Does evolution in body patterning genes drive morphological change—or vice versa?

Graham E. Budd

Summary

Increased understanding of the regulation of body patterning genes in development, especially the homeotic genes, has led to the revival of ideas suggesting that ''saltational'' modes of evolution are important. However, such models are problematic on the grounds of functional continuity and population genetics, and the more dramatic scenarios rely on an overinterpretation of the taxonomic hierarchy. This article proposes an alternative model for the evolution of *Hox* gene expression, stressing the need for incremental functional integration. One surprising implication of the model would be that mutations in *Hox* genes and their regulators have virtually no primary role in driving morphological evolution. Rather, morphological change through microevolutionary adaptation comes first, with *Hox* expression shifting only afterwards, presumably to make the building of the new body pattern more efficient or more stable. Such a model has affinities to Waddington's ''genetic assimilation'' but invokes discrete rather than continuous shifts in control of a particular morphology. *BioEssays* 21:326–332, 1999.

Introduction

The evolutionary emergence of the principal ''body plans'' of the animal phyla has, after a period in the doldrums, regained the central interest[1–3] it commanded at the end of the last century.[4,5] Today, a battery of data—from paleontology to developmental and cell biology—is being assembled to attack the problems it poses. Continued elucidation of the developmental processes that lead to the adult form of a metazoan has, in particular, led to a relatively common and distinctive view of how such a form might have evolved. Briefly, although gene interactions are actually highly complex, involving feedback and repeated gene expression at different times, the process may be viewed as a hierarchical cascade of transcriptional activation or silencing, which becomes more specific during the course of development.[6]

Correspondence to: Graham E. Budd, Department of Earth Sciences, Historical Geology and Palaeontology, Uppsala University, Norbyvägen 22, Uppsala S-752 36, Sweden. E-mail: graham.budd@pal.uu.se

Early genes set up domains that determine fundamental axes such as the anteroposterior and dorsoventral, along which broad body regions, segments, and specific segment identities are successively determined. The *Hox* genes have been a focus of particular interest, which act as intermediaries between the ''higher'' body-patterning genes and the downstream gene products that actually build structures such as limbs and eyes.[7] In such a hierarchy, mutations at a particular level necessarily affect processes that are initiated later in development, but not before. Thus, the higher the level of mutation, the more profound the effects one might expect it to have on adult phenotype.[3] Whereas mutations in downstream genes (and low-level *Hox* genes) might cause subtle shifts in morphology, *Hox* gene mutations can also cause wholesale reassignment of local identity (e.g., the transformation of an arthropod segment into a different one). Even higher mutations, at the level of the ''segmentation'' genes, for example, cause more or less grotesque malformations of the entire body pattern.[8]

Although these mutations and their effects are easily demonstrated in the laboratory, have they had any effect on

BioEssays 21:326–332, © 1999 John Wiley & Sons, Inc.

the actual evolution of body plans through time, from their apparent establishment near the beginning of the Phanerozoic[1]? The literalist answer has been to invoke such massive mutations as indeed providing the basis for new body plans.[2,9,10] Given that differences in the homeotic gene hierarchy broadly correspond to the taxonomic hierarchy, there has been a temptation to equate the two.[2,10] It has also been recently argued that changes in large-scale expression of *Hox* genes—or even in numbers of *Hox* genes—were causally effective in driving profound reorganization of the body plan.[10] In such a view, ''class'' as well as ''phylum'' level differences would be established first in an evolutionary divergence, followed by differences at the ''order,'' ''family,'' and ''genus'' levels—shifts that become increasingly trivial.

Some support for such views has been gleaned from studies demonstrating (for example) correlation between identity of crustacean thoracic limbs and homeotic domain boundaries,[11] evidence that has been taken to suggest that changes in homeotic gene expression are causally correlated with evolution of arthropod body plans. Certainly, the fossil record strongly demonstrates a history of segment diversification in arthropods,[12,13] which could be read as documenting the history of homeotic mutations.

Overall, such ''neo-Goldschmidtian'' models are attractive, in that they suggest that dramatic homeotic transformations may play a crucial role in evolution. However, they create severe difficulties from an adaptive point of view, as they run foul of ''Fisher's principle''[3,14] that macromutations are unlikely to convey adaptive advantage. For example, the feeding appendages of crustaceans typically form functionally complex and integrated units,[15] and it would require modification to an entire set of appendages before a homeotically transformed appendage could be functionally integrated into them to advantage. Thus, even if single mutations in homeotic genes can provide a working and radically different appendage morphology in a particular segment, this is only half the story of evolutionary success. At least as important would be integration of the new morphology into the functional complex that is an animal as a whole. Sudden acquisition of a new feeding appendage would be extremely unlikely to be advantageous unless the other feeding appendages were themselves adapted to incorporate it into their often complex mode of operation, to say nothing of corresponding changes to nerves and muscles that would be required.[16,17] As a further example, ''bithoracic'' flies are incapable of flight, not only because homeotic mutations that generate the extra pair of wings do not also transform the appropriate musculature,[16] but also because the overall aerodynamic design of the fly has evolved for one and not two pairs of wings.

The concatenation of simultaneous change required to generate a successful saltationally evolved organism thus seems to stretch to the limit the idea that, give enough time, such a felicitious combination of mutations will occur. These problems become even more acute at higher levels, where the literalist reading of homeotic or segmentation gene mutations or duplications would seem to imply dramatic new body plans suddenly emerging and, at the extreme, scenarios such as the development of, for example, a ''proto-mollusk'' from a nematode have been contemplated.[10]

Objections to saltational models

Apart from the difficulties posed by functional arguments discussed above, saltational models also seem to rely on certain important (but rarely discussed) conceptual misapprehensions, of which there are at least four. The first is that one can doubt whether appeal to very rare combinations of events should be counted as a scientific hypothesis at all. Clearly, the logical possibility of a complex ''body plan'' suddenly appearing cannot be ruled out. Nevertheless, models that invoke such events are in one sense only narrative and ad hoc: by treating the origins of body plans as a ''black box'' of extremely rare random change, they are not thereby offering an explanation of these events, merely postulating that they might occur— often in the face of what other evidence might suggest. Worse, because they make no appeal to general principles, such ''explanations'' cannot have any predictive power. More satisfactory would be a model of body plan origin that involves an appeal to more general evolutionary principles, such as population genetics and natural selection.

The second problem is the invalid application of explanations appropriate to population genetics to evolutionary patterns. If a fly with one pair of wings produces an offspring that possesses two pairs, one very likely explanation is that one or more homeotic mutations have occurred. However, consider two families of flies, one characterized by one pair of wings, the other with two wings: are we to say that a homeotic mutation was responsible for the shift between the two states? Without knowledge of the detailed evolutionary routes by which each family came about, such a suggestion could be ruled neither in nor out. In other words, mere documentation of a homeotic pattern in a set of animals is not the same as demonstrating homeotic mutations.

Third, these models place an over-reliance on the taxonomic hierarchy as a guide to evolution (Fig. 1), suggesting that the origins of phyla involve ''big'' mutations, origins of classes slighter ''smaller'' mutations, and so on.[3,10] Classifications by definition embody perceived differences (or, more properly, shared similarities) between organisms. It is therefore no surprise that higher levels of a taxonomic hierarchy embody relatively profound, and the lower levels, relatively minor differences: by definition that is the job they are required to perform. However, this purely classificatory scheme cannot give any clues as to how these differences emerged through time, and the fact that differences at the various developmental levels can be broadly correlated with ''classes,'' and so on tells us nothing on this subject. In fact, it would be

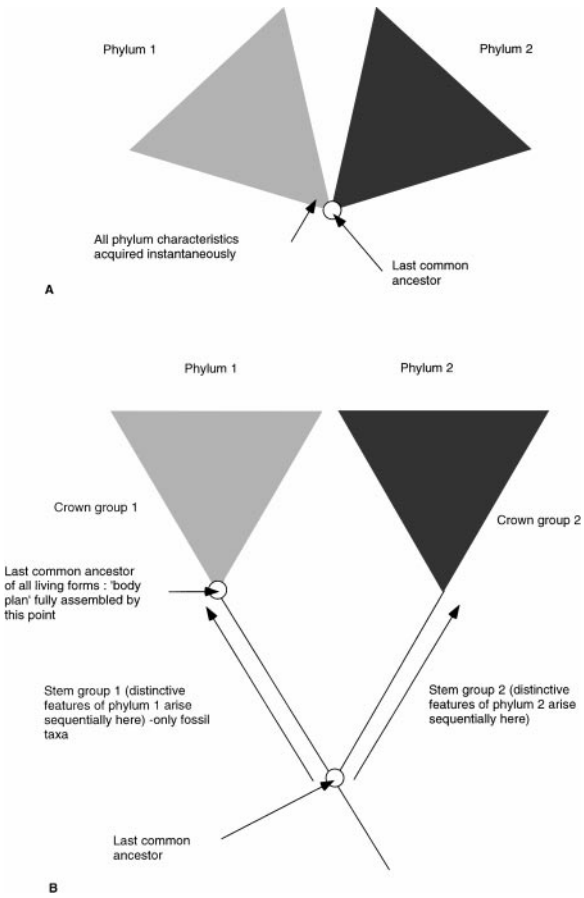

Figure 1. Differing perspectives of classification alter notions of how body-plans evolve. **A:** By considering the classification scheme as being all-encompassing, the essential features of body plans are forced suddenly to accumulate at the base of the new group, giving an emphasis to saltational notions of evolution. Here, the postulated sudden appearance of a phylum might imply a dramatic mechanism such as a change in the number of Hox genes.[8] B: Crown- and stem-group concepts. Although the extant phyla are still defined as they are in A, here it is recognized that they each have a stem group of entirely extinct taxa leading up to them, from an initially identical ancestral form. The stem group provides an ''evolutionary space'' in which major body-plan differences can progressively accumulate without sudden reorganizations.

more surprising if systematic developmental differences were not broadly correlated with the taxonomic hierarchy. There is a logical flaw in attempts to talk about ''phylum level evolution''(e.g., refs. (3) and (10)). If the taxonomic hierarchy truly reflected a ''top-down'' pattern of evolution, so that ''body plans'' were suddenly generated, one would be entitled to ask what sorts of animals were being thus generated. If a proto-mollusk suddenly emerged, one might want to inquire as to what the details of its morphology were—where it had sensory papillae or a shell, what shape its gills were, and so on. In this scenario, there could be no answer to these questions, because these would be ''order'' or ''family'' features, yet to emerge. Even so, such a model of evolution might be the only one available if the modern classificatory system were all-encompassing, so that all organisms could be placed in a particular extant phylum, or a particular class. However, it must be stressed that many fossil organisms do not lie in these groupings, instead forming ''stem groups'' to the extant ''crown groups,'' which consist of the last common ancestor of all the living forms plus all its descendants.[18] Unlike the static concept of a phylum, stem groups can be shown to demonstrate successive evolutionary divergence from a last common ancestor.[19–21] Thus they offer an ''evolutionary space'' in which major body-plan differences may accumulate without postulating dramatic morphological shifts suddenly occurring at the bases of the phyla or classes (Fig. 1).

The fourth flaw in such saltational models—and the potential key to their replacement—is that they assume that the effects of mutations at a particular level in the developmental hierarchy have always been the same. The very nature of regulatory genes, however, means that their mutations do not have direct morphological effects, but only affect the expression of genes that are regulated by them. If these lower genes have themselves evolved, the effects of mutations in the regulatory framework will also have shifted through time. For example, demonstration of dramatic morphological effects of a particular mutation such as *Haltere mimic*, which converts the wing of *Drosophila* into a haltere[22] does not mean that a mutation at this locus has always had this effect. Before halteres originally evolved, it would clearly have caused a different morphological change. As will be proposed below, not only can scenarios be imagined in which, for example, homeotic mutations have little or no morphological effect, these situations might arise through selective pressures. Even if homeotic genes have always played a role in setting up a positional grid within which morphogenesis takes place, their ''homeotic'' nature (in the sense that their mutations cause large-scale ectopic expression of morphology) is not something essential to the genes themselves but is rather a matter of contingency. This in turn suggests that the ''homeotic'' character of homeotic genes could be a derived feature in particular lineages.

The idea that quantum large-scale changes in *Hox* gene expression are causally effective for morphological change is therefore at best problematic. It invokes implausible simultaneous shifts in form and function (of which the homeotic transformation itself might be only one among many), and it embodies the fallacy that mutations in regulative genes and their enhancers had the same morphological effect in the past as they do now, whereas in fact their effect really relies on the

role of the genes they regulate, a role that is sure to have shifted.

Gradual modification of homeotic domains?

There are two broad alternatives to this scenario. The first is that *Hox* spatiotemporal expression has shifted gradually in the evolution of development, suggesting that the apparently distinct and well-defined domains of gene expression in extant animals have in previously been much more blurred,[11,23] a model supported by of the low degree of penetrance of many homeotic mutations. In other words, the traditional ''selector gene'' model of homeotic gene function is to a certain extent illusory (see discussion in ref. (21)), and a model of more fluid homeotic gene evolution involving changes in both region of expression and degree of penetrance should be contemplated.

Although attractive, this model nevertheless presents some problems. First, it implies that the relatively clear-cut nature of homeotic domains in living organisms is a feature that has been separately derived every time a segment has changed identity, and it is unclear, under this scheme, what the benefits of such sharpness of segment definition would be. Indeed, if the evolution of segment identity has occurred only by this gradual process, why is it possible to recognise distinct body region boundaries at all? Second, it is unclear how such a model could, in principle, be applied to the evolution of other body-patterning genes such as the ''segmentation'' genes,[8] which present essentially the same problems. Third, the very existence of homeotic mutations that do decide the identities of entire regions, coupled with this generally distinct nature of homeotic domains, suggests that a real level of regulation exists that is independent of minor ectopic gene expression,[10] and apparent evolution at this level requires explanation. Even in this case, though, there is still no need to invoke implausible macromutation. It is possible to maintain the precision of homeotic domains while only allowing adaptive microevolution. In the following model, rather than changes in regulation of homeotic genes causing segment identity shift, they are enabled by them, so that the morphology changes before the underpinning genetic mechanism does.

''Homeotic takeover''

The key to such a model is the recognition that although morphology is determined hierarchically, each level of the hierarchy has a certain degree of flexibility built into it. This flexibility arises both because of varying degrees of gene penetrance[23] and because of changes that might occur at lower levels in the developmental hierarchy. If as a result of this flexibility, a particular level is able to mimic the morphological effects normally produced by a level above it, the function of effective cause of the morphology may be transferred from the lower level to the higher, which here is referred to as ''homeotic takeover.'' For example, in the case of the crustacean data previously discussed,[11] adaptive modification of a combination of more localized homeotic gene regulators and downstream gene expression, which are known to be able to produce variations in segment and limb morphology,[24] could lead to a thoracic appendage adaptively evolving to mimic a more anterior feeding appendage, while maintaining a broader homeotic identity in common with other, unmodified, thoracic limbs (Fig. 2). Crucially, during this transformation, the pre-existing feeding appendages, neural structure, and other relevant morphological features could also progressively be modified in order to accommodate the new feeding appendage. At such a point when the transforming appendage is functionally and morphologically virtually identical to the original feeding appendages, the shift in overall homeotic domain may occur, with minimal morphological and functional disruption (Fig. 2).

Some concrete data supporting this view come from the experimental work of Waddington and colleagues (see ref. (25) and references therein). This work stresses that the same phenotype can be produced by different genetic mechanisms, as well as environmentally. For example, his work on genetic assimilation of *Bithorax* suggests that two wing-pair flies can be progresssively produced by polygenic changes spread across three chromosomes, as well as by the more familiar route of loss-of-function mutations at the *Ultrabithorax* locus. In evolutionary terms, one can envisage the first process occurring over several generations, involving many minor mutations (or the bringing to light of hitherto unexpressed genetic variation[25]), with the accumulated changes fixed in the population either by slight action of natural selection or, if they are more neutral in effect, by drift. When phenocopies of extreme bithoracic flies have been formed in this way, control of the phenotype could then shift to the *Ubx* locus by the appropriate mutations, without any dramatic morphological shifts.

Such a hypothesis allows the quantized shifts in homeotic gene expression known from mutation studies in the extant fauna to be integrated into a microveolutionary model of evolution that requires major regulatory reorganization to be anticipated by adaptive changes. When homeotic mutations occur, their morphological effects are minimized. These shifts in regulatory organization might themselves be selectively favored, not because of their (minimal) morphological consequences, but because they would represent the most efficient, stable and simple way of building a particular body plan. Indeed, this selection pressure may in principle explain both the initial assembly and subsequent maintainance of *Hox* gene clusters and their *cis*-regulation.[3,26]

One difficulty with this model is that the original differentiation of segments within a broad homeotic domain relies on there being some sort of initial developmental difference existing between segments (selective pressures could other-

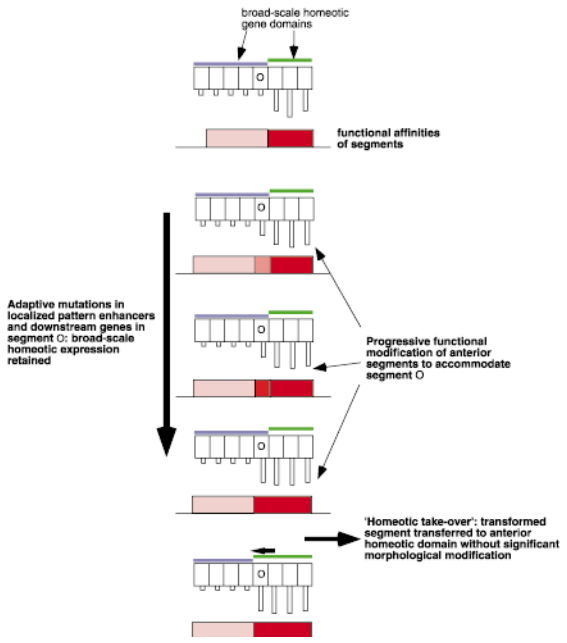

Figure 2. "Homeotic takeover." Morphological anticipation of homeotic transformation in the evolution of arthropod trunk segments. Schematic diagrams represent trunks with appendages, divided into two homeotic domains. Graphs depict level of functional integration: *pink–red*, functional affinity of different segments. Modulation of local homeotic gene regulation and downstream genes enables gradualistic adaptive modification of segment O while also allowing it to be functionally integrated with other appendages. Shift in large-scale homeotic domains (caused by mutations in high-level regulation) can then occur and be selected for on efficiency grounds without morphological or functional disruption.

wise only act on all the segments at once, and no differentiation could occur). This additional information could conceivably come from three sources: nonhomeotic, cues from other morphological structures such as the gut[27]; by genetic assimilation of original developmental plasticity; or by use of a simple anteroposterior gradient along the animal. In many basal arthropods, such as the trilobites, although the trunk segments are all essentially identical (and plausibly belong in the same homeotic domain), they nevertheless show a distinct anteroposterior size gradient.

The "homeotic takeover" model could be tested in two ways. First, it is important to discover whether or not the regulatory roles of *Hox* genes are organized in a genuinely hierarchical manner, as has been suggested.[10] This is an important empirical question, and answering it would allow the question of whether minor changes at the boundaries of homeotic domains are variations within a particular domain (but at a lower level in the hierarchy), or modifications of it to be addressed.[23] For example, arthropod segments of intermediate morphology, such as that bearing the second thoracic limb of the mysid *Mysidium colombiae*[11], require investigation to see what homeotic identity they possess. The report of weak *Ubx-abdA* expression in this limb[11] implies that a quantum change in identity in this case may be ruled out. What remains to be seen is whether such intermediate *Hox* gene expression should be seen as the result of blurring between two homeotic domains (perhaps by a mosaic of expression patterns [23]) or whether it indicates gene expression shifts at lower homeotic levels, whilst still retaining a broader thoracic identity determined at a higher level in the homeotic hierarchy.[10] A clearer cases is provided by the hind limbs of grasshoppers, which belong to segments that clearly retain an overall thoracic identity while exhibiting a subset of morphology that cannot be easily regarded as the result of a mosaic expression of different homeotic domains. If, instead of enlargement and specialization, these limbs had been progressively modified to become smaller, and eventually to disappear, would this process be seen as identical to the one that made them larger, or would it be seen as progressive mosaic mixing between the thoracic and abdominal domains? In the first case, the segment would still have a thoracic identity; in the second an abdominal one. These examples suggest that the interaction between homeotic genes and the downstream genes they regulate is complex, and may be the important determining factor that would allow large-scale homeotic mutations to be anticipated morphologically. A second test of this model, then, would be to look for cases of "homeotic mimicry" in which segments mimicked a new homeotic domain while retaining their own homeotic identity. Such cases may be difficult to find, because selective pressures may make such a state inherently unstable. The change to a more efficient manner of building that body plan would be adaptively advantageous.

Finally, this model predicts that evolutionarily significant homeosis should only take place at the boundaries between homeotic domains where segments can be transferred between functional complexes (mutations such as *Antennapedia* could not take place by this route). Homeotic identity should be continuous and not split up among many segments. The rare exceptions to this rule (e.g., modification of the seventh trunk limb pairs of diplopods into gonopods, between two presumably different homeotic domains, which nevertheless share an identical morphology (e.g., 28, fig.1) provide cases to test whether different homeotic domains can nevertheless support identical morphologies.

Discussion and conclusions

Evolution of complex biological systems, of which animals are an excellent example, presents two related problems. The first is how function of a particular component may shift while its old function is still maintained in the system as a whole.

The second is how new integrated complexes evolve without the sudden appearance of their interdependent parts. Evolution of the genetic hierarchy poses one further problem, that changes within it appear to be at least partially quantized, suggesting that the morphological changes associated with them are similarly modular (and thus an aspect of the second problem). The key to resolution of these problems in general is the recognition of the importance of redundancy,[29,30] typically developing by gene duplication, or by the evolution of repeated expression domains without loss of previous function. These scenarios solve the first problem because the old function of a gene complex may by maintained by a duplicate, or by the same gene with a multiple spatiotemporal expresssion pattern. However, they do not solve the second problem, of how the subsequent evolution occurs adaptively if genetic change is modular. The answer that is often given—that lower levels in the morphogenetic hierarchy shift after high-level mutations to accommodate them[3,10]—seems to rely on a degree of teleological foresight on the part of the organism. Being en route to a highly effective morphology in the future does not count against being extremely maladaptive in the present.

The model proposed here attempts to obviate this difficulty by extending the concept of redundancy, not within the same level of the developmental hierarchy (as would a gene duplication model), but between levels. Mutations that would in an ancestral form have a large morphological effect can in this case be ameliorated by their effects progressively evolving to resemble closely the normal morphology. This shift comes about by mutations at lower levels in the hierarchy that are more likely to be adaptively advantageous.

Given that the broad hierarchy of homeotic gene expression at least crudely corresponds to the traditional taxonomic system of phyla, orders and classes, this mechanism also offers a route by which microevolutionary changes may be passed up through the taxonomic hierarchy. In each case, the morphological effects of a particular high-level mutation could be mimicked by adaptive change in downstream genes, thus minimising the morphological effect of such a mutation. This resolves the paradox that the broadest divisions of animals are often distinguished by differences at high levels in the developmental hierarchy,[3,10] even though it would seem that changes in the responsible genes should cause calamitously maladaptive change, without having effectively to deny that such a hierarchy exists.[23] Thus, although the model discussed herein has been developed primarily with reference to the homeotic genes, it is also broadly applicable at all levels of the developmental hierarchy. One further point of interest is that an unusual form of constraint may be implied by this model. It is possible that only those high-level mutations whose effects can be mimicked by changes below them in the hierarchy do actually occur in evolution, and that some forms of high-level mutation are in effect only achievable by the production of ''monsters''—and thus impossible. However, it is unclear whether in fact any mutations do fall into this catagory. Nevertheless, investigation of the differing effects of high-level mutations—from vertebrate limbs [31] to early insect[32] and echinoderm [33] development—may elucidate the ways in which such mutations can occur without undue morphological disruption. As a corollary, the commonly expressed view that a ''selector gene'' view of the developmental hierarchy implies that once the hierarchy was set up, animal development was effectively frozen[3,30] appears to be incorrect. Although the hierarchy poses a short-term constraint on the sorts of evolution of allowed (hopeful monsters would be banned), the model suggests how in the long-term this barrier could be surmounted (microevolutionary changes can pass up through the hierarchy).

The model of ''homeotic takeover'' proposed here offers some interesting parallels to Waddington's ''genetic assimilation'' whereby environmentally enduced morphology can be assimilated into the genome (through selection) by progressive shifting of the causal basis of the morphology from environmental triggers to downstream genes.[25] However, whereas genetic assimilation models envisage a gradual shift of control toward the genome, homeotic takeover suggests a quantized step of control from downstream up to homeotic and other genes. It nevertheless offers a route by which recently genetically assimilated characters can become further stabilized within the genome, and may thus plausibly be seen as the ''end result'' of the genetic control of once environmentally controlled characters.

Macromutations may be inducible in laboratory animals, but in this scenario, there is no need to consider them as the mechanism behind a neo-Goldschmidtian ''hopeful monster'' style of evolution. Rather than ''[driving] deep reorganizations of the body plan'', or ''diversity [being] dependent on a spectacularly successful evolutionary experiment with *Hox* domains of expression,''[10] homeotic transformations (and, by implication, other changes in the developmental hierarchy) may evolutionarily have had very little to do with causing morphological change. Rather, mutations in regulatory architecture may have been selected for because they increased the efficiency of building a particular body plan, after morphological innovation and reorganization has already occurred.

Acknowledgments

I gratefully acknowledge fruitful discussions with many colleagues, including Michael Akam, Lennart Olsson, Simon Conway Morris, and Ruth Dewel (who criticized earlier drafts), and Sean Carroll, which is not to imply that they necessarily endorse the views herein. Two referees and the Editor made many constructive comments on the manuscript. This work was carried out during tenure of an international research fellowship from the Palaeontological Museum, Uppsala University.

References

1. Conway Morris S. The crucible of creation. Oxford: Oxford University Press; 1998.
2. Gerhart J, Kirschner M. Cells, embryos, and evolution. Malden, MA: Blackwell Scientific; 1997.
3. Arthur W. The origin of animal body plans. Cambridge: Cambridge University Press; 1997.
4. Haeckel E. Generelle Morphologie der Organismen: Allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformite Descendenz-Theorie. 2 vol. Berlin: Georg Reimer; 1866.
5. Bateson W. Materials for the study of variation. London: Macmillan; 1894.
6. Lawrence PA. The making of a fly. Oxford: Blackwell Scientific; 1992.
7. McGinnis W, Krumlauf R. Homeobox genes and axial patterning. Cell 1992;68:283–302.
8. Nüsslein-Volhard C, Wieschhaus E. Mutations affecting segment number and polarity in *Drosophila.* Nature 1980;287:795–801.
9. Jacobs DK. Selector genes and the Cambrian radiation of *Bilateria.* Proc Natl Acad Sci USA 1990;87:4406–4410.
10. Gellon G, McGinnis W. Shaping animal body plans in development and evolution by modulation of *Hox* expression patterns. BioEssays 1998;20: 116–125.
11. Averof M, Patel NH. Crustacean appendage evolution associated with changes in *Hox* gene expression. Nature 388:682–686.
12. Wills MA, Briggs DEG, Fortey RA. Evolutionary correlates of arthropod tagmosis: scrambled legs. Syst Ass Spec Vol 1997;55:57–65.
13. Budd GE. Ecology of Cambrian lobopods and non-trilobite arthropods. In: Riding R, Zhuravlev A, editors. Ecology of the Cambrian radiation. Cambridge: Columbia University Press; (in press).
14. Fischer RA. The genetical theory of natural selection. Oxford: Clarendon Press; 1930.
15. Barker PL, Gibson R. Observations on the feeding mechanism, structure of the gill and digestive physiology of the European Lobster *Homarus gammarus.* Exp Mar Biol Ecol 1977;26:297–324.
16. Roy S, Shashidhara LS, Vijayraghavan K. Muscles in the *Drosophila* second thoracic segment are patterned independently of autonomous homeotic gene function. Curr Biol 1997;7:222–227.
17. Schneiderman AM, Tao ML, Wyman RJ. Duplication of the escape-response neural pathway by mutation of bithorax-complex. Dev Biol 1993;157:455–473.
18. Jefferies RPS. The origin of the chordates—a methodological essay. In: House MR, editor. The origin of the major invertebrate groups. London: Academic Press; 1979, p 443–477.
19. Valentine JW, Hamilton H. Body plans, phyla and arthropods. Syst Ass Spec Vol 1997;55:1–9.
20. Budd GE. The morphology of *Opabinia regalis* and the reconstruction of the arthropod stem-group. Lethaia 1996;29:1–14.
21. Budd GE. Arthropod body plan evolution in the Cambrian with an example from anomalocaridid muscle. Lethaia 1998;31:197–210.
22. Duncan I. The Bithorax complex. Annu Rev Genet 1987;21:285–319.
23. Akam M. *Hox* genes, homeosis and the evolution of segment identity: no need for hopeless monsters. Int J Dev Biol 1998;42:445–451.
24. Carroll SB, Weatherbee S, Langeland J. Homeotic genes and the regulation and evolution of insect wing number. Nature 1995;375:58–61.
25. Waddington CH. Genetic assimilation. Adv Genet 1961;10:257–293.
26. Brooke NM, Garcia-Fernàndez J, Holland PWH. The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. Nature 1998;392:920–922.
27. Coates MI, Cohn MJ. Fins, limbs and tails: outgrowths and axial patterning in vertebrate evolution. BioEssays 1998;20:371–381.
28. Causey NB. The phylogeny of the family Paraiulidae (Paraiuloidea: Blaniulidea: Uilida: Diplopoda). Symp Zool Soc Lond 1974;32:23–39.
29. Averof A, Akam M. *Hox* genes and the diversification of insect and crustacean body plans. Nature 1995;376:420–423.
30. Raff RA. The shape of life. Chicago: University of Chicago Press; 1996.
31. Dolle P, Dierich A, Lemeur M, Schimmang T, Schuhbaur B, Chambon P, Duboule D. Disruption of the Hoxd-13 gene induces localized heterochrony leading to mice with neotenic limbs. Cell 1993;75:431–441.
32. Grbic M, Nagy LM, Strand MR. Development of polyembryonic insects: a major departure from typical insect embryogenesis. Dev Genes Evol 1998;2098:69–81.
33. Wray GA. The evolution of cell lineage in echinoderms. Am Zool 1994;34: 353–363.