Exploring Developmental Modes in a Fossil Arthropod: Growth and Trunk Segmentation of the Trilobite *Aulacopleura konincki*

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ABSTRACT: Trilobites offer the opportunity to explore postembryonic development within the fossil record of arthropod evolution. In contrast to most trilobites, the Silurian proetid *Aulacopleura konincki* from the Czech Republic exhibits marked variation in the mature number of thoracic segments, with five morphs with 18–22 thoracic segments. The combination of abundant articulated specimens available from a narrow stratigraphic interval and segmental intraspecific variation makes this trilobite singularly useful for studying postembryonic growth and segmentation. Trunk segmentation followed a hemianamorphic pattern, as seen in other arthropods and as characteristic of the Trilobita; during a first anamorphic phase, segments were accreted, while in the subsequent epimorphic phase, segmentation did not proceed further despite continued growth. Size increment during the anamorphic phase was targeted and followed Dyar's rule, a geometric progression typical of many arthropods. We consider alternative hypotheses for the control of the switch from anamorphic to epimorphic phases of development. Our analysis favors a scenario in which the mature number of thoracic segments was determined quite early in development rather than at a late stage in association with a critical size threshold. This study demonstrates that hypotheses concerning developmental pattern and control can be tested in organisms belonging to an extinct clade.

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> Given the considerable range in segment numbers observed in fossil and extant arthropods, the existence of mechanisms underlying variation in such a central feature of the arthropod body plan is of immense interest. (Carroll et al. 2001, p. 192)

This article is a detailed exploration of growth and segmentation in a Silurian trilobite species, *Aulacopleura konincki* (Barrande, 1846), which showed a homonomous segmented trunk with marked variation in the number of segments allocated to the mature thorax. It provides insight into how a trilobite species controlled the form of the trunk region during development. Dissection of the developmental controls of trunk segmentation will bring us closer to an understanding of the macroevolutionary history of the Trilobita by linking variation in developmental processes to the evolution of major aspects of body patterning. This is important because trilobites offer an opportunity for investigating developmental evolution among early arthropods and because the variable nature of tagmosis within the trilobite trunk region offers a unique window into the evolution of postcephalic body patterning within a major arthropod clade (Hughes 2003*a*, 2003*b*; Minelli et al. 2003).

Trilobites produced biomineralized exoskeletons early in their ontogenies, presumably shortly after hatching. During the earliest well-represented phase of trilobite ontogeny, called the protaspid period, all body segments formed a fused shield (see Chatterton and Speyer 1997). This period typically embraced a small number of instars (= developmental stages = molt cycles). In later instars,

this was succeeded by the appearance of a series of articulations, the first at the cephalic-trunk boundary (fig. 1). The appearance of this articulation marked entry into the meraspid period. During the protaspid and meraspid periods, new trunk segments were generated at a subterminal growth zone. During the meraspid period, trunk segments at the anterior margin of the transitory pygidium were sequentially released into the thorax through development of an articulation along the posterior margin of their tergites (fig. 1). The meraspid period is therefore divided into a series of degrees, defined by the number of freely articulating segments within the thoracic region. Meraspid degrees did not necessarily correspond to meraspid instars because cases of release of more than one segment per molt and cases of intercalation of molts without segment release are known (see Chatterton and Speyer 1997). The rate at which segments were released into the thorax relative to the rate at which segments were expressed in the subterminal growth zone determined the number of segments allocated to the transitory pygidium and varied among species. Progressive release of trunk segments into the thorax continued until the individual entered the final holaspid period of development, characterized by a stable number of thoracic segments.

In most trilobite species, the transition from the meraspid to the holaspid period was achieved at a similar size and a constant number of thoracic segments in all individuals. This pattern suggests that growth and segment release into the thorax were tightly coordinated but provides little insight into the developmental controls involved in this transition. Investigation of the control mechanisms of this transition requires a system in which there was variation in either the size or the number of segments at transition such that alternative models can be formulated and tested. Examples of variation of this kind are rare (Hughes et al. 1999). The Silurian proetid trilobite *A. koninckii* provides the best-documented case of such variation (see fig. C1 in the online edition of the *American Naturalist*). A series of recent studies have considered meraspid and holaspid growth in *A. koninckii* and have documented intraspecific variation in the number of thoracic segments among holaspids that ranges from 18 to 22 (Hughes and Chapman 1995, 2001; Hughes et al. 1999).

In this article, we exploit an expanded data set and utilize additional analytical approaches to explore in detail growth and segmentation in *A. koninckii*. We establish that size increase in *A. koninckii* followed Dyar's rule, the proposition that the growth ratio between molts remained constant during the meraspid period, and that during the same period, there was also a high degree of size control. We then address the nature of meraspid to holaspid transition and demonstrate that despite variation in segment number in the mature thorax, the holaspid period was characterized by a stable number of thoracic segments (contra Hughes and Chapman 1995). Finally, we formulate and test hypotheses concerning determination and control of this transition by using dynamic modeling of life-history descriptors constrained by the results of the former analyses.

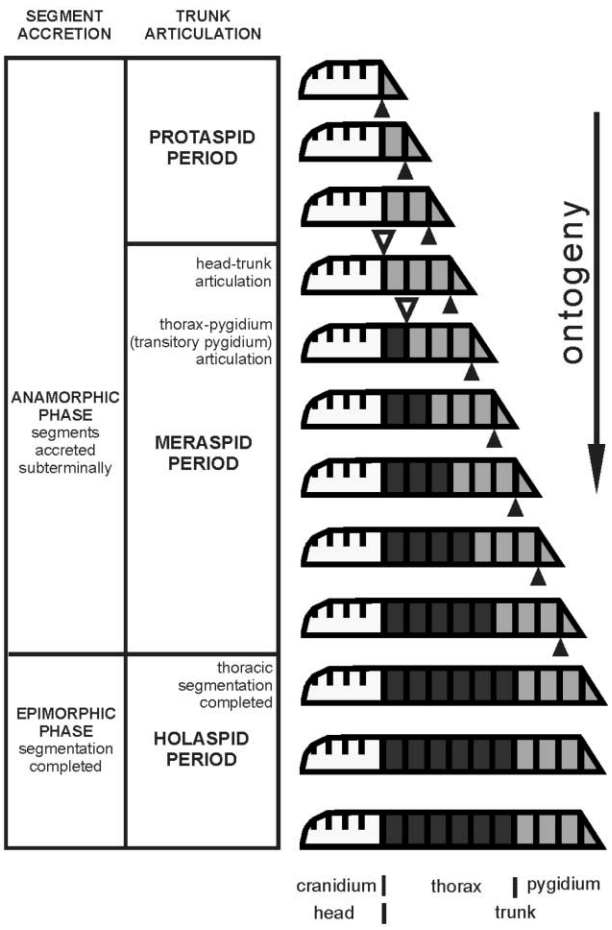

Figure 1: Schematic representation of the ontogeny of a trilobite dorsal exoskeleton. A small solid triangle marks the place where additional segments were first expressed, and a larger open triangle marks points where articulations first appeared. Major developmental events and stages are shown to the left. Depending on the species, the meraspid-holaspid transition could precede, coincide with, or follow the anamorphosis-epimorphosis transition. In this figure, these two transitions are shown to coincide; this is the condition we have determined in *Aulacopleura koninckii*. Fused trunk segments are shown in lighter gray, and freely articulating trunk segments are shown in darker gray. The increase in absolute size of individual segments between molts is not represented.

Material and Measurements

Sample

Measurements were made on 391 complete, articulated meraspid and holaspid dorsal exoskeletons of *Aulacopleura*

koninckí, alive approximately 425 million years ago (fig. 2). All specimens were originally collected in the nineteenth century from within a 1.4-m stratigraphic interval on Na Cernidlech Hill near Loděnice in the Czech Republic (see Hughes and Chapman 1995; Hughes et al. 1999) and are presently housed in museum collections (principally the Museum of Comparative Zoology, Harvard University). The siltstones of the 1.4-m interval are estimated to have accumulated over an interval of a few thousand years (Hughes et al. 1999), but specimens cooccurring on single surfaces confirm that the full range of variation in holaspid thoracic segment number existed at the resolution of individual bedding planes. Taphonomic evidence suggests that such individuals were contemporaries, almost certainly alive during the same season (Hughes and Chapman 1995).

All specimens are currently assigned to a single morphospecies, *A. koninckí*, for the following reasons: the full range of variation in holaspid thoracic segment number within *A. koninckí* is apparently present throughout the collection interval; no meristic characters other than thoracic segment number suggest more than a single *A. koninckí* morphotype; and the total variance in exoskeletal shape exhibited by *A. koninckí* specimens is comparable to that of five other common trilobite species recovered from the same site, which experienced the same broad environmental and preservational history (Hughes et al. 1999, their fig. 3). These other species were all stable in holaspid segment numbers, and each is generally accepted to represent a single morphospecies.

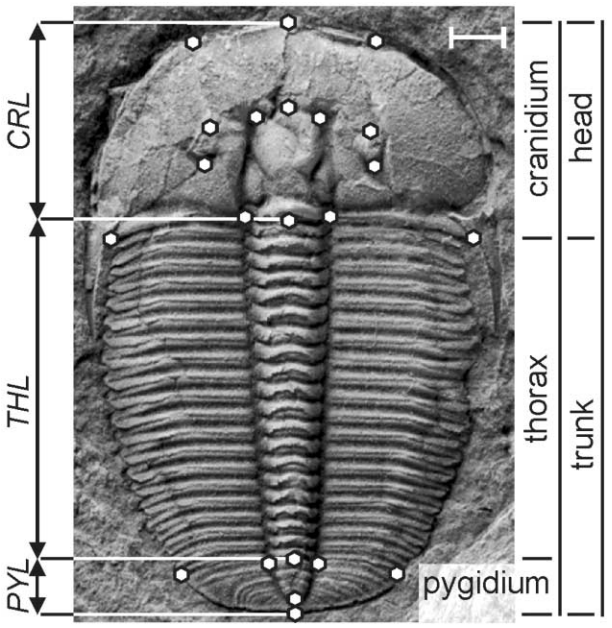

Figure 2: Morphological landmarks, metric dimensions, and major anterior-posterior body divisions measured on *Aulacopleura koninckí*. Scale bar is 2 mm long. The figured specimen is a large holaspid with 20 thoracic segments, BMNH42367.4. *CRL* = cranidium length; *THL* = thorax length; *PYL* = pygidium length.

Measurements

Digital images of specimens were obtained using a video image capture system, and morphometric data were collected using Optimas and National Institutes of Health Image software (see Hughes and Chapman 1995). The Cartesian coordinates of a total of 22 landmarks were digitally recorded on each of 391 specimens. These landmarks (fig. 2) were selected to give a comprehensive representation of the dorsal exoskeleton of the species, optimized by the need for both landmark homology among specimens and replicability in digitizing. Repeated recalibration and redigitization of the same specimen gave a mean value for the coefficient of variation of linear measures of 0.8%. This result suggests that the contribution of measurement error to the size variance recorded in this analysis was quite small.

The landmark configurations provided estimates of body region size based on multiple homologous points rather than linear distances between pairs of landmarks. Analyses were conducted on the landmark configurations covering the dorsal exoskeleton (22 landmarks) and three subsets thereof and summarized the size of the cranidium (anteriormost 15 landmarks), the pygidium (posteriormost seven landmarks), and the thorax (10 landmarks, five shared with the posterior of the cranidium and five shared with the anterior of the pygidium). Centroid size is the square root of the sum of squared distances between each landmark in a configuration and the centroid of the configuration (Bookstein 1991), and it thus summarizes overall size. For the calculations of centroid size, paired homologous landmarks were reflected across an axial midline separately defined for each body region. After reflection, an average position was calculated for each homologous pair of landmarks. This procedure makes the influence of each bilaterally symmetrical character on centroid size determination more comparable to that of individual unpaired landmarks situated along the midline. Following reflection, the dorsal exoskeleton was represented by 14 landmarks and the cranidium by nine landmarks.

For the analysis of absolute growth, five metric variables were extracted from the landmark data. These include two centroid size measures and three traditional (length-based) morphometric distances. The two centroid size measures that capture overall size from different perspective are dorsal centroid size (DCS) and cranidial centroid size (CCS).

The three distance measures were used to illustrate the absolute growth of the three main body regions: cranidium length (CRL), thorax length (THL), and pygidium length (PYL; fig. 2). Raw measures were transformed to their natural logarithms before statistical treatment; they therefore qualify as log-size variables sensu Mosimann (1970).

Two meristic variables, the number of thoracic segments (NTH; fig. 3) and the number of pygidial segments (NPY), were also recorded for each specimen. The NTH provided information pertaining to the developmental stage of each individual during the meraspid period.

Morphometric Analysis

This study is based on cross-sectional and mixed cross-sectional data (Cock 1966). Cross-sectional data consist of a series of static morphometric data, each relating to a different developmental stage and a different set of specimens where the grouping of specimens per stage is done on the basis of a criterion independent of size and shape. When a size-independent criterion of stage assignment is not available, data qualify as mixed cross-sectional.

Postprotaspid developmental stages were numbered sequentially from 0 (corresponding to meraspid degree 0) onward. The constancy of estimated incremental growth rate through the meraspid period suggests that each meraspid degree corresponded to a single instar (i.e., one segment was released into the thorax per molt). For specimens bearing 17 or fewer thoracic segments, the number of thoracic segments therefore provides a direct measure of the number of postprotaspid molt cycles through which an individual had passed. A total of 133 specimens bearing from five to 17 thoracic segments were assigned to corresponding developmental stages in this way (cross-sectional data). These data yield information on the average growth progression of the entire population but do not show the growth progression of individual specimens (longitudinal data). This is a "physiological" limitation in the study of fossil animals lacking persistent structures with accretive growth such as mollusk shells or fish otoliths and scales. Arthropods periodically renew their exoskeleton by molting. Longitudinal data for fossil arthropods can therefore be obtained only when a specimen and its putative exuvia are found together, and, even then, these extraordinarily rare occurrences provide very limited ontogenetic coverage. For the remaining 258 specimens bearing 18–22 thoracic segments, no size-independent criterion for assignment to a particular developmental stage is available (mixed cross-sectional data). These specimens comprise a mix of late meraspids and holaspids at various developmental stages.

Cross-sectional and mixed cross-sectional data require different procedures of statistical analysis. All the procedures adopted here utilize standard statistics (cf. Sokal and Rohlf 1981).

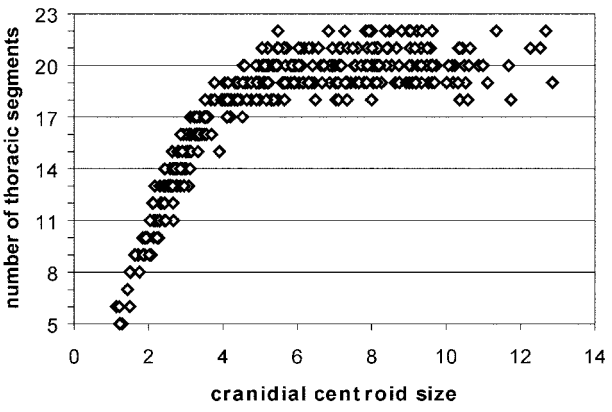

Figure 3: Relationship between thoracic segmentation and cranidial metric growth in *Aulacopleura konincki* ($n = 391$).

Growth Progression and Dyar's Rule

Exoskeletal growth in arthropods occurs in a stepwise manner, postembryonic development being paced by the molt cycle. Of the several rules formulated for describing discrete size increment, Dyar's rule (Dyar 1890) is considered a null model for arthropod growth (Klingenberg and Zimmermann 1992). Dyar's rule assumes a constancy of the postmolt/premolt size ratio between molts (for a linear size variable, this is Dyar's coefficient), while other rules (e.g., Przibram's rule; Przibram and Megušar 1912) predict specific values for Dyar's coefficient. A growth progression conforming to Dyar's rule is a geometric progression that can be expressed by the finite difference equation

$$Y_{i+1} = rY_i,$$

where Y_i is the value of a linear size variable at the ith stage of growth, Y_{i+1} is the value of the same variable at the following stage, and r is the postmolt/premolt ratio (Dyar's coefficient). Log transformation of the former equation gives a linear progression (i.e., a more statistically tractable version of Dyar's rule)

$$X_{i+1} = X_i + \rho,$$

where X is a log-size variable ($\log(Y)$) and ρ is the logarithm of Dyar's coefficient ($\log(r)$).

Allometric growth, caused by differential growth rates among body parts, produces ontogenetic changes in body proportions. Therefore, five metric variables were inde-

Table 1: Dyar's coefficients for five morphometric variables in *Aulacopleura konincki*

Morphometric variable	Label	Dyar's coefficient (95% CI)	r^2 ($n = 13$)
Dorsal centroid size	DCS	1.122 (1.114–1.129)	.992
Cranidial centroid size	CCS	1.096 (1.089–1.102)	.990
Cranidium length	CRL	1.096 (1.088–1.103)	.988
Thorax length	THL	1.174 (1.146–1.191)	.982
Pygidium length	PYL	1.009 (.998–1.020)	.241

Note: CI = confidence interval; r^2 = fraction of the total variance of the variable explained by growth at a constant rate.

pendently tested for their fit to Dyar's rule. Size distributions of all morphometric variables considered herein show marked overlap across different developmental stages in *A. konincki*, so reliable grouping of specimens by developmental stage was possible only for meraspids until degree 17. Metric growth analysis was therefore conducted on these cross-sectional meraspid data (133 specimens, from degree 5 to 17).

In comparative studies (e.g., Cole 1980), Dyar's coefficient is generally calculated as the average of the series of observed growth ratios during ontogeny (provided that these are not sufficiently different as to suggest that growth does not follow Dyar's rule). Because growth ratios of individuals are not available in cross-sectional data, mean growth ratios are indirectly calculated from the mean sizes of two successive instars, and each estimate is thus affected by the combination of two sampling errors. For *A. konincki*, we preferred to estimate Dyar's coefficient as the antilogarithm of the regression coefficient between the mean logarithm of the morphometric variable at a certain stage and the stage number in all stages of the meraspid period. Model I linear regression (least squares) was adopted, and regression residuals were inspected for nonrandom deviation from the expected growth progression.

Results

Except for the morphometric variable relating to the pygidium (PYL), the high values of the coefficients of determination of the regressions (r^2; i.e., the high percentage of variance explained by the regression lines) show that growth in *A. konincki* conformed to a large extent to Dyar's rule (table 1; fig. 4). The sequences of residuals of the five regressions do not reveal any regularity in the pattern of deviation, and residuals are almost exclusively within the range of the 95% confidence intervals of the degree mean. When not within this interval, the residuals are nevertheless very close to its limits (examples in fig. 4; tables B1, B2 in the online edition of the *American Naturalist*). Because PYL does not increase significantly from degree 5 to 17 (two-tailed *t*-test, $P > .088$, $n = 13$; table 1), the coefficient of determination is not a significant index of conformity to Dyar's rule in this case. Despite the dubious meaning of testing the conformity of a zero growth progression to any growth rule, the confidence intervals of the logarithmic PYL growth ratios overlap conspicuously around their mean (0.008).

The estimated Dyar's coefficients vary from 1.01 for PYL (not significantly different from 1, that is, zero growth) to 1.17 for THL, certainly affected by thorax segmental accretion. Overall (DCS) growth, strongly correlated to THL, shows a rate of about 1.12 per stage, significantly higher than cranidial (CCS) growth rate, which is about 1.10.

Growth Regulation

The manner in which growth is regulated during ontogeny can be estimated by quantifying the variance of a morphological trait at successive developmental stages. Assuming a stepwise geometric growth progression (Dyar's rule) for a population, the within-stage variances of a log-size variable in two successive stages are bound by the rela-

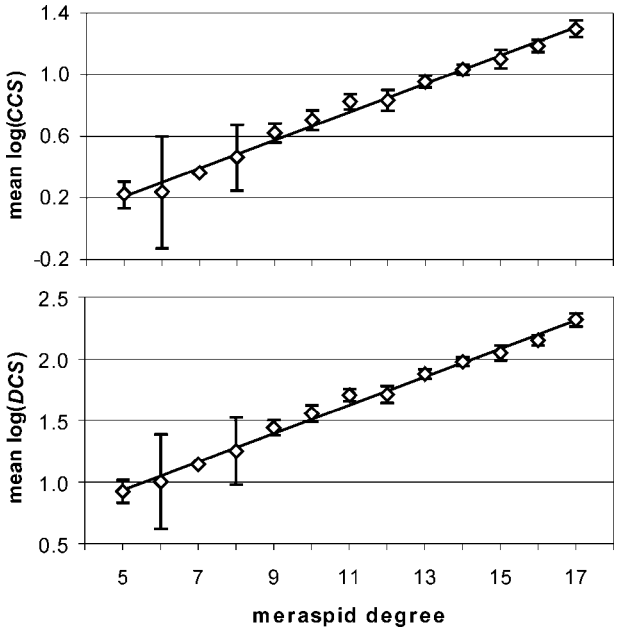

Figure 4: Growth progression during the meraspid period of *Aulacopleura konincki* for two morphometric variables (cranidial centroid size [*CCS*] and dorsal centroid size [*DCS*]). Diamonds are the mean logarithm of the variable for each of the different degrees. Regression lines are shown. Bars are average 95% confidence intervals (not calculated for degree 7, with only one specimen available).

tionship

$$\mathrm{Var}\,(X_{i+1}) = \mathrm{Var}\,(X_i) + \mathrm{Var}\,(\rho) + 2\,\mathrm{Cov}\,(X_i, \rho),$$

where X is a log-size variable and ρ is the logarithm of Dyar's coefficient. Although not directly measurable in a cross-sectional database, the within-stage (between-specimens) Var (ρ) is expected to be larger than 0 for ecological and physiological reasons (see "Discussion"). Assuming a nonzero Var (ρ), if Var (X_i) tends to be constant or to decrease in the observed growth progression, then Cov (X_i, ρ) must be negative. A negative covariance between premolt size and growth ratio indicates that at a given stage, the larger specimens tend to grow less than the smaller ones and vice versa. This phenomenon of regulative growth (negative feedback) has been variously named compensatory, targeted, or convergent growth (Klingenberg 1996).

Within-stage variance was calculated for size variables at each meraspid degree 9–17 (123 of the 133 meraspid specimens). Within-stage variance estimation is not reliable for degrees 5, 6, and 8 (three specimens each) and is impossible for degree 7 (one specimen). For each of the five size variables, the significance of the regression coefficient of the within-stage variance of the logarithm of the variable on the meraspid degree (Model I linear regression) was determined to test whether variance increased or decreased through meraspid ontogeny.

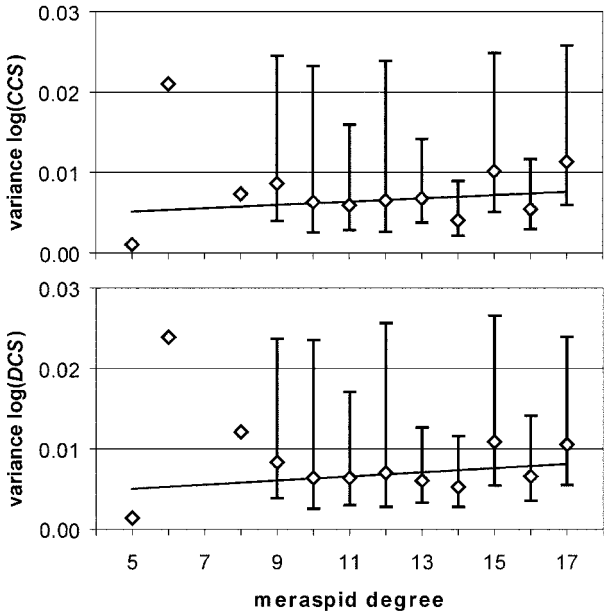

Figure 5: Ontogenetic progression of the variance of two morphometric variables (cranidial centroid size [*CCS*] and dorsal centroid size [*DCS*]) in the different meraspid degrees of *Aulacopleura konincki* (variance not calculated for degree 7, with only one specimen available). Regression lines are shown. Bars are variance 95% confidence intervals (not shown for degrees 5, 6, and 8, excluded from regression analysis).

Results

For each of the five morphometric variables, the regression coefficient of the within-degree variance on meraspid degree is not significantly different from 0 (two-tailed t-test, $n = 9$, DCS: $P > .317$; CCS: $P > .471$; CRL: $P > .969$; THL: $P > .461$; PYL: $P > .076$). Notably, t sample values are well outside standard significance levels at $\alpha = 0.05$, except in the case of PYL. For the other four variables, this behavior shows that there was no systematic increase of their variance with growth (examples in fig. 5), an unequivocal sign of targeted growth.

Dynamics of Thoracic Segment Accretion

The thorax of most (possibly all) trilobites developed in two phases. The early molts were accompanied by addition of new segments released from the transitory pygidium (accretive phase of thoracic development, meraspid period), and then the animal continued to molt and grow without further addition of segments to the thorax (segment-invariant phase of thoracic development, holaspid period; fig. 1). *Aulacopleura konincki* shows marked variation in segment number among large individuals, with 18–22 thoracic segments found among large specimens (fig. 3). The intraspecific polymorphism for the number of thoracic segments in maturity could be interpreted as the result of two alternative dynamics of thoracic segment accretion (fig. 6).

Dynamic 1. In dynamic 1, the development of the thorax is in two phases, as generally in trilobites, but is coupled with intraspecific variability for the stage (and therefore number of thoracic segments) at which the animal turned from the accretive to the segment-invariant phase of thorax development.

Dynamic 2. In dynamic 2, the accretive phase of thorax development is protracted throughout the whole life of the trilobite, with an ontogenetic threshold at which the rate of thoracic segment addition dropped significantly (but with individual variation) regardless of the number of segments the animal possessed when reaching that threshold. Hughes and Chapman (1995) formulated this hypothesis and suggested that the threshold could correspond to a given body size.

Specimens with up to 17 thoracic segments are unequivocally meraspids, whereas those with segment numbers between 18 and 21 are a mixture of meraspids and holaspids. Groups of specimens with the same number of thoracic segments are here referred to as morphs. The

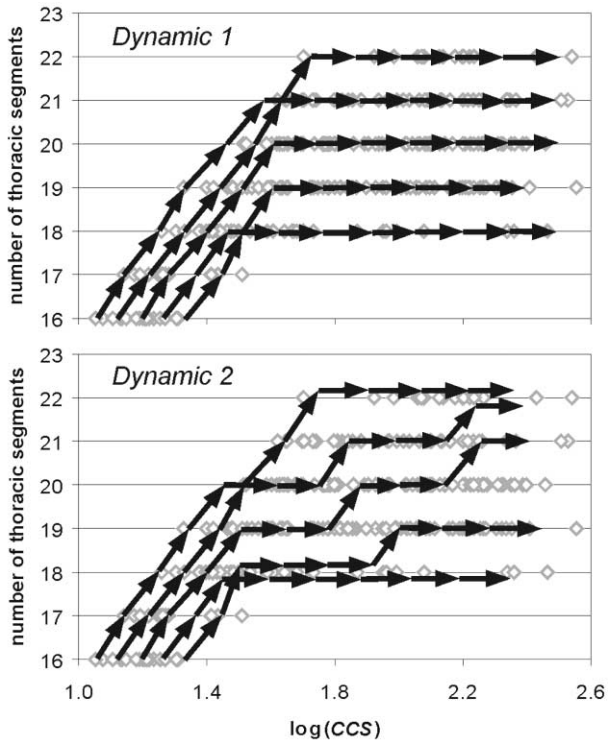

Figure 6: Two models of thoracic segment accretion in *Aulacopleura konincki* ontogeny. Background diamonds correspond to those in the top of figure 3 (but log transformed). *Top*, Some possible growth trajectories for a classical two-phase development coupled with intraspecific variation for the stage of meraspid-holaspid transition (dynamic 1). *Bottom*, Some possible growth trajectories for accretive phase of thoracic development continuing until later stages (dynamic 2).

extreme morph with 22 thoracic segments (morph 22) is by definition composed exclusively of holaspids.

Under dynamic 1, no correlation is expected between the number of thoracic segments and developmental stage, and for all five morphs, 18 and 22 included, constant relative stage frequencies are expected. Under dynamic 2, a small increase in the average number of thoracic segments with age is expected, albeit with individual variation in segmentation schedule. Moreover, although the expected stage distributions of morphs 19, 20, and 21 are difficult to calculate because they depend on additional hypotheses about the individual variation of growth and segmentation trajectories, those of morphs 18 and 22 are easier to anticipate, at least qualitatively. For morph 18, a progressive decrease of relative frequency with stage is expected, while the opposite is expected for morph 22.

We have no criteria for stage assignment independent of size for morphs from 18 to 22. Therefore, we used the size distribution of log (CCS) as a proxy for the stage distribution beyond stage 17. Cranidial centroid size is a suitable proxy for overall size because cephalic segmentation was complete within the earliest preserved ontogenetic stage and the cranidium was an integrated and well-sclerotized tagma, the segmentation of which was apparently specified independently from the segmentation of the trunk region (Hughes 2003*a*, 2003*b*). We preferred CCS to DCS because allometric analysis showed that DCS is influenced by the number of trunk segments (Hughes and Chapman 1995).

We looked for evidence of a continuing accretive phase of thorax development in a subset of specimens that, with a high degree of confidence ($P > .98$, 1 minus the probability for meraspids to have had log (CCS) larger than a certain value on the basis of estimated growth parameters), had entered the mature growth phase (segment-invariant phase under dynamic 1 or slow accretive phase under dynamic 2); these are the 153 specimens with log (CCS) > 1.80. We tested the significance of the regression coefficient of the log (CCS) on the number of thoracic segments (Model I linear regression) and examined the size-frequency distribution of log (CCS) in the two extreme morphs with 18 and 22 thoracic segments (Kolmogorov-Smirnov test).

Results

The regression coefficient of log (CCS) on the number of thoracic segments for all specimens with log (CCS) > 1.80 is not significantly different from 0 (two-tailed *t*-test, $n = 153$, $P > .285$). This shows that there was no relation between developmental stage and number of thoracic segments in this sample of *A. konincki*.

For log (CCS) > 1.80, size distributions of morph 18 ($n = 8$) and morph 22 ($n = 16$) are not significantly different (Kolmogorov-Smirnov test, $P > .43$). Moreover, morph 18 and morph 22 size distributions are not significantly different from a uniform size distribution (Kolmogorov-Smirnov tests, $P > .69$ for morph 18 and $P > .27$ for morph 22). This shows that developmental progression was not accompanied by the gradual "loss" of morph 18 specimens (in favor of morphs 19–22) and "gain" of morph 22 specimens (from morphs 18 to 21).

Therefore, the thorax of *A. konincki* seems to have developed postembryonically following dynamic 1, coupling a two-phased development with intraspecific variability for the stage (and number of thoracic segments) at which thorax development turned from the accretive to the segment-invariant phase. With a high degree of confidence, the 153 specimens with log (CCS) > 1.80 were in the segment-invariant phase of thoracic segmentation and can be considered to be holaspids.

Developmental Determination of the Mature Number of Thoracic Segments

Studies of extant arthropods show that important developmental events can be determined very early in development (e.g., number of trunk segments in geophilomorph centipedes; Lewis 1981); dependent on the actual ontogenetic trajectory of the individual, which evaluates the developmental progression of some trait (e.g., number of larval instars in several insects; Nijhout 1999); or dependent on an external (environmental) signal once the animal has become competent to respond to that signal (e.g., reproductive morphs in the heterogonic life cycle of aphids; Dixon 1973). It is important to note that in very different ways, with all three mechanisms of developmental control, both hereditary and environmental factors can determine the stage at which a certain developmental event takes place and that all three mechanisms can produce intraspecific variation for a given developmental trait.

External morphology and evidence of targeted growth argue against the existence of five distinct meraspid growth trajectories. The origins of the five holaspid morphs must therefore lie in differences in the point of transition from a common meraspid growth trajectory into holaspid growth. The question is, then, What determines the point of this transition?

The sample of *Aulacopleura konincki* offers a unique opportunity to explore the nature of the developmental control behind measurable aspects of phenotypic growth in an ancient, extinct organism. We ventured into this field of inquiry mindful of the limitations in testing or even constraining the various possible mechanisms. Because longitudinal data are not available, our analysis is restricted to patterns of morphological trait covariation. We considered different hypotheses of developmental regulation for the segmentation process that differ in how the transition from the accretive to the segment-invariant phase of thorax development (meraspid-holaspid transition) was determined. The possibility that the switch between the two phases was dependent on an environmental signal is considered in the discussion.

Early Determination Hypothesis (EDH)

The number of stages of the accretive phase, and, therefore, the number of mature thoracic segments, was precociously determined and, therefore, independent from actual meraspid growth. The population was composed of five distinct cohorts, groups of specimens with a specific number of thoracic segments in the segment-invariant phase. Cohort 18 had 18 meraspid stages (degrees 0–17), cohort 19 had 19, and so on until cohort 22, which had 22 meraspid stages. Therefore, morph 18 was a mixture of holaspids of cohort 18 and meraspids of cohorts 19, 20, 21, and 22 (mainly concentrated on the lower [left] extreme of a size distribution); morph 19 was a mixture of holaspids of cohort 19 and meraspids of cohorts 20, 21, and 22, and so on until morph 22, exclusively composed of cohort 22 holaspids. Because cohorts are not morphologically distinguishable in the meraspid growth phase (we cannot identify five clusters of specimens according to size, shape, or any meristic character state in any of the meraspid degrees), we must hypothesize additionally that although the number of mature thoracic segments was precociously determined, specimen membership within a particular cohort was not manifest morphologically (at least in the dorsal exoskeleton) before the holaspid period.

Later Determination Hypothesis (LDH)

The number of adult thoracic segments was determined by the stage at which a certain critical morpho-physiological condition (a critical state of a trait *X*) was reached. The final number of thoracic segments was not prespecified in any individual. In the population, this resulted in five different adult morphs, each with a different number of thoracic segments. The mechanism was the following. Being a quantitative character, we expect trait *X* to have presented within-stage variation with a certain frequency distribution. At stage 18, the trilobites that had crossed the critical state for *X* (those belonging to the right tail of stage 18 distribution of *X*) entered the segment-invariant phase with 18 thoracic segments, a number they retained throughout subsequent growth, while the remaining individuals still in the accretive phase moved on to stage 19 with 19 thoracic segments. This subgroup (now at stage 19) included specimens that had by now crossed the critical state of *X* (and were thus now within the segment-invariant phase, retaining 19 thoracic segments in all subsequent molts), while the others continued the accretive phase. This process, by which the specimens on the right side of a distribution entered the segment-invariant phase while those of the left side continued accreting thorax segments, progressed until stage 22, when all specimens had crossed the critical state for *X*. Trait *X* could be dependent on (or correlated with) morphological traits accessible to study, but it could also be exclusively physiological or solely expressed in internal anatomy, that is, be uncorrelated with external morphology or dependent on (or correlated with) external morphological traits not accessible to study (e.g., ventral traits). The first case is the only one accessible to morphological testing (but see the discussion for considerations relative to the other cases). For LDH, we considered a very general morphological event, the crossing of a certain (critical) size threshold. Although studies of extant arthropods show that the

critical size value can vary within a population being affected by both genetic and environmental factors (Davidowitz et al. 2003), in modeling LDH, we used an invariant critical size. This is because the estimated size variation in the last meraspid stages, with respect to the growth rate (Dyar's coefficient), was already of the magnitude necessary to produce the observed five morphs without allowing for further sources of variation (see app. A in the online edition of the *American Naturalist*).

We used log (CCS) as a size variable for the reasons explained in "Dynamics of Thoracic Segment Accretion." Two stochastic models of size distributions for the five morphs were developed for comparing EDH with LDH (figs. 7, C2). Several parameters of the models have been estimated from morphometric data (see app. A).

Although growth rates during the holaspid period are unknown, for simplicity, we assumed the meraspid growth rate projected into holaspid period, but any combination of growth rate and maximum number of stages that produces the largest specimens we observed (with log (CCS) close to 2.60) achieves the same predictions in terms of the size-frequency distribution. The comparison between the two models is therefore not biased by the assumed holaspid growth rate.

The saw-tooth profiles of the cohort distributions in the LDH, while illustrating the core differences between the construction of the two alternative models, do not represent a significant trait of the model's predictions (see app. A; fig. C2). However, the tests of goodness of fit consider wider size classes where these irregular profiles do not influence the result (see expected size-frequency distributions in fig. 8).

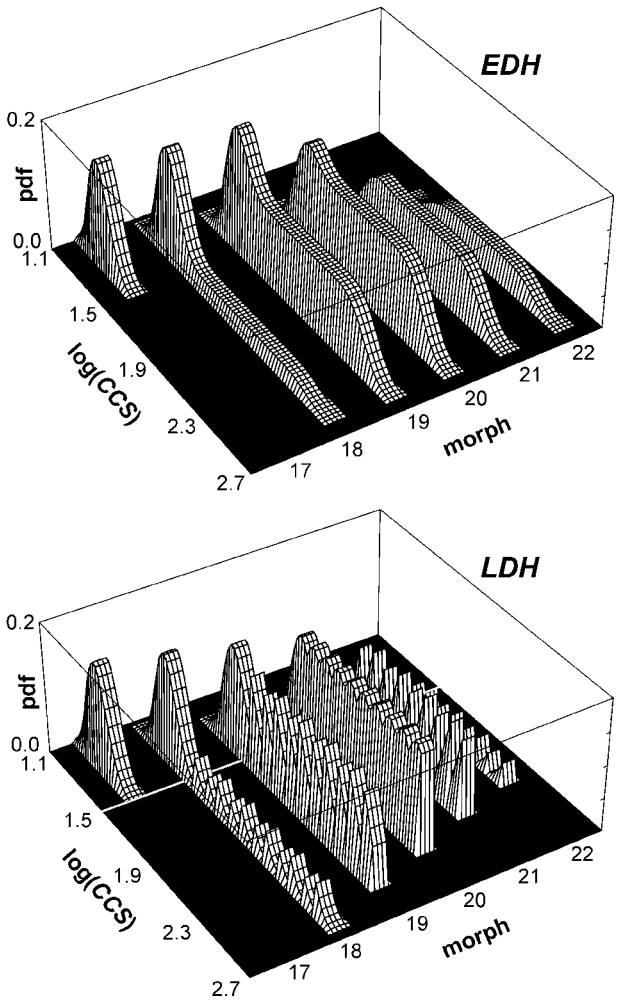

Figure 7: Overall density probability functions of log (CCS) for degree 17 and the five morphs with 18–22 thoracic segments under two different models. *CCS* = cranidial centroid size. *Top*, Early determination hypothesis (*EDH*) of the mature number of thoracic segments. *Bottom*, Later determination hypothesis (*LDH*) of the mature number of thoracic segments, based on the stage of crossing a critical size (*white line*). Details on curves construction are in figure C2 in the online edition of the *American Naturalist.*

Results

EDH. On the whole, size distributions of individual morphs predicted according to EDH fit the observed distributions quite well (fig. 8). The χ^2 tests for the five morphs 18–22 gave the following, in order: morph 18: $P > .428$, df = 3; morph 19: $P > .230$, df = 9; morph 20: $P > .326$, df = 8; morph 21: $P > .233$, df = 6; morph 22: $P > .061$, df = 1. The difference between observed and expected distributions is never significant, and, apart from morph 22 with the smallest sample size, the probabilities are quite large. A χ^2 test of all five morphs together gave a very good fit ($P > .234$, df = 30).

LDH. Overall, size distributions of individual morphs under LDH give a less acceptable fit between observed and expected morph size distributions (fig. 8). The χ^2 tests gave the following for the five morphs 18–22, in order: morph 18: $P > .188$, df = 3; morph 19: $P < .009$, df = 9; morph 20: $P > .330$, df = 8; morph 21: $P > .067$, df = 6; morph 22: $P \ll .001$, df = 1. Calculated χ^2 values are on the whole larger (P smaller) than the respective values calculated on EDH. The fitting of morph 19 and, particularly, morph 22 should be rejected strongly. A χ^2 test of all five morphs together consequently gave a very bad fit ($P \ll .001$, df = 30). A macroscopic trait of LDH predictions (fig. 7) is a progressive decrease in the expected maximum specimen size, from morph 18 to morph 22. This is produced by the progressively reduced number of holaspid stages starting from the same (critical) average size. To assume an increase in the average total number

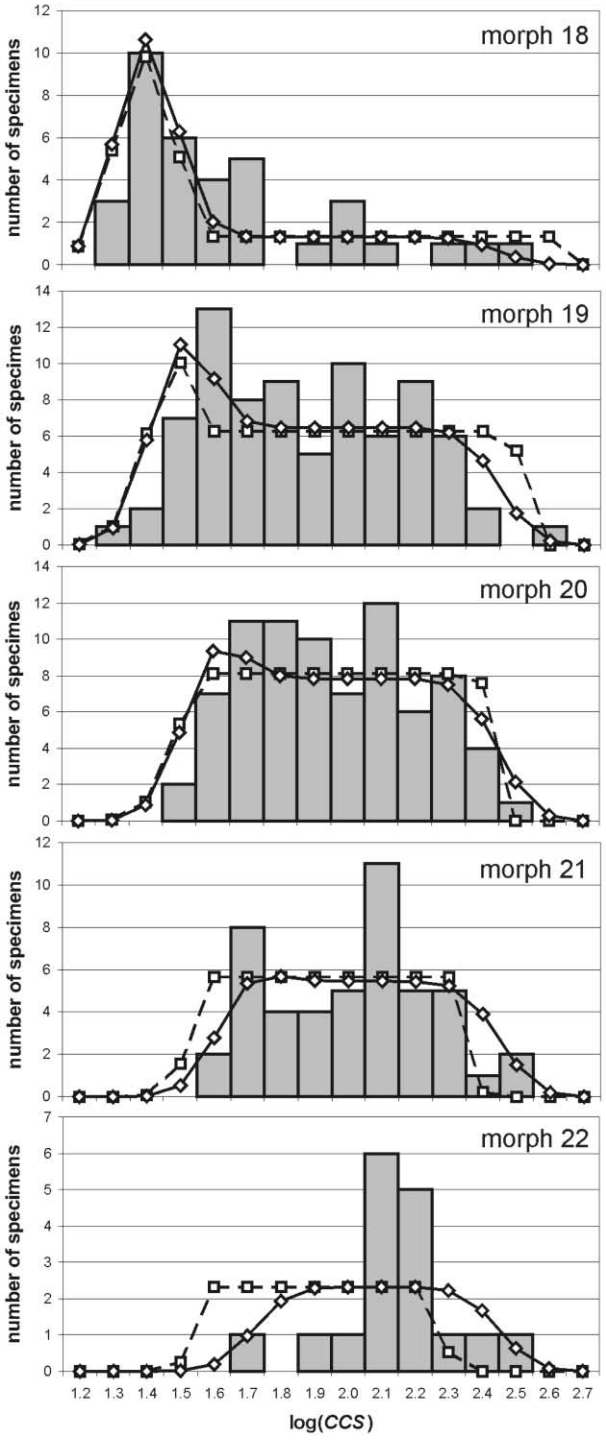

Figure 8: Comparison of observed (*bars*) and expected (*lines*) size-frequency distribution of the five morphs 18–22. *Solid line* = early determination hypothesis (EDH); *dashed line* = later determination hypothesis (LDH). Numbers on the horizontal axis refer to the central value of each size class of width 0.1. Note the better fit of EDH than LDH to the observed data.

of stages from morph 18 to morph 22 (with a one-to-one match between the number of additional segments and the number of additional stages) in order to compensate for this effect seems an ad hoc hypothesis for which there is no compelling justification.

Fit to a theoretical frequency distribution is a severe test for field samples in general and for fossils in particular. Measures of size frequency are quite sensitive to uncontrolled (paleo)environmental and taphonomic factors. Therefore, the goodness of fit to EDH strongly supports this model and suggests that the original distribution has not been obliterated by those external factors. At the same time, the observed distributions are too singular to be explained simply as the result of environmental or taphonomic factors. However, we cannot definitely reject LDH on the basis of this kind of evidence alone. Further evidence that favors EDH over LDH comes from the growth rate of the pygidium.

Pygidial length exhibits an almost zero growth rate up to and including degree 17. Then, quite abruptly, a strong acceleration of growth with respect to other body regions is recorded as a sharp increase in its allometric coefficients with respect to other variables. For example, the allometric coefficient of log (PYL) on log (CCS) turns from 0.58 ($n = 133$) to 1.52 ($n = 258$; Model II, geometric mean regression). If, as seems to have been the case in *A. koninckii*, this change in growth rate was coupled with the meraspid to holaspid transition, EDH, at variance with LDH, predicts a difference in PYL among the five morphs for any given developmental stage (or log (CCS) value) after that transition (fig. 9). This is because while EDH predicts that individuals entered the holaspid period at an average size corresponding to the average size of the stage of the meraspid to holaspid transition (that varies with cohort, smaller for cohort 18 than for cohort 19, etc.), under LDH, all individuals entered the holaspid period at the same (critical) size, and this was independent from the number of meraspid stages that they had experienced. In the five cohorts, regression coefficients of PYL on log (CCS) are not significantly different (GT2 method of comparison, $P > .10$; fig. 10*a*), but there is a notable decreasing trend for the intercept at log (CCS) = 2.20, a convenient value in the middle of the distribution (fig. 10*b*). Not all the pairs are significantly different (GT2 method of comparison, $P < .05$), but the trend is clear, and the extreme morphs are neatly separated, as predicted by EDH.

On more qualitative grounds, LDH predicts a marked decrease in maximum specimen size from morph 18 to morph 22, which is not observed. Moreover, LDH predicts, with a nonnegligible probability (1/360, the residual size frequency of meraspids smaller than the critical size after stage 22), the presence of specimens with more than 22 thoracic segments, which have never been reported. The

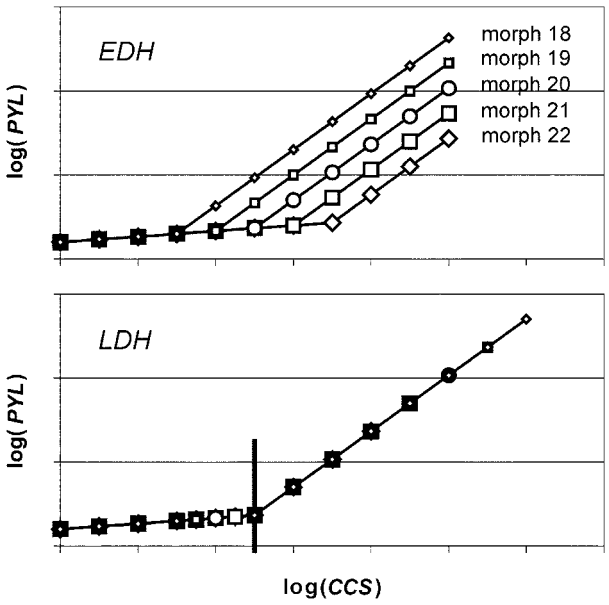

Figure 9: Schematic representation on the ontogenetic allometry between pygidium length (*PYL*) and cranidial centroid size (*CCS*) under early determination hypothesis (*EDH*) and later determination hypothesis (*LDH*). According to EDH (*top*), cohort 18 specimens at any given holaspid size would have longer pygidia than those of the more thoracic segment-rich morphs because a greater proportion of their growth would have been within the holaspid phase. According to LDH (*bottom*), the transition in pygidial growth mode would have occurred at the critical size threshold (*vertical bar*) regardless of the number of thoracic segments generated at that point. Thus, the apparent decreasing growth rate before the critical size is due to the backward shift of the mean of meraspid size distribution caused by the loss of the right tail that entered holaspid period. Furthermore, the different number of holaspid stages produces differential maximum size between morphs, with individuals of morph 18 greater than that of morph 22.

number of holaspid specimens of *A. koninckі* examined from this site since the 1840s numbers in the thousands.

Pygidial Segmentation

In our *Aulacopleura koninckі* sample, the number of pygidial segments (NPY) varies between three and seven. While only five specimens (out of 391) exhibit NPY > 5, variation of NPY between three and five is recorded for almost all stages. For stages 5–17 (cross-sectional data), we studied the progression of within-stage mean and variation of NPY. For the following stages (mixed cross-sectional data), we studied regression of NPY on log (CCS) (Model I linear regression).

Results

During the fraction of the meraspid period documented within the available sample (degrees 5–17), the mean of the number of pygidial segments (NPY) seems to decrease from about 4.5 to about four, but the regression coefficient is not significant (degrees 9–17, two-tailed *t*-test, $P > .264$, $n = 9$). The range of NPY variation is quite stable, matching the observed variation in the following stages (fig. 11). During the holaspid period ($\log(\text{CCS}) > 1.80$), a slight but insignificant increase in NPY is recorded (two-tailed *t*-test, $P > .055$, $n = 153$). Although the regression coefficient is very close to the standard significance region ($\alpha = 0.05$), it can be noted that in the larger specimens, the higher NPY mean is the result of the rising of the lower bound of the range of variation. This pattern is more easily explained by taphonomic considerations rather than by a nonzero probability of pygidial segment addition in the late holaspid period (see "Discussion").

Variation in pygidial segment number was independent of the number of holaspid thoracic segments and therefore not the result of a trade-off of segments between the two regions. This is in accordance with the conclusions of Hughes and Chapman (1995). Individual regression lines

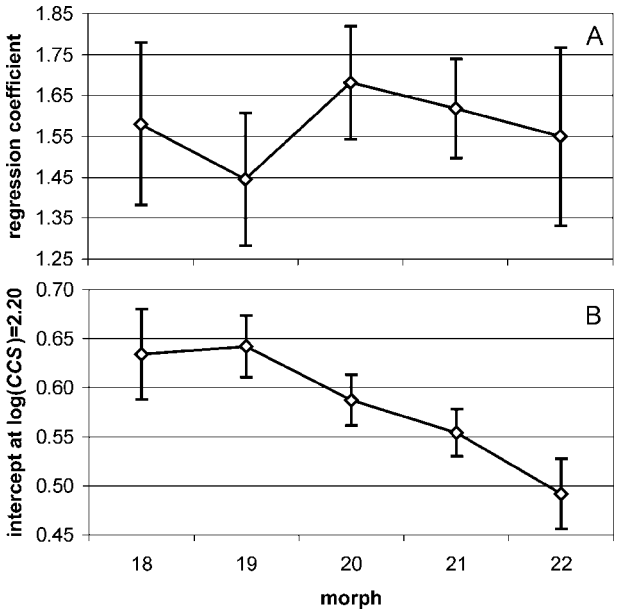

Figure 10: Regression coefficients (*A*) and intercepts (*B*) for the regression of log (PYL) on log (CCS) in the five morphs 18–22 at $\log(\text{CCS}) > 1.80$. *PYL* = pygidium length; *CCS* = cranidial centroid size. Bars are 95% comparison intervals (GT2 method of comparison). Sample statistics whose intervals do not overlap are significantly different. Note that overall the intercepts of the segment-rich morphs are markedly lower than the segment-poor morphs, in accordance with the early determination hypothesis.

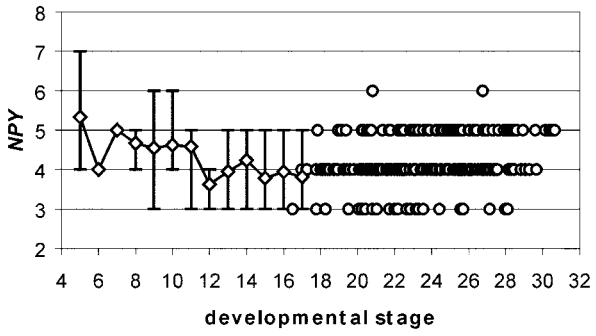

Figure 11: Ontogenetic progression of the number of pygidial segments (*NPY*) in *Aulacopleura konincki*. Because mixed cross-sectional and cross-sectional data are shown together in the figure, the horizontal axes indicate stages based on segment counting until and including stage 17; beyond stage 17, stages are an approximation based on a linear transformation of log (CCS). *CCS* = cranidial centroid size. Diamonds are average NPY for the meraspid degree, and circles are morph 18–22 specimens. Bars are NPY range of variation.

of NPY on log (CCS) in the five morphs 18–22 have the same slopes and the same intercepts (GT2 method of comparison, $P > .10$). Therefore, the variability of *A. konincki* in the number of thoracic segments was not produced by variability in the duration of the phase of segmental release from the transitory pygidium into the thorax. Such a mechanism would have produced morph 22 specimens with pygidia with, on average, four segments less than pygidia of morph 18.

Discussion

Growth

Dyar's rule has proved to be a good model of postembryonic growth for many extant arthropods (e.g., Hartnoll 1982; Klingenberg and Zimmermann 1992) and trilobites (Chatterton and Speyer 1997), and *Aulacopleura konincki* meraspid development conforms well to it.

Among arthropods, several instances of deviation from strict Dyar's rule have been recorded (reviewed in Albert 1982). In most cases, departure from Dyar's rule is simply a tendency for growth ratios to decrease progressively with stage (Hartnoll 1982). For *A. konincki*, the pattern of regression residuals is comparable to random fluctuations around the expected mean of the log-size variables for each stage, and there is no evidence, at least until meraspid stage 17, of a systematic decrease of growth ratios.

We have no data on the absolute growth for stages following degree 17; however, from a preliminary inspection on ontogenetic allometries, we know that most allometric relationships between morphometric variables changed after stage 17 or a few stages later. This means that after those stages, at least some variables changed their absolute growth rates differentially. However, we can exclude drastic increases of growth rates (except for PYL, whose meraspid growth rate was almost 0) because no morphometric variable exhibits appreciable multimodal adult size distribution (cf. Crônier et al. 1998).

In general, comparisons of Dyar's coefficients must be handled with care. This is because the estimation of the coefficients depends on several factors, such as the nature of the sample (laboratory vs. field-collected specimens), the portion of ontogeny considered, or the size variable used (different characters may have different Dyar's coefficients because of ontogenetic allometry). However, the estimated Dyar's coefficients for *A. konincki* are quite low (e.g., 1.10 for CCS) when compared with other arthropods both recent and extinct. For instance, Dyar's coefficients have a median value of 1.52 for holometabolous insects and 1.27 for hemimetabolous insects (Cole 1980) and have a mean value of 1.22 for larval decapods (Rice 1968). Dyar's coefficient has been calculated for several trilobite species (e.g., Palmer 1957; Hunt 1967). Values vary from about 1.08 for the glabella of *Aphelapsis* sp. (Palmer 1962) to about 1.40 for the cephalon of *Trimerocephalus levievrei* (our calculation on Crônier et al. 1998 data), with a mode of about 1.20.

Several explanations for Dyar's rule and for specific values of its coefficient have been offered, and these can be grouped in two broad categories: external causes, such as competitive exclusion (Horn and May 1977; Maiorana 1978), food-finding strategy (Enders 1976), habitat stability (Cole 1980), and maximization of growth efficiency (Hutchinson and Tongrid 1984), where a constant growth rate is promoted and actively maintained by natural selection, and internal causes, such as the mechanism of intermolt hypodermal growth (Bennet-Clark 1971; Freeman 1991) and cell proliferation (Przibram and Megušar 1912; Bodenheimer 1933), where a constant growth rate is a consequence of growth physiology. For *A. konincki*, we have no evidence that the constant growth rate was a response to specific ecological factors; the high degree of conformity of meraspid development to Dyar's rule seen in this species could be the result of a default physiological growth process (Klingenberg and Zimmermann 1992).

The constancy of size variance with growth among meraspid *A. konincki* is evidence of targeted growth. This claim is based on the assumption of a nonzero value for the variance of ρ (the logarithm of the growth ratio), which implies a negative value for $\mathrm{Cov}(X_i, \rho)$, the unequivocal sign of growth compensation. This assumption is justified on the basis of several studies of extant arthropods (reviewed in Hartnoll 1982) that confirm the strong influence on average growth ratios of several external factors, such as temperature, nutrition, or parasitism. The relative im-

portance of these factors is highly variable with species and developmental stage, and there are considerable qualitative differences in the way they influence growth, but their effect is never negligible. Moreover, a sizeable within-stage variation of growth ratios is detectable even when all individuals experience the same environmental and nutritional conditions, as demonstrated in a few longitudinal studies where animals were reared in the laboratory (West and Costlow 1987; Klingenberg 1996). With respect to size variation at hatching, Var (ρ) is in the order of 20% for the centipedes *Lithobius forficatus* (G. Andersson, unpublished data) and between 30% and 40% (depending on water temperature and salinity) for the crab *Rhithropanopeus harrisii* (our calculation on Hartnoll's [1978] data). In absence of compensation, values of this magnitude imply a considerable increase in logarithmic size variance during ontogeny. This variation, not explained by external factors, is possibly attributable to internal ones (i.e., individual variation in physiologically relevant characters as well as developmental instability). There seems to be no reason for arguing that growth in *A. konincki* was less sensitive to environmental factors or more developmentally stable or that relevant physiological parameters were less variable within populations than in extant arthropods, which would result in negligible Var (ρ).

Aulacopleura konincki is the oldest example known to us of targeted growth in the Metazoa. Targeted growth has been documented in several arthropods, although the degree of regulation varies from species to species. Several studies have found targeted growth only among certain stages of development, for instance, in insects (Tanaka 1981; Klingenberg 1996) and crustaceans (Hartnoll and Dalley 1981; Freeman 1990). These results suggest ontogenetically intermittent compensation, but in barnacles, West and Costlow (1987) observed compensation among almost all instars. However, in all cases, the underlying developmental mechanism of growth compensation (i.e., the nature of the feedback mechanism) is poorly understood. Hunt and Chapman (2001) analyzed two rare cases of size clustering in holaspid-stage trilobites and interpreted these clusters to represent instars. These authors then suggested, among other possibilities, that group separation could be explained as targeted growth precise enough to keep within-stage size variation below between-stage size increment, thus limiting overlap of size distributions among stages.

Segmentation

Among recent arthropods, the number of adult trunk segments is generally constant not just at the specific level but also within larger clades such as Eumalacostraca. Intraspecific variability, generally coupled with a relatively high number of segments, is known only in a few clades, such as notostracans, geophilomorph centipedes, and juliform millipedes (Linder 1952; Minelli and Bortoletto 1988; Enghoff et al. 1993). The list of clades in which variation is associated with a first phase of segment accretion followed by a phase with constant segment number is further restricted to an undetermined number of species belonging to a few millipede families and possibly to notostracans, whose segmentation is not easy to interpret. A fixed thoracic segment number is also characteristic of the holaspids of most derived trilobite clades (Hughes and Chapman 1995).

For *A. konincki*, bivariate analyses of the number of thoracic segments versus adult size support a traditional two-phased (meraspid-holaspid) dynamic of thoracic segment addition (dynamic 1) rather than a lifelong phase of thoracic segment accretion (dynamic 2). However, we can offer some additional arguments for rejecting continuous thoracic segment accretion on the basis of comparisons with extant arthropods.

Continuous accretion requires an increase in the number of thoracic segments during the mature phase of development but at a lower rate than in the earlier (meraspid) phase (Hughes and Chapman 1995). This implies that some mature molts resulted in segment addition while some others did not because the meraspid rate of segment addition in *A. konincki* was one new thoracic segment per stage. Inspection of the bivariate distribution NTH versus log (CCS) shows that this had to be accompanied by a high level of intraspecific variability so as to produce an irregular schedule of segment accretion able to spread throughout the mature section of the morphospace as observed (fig. 6). An irregular (uncontrolled) and iterated alternation between segment accretion and stasis producing variation in adult segment number is unknown among recent arthropods. When lifelong segment accretion produces variation in adult segment number, as in many millipedes, this is obtained through variable schedules of segment addition that do not include steps of zero segment addition (Enghoff et al. 1993). Moreover, this "anarchical" accretive phase does not accord with the regular growth of *A. konincki* during the meraspid period.

A conventional two-phase development, with the holaspid period corresponding to a segment-invariant phase of thoracic segmentation, our preferred interpretation, is also supported on comparative grounds. This mode of development is the way many extant arthropods develop postembryonically and certainly occurred in many other trilobites (Hughes and Chapman 1995). Because the number of holaspid thoracic segments was generally invariable within trilobite species (Hughes et al. 1999), some form of developmental control for this character must have been active in their ontogenies. Developmental instability or

hereditary variation in the trigger mechanism for the passage from the accretive to the segment-invariant phase of thoracic development is thus a more reasonable explanation for the pattern seen in *A. koninckí*. Hence, dynamic 1 is more similar to the growth pattern in other trilobites and fits better with modes of phenotypic variation seen in living arthropods.

In trilobites, it is known that in the pygidium, irrespective of whether we count axial rings or pleural ribs, there can be a mismatch between the number of dorsally expressed segments and the number of pairs of ventral appendages (reviewed in Hughes, in press; Minelli et al., in press). However, regardless of the true segmental nature of the caudal unit, our understanding of segmentation is poorer within the pygidium than in the thorax. We lack information on the key protaspid and early meraspid phases, we have no reliable measures of intrastage variation before degree 9, and part of this variation could be due to poor preservation. Moreover, we cannot determine whether the observed intraspecific variation in pygidial segment number was due to relatively flexible control of individual developmental trajectories (hence, to variable segment production schedules during anamorphosis) or whether the developmental schedule instead was already specified at birth but variable among individuals.

To explain the observed combination of numbers of pygidial and thoracic segments, we have to couple the ontogenetic variation in the rate of segment production at the posterior end of the pygidium with a certain level of intraspecific variation in segment production at any given stage. A possible explanation of this puzzling dynamic may be as follows. We hypothesize an early phase (not documented) before meraspid degree 5 during which segments accumulated in the protopygidium (the pygidium of protaspids) and in the early transitory pygidium (the pygidium of meraspids). During this phase, new segments were expressed at an average rate faster than they were released into the thorax at the front end. At the end of this phase, the level of within-stage intraspecific variation observed in later stages was already determined. A possible short declining phase, with an average rate of segment production less than one segment per stage, was followed by a phase of equilibrium between segment expression and thoracic segment accretion at a pace of one segment per stage. The stability of the previously established mean and variance of the number of pygidial segments suggests very low (or zero) intraspecific variation. During the holaspid period, after thoracic segmentation was completed, no new pygidial segments were added. The rising of the lower bound of the range of variation in pygidial segment numbers in later holaspids can be explained by the fact that terminal segments became more clearly expressed at a larger size. Much of the meraspid ontogeny of *A. koninckí* (at least beyond degree 9) showed a consistent, one-to-one balanced relationship between the appearance of new trunk segments and their release into the thorax. This contrasts with the situation in some other trilobites in which the balance of segment appearance and release was unequal and variable during ontogeny (e.g., Kopaska-Merkel 1987).

Following Enghoff et al. (1993), postembryonic development, where the final and fixed number of body segments is reached after a series of molts (anamorphic phase), followed by further growth molts without further increase in the number of body segments (epimorphic phase), is called hemianamorphosis. For *A. koninckí*, the two-phase development of the thorax combined with the stability of number of pygidial segments from late meraspids onward allows us to classify its postembryonic development as heminamaorphic (see fig. 1). In this species, the separation between the accretive and the segment-invariant phases of thoracic development (meraspid-holaspid transition) coincided with the passage from the anamorphic to the epimorphic phase of hemianamorphic development.

Anamorphosis to Epimorphosis Switch Control

Exploring developmental control in an extinct clade is a difficult task, and we have to anticipate that our hypotheses are not mutually exclusive nor exhaustive because many different dynamics could be modeled and additional sources of variation could be considered. However, among our selected hypotheses, EDH was the best-supported one. In this hypothesis, the number of anamorphic stages, and therefore the number of adult thoracic segments, was determined precociously. The population was composed of five distinct cohorts. Membership of a particular cohort could be under either hereditary (e.g., genetic) or environmental influence.

The alternative hypothesis (LDH) suffers from several different kinds of drawbacks when its expectations confront the morphometric data. A later determination of a key developmental event on the basis of a size threshold is a well-known mechanism of regulation among recent arthropods (e.g., Tanaka 1981), but for *A. koninckí*, this mechanism predicts size-frequency distributions that fit poorly with the data, and it does not predict the observed different allometric relationship between pygidium length and cranidium centroid size for the five holaspid morphs. Thus, there is evidence for rejecting this version of the LDH.

However, some other versions of LDH cannot be ruled out because in terms of patterns of size distribution, their predictions are indistinguishable from those of EDH. In the case of later determination of the first epimorphic

stage, on the basis of the recognition of an exclusively physiological trait or internal anatomy (uncorrelated with any morphological accessible trait), predictions do not differ from those of EDH because within-stage distribution of the state value of the trait is uncorrelated with size, just as cohort membership is also uncorrelated with size. This is certainly a possibility, and in such a case, the population would have not been divided into early determined cohorts. The possibility of a later determination of the first epimorphic stage on the basis of external morphological traits not accessible to study (e.g., a ventral trait, perhaps), while producing the same predictions, seems instead less likely. It is quite improbable that there was a (now) nonobservable morphological trait uncorrelated with dorsal morphology that was solely responsible for the developmental switch.

We also considered additional hypotheses of regulation based on the response to a specific environmental cue, although we did not produce mathematical models to address this issue specifically. We are not aware of studies specifically addressing the passage from anamorphosis to epimorphosis in living hemianamorphic arthropods with respect to these types of controls. Nevertheless, studies of other developmental decisions show that these sorts of mechanisms are feasible. These hypotheses can be viewed as variations on EDH and LDH. In such cases, the developmental trait to be determined is not the stage at which the trilobite thorax switched from anamorphosis to epimophosis but the stage at which it acquired competence to respond to an environmental signal for switching to epimorphosis. Predictions are obviously more diffuse because in addition to trait variation, we should also consider the unpredictability of the stage at which the hypothetical signal was actually received. Dependence on such an environmental signal inevitably increases the level of variation of the mature number of thoracic segments with respect to the corresponding hypotheses.

An early division into cohorts (as in EDH) with response competence acquired at different stages also is consistent with the data, but, unfortunately, predictions with respect to accessible morphology are no different from those of EDH. Because of the unknowable variability due to the reception of the signal, we cannot even establish the number of cohorts; these could have been anywhere from one (no cohort division) to five (e.g., signal always present, so environmental effects did not increase NTH variation). However, it should be considered that with this kind of regulation, there is no way to limit the maximum number of thoracic segments to 22.

Linking acquisition of signal response competence to an ontogenetic threshold (as in LDH) would inevitably increase the level of predicted variation of NTH in respect to LDH. Because of differential growth, different individuals could acquire competence at a different stage and then switch to epimorphosis in the same stage or 1, 2, 3, … *n* stages later. An increase of NTH variation is incompatible with LDH because the estimated range of size variation in the last meraspid stages in respect to the growth rate (Dyar's coefficient) was already at its maximum for producing exactly five morphs (this is the reason why we have regarded LDH as an acceptable hypothesis).

Conclusions

This study shows that through the morphometric analysis of appropriate samples, it is possible to address questions of high interest for evolutionary developmental biology using data from fossils, permitting the direct comparison of ancient developmental processes with recent ones. The development of *A. koninckí* was tightly regulated with respect to size and thoracic segmentation. It is commonly held that marked variation is associated with primitiveness both in trilobites specifically (see Hughes et al. 1999) and as a general macroevolutionary principle (an expression of this with respect to meristic variation is the so-called Williston's law; for a critical assessment, see Minelli et al., in press). In *A. koninckí*, the opposite is found; the variable thorax segmentation was associated with a high degree of developmental control. Whether this precise developmental control was related to this species' status as a relatively derived trilobite (Hughes et al. 1999) remains an open question. Further investigation of the development of *A. koninckí*, currently in progress, will assess patterns of relative growth and ontogenetic trajectories of shape change.

Acknowledgments

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APPENDIX A

Modeling of Size-Frequency Distributions

Models have been implemented with the software package *Mathemathica*. Morph size distributions (fig. 7) were obtained by combining the size distributions of all the developmental stages calculated under the two hypotheses (fig. C2).

Assumptions Common to the Two Models

- Log(CCS) is the reference log-size variable (X).
- For each meraspid stage, log-size (X_i) is a normally distributed random variable; that is untransformend size distribution is lognormal. This is a typical size distribution for specimens of the same stage (Sokal and Rohlf 1981).
- Growth follows Dyar's rule: $X_{i+1} = X_i+\rho$, the geometric progression assessed for meraspids.

Parameters Common to the Two Models Estimated from Morphometric Data

- Mean of the logarithm of Dyar's coefficient is $E(\rho) = 0.091$ (the value for meraspids).
- Variance of the logarithm of Dyar's coefficient is $var(\rho) = 0.0$. We have no direct estimation of $var(\rho)$, but because linear regression of var(log(CCS)) on meraspid degree was not significant ($var(\rho)$ was compensated by $cov(X_i, \rho)$), ρ can be treated as a constant of value $E(\rho)$ rather than as a random variable.
- Variance of log(CCS) at a given stage: $var(X_i) = 0.0072$ (the average value for degrees 9 to 17).
- Mean of log(CCS) at stage 1: $E(X_1) = -0.1559$ (extrapolation from the meraspid regression line).
- Maximum stage is 29. This value is not an inference about the biology of *Aulacopleura konincki*. The actual number of stages is unknown because it depended on unknown growth rates during the holaspid period. For simplicity we assumed the Dyar's coefficients for meraspids projected into holaspid period (this, by extrapolation, produces the value 29), but any combination of growth rate and maximum number of stages that produces the largest specimens we observed (with log(CCS) close to 2.60) is equally reasonable and produces the same predictions in terms of the size frequency distribution.

Parameters of EDH Estimated from Morphometric Data and Additional Notes

- The relative abundance of the five cohorts, reflecting the actual proportion of forms in the sample, is a parameter of the model, not a prediction. Cohort 18-22 relative abundances are set at: 0.052, 0.281, 0.327, 0.235, 0.105, corresponding respectively to the observed morph relative abundance from the 153 specimens of the five morphs with log(CCS)>1.80. For this part of the distribution the model predicts that (P>0.98, 1- the probability of log(CCS)>1.80 for meraspids) specimens of all cohorts have already entered the holaspid period, for which morph relative abundance equates to cohort relative abundance.
- For each stage (whether meraspid or holaspid) log(CCS) is a normally distributed random variable. In a bivariate distribution of number of thoracic segments versus log(CCS), the five cohorts overlap until the seventeenth stage (degree 17); thereafter, they begin to separate.

Parameters of LDH Estimated from Morphometric Data and Additional Notes

- The critical size is a parameter of the model, not a prediction. Critical log(CCS) = 1.53, the value for which there is the best fit between expected and observed morph relative abundance for the 212 specimens with log(CCS) in the range 1.55-2.35. The model predicts that, in this part of the distribution, the relative abundance of the five morphs equates to the probabilities of crossing the critical size in the five stages from 18 to 22. All specimens with log(CCS)>1.53 can be considered holaspids.
- In modeling LDH, we used an invariant critical size because the estimated size variation in the last meraspid stages with respect to the growth rate (Dyar's coefficient) was already of the magnitude necessary for producing almost exactly five morphs. Genetic variation for the critical size would have produced a wider range of morphs because, for any class of size, the holaspid period could have been entered at a variable

(critical) size, and therefore at a variable developmental stage. As for environmental factors, if the actual (environment-dependent) growth rate had been positively correlated with the value of the critical size (faster rate - higher critical size), this would have reduced the range of morphs, compensating size variation. On the other hand, if the actual growth rate had been negatively correlated with the value of the critical size (faster rate - lower critical size), this would have widened the range of morphs.

- Because only the largest specimens of a certain stage enter epimorphosis, there is a strong reduction of the within-stage size variance for a certain morph, and, at least for the first epimorphic stages, size is not normally distributed.
- Once a group of specimens enters the holaspid period at a certain stage, it inherits the size distribution of the preceding stage, but because of the cut at the critical size, this distribution is not expected to be normal. Rather, each cohort represents a sector, about one fifth of the log-size range, of a normal distribution of the sample in the last meraspid instar. We show the distribution of a certain morph in the holaspid stage as the sum of different stage distributions, each obtained by translating this initial distribution according to the logarithmic growth coefficient. The very skewed distributions close to the transition threshold (e.g., of the nineteenth stage of morph 18) are expected to become more symmetrical with growth because of individual variation and environmental influences. However, in order to precisely model the change of the shape of the size distribution with growth we need to know the magnitude of var(ρ) and the mechanism of growth compensation, but this information is not available. Therefore, we presented curves with saw-tooth profiles (fig. 7, fig. C2) in order to clarify the core differences between the two alternative models, but the tests of goodness of fit considered wider classes of size, where these irregularities could not influence the result (see fig. 8).
- Because all the specimens enter epimorphosis at the same size and have experienced a variable number of anamorphic stages according to their number of thoracic segments, holaspid specimens of the same size but belonging to different morphs are on average expected to belong to different stages. For instance, a morph 18 specimen of a certain size will on average have experienced four fewer molts than a morph 22 specimen of the same size.

APPENDIX B

Table B1: Growth progression of cranidial centroid size (CCS) during the meraspid period of *Aulacopleura konincki*.

Meraspid degree	n	Observed mean log(CCS) (95% CI)	Expected mean log(CCS)	Mean postmolt/premolt CCS ratio (95% CI)
5	3	0.225 (0.146-0.305)	0.210	
6	3	0.240 (-0.120-0.600)	0.301	1.015 (0.800-1.288)
7	1	0.362 (-)	0.392	1.130 (-)
8	3	0.464 (0.251-0.677)	0.484	1.107 (-)
9	11	0.621 (0.559-0.684)	0.575	1.171 (1.028-1.333)
10	8	0.705 (0.640-0.770)	0.667	1.087 (0.998-1.185)
11	12	0.826 (0.777-0.875)	0.758	1.129 (1.047-1.216)
12	8	0.835 (0.769-0.900)	0.849	1.009 (0.936-1.088)
13	19	0.955 (0.915-0.995)	0.941	1.128 (1.051-1.211)
14	17	1.033 (1.001-1.066)	1.032	1.081 (1.028-1.137)
15	14	1.102 (1.044-1.160)	1.124	1.071 (1.008-1.139)
16	18	1.187 (1.151-1.224)	1.215	1.089 (1.022-1.159)
17	16	1.298 (1.241-1.355)	1.306	1.117 (1.048-1.190)

Note: The mean log(CCS) expected on the basis of Dyar's rule (log(Dyar's coefficient) = 0.091) are within the 95% confidence intervals of observed means but for meraspid degree 11. The 95% confidence intervals of postmolt/premolt CCS ratio include the expected Dyar's coefficient (1.096) but for the passage from meraspid degree 11 to degree 12. Confidence intervals not calculated for mean log(CCS) of degree 7 and for the grow ratios degree 7/degree 6 and degree 8/degree 7 because there is only one degree 7 specimen available.

Table B2: Growth progression of dorsal centroid size (DCS) during the meraspid period of *Aulacopleura konincki*.

Meraspid degree	n	Observed mean log(DCS) (95% CI)	Expected mean log(DCS)	Mean postmolt/premolt DCS ratio (95% CI)
5	3	0.923 (0.830-1.016)	0.933	
6	3	1.002 (0.619-1.386)	1.048	1.082 (0.839-1.396)
7	1	1.143 (-)	1.163	1.152 (-)
8	3	1.251 (0.977-1.524)	1.278	1.113 (-)
9	11	1.441 (1.379-1.502)	1.393	1.209 (1.057-1.383)
10	8	1.555 (1.490-1.621)	1.508	1.122 (1.030-1.221)
11	12	1.704 (1.654-1.755)	1.623	1.161 (1.075-1.253)
12	8	1.710 (1.642-1.778)	1.738	1.006 (0.930-1.087)
13	19	1.875 (1.837-1.912)	1.853	1.179 (1.101-1.264)
14	17	1.977 (1.940-2.015)	1.968	1.108 (1.053-1.166)
15	14	2.049 (1.989-2.110)	2.082	1.075 (1.007-1.147)
16	18	2.150 (2.110-2.190)	2.197	1.106 (1.034-1.182)
17	16	2.315 (2.260-2.370)	2.312	1.179 (1.106-1.258)

Note: The mean log(DCS) expected on the basis of Dyar's rule (log(Dyar's coefficient) = 0.115) are within the 95% confidence intervals of observed means but for merspid degree 11 and 16. The 95% confidence intervals of postmolt/premolt DCS ratio include the expected Dyar's coefficient (1.122) but for the passage from merspid degree 11 to degree 12. Confidence intervals not calculated for mean log(DCS) of degree 7 and for the grow ratios degree 7/degree 6 and degree 8/degree 7 because there is only one degree 7 specimen available.

APPENDIX C

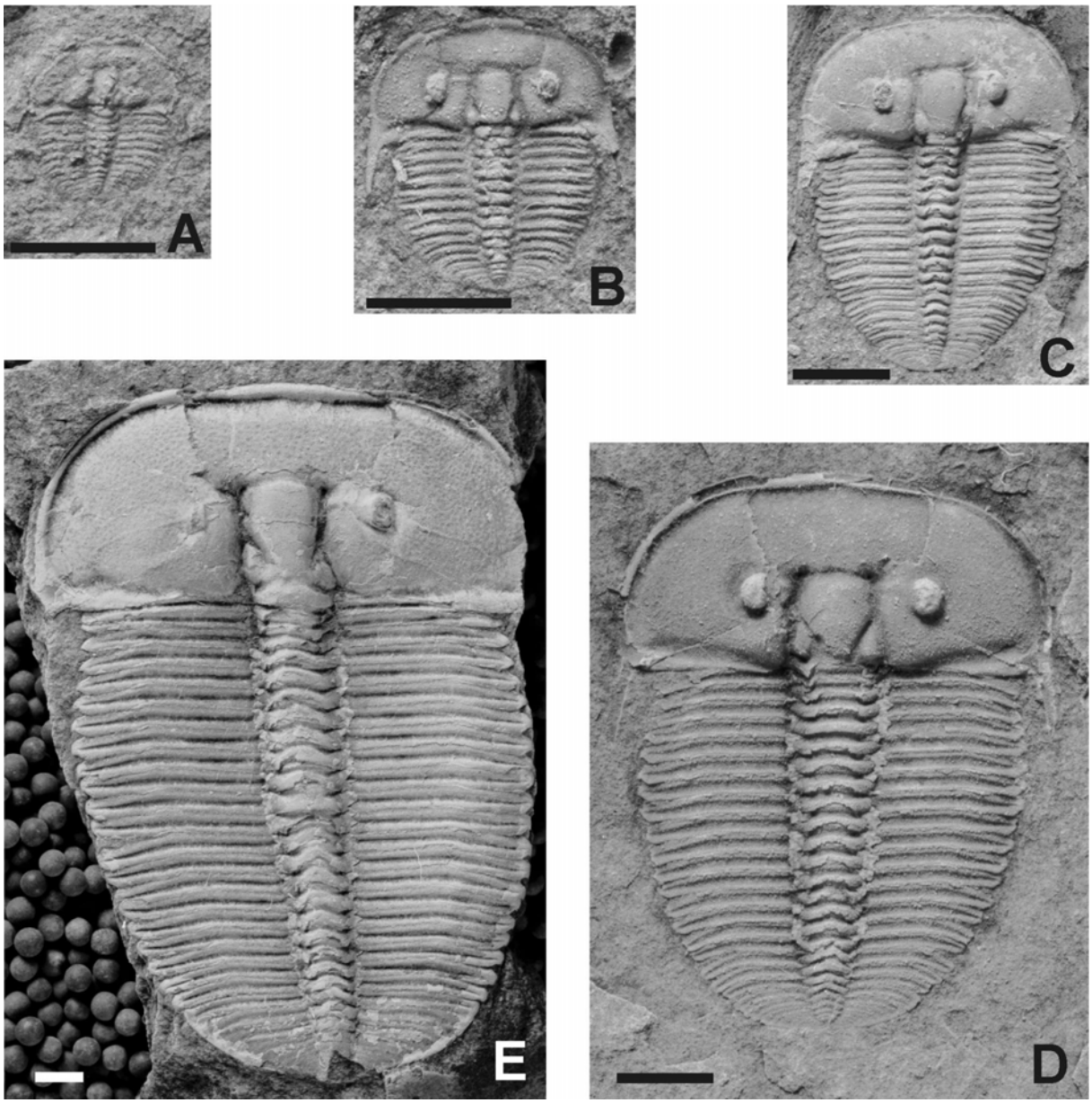

Figure C1: Selected specimens of *Aulacopleura konincki* illustrating the ontogeny of this trilobite. The major ontogenetic shape change relates to elongation of the trunk region as additional segments were accreted to the thorax, accompanied by a relative decrease in the size of the caudal region (transitory pygidium). The majority of shape change was concentrated in the earlier portions of meraspid ontogeny. All specimens are from Barrande's pits at Na Cernidlech Hill near Loděnice in the Czech Republic in the Upper Wenlockian (Silurian) Motol Formation. These, with their total length (TTL) are: A) USNM475170, TTL = 2.3 mm, eigth thoracic segments, four pygidial segments; B) BMNH42363.2, TTL = 3.7 mm, 11 thoracic segments, five pygidial segments; C) USNM475179, TTL = 7.2 mm, 17 thoracic segments, five pygidial segments; D) MCZ103490, TTL = 11.7 mm, 19 thoracic segments, four pygidial segments; E) MCZ103496, TTL approximately 30.5 mm, 21 thoracic segments, five pygidial segments (specimen not included in analysis as rear of pygidium damaged). Scale bars are 2 mm long.

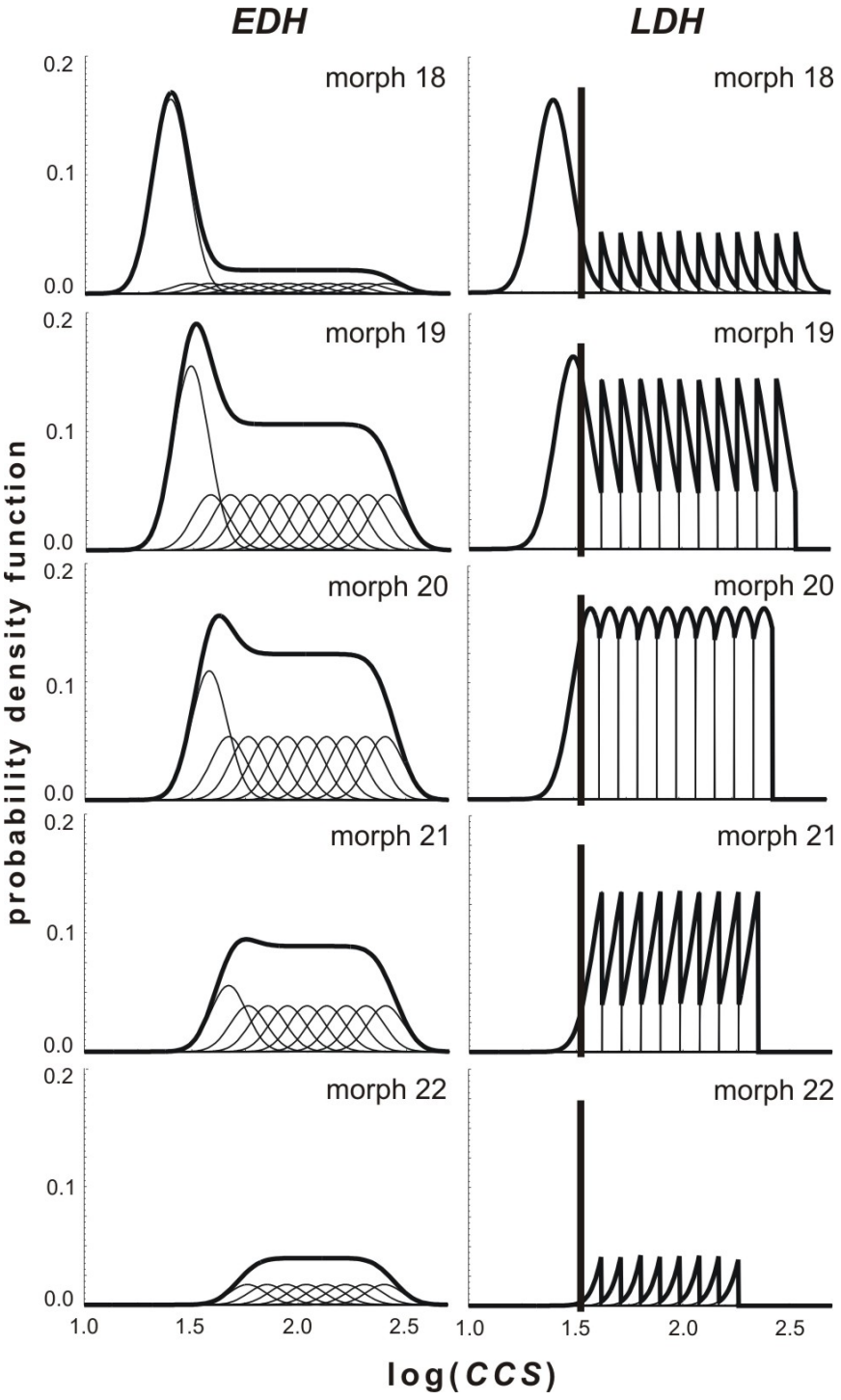

Figure C2: Construction of density probability functions of the five morphs with 18-22 thoracic segments under the two alternative hypotheses, early detemination hypothesis (EDH) and later determination hypothesis (LDH). *Thick line*, overall morph density function; *thin lines*, density functions of the different developmental stages of the morph. *Left column*, early determination hypothesis of the mature number of thoracic segments (EDH). The leftmost thin-line bell-curve of each morph includes meraspid stages, but for morph 22. *Right column*, later determination hypothesis of the mature number of thoracic segments on the basis of actual size (LDH). Vertical bar represents the critical size.