When is homology not homology?

Gregory A Wray* and Ehab Abouheif†

Although genes have specific phenotypic consequences in a given species, this functional relationship can clearly change during the course of evolution. Many cases of evolutionary dissociations between homologous genes and homologous morphological features are now known. These dissociations have interesting and important implications for understanding the genetic basis for evolutionary change in morphology.

Addresses
Department of Ecology & Evolution, State University of New York, Stony Brook, New York 11794-5245, USA
*e-mail: gwray@life.bio.sunysb.edu
†e-mail: ehab@life.bio.sunysb.edu

Current Opinion in Genetics & Development 1998, **8**:675–680

http://biomednet.com/elecref/0959437X00800675

Introduction

One of the fascinating realizations to emerge during the past two decades of developmental genetics is the extraordinary complexity of the relationship between genotype and phenotype. This complexity has important, and quite interesting, implications for understanding homology, which is the central concept of comparative biology. Gavin de Beer was among the first to recognize the evolutionary implications of the complexity of the genotype–phenotype relationship: in an insightful essay published in 1971 [1], he noted that homologous genes do not necessarily encode homologous structures and that homologous structures need not be encoded by homologous genes. These were remarkably prescient inferences, as they were made at a time when relatively few pertinent data were available, and before molecular techniques transformed developmental biology.

In the quarter-century since de Beer published his essay, many additional examples have corroborated his conclusions. It is now clear that several distinct kinds of dissociations can evolve between homologous genes and homologous aspects of morphology [2,3•,4•,5] (Figure 1). In such cases, homology at one level of biological organization does not reflect homology at another [6,7••]. Furthermore, evolutionary dissociations of this kind may be more common than is generally appreciated, particularly when comparisons are made across deep phylogenetic divides. Evolutionary dissociations between genotype and phenotype limit to some extent the usefulness of gene expression domains for making inferences about the evolutionary history of morphological structures [4•,5,6]. On the other hand, these same dissociations provide an extraordinarily valuable window into understanding the genetic basis for morphological evolution [2,3•,4•,5].

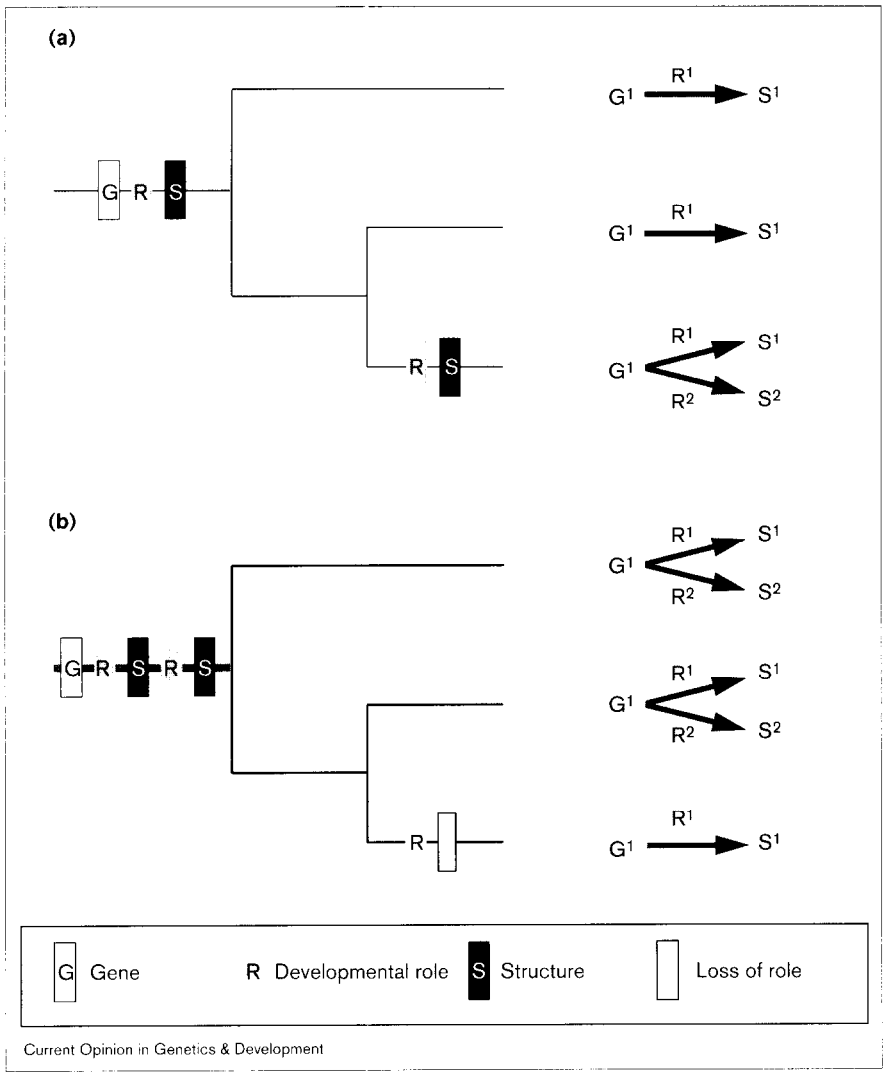
It is worth pausing briefly to consider what is meant by the term 'homology' before proceeding to a discussion of these issues. Although most biologists seem to have a good intuitive feel for the concept of homology, the literature on homology is famously full of philosophical and methodological debates. The clearest, most practical, and most widely accepted definition of the term homology is simply the presence of a feature in the most recent common ancestor of two species [8,9]. This definition has the added virtues of being applicable to any feature of biological organization (molecular, behavioral, developmental, etc.) and of forcing one to be explicit about the phylogenetic history of the features of interest [7••,10]. As ancestors are rarely available for direct examination, homology is usually a hypothesis about evolutionary history rather than a direct observation [6,7••,9,10]. We will use the term 'homology' in this formal sense, to mean a hypothesis that a particular similarity in two extant species predates their evolutionary divergence.

Homologous genes, non-homologous morphology

The more that is learned about regulatory genes, the clearer it becomes that few, if any, are dedicated to a single developmental task [3•,4•,5]. For instance, the Notch signalling system is utilized on many separate occasions during the development of *Drosophila melanogaster*. These include the production of structures that are clearly not homologous, such as wings, ommatidia, and bristles [3•,11,12]. In other animals, homologous elements of this signalling pathway are also used repeatedly during development, again in structures that are not homologous, such as feathers and T-lymphocytes (Table 1) [3•,13,14]. Given the diversity of uses to which this signalling system has been put during the course of animal evolution, it is difficult (at least from existing data) to guess what its ancestral role may have been. (Note that Table 1 is a only partial list of the known developmental roles of the Notch signalling system.) The same conclusions emerge from a consideration of other intercellular signalling systems, such as those mediated by hedgehog, TGF-β, and Wnt family members [3•,5]. There are almost certainly many more intercellular signalling events than there are intercellular signalling systems in most metazoans, implying numerous cases where a homologous gene has become involved in the development of a non-homologous structure (Figure 1a).

This situation is not unique to signalling proteins. For example, *engrailed* — which encodes a homeodomain transcription factor — regulates embryonic patterning, gut differentiation, and neurogenesis (among other things) in *Drosophila* [15,16]; in *Mus musculus* it is involved in patterning the brain and somite differentiation (among other things) [17]; and in the echinoderm *Amphipholis squamata*, its expression is associated with skeletogenesis and neuronal

Figure 1



Evolutionary dissociations between homologous genes and homologous structures. The evolutionary histories of developmental regulatory genes, their developmental roles, and the structures to which they give rise are not always congruent. Most developmental regulatory genes of metazoans are clearly more ancient than some of their current developmental roles. For instance, homeodomain transcription factors predate the origin of the metazoans [47] but are involved in patterning many structural features unique to particular metazoan groups [14,15,18•,22••,45,46•]. Developmental roles have been gained (**a**) and lost (**b**) on many occasions (for examples, see text and Figure 2). The acquisition of new developmental roles may be important in the origin of evolutionary novelties, but can confound the use of gene expression to identify homologous structures. G, gene; R, developmental role; S, structure.

differentiation [18•]. Similarly, the transcription factor encoded by *hunchback* is involved in embryonic pattern formation and development of the central nervous system in *Drosophila*, whereas its expression in the leech *Helobdella triserialis* implies a different set of roles [19]. Both transcription factors participate in developmental processes that produce structures that are certainly not homologous and, again, it is not immediately clear what the ancestral roles of these genes may have been. The same general conclusions emerge from a comparison of many other transcription factors for which detailed studies have been carried out. As with signalling systems, transcription factors are used over and over again, not just during the development of a single organism but throughout evolution [4•,5].

It is worth emphasizing that none of the examples above is incompatible with the observation that developmental roles are often conserved in evolution. Many examples of role conservation are known and have been discussed widely [3•,20]. The most famous example is the role that *Hox* genes play in patterning the anteroposterior axes of insects and vertebrates [21]. Even in cases such as this, however, only one developmental role appears to have been conserved while several other roles are clearly not. For instance, *Hox* genes are involved in patterning appendages in vertebrates but not in arthropods. In addition, in vertebrates they exhibit the classic nested domains of expression in somites, rhombomeres, and the reproductive tract [21,22••,23], structures that are not homologous to each other and some of which are unique to vertebrates (therefore necessarily representing recruited, or novel, developmental roles). The one conserved role is memorable because it is so striking but roles that are not conserved between phyla are more numerous than the ones that are. Although much attention has been devoted to conserved roles, non-conserved roles are clearly also significant, both developmentally and evolutionarily.

As de Beer wrote nearly 30 years ago [1], "characters controlled by identical genes are not necessarily homologous".

Table 1

A partial list of the many known roles of the Notch signalling pathway.

Phylum	Developmental processes[a]
Arthropoda	Patterning wing imaginal disks. Specifying distinct bristle organ cell fates. Specifying distinct ommatidial cell fates.
Nematoda	Specifying distinct AB lineage cell fates. Specifying vulval cell fates.
Chordata	Patterning feather primordia. Specifying distinct T-lymphocyte cell fates. Specifying various neuronal cell fates.

[a]From [3•,11–13].

The association between a homologous gene and a homologous aspect of phenotype can be conserved but often it is not. Even leaving aside evolutionary comparisons, the repeated use of a gene during the development of a single organism to build non-homologous structures is probably the rule rather than the exception [3•,4•]. Although instances of conservation are often impressive, they are only part of the complex evolutionary relationship between genotype and phenotype.

Non-homologous genes, homologous morphology

A growing number of cases demonstrate that the inverse situation, where genes that are not homologous encode a homologous morphological feature, can also occur. One of the first cases to be recognized involves evolutionary changes in the developmental roles of *even-skipped* (*eve*), which encodes a homeodomain transcription factor.

The eponymous role of *eve* in *Drosophila*, where it was first identified and characterized, is pattern formation: expression occurs in a 'pair-rule' pattern during embryogenesis and is required for the correct development of every other segment [24]. Like other regulatory genes, *eve* has additional developmental roles in *Drosophila*, including neurogenesis [25] and less well-characterized roles in the anal pad and dorsal mesoderm [26].

Surprisingly, *eve* has lost its pair-rule patterning role within the insects: in the locust *Schistocerca americana* and in the wasp *Aphidius ervi* there is no segmentally reiterated pattern of expression within the ectoderm, although the later neurogenic role is present (Figure 2a) [27,28•]. In both cases, homologous structures (segments) are present but at least one homologous gene no longer contributes to their development. Indeed, in the case of the wasp, the relatively closely related species *Bracon hebetor* has the usual pair-rule pattern of expression within the ectoderm [28•]. The loss of a segmentation role for *eve* in *Aphidius* is probably a result of its highly modified early development as an endoparasite rather than with any modification in adult morphology [28•].

Other cases have been documented within the insects. *Sex-lethal* (*Sxl*) is a 'master regulatory gene' that controls sex determination in *Drosophila melanogaster* through a well-characterized pathway of alternative splicing [29]. This pathway appears to be present in at least two other *Drosophila* species, based on alternate splicing of transcripts [30]. In several other dipterans — including *Ceratitis capitata* and *Musca domestica* — however, *Sxl* is almost certainly not involved in sex determination: although the gene is present, it is not alternatively spliced and is not expressed at the correct time [31•,32•]. On the basis of the phylogenetic distribution of these data, the sex-determination role of *Sxl* in *Drosophila* is almost certainly the derived case (Figure 2b). This is the reverse situation from the *eve* example: here a gene has become involved in a developmental process after that process first evolved.

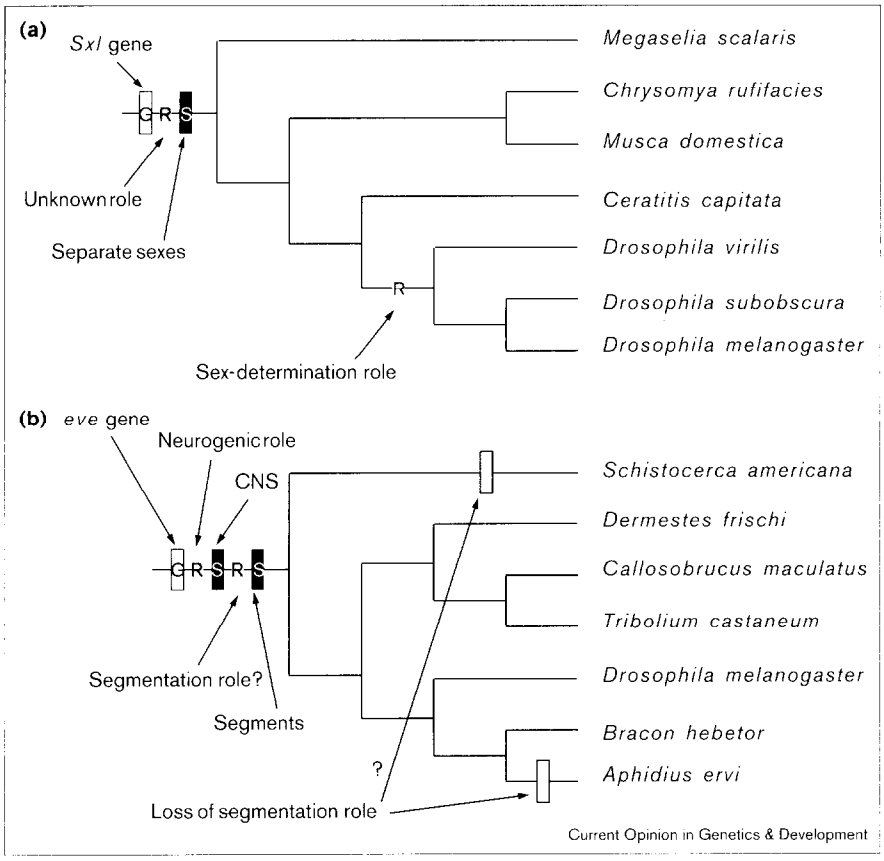
In these examples, a homologous gene and a homologous phenotypic feature are present in all of the species mentioned, but in only some of them does the gene contribute to the development of the feature. Other cases include apparent losses of segmentation and homeotic patterning roles for *fushi-tarazu* [33,34] and *zen* [35] during arthropod evolution. In none of the cases discussed here is the genetic basis for development known in species other than *Drosophila melanogaster*, reflecting the significant technical difficulties intrinsic in making such assessments. It also points to an important, but rarely acknowledged, bias in our knowledge of comparative developmental genetics: most of the molecular methods used to study development in non-model organisms rely on sequence similarity to work. This means that finding differences is inherently much more difficult than finding similarities, and inherently more difficult to interpret. This technical bias is reinforced by an apparent bias in interest, in that many molecular biologists seem more excited by similarities than by differences (while many evolutionary biologists would have the opposite bias).

Once again, we can summarize these examples with a quotation from de Beer [1], "homologous structures need not be controlled by homologous genes". Relatively few examples of this phenomenon have been described but the inherent difficulty of detecting this kind of evolutionary dissociation, combined with some clear examples of its existence, suggests that it is not sufficiently rare that it can be safely ignored. Although this kind of evolutionary dissociation between genotype and phenotype may be relatively uncommon when comparing closely related species, both the *eve* and *Sxl* examples demonstrate that the genetic basis for an important developmental process can change even among quite closely related taxa.

Implications for understanding the evolution of morphology

The examples discussed above, along with many other similar cases, are beginning to provide a clearer understanding of the complex, and often surprising, evolutionary

Figure 2



Evolutionary history of **(a)** *Sex-lethal* (*Sxl*) and **(b)** *even-skipped* (*eve*) in insects. The acquisition and loss of developmental roles can occur without simultaneous gains and losses of the phenotypes they encode. (a) Acquisition of a developmental role. The gene *Sxl* is the 'master regulatory gene' in the sex-determination pathway of *Drosophila melanogaster* and at least some other *Drosophila* species [29,30]. *Sxl* is present in other flies, all of which have separate sexes and sexual dimorphism, but does not appear to be involved in sex determination [31•,32•]. As a result of the relatively derived phylogenetic position of the genus *Drosophila*, a role in sex determination is almost certainly a new function for *Sxl* [31•,32•]. (b) Loss of a developmental role. The gene *eve* is involved in segmentation, neurogenesis, and other developmental processes in *Drosophila* and some other insects [24–26]. In the locust *Schistocerca americana* and the wasp *Aphidius ervi*, both of which have segments, the segmenation role is absent [27,28•]. As a result of the derived phylogenetic position of *Aphidius*, the absence of pair-rule *eve* expression in this species almost certainly represents the loss of a developmental role [28•]. Whether the absence of pair-rule expression in *Schistocerca* also represents a loss, or is the ancestral condition for insects, can only be determined by examining *eve* expression in other, more distantly-related arthropods (hence the question marks). G, gene; R, developmental role; S, structure.

relationship between genotype and phenotype. Evolutionary dissociations between genotype and phenotype in particular provide two important messages.

The first is a clearer understanding of the circumstances under which gene expression data can be used to make inferences about homology of morphological structures. This research program offers great promise [20,36]. It has been applied with considerable success, for example, to unraveling the evolutionary history of the vertebrate brain through comparisons of gene expression among taxa [37,38•]. The relative positions of expression domains for several transcription factors is concordant in the anterior central nervous systems of vertebrates, urochordates, and cephalochorates — providing molecular 'landmarks' for inferring homologies among morphological regions of the brain. This approach has also worked well for identifying homologous body regions in crustaceans [39•] and homologous regions of vertebrate fins and limbs [22••,40].

Gene expression, however, is certainly not an infallible guide for determining the homology of structures. No one would interpret the Notch expression data summarized in Table 1 to mean that the vulva of *Caenorhabditis elegans* is homologous to the eye of *Drosophila* and to T-cells of humans. The simple fact that regulatory genes have multiple expression domains and play multiple developmental roles within single organisms makes such facile inferences absurd. Discussion of this difficulty, along with recommendations for more rigorous approaches to using gene expression to discriminate among competing hypotheses of morphological homology have been presented by several authors [5,6,7••,10,41,42]. In general, this approach will be most reliable when applied to relatively closely related species. In such cases, anatomy will be broadly similar, providing landmarks for realistic interpretations of gene expression data; in such cases, there will be less likelihood of evolutionary dissociations between homologous genes and homologous morphological structures [5,42].

The second, more exciting, message concerns the genetic basis for evolutionary change in morphology. The origin of morphological novelties — such as chordate somites or insect wings — has long puzzled evolutionary biologists [43,44]. As heritable new phenotypes must have a genetic basis, it is assumed that the origin of novel structures of any complexity will require a set of new alleles or even new genes. This poses the problem of how several new alleles (or genes) could all become established in a population before they produce a functionally advantageous phenotype. The fact that regulatory genes are typically involved in several distinct developmental processes within single species provides an important clue to the resolution of this apparent paradox. Changes

in interactions between regulatory genes and their targets — rather than changes in the biochemical activities of genes — may underlie many evolutionary changes in morphology, including the origin of novel structures.

Regulatory genes can clearly take on new developmental roles and lose old ones during the course of evolution. This leads to the hypothesis that the origin of morphological novelties relies, at least in part, on the acquisition of new developmental roles from existing genes [4•,18•]. Several cases where such role recruitment is phylogenetically correlated with the origin of a morphological novelty have been identified (e.g. [18•,22••,35,45,46•]), providing cases for testing this hypothesis directly. At present, these cases all involve guilt by association: in none has the gene in question been demonstrated to play a role in the development of the novel structure. As methods for experimentally disrupting gene expression and inducing ectopic expression in non-model taxa become increasingly practical, it should be possible to delve into the genetic basis for these and other evolutionary changes in morphology to get increasingly fruitful results.

Conclusions

Regulatory genes provide important insights into both the unity and diversity of animal morphology. To date, the unifying aspects have attracted far more attention. Regulatory genes have extreme promise for use as molecular indices of morphological homology, although this approach has clear limitations. Far less attention has been paid to the role that these genes have played in generating diversity of morphology, a process that is inherently more difficult to study.

As analyses of regulatory genes are extended to more and more species, numerous cases of evolutionary dissociation between homologous genes and homologous structures have come to light. These dissociations mean that homology at one level of biological organization does not always imply homology at another. Such cases should not be viewed simply as 'noise' that interferes with the identification of morphological homologies. They also provide an exciting window into the genetic basis for morphological evolution.

Acknowledgements

Thanks to Sherryl Broverman for constructive comments. Research in GA Wray's lab is supported by grants from the National Science Foundation and AP Sloan Foundation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. de Beer GR: *Homology: an Unsolved Problem.* Oxford: Oxford University Press; 1971.

2. Zuckerkandl E: **Molecular pathways to parallel evolution: I. Gene nexuses and their morphological correlates.** *J Mol Evol* 1994, **39**:661-678.

3. • Gerhart J, Kirschner M: *Cells, Embryos, and Evolution.* Boston: Blackwell Science; 1997.
A central theme of this book is the multiple uses to which regulatory genes have been put during the course of animal evolution.

4. • Duboule D, Wilkins AS: **The evolution of 'bricolage'.** *Trends Genet* 1998, **14**:54-59.
This review discusses the recruitment of existing genes to new developmental roles in terms of the idea of evolution as a 'tinkering' process.

5. Wray GA: **Evolutionary dissociations between homologous genes and homologous structures.** In *Homology* (Novartis Foundation Symp 222). Chichester: Wiley; 1998:in press.

6. Dickinson WJ: **Molecules and morphology: where's the homology?** *Trends Genet* 1995, **11**:119-121.

7. •• Abouheif E, Akam M, Dickinson WJ, Holland PWH, Meyer A, Patel N, Raff RA, Roth VL, Wray GW: **Homology and developmental genes.** *Trends Genet* 1997, **13**:432-433.
This short paper outlines some pitfalls associated with using regulatory genes as indices of homology at the morphological level: failure to distinguish genes which are orthologous (gene copies derived from speciation) and paralogous (gene copies derived from duplication); use of the term 'functional homology' which confuses similarity because of a common evolutionary origin (homology) with similarity due to functional convergence (analogy); and conflicting comparisons across different levels of biological organization, such as genes, their expression patterns, their developmental roles, and the structures to which they give rise. The paper provides practical solutions to avoid these.

8. Mayr E: *The Growth of Biological Thought.* Boston: Belknap Press; 1982.

9. Hall BK (Ed): *Homology: The Hierarchical Basis of Comparative Biology.* San Francisco: Academic Press; 1994.

10. Abouheif E: **Developmental genetics and homology: a hierarchical approach.** *Trends Ecol Evol* 1997, **12**:405-408.

11. Bang AG, Bailey AM, Posakony JW: **Hairless promotes stable commitment to the sensory organ precursor cell fate by negatively regulating the activity of the Notch signaling pathway.** *Dev Biol* 1995, **172**:479-494.

12. Kim J, Irvine KD, Carroll SB: **Cell recognition, signal induction, and symmetrical gene activation at the dorsal-ventral boundary of the developing *Drosophila* wing.** *Cell* 1995, **82**:795-802.

13. Robey E: **Notch in vertebrates.** *Curr Opin Genet Dev* 1997, **7**:551-557.

14. Crowe R, Henrique D, Ish-Horowicz D, Niswander L: **A new role for Notch and Delta in cell fate decisions: patterning the feather array.** *Development* 1998, **125**:767-775.

15. Patel NH, Martin-Blanco E, Coleman KG, Poole SJ, Ellis MC, Kornberg TB, Goodman CS: **Expression of engrailed proteins in arthropods, annelids, and chordates.** *Cell* 1989, **58**:955-968.

16. Rogers BT, Kaufman TC: **Structure of the insect head as revealed by the EN protein pattern in developing embryos.** *Development* 1996, **122**:3419-3432.

17. Davis CA, Holmyard DP, Millen KJ, Joyner AL: **Examining pattern formation in mouse, chicken and frog embryos with an EN-specific antiserum.** *Development* 1991, **111**:287-298.

18. • Lowe CJ, Wray GA: **Radical alterations in the roles of homeobox genes during echinoderm evolution.** *Nature* 1997, **389**:718-721.
This paper provides several examples of probable gains and losses of developmental roles for three transcription factors (distal-less, engrailed, and orthodenticle). These factors play some developmental roles that are distinct from those in either arthropods and chordates, and play different developmental roles from class to class within echinoderms.

19. Savage RM, Shankland M: **Identification and characterization of a *hunchback* orthologue, *Lzf2*, and its expression during leech embryogenesis.** *Dev Biol* 1996, **175**:205-217.

20. DeRobertis EM, Sasai Y: **A common plan for dorsoventral patterning in Bilateria.** *Nature* 1996, **380**:37-40.

21. McGinnis W, Krumlauf R: **Homeobox genes and axial patterning.** *Cell* 1992, **68**:283-302.

22. •• Shubin N, Tabin C, Carroll S: **Fossils, genes, and the evolution of animal limbs.** *Nature* 1997, **388**:639-648.
This review provides instructive analyses of comparative gene expression data during limb development in arthropods and chordates. The discussions highlight the importance of a robust phylogenetic framework and a detailed

understanding of comparative anatomy for understanding the role that particular genes have played in the course of morphological evolution.

23. Satokata I, Benson G, Maas R: **Sexually dimorphic sterility phenotypes in *Hox a-10* deficient mice.** *Nature* 1995, **374**:460-463.

24. Nüsslein-Volhard C, Wieschaus E: **Mutations affecting segment number and polarity in *Drosophila*.** *Nature* 1980, **287**:795-801.

25. Doe CQ, Smouse D, Goodman CS: **Control of neuronal fate by the *Drosophila* segmentation gene *even-skipped*.** *Nature* 1988, **333**:376-388.

26. Patel NH, Condron BG, Zinn K: **Pair-rule expression patterns of *even-skipped* are found in both short- and long-germ beetles.** *Nature* 1994, **367**:429-434.

27. Patel NH, Ball EE, Goodman CS: **Changing role of *even-skipped* during the evolution of insect pattern formation.** *Nature* 1992, **357**:339-342.

28. • Grbic M, Strand MR: **Shifts in the life history of parasitic wasps correlate with pronounced alterations in early development.** *Proc Natl Acad Sci USA* 1998, **95**:1097-1101.
This paper provides a good example of how the developmental role of a regulatory gene can change in association with shifts in an organism's life history. It describes the surprising evolutionary loss of the well-studied segmentation role of *eve*.

29. Cline T: **The *Drosophila* sex determination signal: how do flies count to two?** *Trends Genet* 1993, **9**:385-390.

30. Bopp D, Calhoun G, Horabin JI, Samuels M, Schedl P: **Sex-specific control of *Sex-lethal* is a conserved mechanism for sex determination in the genus *Drosophila*.** *Development* 1996, **122**:971-982.

31. • Meise M, Hilfikerkleiner D, Dubendorfer A, Brunner C, Nothiger R, Bopp D: ***Sex-lethal*, the master sex-determining gene in *Drosophila*, is not sex-specifically regulated in *Musca domestica*.** *Development* 1998, **125**:1487-1494.
This, and the following report [32•], demonstrate that a regulatory gene (*Sxl*) can become involved in a developmental process (sex determination) after that process first evolved. It also demonstrates that *Sxl* is probably not a 'master regulatory gene' for sex deteremination in most flies.

32. • Saccone G, Peluso I, Artiaco D, Giordano E, Bopp D, Polito LC: **The *Ceratitis capitata* homologue of the *Drosophila* sex determining gene *sex-lethal* is structurally conserved, but not sex-specifically regulated.** *Development* 1998, **125**:1495-1500.
See annotation [31•].

33. Dawes R, Dawson I, Falciani F, Tear G, Akam M: ***Dax*, a locust *Hox* gene related to *fushi-tarazu* but showing no pair-rule expression.** *Development* 1994, **120**:1561-1572.

34. Brown S, Hilgenfeld RB, Denell RE: **The beetle *Tribolium castaneum* has a *fushi tarazu* homolog expressed in stripes during segmentation.** *Proc Natl Acad Sci USA* 1994, **91**:12922-12926.

35. Falciani F, Hausdorf B, Schroder R, Akam M, Tautz D, Denell R, Brown S: **Class 3 *Hox* genes in insects and the origin of *zen*.** *Proc Natl Acad Sci USA* 1996, **93**:8479-8484.

36. Slack JMW: **A Rosetta Stone for pattern formation in animals?** *Nature* 1984, **310**:364-365.

37. Holland ND, Panganiban G, Henyey EL, Holland LZ: **Sequence and developmental expression of *AmphiDll*, an amphioxus *Distal-less* gene transcribed in the ectoderm, epidermis and nervous system: insights into the evolution of the craniate forebrain and neural crest.** *Development* 1996, **122**:2911-2920.

38. • Wada H, Saiga H, Satoh N, Holland PWH: **Tripartite organization of the ancestral chordate brain and the antiquity of placodes: insights from ascidian *Pax-2/5/8*, *Hox*, and *Otx* genes.** *Development* 1998, **125**:1113-1122.
The authors illustrate how the expression domains of several genes provide a more secure interpretation of morphological homologies among species than does a single gene. The discussion provides a clear example of applying expression data to understanding the history of morphology.

39. • Averof M, Patel N: **Crustacean appendage evolution associated with changes in *Hox* gene expression.** *Nature* 1997, **388**:682-686.
This paper provides another careful application of gene expression data to understanding the history of morphological change.

40. Sordino P, Duboule D: **A molecular approach to the evolution of vertebrate paired appendages.** *Trends Ecol Evol* 1996, **11**:114-119.

41. Bolker JA, Raff RA: **Developmental genetics and traditional homology.** *Bioessays* 1996, **18**:489-494.

42. Holland PWH: **How gene duplication affects homology.** In *Homology* (Novartis Foundation Symp 222). Chichester: Wiley; 1998:in press.

43. Nitecki M (Ed): *Evolutionary Innovations*. Chicago: University of Chicago Press; 1989.

44. Müller GB, Wagner GP: **Novelty in evolution: restructuring the concept.** *Ann Rev Ecol Syst* 1991, **22**:229-256.

45. Carroll SB, Gates J, Keys DN, Paddock SW, Panganiban G, Selegue JE, Williams JA: **Pattern formation and eyespot determination in butterfly wings.** *Science* 1994, **265**:109-114.

46. • Panganiban G, Irvine SM, Lowe C, Roehl H, Corley LS, Sherbon B, Grenier JK, Fallon JF, Kimble J, Walker M *et al.*: **The origin and evolution of animal appendages.** *Proc Natl Acad Sci USA* 1997, **94**:5162-5166.
Apparent cases of parallel recruitment of *Distal-less* into the development of appendages that are not themselves homologous are documented in this paper. The data illustrate one of the pitfalls of using regulatory gene expression to infer homology of structures.

47. Bharathan G, Janssen B-J, Kellogg EA, Sinha A: **Did homeodomain proteins duplicate before the origin of angiosperms, fungi, and metazoa?** *Proc Natl Acad Sci USA* 1997, **94**:13749-13753.