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Evo-devo perspectives on segmentation: model organisms, and beyond

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Bilaterian animals show a diverse array of segmental patterns and segmentation processes. Differences in pattern and process emerge both in comparisons of taxa and among sets of serial structures within one animal. Diversity in developmental mechanisms of segmentation and their genetic control is reflected in the modes in which segmentation evolves, which are difficult to accommodate within the traditional concept of segments as modular building blocks. Thus, in spite of the apparent simplicity of segmental patterns, studying the evolution of segmentation requires an approach that, in an adequate comparative framework, combines the efforts of researchers of genes, cells, embryos and post-embryonic stages.

The concept of SEGMENTATION (see Glossary) has its origin in commonplace observations of external morphology; for example, Aristotle recognized a class of *entoma* (insects) as animals presenting serially arranged incisions along their dorsal and sometimes their ventral side. Such external segmentation is often mirrored by the serial arrangement of internal structures, such as the nervous ganglia or the excretory organs. In these cases, one might be tempted to describe the animal as comprising a chain of basically identical units repeated along the main body axis. This led Cuvier (during the early 19th century) to group annelids and arthropods in his *embranchement* of the Articulata. Zoologists then extended the list of segmented animals to include the vertebrates. However, other than the arthropods (plus onychophorans and tardigrades), annelids (perhaps extending to, or including echiurans, pogonophorans and vestimentiferans) and vertebrates plus cephalochordates, all of which are commonly considered segmented, there is a heterogeneous array of other BILATERIANS with diverse serial structures running along the main body axis (Box 1) [1–3].

Segmentation is both a morphological feature and a developmental process and, even limiting our perspective to annelids, arthropods and chordates, the diversity of segmentation mechanisms is remarkable (Box 2). Developmental genetics has shown that this diversity is richer and more taxonomically scattered than was previously supposed, to the extent that the old simple models for the evolution of segmentation are now inadequate.

A tour across segmentation diversity in developmental mechanisms, their genetic control and evolutionary patterns shows the need to revise the traditional concept of

Glossary

Anamorphosis: a mode of post-embryonic development. Juveniles hatch with an incomplete complement of segments; the final adult number of segments is reached later during ontogeny.

Bilaterians: all multicellular animals, excluding sponges, placozoans, cnidarians and ctenophores.

Compartment: cell population with different self-recognition properties such that the progeny of the founding cells of one compartment do not intermingle with those of neighbouring compartments.

Epimorphosis: a mode of post-embryonic development. The full complement of segments is produced during embryogenesis.

Gastrulation: process in early animal embryogenesis by which the germ layers (endoderm, ectoderm and, in bilaterians, mesoderm) are established.

Germ-band: early insect embryo. Insects are classified as short (e.g. the grasshopper *Schistocerca*), intermediate (e.g. the beetle *Necrobia*) or long germ-band (e.g. the fruit fly *Drosophila*) according to the length of the embryo relative to the length of the entire egg and the number of segments specified before gastrulation.

Molecular oscillator: chemical system where the concentration of one or more molecules varies periodically with time.

Ontogeny: development of an organism from the fertilized egg to the adult.

Parasegment: ontogenetically transient (embryonic) segmental unit, corresponding to one compartment, spanning the same length as a segment, but shifted in phase with respect to the morphologically distinct segments found in juvenile and adult.

Proglottid: segmental unit of tapeworms.

Proliferative zone (also **growth** or **progress zone**): ambiguous designation referring to a posterior (subterminal) region where segments develop sequentially. In common usage, it does not strictly indicate a zone of localized cell proliferation by mitosis that produces segmented tissue.

Pseudosegment: in traditional textbook zoology, serial body unit along the main body axis in animals other than annelids, arthropods or vertebrates.

Resegmentation: in vertebrates, secondary segmentation that follows somite formation, occurring in the ventral half of the somite (sclerotome) to form vertebrae. Vertebrae are out of phase with the dorsal half of the somite (dermomyotome).

Rhombomere: segmental compartment of the developing hindbrain (rhombencephalon) in vertebrates.

Segment: one of several repetitive body units along the antero–posterior body axis.

Segmentation: developmental process of segment formation (segmentation mechanism). Condition of a segmented animal, a form of body symmetry (translational symmetry).

Somite: in vertebrate and cephalochordate embryos, this is a segmental block of mesodermal cells on either side of the neural tube that, later in ontogeny, will produce segmental and non-segmental structures.

Somitogenesis: in vertebrate and cephalochordate embryos, this is the process of segmentation of the paraxial mesoderm producing the somites.

Zooid: growing and/or redifferentiating part of the body of an asexually reproducing animal (e.g. a microstomid flatworm; Box 1) forming a new individual. When new individuals are formed in an antero–posterior direction, segmental chains of zooids are produced before they detach to become autonomous. Also an individual in a colony of a modular organism.

Zonite: segmental unit of kinorhynchs (Box 1).

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Box 1. Segmental structures in 'non-segmented' animals

The following taxa are usually described as being PSEUDOSEGMENTED rather than segmented. However, different traits of their morphology are repeated according to a periodic pattern along the main body axis.

Orthonectida

A group of small parasites of uncertain affinity, with species having transversal rings of epidermal cells. In *Ciliocincta sabellariae*, the series of rings is patterned with wreaths of cilia, and there are alternate rings with and without granular inclusions. Females have 38–39 rings, males, ∼20 (Figure Ia: female, 270 μm; reproduced with permission from [44]).

'Turbellaria'

A paraphyletic group of free-living Platyhelminthes (flatworms) with species having serially repeated gut diverticula, protonephridia and transverse nerves. Catenulids, stenostomids and microstomids reproduce asexually, producing a chain of up to 16 ZOOIDS, sometimes more (Figure Ib: *Microstomum lineare,* the zooid chain is 8 mm in length).

Cestoda

Parasitic Platyhelminthes (tapeworms) whose bodies are articulated into a chain of PROGLOTTIDS (up to 4500) complete with excretory and reproductive apparatus, which are often detachable, but have limited autonomy (Figure Ic: *Taenia solium*, up to 8 m in length).

Bdelloidea

Benthic parthenogenic rotifers with 15–18 (typically 16) telescopic rings of intraepithelial skeletal laminae (Figure Id: *Philodina roseola*, 430 μm).

Nemertini

The ribbon worms, whose segmental gonads (each with its independent external opening) alternate with the lateral diverticula of the gut. Some species can grow up to several meters in length (Figure Ie: *Prostoma graecense*, 15 mm).

Mollusca

Seven or eight transverse rows of dorsal calcareous spicules or plates in larval Solenogastres, Caudofoveata and Polyplacophora. Eight dorsal plates and 6–88 pairs of ctenidia in adult Polyplacophora. Three-six pairs of ctenidia and three-seven pairs of metanephridia in Monoplacophora. A series of foot retractor muscles in Monoplacophora, Polyplacophora and *Babinka* (Bivalvia, Ordovician) [45] (Figure If: the polyplacophoran *Chiton olivaceus,* 38 mm).

Gastrotricha

Microscopic aquatic bilaterians. Many species with rows of cuticular spines, scales, or plates with species-specific number and arrangement (Figure Ig: *Chaetonotus brachyurus*, 130 μm).

Kinorhyncha

Tiny marine animals with a body divided into 13 (exceptionally 14, genus *Campyloderes*) ZONITES, segmental units with repetitive ganglia, intersegmental muscles and epidermal and cuticular structures. Juveniles hatch with fewer zonites; the full complement is achieved via a series of moults (anamorphic post-embryonic development) (Figure Ih: *Echinoderes dujardini*, 400 μm).

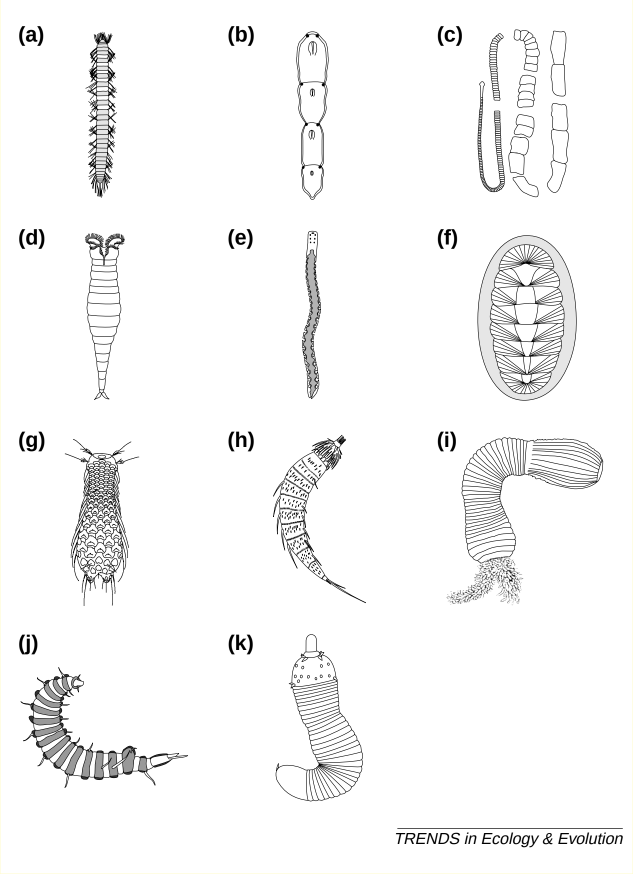

Figure I. Examples of pseudosegmented taxa.

Priapula

Species with cuticular annulation. More conspicuous segmental exoskeletal structures were present in the Palaeozoic *Palaeoscolex* [46] (Figure Ii: *Priapulopsis caudatus*, 50 mm).

Nematoda

Free-living, typically marine, nematodes of the family Desmoscolecidae having tyre-like cuticular rings, sometimes made more conspicuous by aggregated materials (desmen). The number of desmen, up to hundreds and variable in some species, is lower and fixed in others (e.g. 17 in *Desmoscolex falcatus*, Figure Ij: 400 μm; reproduced with permission from [47]). Erratic root ectoparasites of the family Criconematidae with ornate embricated cuticular rings.

Gordioidea

In this order of horsehair worms (Nematomorpha), free-living larvae have an annulated cuticle (Figure Ik: larval *Gordius aquaticus*, 145 μm).

segmentation and to exploit fully the powerful approach now suggested by evolutionary developmental biology, or 'evo-devo' [4]. Focussing on the evolution of ONTOGENY as a whole rather than on its individual components, and completing a comparative analysis that is not limited to a few model species, will increase the explanatory power of our models of the evolution of segmentation.

Mechanisms of segmentation

Developmental mechanisms of segmentation are diverse, and differences are often found between even phylogenetically close species; distantly related species can instead exhibit unexpected similarities.

Body segmental units can originate almost simultaneously within a closed system (i.e. one with already determined anterior and posterior ends, as in the long GERM-BAND insect *Drosophila*), but often originate sequentially, mainly in an antero–posterior progression. The sequential process can either be associated with the activity of a subterminal PROLIFERATIVE ZONE, as in annelids [5], malacostracan crustaceans [6] and vertebrates (SOMITOGENESIS) [7], or not, as is the case in insects, where, even in short germ-band species, there is no evidence that the germ band elongates by activity of a posterior proliferative zone [8].

Other than the superficial similarity of the sequential appearance of molecular or morphological segmental markers, little is known about the mechanism of segment specification at the posterior end of the developing embryo in animals other than vertebrates, where the association with the activity of a MOLECULAR OSCILLATOR has been demonstrated [9]. Moreover, in insects, the earliest expression of segmentation is in the ectoderm, whereas, in annelids and vertebrates, segmentation is driven by the mesoderm.

Although more than one mechanism can give rise to sequential segmentation, in arthropods, simultaneous and sequential segmentation are perhaps not so different, because there are many insects that are intermediate between the two extreme short and long germ-band types [10]; the diversity in germ length is sometimes high even within one order, as in beetles [11]). In the lancelet *Branchiostoma lanceolatum*, the first five or six SOMITE pairs form simultaneously, whereas the rest form sequentially [12]. Both simultaneous and sequential segmentation can produce different segmental units within the same animal, as seen in the step-wise subdivision of the hindbrain in RHOMBOMERES [13] versus sequential somitogenesis in vertebrates [9].

Other variables in segmentation processes include the involvement of COMPARTMENT formation and the relationship between compartment boundaries and segment margins. Compartments of the same length as a segment but shifted posteriorly about half a segment (called 'PARASEGMENTS') are formed, for example, in insect embryos, but also in crustaceans [6] and spiders [14]. These are the primary segmental units of the embryo, the posterior half of one parasegment and the anterior half of the following parasegment eventually forming one of the segments as defined by anatomists. However, in the centipedes *Lithobius* [15] and *Strigamia* [16], the primary units of segmentation of the blastoderm are the prospective definitive segments. In vertebrates, each vertebra is formed by materials from two contiguous somites; thus, the vertebral series is also shifted by one-half unit in respect to the somites. The developmental units involved in this process (called 'RESEGMENTATION') are compartments in the chick, but not in zebrafish [17].

Fundamental differences in segmentation emerge in comparisons of different taxa and between different sets of serial structures within one animal. There are two pieces of evidence for the existence of multiple distinct segmentation processes within the same animal. First, units belonging to different serially repeated structures do not necessarily follow the same periodicity along the body axis. Examples are muscles blocks versus gill slits in the lancelet, and legs versus exoskeletal rings in the tadpole shrimp *Triops cancriformis* (Box 2). Second, a small set of segments at the front end of the embryo can be the product of processes other than those that generate the remaining segments. This has been shown in insects [18] chelicerates [19] and millipedes [20] and is suggested to be a general feature of all arthropods [21].

Resulting from this diversity in segmentation mechanisms is intra-specific variation in the level of developmental precision of the number of segments produced, as well as the ability to regenerate parts of the segmented body axis [22]. However, it is not possible to reduce such diversity to simple contrasts between a few model species to then be extrapolated to whole phyla of segmented metazoans.

Segmentation genes

If a model-system approach fails to account for the diversity and taxonomic distribution of segmentation mechanisms, does the picture become simpler if segmentation is considered at the gene expression level?

So-called 'segmentation genes' were first discovered in the fruit fly *Drosophila*, where they are classified into gap, pair-rule and segment-polarity genes. These genes occupy different levels in a hierarchical cascade leading from the early gap genes to the later-expressed pair-rule and segment-polarity genes that encode proteins that are eventually arranged in the embryo as stripes with segmental periodicity. Homologues of most of these *Drosophila* segmentation genes have been discovered in other bilaterians, but their actual developmental role is often different.

For example, the segment-polarity gene *engrailed* (*en*) is expressed in a series of transversal stripes in arthropods, leeches, chitonid molluscs, onychophorans, vertebrates and the lancelet [23]. However, in *Drosophila*, *en* is expressed exclusively in the ectoderm, marks compartment boundaries and is involved in patterning the nervous system; in the leech, it is expressed both in ectoderm and mesoderm and does not play any role in the segmentation of the nervous system [24]. In most arthropods, *en* marks the posterior end of the developing segment, but, in vertebrates, its homologue is expressed in the somites only after these are formed [25]. In the millipede *Glomeris*, *en* marks different intra-segmental locations in the dorsal and ventral structures of the same animal [20]. Homologues of *en* have also been found in animals without any overt segmentation, such as the mollusc *Patella* [26]. Ectodermal expression of *en* is also associated with skeletal development across a range of bilaterian clades (segmented and not), from arthropods to molluscs to ophiuroid echinoderms [23].

The role of a segmentation gene can vary widely within a clade, and the same developmental task can also be accomplished by different segmentation genes. This is evident even in a clade with scarce morphological variation in segmental patterns, as occurs in insects. Actual diversity in insect segmentation largely exceeds that seen in germ-band type [8]: germ-band development combines with timing in segment specification, blastoderm cellularization and GASTRULATION in many different ways. In *Drosophila* and the honeybee *Apis mellifera*, the pair-rule gene *even-skipped* (*eve*) shows first a two-segment periodicity, which is typical of pair-rule genes and, later during embryonic development, a one-segment periodicity, similar to segment-polarity genes. However, in the dipteran *Clogmia*, *eve* is expressed only in a pair-rule pattern, in the parasitic wasp *Copidosoma* only in segment-polarity pattern, whereas in the grasshopper *Schistocerca*, it is not expressed in a periodic pattern at all. The double-segmental pattern of a pair-rule gene can secondarily develop into a segmental pattern in two different ways even in the same insect (*Drosophila*). Early stripes get narrower and new intercalary stripes (midway between any two of the earlier stripes) appear *de novo* (*eve* and *runt*), or early stripes split in two through loss of expression in the middle (*paired*) [8].

In vertebrates, somitogenesis involves a molecular oscillator, consisting of the alternate expression of a set of so-called 'cyclic genes', among which are genes of the *Delta-Notch* signalling pathway [9]. The recent finding that homologues of these genes are involved in spider segmentation has been interpreted as evidence of a genetic programme that is common to arthropods and vertebrates [27]. However, as pointed out by the authors themselves, in bilaterians, the *Delta-Notch* system is involved in boundary formation in many tissues independent of segmentation and it is not known whether, in spiders, the dynamic expression of these genes is a periodic oscillation.

At the level of segmentation genes, we find the same pattern that is seen at the level of developmental mechanisms. The same outcome can be the product of different genes in different animals, and the role of the same segmentation gene can vary widely even among close relatives.

Evolutionary patterns

The phylogenies of segmented bilaterians show evolutionary changes in segment number by either increasing or decreasing the number of segments [28]. However, developmental constraints on the evolution of the number of segments have occurred in different lineages. In some arthropod taxa, the adult number of segments is invariable (e.g. decapod crustaceans), whereas in others, intraspecific variation is the norm (e.g. spirostreptid millipedes). In geophilomorph centipedes, the number of leg-bearing segments varies from 27 to 191, but, intriguingly, only odd numbers are observed. The number of segments might change more easily within some body regions, as in mammals, where the thoracic vertebrae vary between nine and 25, whereas the cervical vertebrae are usually seven.

Differences in the temporal schedule of segment production can affect embryonic and post-embryonic development. Development is ANAMORPHIC in basal centipedes (scutigeromorphs, lithobiomorphs and craterostigmomorphs) and EPIMORPHIC in the more-derived scolopendromorphs and geophilomorphs. Different kinds of anamorphosis, qualitatively diverse in terms of post-embryonic segment addition, are found in millipedes [29].

This first set of modes of evolutionary change easily fit the stereotype of segmented animal as being comprised of a series of repetitive units evolving in number, specialization and developmental scheduling. However, the list of modes in which segmentation evolves does not end here.

Structures with a periodic arrangement can be independently acquired and lost. Some leeches have acquired a segmented gut, whereas the enchytraeid oligochaetes have lost coelomic segmentation. More or less extensive loss of segmental arrangement in the nervous system has occurred independently in several arthropod lineages. In chordates, segmentation of the (mesodermal) dermomyotome was progressively restricted to the dorsal part in the lineage leading to fish and terrestrial vertebrates [30]. Serial structures can independently change period and phase. This has occurred in several arthropod lineages, producing a mismatch in segmental arrangement among dorsal and ventral structures, such as in pauropods, symphylans, many millipedes and crustaceans [31].

Segmentation is not only a feature of the 'definitive' organization of the animal, but also a feature of the embryo. As such, its significance is not necessarily or uniquely to be seen in relation to the 'finished product'. Regular spacing of a transient segmental structure (the precursors of ventral motor neurons) is found even in the nematode *Caenorhabditis elegans* [32], an animal whose adult anatomy exhibits no segmentation. This 'transient segmentation' evolved possibly as a functional

Box 2. Ten 'conceptual' models of segmentation

Other than the more conspicuous differences in segment production (e.g. simultaneous versus sequential), segmentation processes are also very diverse with respect to the type of control mechanism, level of coordination, developmental scheduling and embryonic tissues involved (Table I; Figure I).

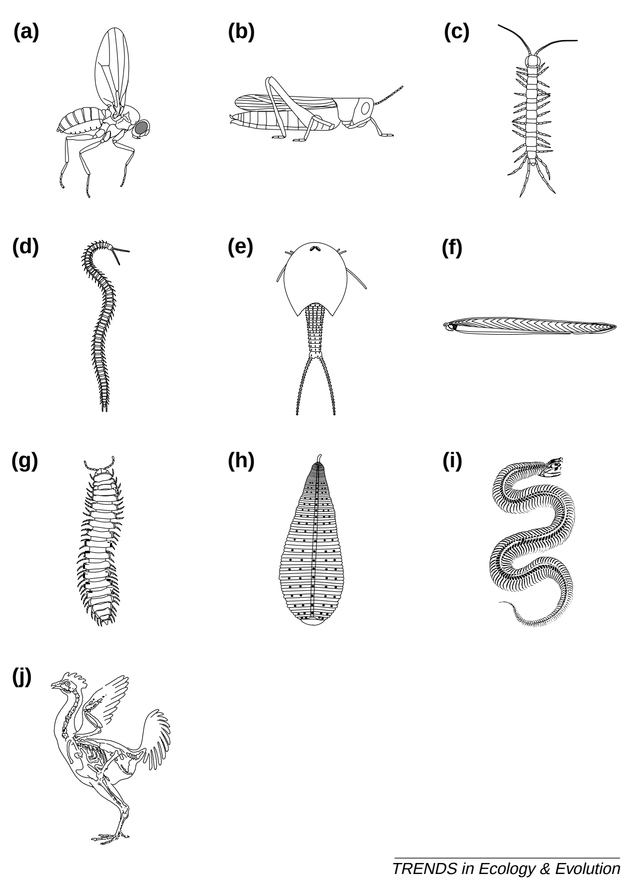

Figure I. Conceptual models of segmentation.

Table I. 'Conceptual' models of segmentation

Taxon		Figure	Refs
Drosophila melanogaster **(Insecta: Diptera; fruit fly)**	Long germ-band insect; segmental units (parasegments) originate almost simultaneously; blastoderm (the external embryonic layer, initially syncytial, surrounding the yolk mass) achieves a final 14-stripe pattern of gene expression by stepwise subdivision of broader segmental units through a cascade of activation of segmentation genes	Ia	[48]
Schistocerca gregaria **(Insecta: Orthoptera; desert locust)**	Short germ-band insect; few anterior segments originate synchronously in the cellular blastoderm of the early embryo, the remaining segments are sequentially specified in antero–posterior progression	Ib	[49]
Lithobius atkinsoni **(Chilopoda: Lithobiomorpha; brown centipede)**	Juvenile hatches with an incomplete complement of segments. The final number is reached after a series of moults (anamorphic development). The number of adult segments does not vary within lithobiomorphs	Ic	[15]
Strigamia maritima **(Chilopoda: Geophilomorpha; centipede)**	Juvenile hatches with the full complement of segments (epimorphic development). Intraspecific within-sex variation for the number of trunk segments; females have two leg-bearing segments more than do the corresponding males. Individuals with more segments are the same length as those (of the same sex) with fewer segments (segment length compensation). There is a significant heritable component in segment number variation	Id	[50]
Triops cancriformis **(Crustacea: Notostraca; tadpole shrimp)**	Mismatch in segmental arrangement between different segmentally repeated structures: the series of legs is shorter than the series of exoskeletal rings; leg series has different periodicity along the body axis; longitudinal dorsal and lateral muscles are in phase with rings, whereas longitudinal ventral muscles are in phase with legs; rings and legs have independent post-embryonic developmental schedules	Ie	[51]
Branchiostoma lanceolatum **(Cephalochordata; lancelet)**	Two independent systems of repetitive structures: branchiomery (gill slits, excretory organs and haemal system) and myomery (muscle blocks, coelomic sacs and nervous system), myomery has left-right asymmetry (left myomers shifted one half unit forward with respect to the right ones)	If	[12]
Polydesmus complanatus **(Diplopoda; millipede)**	Anterior tergites (dorsal sclerites) of the trunk corresponding to one pair of legs each (first three trunk segments), followed by tergites corresponding to two pairs of legs each (diplosegments)	Ig	[29]
Helobdella triserialis **(Annelida: Hirudinea; leech)**	Sequential production of segment anlagen results in an invariant segment number (32); segments are founded by cells derived from blast cells through an invariant series of divisions; rows of founder cells are brought into alignment through cell migration (morphogenetic assembly); epidermis and cuticle further subdivided, with three annuli per segment	Ih[a]	[5]
Python sebae **(Vertebrata: Serpentes; snake)**	Mesodermal segmentation (muscles and vertebrae) projects onto ectoderm derivatives (shields and scales); in most snakes, there is a ~1:1 ratio between the number of ventral shields and the number of vertebrae	Ii	[52]
Gallus gallus **(Vertebrata: Aves; chick)**	Somitogenesis of paraxial mesoderm associated with a molecular oscillator; presomitic mesoderm (PSM) formed in antero–posterior direction by a population of posterior stem cells, whereas somites differentiate at the anterior border of PSM for the alternate (in time) expression of cyclic genes; temporal prepattern of progress zone is transformed into the spatial pattern of somites	Ij[b]	[9]

[a] Image reproduced with permission from [53].

[b] Image reproduced with permission from [54].

feature of the developing animal, as a specific way to arrange clusters of cells [33], to optimize resource utilization during development, or to simplify cell fate specification [34].

This second set of modes of evolutionary change in segmentation is more difficult to accommodate within a traditional, modular view of segmented animals. New comparative data from molecular, cellular and developmental biology requires us to abandon the traditional view of segments as elements of a segmented archetype, to embrace instead a concept based on the actual diversity of processes through which animals develop.

Studying the evolution of segmentation

Our recipe for the study of segmentation is based on a less restrictive concept of segmentation and on a deeper integration of comparative data at many levels. The concept of segmentation should be relaxed in several directions.

First, segmentation applies to organs (or tissues) rather than to organisms [1]. A segmented organism results when several different structures present a repetitive pattern with the same period and phase [35]. What zoologists have traditionally described as 'true segments' are perhaps no more than a largely coincident array of serial features, separately involving nervous system (neuromeres), musculature (myomeres), excretory apparatus (nephromeres) and other organs. Second, segmentation appears to be a 'generic' property of bilaterians [36], that is, a feature related to the general physicochemical properties of living tissue, regardless of its specific molecular makeup. As such, segmentation would be expected to have evolved numerous times. Third, a periodic feature is simultaneously both structural pattern and developmental signal. This enables stabilization of transient segmental expression and the exploitation of early segmental patterns as pre-patterns for further morphogenetic processes. An origin of 'true' segmentation via stepwise inclusion of segmental structures has been recently suggested [3].

What then is segmentation? What remains after our discussion could appear a trivial and useless geometric definition of segmentation as a form of symmetry [37]; that is, translational symmetry: a definition that is not suitable for coding the character 'segmentation' in a matrix for phylogenetic reconstruction. However, reconstructing phylogeny does not exhaust the research agenda of evolutionary biologists. Mechanisms of evolutionary change and the origin of evolutionary novelties must also be elucidated. Sometimes, definitions perform more as conceptual traps than as starting points for further investigations. If we shoehorn phenomena into our preconceived categories, we will lose the wealth of information about them. Such is the case for segmentation.

A recent paper about the expression of segmentation genes in the pill millipede *Glomeris* [20] offers a strong argument for the value of the approach that we are defending here, because the paper demonstrates that segments, obvious as they might seem in millipedes, are not a kind of preformed building blocks that are only waiting to be produced, or arranged, in a linear series. Instead, they are the result of a tentative matching of independently established dorsal and ventral series of repetitive parts. Moreover, the genes involved in establishing the dorsal and the ventral segmental pattern are not all the same, and one of these genes (*en*), although expressed both dorsally and ventrally, appears to have different roles and significance in the two serial patterns. Finally, there are large differences between the expression patterns of these genes in different regions along the main body axis and – to complete the 'deconstruction' of its segmental nature – the anterior half of the developing head does not show any evidence of dorsal segmentation.

By adopting an evo-devo approach, we can gain a better understanding of how periodic patterns and processes operate and change during evolution. This does not reduce to deciding whether *Urbilateria*, the last common ancestor of bilaterians, was segmented [2,38–41]. What we can achieve is a deeper understanding of how bilaterian segmentation evolves and produces novelties and constraints that shape the arena of phenotypic variation on which natural selection operates [42,43]. Beyond the scope of this paper, this view unravels connections between segmentation and other key life processes (e.g. asexual reproduction, see catenulids in Box 1) and major features of animal organization (e.g. other body symmetries as in echinoderms, segmented appendages as in arthropods and colonial organization as in corals), thus unitarily addressing the evolution of animal form.

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References

1 Budd, G.E. (2001) Why are arthropods segmented? *Evol. Dev.* 3, 332–342

2 Balavoine, G. and Adoutte, A. (2003) The segmented *Urbilateria*: a testable scenario. *Integr. Comp. Biol.* 43, 137–147

3 Scholtz, G. (2003) Is the taxon Articulata obsolete? Arguments in favour of a close relationship between annelids and arthropods. In *The New Panorama of Animal Evolution* (Legakis, A. *et al.*, eds), pp. 489–501, Pensoft Publishers

4 Hall, B.K. (1998) *Evolutionary Developmental Biology*, Chapman & Hall

5 Zackson, S.L. (1982) Cell clones and segmentation in leech development. *Cell* 31, 761–770

6 Dohle, W. and Scholtz, G. (1988) Clonal analysis of the crustacean segment: the discordance between genealogical and segmental borders. *Development* 104, 147–160

7 Palmeirim, I. *et al.* (1997) Avian *hairy* gene expression identifies a molecular clock linked to vertebrate segmentation and somitogenesis. *Cell* 91, 639–648

8 Davis, G.K. and Patel, N.H. (2002) Short, long and beyond: molecular and embryological approaches to insect segmentation. *Annu. Rev. Entomol.* 47, 669–699

9 Pourquié, O. (2003) The segmentation clock: converting embryonic time onto spatial pattern. *Science* 301, 328–330

10 Salazar-Ciudad, I. *et al.* (2001) Phenotypic and dynamical transitions in model genetic networks. II. Application to the evolution of segmentation mechanisms. *Evol. Dev.* 3, 95–103

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 Conklin, E.G. (1932) The embryology of the *Amphioxus*. *J. Morphol.* 54, 69–118

13 Lumsden, A. and Krumlauf, R. (1996) Patterning the vertebrate neuraxis. *Science* 274, 1109–1115

14 Damen, W.G.M. (2002) Parasegmental organization of the spider embryo implies that the parasegment is an evolutionary conserved entity in arthropod embryogenesis. *Development* 129, 1239–1250

15 Hughes, C.L. and Kaufman, T.C. (2002) Exploring myriapod segmentation: the expression patterns of *even-skipped*, *engrailed*, and *wingless* in a centipede. *Development* 129, 1225–1238

16 Chipman, A.D. *et al.* (2004) Early development and segment formation in the centipede *Strigamia maritima* (Geophilomorpha). *Evol. Dev.* 6, 78–89

17 Morin-Kensicki, E.M. *et al.* (2002) Segmental relationship between somites and vertebral column in zebrafish. *Development* 129, 3851–3860

18 Rogers, B.T. and Kaufman, T.C. (1997) Structure of the insect head in ontogeny and phylogeny: a view from *Drosophila*. *Int. Rev. Cytol.* 174, 1–84

19 Dearden, P.K. *et al.* (2002) Expression of pair-rule gene homologues in a chelicerate: early patterning of the two-spotted spider mite *Tetranychus urticae*. *Development* 129, 5461–5472

20 Janssen, R. *et al.* (2004) Gene expression suggests decoupled dorsal and ventral segmentation in the millipede *Glomeris marginata* (Myriapoda: Diplopoda). *Dev. Biol.* 268, 89–104

21 Minelli, A. (2001) A three-phase model of arthropod segmentation. *Dev. Genes Evol.* 211, 509–521

22 Minelli, A. (2003) *The Development of Animal Form*, Cambridge University Press

23 Jacobs, D.K. *et al.* (2000) Molluscan *engrailed* expression, serial organization, and shell evolution. *Evol. Dev.* 2, 340–347

24 Shankland, M. (2003) Evolution of body axis segmentation in the bilaterian radiation. In *The New Panorama of Animal Evolution* (Legakis, A. *et al.*, eds), pp. 187–195, Pensoft Publishers

25 Holland, L.Z. and Holland, N.D. (1998) Developmental gene expression in amphioxus: new insights into the evolutionary origin of vertebrate brain regions, neural crest, and rostrocaudal segmentation. *Am. Zool.* 38, 647–658

26 Nederbragt, A.J. *et al.* (2002) Expression of *Patella vulgata* orthologs of *engrailed* and *dpp-BMP2/4* in adjacent domains during molluscan shell development suggests a conserved compartment boundary mechanism. *Dev. Biol.* 246, 341–355

27 Stollewerk, A. *et al.* (2003) Involvement of *Notch* and *Delta* genes in spider segmentation. *Nature* 423, 863–865

28 Richardson, M.K. *et al.* (1998) Somite number and vertebrate evolution. *Development* 125, 151–160

29 Enghoff, H. *et al.* (1993) Anamorphosis in millipedes (Diplopoda). The

present state of knowledge and phylogenetic considerations. *Zool. J. Linn. Soc.* 109, 103–234

30 Mazet, F. and Shimeld, S.M. (2003) Characterization of the amphioxus *Fringe* gene and the evolution of the vertebrate segmentation clock. *Dev. Genes Evol.* 213, 505–509

31 Minelli, A. and Fusco, G. (1995) Body segmentation and segment differentiation: the scope for heterochronic change. In *Evolutionary Change and Heterochrony* (McNamara, K.J., ed.), pp. 49–63, John Wiley & Sons

32 Walthall, W.W. (1995) Repeating patterns of motoneurons in nematodes: the origin of segmentation? In *The Nervous System of Invertebrates: An Evolutionary and Comparative Approach* (Breidbach, O. and Kutsch, W., eds), pp. 61–75, Birkhäuser

33 Lawrence, P.A. (1992) *The Making of a Fly*, Blackwell

34 Braun, V. *et al.* (2003) ALES: cell lineage analysis and mapping of developmental events. *Bioinformatics* 19, 851–858

35 Holland, P.W.H. (1990) Homeobox genes and segmentation: co-option, co-evolution, and convergence. *Semin. Dev. Biol.* 1, 135–145

36 Newman, S.A. (1993) Is segmentation generic? *Bioessays* 15, 277–283

37 Beklemishev, W.N. (1969) *Principles of Comparative Anatomy of Invertebrates*, Oliver & Boyd

38 De Robertis, E.M. and Sasai, Y. (1996) A common plan for dorsoventral patterning in Bilateria. *Nature* 380, 37–40

39 Davis, G.K. and Patel, N.H. (1999) The origin and evolution of segmentation. *Trends Genet.* 15, 68–72

40 Erwin, G.H. and Davidson, E.H. (2002) The last common bilaterian ancestor. *Development* 129, 3021–3032

41 Seaver, E.C. (2003) Segmentation: mono- or polyphyletic? *Int. J. Dev. Biol.* 47, 583–595

42 Fusco, G. (2001) How many processes are responsible for phenotypic evolution? *Evol. Dev.* 3, 279–286

43 Müller, G.B. and Newman, S.A. eds (2003) *Origination of Organismal Form. Beyond the Gene in Developmental and Evolutionary Biology* (Vienna Series in Theoretical Biology), Bradford Books

44 Ax, P. (1995) *Multicellular Animals*, Springer-Verlag

45 McAlester, A.L. (1965) Systematic, affinities, and life habits of Babinka, a transitional Ordovician lucinoid bivalve. *Paleontology* 8, 231–246

46 Conway Morris, S. (1997) The cuticular structure of the 495-Myr-old type species of the fossil worm *Palaeoscolex*, *P. piscatorum* (?Priapulida). *Zool. J. Linn. Soc.* 119, 69–82

47 Plat, H.M. and Warwick, R.M. (1988) *Synopsis of the British Fauna (New Series). Free Living Marine Nematodes II. British Chromadorids*, E.J. Brill

48 Bate, M. and Martínez Arias, A. (1993) *The Development of Drosophila melanogaster*, Cold Spring Harbor Laboratory Press

49 Davis, G.K. *et al.* (2001) Pax group III genes and the evolution of insect pair-rule patterning. *Development* 128, 3445–3458

50 Kettle, C. *et al.* (2003) The pattern of segment formation, as revealed by *engrailed* expression, in a centipede with a variable number of segments. *Evol. Dev.* 5, 198–207

51 Linder, F. (1952) Contributions to the morphology and taxonomy of the Branchiopoda Notostraca, with special reference to the north American species. *Proc. U.S. Nat. Mus.* 102, 1–69

52 Alexander, A.A. and Gans, C. (1966) The pattern of dermal-vertebral correlation in snakes and amphisbaenians. *Zool. Med.* 41, 171–190

53 Nesemann, H. and Neubert, E. (1999) *Süsswasserfauna von Mitteleuropa 6/2. Annelida, Clitellata*, Spektrum Akademischer Verlag

54 Lecointre, G. and Le Guyader, H. (2001) *La Classification Phylogénétique du Vivant*, Editions Berlin