[26] bear little relation to those in vertebrates,

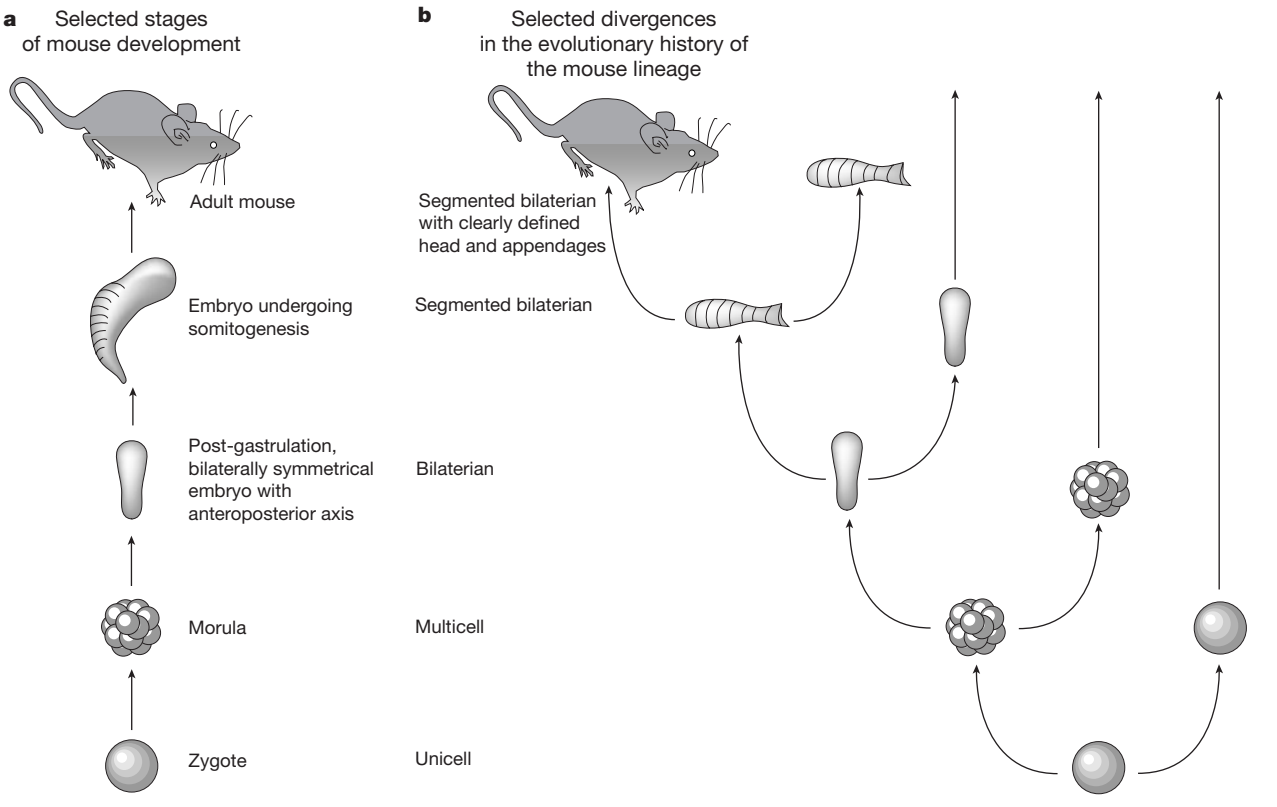

Figure 1 The recapitulatory aspect of the evolution of development in lineages that exhibit increasing phenotypic complexity. The ontogeny of an individual mouse (**a**) recapitulates the broad levels of complexity, but not the precise ancestral forms (either embryonic or adult), that the mouse lineage passed through in the course of its evolution (**b**).

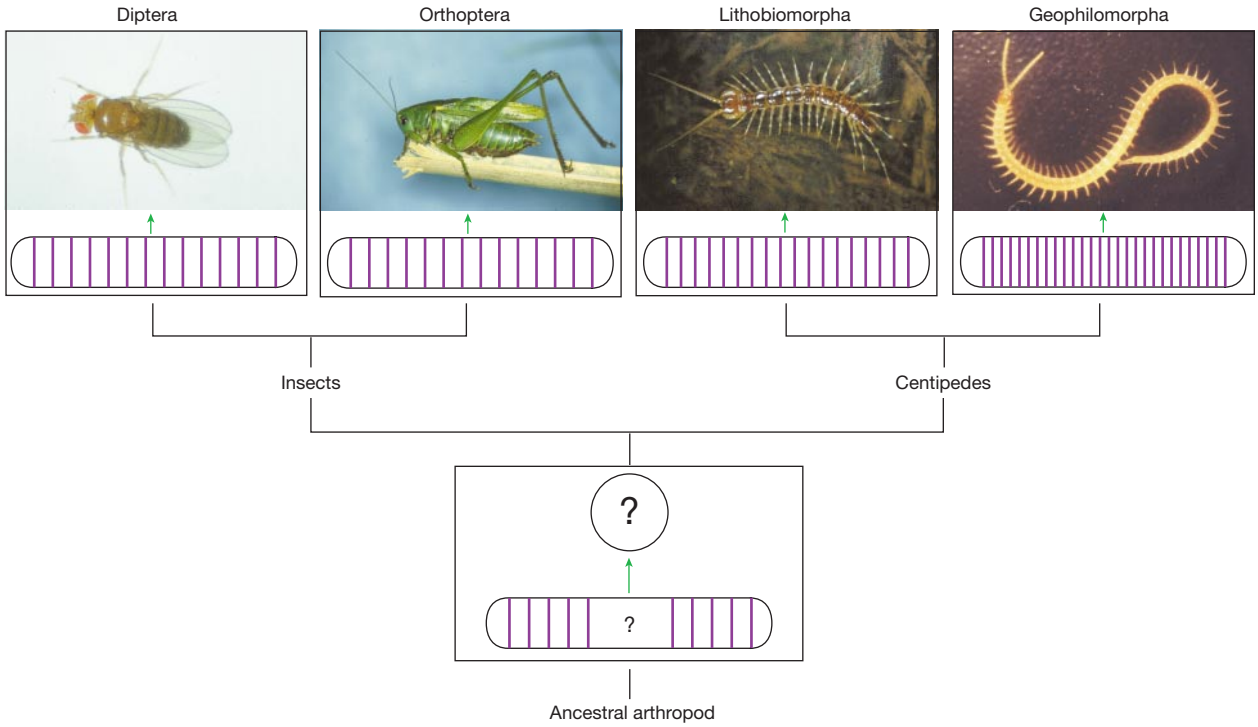

Figure 2 Conservation and change in a developmental mechanism. In all arthropods studied, segments are generated through a cascade of gene interactions, an important component of which is the production of segmental stripes of expression of the segment polarity gene *engrailed*. Starting from an arthropod stem species of unknown embryonic segment number and unknown adult morphology, numerous divergences have occurred in the following: (1) the number of *engrailed* stripes/segments (compare centipedes with each other and with insects); (2) the way in which stripes of expression are generated in development (roughly simultaneously in flies but in anteroposterior sequence of varying duration in the others); and (3) segment identities, which are controlled separately by *Hox* genes. The source of information for insects is from refs 24 and 25. The centipede *engrailed* images were based, at the time of writing, on extrapolation from the known segmental expression patterns of this gene in other arthropods. Recent work has confirmed that such extrapolation is justified (C. Hughes, T. Kaufman and C. Kettle, unpublished data).

as perhaps might be expected given the fundamental gap between bilateral and pentaradial symmetry. The dorsoventral axis inversion that accompanied the divergence of protostomes and deuterostomes[27,28] is intermediate between the above two examples.

Evo-devo and general evolutionary theory

How do all these studies impact on evolutionary biology, a conceptually driven discipline? Perhaps comparative studies of expression patterns are just 'filling in the details'. After all, if morphology evolves, these things that are the underlying causes of morphology must also be evolving. So it might be argued that the accumulating data of evolutionary developmental biology are of no particular consequence for the overall structure of evolutionary theory. However, I will now put forward a rather different view.

As is widely acknowledged, development had a minor role both in the work of Darwin[29] and in the establishment of the modern synthesis of the mid-twentieth century[30–32]. One reason is that even as late as the completion of the synthesis, developmentalists were conceptually isolated from geneticists and evolutionists, for whom the gene had by then assumed a central importance. Developmental concepts of the day, such as induction, gradients and fields, were hard to visualize from a genetic perspective, and thus hard to accommodate within a gene-centred view. Today all that has changed, and developmental genetics is a very large part of developmental biology. Now that the barrier has disappeared, does the new developmental perspective have a conceptual contribution to make to our overall understanding of evolution?

In fact, several groups of concepts have emerged recently from evolutionary developmental biology. Some are new, whereas others are resurrections or elaborations of much older concepts (going back to von Baer and beyond). These concepts are listed in Table 1, along with key references. There are too many to discuss in detail so it will be necessary to be selective. Below I discuss the first two groups of concepts in Table 1: one centred around the idea of developmental reprogramming, the other around the idea of gene co-option.

Developmental reprogramming and bias

Given that development has long been the 'missing link' of evolutionary theory, where and how does that link fit, in terms of conceptual structure? There is a very simple answer to this question (Table 2). Evolution is often portrayed as an interplay between mutation and selection, with the former providing a supply of variation, and the latter acting as a fitness-based sieve. But this picture has a major flaw, because mutation provides new genes, whereas selection acts not on genes but on phenotypes. The missing link, then, is how we get from altered gene to new phenotype. This is the process of developmental reprogramming[33]—that is, a mutationally driven change in something that is itself a state of change. If a particular ontogeny is represented by a trajectory through multidimensional phenotypic space, then after reprogramming we have a different trajectory. Many types and degrees of difference are possible.

The term reprogramming should be interpreted with care. Looked at in one way, development is programmed by genes. But this is too limited a view[34]. There is a complementary process, the epigenetic programme, through which genes are controlled by developmental agents of diverse kinds, including transcription

Table 1 Key concepts of evolutionary developmental biology

General theme	Specific concept	Selected references
The nature of developmental variation	Developmental reprogramming	33, 72
	Developmental bias/mutation bias	41, 42
	Developmental constraint	43, 44
	Developmental drive	45
	Developmental reaction norms	36, 37, 71
Re-use of developmental genes in evolution	Co-option	55, 56, 58, 63
	Exaptation	54, 55
	Cassettes of developmental genes	56, 57
	Paramorphism	57
Aspects of evolutionary conservation	Body plans	9
	Evolutionarily stable configurations	73
	Phylotypic stage/period	10–12
	Zootype	74
	Homology	75–77
Factors promoting evolutionary change	Modularity	78
	Dissociability	78
	Evolvability	79
	Duplication and divergence (of body parts and of genes)	78, 80
Evo-devo aspects of natural selection	Developmental/internal selection	81
	Generative entrenchment/burden	82, 83
	Co-adaptation of developmental processes	9
	Unmasking of hidden variation	84
	Genetic assimilation	20

The concepts listed, both old and recent, are grouped according to the general theme to which they relate. Selected references are indicated. The list is not intended to be exhaustive.

factors and secreted morphogens. The delayed entry of the zygotic genome into the control of early development in most animals[35] reveals that the very beginnings of the developmental process are controlled epigenetically. Finally, there is an ecological aspect to the overall programme, because the ontogenetic trajectory is often influenced by environmental factors (the reaction norm[36]), sometimes in an abrupt way, as in the case of aquatic plants that make different kinds of leaves above and below water (heterophylly[37]), or the insects that produce winged and wingless forms at different population densities. More often, the whole ontogenetic trajectory is deflected in a continuously variable way in response to environmental conditions.

What constitutes developmental reprogramming thus becomes clear. It is a mutation-based, and thus inherited change in the overall genetic/epigenetic/ecological programme through which we get from genome to phenome. Importantly, it is a theory-neutral process, to which even the staunchest neo-darwinian is unlikely to object. Reprogramming becomes controversial only if we propose that it exhibits intrinsic biases (see below), which may provide an additional evolutionary mechanism. Reprogramming can be examined at many different levels, from the alteration of a developmental gene's product through its ontogenetic consequences to (in some cases) its ultimate effects on the adult phenotype. At any one level, there are four possibilities: changes in timing, spatial distribution, quantity and type; respectively heterochrony[38–40], heterotopy[39,40], heterometry[33] and heterotypy[33]. However, a change of one of these kinds at the molecular level may well give rise to other kinds of change at the phenotypic level.

The conclusion is that intraspecific evolutionary change in morphology requires, at minimum, the three processes of mutation, reprogramming and selection, whereas trans-specific evolution requires, in addition, reproductive isolation. The only barrier to the general inclusion of reprogramming as a logically necessary component of the evolutionary process is a passive one, namely inertia. There is no element of antagonism in the sense that there is no branch of evolutionary thought that is anti-development *per se*.

The picture changes radically if it is proposed that reprogramming is, at least in some cases, systematically biased, in that mutation more readily produces changes in certain directions than others, including the extreme case of some directions being apparently 'prohibited'. Such a state of affairs in general has been referred to as mutation bias[41] or developmental bias ("evolution biased by development"[42]). Negative biases, both relative and absolute, constitute constraint[43,44], whereas positive biases have recently been termed developmental drive[45] (quite distinct from meiotic drive[46], molecular drive[47] and dominance drive[48]). Proposals that these biases can potentially lead to the direction of evolutionary change being determined by developmental dynamics as well as by population dynamics are in contrast with the historical thrust of darwinism and neo-darwinism, that the direction of change is determined exclusively by selection[49,50].

The main problem facing proponents of a directional evolutionary role for developmental bias is that evidence for this is both very limited at present and harder to acquire than evidence for selection. The predominance of certain leaf-arrangement[51] and floral-symmetry[52] patterns in angiosperms have been proposed as examples of bias-led evolution. The fact that all 3,000 or so species of centipede have odd numbers of leg-bearing segments (from 15 to 191) also suggests developmental bias[53], and in this case an alternative selective explanation is highly implausible.

Table 2 Processes causing evolutionary change at four levels of biological organization

Level	Initial state	Process causing change	Altered state
Gene	Gene making morphogenetic protein	Mutation	New version of gene and new protein
Organism	Organism with particular ontogenetic trajectory	Developmental reprogramming	New ontogenetic trajectory
Population	Population with certain relative frequencies of 'old' and 'new' ontogenies	Natural selection	Population with altered frequencies of the two ontogenies
Species	Genetically distinct populations	Reproductive isolation	Pair or group of daughter species

Attention is focused here on the main process at each level; this should not be taken as a denial of a role for additional processes (for example, genetic drift at the population level). The source of information for this table is ref. 33.

Are such examples exceptions to a general rule that selection on its own determines evolutionary directionality, or are they an indication of a general but as yet largely undiscovered role for developmental bias? This is an entirely open and very important question, which is likely to be a major focus of future studies. These should include both further studies of unusual phylogenetic distributions of character states, including excesses, deficiencies and absences of particular states for which selective explanations seem implausible, and studies that attempt to quantify the tendency of mutations in model-system organisms to produce reprogramming in some directions rather than others. Considerable care will be needed in the design of these studies, however, if explanations other than developmental bias are to be excluded.

Co-option, exaptation and paramorphism

A central concept that is emerging from comparative studies of developmental genes and their interactions is the co-option of these for different functional roles as evolution proceeds. This is essentially a molecular version of the concept of exaptation[54,55], in which a structure evolved for one adaptive reason is later 'exapted' (or co-opted) for some other role. Variations on this theme are: the idea that genes are co-opted not individually but as interacting cassettes[56], and the idea that co-option accompanies and helps to cause the formation of new structures (paramorphism[57]; see below) rather than occurring afterwards to refine them.

The 'pattern-before-process' argument that emerged from cladistics (see above) is particularly important in considering the possible co-option of developmental genes[58]. For example, overt metameric segmentation is pervasive in three high-level taxonomic groups: annelids, arthropods and chordates. It was originally thought that annelids and arthropods were sister groups[59], and thus that segmentation had originated twice—in an annelid/arthropod common ancestor and in a primitive chordate. More recently, it was suggested that the bilaterian stem group (Urbilateria) was segmented[60], and that segmentation had thus originated only once, although this seems implausible given the large number of secondary losses it implies in unsegmented phyla. But with the recognition of the superphyletic groups Ecdysozoa and Lophotrophozoa[61] in addition to Deuterostomia, it is now generally thought that segmentation arose on three separate occasions. If this is true, why is some of the developmental–genetic machinery underlying segmentation shared between two or all of the three metameric phyla? For example, why is there segmentally reiterated expression of an *engrailed* homologue in some chordates[62], resembling—at least in broad terms—the pattern that is well known in arthropods[24,25]? Is this a case of independent co-option?

Before attempting to answer this question, it is worth noting the parallel situation in limb formation[63]. Arthropod and vertebrate limbs are not regarded as homologous, yet the formation of both is characterized by expression of *Distal-less (Dll)* in the prospective limb-tip region. And indeed, clearly non-homologous outgrowths in other animals, for example the tube feet of echinoderms, are also characterized by *Dll* expression. So again, does this represent independent co-option of homologous developmental genes for similar topographic roles in different evolutionary lineages? Is such co-option not rather improbable?

A possibility that is gaining favour[58,63] is that the genes concerned may have had a function in a common ancestor that was of the same general kind as now observed, but in a different developmental context. For example, in an ancestral bilaterian that had neither segments nor legs, there may nevertheless have been subdivision of some internal body part (*engrailed*) or some rudimentary outgrowth (*Distal-less*). If, following some subsequent lineage divergence, two or more descendant species independently become segmented, or independently develop limbs, the easiest way to do so may be to make use of a developmental system already in existence.

This is unlikely to happen on a piecemeal basis, with one gene at a time being co-opted from the original to the new function. Why keep using *engrailed* in segmentation and *Distal-less* in limb outgrowth? It is not just the fact that the products of these genes and of their upstream controllers have gene-regulatory roles that is important; rather it is the specificity of those roles. If a gene whose product acts at a particular point in an interaction cascade can be expressed at an ectopic location, then the whole cassette downstream of that may be expressed in the new location too. Notably, *engrailed* and *Distal-less* are both fairly far downstream in their respective pathways, so their activation in new locations may be just such a downstream consequence of the ectopic activation of their upstream controllers. However, this apparently neat model may be deceptive (see Box 1).

This line of argument has been taken one stage further in relation to limbs. It has been argued that the initial formation of proto-limbs in an originally limbless lineage is not something that will occur before, and in isolation from, the co-option of a cassette of outgrowth-inducing genes. Rather, these things will happen together[57], with the result that limbs can be thought of as 'axis paramorphs', that is, scaled-down, rotated, and simplified (for example, lacking endoderm) versions of the main anteroposterior body axis.

The key question now becomes: what kind of mutation causes the initially ectopic expression of cassettes of developmental genes to occur in a spatiotemporal pattern that affords some possibility of functional improvement and thus of being favoured by selection? Although we cannot yet answer this question, it is interesting to consider a possible link with developmental bias. The best way to do this is to contrast the evolutionary initiation of limb formation with an alternative case of developmental evolution often dealt with by quantitative geneticists and evolutionary ecologists, namely evolution of body size. There are two important differences between these two systems. Increased or decreased body size is a rather generalized change, and there is almost always heritable variation in a population around the mean size. Thus the situation is perhaps not one in which developmental bias is likely to have a role in determining the direction of evolutionary change; rather, this is set by selection. But the ectopic activation of whole cassettes of developmental genes involved in outgrowth formation in a particular spatiotemporal pattern is both much more specific and much less likely to be based on routinely occurring intrapopulation variation. Both of these features make it much more probable that developmental bias will have a role here. Indeed, we can extrapolate from this comparison and argue that developmental bias is most likely to have an important role when major evolutionary innovations are taking place.

Intraspecific origins

Although much of evolutionary developmental biology concerns large-scale comparisons, often between classes and phyla, it seems probable that all evolutionary changes ultimately begin as intraspecific variation—whether routine, as in the case of body size, or exceptional, as in the case of the origin of limbs. This variation occurs within particular populations living in particular environments. Thus, to investigate the evolutionary origins of interspecific differences, either in developmental genes or in the corresponding ontogenies and adult phenotypes, it is desirable to examine intraspecific variation in the same genes, ontogenies or phenotypes. So far, work in this area is rather limited. Nevertheless, two different approaches can already be distinguished. The first is to take a classic evo-devo model system and to add a population dimension. The second is to take a classic ecological genetics model system and to add a developmental dimension. We can now look at examples of these in turn.

Segmentation provides a good system for the evo-devo to population approach. An important finding here is that one of the *Hox* genes determining segment identity, *Ultrabithorax*, exhibits intraspecific variation, in the form of polymorphism, within

Box 1
Developmental pathways and their evolution

The concept of an interaction pathway — and the group of genes that encode its components — as comprising a sort of developmental cassette[56] that can be treated as a unit of evolutionary change is an attractive one. Different kinds of evolutionary process involving such units can be considered, including their divergence in separate lineages after speciation and their co-option for a new developmental purpose within a lineage, possibly coincident with the appearance of new morphological structures such as limbs (paramorphism[57]; see text). However, development is a complex process, and evolution, because of its stochastic and historical aspects, works in a messy way. Therefore, we need to be cautious in attempting to apply neat concepts like co-option of whole cassettes. Here, I outline some of the relevant complexities.

Complexities relating to development itself. A single developmental gene can have a very complex pattern of expression[85]. This may be aided by different promoters or other controlling elements regulating expression in different tissues or at different times, such as occurs in the *Pax6* gene, which is involved in eye development[86]. When we consider a whole pathway, another level of complexity appears. In the *Drosophila* segmentation pathway, the four groups of genes involved — maternal, gap, pair rule and segment polarity — were well characterized more than a decade ago[87]. It now seems that there are about ten genes per group, and so about forty in total. But the pattern of interaction is far from being a linear cascade. Some of the genes fall into two groups. For example, *hunchback*, which is normally thought of as a gap gene, is expressed both maternally and zygotically. And a newly discovered gene, *lilliputian*, seems to be maternal, yet mutants exhibit a pair-rule phenotype[88]. Also, even without this complication, the pair-rule group of genes is a double group in the sense that some of its members are 'primary' and some 'secondary'. Furthermore, as well as interactions between groups, there are interactions within them, and these take a variety of forms. At a smaller scale, all interactions are likely to involve variation in such parameters as the stability of RNA and protein molecules, although computer modelling[89] suggests that the overall result, in terms of the pattern of expression of segment polarity genes like *engrailed*, may be robust in the sense of being broadly unaffected by such variation.

No such pathway exists in isolation from the rest of the developing organism. This makes the use of the terms upstream and downstream problematic as descriptors of developmental genes and their products. It is essential to recognize that these are relative terms. For example, *engrailed* is often seen as a downstream gene because it falls into the segment polarity group. But a vast amount of morphological detail lies between the rudimentary proto-segments that exist when *engrailed* stripes first appear, and their final, fully elaborated form. So, from another perspective, *engrailed* is an upstream gene. The same argument can be used regarding *Distal-less*, which comes at the 'end' of a series of interactions (with *decapentaplegic* and *wingless* being immediately upstream[90]), but whose product goes on to control downstream processes. A converse argument can be made for *Pax6*, which is normally considered to be upstream: it is downsteam from another perspective[91].

Complexities that emerge from comparative studies. Broad-scale comparative studies extending beyond model systems to encompass many taxa usually start with, or have a bias towards, a particular gene in the pathway concerned. Often, this is for methodological reasons, such as the use of the monoclonal antibody 4D9 in detecting homologues of *engrailed*[24,25]. Given the widespread conservation of the segmental expression of this gene, it would have seemed reasonable to predict an equal or even greater degree of conservation of those genes upstream of it. This would be the pattern expected on the basis of a genetic version of von Baer's laws. So far, however, this prediction has not been borne out. One of the earliest-acting genes in the pathway — the anterior determinant *bicoid* — seems to be a relatively recent evolutionary innovation found only in a smallish clade[92]. Furthermore, both *bicoid* itself and its interaction with *hunchback* seem to be capable of quite rapid evolution[93,94]. It is also possible that the pair-rule component of the pathway is not highly conserved. However, the details of this pathway are still being investigated. It was initially thought that in the orthopteran *Schistocerca* (an insect with short-germ development) there was no two-segment periodicity of expression of homologues of the *Drosophila* pair-rule genes[95]; however, such expression has recently been demonstrated[96]. A similar expression pattern of pair-rule homologues has been confirmed for the beetle *Tribolium*[97], which is phylogenetically intermediate between *Schistocerca* and *Drosophila*.

Caution, but not despair. An understanding of the extraordinary complexity of ecosystems did not deter Darwin from his belief in a single, simple idea (natural selection) that we still regard as the central, 'external' principle of evolution, that is, the main mechanism responsible for adaptation of organisms to their environment. Our growing understanding of the many complexities of development similarly should not deter us from searching for simple general concepts that relate to the 'internal' aspect of evolution, including the ways in which developmental pathways evolve (such as an evolutionary trend towards hierarchical patterns of interaction[98,99]), and the ways in which the internal co-adaptation of organisms is maintained and enhanced.

outbred populations of *Drosophila melanogaster*[64]. This connects with a possible role for *Ultrabithorax* in the evolution of morphological differences between congeneric species[65] (*D. melanogaster* and *D. simulans*). Also, in relation to genes determining segment number rather than identity, a few groups of arthropods exhibit intraspecific variation in segment number, based on different numbers of *engrailed* expression stripes, in turn generated by variation in upstream genes. In one case (the centipede *Strigamia maritima*) comparative studies of populations living at different latitudes reveal a cline[66], and consequently suggest the action of selection.

Naturally occurring variation in a plant developmental gene provides another example of the evo-devo to population approach. Populations of *Arabidopsis thaliana* show considerable molecular variation at the CAULIFLOWER locus, which encodes a transcription factor (of the MADS-box family[67]) that controls the development of inflorescence meristems[68]. The pattern of variation found suggests the action of natural selection. Also, it appears that the artificial selection used by farmers to produce the domestic varieties of *Brassica oleracea* (cauliflower and broccoli) caused changes at the CAULIFLOWER locus[69], and presumably at other loci as well.

Butterfly wing pigmentation patterns provide a good system for the ecological genetics to evo-devo approach. Early studies (for example, on mimicry[70]) had both ecological and transmission genetics dimensions, but lacked information on developmental mechanisms. Recent studies of eyespots in the butterfly *Bicyclus anynana*[71] have added this missing dimension. One notable result to emerge from these studies is that the gene *Distal-less*, a familiar component of limb formation, is also involved in the generation of eyespots. Selection experiments reveal that this system is readily modifiable, and it looks as though the cassette concept may apply here, with changes in the expression patterns of interacting genes.

Towards a synthesis?

Thus far, I have discussed two of the five groups of concepts in Table 1 in detail, but will now briefly address the connections between all five of these groups, in addition to looking at approaches that might be most productive for the future.

The third, fourth and fifth groups of concepts in Table 1 relate to the interaction between conservation and change. Unsurprisingly, certain features favour one, while their opposites favour the other. For example, an early stage of development in which the embryo is developing as a coordinated whole (for example, the vertebrate neurula) is highly entrenched, resistant to selection, and thus widely conserved, although there are many exceptions to this pattern, especially in multi-stage life histories with free-living larval forms. In contrast, later stages (for example, limb buds) are often quasi-autonomous modules of enhanced evolvability, although caution is necessary because some of the genes involved may also have other developmental roles. When selectively driven change is possible, it is sometimes aided by mechanisms for the unmasking of variation; the selection itself may relate to external adaptation, internal co-adaptation, or a mixture of the two. In all cases of evolutionary change in ontogeny, developmental reprogramming is a necessary part of the process, and one way of achieving it is co-option of existing developmental genes for new roles. Developmental bias may interact with selection to govern the direction of change, although this is just an intriguing, but largely untested possibility.

With regard to future work, three approaches stand out as being particularly desirable. First we need to develop rigorous tests for developmental bias, as noted above. Second, we need some thorough case studies of downstream developmental genes to contrast with their upstream counterparts. (Even the most downstream of those that I have used as examples are quite far upstream in relation to the totality of development; see Box 1.) We need to ask whether both DNA sequences and gene functions are less conserved in downstream genes, especially those that can be shown to have no cryptic early role in addition to their late one. Third, we need to focus experimental work on the important concepts of co-option, cassettes and paramorphism. This can include both high-level comparative studies and studies focusing on mutations (for example, in *Drosophila*) that show ectopic expression of developmental genes that could provide a basis for the establishment of new roles. In other words, we should attack the problem at both ends—its origins in terms of mutation and reprogramming within species, and its long-term results, manifested as accumulated evolutionary divergence over hundreds of millions of years. □

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