

# Trunk segment numbers and sequential segmentation in myriapods

Giuseppe Fusco

Department of Biology, University of Padova, Via U. Bassi 58/B, I-35131 Padova, Italy

Correspondence (email: giuseppe.fusco@unipd.it)

**SUMMARY** Sequential segmentation from a posterior “proliferative zone” is considered to be the primitive mechanism of segmentation in arthropods. Several studies of embryonic and post-embryonic development and gene expression suggest that this occurs in all major arthropod taxa. Sequential segmentation is often associated with the idea of posterior production of body units that accumulate along the main body axis. However, the precise mechanism of sequential segmentation has not been identified yet, and, while searching for the genetic circuitry able to generate a first periodic pattern in the embryo, we can at least outline

the distinctive role in segmentation of a proliferative zone. A perusal of myriapod segmentation patterns suggests that these patterns result from multi-layered developmental processes, where gene expression and epigenetic mechanisms interact in a nonstrictly hierarchical way. The posterior zone is possibly a zone of periodic signal production, but, in general, the resulting segmental pattern is not completely attributable to the activity of the signal generator. In this sense, a posterior proliferative zone would be more a “segmental organizer” than a “segment generator.”

## INTRODUCTION

In arthropods, segmentation patterns and processes are very diverse (Minelli and Fusco 2004). Body segmental units originate almost simultaneously in the *Drosophila* embryo, but more often originate sequentially in an anteroposterior progression from a sub-terminal region, often referred to as a “proliferative zone.” Although the logics of these two mechanisms might seem diametrically opposed, simultaneous and sequential segmentation can occur in combination, as, for instance, in many insects with the embryo intermediate between short and long germ-band types. Here, the most anterior segments originate synchronously, whereas the remaining segments are sequentially specified from a sub-terminal zone (Davis and Patel 2002). At least for a significant posterior portion of the main body axis, sequential segmentation is generally considered the primitive condition in arthropods (Peel 2004). Theoretical mechanisms for the evolutionary change from sequential to partially, or totally simultaneous segmentation have been recently proposed. These are based on a gradual cellular-to-syncytial transition of the context (the blastoderm) where the same segment-forming gene network operates (Salazar-Ciudad et al. 2001), or on a progressive increase (from the anterior) of the segmental units falling under the control of gap genes (Peel 2004).

In vertebrates, the mechanism of sequential segmentation of the paraxial mesoderm (somitogenesis) is based on a molecular oscillator localized in a posterior “progress zone.” The temporal alternate expression of a set of “cyclic genes” is

transformed into the spatial pattern of somites (Pourquié 2003). Homologs of these genes are involved in segmentation of the spider *Cupiennius*, and this has been interpreted as evidence for a mechanism of segmentation based on a “segmentation clock,” which would be common to arthropods and vertebrates (Stollewerk et al. 2003). However, in arthropods, a clock-like mechanism remains to be demonstrated (Peel and Akam 2003), and beyond the posterior serial appearance of some molecular or morphological segmental marker in the developing embryos of a few model systems, very little is known about the mechanism of segment sequential specification.

Myriapoda is the taxon of many-legged arthropods that groups pauropods (Pauropoda), symphylans (Symphyla), centipedes (Chilopoda), and millipedes (Diplopoda). In this group, with the possible exclusion of a small set of anterior head segments, a sequential mechanism of segmentation is supported by several lines of evidence. In a few model species, studies of descriptive embryology (Sograff 1883; Heymons 1901; Tiegs 1940, 1947; Dohle 1974; Aerteel 1984) provide evidence of sequential production, from a posterior zone, of morphological segmental units. Studies of gene expression in the early phases of embryogenesis (Hughes and Kaufman 2002; Janssen et al. 2004; Chipman et al. 2004a) show stripes of expression of segmentation genes that appear sequentially from a posterior region of the germ-band. Moreover, in most myriapods, only a part of the series of segments is produced during embryogenesis. In these arthropods, during post-embryonic development, there is an increase in the number of

trunk segments that appear to emerge from a zone close to the rear of the body in a series of discrete increments in correspondence of molts.

Apparently, the only job left to do is the identification of the precise mechanism(s) of segment specification, in order to trace homologies for reconstructing myriapod and arthropod evolution, or, (more ambitiously) to shed light on the origins of metazoan segmented body plans. However, segmentation does not end with the first appearance of a series of stripes of gene expression, nor does it coincide with the emergence of more or less completely formed body units close to rear of the body. Segmentation is a process, not an event, and the complexity of arthropod segmentation patterns clearly indicates a multi-layered process, where gene expression and epigenetic mechanisms interact in a nonstrictly hierarchical way.

## SEGMENTS AND SEGMENTATION

A segmental pattern is a form of body symmetry (Beklemishev 1969). It refers to the serial occurrence of homologous body structures along the main body axis. However, when translational symmetry is referred to the whole body, rather than to a set of body structures, the idea of a body “comprised of” a certain number of body blocks will result (Budd 2001). This concept of a segment, as a body unit repeated along the main body axis, can certainly provide a useful unit of description, but the value of this unit is not universal within the arthropods, and even within some of their sub-groups. In particular, descriptive difficulties arise when different serial structures present discordant arrangement within the same animal.

Enghoff et al. (1993), introducing a review of millipede post-embryonic segmentation, warned of the inconsistent usage of the term “segment.” Making an effort to “avoid the term ‘segment’ as far as possible,” these authors referred directly to the individual serial structures, such as rings, plates, and leg pairs. But more often, the descriptive scheme adopted is clearly typological. Some structures are entrusted to lead the description as the identifiers of “true segments,” either because a sizable number of different structures are repeated with the same periodicity (e.g., Scholtz 2002), or because these diagnostic features are those that emerge first in development (e.g., Janssen et al. 2004). In contrast, other structures that do not match with the former are described separately, trying as much as possible to “derive” these elements from specific “true segments.” This is perhaps a useful trick in descriptive morphology, but it is clearly unsatisfactory as a starting point for studying the evolution of segmentation. In millipedes, there have been years of debate over the segments to which dorsal and ventral structures that do not match exactly may belong. Now, in a recent article on the expression of segmentation genes in the pill millipede *Glomeris*, it has been dem-

onstrated that dorsal and ventral series of repetitive units are independently established in the embryo germ-band (Janssen et al. 2004).

Here, pragmatically, for the part of the article concerned with the description of segmentation in different myriapods, segments coincide with the units of traditional descriptive morphology, generally defined by the periodic concordance of several structures (details on the segmental constitution of the different myriapod taxa are reported in the Appendix). A more critical use of the term “segment” is instead adopted in the following discussion on myriapod segmentation processes.

## THE “SPACE” OF MYRIAPOD SEGMENTATION

Although the segmental composition of the trunk is generally a part of the original description of a species, information on segmentation pattern in myriapod literature is not as detailed as one might suppose. Taxonomists are mainly interested in characters that are stable within a taxon. Therefore, information about intraspecific variation in adult segmental arrangement is often incomplete, and the post-embryonic segmentation schedule is unknown for many species, not to speak of its possible intraspecific variations. Although phylogenetic relationships within a few clades, at different taxonomic levels, have been recently investigated (e.g., Bonato et al. 2003; Edgecombe and Giribet 2004), in general, myriapod phylogeny is so poorly resolved as to rule out the possibility to map the transformations of segmentation processes and patterns on a reliable and comprehensive phylogenetic tree. However, morphological data can at least contribute to delineate a “space” of segmental patterns and segmental post-embryonic processes. This can give valuable indications of the developmental mechanisms of segmentation and, in particular, of the required performances of a putative posterior proliferative zone.

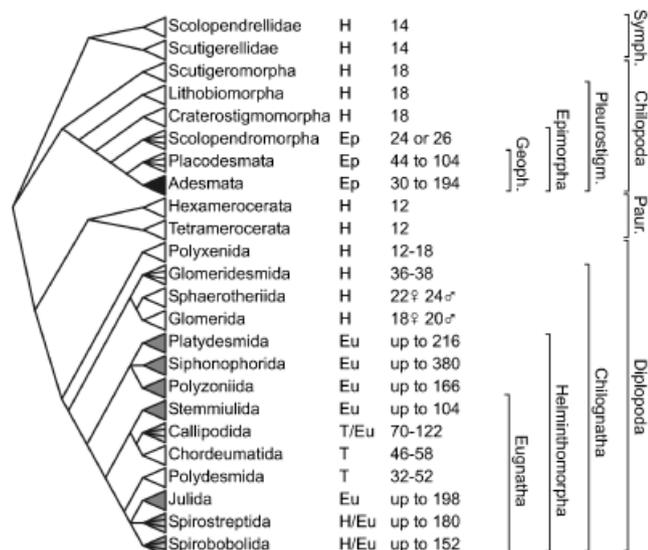
The space outlined here does not represent a formal (geometric) space of segmentation patterns and processes. It is intended to capture only the basic aspects of the constitution of a segmented trunk, that is, those related to the number and the possible concordance of serially repeated structures. Other aspects, generally referable to regional specification, or to segmental identity or specialization (degree of heteronomy), are left aside.

## Embryonic and post-embryonic segmentation

Segmentation can variably affect embryonic and post-embryonic development. In “epimorphic” development, the full complement of segments is produced during embryogenesis, whereas in “anamorphic” development, juveniles hatch with an incomplete complement of segments, and the expected adult number of segments (when fixed) is reached later in

ontogeny. Three different kinds of anamorphosis can be distinguished (Enghoff et al. 1993): “euanamorphosis,” when the addition of new segments continues until the last molt the animal undergoes, without any evidence of an expected fixed terminal number; “teloanamorphosis,” when the animal does not molt any more after it has reached the final number of segments; and “hemianamorphosis,” when the final and fixed number of segments is reached after a number of molts, but growth continues through further molts without a further increase in the number of body segments.

The transition from anamorphic to epimorphic development has apparently occurred only once within myriapods (Fig. 1). Development is epimorphic in the derived centipede clade formed by Scolopendromorpha plus Geophilomorpha, whereas it is hemianamorphic in more basal clades (Scutigermorpha, Lithobiomorpha, and Craterostigmomorpha).



**Fig. 1.** Taxonomic-phylogenetic distribution of segmental characters in myriapods. The tree-like diagram was obtained by collating symphylan (Edwards 1959) and pauropod (Scheller 1982) classifications with centipede (Edgecombe and Giribet 2002) and millipede (Enghoff 1984, modified in Enghoff et al. 1993) cladograms. Terminal taxa are represented as triangles. Empty triangles: no intraspecific and intrasex variation for the number of trunk segments; black triangle, intraspecific and intrasex variation in almost all species; striped triangles, intraspecific and intrasex variation in some sub-taxa; gray triangles, euanamorphic mode of development; disappearing gray triangles, euanamorphic mode of development in some taxa. Mode of development. Epi, epimorphosis; Eu, euanamorphosis; H, hemianamorphosis; T, teloanamorphosis. Number of trunk segments. Published data on the number of segmental units in different myriapod classes are not directly comparable. For a general examination, original data have been transformed into “quasi-equivalent” segmental units (see Appendix for details). Nonterminal taxa. Geoph., Geophilomorpha; Paur., Pauropoda; Pleurostigm., Pleurostigmophora; Symph., Symphyla.

Hemianamorphic development is typical of all pauropods, symphylans, and basal millipedes (although not exclusively), but it is also found in basal chelicerates (Pycnogonida), basal insects (Protura), most crustaceans, and it was the typical mode of post-embryonic development in trilobites (Minelli et al. 2003).

### Number of trunk segments

The number of adult segments is highly variable within myriapods (Fig. 1, Appendix), but we know almost nothing about how these numbers are determined and controlled during ontogeny (Hughes and Kaufman 2002).

In centipedes, the number of leg-bearing trunk segments varies between 15 in the most basal groups, and 191 in very polypodous specimens of the geophilomorph *Gonibregmatius plurimipes*. In this range, only odd numbers are observed (Minelli et al. 2000). In millipedes, although segment numbers are difficult to define, because of the marked mismatch in segmental arrangements in different structures as described below, the length of the segmental series varies from 11 leg pairs in some pincushion millipedes (Polyxenida) up to 375 leg pairs in the siphonophorid *Illacme plenipes* (Enghoff et al. 1993). The number of leg pairs is reduced to 8 in some brachypauropodid pauropods, the minimum number in myriapods, but the number of trunk segments seems to be invariably 12 in pauropods (Scheller 1985). Fourteen trunk segments and 12 leg pairs is the standard condition in symphylans (Dunger 1993).

Evolutionary change toward considerably higher numbers of segments has occurred at least twice: in helminthomorph millipedes, coincident with the advent of euanamorphosis, and in the epimorphic centipede clade Geophilomorpha. The possibility of evolutionary transitions toward very polypodous forms is apparently not related to the developmental timing of segmentation, as epimorphosis and euanamorphosis, in some way, represent opposite ends of a continuum of segmentation schedules. Beyond these two major transitions, both an increase and a decrease in segment numbers seem to have occurred with nonnegligible frequency. This contradicts the so-called Williston’s rule (Minelli 2003), which predicts a macroevolutionary trend toward series less numerous and less numerically variable for a segmented body (see Fusco and Minelli 2000a).

### Intraspecific variation in the number of trunk segments

A comparative evaluation of intraspecific variation in the number of trunk segments is limited to species with epimorphic, hemianamorphic, and teloanamorphic development (Fig. 1, Appendix). For euanamorphic species, although the range of variation is often reported in taxonomic literature, the apparently “open” addition of new segments in

ontogeny does not allow us to disentangle segment number variation from variation in the post-embryonic segmentation schedule. Therefore, the “meaning” of this variation is not directly comparable with that in species with a “targeted” segmentation.

Intraspecific variation in the number of trunk segments has evolved independently several times within myriapods. This is remarkable in the panorama of arthropod segmentation, where—outside the myriapods—the number of segments is generally stable even within high-rank taxa, and cases of intraspecific variation are relatively rare (see Linder (1952) for branchiopod crustaceans, and Hughes et al. (1999) for trilobites).

In broad terms, intraspecific variation is positively correlated with the mean number of segments (Fusco, unpublished data), but, in terms of serial homology, variation does not have the same implications in all taxa. There is no single answer to the question of whether it is possible to homologize trunk segments with the same ordinal position among series with different numbers of elements (Minelli 2001).

In series of otherwise homonomous segments, unique morphological markers may allow the alignment of segmental series. In millipedes, which, like pauropods and symphylans, are progoneate, the position of the genital opening is the most obvious guide. Independent of the length of the series, this position is stable within the group at the level of the second leg pair. Centipedes are opisthogoneate, that is, the genital opening is located close to the anus, and some other segmental marker should be used. In this group, in terms of serial homology, there are two different situations (Fusco and Minelli 2000a).

In the basal Pleurostigmophora, the number of leg-bearing trunk segments is either 15 (Lithobiomorpha and Craterostigmomorpha), or 21 or 23 (Scolopendromorpha). Although this variation is mostly interspecific, an interesting segmental marker shows an invariant ordinal position. Irrespective of the length of the series (with 15, 21, or 23 leg-bearing segments), the otherwise regular alternation of long and short dorsal plates (tergites) has a discontinuity at the level of leg-bearing segments VII and VIII, both with a long tergite (Demange 1969). Here, the possibility to align segmental series counting segments from the head mirrors the condition in progoneate myriapods.

The worm-like geophilomorph centipedes are different. Berto et al. (1997) compared the segmental trend of metric traits in tergites and sternites between specimens of the same species and sex, but with different numbers of trunk segments. They showed that the overall shape of the segmental pattern is largely independent of the number of segments. The metric features of a segmental pattern are not linked to the absolute ordinal position of a given trunk segment but rather depend on its relative position within the trunk. This is in agreement with Minelli's (1992) observation on the sternal grooves in the

geophilomorph *Sigmatogaster gracilis* (Himantariidae), where the segmental position and extent of the segmental domain of this morphological marker are proportional to the number of trunk segments. Moreover, in a few cases studied (Berto et al. 1997; Kettle and Arthur 2000; Fusco, unpublished data), the length of the whole trunk is not correlated with the number of trunk segments, that is, in specimens with more segments, these are on average shorter.

### Developmental precision

Nijhout and Davidowitz (2003) distinguish two main kinds of phenotypic variation. The first is the systematic variation of the target phenotype (the phenotype specified by a given genetic makeup and environmental conditions) with genetic or environmental variation. The second is the variation around the target phenotype, or “developmental instability.” This is a form of nonheritable variation caused by stochastic events in ontogeny, not compensated by the developmental system.

In myriapods, when the number of segments is variable within a species, one can question as to what the source of such a variation is. Considering individuals of the same age and from the same locality allows a certain control for environmental variation, but available information, in general, does not allow us to evaluate the relative contribution of genetic variation and developmental instability. However, considering epimorphic and hemianamorphic species with an invariant number of segments in adults (so as to exclude the genetic component of variation), we can gain at least an idea of the level of precision that developmental systems can reach.

Among the epimorphic myriapods, the highest species-fixed number recorded is 65 leg-bearing segments in the geophilomorph centipede *Mecistocephalus mirandus*, although this is only known for a small sample, and its taxonomic status is currently under revision. An uncertain taxonomic status also concerns a few nominal species credited with fixed numbers between 53 and 63 (Bonato, pers. comm.). However, there are tens of other *Mecistocephalus* species, known from very large samples from different localities, that exhibit invariably 49 (many species) or 51 (a much smaller number of species) leg-bearing segments. Among the anamorphic myriapods, the highest species-fixed number recorded is 54 leg-bearing segments in the females of chordeumatid millipedes such as *Peterjohnsia* (Enghoff et al. 1993). They hatch with three pairs of legs, and invariably reach the number of 54 leg pairs within seven molts.

In arthropods, anamorphic development represents a mystery in terms of developmental precision. How an unerring biological mechanism of segment counting, active for several months of exposure to variable environmental conditions, is able to produce a programmed number of segments in the order of tens remains an unanswered question.

### Forms of segmental mismatch

We speak of segmental mismatch when different views of the same animal (e.g., dorsal vs. ventral, or external vs. internal) show dissimilar segmental patterns.

Among the arthropods, the most well-known case of segmental mismatch is that of the tadpole shrimps (Crustacea: Notostraca), with their marked differences in periodicity, length of the series, and post-embryonic segmentation schedule among dorsal and ventral structures (Linder 1952; Minelli and Fusco 2004). However, among myriapods, cases of mismatch are no less intriguing and, at the same time, they are very numerous and diverse in their combinations.

In symphylans, tergites are more numerous than trunk segments (14). Two families are currently recognized. In Scutigereleididae, there are 15 tergites, whereas in Scolopendrellidae, the tergite number is 17 or 21–24, depending on the species. In post-embryonic development, termination of segment addition may precede the completion of tergite arrangement (Dunger 1993).

In centipedes, there are two classic cases of dorso-ventral mismatch (Lewis 1981). In Scutigermorpha, the 15 leg-bearing trunk segments are covered by only seven tergites, interpreted to have resulted from fusion of originally separate tergites (I, II+III, IV+V, VI+VII+VIII, IX+X, XI+XII, XIII+XIV, no tergite XV is present). In Craterostigmomorpha, the 15 leg-bearing trunk segments instead show 21 tergites, as tergites III, V, VII, VIII, X, and XII (corresponding to the long tergites in scolopendromorphs and lithobiomorphs) are split transversely into two. The tergites of leg-bearing trunk segments are split transversely into two in all Geophilomorpha as well, whereas sternites can be entire or fragmented depending on the species and the segmental position along the trunk.

In centipedes, the segmental arrangement of tracheal openings (spiracles) is also very interesting. In Notostigmophora (Scutigermorpha), spiracles open medio-dorsally, in the posterior part of each tergite, whereas in all other centipedes (Pleurostigmophora) spiracles open in the pleural region. Leaving aside the derived Geophilomorpha, in Pleurostigmophora with 15, 21, or 23 leg-bearing segments, long (L) and short (S) tergites alternate in the following way: L-S-L-S-L-S-L-L-S-L-S-L-S-L-S(-L-S-L-S-L-S(-L-S)). Spiracles are generally present on most segments with long tergites: III, V, VIII, X, XII, XIV, (XVI, XVIII, XX, XXII)), but (a) in the lithobiomorph genus *Lamyctes*, there is a pair of spiracles also on segment I; (b) in ten scolopendromorph genera, not closely related and with either 21 or 23 leg-bearing segments, there is also a pair of spiracles on segment VII, and (c) in the scolopendromorph genus *Plutonium*, spiracles are present from the second to the penultimate leg-bearing trunk segment (II–XX), mimicking the condition found in the seg-

mentally quasi-homonomous Geophilomorpha (Minelli et al. 2000).

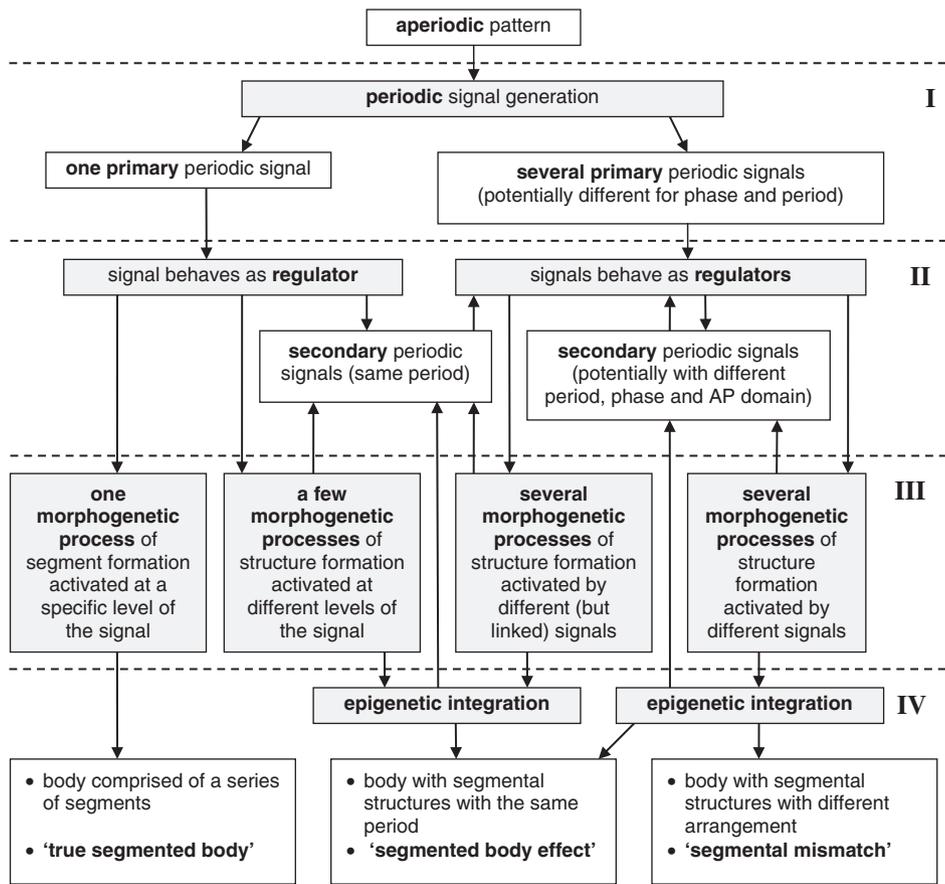
In Tetramerocerata, one of the two pauropod major clades (Scheller 1982), the number of tergites is smaller than the number of trunk segments (12). There are generally six tergites on segments II, III, V, VII, IX, and XI, segments II and III representing a serial anomaly (to be compared with the one in basal pleurostigmophoran centipedes) in the otherwise regular alternation. In the family Brachypauropodidae, the six tergites are variably sub-divided, transversely and/or longitudinally, but not all the tergites of the same animal are equally split. In two genera, this produces the effect of 9 or 10 undivided tergites (Scheller 1985).

In millipedes, the most conspicuous form of mismatch is the presence of two leg pairs and only one tergal or pleurotergal plate for each (diplo)segment. The pattern is complicated by the fact that the first apodous segment (the collum) and the following three segments, bearing one leg pair each, are apparently of “normal” segmental constitution. But the situation is even more complicated. In most species where dorsal, pleural, and ventral sclerites do not fuse to form a ring, there is no molt-to-molt consistent rule of progression for the number of dorsal, pleural, and ventral structures. The result is that, even in adults, the number of leg pairs cannot be inferred from the number of (pleuro)tergites and vice versa. The diversity in diplopod segmental arrangement is simply too complex to be summarized here; a very good review is provided by Enghoff et al. (1993). These complex patterns are in agreement with the recent finding that in the pill millipede *Glomeris*, dorsal and ventral serial structures are independently established in the embryo (Janssen et al. 2004). Developmental genetics of post-embryonic segmentation still need to be explored.

### MYRIAPOD SEGMENTATION LESSON

According to a naïve view of the process of segmentation, modular morphogenetic processes for the production of repetitive body units are activated by some periodic signal developing along the main axis. Simultaneously, overimposition of regional differentiation along the same axis is converted into the attribution of specific segmental identity. This simple view does not match with the complexity of segmental arrangement in arthropods and with the several modes of evolutionary change that one can infer on the basis of the taxonomic distribution of segmental patterns (Minelli and Fusco 2004). In this respect, myriapods, with their wide assortment of segmentation patterns and processes, suggest a different view.

Different layers of segmentation should be distinguished (Fig. 2). These are not necessarily sequential steps in an ontogenetic succession, nor do they represent a hierarchical



**Fig. 2.** Schematic representation of the “segmentation layers.” Each layer is intended as a “logical” component of the developmental process of segmentation. Gray boxes are processes and white boxes indicate patterns. A “signal” is any feature along the main axis which can be exploited as a pre-pattern to regulate further developmental processes. See text for details (Myriapod segmentation lesson).

cascade of developmental processes. They are just intended to identify the principal elements of the “logic” of the developmental process of segmentation, with their relationships. The term “signal” is used here with the generic meaning of any feature (level of gene expression, molecule concentration, but also the physical state of cells and extracellular matrix) that is not homogeneous along the main axis and that can be exploited as a pre-pattern to regulate further developmental processes. Any periodic feature is simultaneously a structural pattern and (potentially, at least) a developmental signal.

**First layer: emergence of primary periodic signals**

The first and most important layer is the emergence of a spatially periodic signal in a field that is differently patterned. In *Drosophila*, the seven stripes of expression of the primary pair-rule genes appear almost synchronously in the syncytial blastoderm. This is made possible by the relationship between different binding sites of the *cis*-regulatory region of the primary pair rule genes, which is dominated by the logic operator “OR,” as each stripe of gene expression is activated by a different combination of gap genes (Rivera-Pomar and Jäckle 1996). For arthropods with sequential segmentation, we do not have a comparable level of understanding, although a

mechanism based on the spatial translation of a temporal oscillation, similar to that discovered in vertebrates, has been proposed (Newman 1993; Stollewerk et al. 2003). Irrespective of the precise mechanism of segmentation, the distinctive quality of this first layer is the emergence of a periodic pattern from a different spatial configuration. This feature defines a starting point for considering the development of segmentation.

This first signal may be alone or may be accompanied by other independently emerging signals with potentially different period, phase, or anteroposterior localization. This is the case when simultaneous segmentation and sequential segmentations generate a periodic pattern in different domains along the same axis. In chelicerates (Dearden et al. 2002), millipedes (Janssen et al. 2004), and insects (Rogers and Kaufman 1997), a few anterior segments in the embryo are produced by processes other than those that generate the remaining segments. Minelli (2001) suggested a different segmentation process for a few anterior “naupliar” segments as a general feature of all arthropods. Probably, distinct primary signals are at the origin of different dorsal and ventral segmentation in millipedes (Janssen et al. 2004; Minelli 2004), and perhaps of the segmental arrangement of other myriapods with marked segmental mismatch.

## Second layer: emergence of secondary periodic signals

Wherever periodic patterning is present, it can be exploited as a pre-pattern to produce more elaborate or otherwise differentiated periodic patterns. A second layer consists of those periodic signals that are produced as an elaborate refining of pre-existing periodic signals, or from their combination. Pre-existing signals can be the primary signals of the first layer, but also earlier periodic signals from any other layer, this second one included.

In *Drosophila*, primary pair-rule genes regulate the expression of secondary pair-rule genes and together they regulate the expression of downstream segment-polarity genes. Widespread occurrence of gene expression with a “two-segment” periodicity in noninsect arthropods is still controversial (Davis and Patel 2003), but in a few model organisms evidence is quite compelling, as in the mite *Tetranychus* (Dearden et al. 2002) and in the geophilomorph centipede *Strigamia* (Chipman et al. 2004b). Indications that, in some myriapods, segments can be specified at least in pairs also come from the “forbidden” even numbers of leg pairs in geophilomorph centipedes (Minelli et al. 2000) and the forbidden numbers of leg pairs in some polyzoid millipedes, either even or odd, depending on the species (Enghoff et al. 1993). Other indications come from sexual dimorphism in trunk segment numbers in geophilomorphs (females generally with two segments more than males), in glomerid and sphaerotheriid millipedes (males with two extra segments), and some species of polydesmid millipedes (females with two extra segments).

The possibility of determining the final number of segmental units by a stepwise refining of a first signal with a larger period, can partially downgrade the problem posed by the observed levels of developmental precision in the number of trunk segments. Similar processes can work even “below the segment level.” In geophilomorphs, there are two tergal plates for each leg-bearing trunk segment. In *Strigamia maritima*, tergites are already split into two at the first post-embryonic stadium (peripatoides), but they are undivided at their first appearance in the germ-band (C. Brena, pers. comm.).

Mechanisms can be different (e.g., of the reaction-diffusion type), but in modern organisms, more probably, the process is accomplished and controlled by differential gene expression (Newman 1994). A growing body of evidence is shedding light on the structural and “logical” complexity of gene *cis*-regulatory apparatus (Yuh et al. 1998; Davidson 2001). The modular constitution of the enhancer region of a candidate segmental gene would make possible the combination of two or more pre-existing periodic signals (which behave as regulators) into a new signal, in a way that mimics the simple mathematical combination of goniometric functions.

## Third layer: initiation of a morphogenetic process

Initial formation of periodic structures (e.g., appendages, dorsal, ventral and pleural plates, spiracles, and tracheal branches) is set off by the activation of specific morphogenetic processes. Different morphogenetic processes can potentially “read” (be regulated by) different signals or signal combinations, thus producing structures that can exhibit concordant, partially concordant, or discordant segmental arrangements. In this respect, the scolopendromorphs offer an interesting case (Lewis 1981). The morphogenetic processes for the formation of tracheal branches exhibit different “reading options” of a pre-existing pre-pattern. Spiracles can be present on all leg-bearing segments with a long tergite to the exclusion of the VII (most common), including the VII (some genera), or, ignoring tergite alternation, on all segments from the second to the penultimate (one species). Similar options are available for color pattern. Most scolopendromorphs present a more or less uniform coloration along the trunk, but some species (e.g., *Scolopendra heros*) present different colors in different portions of the body (“reading” of a regional signal). In *Scolopendra hardwicki*, the color pattern reflects the alternate disposition of long and short tergites, VII–VIII anomaly included: segments with short tergites are black (“reading” of a periodic signal).

## Fourth layer: epigenetic interactions

Irrespective of the concordant or discordant periodic arrangement of different morphogenetic modules, the development of a functional integrated body system relies on an epigenetic dialogue between the structures that are growing and differentiating. This obviously applies to both segmented and non-segmented structures. For instance, dorso-ventral muscles grow often between segmentally different “worlds,” in terms of sclerites with different arrangement, number, size, pattern, and degree of overlap when they are imbricated.

Epigenetic interactions could also explain some very complex segmental patterns. In geophilomorph centipedes, the pleural region of each segment presents many sclerites forming a specific mosaic-like pattern. It is quite unparsimonious to think of a combination of periodic signals able to produce a complex alternation between sclerites and arthroal membranes in the cuticle. It is more parsimonious to invoke interactions with the internal development of segmental muscle insertions, in a manner that recalls the combination between formation of arthroal membranes and development of muscle insertions in regenerating arthropod appendages (Maruzzo et al. 2005). That the borders of different sclerites of the same segment can be shaped by different mechanisms within the same animal is also in agreement with the dissimilar developmental stability (measured as a kind of fluctuating asymmetry, but working on another axis, “translational asymmetry”) exhibited by different series of ventral sclerites

in the trunk of the geophilomorph centipede *Pleurogeophilus mediterraneus* (Fusco and Minelli 2000b).

Segmental patterns delineated by epigenetic mechanisms, in their turn, can provide periodic pre-patterns for subsequent morphogenetic processes.

### Segmental patterns

Body patterns resulting from a developmental process of segmentation can be grouped into three broad categories (Fig. 2).

When a single primary periodic signal regulates a single morphogenetic process of segment formation, which accounts for all the segmental structures of the body, the result is a body actually “comprised” of a series of segments that can be considered body-building blocks. A “true segmented body” seems to be a purely hypothetical category for arthropods (Minelli and Fusco 2004).

When a more complex network of signals and regulative interactions is involved, the resulting segmental pattern depends on the level of concordance between different segmental structures. When many structures present the same period, the effect of a body comprised of a series of segments is misleadingly obtained (Holland 1990; Budd 2001; Minelli and Fusco 2004). To some extent, this could be the case for most centipedes. On the contrary, when different structures present different segmental arrangements, the resulting body pattern is affected by segmental mismatch, as in the case of millipedes. Notably, the same type of pattern can result from different combinations of developmental processes, and no process is intrinsically decisive for the final pattern.

### CONCLUSIONS

Nontrivial difficulties emerge in comparing segmentation patterns and processes across myriapods. Structures arranged serially with the same period along the main axis inevitably produce the effect of a series of body modules, but we cannot assume that these morphological modules correspond to developmental modules (*sensu* Wagner 1996). When a segment is merely a descriptive unit, but at the same time, is not a developmental module, it is also not an independent evolutionary unit. Studies on the evolution of segmentation need a more development-based concept of segments and segmentation, and this initially requires a logical decomposition of the process of segmentation itself.

Consequently, what is, or what should be a proliferative zone? It is not necessarily a zone of particularly intense mitotic activity (a zone of cellular proliferation), as, in the embryo, germ elongation can be produced by cell rearrangement (e.g., convergent extension) as well (Davis and Patel 2002; Peel 2004). It is not necessarily the zone of segment proliferation, as segmental structures can define their arrangement

much later than the production of the first periodic signal. What is it then? When sequential segmentation is not completely replaced by simultaneous segmentation, it is the zone where primary periodic signals are forming, the zone where a spatially periodic pattern is produced by a temporal oscillation or by a black-box nonperiodic pre-pattern still to be discovered. It is the zone of sequential production of one or few signals that initiate a process whose results in terms of translational symmetry are not completely attributable to the performances of the signal generator. In this sense, a proliferative zone is more a “segmental organizer” than a “segment generator.” The manner in which the first periodic pattern is produced in the embryo is possibly a qualifying trait of a segmental body plan, but it is not necessarily the most important in terms of resulting morphology, functional anatomy, and life history.

It is always dangerous to infer process from pattern, as there are generally many different processes that can produce the same pattern, and segmentation should be probably studied case by case. However, a more articulated and critical view of segmentation can provide a more development-based conceptual framework for comparing and explaining the variety of arthropod segmentation patterns.

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Table A1. Adult trunk segment numbers in myriapods

Taxon	Development	Number of adult trunk segmental units	Intrasex segmental variation (range)	Sexual segmental variation	General reference
Symphyla					
Scolopendrellidae	H	14 TS <sup>1</sup>			Dunger (1993)
Scutigerepidae	H	14 TS <sup>2</sup>			Dunger (1993)
Chilopoda <sup>3</sup>					
Scutigermorpha	H	15 LBS			Lewis (1981)
Lithobiomorpha	H	15 LBS			Lewis (1981)
Craterostigmomorpha	H	15 LBS			Lewis (1981)
Scolopendromorpha	Ep	21 or 23 LBS	One species <sup>4</sup> (2 LBS)		Lewis (1981)
Geophilomorpha					
Placodesmata	Ep	41 to 101 LBS <sup>5</sup>	One species <sup>6</sup> (8 LBS)		Bonato et al. (2003)
Adesmata	Ep	27 to 191 LBS <sup>5</sup>	Almost all species (up to 82 LBS)	♀ generally with 2 segments more than ♂ <sup>7</sup>	Minelli et al. (2000)
Pauropoda					
Hexamerocera	H	12 TS <sup>8</sup>			Scheller (1982)
Tetramerocera	H	12 TS <sup>9</sup>			Scheller (1982)
Diplopoda					
Polyxenida	H	11, 13 or 17 LP			Enghoff et al. (1993)
Glomeridesmida	H	35–37 LP	One species (2 LP)		Enghoff et al. (1993)
Sphaerotheriida	H	21 LP ♀ and 23 LP ♂		♀ with 2 LP more than ♂	Enghoff et al. (1993)
Glomerida	H	17 LP ♀ and 19 LP ♂		♂ with 2 LP more than ♀	Enghoff et al. (1993)
Platydesmida	Eu	up to 110 PT <sup>11</sup>			Hoffman (1982)
Siphonophoriida	Eu	up to 192 T <sup>11</sup>			Hoffman (1982)
Polyzonida	Eu	up to 85 T <sup>11</sup>			Hoffman (1982)
Stemmiulida	Eu	up to 34 PT <sup>11</sup>			Enghoff et al. (1993)
Callipodida	Te/Eu	37–63 PT <sup>11</sup>			Enghoff et al. (1993)
Chordeumatida	Te	25–31 PT <sup>11</sup>	Several species (up to 3 PT <sup>12</sup> )	♀ with 2 PT <sup>12</sup> more than ♂ (some species)	Enghoff et al. (1993)
Polydesmida	Te	18–28 R <sup>11</sup>		♀ with 1 R <sup>12</sup> more than ♂ (some species)	Enghoff et al. (1993)
Julida	Eu	up to 101 R <sup>11</sup>			Enghoff et al. (1993)
Spirostreptida	H	47–77 R <sup>11</sup>			Enghoff et al. (1993)
Spirostreptidea	Eu	up to 92 R <sup>11</sup>	Several species (up to 18 R <sup>12</sup> )		Enghoff et al. (1993)
Other Spirostreptida	H/Eu	up to 78 R <sup>11</sup>	Several species (up to 10 R <sup>12</sup> )		Enghoff et al. (1993)
Spirobolida					

<sup>1</sup>12 leg pairs (II–XIII segment), 15 tergites.

<sup>2</sup>12 leg pairs (II–XIII segment), 17, or 21–24 tergites.

<sup>3</sup>Total number of trunk segments is possibly the number of LBS plus three: one anterior trunk segment bearing a pair of poisonous massillipedes (forcipular segment), plus (possibly) two terminal apodous segments of the genital region (Minelli 1993).

<sup>4</sup>*Rhoda thayeri* Meinert, 1886 (with 21 LBS) has been recently demonstrated to be a junior synonym of *S. bahiensis* (Brandt, 1841), originally credited with a fixed number of 23 LBS (Schilevko in press). *Scolopendropsis bahiensis* is thus a segmentally dimorphic species.

<sup>5</sup>Only odd numbers of leg-bearing segments in the specified interval.

<sup>6</sup>Other few cases of possible intraspecific variation (limited anyway to two LBS) are currently under study (L. Bonato, pers. comm.).

<sup>7</sup>There are species where females have 0, 4, 8, 10, or 16 more segments than males (Berto et al. 1997).

<sup>8</sup>11 leg pairs (II–XI segment), 12 tergites.

<sup>9</sup>8–10 leg pairs (II–IX/XI segment), typically six tergites.

<sup>10</sup>As dorsal and ventral segmental sclerites do not match, numbers of serial elements of specific body parts are recorded here. As a rule, and in simplified terms, the diplopod trunk is composed of a first apodous segment (collum), followed by three segments with one tergite and one leg pair each, followed in turn by a series of variable length of pairs of leg-bearing segments with one tergite each (diplosegments). In euamorphic species, a number of terminal apodous segments, following the series of leg-bearing segments, persists in adult stages. Dorsal sclerites may be free (tergites), fused to pleural sclerites (pleurotergites), or fused to pleural and ventral sclerites (rings).

<sup>11</sup>The approximate number of trunk segments can be calculated as  $2N - 4$ , where  $N$  is the number tergites, pleurotergites or rings.

<sup>12</sup>Equivalent to a double number of trunk segments and leg pairs.

Development: Epi, epimorphosis; Eu, euamorphosis; H, hemiamorphosis; Te, teloanamorphosis; Tr, trunk segments; LBS, leg-bearing segments; LP, leg pairs; T, tergites; PT, pleurotergites; R, rings.