Trilobite body patterning and the evolution of arthropod tagmosis

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Summary
Preservation permitting patterns of developmental evolution can be reconstructed within long extinct clades, and the rich fossil record of trilobite ontogeny and phylogeny provides an unparalleled opportunity for doing so. Furthermore, knowledge of Hox gene expression patterns among living arthropods permit inferences about possible Hox gene deployment in trilobites. The trilobite anteroposterior body plan is consistent with recent suggestions that basal euarthropods had a relatively low degree of tagmosis among cephalic limbs, possibly related to overlapping expression domains of cephalic Hox genes. Trilobite trunk segments appeared sequentially at a subterminal generative zone, and were exchanged between regions of fused and freely articulating segments during growth. Homonomous trunk segment shape and gradual size transition were apparently phylogenetically basal conditions and suggest a single trunk tagma. Several derived clades independently evolved functionally distinct tagmata within the trunk, apparently exchanging flexible segment numbers for greater regionally autonomy. The trilobite trunk chronicles how different aspects of arthropod segmentation coevolved as the degree of tagmosis increased. *BioEssays* **25:386–395, 2003.**
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Introduction
The characterization of domains of early Hox gene expression across the major groups of arthropods[1] provides a new context in which to evaluate the morphological homologies of arthropod anteroposterior (ap) body patterning. In a complementary fashion, the ap patterning of extinct arthropod clades is also receiving renewed attention for the light it may throw on the early stages of arthropod diversification.[2] This paper identifies features of ap body plan common to all members of the richly fossiliferous, extinct arthropod clade Trilobita and considers these in relation to controls of body patterning known among extant arthropods. It emphasizes those aspects of trilobite ap patterning that varied within the group, and highlights unusual features of the trilobite trunk region that may provide a model system in which to explore aspects of the

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evolution of arthropod tagmosis. The paper aims to demonstrate how knowledge of developmental genetics can illuminate patterns seen within extinct clades, and how information from fossils may yield unique insights into the evolution of developmental regulation.

Taxonomic variety, stratigraphic continuity and accessibility of ontogenetic information distinguish trilobites from non-biomineralized fossil arthropods that are known only from cases of exceptional preservation, such as the Burgess Shale or Chengjiang.[3] During the 270 million years of trilobite evolution chronicled within the fossil record, the clade displayed marked diversity; some 10,000 species have been described to date. Their durable calcitic exoskeleton, synapomorphic for the group and first expressed shortly after hatching, has enabled the reconstruction of ontogenetic sequences for many tens of species, including representatives of all the major trilobite clades. These ontogenies permit analysis of the mode of segment expression, at least with respect to the dorsal exoskeleton. Such dynamic information, coupled with conserved features of adult body organization, and knowledge of the ventral appendages gleaned from some 20 species, allows identification of those features of ap body patterning characteristic of the group as a whole.[4]

AP divisions of the trilobite body plan
A variety of patterns of variation among segments occurred within the Trilobita. Adjacent segments may appear to be identical or they may differ in size, shape, or both. The degree of size or shape difference between adjacent segments was either static throughout ontogeny, or it varied dynamically. Boundaries between exoskeletal segments were either fused or separated by an articulation, and this feature also varied during ontogeny in certain portions of the animal. The principal divisions of the trilobite ap body plan are here identified with respect to these varied styles of structural difference. The aim is to integrate the various types of information into a general model of trilobite ap body patterning that reflects not only the overall characteristics of the group, but also the variation in body patterning displayed within it.

Adult dorsal exoskeleton
The dorsal exoskeleton of all mature trilobites showed two distinct boundaries along the ap axis which relate to the boundaries between sets of fused and sets of articulating

Glossary

Interpretation of trilobite biology, based almost entirely on morphology, requires a rich descriptive vocabulary. Terms introduced in the text are defined here and in Figs. 2,3.

ADULTHOOD—Conventionally considered to begin at the onset of epimorphic growth in trilobites following completion of thoracic segmentation,[36] and used in this sense herein for descriptive convenience. Although most epimorphic trilobites would have been sexually mature, there are no strong arguments for a specific link between the onset of epimorphosis and the advent of sexual maturity.[37]

ANAMORPHIC GROWTH—In which moulting is accompanied by the sequential appearance of additional segments.

AXIS—Central, inflated portion of the dorsal exoskeleton.

CEPHALON—The anteriormost or "head" division of the trilobite body comprised of a set of fused segments whose identity is expressed axially but whose boundaries are unclear in the pleural region.

GLABELLA—Central inflated portion of the cephalic axis which served as the stomach capsule.

HOMONOMOUS—Two or more segments being identical or closely comparable in shape.

EPIMORPHIC GROWTH—In which sequential moults retain a constant number of segments.

MACROPLEURAL—Trunk segments of similar axial and overall structure to adjacent segments, but whose pleural proportions are significantly exaggerated in some manner.

MANDIBULATES—Arthropods with specialized jaw apparatus including myriapods, crustaceans, and insects.

PLEURAE—Lateral portions of the dorsal exoskeleton bounding the central axis.

PODOMERES—Segmented units of the walking limb separated by articulated joints.

PREAXIAL—The portion of the cephalon anterior to the glabella.

PYGIDIUM—The posterior, caudal portion of the body of adult trilobites containing fused trunk exoskeletal segments.

TAGMOSIS—Division of the body into structurally and functionally distinct segments or batches of segments individually called tagma.

TERATOLOGICAL SEGMENT—A segment showing evidence of a developmental malformation.

THORAX—The central portion of the body of trilobites containing freely articulating trunk segments.

TRUNK—The posterior region of the trilobite body comprising a set of segments whose identity is clearly expressed both axially and pleurally.

segments (Figs. 1, 2). The anterior boundary separated the fused segments of the cephalon from the articulating segments of the trunk region. The cephalon was characterized by four or five segments demarcated by furrows within the central axis (Figs. 1–3). Any additional anterior cephalic segments were not clearly defined in the exoskeleton, and the preaxial portion of the cephalon showed marked ontogenetic and phylogenetic variability within the clade (Figs. 1, 2), as may be the case in other early arthropods.[2] Adjacent cephalic segments commonly differed markedly in shape and size, both within species and across the group as a whole. In striking contrast, the mature trunk consisted of segments whose boundaries were expressed in both the axial and pleural regions (Figs. 1, 2), were freely articulating in the anterior portion of the trunk, and were broadly homonomous in form (notable but relatively rare exceptions being macropleural segments—Fig. 1A). The number of adult trunk segments varied markedly across the clade with a range of about 40 segments. The adult trunk was divided into an anterior set of freely articulating segments that constitute the trilobite thorax and a posterior set of fused segments that comprise a caudal region, known as the pygidium. The size of trunk segments generally decreased towards the rear, in some cases gradually (Figs. 1C, 2A), in others with more abrupt transitions (Fig. 1A). Likewise, the shapes of segments were either homonomous throughout the trunk (Figs. 1C, 2A), or differentiated into sets or "batches" of similar segments (Figs. 1A, B,D, 2B). The mature cephalon, thorax, and pygidium have long been considered to define three distinct tagmata of the trilobite ap body plan.[5]

Ontogeny of dorsal exoskeleton

Trilobite ontogeny was characterized by the increase in size and morphological modification of those segments present in earlier instars, and by the appearance of new segments. Among preserved juvenile stages, the number of segments expressed in the cephalic exoskeleton was constant, just as it remained constant among adults throughout the clade. By contrast, segment expression within the trunk region was markedly dynamic, both ontogenetically and phylogenetically. During early growth, ontogenetic sequences displayed stepwise, anamorphic appearance of new trunk segments.[6] This was followed by a terminal epimorphic phase, commonly equated with sexual maturity, during which growth and moulting continued but in which no additional segments were expressed (Fig. 4). Tracking the position of a distinctive macropleural segment through ontogeny demonstrated that the site at which new trunk segments were first expressed was the anterior of the terminal segment of the trunk region[7] (Fig. 4). This suggests a posteriormost division of the trilobite body plan—between a terminal region, the anterior of which marked the site of segment expression, and the trunk segments themselves. The expression of new segments was not
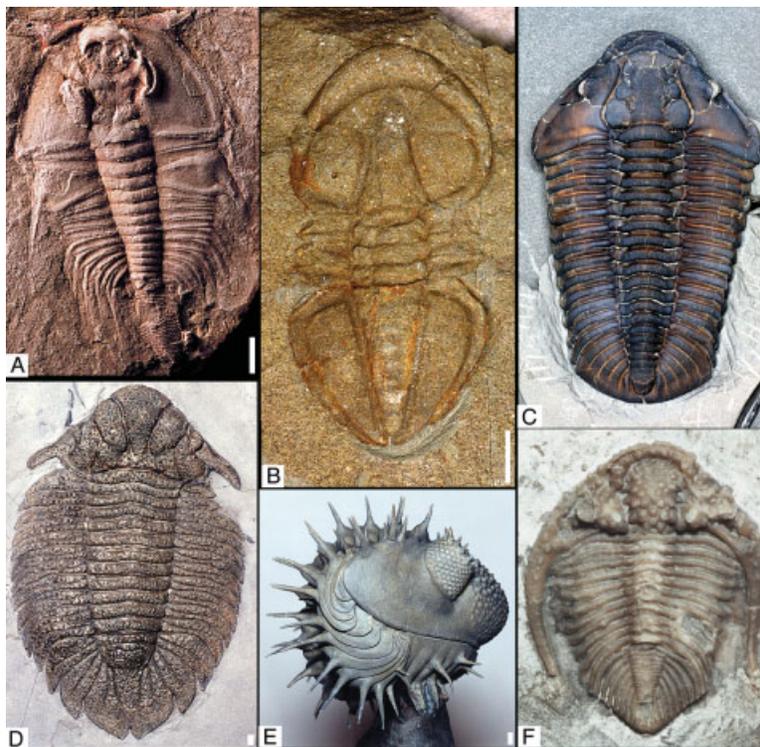
Figure 1. Adult trilobite exoskeletons showing major features of anteroposterior body patterning. **A:** *Bristolia insolens* with the third trunk segment macropleural and the 14th trunk segment marking the divide between large prothoracic and smaller opisthothoracic segments (see Fig. 3), from Early Cambrian rocks of San Bernardino County, California, Norm Brown collection. **B:** *Serrodiscus speciosus* with three thoracic segments and at least eight pygidial segments from Early Cambrian rocks of Columbia County, New York, USNM156592. **C:** *Flexicalymene* aff. *F. granulosa* showing a single trunk segment morphotype from Late Ordovician rocks of Covington, Kentucky, CMCP2504. **D:** *Dicranopeltis nereus* showing a marked transition in trunk segment proportions across the thoracic/pygidial divide, from Early Silurian rocks of Orleans County, New York, Kent Smith collection. **E:** *Drotopos armatus* showing encapsulated enrolment from Middle Devonian rocks of Morocco, Guy Darrough collection. **F:** *Erratencrinurus vigilans* showing mismatch of axial and pleural segmentation in the pygidium, from Middle Ordovician rocks at Brechin, Ontario RMSC12001.46.1. A, B, and D are marked examples of the "two-batch" trunk condition. A exemplifies the condition shown in Fig. 6B1; B and D illustrate the condition in Fig. 6B2. Scale bar is 2 mm long for all specimens.
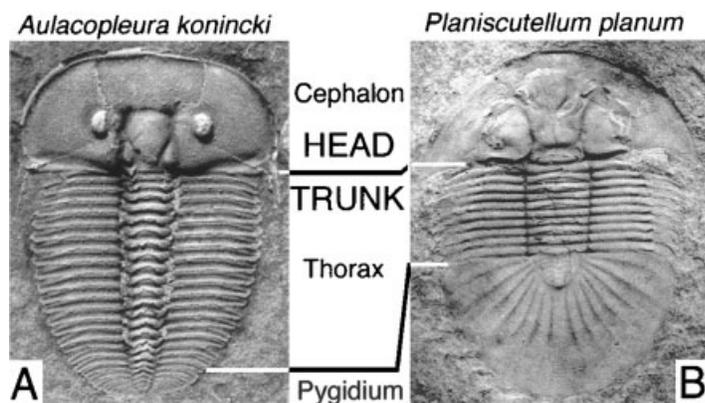

Figure 2. Major ap articulation divisions of the adult body of trilobites. The trunk region is divided into the freely articulating segments of the thorax and the fused segments of the pygidium. **A:** *Aulacopleura konincki*, the segments of the adult thorax and pygidium are similar in morphology and size (the specimen is about 2 cm long). This species typifies the homonomous trunk segment condition. **B:** *Planiscutellum planum*, adult thoracic and pygidial segments bear strikingly different morphologies and sizes (the specimen is about 2 cm long). This species typifies the "two-batch" trunk condition. Both specimens are from Early Silurian rocks near Loděnice in the Czech Republic.
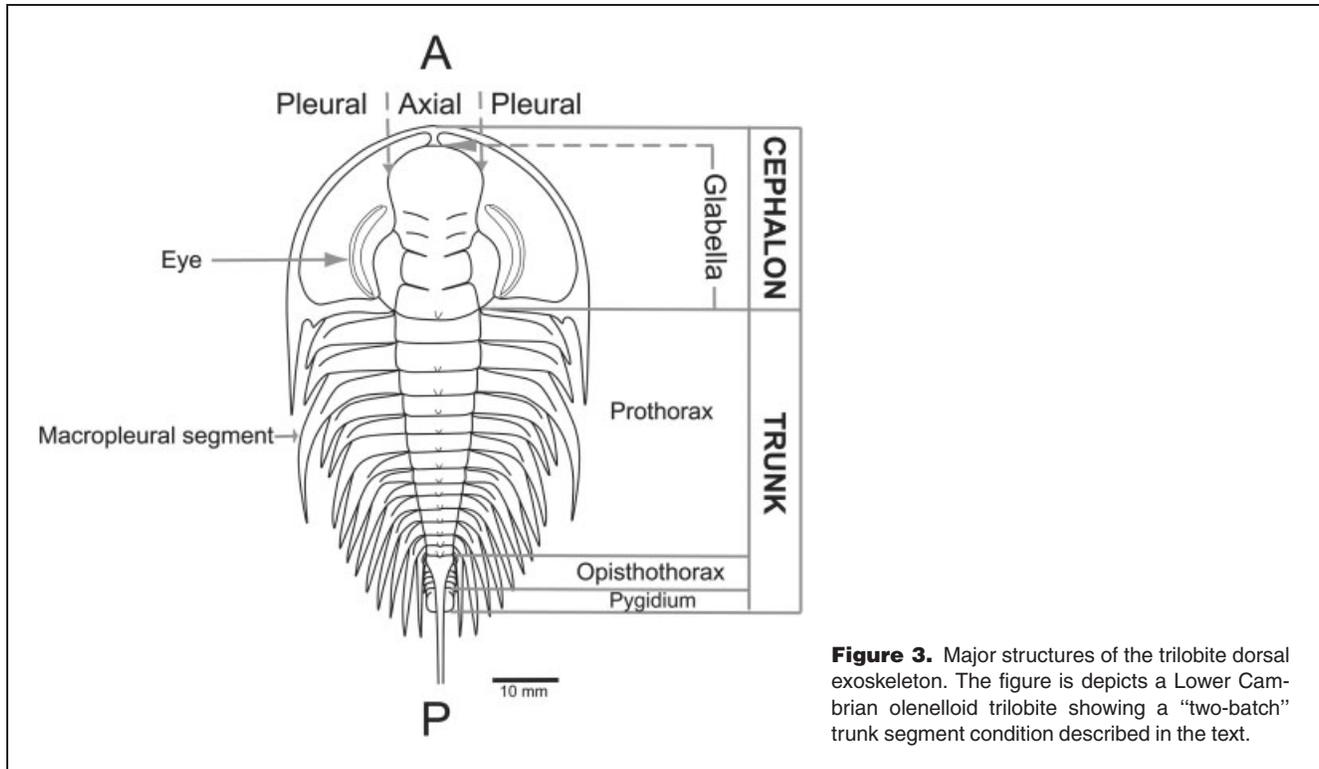
Figure 3. Major structures of the trilobite dorsal exoskeleton. The figure is depicts a Lower Cambrian olenelloid trilobite showing a "two-batch" trunk segment condition described in the text.

necessarily coincident with the cellular differentiation of segment primordia,[6] and mismatches in the numbers segments expressed ventrally and dorsally, or even between the dorsal axis and pleurae, are evident within the caudal region of some trilobites (Fig. 1F).

During the earliest known stages of trilobite ontogeny, all body segments formed a fused shield. In later instars, this was succeeded by the appearance of a series of articulations, the first at the cephalic−trunk boundary (Fig. 4). At the same time that new segments were expressed adjacent to the terminal region, the boundary between freely articulating and fused trunk segments migrated posteriorly, with the result that the number of articulating segments within the thorax increased progressively during growth (Fig. 4). The specific balance between the rates of segment expression near the posterior of the fused caudal region and segment release at the anterior of that region could vary during ontogeny and between taxa. The dynamic nature of segment exchange between the caudal region and the thorax in early ontogeny is a striking aspect of trilobite body patterning, and is considered further below.

Adult ventral appendages
The rare preservation of trilobite ventral appendages confirms that there was a pair for every segment expressed within the dorsal axis, except within the poorly differentiated posteriormost caudal region of some species.[4] With the exception of the anteriormost and, in one case, posteriormost pairs, all trilobite appendages were biramous, with a dorsal filamentous component and a ventral leg. The size of these biramous appendages varied along the ap axis in broad accord with dorsal segment size, but the shape apparently remained constant, even across the cephalic/trunk divide. The only notable ap variations among the biramous appendages of species that were unequivocally Trilobita were slight variations in the spinosity of the leg podomeres, and these differences cannot be compared to the functionally distinct appendage morphotypes common among derived mandibulate arthropods. Variations in biramous appendage structure among trilobite taxa were comparably slight. The anteriormost appendage pair consisted of uniramous antennae, and one species also had a pair of similar structures as the posteriormost appendage pair,[8] probably associated with the terminal exoskeletal segment. The antennae correlated spatially with the anteriormost dorsal cephalic segment. Interestingly, marked transitions in appendage morphology did not correlate with the exoskeletal articulation boundaries traditionally used to designate trilobite tagma,[5] nor was there marked differentiation among the biramous appendages of the head. On the contrary, where the limb structure is known, it was homonomous throughout the posterior head and all but the last trunk segment.

The trilobite ap body plan
This review of trilobite ap body patterning suggests a model that can be applied to all trilobite taxa (Fig. 5). From anterior to
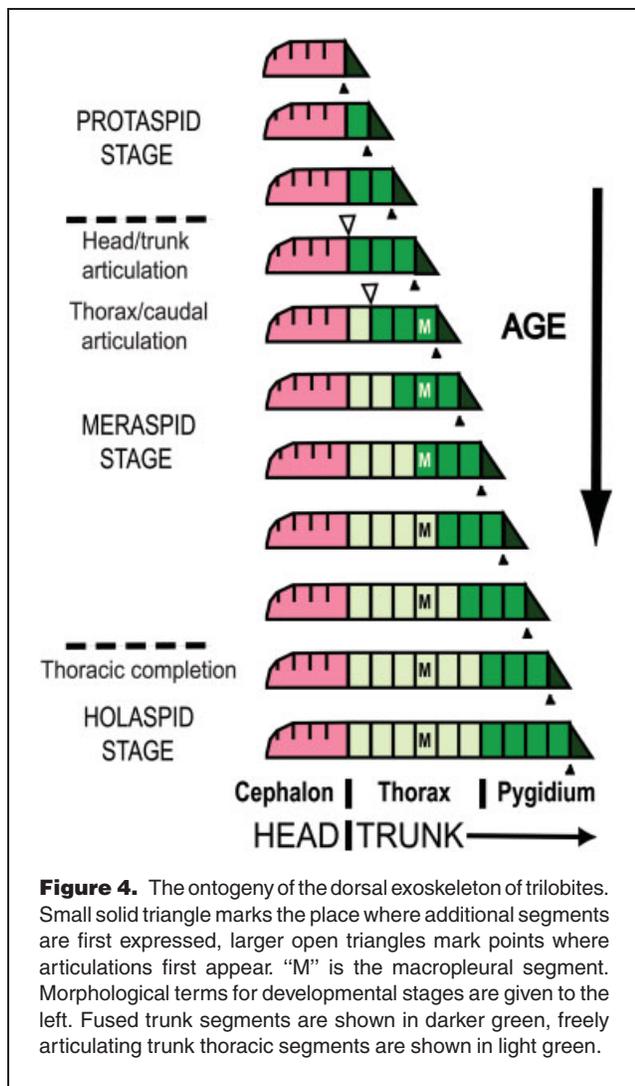
Figure 4. The ontogeny of the dorsal exoskeleton of trilobites. Small solid triangle marks the place where additional segments are first expressed, larger open triangles mark points where articulations first appear. "M" is the macropleural segment. Morphological terms for developmental stages are given to the left. Fused trunk segments are shown in darker green, freely articulating trunk thoracic segments are shown in light green.

posterior along the axis, segments were scored if the state in each category differed from that of the preceding segment. This procedure highlights how some ap divisions affect more segmentation categories than others, and also documents variability in aspects of body patterning within the group. This attempt to differentiate among aspects of segmentation is not intended to imply mutual independence among all categories, or that each category has equal "weight" in terms of its significance to body plan organization. It is simply an illustrative device to show how aspects of segmentation differed, and to emphasize that trilobite modularity can be viewed in various ways.

Based on the minimal condition, common to all trilobites, six distinct ap regions can be recognized (Fig. 5). These include the inference of a zone of preantennal segments, whose exoskeletal signature was obscure or absent in adult trilobites.

The head showed more concentrated differentiation among segments than the trunk, and a greater range of conditions. The antennal−biramous divide appeared consistently across the group. The division that reflected transition in the highest number of categories, and the smallest range within the group, was the cephalic−trunk divide, and this matched the contrast in the stability of segment numbers within the two regions as discussed above. The thoracic−pygidial divide was less prominent in the minimal condition but showed a marked range. The terminal region differed both developmentally and ontogenetically in some cases.

The consistency of these major body regions among trilobites brings additional aspects of trilobite segmentation into focus. Firstly, how do these body regions relate to those of other arthropods, and can aspects of the developmental basis of trilobite ap body patterning be inferred on the basis of pan-euarthropod homology? Secondly, which aspects of trilobite body patterning varied within the group, and what relevance may these have for understanding the evolution of arthropod body patterning? Among the Trilobita, there was considerable variation in the sizes and shapes of a constant number of cephalic segments. The trunk region varied not only in the number of segments, but also in aspects of their morphology, such as the occurrence of the macropleural segment or segments, and the nature of the thoracic−pygidial boundary.

The cephalon was able to sustain a free-living existence immediately after hatching, prior to development of the trunk, and vital organs involved in feeding, digestion and cognition are known to have been concentrated within that region. Cephalic segments generally showed greater shape change per moult than trunk segments and, because this was the site of ingestion and food processing, the dynamic cephalic morphology may have related to changing feeding habits during growth, as larger individuals were able to manipulate food particles of a greater size range.

The occurrence of macropleural segments was sporadic but widespread within the Trilobita, commonly varying at low taxonomic levels, both in terms of morphological expression and in topological position within the trunk. The functional significance of macropleurae remains unclear. The ap boundary that shows the greatest range of variation among the Trilobita is that between the adult thorax and pygidium. This issue is discussed in detail below.

Trilobite ap patterning in the context of other euarthropods

Recent phylogenetic analyses place the trilobites as a monophyletic group within a broad arachnate clade that contains the extant chelicerates.[9,10] This clade, together with mandibulate arthropods, form the Euarthropoda. The position of the uniramous insects and myriapods within the Mandibulata has been contentious, but recent data suggest myriapods as the
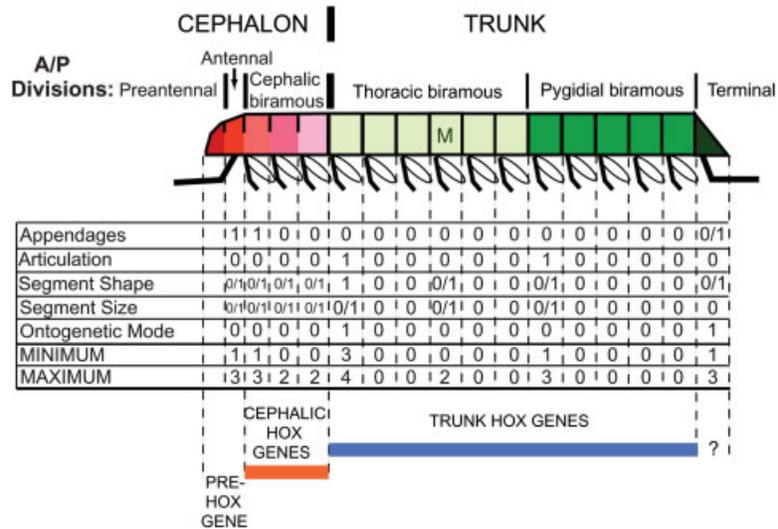
CEPHALON | TRUNK

A/P Divisions: Preantennal | Antennal | Cephalic biramous | Thoracic biramous | Pygidial biramous | Terminal

M

Appendages	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0/1
Articulation	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Segment Shape	0/1	0/1	0/1	0/1	1	0	0	0/1	0	0	0/1	0	0	0	0	0/1
Segment Size	0/1	0/1	0/1	0/1	0/1	0	0	0/1	0	0	0/1	0	0	0	0	0
Ontogenetic Mode	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
MINIMUM	1	1	0	0	3	0	0	0	0	0	1	0	0	0	0	1
MAXIMUM	3	3	2	2	4	0	0	2	0	0	3	0	0	0	0	3

CEPHALIC HOX GENES | TRUNK HOX GENES ?

PRE-HOX GENE

Figure 5. The major ap regions of the trilobite body plan, and their relations to transitions in various categories of segment identity. The trilobite cephalon is shown as containing three pairs of biramous appendages, the most common, but perhaps not universal, condition. In the matrix, a segment received a score of 1 if its condition differed markedly from that of the preceding segment, and 0 if it was the same. "Appendages" refers to the ventral limbs, all other categories refer to aspects of the dorsal exoskeleton. Significant boundaries occur at the anterior of those segments that scored highly. States that varied within the Trilobita are denoted by a 0/1 score. The minimum condition defines the basic ap body plan for the whole group; the maximum condition illustrates the degree of variation in aspects of body patterning and modularity within the group. Zones of cephalic (*labial* to *fushi tarazu*) and trunk (*Antennapedia* to *Abdominal-B*) Hox gene expression are inferred based on expression patterns in modern arthropods and their morphological homologies with trilobites. Ontogenetic mode defines the manner in which segments were specified; cephalic segments—embryonic and likely en masse, trunk segments—larval and sequential. At the level of all Trilobita, ontogenetic trajectory mirrors segment size, and so is not included as a separate field.

most basal mandibulate clade and insects as the most derived.[11] Three aspects the trilobite ap body plan are sufficiently similar to morphological divisions seen across extant euarthropods to warrant the inference of homologous developmental controls. These are the differentiation of cephalic segmentation into an anterior antennal segment and posterior biramous appendages, the cephalic–trunk divide, and the terminal region.

All euarthropod clades show a basal condition of ten Hox genes.[1] Given this, it is reasonable to consider how this set of Hox genes might have been deployed in trilobites, based on conserved patterns among all euarthropods. The purpose of this approach is not to invoke the operation of particular genes in trilobites, as in the recent attempt to relate the eight Hox genes of *Drosophila* to eight putative ap regions of the trilobite body plan,[12] critiqued elsewhere,[4] but rather to assess trilobite body patterning and its evolution in the context of the entire euarthropod clade.

Among extant arthropods the anteriormost appendage pair is antennal or chelate. This first appendage, and regions anterior to it, lack Hox gene expression in the developing segments. Given that the first appendage of trilobites is also antennal and differs from the adjacent biramous appendages, and that antennae are the default appendages expressed in

other body regions of the beetle *Tribolium* when Hox gene expression is suppressed,[13] it seems likely that the posterior boundary of the antennal segment marks the anterior boundary of Hox gene expression in trilobites. Similarly, the cephalic–trunk divide in trilobites mimics a boundary seen among all extant arthropods. Uncertainties about the homologies of specific cephalic segments and their relations to particular Hox gene expression domains among modern arthropods hinder more precise inference, but the division of Hox genes among extant arthropods into a cephalic set— *labial* to *fushi tarazu* orthologs, and a trunk set, *Antennepedia* to *Abdominal-B* orthologs, probably also pertained in trilobites (Fig. 5). The terminal region appears comparable to the trunk teloblastic region of modern arthropods, such as in short-germ-band insects. The terminal uniramous antenniform cerci seen in one trilobite species were so different from adjacent biramous appendages and so similar to the first appendage pair that the possibility that they were developed in the absence of Hox gene expression should be entertained.[4]

Extant arthropods commonly show overlapping zones of Hox gene expression within the trunk, the boundaries of which bear no obvious general relationship to morphology.[1] However, the evolution of specialized appendage morphologies in the mandibulate cephalon correlates with the restriction

of the expression domains of certain cephalic Hox genes such as *proboscipedia* and *Sex combs reduced*. The homonomous nature of trilobite biramous appendages is of interest in this regard, for it is consistent with the lower degree of cephalic limb tagmosis witnessed in chelicerates and basal mandibulates, such as myriapods, in which cephalic Hox gene expression domains overlapped.[1] Trilobite cephalic limb patterns thus support the notion that mandibulate evolution was characterized by increased cephalic limb tagmosis and associated restriction of Hox expression patterns, perhaps by the evolution of *cis*-regulatory inhibition of particular Hox genes within specific segments. Such an explanation accords with observations of an increased degree of limb tagmosis among modern, as opposed to ancient, arthropods.[13–15] This transition reflects both the relative decline of the weakly tagmatized marine arachnates and the increased dominance of derived, highly tagmatized mandibulates.

What kept the trilobites and other arachnates from greater regionalization of their cephalic appendages, despite their ability to specify distinct antennae from biramous appendages, and to differentiate the shapes of exoskeletal segments? The overall tempo and mode of arachnate ontogenetic change is apparently gradual and linear compared to the intermittent and markedly non-linear changes that characterize many derived mandibulates (of which insect metamorphosis is a prime example).[16] Thus, the ability to modify limb morphology markedly during ontogeny, with associated changes in Hox gene expression, seems to have been limited in arachnates. The extent to which this inability to modularize cephalic appendages contributed to the demise of marine arachnates, and extinction of the Trilobita, is unknown. Trilobites were evolutionarily plastic both in overall morphology and the varieties of ecological strategies that they pursued,[17,18] which were probably broadly comparable to those occupied by marine arthropods today. Nevertheless, given the functional importance of cephalic appendage specialization for feeding in modern marine euarthropods, the homonomous structure of most trilobite limbs can be seen as "archaic".[19] The presence of developmental constraints on the evolution of trilobite cephalic regionalization[20] is worthy of consideration and, despite their morphological diversity,[21] Cambrian arthropods in toto can be viewed as less strongly modularized than their recent counterparts.[13,14,19]

The evolution of trilobite trunk tagmosis

As both the number and form of exoskeletal segments varied within the trilobite trunk but all were specified sequentially in a common developmental mode, this region offers the opportunity to explore further the relationships between different aspects of segment specification. Such a line of research is likely to be profitable because long-term evolutionary trends in both the number and form of trunk segments are well known in the Trilobita. In addition, there is an adaptive context in which to view the evolution of the form of the dorsal exoskeleton, i.e. the increased premium upon encapsulated enrolment as a response to the evolution of more efficient predators. Different aspects of trunk evolution are considered below, but at present assessment of the underlying controls behind these trends is hindered both by incomplete compilation of data for each category, and by the need for better resolved phylogenies. The latter difficulty limits our ability to assess the extent to which trends were convergent among multiple clades. Accordingly, what follows is intended in the spirit of a research prospectus.

Overall trends seen in trilobite trunk evolution include: (1) a decrease in the range in number of trunk segments despite increased taxic diversity (compare Fig. 1A,B with Fig. 1C,F), (2) an increase in the proportion of trunk segments allocated to the pygidium, known as "caudalization",[23] (3) increased prominence of body shapes that permitted encapsulated enrolment (Fig. 1E), accompanied by the evolution of locking structures that secured individuals when in an enrolled state[24] and (4) increased regionalization of the trunk into batches of similar segments.

The first three trends describe major aspects of trilobite trunk evolution and were all directly related to development because the trunk was constructed from segments that appeared sequentially at a common point of origin. For example, the reduction in overall range and increased stability of trunk segment numbers among derived clades [23,25,27] suggests an increased premium upon precise control of trunk segment expression, and this has been dubbed the "paradigmatic" example of developmental entrenchment associated with the Metazoan radiation.[28] In this case recent work suggests that any such canalisation was powered by external selection on life habits, rather than by internal developmental entrenchment.[26] Nevertheless, important as these trends are, their relation the genetic basis of arthropod body patterning is presently unclear. This is because our ability to infer links between body form and developmental controls relies largely on distinctions in segment morphology rather than segment number or articulation state *per se*. Accordingly, although caudalization perhaps provided increased mechanical strength within the trunk region in the face of more efficient predators, there is no clear basis on which to interpret this trend in terms of shifting expression domains of body patterning genes. On the other hand if the adult thoracic-pygidial boundary coincided with a marked distinction between "batches" of morphologically distinct thoracic and pygidial segments, such an inference would be more reasonable because batches of distinct trunk segments commonly relate to zones of Hox gene expression among modern arthropods.[29] Hence this review will focus primarily on the fourth trend, morphological distinctions among segments within the trilobite trunk region, and consider aspects of the first three trends in that context.

The trilobite trunk region showed marked variation in segment sizes and shapes (Figs. 1, 5). Myriad local variations in features such as surface ornament, and the presence and position of the macropleural segment and axial spines occurred within the trunk region (Figs. 1A,E, 3). In some cases such features were ontogenetically dynamic with those prominent in one portion of ontogeny becoming obscure at others, and this variability hinders interpretation of these features in terms of developmental processes. A more tractable issue, for reasons given above, is the repeated trend toward division of trunk segments into discrete sets or "batches" of segments based on differences in segment size and/or shape and, in some cases, also in articulation state (Figs. 1A,B,D, 2B).

In most trilobites, the transition between the adult thorax and pygidium marked the boundary between freely articulating and fused segments that were homonomous in size and shape (Figs. 1C, 2A, 5). This appears to have been the basal condition of the clade, and species that varied in the numbers of adult thoracic segments[16,24] were of this kind. In such cases, allocation of segments to the thorax or pygidium may have had limited functional significance. Indeed, it is presently unclear whether the termination of thoracic construction in these species was directly related to numbers of segments *per se*, or to other factors such as the overall size and shape of the animal. In situations such as this, in which the division of the trunk into thorax and pygidium appears to be of minor morphological significance, there is no clear basis for recognising the two regions as functionally distinct tagmata.[6]

In trilobites with two batches of trunk segments such as the scutelluids (Fig. 2B), the adult thoracic–pygidial boundary coincided with a change in segment articulation, size, shape and ontogenetic trajectory. The segments that ultimately constituted the adult scutelluid pygidium differed in early ontogeny from those that ultimately formed the adult thorax.[30] This implies that the adult articulation state and segment morphology were intimately linked early in development, long prior to the onset of epimorphosis. Since the axial region of the scutelluid pygidium, to which appendages were attached, was small despite containing numerous segments, it is probable that pygidial appendages were similarly reduced in this taxon, perhaps associated with a novel functional use of the pygidium in propulsion.[22,30] Hence, in this clade there were four structural and developmental differences between segments of the adult thorax and pygidium. Furthermore, scutelluids consistently showed 10 thoracic segments in adulthood. All these attributes support interpretation of the mature thorax and pygidium as functionally differentiated tagmata, with the coevolution of the different aspects of segmental variation contributing to the stability of thoracic segment numbers within this clade. There appears to have been an evolutionary trade-off between flexibility in the number of adult thoracic segments and increased morphological differentiation among batches of trunk segments. Advantages of phylogenetically basal flexibility in numbers of thoracic segments could include a more malleable transition point into epimorphosis, perhaps suggestive of an adaptively flexible life history strategy. It appears that this flexibility was surrendered for the advantages of greater regional autonomy within the trunk in "two-batch" forms.

The contrast of the "two-batch" condition with the simple articulation boundary seen in other trilobites suggests that the trilobite fossil record chronicles the evolution of a distinct posterior arthropod tagma. While patterns of trunk tagmosis clearly differ among modern arthropods, for example within Crustacea, segments are not exchanged between tagma during ontogeny. Thus, a key aspect of trilobite evolution, inaccessible in extant arthropods, is the opportunity to explore the ontogenetic and evolutionary sequences of changes in different aspects of segmentation that together contributed towards formation of a functionally distinct pygidium. In this regard, trilobites may serve as a useful proxy for understanding the evolution of arthropod trunk tagmosis in the aftermath of the Cambrian radiation. While the major arthropod clades and body plan divisions, and their underlying developmental basis, diverged prior to the appearance of trilobites in the rock record, the Palaeozoic history of trilobites may provide some insight into how the increased level of tagmosis seen in modern, as opposed to "archaic",[19] arthropods came about.

A "two-batch" trunk condition was achieved independently in different trilobite clades (Fig. 6). The extent of size difference between the segments of the anterior and posterior trunk batches varied considerably. In some cases, such as in some Cambrian olenelloids, a sharp posterior decrease in segment size formed the most marked variation between batches (Figs. 1A, 6B1). In others, such as in many post-Cambrian scutelluids and lichiids, the principal difference was one of shape (Figs. 1D, 2B, 6B2). Furthermore, the boundary between trunk segment batches did not correlate with the boundary between articulating and fused trunk segments in all mature trilobites. In the "two-batch" olenelloids, the adult pygidium consisted of only a few segments, and the anterior pygidial segment was morphologically similar to those preceding it. The boundary between trunk segment batches occurred anterior of this, between the large segments of the prothorax, and the small segments of the opisthothorax (Figs. 1A, 3). These differences emphasize that transitions in segment morphology, size, and articulation pattern occurred repeatedly and in different manners amongst trilobite clades. The premium upon enrolment may have constrained the overall shape of the pygidium in derived clades, but there is no obvious connection between enrolment and the two-batch condition. What is striking among derived trilobites is the extent to which the tight and functionally required correlation between the proportions of the pleural and axial regions of each thoracic segment relaxed within the pygidium, a region in which pleural
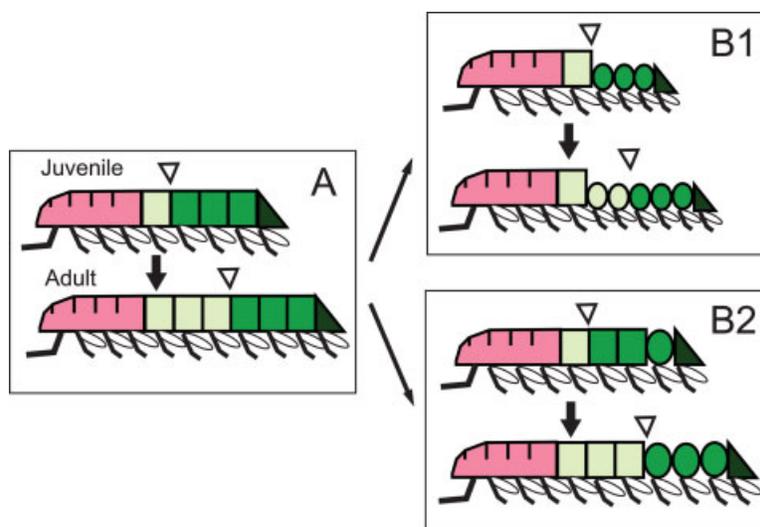
Figure 6. Contrasting modes of "two-batch" trunk regionalization in trilobites. Open arrows and the change from light to dark green represents the boundary between fused and articulating segments. Each box shows a juvenile as the upper figure, with four trunk segments plus the terminal piece (triangular). The lower figure represents an adult with three thoracic segments and three pygidial segments plus the terminal piece. **A:** In the most common and apparently basal condition of the clade (Fig. 2A), all trunk segments share a similar morphology, represented by rectangular cells. **B1,B2:** Two trunk segment morphotypes are expressed during ontogeny, the second of which represented by oval cells. B1: The position of the transition between morphotypes did not coincide with the boundary between freely articulating and fused trunk segments in adulthood, but marks a transition in both segment size and shape (e.g. Fig. 3). This condition characterized some trilobites known from Lower Cambrian rocks. B2: The transition between segment morphotypes was coincident with the boundary between freely articulating and fused trunk segments in adulthood (Figs. 1B,D, 2B). This condition characterized certain derived trilobite clades. The numbers of trunk segments in the adults of these stylized examples have been reduced for clarity.

and axial portions commonly expressed markedly different number of segments (Fig. 1F).

Can we interpret the two-batch condition in regulatory terms? Given that batches of trunk segments tend to correlate with Hox gene expression patterns among modern arthropods it seems likely that this was also the case in trilobites. Inference about the specific manner of Hox gene deployment within the trilobite trunk is difficult because trunk Hox gene expression zones commonly overlap within living arthropods[1] and expression boundaries do not always result in different segment morphologies. Nevertheless, the Hox gene most likely involved in the specification of a distinct caudal region in trilobites was *Abdominal-B*. Since the common ancestor of trilobites and other euarthropods shared *Abd-B* (and other arthropod trunk Hox genes) it is difficult to determine whether the two-batch condition represents the evolution of a regionalized zone of trunk Hox gene expression, or simply the evolution of downstream morphological consequences of a posterior trunk expression domain that was present throughout Trilobita. Several kinds of analyses will provide further insight into the evolution of the different aspects of the trunk region. Analyses of teratological segments,[31] ontogenetic studies of the rate and manner of segment growth,[32] and metrics that examine the complexity of shape and size

variation among segments[33] will be combined to illuminate aspects of the developmental specification and trajectories of individual segments, and the development of differences among segments.

Concluding remarks

This study illustrates how data on gene expression in contemporary organisms can be used to formulate hypotheses about developmental evolution in extinct relatives. Such an approach also helps highlight unique attributes of fossil organisms critical for a more complete understanding of evolutionary history. Recent experimental work suggests mechanisms by which arthropod body patterning might have evolved[34] and there is now a vital need to evaluate such mechanisms in the light of the actual record of evolution.[35] By exploring the manner in which the "two-batch" condition evolved in different trilobite clades using developmental information from fossils, we will be able to compare the progression of changes that lead to the increased tagmosis of the posterior trunk region. In doing so we will illuminate how different aspects of segment specification coevolved to produce a functionally distinct posterior tagma in the aftermath of the Cambrian radiation, and evaluate the evolutionary trade-offs that this transition entailed. This opportunity is exciting

because modern arthropods are famously stable in their patterns of tagmosis. Cases such as trilobite trunk evolution and the fin-to-limb transition in earliest tetrapods, in which fossils provide the empirical record of the development of a major evolutionary innovation, demonstrate palaeontology's unique and integral role within evolutionary developmental biology.

Acknowledgments

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