Embryonic development and the understanding of the adult body plan in myriapods

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Abstract

The adult body plan is laid down during embryonic and post-embryonic development of an organism. Here we review two examples for how data on gene expression during embryonic development have changed our understanding of the adult body plan of myriapods. Gene expression studies in the geophilomorph centipede *Strigamia maritima* (Leach, 1817) have demonstrated that a developmental constraint underlies the always-odd number of leg bearing segments in geophilomorph centipedes. Similarly, data on gene expression in the millipede *Glomeris marginata* (Villers, 1789) have demonstrated a decoupling of dorsal and ventral segmentation, which provided an explanation for the discrepancy in dorsal and ventral structures in the body of millipedes. Knowledge on the molecular mechanisms underlying embryonic development therefore significantly contributes to understanding morphological features of the adult myriapod body.

Keywords: centipedes, millipedes, segmentation, embryology, *Glomeris marginata*, *Strigamia maritima*

1. Introduction

A segmented body is one of the hallmarks of arthropods. But, the segmented body also shows a lot of variation among the various arthropod groups. There is, for instance, variation in the number of segments per se, but also in the joining of segments to form tagmata (e.g. head-thorax-abdomen in insects, prosoma-opisthosoma in chelicerates and head-trunk in myriapods). Moreover, the segmental organisation is not always obvious in adult arthropods. In some arthropod groups the segments are fused in adults and the individual segments are not always apparent anymore, as for instance is the case in the abdomen of some insects or the opisthosoma of most spiders. While the segmented body is externally not always obvious in adults, the segmental organisation is usually clear in arthropod embryos. Also the way the segments form seems to be almost as diverse as the arthropods themselves. In some arthropods, all segments are laid down at the same time during embryonic development; the

well-studied fly *Drosophila melanogaster* Meigen, 1830 is an example of this. Many arthropods, however, do not form all segments simultaneously but add their posterior segments one after the other. There are even arthropod groups that only develop a particular number of segments during embryonic development, and add post-embryonically additional segments to their body. There is thus quite some variation in the segmented body and the modes of segmentation among arthropods.

In this paper we focus on myriapods. Even within the myriapods, there is a lot of variation. Most of our knowledge of the myriapod body plan comes from adults. And by looking at the adult body plan some peculiarities are obvious. For instance, segment number may vary. Furthermore, geophilomorph centipedes display a morphological oddity in that they always have an odd number of leg bearing segments and never an even number. Another peculiarity is observed in diplopods, but also in pauropods and symphylans, that have differences in the number of segmental structures along their dorsal side compared to their ventral side.

Here we discuss two examples how studies on the embryonic development, and more specifically studies on gene expression, helped us to better understand some phenomena related to the segmented body of myriapods. First, we review how gene expression patterns in the early embryo of the geophilomorph *Strigamia maritima* (Leach, 1817) helped us to understand why centipedes are made up of an odd number of leg-bearing segments. Then, we review how gene expression studies in the embryo of the millipede *Glomeris marginata* significantly increased our understanding of the diplosegmentation of the diplopod body plan, and shortly discuss the implications this work has for our way of thinking of segments in arthropods in general.

Why are centipedes odd?

Centipedes show a remarkable morphological feature in that the adults always have an odd number of leg-bearing trunk segments (LBS). Of the four major centipede orders, the Lithobiomorpha and Scutigeromorpha have 15 LBS, the Scolopendromorpha have 21 or 23 LBS, with one exception, *Scolopendropsis duplicata* Chagas-Junior et al., 2008, a scolopendromorph species with 39 or 43 LBS, and the Geophilomorpha have 27–191 LBS, but this range includes odd numbers only (Arthur 1999, Minelli & Bortoletto 1988, Minelli et al. 2000, Chagas-Junior et al. 2008). The range of LBS numbers in the geophilomorph centipedes is even more remarkable, as the number of segments can vary between individuals of different populations of a species and even can vary between individuals within one population. But despite the variation, the number of LBS is always odd. Why are centipedes odd, and never have an even number of LBS? One possible explanation that comes to mind is that this is the result of natural selection; another possible explanation is that it reflects a developmental constraint (Arthur 1999, Damen 2004).

The elegant work of Ariel Chipman, Wallace Arthur and Michael Akam on the geophilomorph *Strigamia maritima* (Chipman et al. 2004, Chipman & Akam 2008) provides evidence that a developmental constraint is the most likely explanation of the always-odd LBS number. The developmental constraint concept assumes that some morphological forms are absent because they are inconsistent with existing developmental mechanisms, rather than that they are non-adaptive. More specifically, for the always-odd LBS numbers this means that the developmental mechanism generating the segments impedes development of an even

number of LBS rather than that centipedes with an even number of LBS have a selective disadvantage over those with an odd number of LBS and therefore did not persevere in evolution (Arthur 1999, Arthur & Farrow 1999).

Chipman and co-workers took advantage of the wealth of data that are available on the mechanism of segmentation in arthropods. Work on the fruit fly *Drosophila* during the last three decades has taught us the molecular and genetic pathways that control segment formation in the fly (Nüsslein-Volhard & Wieschaus 1980, Sanson 2001). Many of the genes that are known from the fly are also involved in segment formation of other arthropods (Tautz 2004, Peel et al. 2005, Damen 2007). During embryogenesis segments of most arthropod species form from the unsegmented tissue of the posterior disc that buds off segments towards the anterior end. Although the segments bud off one after the other in other arthropods (e.g. Schoppmeier and Damen 2005), gene expression analyses in *Strigamia* have shown that during the initial phases of segment specification the genes are expressed in a two-segment periodicity and thus are defining double segmental units rather than single segmental units (Chipman et al. 2004, Chipman & Akam 2008).

This is probably most obvious from the expression of the *Strigamia caudal* (*Stm-cad*) and *even-skipped* (*Stm-eve1*) genes, which both are expressed in stripes in the unsegmented tissue of the posterior disc (Chipman et al. 2004, Chipman & Akam 2008). Only just before the segments are budded off from the posterior disc, a new stripe of *Stm-cad* and *Stm-eve1* gene expression intercalates between the initial stripes of gene expression. The final pattern thus is clearly in a one-segment periodicity, but the initial *Stm-cad* and *Stm-eve1* stripes were in a two-segmental periodicity. Expression patterns of various other genes are in complete agreement with this notion (Chipman & Akam 2008). In fact, the gene expression data show that three distinguishable phases exist: in the posterior disc the expression patterns are in a double segmental periodicity, then in a transition zone where the segments are emerging, the expression patterns transform into a single segmental periodicity, and finally, in the newly formed segments, expression patterns are in a segmental periodicity.

The molecular specification of further segments therefore always takes place in quanta of two segments, providing a molecular basis for the always-odd number of LBS: this double segmental mechanism will always produce even numbers (2n) of trunk segments, but because the first trunk segment does not bear a leg (it bears the poison claw and is not counted as LBS) the number of LBS is always odd (2n–1). The developmental mechanism that initially specifies the segments in double segmental units thus impedes the addition of single segments and is the reason for the developmental bias or constraint that causes the always-odd phenotype. The gene expression studies in *Strigamia* form an excellent example on how the understanding of the embryology helped to explain a remarkable morphological characteristic of geophilomorph centipedes (Damen 2004). Why does the geophilomorph posterior disc deviate from the normal arthropod posterior disc by producing double segmental units instead of normal segments? A number of genetic factors have been identified that are involved in geophilomorph double segmentation (e.g. caudal, Notch), but these genes are involved in normal segmentation in other arthropods as well, and thus cannot be the cause of the double segmentation mode. The actual regulator of double segmentation versus normal segmentation likely acts upstream of these known factors and has not been identified yet. This is the starting-point for future studies to deepen our understanding of the evolution of developmental constraints.

The segmented body of myriapods or what is a segment?

Another peculiarity is the discrepancy in numbers of dorsal and ventral structures in millipedes, pauropods and symphylans, which even leads to confusion on what a segment is. Whatever myriapod one takes, it is obvious that its body is constructed of repetitive units. In centipedes, the situation is relatively simple and there seems to be not much discussion on what a segment is. The other main myriapod groups, however, are more variable in terms of dorsal versus ventral segmental structures, obscuring the view of what a segment is. In millipedes there is a clear discrepancy in the numbers of ventral segmental structures (e.g. leg pairs, sternites) and dorsal segmental structures (e.g. tergites). What should be considered as a segment in millipedes? Looking from the dorsal side would result in another judgement than if one looks from the ventral side. A number of millipedes fuse dorsal tergites with ventral sternites and leg pairs into rings. From these ring-forming millipedes it becomes clear that apart from the first three rings, which each consist of a single tergite and a single leg pair, all more posterior rings each consists of a single tergite that is fused to two leg pairs. Should each such ring be considered as a segment? In that case we have to deal with two types of segments (rings): haplosegments that consist of a tergite with a single leg pair and diplosegments that consist of a tergite with two leg pairs. In millipedes that do not form rings (e.g. Glomeriids) it is very hard to make such correlations, but the posterior tergites appear to cover two leg pairs each, which would correspond to what is seen in ring–forming millipedes. Also in the Symphyla and Pauropoda differences exist in the number of ventral and dorsal segmental structures (e.g. Janssen et al. 2006a).

These dorso-ventral discrepancies still form one of the most intriguing open questions concerning the segmented myriapod body plan. Like in the above example of the odd number of leg segments in the centipedes, the analysis of gene-expression patterns in a millipede, namely the pill millipede *Glomeris marginata*, provided significant new insights into these issues.

Decoupled pattern formation in dorsal and ventral tissue in millipedes

Our studies on gene expression patterns during embryonic development of the pill millipede *Glomeris marginata* revealed major new insights into the differences in dorsal and ventral segmentation of millipedes. We also took advantage of the knowledge on segmentation genes from *Drosophila* and other arthropods, and analysed the expression patterns of these genes in embryos of *Glomeris*. Unexpectedly, we detected major differences in the way these genes are expressed in ventral and dorsal tissue in *Glomeris* (Janssen et al. 2004, Janssen et al. 2006b, Janssen et al. 2008). We used genes that belong to the segment-polarity class of segmentation genes. These genes define segmentally reiterated boundaries in the *Drosophila* embryo (Martinez-Arias & Lawrence 1985, Lawrence 1992) as well as in other arthropods (Damen 2002, Hughes & Kaufman 2002). The parasegment that is defined by these boundaries is considered as a segmental functional unit in the early embryo. The *Glomeris* gene homologs of segment-polarity genes, like *engrailed*, *wingless*, *hedgehog*, *cubitus interruptus* and *patched,* are expressed in the ventral tissue in similar patterns as in *Drosophila* and other arthropods (Janssen et al. 2004, Janssen et al. 2008). More specifically, they molecularly define segmentally reiterated ventral units that each are associated with one pair of legs (in the trunk). The genetic network underlying the generation of segmentally

reiterated boundaries and structures on the ventral side of the *Glomeris* embryo thus is the segment-polarity gene network that is used in segmentation of all arthropods studied to date. Genetic data from *Drosophila* revealed that this network defines a genetic feedback loop between *wingless* and *hedgehog* expressing cells and in this way generates the segmentally reiterated boundaries.

To our surprise, we found that the expression patterns of these genes in the dorsal tissue of the *Glomeris* embryo were significantly different and were not compatible with the use of the same genetic network (Janssen et al. 2004, Janssen et al. 2008). For instance, although the *engrailed* and *hedgehog* genes are expressed in segmentally reiterated stripes in the dorsal tissue, they do not align to the ventral stripes. Furthermore, no *wingless* gene expression was found dorsally, and also the relative position of other genes was different compared to their ventral expression. A genetic feedback loop that usually defines the segmentally reiterated boundary between *wingless* and *hedgehog* expressing cells and that also seems to act ventrally in *Glomeris* cannot act dorsally because the dorsal expression patterns are incompatible with such a mechanism (Janssen et al. 2004). Instead the dorsal gene expression patterns suggest that a different genetic mechanism acts in generating segmentally reiterated boundaries there (Janssen et al. 2008). The significant differences in gene-expression patterns in dorsal and ventral tissue of the millipede imply that the dorsal and ventral segmentally reiterated boundaries and structures are independently established in the embryo (Fig. 1). This has major implications for our understanding of what a segment is in millipedes.

A new view on the correlation of ventral and dorsal structures

The analysis of gene expression patterns during *Glomeris* embryology helped us to better understand the discrepancy in numbers of dorsal and ventral segmental structures in millipedes (Fig. 1A–C). Most importantly, they show that the establishment of dorsal and ventral segmental structures is decoupled and that their specification takes place independently from each other. There is thus no necessary reason why they should produce identical numbers of segmental structures. This has implications for the adult body plan. It not only explains the difference in numbers of dorsal and ventral structures in *Glomeris* but also contributes a solution to the problem of tergite to leg pair correlation (Fig. 1C). There is no direct developmental connection between the dorsal and ventral segmental structures, and consequently, the borders between tergites do not necessarily need to align with other segmental boundaries. The fusion into rings, as seen in ring-forming millipedes, therefore presumably is a process that takes place secondarily (discussed in detail in Janssen et al. 2006a). The decoupling model also explains the discrepancy in dorsal and ventral structures in pauropods and symphylans, and can be extrapolated to particular crustaceans (Notostraca) that also display differences in the number of dorsal and ventral segmental structures (Janssen et al. 2004, Janssen et al. 2006a).
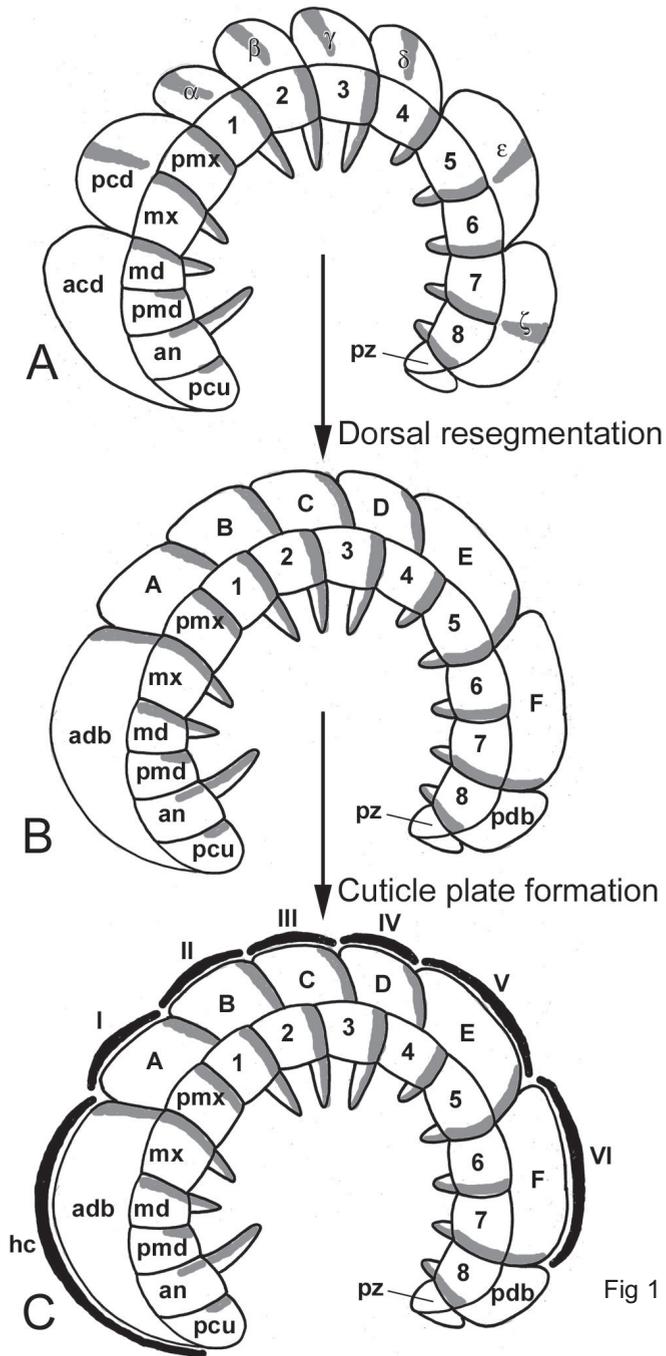
Fig 1

Fig. 1   Correlation of ventral and dorsal segmental elements in millipedes revealed by developmental genetic studies. Schematic drawings of a pill millipede (*Glomeris marginata*) embryo in lateral view, anterior to the left. A: The embryo is subdivided into primary units that we generally call 'segments'. The number of ventral segments is higher than the number of dorsal segments. The head consists of five ventral segments (an, pmd, md, mx, pmx), two dorsal segments (acd, pcd) and a protocerebral unit (pcu) the segmental nature of which is debated. The trunk consists of eight ventral segments (labelled with Arabic numerals), six dorsal segments (labeled with Greek numerals), and a posterior zone (pz), which will give rise to further segments during postembryonic development. All segments, ventral as well as dorsal ones, are separated by morphological grooves. The expression of the gene *engrailed* is indicated in grey in all panels; B: Later in development the morphological grooves between the dorsal segments disappear and new dorsal morphological grooves appear at the position of the dorsal *engrailed* stripes. We call this process 'dorsal resegmentation'. The result is that the primary dorsal segments are transformed into new segmental units that we call 'dorsal blocks'. There are an anterior and a posterior dorsal block (adb, pdb) and six blocks in between (labelled with upper case letters); C: Finally, the epidermis of these dorsal blocks (except for the posterior one) secretes cuticle that will form rigid plates (tergites) in the adults. The primordial tergites are drawn in black in the figure. The most anterior cuticle plate will form the head capsule (hc), followed by six primordial tergites (labeled with Roman numerals). This peculiar developmental mode leads to the well-known mismatch between dorsal and ventral segmental structures. For example, the trunk in dorsal aspect has six cuticular plates and one could expect to find six segments underneath corresponding to these six plates. In fact, however, plates I to V cover adjacent portions of pairs of ventral segments and plate VI even covers two ventral segments. The result is a complex correlation between dorsal and ventral segmental structures. How is this possible? Recent work has revealed that the genetic patterning mechanisms in dorsal and ventral segments are very different. An example is the *engrailed* gene shown in the drawing (grey stripes) that is expressed in the posterior of the ventral segments, but in the middle of the dorsal segments. In the meantime, differential dorso-ventral gene expression has been demonstrated in a number of additional genes including *wingless*, *hedgehog*, *cubitus-interruptus*, *H15*, *optomotor-blind*, *decapentaplegic*, *Notum* and *patched* (Janssen et al. 2004, Janssen et al. 2008). These data show that dorsal and ventral segments are established independent from each other through different mechanisms. We have called this 'decoupled segmentation' and it leads to a different final number of dorsal and ventral segments. Abbreviations: acd, anterior cephalic dorsal segment; pcd, posterior cephalic dorsal segment; pcu, protocerebral cephalic unit; an, antennal segment; pmd, premandibulary segment; md, mandibulary segment; mx, maxillary segment; pmx, postmaxillary segment; 1-8, ventral segment 1–8; alpha-zeta, dorsal segments in the trunk; pz, posterior zone; adb, anterior dorsal block; pdb, posterior dorsal block; A–F, intermediate dorsal blocks; hc, head capsule; I–VI, dorsal cuticular plates.

What is a segment?

Finally, these data begin changing our view of segments as building blocks of arthropods. The segments (or parasegments) are often considered as a kind of preformed building blocks that will give rise to all segmental structures. The *Glomeris* data indicate that segments should not be considered as preformed building blocks. Due to the dorsal and ventral uncoupling one would have to consider dorsal and ventral building blocks. Rather than defining such preformed building blocks, the genes seem to provide a framework of repetitive positional information. The term segmental boundary, as used in this paper (and in many other papers), is referring to something defined by gene expression patterns and gene functions that provide a framework of positional information. Different morphological processes then use this positional information as a reference system. *Glomeris* apparently uses dorsally and ventrally distinct genetic mechanisms to provide uncoupled positional information. This positional information then leads to dorsal and ventral distinct reference systems that do not align in a one-to-one fashion. Further discussions on this issue go beyond the scope of this paper. We therefore refer to Budd (2001), Minelli (2004), Minelli & Fusco (2004), and Fusco (2008) for detailed discussions.

2. Conclusion

There are still many open questions concerning the segmented body plan. Here we have described how two studies on gene expression patterns during embryology have provided us with new insights into the adult body plan of myriapods. Since the body plan is laid down during embryonic and post-embryonic development, such studies significantly contribute to our understanding of the adult body. The two examples reviewed in this paper demonstrated the power of such studies. A better understanding of the molecular mechanisms underlying embryology therefore may solve some more of the open questions in the future.

3. Acknowledgements

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