Myriapod metamerism and arthropod segmentation

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Outstanding progress in understanding segmentation of tracheate arthropods (Atelocerata), i.e. Chilopoda, Diplopoda, Pauropoda, Symphyla and Insecta, has been gained through experimental studies carried out on a single, very derivative organism, i.e. Drosophila. We stress the need for a broader comparative approach. We have studied the segmental structure of the trunk in geophilomorph centipedes, where we can identify morphogenetic units of two, four, eight or 16 segments. Accordingly, we sketch an improved model for arthropod segmentation, with the following initial steps: (a) biochemical marking of a very few repetitive units (cosegments); (b) iterative duplications of this first periodicity, until the embryo acquires an array of biochemical markings matching the whole number of segments of the future larva or juvenile specimen; (c) transpatterning, stabilization and interpretation of this 'segmental' arrangement; (d) possible repatterning, to give a final repetitive pattern we define as metasegmental. Finally, we express some doubt about the homology between annelid and arthropod segmentation.


INTRODUCTION

Experimental studies on the developmental origin of body segmentation in Drosophila occupy many pages in recent issues of scientific journals such as Nature, Science, Developmental Biology, Cell, or The EMBO Journal. This exciting...
research has shown unexpected regularities at levels above the segmental one, controlled by numerous 'segmentation genes'.

The discovery of these regularities has led to the development of new models designed to explain the origin of segmentation (see for instance Lawrence, 1981; Martinez-Arias & Lawrence, 1985; Meinhardt, 1986).

These models are drawn from experiments on *Drosophila*, an insect with very advanced body structure. This is possibly the cause of some idiosyncrasies in the current models of segmentation, which may be partially overcome by a broader comparative survey of ontogenetic processes of segmentation in Arthropods, especially in those with more primitive segmental patterns, such as centipedes and millipedes.

As a first contribution to this project, we have studied some numerical regularities of the segmental structure in centipedes, with special reference to geophilomorphs.

Our choice is easily justified. Insects together with the so-called 'myriapods' form a monophyletic taxon, recognized in such different arthropod phylogenies as those suggested by Sharov (1966), Manton (1973, 1974) and Boudreaux (1979). Within this taxon, named Atelocerata after Heymons (1901), the embryo possibly becomes segmented according to quite general rules. Therefore, any evidence concerning centipedes (or millipedes) may also be useful in understanding insect segmentation, and vice versa.

In the past, the developmental origin of body segmentation in centipedes and millipedes has been studied by a few authors. In the following lines we briefly summarize the most interesting speculations on the subject, due to Pflugfelder (1932), Maynard Smith (1960) and Demange (1967, 1969).

By studying the embryonic development of a millipede (*Platyrrhacus amauros* Attems), Pflugfelder (1932) was inclined to describe segmentation as the outcome of a hierarchical, three-step splitting of major embryonic territories he calls "first order macrosomites", i.e. an archicerebral, an intermediate and a caudal or proliferative region. These units progressively become subdivided into "second order macrosomites" which in turn give rise to "third order macrosomites". These last units may finally split into two micromesites each; for instance, a typical diplosegment of the trunk is regarded by Pflugfelder as a third order macrosomite incompletely split into two micromesites. First appearance and subsequent differentiation of the segmental units are not synchronous along the trunk but regularly proceed from the head towards the posterior end of the body; for instance, the proliferative region gives rise to a new caudal 'somite' at each post-embryonic stage, until the final complement of segments is achieved.

Three features of this developmental model deserve major attention. First, it assumes that the animal gets its final segment number through a multiplicative process of higher order units. Second, it postulates that the \( n \)-level units do not give rise to a constant number of \( (n+1) \)-level units: for instance, Pflugfelder assumed that some second order macrosomites give rise to three third order macrosomites, others only to two. Third, it assumes an heterochronous segmentation of the body, the posterior trunk segments progressively differentiating during the post-embryonic development. One or more of these features are incorporated in the following models.
Another multiplicative model of segmentation has been proposed by Maynard Smith (1960) to explain the origin of what he calls modal variation, i.e. intraspecific variation where some modal number of structures is present in almost all individuals. Maynard Smith explicitly refers to segment number in centipedes and millipedes and assumes that it can be the outcome of a multiplicative process, where a small number \( n_1 \) of primary units is laid down first, under strict morphogenetic control, so that this number is virtually fixed within the population (or species, or even higher taxon); subsequently, each first order unit gives rise to \( n_2 \) second order units; the last process can be also well controlled, so that a nearly fixed number of \( n_1 \times n_2 \) units finally results. Further multiplicative steps are obviously possible.

At variance with Pflugfelder, Maynard Smith seems to assume that all first order units split into an equal number of second order units, in so far as no developmental constraints are at work. For the myriapods with anamorphic development, Maynard Smith assumes that segments are laid down in groups, following a mechanism controlling the ratio between the rates of segment formation and moulting, and that some mechanism should be at work to ensure that development ceases after a given number of moults or when a given size is reached.

Demange's (1967, 1969) approach to segmentation of chilopods and diplopods mostly rests on comparative morphology of trunk sclerites and musculature. He accepts Pflugfelder's model of segmentation by subsequent splittings of primary embryonic regions and applies it also to centipedes. The most original feature of Demange's model is his concept of 'metameric reduction', i.e. an evolutionary trend towards more or less complete reduction of the posterior part of higher order segmental units (macrosomites). This process induces more or less evident deviations from a regular arrangement of segments.

We believe that these segmentation models proposed for the myriapods can profitably be matched with some developmental models recently proposed for Drosophila and supported by experimental work on this insect.

Kauffman, Shymko & Trabert (1978) suggested that an increasing number of territories become identified in Drosophila embryo through an early sequence of binary decisions. Their model might also be called a multiplicative one, like those just summarized; however, at variance with them, it assumes that all developmental choices of a given rank are synchronous.

This model has gained experimental support through the discovery of the so-called pair-rule genes, whose transcripts and translation products are laid down in the embryo early on, to give the first cues to body segmentation (see, for instance, Carroll & Scott, 1985, 1986; DiNardo, Kuner, Theis & O’Farrell, 1985; Ingham, Howard & Ish-Horowicz, 1985; Kilchherr, Baumgartner, Bopp, Frei & Noll, 1986; Lawrence, 1981; Lawrence & Struhl, 1982; Nüsslein-Volhard & Wieschaus, 1980; Weir & Kornberg, 1985; some details are given in the Discussion section of this paper).

Another model of segmentation in Drosophila has been proposed by Meinhardt (1986). It underlines the primacy, in ontogeny, of establishing the full complement of segments in the embryo over specifying the identity of each segment; furthermore, it assumes that the boundaries between segments are defined through a hierarchical induction of cell stages by sequential activation
of a few genes. According to Meinhardt, this hierarchical process possibly allows a simultaneous formation of segments without the time-consuming sequential segment formation seemingly occurring in other insects.

The present knowledge about arthropod development hardly allows us to decide whether a generalized model of segmentation applies to the whole phylum or, at least, to all tracheate arthropods. However, we believe that a comparison of insects with myriapods may improve our current understanding.

Unfortunately, in comparison with *Drosophila*, centipedes and millipedes are much less suitable to experimental studies of development; however, as Maynard Smith (1960: 408) said while proposing models for the developmental origin of segmentation:

> evidence for multiplicative processes can be obtained from purely descriptive embryology; even in the absence of such embryological evidence, the presence of such processes can be inferred from certain patterns of variation within or between species. In the myriapods both lines of evidence suggest the presence of multiplicative processes.

### THE SEGMENTAL ARRANGEMENT OF CENTIPEDES

The centipede body includes only two regions, i.e. a head and a trunk. Embryology (Heymons, 1901) and morphological evidence concerning segmental appendages (Lauterbach, 1973) suggest that the centipede head is composed of the same number of segments as the insect head. This number seems to be six, in spite of some recent discussion for insects (but see Struhl (1981) for a reassessment of the question). The first trunk segment bears a pair of specialized appendages (poison-claws or forcipules) and is commonly known as the forcipular segment; it is followed by numerous leg-bearing segments, always odd in number. Leg-bearing segments are 15 in adult Lithobiomorpha, Craterostigmomorpha and Scutigeromorpha, 21 or 23 in Scolopendromorpha and between 29 and 191 in Geophilomorpha. In this last taxon, segment number commonly varies within a single species. After the last leg-bearing segment there is an apodous genital region, possibly consisting of two segments plus the telson. Segment number is already fixed at birth in epimorph centipedes (scolopendromorphs and geophilomorphs), whereas it increases during the post-embryonic development in anamorphs (lithobiomorphs, craterostigmomorphs and scutigeromorphs).

### MATERIALS AND METHODS

We have gathered data on segment numbers both from the literature and from an extensive survey of unpublished materials. A check-list of world geophilomorphs, with data on segment number, has been established by updating the old monograph published by Attems in 1929.

There are some difficulties in compiling the data on segment numbers, because most descriptions only quote the extreme values of the range of intraspecific variability, without specifying whether all intermediate classes (we mean 'possible' classes, excluding those with even numbers of leg-bearing segments) are actually documented in their material. This must be stressed,
because we have positive evidence of many instances of discontinuous or, at least, of bimodal distributions (see below, Fig. 8). However, in the absence of positive knowledge of more restricted distributions, we could not but follow a conventional procedure, i.e. to include a given species in every class defined by odd numbers of leg-bearing segments for the range given by the authors, extremes included. A further difficulty lies in the frequent lack of information about sex differences; therefore, we have done most work on distributions cumulated over both sexes.

More detailed data on the variability in segment number have been obtained for several species through a perusal of the literature and an *ad hoc* examination of long series. In this way we have gained information about 16 species. The original protocols are not reproduced here; they may be obtained from the authors.

RESULTS

Number of trunk segments in geophilomorph centipedes

The number of leg-bearing segments varies within geophilomorphs from 29 (*Dinogeophilus oligopodus* Pereira and some male specimens of *Geophilus richardi* Brölemann) to 191 (*Gonibregmatus plurimipes* Chamberlin). All detailed observations confirm the well established fact, that this segment number is always odd.

It is worth saying, that this pattern still remains in all teratological specimens known to date (see Balazuc & Schubart, 1962; Minelli & Pasqual, 1986): no specimen with an even number of leg-bearing segments has ever been described; on the contrary, trunk segmentation troubles (helicomerism) seem sometimes to underline the difficulty of splitting a two-segment set into two one-segment units (Fig. 1).

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Figure 1. Segmentation troubles in geophilomorphs. A two- or four-segment unit lies between two regions of the trunk with defects of opposite polarity. A, *Himantarium gabrielis*, ventral side, after Brölemann (1894), redrawn; B, the same, dorsal side, after Minelli & Pasqual (1986), redrawn; C, *Plurigeophilus takakawai* Verhoeff, ventral side, after Shinohara (1949), redrawn. S = sternite; T = tergite.
The number of leg-bearing segments varies broadly within each family and, generally speaking, also within each species. However, this variability is far from random; on the contrary, it follows some patterns we will try to explain below in terms of possible morphogenetic mechanisms of segmentation.

As we will see in the following paragraphs, the size of the repetitive units defining these patterns is 2 or 4 or 8 or 16 segments. We can recognize such patterns at family level as well as within the single species. At family level, modal values in frequency distributions of segment numbers occur at evenly spaced intervals. At species level, segment number distributions frequently show absolute or relative maxima at preferred values also occurring with similar regularity. These numerical patterns are sometimes linked with sexual dimorphism.

**Interspecific variability**

A frequency distribution of numbers of leg-bearing segments cumulated over the entire order (Fig. 2) is poorly informative, because of the overlapping of many family distributions, whose analysis is more interesting for our problem.

Geophilomorph families fall into three main groups, according to trunk segment number and variation.

To a first group belong four families of very elongated, polypodous geophilomorphs, i.e. Himantariidae, Oryidae, Gonibregmatidae and Eriphantidae. For most species belonging to these families the number of trunk segments has both a high mean value (often larger than 100) and high variability, as customary in repetitive structures of high multiplicity (Remane, 1952). Most interesting is the distribution of segment numbers \( n \) in Oryidae (Fig. 3), where the relative maxima occur at 16-segment intervals \( n = 81 + 16k \), with \( k \) = integer.

Dignathodontidae and Ballophilidae are somehow intermediate between the first and the second group: in these families, the number of leg-bearing segments

![Figure 2. Frequency distribution of numbers of leg-bearing segments within the order Geophilomorpha. Data in Figs 2-5 were recorded by the methods explained in the text (Materials and Methods).](image-url)
is smaller than in the first group, but not so reduced as in the following one; moreover, intraspecific variability is still large.

Typical representatives of the second group are Schendylidae (Fig. 4), Geophilidae (Fig. 5) and Linotaeniidae. For instance, in Schendylidae the modal value is as low as 47 and intraspecific variability mostly includes three to four contiguous classes.

The third group comprises only the family Mecistocephalidae. These geophilomorphs differ from all the others because their segment number is fixed within a single species and identical between the sexes. More than half the known mecistocephalid species possesses \( n = 49 \) pairs of legs, but the whole frequency distribution (Fig. 6) is anything but random: 84 species out of 104 have \( n = 49 \pm 4k \), where \( k \) is an integer, whereas only 20 species have \( n = 49 + 2 \pm 4k \); a quadrisegmental pattern seems also to occur in this family, in addition to the customary bisegmental one.

The modal value of 47 leg-bearing segments we find in Schendylidae deserves more than a cursory look. In fact, another family (Linotaeniidae) also reaches its maximum at the same value and a third one (Dignathodontidae) has a relative maximum again at 47.

Figure 3. Frequency distribution of numbers of leg-bearing segments within the family Oryidae. See Fig. 2.

Figure 4. Frequency distribution of numbers of leg-bearing segments within the family Schendylidae. See Fig. 2.
This is possibly only the beginning of the story, because Geophilidae (Fig. 5) have a maximum at $47 + 8 = 55$ segments, and 55 is also a relative maximum in the frequency distributions for Linotaeniidae and Ballophilidae.

Moreover, further numbers $n$ of the kind $n = 47 \pm 8k$ (where $k$ is an integer) appear in the distributions we have recorded: 39 is a relative maximum for Ballophilidae and Schendylidae; 63 is a relative maximum for Linotaeniidae; 71 and 79 are both relative maxima for Geophilidae and Dignathodontidae; 95 is the frequency maximum for Himantarriidae; and 111 and 143 are further relative maxima for Dignathodontidae.

This picture looks even more impressive if we also take into account two smaller numbers, i.e. $47 - (3 \times 8) = 23$ and $47 - (4 \times 8) = 15$: 23 is the number of leg-bearing segments in some species of the order Scolopendromorpha (although most species of that order only have 21 leg-bearing segments), whereas 15 is the adult number of leg-bearing segments in all anamorph centipedes, i.e. Lithobiomorpha, Craterostigmomorpha and Scutigeromorpha.

Summing up, in the whole class Chilopoda we recognize an octonary regularity, which does not seem to match very well with the universal ‘oddity’ of leg-bearing segments. However, the difficulty is easily overcome (see also...
Maynard Smith, 1960) by observing that the leg-bearing segments of the trunk are but a part of the whole animal: they are preceded by seven segments (six head plus the forcipular segments) and followed by two further segments (genital region). Therefore, the all-inclusive number of segments \( N \) composing a centipede body may be calculated as follows: \( N = 6 + 1 + n + 2 \), where \( n \) is the number of leg-bearing segments. By applying this formula to the cases just discussed, we obtain (Table 1) a set of even values, all multiples of eight!

This regularity suggests that the metameric body plan is fully defined before (or, at least, independently from) any beginning of tagmosis.

The figures in Table 1 also supports the contention that metamerism is accomplished through a mechanism of iterative duplication of primary serial units (see Discussion, p. 333).

**Intraspecific variability**

Most geophilomorph species exhibit an intraspecific variability in segment number, the main exception being represented by the species of family Mecistocephalidae, as already seen. Segment number varies both between and within sexes.

Variability is obviously larger among many-segmented species than among shorter ones. In the males of *Himantarium gabrielis* (Linné) a significant set of

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<td>1</td>
<td>143</td>
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frequency maxima occurs at \( n = 85 + 16k \) (Fig. 7); in females the values shift by eight segments towards higher numbers. Once more, the segmental pattern of these centipedes exhibits regularities following a power of two: a 16-segment pattern within each sex and an eight-segment relation between the sexes.

Octonary segmental patterns also recur in *Ribautia campestris* Demange (Fig. 8A) and *Stigmatogaster gracilis* (Meinert) (Fig. 8B).

Our data are less complete for other species, but the frequency distributions for *Strigamia acuminata* (Leach) (Fig. 8C) and *Ballophilus smaragdus* Demange (Fig. 8D) strongly suggest that individual differences by sets of eight or 16 segments are at least as probable as two-segment differences.

**Sexual dimorphism**

Prunescu & Capuse (1971) have observed many broods of *Henia illyrica* (Meinert), *Pachymerium ferrugineum* (C. L. Koch), *Strigamia crassipes* (C. L. Koch) and *Str. acuminata* (Leach), where the new-born females always had the same
segment number \( n \) as the mother and the new-born males always had \( n' = n - 2 \). In these species, as in many other geophilomorphs, males and females of the same species differ by a two-segment unit. This difference does not seem to be balanced by a difference in the segmental origin of the apodous genital region of the two sexes.

In the geophilomorph species with larger \( n \), the segmental differences between the sexes is also generally larger: the case of *Himantarium gabrielis* (sexual difference = eight segments) has been just quoted; the same difference occurs in *Orya panousei* Demange (Demange, 1961), whereas it is as large as 16 in *Lamottephilus spinosus* Demange (Demange, 1963).

**DISCUSSION**

*A revised model of arthropod segmentation*

The data given in the previous section all point towards the existence of regularities of order 2, 4, 8 . . ., in the segmental organization of centipedes. These regularities cannot but be the expression of morphogenetic rules of the kind recently revealed in *Drosophila* as the phenotypic effects of the so-called ‘pair-rule genes’ and incorporated in the already quoted model of Meinhardt (1986). Four loci will be quoted in the following discussion: engrailed (\( en \)), fushi-tarazu (\( ftz \)), hairy (\( h \)) and paired (\( prd \)).

The segmental patterns we have described in centipedes may be matched with the very large amounts of information from molecular embryology and developmental genetics of *Drosophila* to obtain the following model of arthropod segmentation, to be generalized within Atelocerata (insects and ‘myriapods’) at least.

**Step 1.** Maternal genes in the egg define a reference system specifying the antero-posterior and the dorso-ventral polarity axes of the future embryo (Carroll, Winslow, Schupbach & Scott, 1986; North, 1986; Perrimon & Mahowald, 1986; Frohnhoffer & Nüsslein-Volhard, 1986).

**Step 2.** Transcripts of other genes give a biochemical marking to a very small number of repetitive units, in the form of more or less complete rings across the antero-posterior axis of the embryo. We do not definitely know whether this primary pattern is built through the activity of maternal or zygotic genes. However, a very early marking of two primary units has been demonstrated at least for some zygotic genes, as \( ftz \) and \( en \) (Weir & Kornberg, 1985). Another question which remains open concerns whether this first pattern arises from localized synthesis of specific metabolites or from differential accumulation in the ring-like sites where we find them.

**Step 3.** The regularity defined through these first repetitive belts is now:

(a) reproduced by other metabolites, which become arranged according to the same pattern, but possibly with a phase difference in respect to their reference pattern (see for instance the phase shift of the \( ftz \) transcripts, when in seven-rings stage, in respect to the seven \( h \) rings; Ingham *et al.*, 1985);

(b) stabilized through interactions between metabolites (Carroll & Scott, 1986; Harding, Rushlow, Doyle, Hoey & Levine, 1986; Jäckle, Tautz, Schuh,
Seifert & Lehmann, 1986), whose number probably increases during this developmental step; such interaction are also a key feature of Meinhardt's model; and, last but not least,

c) duplicated, once or more.

The metabolites we refer to can be identified as the products of the so-called pair-rule genes. It is quite probable that they are sequentially synthesized and subject to complex regulatory interactions.

At this developmental stage, embryo segmentation only rests on the arrangements of biochemical markers; any morphological evidence is still lacking. However, these markers identify a lot of discrete units (cells or nuclei) and endow them with some stability and prospective value. In Drosophila these events occur during the process of cellularization of the blastoderm.

Subsequent duplication of the biochemically marked rings has no relation to mitotic cycles (cf. the experiments done by van der Meer, Kemmner & Miyamoto, 1982, on the beetle Callosobruchus). Iterative doubling of the primary pattern may be accomplished by either of the following mechanisms: splitting of the previous rings, or intercalary formation of new rings between those already extant. In the first instance, a broad marked band is split into two narrower ones, because the marking disappears in the middle; in the alternative mechanism intercalary bands appear midway between couples of pre-existing ones. Both mechanisms can be easily interpreted in biochemical terms; both seem to be supported by experimental evidence from Drosophila: the first behaviour has been described for the transcripts of the gene fiz (Weir & Kornberg, 1985) and for those of prd (Kilchherr et al., 1986); the second one for the transcripts of the gene en (Weir & Kornberg, 1985).

This biochemical step in segmentation ends up by defining a number of segmental units, which corresponds approximately to a power of 2, i.e. 2^n, where n is the number of duplication cycles undergone by the system of ‘biochemical metameric markers’ (BMMs). There is possibly no single BMM undergoing the whole duplication process, because an already established multiplicity may be the beginning of the cycles undergone by the BMMs interested in the final steps of the process (Fig. 9).

The antero-posterior and dorso-ventral gradients already present in the developing embryo before any appearance of biochemical segmentation are possibly the cause of heterochronies in the duplication cycles.

For instance, a retardation on the dorsal side of the embryo with respect to the ventral one may be involved in the origin of diplosegments in millipedes. Genes affecting dorso-ventral polarity in Drosophila are well known; they mostly have a dorsalizing effect, but a ventralizing gene ToI is also known (for a review, see Anderson & Nüsslein-Volhardt, 1984).

An analogous argument possibly applies to the retardation in duplication cycles of the hindmost segments of the body; in fact, a segmental heterochrony has been described for the appearance of transcripts of the gene en in Drosophila (DiNardo et al., 1985). Heterochronies will be discussed below (p. 335) in relation to anamorphosis.

This antero-posterior polarity is possibly the cause of the main shifts from powers of 2 as a comprehensive number (n) of body segments in several Atelocerata.

Step 4. This segmentation defined by biochemical markers acts as a prepattern
for establishing the morphological segmentation of the embryo and of the post-embryonic stages.

In this model segmentation is acquired through an initial appearance of a very small number of repeated units which increase in number during a few cycles of iterative doublings. This process seems to require a small amount of information: a feature that increases the likeliness of the model. Moreover, control over the numerical outcome of such a multiplicative process seems to be very simple, as already observed by Maynard Smith (1960).

We are now ready to do a comparison between our model and those by Kauffman et al. (1978) and by Meinhardt (1986).

We suggest that the embryo becomes divided into an increasing number of territories identified through a small number of binary decisions, as foreseen by Kauffman et al. (1978). However, their model aimed to explain the origin of the positional information reference system in the embryo rather than the origin of the segmental body plan: this is possibly the cause of the silence regarding this interesting model in recent literature on segmentation (but see Weir & Kornberg, 1985, for a valuable exception).

On the other hand, our model shares with Meinhardt’s (1986) the emphasis on biochemical marking of a serial set of embryonic districts. However, Meinhardt’s model does not foresee an increase in multiplicity of segmental units through doubling cycles.

**Epimorphosis vs. anamorphosis**

While defining our segmentation model, we did not specify whether the doubling cycles generating the segmental pattern are necessarily synchronous along the whole body length or not.
From *Drosophila* embryology we know that the rings of proteins coded by the pair-rule gene *en* appear in antero-posterior sequence (DiNardo et al., 1985), but this heterochrony does not extend over more than one cell cycle and the whole biochemical marking of the embryo is completed before the first morphological evidence of segmentation.

On the contrary, much more important heterochronies apparently occur in the course of segmentation of anamorphic arthropods (see, for instance, the already quoted work by Pflugfelder, 1932), but also of insects with short germ band (Kurzkeime) (Sander, 1976; Meinhardt, 1986).

Of course, we do not dispute the morphological evidence concerning the embryonic development of short germ band insects or that concerning the embryonic and post-embryonic development of anamorphic myriapods; the hindmost segments of the body obviously appear in sequence. However, we can not rule out the possibility that in these cases too, (a) the whole complement of segments has been already marked biochemically during an early developmental stage, and (b) the first appearance of the segments as morphologically distinct units is only the expression of a delayed interpretation of already determined segmental identities.

Some arguments seem to support this point of view. First, the fact that in *Drosophila* the BMMs are laid down before the blastoderm shows the first structural evidence of segmentation suggests that the timing of segment appearance as embryonic anlagen is not necessarily a good cue to recognize the timing of the more basic biochemical marking of the segmental pattern.

Second, in so far as the segments are primarily defined by segmentation genes, a delayed identification of posterior segments implies that the activity of the pair-rule genes and of other segmentation genes can be much prolonged in time without losing in accuracy. In the case of anamorphic myriapods this activity should last many months or even some years! Otherwise, we should suppose that the posterior segments are primarily identified through a mechanism controlled by another set of genes, or even derive from a not-multiplicative mechanism, for instance a chemical counting device. Both explanations seem to be quite unlikely.

Third, the number of the new segments differentiating at each stage at the posterior body end of an anamorphic myriapod is quite variable, both in the life history of a single animal or between species with the same adult segment number (Table 2) and between individuals of the same species which will later converge towards similar adult numbers (see, for instance, the developmental schedules of hundreds of specimens of *Proteroiulus fuscus* (Am Stein) accurately analysed by Peitsalmi (1981)).

This problem must be studied in depth with special reference to the diplopods, with their varied developmental schedules. We plan to investigate this topic in the near future. In this paper, we just recall that in millipede larvae new segments differentiate in quite a large legless region following the last complete diplosegment; this region could possibly contain, in a temporarily ‘freezed’ form, the biochemically marked anlagen for all further segments to be expressed in the following developmental stages.

It is also possible that some groups have modified their developmental schedule to such an extent as to become unable to express fully all segmental anlagen they have in their hindmost region. A similar opinion is expressed by
Table 2. Developmental schedules of some hemianamorphic (A, B) and anamorphic (C) myriapods. In A and B the final (epimorphic) developmental stages are not considered. A, Comparison between two hemianamorphic centipedes, both with 15 pairs of legs in the adult stage; B, comparison between two hemianamorphic millipedes, both with 17 pairs of legs in the adult stage (females); C, Developmental schedules of some anamorphic millipedes, with various but species-specific numbers of leg pairs in the adult stage.

<table>
<thead>
<tr>
<th></th>
<th>Pairs of legs at birth</th>
<th>Pairs of legs added at each stage</th>
<th>Final no. of leg pairs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scutigera coleoptrata (Linné)</td>
<td>4</td>
<td>1 2 2 2 2 2</td>
<td>15</td>
<td>Verhoeff (1902–25)</td>
</tr>
<tr>
<td>Lithobius forficatus (Linné)</td>
<td>7</td>
<td>1 0 2 2 3</td>
<td>15</td>
<td>Verhoeff (1902–25)</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomeris spp., ♂♀</td>
<td>3</td>
<td>5 2 3 2 2</td>
<td>17</td>
<td>Verhoeff (1906)</td>
</tr>
<tr>
<td>Trachysphaera spp., ♂♀</td>
<td>3</td>
<td>3 2 3 3 2 1</td>
<td>17</td>
<td>Tabacaru (1963)</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polydesmus angustus Latzel, ♂</td>
<td>3</td>
<td>3 5 6 6 4 2 2</td>
<td>31</td>
<td>Verhoeff (1928–32)</td>
</tr>
<tr>
<td>Opisthocherion canayerensis Mauriès et Geoffroy, ♂</td>
<td>3</td>
<td>2 5 6 8 8 6 4</td>
<td>42</td>
<td>Geoffroy (1984)</td>
</tr>
<tr>
<td>Nanogona polydesmoides (Leach), ♂</td>
<td>3</td>
<td>2 5 6 8 8 8 6 4</td>
<td>50</td>
<td>Geoffroy (1984, sub Polymicrodon polydesmoides)</td>
</tr>
</tbody>
</table>

Demange (1969: 536), when saying that “l’adulte des Diplopodes Iuloidea possède... en puissance toutes les ébauches des métamères qui n’apparaîtront jamais par suite de l’arrêt des processus de développement segmentaire de la zone de croissance”.

Last but not least, no arthropod can regenerate even a single body segment; that is also true for the anamorphic ones: at the posterior end of the body, the larvae have a growth and differentiation zone, rather than a true blastema with embryonic character.

We are aware of the speculative character of our interpretation; however, it seems that it can explain many facts which do not easily fit into the current model. At any rate, our contentions are amenable to experimental testing.

In this perspective, the contrast between anamorphosis and epimorphosis does not seem to be very large, but anamorphosis appears to require additional information, because of the subtle timing of the processes to be controlled. Therefore, we believe that epimorphosis could be more primitive than anamorphosis, in spite of the generalized occurrence of epimorphosis in insects, i.e. the highest Atelocerata.
We acknowledge the difficulty of fitting these ideas within the phylogenetic framework developed by Dohle (1980a, 1985), but it is fair to note that the developmental pattern of non-epimorphic myriapods is not uniform. Within Diplopoda, there are truly anamorphic groups such as julids where new additions of segments proceed until the adult stage and beyond, and groups such as pill millipedes with hemianamorphic development where the stage attaining the final complement of segments is followed by some epimorphic stages leading to the mature one. As far as (hemi)anamorphic Chilopoda are concerned, “the course of anamorphosis is not exactly the same in Scutigeromorpha and Lithobiomorpha”, as remarked by Dohle himself (Dohle, 1985: 61).

Are all instances of anamorphosis within arthropods actually homologous? At present, it is not easy to answer this question. At any rate, the taxonomic distribution of epimorphosis and anamorphosis within arthropods implies the polyphyly of anamorphosis, if the common ancestor of all recent arthropods was epimorphic, or the polyphyly of epimorphosis, if the common ancestor was anamorphic. And it seems to us more probable that a modus requiring complex controls, like anamorphosis, should be the derived one.

**Compartments and parasegments**

By studying cell lineages in *Drosophila*, Garcia-Bellido, Ripoll & Morata (1973) discovered that the epidermis of each segment derives from two groups of clones (polyclones) separated very early-on, both from a spatial and a genealogical point of view, so as to define two compartments whose boundaries are not crossed by cells of either side. This concept of compartment has been of fundamental importance in understanding insect morphogenesis.

More recently, Martinez-Arias & Lawrence (1985) have defined as parasegments the repetitive units corresponding to a couple of compartments, where the anterior one will be a posterior compartment in a future segment of the larva and the adult, and vice versa. According to these authors, the metameric structure defined by the BMMs we have discussed in the previous paragraphs is parasegmental. For instance, the prospective posterior compartments are identified by the presence of transcripts of the gene *en* (Lawrence & Struhl, 1982; Lawrence, 1985), which seem to be necessary for the formation of the segmental boundary. However, some problems arise.

First, compartmentalization seems only to affect the ectoderm. Ferrus & Kankel (1981) and Lawrence & Johnston (1984) failed to find any trace of compartments in the musculature and in other mesodermal derivatives. Accordingly, they only refer to ectodermal structures while defining segmental or parasegmental boundaries, in contrast to the customary definitions of comparative morphology and embryology, where segments are primarily identified in terms of longitudinal musculature and other mesodermal derivatives.

Second, the phase shift between biochemically marked embryonic rings and morphological segments does not guarantee that the first repetitive units occurring in the embryo are actually parasegmental, as already remarked upon by Carroll & Scott (1985).

We do not know whether the concepts of compartment and parasegment also apply to centipedes and millipedes; at any rate, we believe that present-day
models contrasting the parasegmental structure of embryos with the final segmental organization of insect larvae and adults do not adequately fit the descriptive and experimental data available to date.

Segmentation in Annelida and Arthropoda, or are Articulata truly monophyletic?

How can the phylogenetic origin of a system of regular biochemical markings on the epidermis by iterative doubling of an elementary structure be explained? We have come across such a process by investigating the origin of segmentation in arthropods, but the process itself may prove to be older than the arthropodan body plan and not to have evolved primarily as a system for controlling the number of segments by controlling the process generating them, but as a device for obtaining evenly spaced epidermal structures, whatever their number.

In fact, the doubling processes described above (step 3 of our sequence, see p. 333), guarantee a regular periodicity of markings, in the absence of foreign disturbing agencies. Which epidermal (or at least 'external') structures are possibly most affected by troubles in regular spacing? An obvious answer is: locomotory ones, not necessarily arthropodan appendages with their supporting musculature, or well-defined polychaete parapods, but also simple ciliary bands encircling the body. This is possibly a way to explain the distribution of locomotory bands of cilia in animals lacking 'true' segmentation, as in some of the so-called Archiannelida. In a species of *Dinophilus* studied by Jägersten (1944), the young just hatched from the egg has only a single ciliary band around each segment, whereas in the adult there are two bands per segment, to give an array of evenly spaced ciliary units. This is possibly only a first hint to a wider perspective, in so far as we can agree with Beklemishev (1969: 1:204) when he says that:

> the metamerism of the ectodermal organs of *Dinophilus* is..., point by point, the same as we have seen in lower worms, e.g. in *Procerodes lobata*. From the embryological point of view there are also no objections to this interpretation of the metamerism of *Dinophilus*: the larval segments in *Dinophilus*, in all cases where their development is known, mostly originate simultaneously along the whole length of the larval body.

An investigation of the control process over the morphogenesis of the ciliary pattern in these animals may prove of the utmost importance in gaining an understanding of the origin of segmentation in Articulata, i.e. Annelida and Arthropoda *sensu lato*.

In fact, our model seems to be in accordance with comparative morphological and experimental evidence concerning insects, centipedes and, possibly, millipede, but is it justified to apply it also to the other arthropods and to annelids too? Present knowledge forces us to refrain from generalizations, but we want to stress the broad phylogenetic implications of the matter. The question encompasses arthropod monophyletism as well as the monophyletism of Articulata as a whole.

The mechanisms we begin to understand in Atelocerata possibly apply to all arthropods: Martinez-Arias & Lawrence (1985) have suggested that a re-organization of parasegments into segments may be a common feature of all
Figure 10. Origin and developmental fate of segments in *Drosophila*, according to current interpretations (left; mainly based on Martinez-Arias & Lawrence, 1985) and in our view (right). The four boxes schematically represent an embryo in subsequent developmental stages. Each box is crossed by lines corresponding to biochemically or morphologically distinct units: what matters are their numerical and spatial relationships. The dots indicate mesodermal (musculature) units, phase-adjusted according to Martinez-Arias & Lawrence (1985).

arthropods, as supported by some observations on crustaceans (Dohle, 1976, 1980b).

Matters are surely different in annelids, at least in polychaetes, where trunk segments sequentially arise by mesodermal proliferation. We may even doubt whether annelid segments are truly homologous with those of arthropods.

In annelids, segments are primarily mesodermal units arising in serial sequence by steps punctuated by mitotic cycles, whereas in Atelocerata (possibly in all arthropods) they are primarily ectodermal units, not arising in sequence, but by iterative doubling of biochemical markers laid down independently from mitotic cycles.

In spite of these remarkable differences, a homology still possibly exists at the level of mesodermal derivatives, in particular in the arrangement of the longitudinal musculature. If a basic segmental pattern of all Articulata is to be identified in the repetitive units of the musculature or other mesodermal structures, then all other features, including secondary annulation of segments in leeches, diplosegments of millipedes and resegmentation of insect ectoderm (in the sense of Martinez-Arias & Lawrence, 1985), should be regarded as apomorphies defining a single phylum, or class.

Therefore, as long as we call *segments* the repetitive body units of annelids, *segments* are also the repetitive units of insect musculature, whether or not their compartmental origin is 'parasegmental'. But, from the same point of view, the new 'segmental' units displayed by the ectoderm of insects (possibly, of all arthropods) are not 'true' segments, but a kind of *metasegment* (Fig. 10).
At this point, we might even suggest a new name, *eosegments*, for the earliest repetitive units identified in *Drosophila* embryo and also postulated by our model, as a starting point in the segmentation of insects, centipedes and millipedes.

At the very end of this discussion, what truly matters are not new names, but the development of a new comparative phylogenetic approach to the segmentation problem, in order to complement and fully exploit the remarkable results of the experimental work done and still in progress on *Drosophila*.

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