COMMENTARY ON *J. GENET*. CLASSIC

Towards a genetic architecture of cryptic genetic variation and genetic assimilation: the contribution of K. G. Bateman

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Introduction

Clearly spurred on by Waddington, Bateman (1959) continued the work on the crossveinless phenocopy in *Drosophila melanogaster* and helped to establish it as a model system in quantitative genetics for threshold traits. In this commentary, I will discuss the context of this work examining the genetic architecture of the crossveinless phenocopy both within the light of current studies of evolutionary genetics, and modern evolutionary-developmental biology. Far from being a historical curiosity, it appears that this phenotype may be a relevant and powerful model system for current studies of cryptic genetic variation and possibly for examining allelic architecture of complex diseases. I will discuss what future research directions will allow for this possibility.

Genetic assimilation

In a series of papers, C. H. Waddington (1942, 1948, 1952, 1953) described a particular model of evolved developmental buffering where development was channelled in one of several particular outcomes. With the appropriate evolutionary forces, the genetic system would evolve such that the phenotype would be 'canalized', that is, the target phenotype would be the most likely outcome even under many common environmental or genetic perturbations. Waddington further suggested that selection for an alternative phenotype would occur in two steps: a change in the threshold for the new trait, followed by a re-canalization under the new system. To demonstrate this idea, Waddington (1952, 1953) selected for a high temperature induced phenotype that mimicked the crossveinless phenotype (phenocopy) in *D. melanogaster*. Waddington observed that the population could respond to selection on this phenotype, demonstrating that there was genetic variation for what was virtually an invariant phenotype under normal environmental conditions. In modern parlance this variation is described as potential or 'cryptic' genetic variation for a trait (Gibson *et al*. 1999; Dworkin *et al*. 2003; Gibson and Dworkin 2004).

While these results were intriguing, the most fascinating finding is that individuals expressing the crossveinless phenotype began to appear in the selected population without the required high temperature stimulus during pupal development (Waddington 1952). Via selection on these individuals, populations of flies with complete penetrance of the crossveinless phenotype were produced. Waddington described this phenomenon as genetic assimilation of the environmentally induced trait, involving the evolution of the threshold for a trait. Waddington followed this up by repeating the experiment for a much more profound phenotypic change, a homeotic transformation of the third thoracic segment to the second thoracic segment, resulting in a transformation of the balancing organ (halteres) to a second set of wings (Waddington 1956).

While Waddington set the stage for the study of canalization, many unanswered questions remained. In particular was a concern that the observed genetic assimilation may have been an artefact of the selection process. Selection for increased penetrance of the phenocopies could have resulted in selection for new mutations that caused the same phenotypic abnormality. If this was the case, then genetic assimilation would not have been the result of selection on standing genetic variation, but on new mutations that occurred during the selection phase. Thus, genetic assimilation would not be the result of many mutations of small effect acting in concert, and resulting in the fixation of the trait, but selection for the rare (and likely deleterious) mutation of large effect.

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Under the guidance of Waddington, K. G. Bateman (1959) repeated and extended the original experiments on the selection of the vein phenocopies in a number of important ways to address this and other questions. First, by repeating the selection experiment for the same phenocopy, the loss of the posterior crossvein (pcvl), in two independently derived populations. In addition, Bateman performed the same selection process, but on a population consisting of genetically identical (isogenic) individuals. Finally, selection experiments were performed on a number of additional venation phenocopies; the loss of the anterior crossvein (acvl) as well as the ectopic expression of additional crossveins.

Bateman did indeed demonstrate that Waddington's initial observations for an increase in pcvl phenocopy penetrance could be replicated in both populations, and that after 15 generations 13% of individuals had the pcvl phenotype without high temperature induction. As Waddington had shown, these genetically assimilated individuals could be selected upon to create lines with complete penetrance even without the environmental stimulus. Most importantly though, Bateman was unable to increase the penetrance of the phenocopy in the isogenic population, and no genetically assimilated line could be generated. These results were inconsistent with the hypothesis that selection on the phenocopy unwittingly also selected upon new mutations of large effect. In effect, this suggested that both the increase in phenocopy frequency, and the genetic assimilation, were the result of selection on the standing genetic variation in the populations. From some genetic analyses between the two independent populations, it was also clear that the same alleles were not being selected upon in each population, consistent with a model of different initial frequencies of the alleles. While it is possible that this was a result of selection for rare alleles of major effect in the population, as opposed to common alleles of a number of genes, further work was largely consistent with the latter proposition (Bateman 1959; Milkman 1960, 1961; Mohler 1965a,b). Indeed, this later work demonstrated that the alleles increasing the susceptibility to the crossveinless phenocopy were geographically widespread, with different populations having different combinations of alleles.

Contrary to Waddington's model for a change in the threshold of a trait followed by re-canalization, Bateman suggested her results favoured a model where there was a change in the mean underlying the effect; in quantitative genetics this is described as liability (Falconer and Mackay 1996). In modern genetic parlance, perhaps the best way to describe Bateman's model, and results is as follows. There are common alleles of a number of genes that can increase the relative risk (relative to individuals without the alleles) for the crossveinless phenocopy via environmental induction. Selection for this phenocopy will increase the individual frequencies of each of these alleles, as well as the joint probability of the 'disease' causing alleles occurring together. These alleles will increase in frequency such that the rare combinations will arise resulting in genetically assimilated individuals for the phenotype. These combinations can then be indirectly selected upon in the genetically assimilated individuals, resulting in populations that are fixed for the crossveinless phenotype without any environmental induction. This sort of hypothesis, which still requires explicit genetic testing, is exactly the sort of formulation that occurs with respect to diseases with complex genetic architecture: in particular, the question of whether a given disease is caused by rare combinations of common alleles (Pritchard 2001; Pritchard and Cox 2002)? Given the amount of effort that has gone into addressing this question for the crossveinless phenotype (Bateman 1959; Milkman 1960, 1961; Mohler 1965a,b), it is worth considering it as an empirical model system for the study of such threshold models for diseases.

One important coda to the selection experiments that Bateman performed was that selection was relaxed on populations as they reached variably increasing penetrance. In populations where the penetrance was not yet 100%, relaxation of selection did result in the gradual decline in the frequency of the phenocopy. This suggests that there must have been some fitness cost associated with the high frequency of the alleles, although this was not specifically addressed. In lines that reached complete penetrance, relaxation of selection did not result in a decrease in the phenotype frequency consistent with the fixation of the alleles causing the phenotypes.

The crossveinless phenocopy as a model system for evolutionary developmental genetics

Instead of relegating all of this research to a bygone era of genetics without genes (or at least molecular biology), it is worth considering the *D. melanogaster* crossveinless phenocopy as a model system to study the evolution of threshold traits and disease, and as a means toward an understanding of cryptic genetic variation. While the phenocopy induction by rearing under high temperatures during pupal development may not have been thought of as being likely to occur under nature, recent work has shown that natural populations do experience such temperatures (Feder *et al*. 1997), and that flies reared in the wild can show a variety of morphological defects triggered by temperature stress (Williams *et al*. 2003).

As mentioned above, the early work on the genetic architecture of this trait suggests that the genetic assimilation is the result of rare combinations of common alleles. Can this be verified, and the responsible polymorphisms found? There are a large number of approaches that allow for the direct estimation of the underlying gene

expression or protein activity, thus providing a direct approach to determining whether the liability (gene expression), and/or the threshold underlying trait expression were the indirect targets of selection. Indeed, it has been observed that the ether induced phenocopy of the wing to haltere transformation is in part a result of reduced expression of the *Ultrabithorax* gene (Gibson and Hogness 1996). If the observed phenotypic changes in the population are due to rare combinations of common alleles, then it is feasible to examine the frequencies of these alleles in natural populations, as well as to examine their individual and joint dynamics under selection. From this a number of questions could be addressed. (1) Are the same alleles responsible for an increase in phenocopy frequency also responsible for the genetic assimilation of the phenotype? (2) Is it one particular combination of naturally occurring alleles that allows for genetic assimilation, or are there many different combinations of a subset of a larger pool of alleles that allow for this phenotype? (3) Why do these alleles persist in the population? Are they simply conditionally neutral variants that allow the expression of the cryptic genetic variation, or is it possible that they have pleiotropic effects elsewhere? While mapping the polymorphisms responsible for a phenotype can be notoriously difficult (Dworkin *et al*. 2005), there are a number of factors that may facilitate this. In addition to all of the general tools for linkage mapping in *Drosophila*, the developmental biology of the crossveins has been explored in depth recently, resulting in a number of excellent candidate genes that could harbour genetic variants for the phenotype (Marcus 2001). While the Egfr signalling pathway seems to play a dominant role in the specification of the longitudinal veins of the wing (Bier 2000), TGF-*b* (Decapentaplegic) signalling appears to be one of the major developmental events for making the crossveins (Ralston and Blair 2005). Indeed, a number of TGF-*b* related signalling molecules seem to have very specific functions in the development of the crossveins. The Decapentaplegic-like ligand glass-bottom boat (*gbb*) causes loss of crossveins in viable mutant combinations (Yu *et al*. 2000). The metalloprotease Tolloid-related (Tlr) appears to be particularly involved with cleaving the Short gastrulation (Sog) protein which releases TGF-*b* ligands from an inhibitory complex. *Tlr* mutants have a pcvl phenotype that is rescued in double mutant combinations with *sog* (Serpe *et al*. 2005). In addition the *crossveinless-2* gene was recently cloned, and found to encode a Sog like protein (Conley *et al*. 2000), and *crossveinless* (*cv*) was found to encode a Twisted gastrulation (Tsg) like modulator of TGF-*b* ligands (Shimmi *et al*. 2005; Vilmos *et al*. 2005). While a large number of potential candidates involved in the formation of the crossvein can be examined, only a few of those appear to fail to complement alleles that increase the penetrance of phenocopy induction. One particular candidate, *detached*, has still not been cloned. However, other genes such as *crossveinless-c* have been cloned recently (Denholm *et al*. 2005), and appear to suggest that a diverse array of developmental functions are involved in crossvein expression. While there is still much important work to be done to understand the genetics of crossvein development, this provides a wide array of candidate genes that may harbour natural variation for sensitivity to phenocopy induction.

Indeed, an approach to re-examining the genetic basis of phenocopy induction should begin by replicating the original selection experiments, followed by examining the changes in allele frequencies for polymorphisms in these candidate genes, as well as SNP's in candidate regions as suggested by earlier work (Waddington, 1953; Bateman 1959; Milkman 1962; Mohler 1965b). In addition, further linkage mapping could be used to confirm some of these candidate genes, while *in-situ* hybridization and candidate gene expression analysis could be used to examine whether particular candidate genes might be involved via the phenocopy induction. These approaches will hopefully allow for the verification of the roles of these candidate genes, which could be followed up by linkage disequilibrium mapping to localize the causal polymorphisms (Dworkin *et al*. 2003). Even without identifying the actual polymorphisms, having several candidate genes will allow a more complete examination of other populations to determine whether alleles for these genes also segregate, and affect phenocopy induction. Indeed, the crossveinless phenocopy may not only be a useful model system for evolutionary developmental genetics, but may provide a future model for complex diseases whose effects are the result of interactions between both genetic and environmental factors.

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