Commentary

Analysing phenotypic variation: when old-fashioned means up-to-date

Phenotypic variation has been the subject of evolutionary studies since Darwin's theory was established. However, when the molecular basis of heritable variation was discovered, a shift towards what was thought to be the evolutionary theory's holy grail eclipsed traditional morphological studies. Paleontology, zoology and anatomy then became (and remained frequently) considered as old-fashioned and irrelevant for bringing in new data and theories. Focusing on genome sequencing, proteomic analysis and transgenic manipulations is now considered as the best – and only – way of making fundamental advances in the biosciences. New questions however appear with technical advances, such as: what is behind the origin and maintenance of genetic variation? To what extent does molecular polymorphism reflect genetic variation? What is the link between one-dimensional DNA sequences and three-dimensional phenotypes? What are the relative weights of genetic and environmental influences in building a living organism?

Facing the apparently endless complexity of molecular organization of developmental, physiological and cellular processes, doubts are now being raised concerning the possibility of a purely deterministic logic for biological research.

In 1917, D'Arcy Wentworth Thompson published his famous book '*On Growth and Form*', in which he developed ideas and methodologies based on the mathematical appraisal of organic forms. Eighty-five years later, after fluctuating periods of deep influence and deep oversight, the significance of D'Arcy Thompson's work has been brightly acknowledged through the emergence of geometric morphometrics (Bookstein 1991).

Geometric morphometrics is based on the following principle: considering that traditional morphometrics focusing on linear measurements of biological objects only provides a fragmentary vision of organic structures, one should try mathematically to appraise global shapes, considering whole objects as integrated units (Dryden and Mardia 1998). The emergence of highly powerful methodologies within the past few decades has generated an increasing number of morphometric studies in fields ranging from paleontology (Crônier *et al* 1998) to developmental biology (Klingenberg and Zaklan 2000) and quantitative genetics (Klingenberg *et al* 2001). Using landmark configurations or coordinates of equally spaced points around an outline as shape descriptor variables, multivariate statistics allows one to precisely and objectively characterize mean shapes and quantify shape variation. This variation may in turn be decomposed into independent components which can be interpreted in terms of evolutionary processes (Debat *et al* 2000). Such a methodology has proven to be promising in addressing classical unresolved problems (e.g. the importance of heterochronic processes in hominid evolution (Ponce de Leon and Zollikofer 2001), or the analysis of the effects of inbreeding and hybridization on phenotypes (Auffray *et al* 1996).

When external conditions change, individuals and populations have to change themselves in order to cope with the stress of change. One of the most consistent trends recorded in analyses of phenotypic response to stress is an increase in phenotypic variation (Hoffmann and Parsons 1997). Two explanations for this increase have been advanced: (i) Genetic variance may increase in unfavourable conditions (Nevo 2001), although it is not clear what causes this increase. (ii) Stress can disrupt developmental stability leading to an increase in fluctuating asymmetry (FA) (Parsons 1993). Hsp90 has been suggested to be a potential molecular buffering system: Rutherford and Lindquist (1998) have shown that this molecular chaperone may mask cryptic mutations which become

phenotypically expressed under stressful conditions. However, the link with FA remains hypothetical (Rutherford *et al* 2000; Milton *et al* 2001), and more precise data are definitely required.

Adaptive phenotypic plasticity is believed to represent an alternative mode of adaptation to fluctuating environmental conditions. Defined as 'a set of processes historically selected in order to produce different phenotypes in relation to some environmental parameter' (Debat and David 2001), plasticity is generally appraised through the analysis of response curves or reaction norms (Karan *et al* 1999). Simple polynomial adjustments may be used to characterize this response (Gibert *et al* 1998), allowing comparisons among groups. The genetic basis of plasticity has been extensively studied and a consensus has been achieved; genes that affect a trait's mean value may also be involved determining its environmental sensitivity (reviewed in Via *et al* 1995). However, its developmental basis remains largely obscure.

Recent advances in molecular biology suggest that identifying genes involved in the regulation of shape variation is possible. However, from genes to phenotypes, the link is still missing. One should thus focus on the developmental processes which are influenced by such molecular phenomena in order to understand the basis of phenotypic variation.

An exhaustive list of the perspectives opened by a convergence of geometric morphometrics with molecular genetics is largely beyond the scope of our contribution. However, four main issues related to the questions presented above may be outlined.

(1) The origin of the apparent increase in genetic variance under stressful conditions may be addressed. Two hypotheses have been advanced to explain this increase, which are not mutually exclusive.

Firstly, stress could enhance the expression of cryptic genetic variation. This hypothesis may be in turn decomposed into two sub-hypotheses: (i) Cryptic variance affects *genes which are involved in the trait formation under normal conditions*. Using geometric morphometrics, we could compare phenotypic variation patterns among stressed and controlled samples. According to the hypothesis, one should expect variation patterns to be highly correlated among groups. (ii) Alternatively, if no congruence is observed, one might suspect stress to affect specific genes, different from those expressed under usual conditions, that is, *stress-induced genes*. Such an approach should obviously be coupled with the analysis of candidate genes, and Hsp90 appears as particularly relevant.

Secondly, stress could directly enhance the frequency of mutations (e.g. through direct influence on the SOS response) and lead to the recorded increase in genetic variance. The activity of transposable elements (TEs) has been shown to be modified under stressful conditions, and this has been suggested to promote adaptive change (Capy *et al* 2000). However, the mechanism responsible for the induction of TE activation remains debated. The origin of the increase in genetic variance under stressful conditions could then be considered as a key problem: its investigation requires an approach combining molecular and morphometric tools.

(2) The use of geometric morphometrics in the analysis of buffering mechanisms has already shown to be promising. Recently, the relationship between canalization and developmental stability has been studied using landmark superimposition (the so-called Procrustes superimposition method) (Debat *et al* 2000). Since no consensus has been achieved, additional data are needed. The same approach should be used to investigate the relationship between genetic and environmental canalization: are there two distinct mechanisms?

(3) Since phenotypic plasticity is often studied through reaction norms focusing on standard metric characters, its developmental bases are difficult to analyse. We would like to put forward the use of geometric techniques to build "geometric reaction norms". Focusing on a phenotypic trait whose developmental basis has been extensively studied – such as the *Drosophila* wing – one could analyse the global shape change associated with an environmental parameter (e.g. temperature). Comparing such a geometric reaction norm to phenotypes of known mutants should at least suggest some developmental pathways that are involved in the response to stress, and therefore, in the plastic phenomenon.

(4) Finally, the combined use of geometric, morphometric and molecular quantitative genetics [i.e. quantitative trait loci (QTL) analysis] should provide new insights into the genetic bases of phenotypic variation. Such an approach has been successfully applied to the study of fluctuating asymmetry (Klingenberg *et al* 2001).

Advances in molecular biology, particularly when applied to QTL analysis, are incontestably promising. We do not argue against the importance of making efforts in that direction. However, molecular advances cannot provide significant insights in biology if not confronted with historical knowledge in morphology. We rather suggest that new approaches, such as geometric morphometrics, rooted in the traditional approach of comparative anatomy, should be combined with molecular techniques. Real integrative studies, i.e. those that *equally* integrate different techniques and levels of investigation, are needed. They will both provide biologists with modern skills required to solve old questions and help them to address new problems within the frame of the knowledge gathered by traditional naturalists.

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