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INTEGRATION AND REGULATION OF DEVELOPMENTAL SYSTEMS IN TRILOBITES

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INTRODUCTION

Developmental systems determine whether selectively useful variation arises (e.g., Maynard Smith *et al.*, 1985; Wagner, 1988; Gerhart and Kirschner, 2007). They can reduce that variation via canalization or by structuring it into integrated complexes, thereby preventing organisms from varying one part without interfering with other parts (Wagner *et al.*, 2007). Temporal or phylogenetic changes in intrinsic developmental factors may explain macroevolutionary trends in intraspecific variation (e.g., Webster, 2007) and clade diversification history, as proposed by the developmental or "genomic" hypotheses regarding the unique Cambrian radiation (Riedl, 1978; Valentine, 1995; Wagner and Laublicher, 2004; Budd, 2006; Davidson and Erwin, 2006; Erwin, 2007). Techniques for assessing and comparing patterns and levels of integration are becoming increasingly refined and powerful, but have yet to be applied to fossil taxa. As a result, the extent to which changes in developmental canalization and integration could have influenced macroevolutionary diversification is currently unclear. In this short paper, we discuss methods appropriate for quantifying the level and structure of variation in fossil taxa, permitting unparalleled insight into their developmental systems.

CANALIZATION, FLUCTUATING ASYMMETRY, AND MODULARITY

Canalization refers to the buffering of developmental systems, ensuring that the same phenotype is produced despite genetic and environmental perturbations (Waddington, 1942, 1952). Types of canalization are now typically distinguished according to the source of the perturbations being buffered. Thus genetic and environmental canalization are distinguished from each other, and environmental canalization is further subdivided into "macroenvironmental" canalization (the converse of phenotypic plasticity) and "microenvironmental" canalization (which is buffering against random perturbations within a constant environment; Debat and David, 2001). Theoretical studies have shown that genetic canalization evolves by natural selection only under highly restrictive conditions, in striking contrast to environmental

canalization (Hermisson and Wagner, 2004). The distinction between macro- and microenvironmental canalization is equally important in that macroenvironmental canalization is expected only when the optimal phenotype is constant over the environmental range of a species, but microenvironmental canalization (resistance to developmental noise) is expected under all environmental conditions (Debat and David, 2001; Nijhout and Davidowitz, 2003).

Low levels of microenvironmental variation provide the primary evidence for well-buffered developmental systems, which can be measured in fossil taxa by fluctuating asymmetry (FA), i.e., random deviations from bilateral symmetry of a symmetric structure (e.g., Van Valen, 1962; Scheiner *et al.*, 1991; Santos *et al.*, 2005). One major advantage of using FA as a measure of microenvironmental variation is that its expected value is known—it is zero regardless of genotype and environment. A second major advantage is that it enables us to control for both genetic and macroenvironmental variation because both sides of an organism have the same genotype and develop within nearly identical environments.

FA has become of great interest in evolutionary developmental biology not only because it is regarded as a useful indicator of developmental noisiness, but also because it is now viewed as especially informative about modularity (Klingenberg and Zaklan, 2000; Klingenberg *et al.*, 2003; Badyaev *et al.*, 2005). The rationale is that FA can distinguish between two developmental causes of integration: (1) a process acting in parallel in different modules, and (2) direct interactions along or between intramodular pathways (e.g., Klingenberg and Zaklan, 2000; Klingenberg *et al.*, 2003; Klingenberg, 2005). Only direct interactions are likely to explain correlated FA because the causes of parallel variation (i.e., common genetic or environmental factors) are controlled. Therefore, correlations found between (signed) asymmetries indicate direct intramodular interactions.

METHODS FOR STUDYING TRILOBITE DEVELOPMENTAL SYSTEMS

The methods of landmark-based geometric morphometrics (Bookstein, 1991; Zelditch *et al.*, 2004) can be used to analyze phenotypic variation and FA. These methods extract information about shape variation by analyzing coordinates of landmarks (i.e., homologous, discrete, anatomical loci; Fig. 1). These methods retain information about the spatial relationships among landmarks, making it possible to relate abstract results of statistically powerful analyses to the physical structure of the organisms (Bookstein, 1991; Zelditch *et al.*, 2004; Slice, 2007).

Analyzing FA and symmetrical variation of shape

The favored method for analyzing FA is the two-factor mixed-model analysis of variance, with the two main effects being "individuals" and "sides" (Leamy, 1984; Palmer and Strobeck, 1986). According to the recommended protocol, the right and left sides are measured two or more times, then the overall mean (over all replicates and sides) is estimated from the combined data, and deviations from the overall mean are partitioned into components due to the two main effects. The symmetric variation among individuals is quantified by the main effect of "individuals," after correction for asymmetry; directional asymmetry (handedness) is quantified by the main effect of "sides." The interaction term documents variation among individuals in right-left differences, which is FA. The statistical significance of FA is assessed by the F-ratio between the interaction mean square and measurement error mean square (Leamy, 1984; Palmer and Strobeck, 1986). After removing measurement error from the estimate of FA, populations can be compared using Levene's test, which is relatively insensitive to departures from normality (see Palmer and Strobeck,

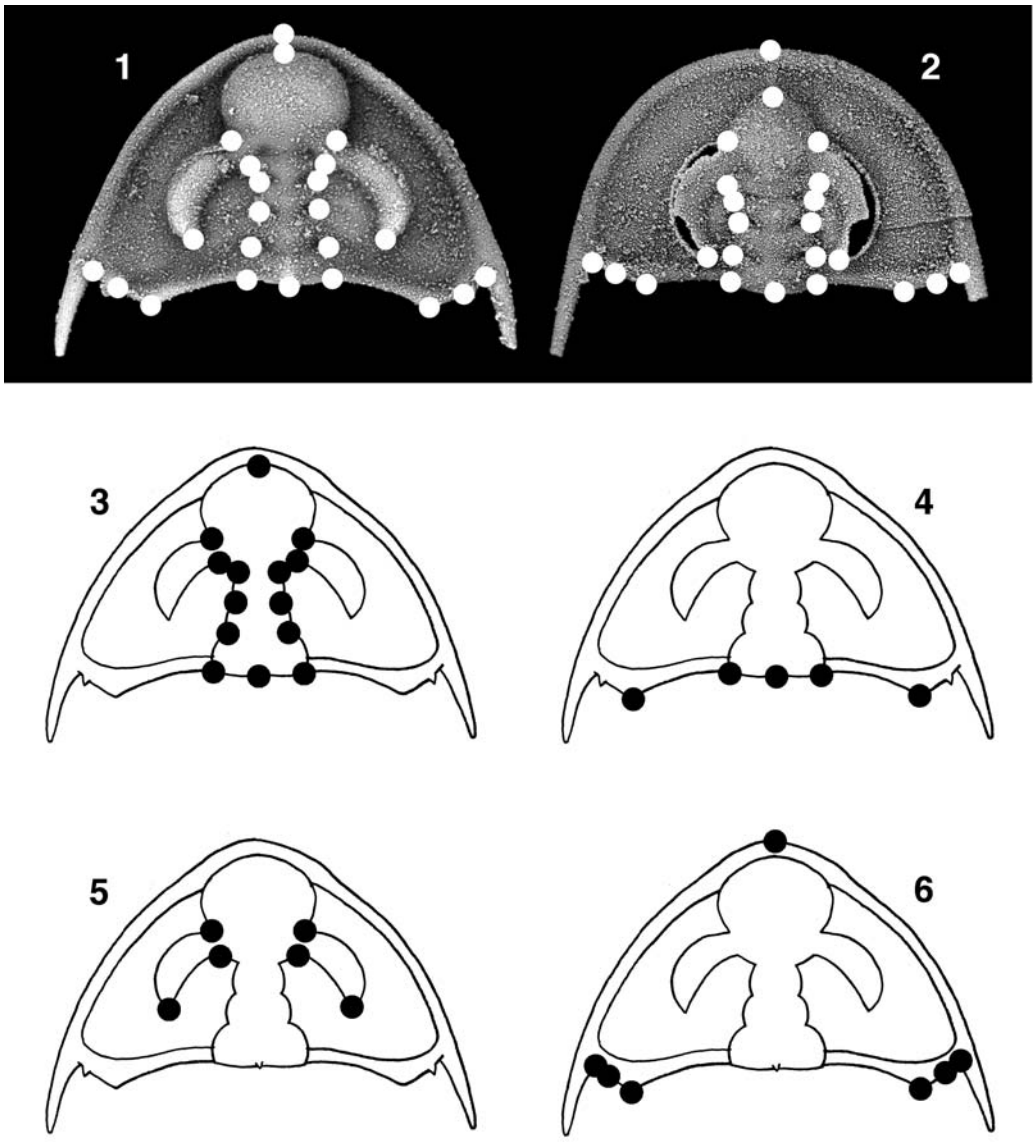

Figure 1. 1-2, Representative silicified cephala of olenelloid trilobites. White circles show location of landmarks appropriate for inclusion in the geometric morphometric study of developmental integration (see text). Both specimens from collection ICS-1173 (1.8 meters below top of Combined Metals Member, Pioche Formation, Dyeran, traditional "Lower" Cambrian) of Hidden Valley, Burnt Springs Range, Lincoln County, Nevada. *1*, *Nephrolenellus geniculatus* Palmer, 1998, UCR 9963.19, x12. *2*, *Olenellus gilberti* Meek in White, 1874, UCR 9963.120, x8. *3-6*, Landmarks associated with hypothesized developmental modules on the olenelloid cephalon, shown on the cephalic outline of *N. geniculatus*. 3, The "glabella module", covering and associated with the anterior portion of the digestive tract. 4, The "articulation module", associated with the functional articulation between the cephalon and first thoracic segment. *5*, The "ocular module", associated with the ocular lobes. 6, The "cephalic margin module", defining cephalic outline and associated with functional interaction with the substrate and feeding/respiratory currents.

1986 for additional details). The statistical approach outlined above has been adapted to the analysis of shape using Procrustes-based methods (Auffray *et al.*, 1996; Klingenberg and McIntyre, 1998; Klingenberg *et al.*, 2002). The same statistical model is used to partition variation. Using geometric data, the first step is to reflect the data for each specimen; the difference between the original and its mirror image quantifies the right-left difference of that specimen. The original and reflected copies are superimposed, with the mean shape being calculated over originals and reflected copies of all individuals. The main effect of "individuals" and sides can then be quantified as above, and the interaction between them estimates FA. The statistical significance of FA can be assessed by a permutation test and estimates for FA can be corrected for measurement error by the procedure outlined above. These estimates are also comparable across populations by Levene's test.

Analyzing modularity/morphological integration

The analysis of FA allows us to examine the extent to which morphological integration is structured by the intrinsic architecture of developmental pathways. Should morphological integration be largely due to that architecture, integration could be internally constrained in its evolutionary potential. That is because altering the structure of signaling interactions is thought to be less feasible than altering alleles acting in parallel within two or more modules (Klingenberg, 2005). Estimating the impact of direct interactions on integration therefore potentially provides important information on both causes and evolutionary potential of integration. This impact can be estimated from the matrix correlation between the FA and symmetric components of variance (e.g., Klingenberg and Zaklan, 2000; Klingenberg, 2005). It is then possible to assess (1) the level of integration, (2) the impact of direct interactions on integration, and (3) the stability of the structure of integration.

1. Measuring the level of integration: The most widely used measure of integration is the variance of the eigenvalues (Wagner, 1984) because high correlations/covariances produce one or few relatively large eigenvalues and many small eigenvalues, whereas low correlations/covariances produce nearly equal eigenvalues. Thus eigenvalues should be highly variable when morphologies are highly integrated but nearly equal when morphologies are weakly integrated. To eliminate the dependence of the variance on the mean eigenvalue, eigenvalues are typically standardized for overall variance before comparisons are made. The comparisons are done using a resampling procedure that repeatedly draws samples from each population; for each iteration, the standardized eigenvalue variance is calculated for each population then the difference is computed, yielding the distribution of differences against which the observed difference is compared.

2. Measuring the impact of direct interactions on morphological integration: The impact of direct interactions on morphological integration can be estimated by the matrix correlation between the symmetric and FA components of variation. If this value is high, then the direct interactions responsible for correlated FAs contribute highly to the structure of morphological integration. The complex morphology of the trilobite cephalon offers many readily identified anatomical loci and outlines ideal for landmark- and semilandmark-based morphometric analysis, especially of FA (Fig. 1). The cephalon can be subdivided into functionally defined structures (Fig. 1.3-6). The mosaic of functionally and anatomically diverse structures on the cephalon increases the likelihood that developmental systems controlling cephalic morphology were integrated into modules. The hypothesis that these cephalic regions associated with distinct functions constitute developmental modules can then be tested. Correlations between the FAs of these partitions can be estimated following the protocol of Monteiro *et al.* (2005), which estimates the pairwise Procrustes

distances between all specimens for each partition, then computes the matrix correlation between those pairwise distance matrices between partitions. Thus, whereas the first test regarding the role of direct interactions involves simply estimating the correlation between the symmetric and FA components of variation, the second involves testing explicit hypotheses regarding the developmental architecture of integration.

3. Assessing the stability of the structure of integration: Interspecific comparison of integration structure can be achieved by comparing covariance matrices across species for both the symmetric and FA components of variance and by comparing correlation matrices for the partitions (as outlined above). Because morphological integration is usually measured in terms of correlations, comparisons should be based on the correlation matrices obtained from the procedure outlined above.

SAMPLING REQUIREMENTS

Given the focus on the measurement and biological interpretation of phenotypic variation and deviations from symmetry, taphonomic overprint on morphology is a particularly serious concern: tectonic or compaction-related deformation of fossils can introduce a strong non-biological signal into morphometric analyses (Webster and Hughes, 1999). To negate such issues, studies should utilize only high-quality, undeformed, three-dimensional fossils, preserved in carbonates either as testate material (recovered as "crackout" by physical breakage of the rock) or as silicified material (recovered as isolated sclerites by acid dissolution of the rock; Fig. 1). Any specimens showing evidence of tectonic or compaction-related deformation should be excluded from the study. Differences in phenotypic variation attributable to environmental heterogeneity, time-averaging, ontogeny, and phylogeny must also be taken into account and impose additional constraints on choice of study system when inter-sample comparisons are to be drawn.

DISCUSSION AND FUTURE WORK

The above methods for rigorously quantifying and comparing the level and structure of developmental integration have been successfully applied to evolutionary questions involving extant taxa (references above). However, the techniques have yet to be employed on fossil taxa, and many key questions involving long-term temporal dynamics of developmental systems therefore remain unanswered. A question of considerable theoretical importance is the direction in which integration evolves. A long favored model posits that integration increases over time (e.g., Cheverud, 1996). The primary alternative proposes that modularity evolves from an initially high level of integration. That second possibility is more difficult to achieve because integration must be decreased *between* modules but maintained or increased *within* modules (Wagner, 1996; Wagner *et al*., 2007). The best-supported theoretical model for the evolution of modularity by natural selection proposes that it evolves as a side effect of selection for canalization (Wagner and Mezey, 2004; Wagner *et al*., 2007). Despite the large number of comparative studies of integration, it is still not known whether integration or modularity tends to increase over time, or even if there is any temporal trend in either direction, much less whether modularity evolves in concert with canalization. Neither is it known whether regulation or integration of developmental systems changes on a timescale commensurate with macroevolutionary diversification trends, or whether they serve as a constraint explaining such trends.

Application of the above techniques to suitably well-preserved fossil taxa within a tightly constrained phylogenetic, stratigraphic, and environmental framework provides an avenue for addressing these questions. Such studies are already under way for several trilobite clades (e.g., Olenelloidea, Agnostina, corynexochines, Phacopida; unpublished data). These, and analogous studies of other fossil clades, will provide a fruitful research area in the evolutionary-developmental biology field over the coming years.

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