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19 Hunter, T. (1987) A thousand and one protein-kinases. *Cell* 50, 823–829

20 Bargmann, C.I. (1998) Neurobiology of the *Caenorhabditis elegans* genome. *Science* 282, 2028–2033

21 Ma, H. (1994) GTP-binding proteins in plants – New members of an old family. *Plant Mol. Biol.* 26, 1611–1636

22 Plakidou-Dymock, S. *et al.* (1998) A higher plant seven-transmembrane receptor that influences sensitivity to cytokinins. *Curr. Biol.* 8, 315–324

23 Riechmann, J.-L. and Meyerowitz, E.M. (1998) The AP2/EREBP family of plant transcription factors. *J. Biol. Chem.* 379, 633–646

24 Goodrich, J. *et al.* (1997) CURLY LEAF: A Polycomb group gene that regulates homeotic gene expression in *Arabidopsis*. *Nature* 386, 44–51

25 Grossniklaus, U. *et al.* (1998) Maternal control of embryogenesis by MEDEA, a Polycomb-group gene in *Arabidopsis*. *Science* 280, 446–450

26 Luo, M. *et al.* (1999) Genes controlling fertilization-independent seed development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 96, 296–301

27 Ohad, N. *et al.* (1999) Mutations in FIE, a WD Polycomb group gene, allow endosperm development without fertilization. *Plant Cell* 11, 407–415

28 DeLange, R.J. *et al.* (1969) Calf and pea histone IV. III. Complete amino acid sequence of pea seedling histone IV; comparison with the homologous calf thymus histone. *J. Biol. Chem.* 244, 5669–5679

29 Kieber, J.J. *et al.* (1993) CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein-kinases. *Cell* 72, 427–441

30 Clark, K.L. *et al.* (1998) Association of the *Arabidopsis* CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. *Proc. Natl. Acad. Sci. U. S. A.* 95, 5401–5406

31 Ichimura, K. *et al.* (1998) Isolation of ATMEKK1 (a MAP kinase kinase kinase) – Interacting proteins and analysis of a MAP kinase cascade in *Arabidopsis*. *Biochem. Biophys. Res. Commun.* 253, 532–543

32 Posas, F., Witten, E.A. and Saito, H. (1998) Requirement of STE50 for osmotic stress-induced activation of the STE11 mitogen-activated protein kinase kinase kinase in the high-osmolarity glycerol response pathway. *Mol. Cell. Biol.* 18, 5788–5796

33 Padgett, R.W. *et al.* (1998) TGF-beta signaling, Smads, and tumor suppressors. *BioEssays* 20, 382–390

34 Pennell, R. (1998) Cell walls: structures and signals. *Curr. Opin. Plant Biol.* 1, 504–510

35 Cox, G.N., Kramer, J.M. and Hirsh, D. (1984) Number and organization of collagen genes in *Caenorhabditis elegans*. *Mol. Cell. Biol.* 4, 2389–2395

36 Kramer, J.M. (1994) Genetic analysis of extracellular matrix in *C. elegans*. *Annu. Rev. Genet.* 28, 95–116

37 Wei, N. and Deng, X.W. (1999) Making sense of the COP9 signalosome – a regulatory protein complex conserved from *Arabidopsis* to human. *Trends Genet.* 15, 98–103

38 Koonin, E.V. *et al.* (1997) Comparison of archaeal and bacterial genomes: computer analysis of protein sequences predicts novel functions and suggests a chimeric origin for the archaea. *Mol. Microbiol.* 25, 619–637



The origin and evolution of segmentation

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Arthropods, annelids and chordates all possess segments. It remains unclear, however, whether the segments of these animals evolved independently or instead were derived from a common ancestor. Considering this question involves examining not only the similarities and differences in the process of segmentation between these phyla, but also how this process varies within phyla, where the homology of segments is generally accepted. This article reviews what is known about the segmentation process and considers various proposals to explain its evolution.

To most of us who are segmented, the advantages of a segmental body plan are perhaps not immediately obvious. However, segments are of fundamental evolutionary and developmental importance to several metazoan phyla, including our own (see Glossary). The history of metazoan life – should we ever be up to the task of writing it – would surely be deficient without at least a chapter on the evolution of segments and the ways in which they are made.

Homology of segmentation and phylogeny

Before considering the evolution of segmentation, we first need to specify what we mean by a ‘segment’. True segmentation, or metamerism, is usually considered to be the repetition along the anterior–posterior axis of a structural unit that comprises a suite of characters involving the entire body¹. Animals and plants, which evolved multicellularity independently, possess simple serial repetition of structure, and serial repetition is also a feature of some animal appendages, such as insect antennae, suggesting that it evolved multiple times in many contexts. Traditionally, however, it is the body segments of arthropods, annelids and chordates that have been accorded special significance as examples of true metamerism.

Historically, intuitive ideas concerning the evolutionary origins of segmentation in arthropods, annelids and chordates have strongly influenced our picture of the evolutionary relationships among the

bilaterally symmetrical metazoans (the bilaterians). At the close of the 19th century, the segmentation observed in these three phyla was commonly held to be homologous, that is, derived from a segmented common ancestor. Thus, segmentation was often used to unite these groups within a single clade. However, the protostome–deuterostome distinction², made at the beginning of the 20th century, asserted that most bilaterian phyla are more closely related to either chordates or annelids plus arthropods than these two groups are to each other. Since then, the deep phylogenetic separation of chordates and annelids plus arthropods has been retained and confirmed, leading many to regard the segmentation in these two groups as having evolved independently³. Similarly, true segmentation traditionally has been regarded as a shared, derived character of annelids and arthropods, uniting these phyla in a clade to the exclusion of unsegmented phyla, such as the molluscs⁴. However, recent analyses of morphological⁴ and molecular^{5,6} data have suggested that these two segmented phyla are actually more closely related to several unsegmented phyla than they are to each other (Fig. 1).

If this most recent version of metazoan phylogeny⁷ is correct, then it represents a direct challenge to the supposed shared, derived characters that previously united annelids and arthropods, segmentation being primary among them. Essentially, we are now faced with three different hypotheses for the evolution of segmentation (Fig. 1). While it is true that mere phylogenetic separation does

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not force us to concede that segments evolved independently (Fig. 1a), any hypothesis of segment homology is less parsimonious because it would require several instances of segmentation loss among the other 20 or so protostome phyla (Fig. 1b,c).

How do we discriminate between these different hypotheses? One way is to rely on the assumption that homologous structures are more likely to employ the same developmental tools than structures that have evolved independently. Thus, the first hypothesis, that segmentation was acquired independently in all three phyla (Fig. 1a), would predict that similarities in the developmental process of segmentation in these different groups should be minimal or merely superficial. The second hypothesis, that segments are homologous within the protostomes, but that chordates evolved segments independently (Fig. 1b), would predict significant differences between chordates and arthropods in the way that they make segments, but significant similarities in the way annelids and arthropods make segments. The third hypothesis, that segmentation is homologous throughout the Bilateria (Fig. 1c), has the heaviest burden of proof because it would predict universal similarities in the process of segmentation that are sufficient to outweigh obvious differences. Additionally, the second and third hypotheses would also predict that, despite the loss of overt segmentation in many phyla, we ought to be able to detect molecular or developmental vestiges of an ancestral segmented state during the embryogenesis of at least some unsegmented phyla.

How do we begin to look for significant similarities and differences in the mechanisms of segmentation in various species? At the molecular and genetic level, we understand the most about segmentation in the arthropod *Drosophila melanogaster*. In this animal, the embryo is subdivided sequentially into smaller and smaller units through the sequential action of maternal coordinate, gap, pair-rule and segment-polarity genes. Many of these genes have now been well characterized, and, for most, their precise patterns of expression reflect their role in segmentation and, indeed, are essential to it. Thus, one approach to addressing questions on the evolution of segmentation has been to compare the expression patterns of orthologues of these *Drosophila* segmentation genes in various organisms. In particular, genes of the segment-polarity and pair-rule class have received the most attention – partly because their expression patterns correlate most easily with the later morphological manifestations of segmentation.

Glossary

Bilaterians

The bilaterally symmetrical metazoans.

Blastoderm

In insect embryogenesis, the stage consisting of a monolayer of cells or nuclei surrounding yolk.

Clade

A group of organisms including all the descendants of its last common ancestor. Also called a monophyletic group.

Deuterostomes

Members of one of two clades of the bilaterians classically characterized by a non-blastoporal origin of the mouth. Includes chordates, echinoderms and hemichordates.

Homology (adjective, homologous)

Morphological or structural similarity due to common ancestry. A shared feature that is present in the common ancestor.

Metazoans

The multicellular animals, including sponges.

Orthology (noun, orthologue)

The relationship of two related genes derived from a speciation event as opposed to a gene-duplication event.

Phylogeny

The evolution of a group of organisms; a branching diagram representing the evolutionary relationships between members of a group of organisms.

Phylum (plural, phyla)

A group of species sharing a common body plan.

Protostomes

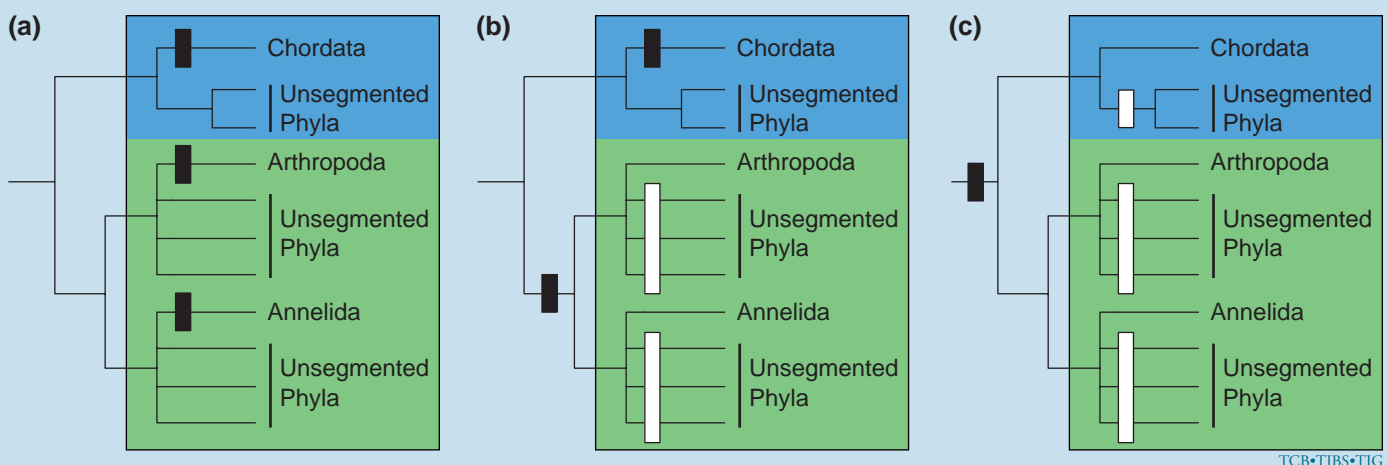
Members of one of two clades of bilaterians classically characterized by a blastoporal origin of the mouth. Includes, among others, arthropods, annelids, molluscs and nematodes.

Syncytium

Tissue containing nuclei not separated by cell membranes.

Segmentation in arthropods

Why begin with a comparison of segmentation in *Drosophila* (Order: Diptera) versus other insects and other arthropods (Fig. 2)? The answer is that, before we can accurately assess the similarities and differences in segmentation between different phyla, we must first consider the extent to which this process varies



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FIGURE 1. Three hypotheses for the evolution of segmentation among the Bilateria. Blue indicates deuterostome phyla, green indicates protostome phyla. Both the acquisition of segmentation (solid black blocks) and the loss of segmentation (solid white blocks) are also indicated. (a) Hypothesis 1 proposes three independent acquisitions of segmentation. (b) Hypothesis 2 asserts the homology of segmentation among protostomes and thus requires later loss of the segmented state. (c) Hypothesis 3 asserts the homology of segmentation across the Bilateria and thus requires even more loss. Exactly how much loss occurs in (b) and (c) and, thus, exactly how much less parsimonious these scenarios are will require further phylogenetic resolution.

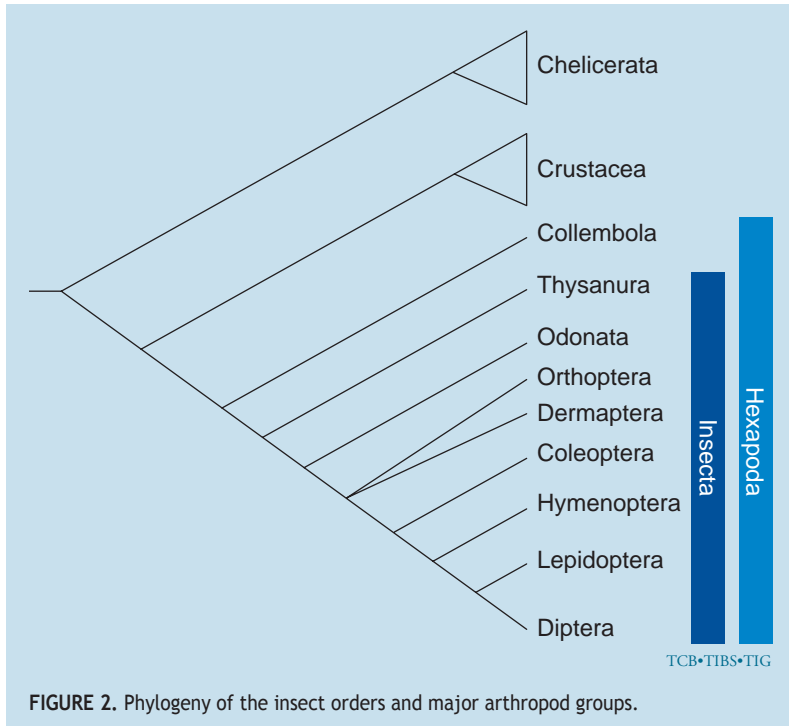


FIGURE 2. Phylogeny of the insect orders and major arthropod groups.

within a single clade, whose members all possess segments that are undoubtedly homologous. It would be premature to compare segmentation in *Drosophila*, leeches and chickens without having some idea of how representative each of these organisms is of the general (and presumably ancestral) mechanisms of segmentation within the arthropods, annelids and chordates, respectively.

Segmentation in insects

All insect embryos begin as a syncytium in which the majority of nuclei eventually migrate to the periphery to form a blastoderm, which subsequently becomes cellularized. However, beyond these early events, embryogenesis varies quite substantially among different insects. In particular, there is the distinction between long-, intermediate- and short-germ modes of embryogenesis⁸. In long-germ insects, such as *Drosophila*, all segments are patterned simultaneously within the blastoderm. In short-germ insects, such as *Tribolium* and *Schistocerca*, only segments of the head are patterned in the initial blastoderm, whereas the remaining segments of the thorax and abdomen form progressively from a posterior growth zone. Intermediate-germ insects fall somewhere between these two extremes. Early patterning and segmentation of the long-germ embryo takes place in a syncytium in which determinants are free to diffuse, whereas much of the patterning and segmentation of short- and intermediate-germ embryos takes place instead in a cellular environment. This, of course, raises questions as to the mechanisms employed by short- and intermediate-germ insects, which show the progressive addition of segments in a cellular environment.

In embryos representative of all three modes of embryogenesis, the segment-polarity genes are expressed just before and during the overtly segmented germ-band stage in a segmentally reiterated pattern. The two that have been most widely studied are *engrailed* and *wingless*. Each is expressed as a single stripe within each individual segment, and these patterns have thus far been found in all insects examined. In contrast to the segment-polarity genes, orthologues to the pair-rule class of genes have tended to exhibit more-divergent patterns. The three that have been studied most widely are *even-skipped* (Fig. 3), *hairy* and *fushi-tarazu*. In *Drosophila*, all three of these genes are expressed in stripes before the onset of gastrulation, with a two-segment periodicity. In addition,

even-skipped also shows a segmental pattern of expression immediately following gastrulation. The expression of all three of these pair-rule genes is conserved in the beetle *Tribolium castaneum* (Order: Coleoptera)^{9–13}. In the case of *even-skipped*, its pair-rule function appears to be conserved as well, because chromophore-assisted laser inactivation (CALI) of the protein results in a pair-rule phenotype¹⁴. However, a deletion mutant of the *Tribolium Hox* complex that includes the *fushi-tarazu* orthologue does not exhibit any pair-rule defects¹⁵, indicating that at least this gene appears to be functioning differently compared with its *Drosophila* orthologue. Additionally, a recent genetic screen in *Tribolium* has revealed at least two mutants that display pair-rule phenotypes¹⁶.

In the more phylogenetically primitive grasshopper *Schistocerca* (Order: Orthoptera), pair-rule expression has been particularly elusive. Neither the *even-skipped* nor *fushi-tarazu* orthologues is expressed in stripes in the early embryo, but rather both are expressed in broad, Hox-like, posterior domains^{10,17} (Fig. 3). In light of the phylogenetic position of *Schistocerca*, it is tempting to view these posterior expression domains as ancestral, existing prior to the evolutionary recruitment of these genes to play a role in segmentation¹⁷. This is perhaps most reasonable for *even-skipped* as *even-skipped* orthologues are linked to the *Hox* clusters in vertebrates^{18,19}, and *even-skipped* orthologues of vertebrates and nematodes are expressed in posterior *Hox*-like domains^{18,20,21}. However, the observation that both earwigs (Order: Dermaptera) and crickets (Order: Orthoptera) possess stripes of a one-segment periodicity (P. Moore, R. Dawes and N. Patel, unpublished), suggests that a pattern of segmental stripes is the ancestral *even-skipped* expression pattern for insects and that the grasshopper perhaps represents a reversion to an even more ancestral pattern shared by nematodes and vertebrates.

Segmentation in non-insect arthropods

In attempting to understand the ancestral expression patterns and functions of segmentation genes in arthropods, it will be especially important to investigate segmentation in other arthropod groups, such as crustaceans (brine shrimp, crayfish, etc.), chelicerates (spiders, scorpions, etc.) and myriapods (millipedes and centipedes) (Fig. 2). In many species of crustaceans, most of the thorax and abdomen develops in a cellular environment from a posterior growth zone following gastrulation and thus, at least superficially, resembles the development of short-germ insects. In the case of the crustaceans examined so far, the expression of *engrailed* is similar to that seen in insects²². We also know that *engrailed* is expressed in segmental stripes in spiders²³. It is now important to characterize the expression patterns of pair-rule genes in various non-insect arthropods in order to establish the ancestral function of these genes within this group. For now, though, the available data on arthropods reveal that, within this group, the process of segmentation is quite variable at the pair-rule level and yet fairly conserved at the segment-polarity level.

Are segments homologous across phyla?

In spite of its prevalence, variation at the pair-rule level is not enough to shake the widely held conviction that the segments of all arthropods are derived from a common segmented ancestor. Taking this variation into account, though, is important in weighing evidence for either the homology or independent evolution of segments between phyla. What, then, are the similarities and differences in the processes of annelid and chordate segmentation when compared with those of arthropods?

Segmentation in annelids (evaluating hypothesis 2)

In the case of annelid worms, the most detailed embryological and molecular data we have come from the leech. In these animals, development proceeds by the anterior-to-posterior progression of

stem cells, which lay down the founder cells for each segment by a series of longitudinal asymmetric divisions, a pattern that is at least superficially similar to that of some crustaceans^{24,25}.

The molecular data we have for annelids concern an orthologue of the segment-polarity gene *engrailed* from the leech *Helobdella triserialis*. The expression pattern of this gene appears to be consistent with a role in segmentation²⁶ because cells that have or will express *engrailed* do come to occupy a narrow stripe in the posterior of each segment, just anterior to the segmental furrow²⁷. More detailed analysis of the N lineage (which gives rise to a large proportion of the nervous system) in the leech *Theromyzon rude*, however, suggests that *engrailed* expression appears only after the ganglionic primordia have separated, casting doubt on its functional role in the segmentation process²⁸. Thus, a firm conclusion on the possible homology of arthropod and annelid segments will require future study, both of leeches and ideally of the more basal annelid groups, such as oligochaetes and polychaetes, to provide a clearer picture of the mechanisms used by these organisms to make segments.

Segmentation in chordates (evaluating hypothesis 3)

What about our third hypothesis – the global homology of segments (Fig. 1c)? Are the similarities between segmentation in arthropods and chordates enough to outweigh the differences? At first glance, the most obvious aspect of vertebrate segmentation – somitogenesis – appears similar to the progressive anterior to posterior formation of segments that we observe both in short-germ insects and in crustaceans. As for genes from the fly segmentation hierarchy, one of the *engrailed* proteins in zebrafish has been localized to the anterior of developing somites. However, this expression appears only after morphological segmentation has occurred, and it is associated with a specific subset of muscle cells within the somite²⁹. Similarly, mouse members of the PAX Group III family of genes (related to the *Drosophila* segment-polarity gene *gooseberry* and pair-rule gene *paired*) are also expressed in a pattern of somitic stripes. However, this restricted expression is associated with a specific tissue type (dermomyotome) and appears only after the somites have formed³⁰.

In support of the third hypothesis, however, transcripts of *engrailed* in *Amphioxus* have recently been localized to the posterior of the first eight somites, and the expression is reported to precede morphological segmentation³¹. However, the simple observation of stripes might be misleading. One analysis of the expression patterns of randomly selected cDNAs from *Drosophila* revealed that 87% are expressed in segmentally reiterated stripes, including ‘housekeeping’ genes such as those encoding ribosomal proteins³². Indeed, segmentally reiterated patterns of gene expression are exactly what one would expect during the development of a segmented organism, but it is likely that only a subset of these genes actually function in segmentation. Thus, it will be important to test experimentally whether the segmentally repeating patterns of *engrailed* in chordates are involved in the actual establishment of segments and not just the specification of cell type.

Even more excitement has been generated by a result from zebrafish in which transcripts of *her1*, a putative orthologue of the pair-rule gene *hairy*, were localized to domains that correspond to future alternating somites before overt morphological segmentation, thus mimicking a pair-rule pattern³³. These results have led to the suggestion that the common ancestor of protostomes and deuterostomes was segmented^{31,34,35} and that segments across the Bilateria are homologous (Fig. 1c). However, one problem with the zebrafish result is that it is not yet clear that *her1* is indeed an orthologue of *Drosophila hairy*³⁶, and it is crucial that we deal with true orthologues if we intend to invoke them to homologize a structure or process. Additionally, while a description of the *her1* mutant is not yet available, a zebrafish double mutant that indirectly eliminates expression of *her1* fails to exhibit any segmentation defects³⁷.

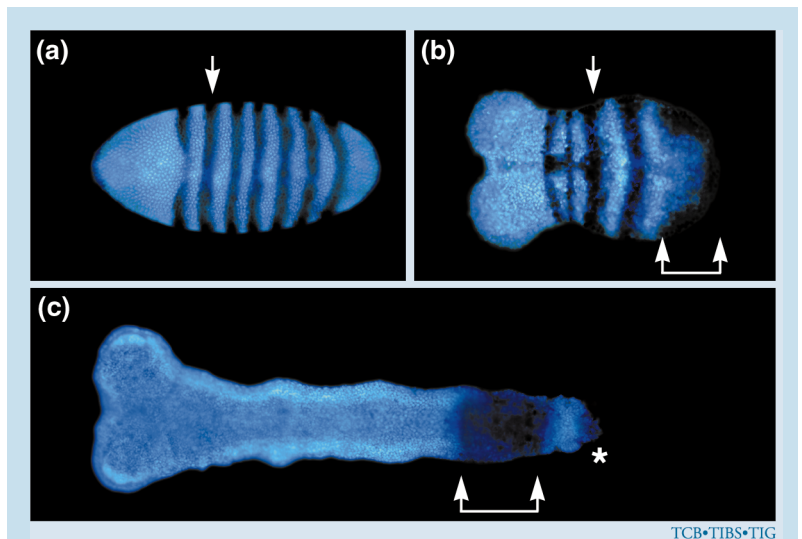


FIGURE 3. Expression of *even-skipped* in (a) *Drosophila melanogaster*, (b) *Tribolium castaneum* and (c) *Schistocerca americana*. *Even-skipped* protein appears in pair-rule stripes (white arrows) in embryos of both the long-germ insect *Drosophila* and the short-germ insect *Tribolium* but not in the embryo of the short-germ insect *Schistocerca*. In addition, the embryos of both *Tribolium* and *Schistocerca* express *Even-skipped* in a broad posterior domain (arrowed bracket). The asterisk indicates *Even-skipped* expression in the anal pad of *Schistocerca*, a pattern also found in older embryos of both *Drosophila* and *Tribolium*.

In chick, too, the situation differs substantially from *Drosophila*. Here, another putative orthologue of *Drosophila hairy*, *c-hairy1*, is expressed in a cyclical segmental pattern and might be involved in the periodic genesis of somites³⁶. The *c-hairy1* expression pattern, however, does not appear to possess any sort of two-segment periodicity. Thus, it will be especially important to isolate a chick or mouse orthologue of zebrafish *her1* to determine whether this pair-rule pattern is conserved in other vertebrates.

It is also clear that much of the impetus to homologize comes from a conviction that the pair-rule patterning mechanism is not a ‘logical’ way to make segments and that, if we find such a mechanism acting in vertebrates, it is unlikely to have evolved independently. However, this argument relies on the assumption that we can judge accurately what is and what is not an obvious evolutionary solution at the level of developmental mechanism. Finally, it is important to note that several vertebrate genes have been implicated in somitogenesis whose *Drosophila* orthologues (*Notch*, *Delta* and *fringe*) play no identified role in segmentation^{38–40}.

For many, these differences in the process of segmentation, whether molecular or embryological, are easily dismissed as ‘negative’ data in the face of astonishing similarities. But excessive focus on isolated similarities, rather than a more balanced consideration of all the evidence, runs the risk of mistaking independent evolution for common ancestry. On the other hand, it is entirely conceivable that more than 600 million years of evolution has left us with only isolated similarities of a once-shared, ancestral segmentation mechanism.

Concluding remarks

It is clear that we need far more data, including more genes analysed in more species spanning a range of phyla, as well as tools to study gene function in non-model systems, to answer the questions we have posed. Although some of the similarities in patterning found between distantly related organisms are indeed surprising, it is perhaps too soon to conclude that segmentation is homologous between the various phyla. Indeed, the opposite conclusion of convergence at the level of developmental mechanism is perhaps more intriguing.

References

- 1 Willmer, P. (1990) Body divisions – metamerism and segmentation. In *Invertebrate Relationships; Patterns in Animal Evolution*, pp. 39–45, Cambridge University Press
- 2 Grobden, K. (1908) Die systematische Einteilung des Tierreiches. *Verh. Zool. Bot. Ges. Wien* 58, 491–511
- 3 Clark, R.B. (1981) Locomotion and the phylogeny of the Metazoa. *Boll. Zool.* 48, 11–28
- 4 Eernisse, D.J. *et al.* (1992) Annelida and Arthropoda are not sister taxa: a phylogenetic analysis of spiralian metazoan morphology. *Syst. Biol.* 41, 305–330
- 5 Adoutte, A. *et al.* (1999) Animal evolution. The end of the intermediate taxa? *Trends Genet.* 15, 104–108
- 6 de Rosa, R. *et al.* (1999) Hox genes in brachiopods and priapulids and protostome evolution. *Nature* 399, 772–776
- 7 Aguinaldo, A.M. *et al.* (1997) Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* 387, 489–493
- 8 Sander, K. (1976) Specification of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol.* 12, 125–238
- 9 Sommer, R.J. and Tautz, D. (1993) Involvement of an orthologue of the *Drosophila* pair-rule gene *hairy* in segment formation of the short germ-band embryo of *Tribolium* (Coleoptera). *Nature* 361, 448–450
- 10 Patel, N.H. *et al.* (1992) Changing role of even-skipped during the evolution of insect pattern formation. *Nature* 357, 339–342
- 11 Patel, N.H. *et al.* (1994) Pair-rule expression patterns of even-skipped are found in both short- and long-germ beetles. *Nature* 367, 429–434
- 12 Brown, S.J. *et al.* (1997) Molecular characterization and embryonic expression of the even-skipped ortholog of *Tribolium castaneum*. *Mech. Dev.* 61, 165–173
- 13 Brown, S.J. *et al.* (1994) The beetle *Tribolium castaneum* has a *fushi-tarazu* homolog expressed in stripes during segmentation. *Proc. Natl. Acad. Sci. U. S. A.* 91, 12922–12926
- 14 Schroder, R. *et al.* (1999) Elimination of EVE protein by CAL1 in the short germ band insect *Tribolium* suggests a conserved pair-rule function for even skipped. *Mech. Dev.* 80, 191–195
- 15 Stuart, J.J. *et al.* (1991) A deficiency of the homeotic complex of the beetle *Tribolium*. *Nature* 350, 72–74
- 16 Maderspacher, F. *et al.* (1998) Pair-rule and gap gene mutants in the flour beetle *Tribolium castaneum*. *Dev. Genes Evol.* 208, 558–568
- 17 Dawes, R. *et al.* (1994) *Dax*, a locust Hox gene related to *fushi-tarazu* but showing no pair-rule expression. *Development* 120, 1561–1572
- 18 Bastian, H. and Gruss, P. (1990) A murine even-skipped homologue, *Evx 1*, is expressed during early embryogenesis and neurogenesis in a biphasic manner. *EMBO J.* 9, 1839–1852
- 19 D'Esposito, M. *et al.* (1990) *EVX2*, a human homeobox gene homologous to the even-skipped segmentation gene, is localized at the 5' end of *HOX4* locus on chromosome 2. *Genomics* 10, 43–50
- 20 Ruiz i Altaba, A. and Melton, D.A. (1989) Bimodal and graded expression of the *Xenopus* homeobox gene *Xhox3* during embryonic development. *Development* 106, 173–183
- 21 Ahringer, J. (1996) Posterior patterning by the *Caenorhabditis elegans* even-skipped homolog *vab-7*. *Genes Dev.* 10, 1120–1130
- 22 Patel, N.H. (1994) The evolution of arthropod segmentation: insights from comparisons of gene expression patterns. *Development* (Suppl.), 201–207
- 23 Damen, W.G. *et al.* (1998) A conserved mode of head segmentation in arthropods revealed by the expression pattern of Hox genes in a spider. *Proc. Natl. Acad. Sci. U. S. A.* 95, 10665–10670
- 24 Weisblat, D.A. *et al.* (1988) Segmentation in leech development. *Development* (Suppl.) 104, 161–168
- 25 Shankland, M. (1991) Leech segmentation: cell lineage and the formation of complex body patterns. *Dev. Biol.* 144, 221–231
- 26 Wedeen, C.J. and Weisblat, D.A. (1991) Segmental expression of an engrailed-class gene during early development and neurogenesis in an annelid. *Development* 113, 805–814
- 27 Lans, D. *et al.* (1993) Cell lineage analysis of the expression of an engrailed homolog in leech embryos. *Development* 117, 857–871
- 28 Shain, D.H. *et al.* (1998) Gangliogenesis in leech: morphogenetic processes leading to segmentation in the central nervous system. *Dev. Genes Evol.* 208, 28–36
- 29 Patel, N.H. *et al.* (1989) Expression of engrailed proteins in arthropods, annelids and chordates. *Cell* 58, 955–968
- 30 Goulding, M. *et al.* (1994) Regulation of *Pax-3* expression in the dermomyotome and its role in muscle development. *Development* 120, 957–971
- 31 Holland, L.Z. *et al.* (1997) Sequence and embryonic expression of the amphioxus engrailed gene (*AmphiEn*): the metameric pattern of transcription resembles that of its segment-polarity homolog in *Drosophila*. *Development* 124, 1723–1732
- 32 Liang, Z. and Biggin, M.D. (1998) *Eve* and *ftz* regulate a wide array of genes in blastoderm embryos: the selector homeoproteins directly or indirectly regulate most genes in *Drosophila*. *Development* 125, 4471–4482
- 33 Muller, M. *et al.* (1996) Expression domains of a zebrafish homologue of the *Drosophila* pair-rule gene *hairy* correspond to primordia of alternating somites. *Development* 122, 2071–2078
- 34 Kimmel, C.B. (1996) Was Urbilateria segmented? *Trends Genet.* 12, 329–331
- 35 De Robertis, E.M. (1997) Evolutionary biology. The ancestry of segmentation. *Nature* 387, 25–26
- 36 Palmeirim, I. *et al.* (1997) Avian *hairy* gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell* 91, 639–648
- 37 van Eeden, F.J. *et al.* (1998) Zebrafish segmentation and pair-rule patterning. *Dev. Genet.* 23, 65–76
- 38 Conlon, R.A. *et al.* (1995) Notch1 is required for the coordinate segmentation of somites. *Development* 121, 1533–1545
- 39 Hrabe de Angelis, M. *et al.* (1997) Maintenance of somite borders in mice requires the Delta homologue *Dll1*. *Nature* 386, 717–721
- 40 Zhang, N. and Gridley, T. (1998) Defects in somite formation in *lunatic* fringe-deficient mice. *Nature* 394, 374–377

WERE THEY RIGHT?

In 1993, *Trends in Genetics* invited five eminent geneticists to speculate on what genetics in the year 2000 would hold. As the quotes below show, they wrote with both humour and insight.

Some brave souls will be embarking on the analysis of polygenic systems. It will still be easier to make a worse mouse (or cow) than a better one.

Anne McLaren

The torrent of new books on ethical issues related to human genetic engineering will have settled down to a steady trickle of one per month, the flood of new journals on molecular medicine will have caused a world shortage of paper, and electronic publishing will be the name of the game. A DNA-sequencing laboratory will have temporarily shut down while the High Court hears a claim for sexual harassment by a robot.

David J. Weatherall

Understanding morphogenesis will therefore require searching for evolutionary invariance. The emerging picture is going to be more akin to a cubist painting than to a realistic portrait. But in that, biology merely falls in line with the abstract hard sciences.

Antonio Garcia-Bellido

I almost forgot to say that genetics will disappear as a separate science because, in the 21st century, everything in biology will become gene-based, and every biologist will be a geneticist.

Sydney Brenner