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PERSPECTIVE: FROM MUTANTS TO MECHANISMS? ASSESSING THE CANDIDATE GENE PARADIGM IN EVOLUTIONARY BIOLOGY

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Abstract.—The generation of mutants in model organisms by geneticists and developmental biologists over the last century has occasionally produced phenotypes that are startlingly reminiscent of those seen in other species. Such extreme mutations have generally been dismissed by evolutionary geneticists since the ''modern synthesis'' as irrelevant to adaptation and speciation. But only in recent years has information on the molecular bases of mutant phenotypes become widely available, and thus work on testing the relevance of such extreme mutations to the generation of phylogenetic diversity has just begun. Here we evaluate whether evolutionary mimics are, in fact, useful for pinpointing the genetic differences that distinguish morphological variants generated during evolution. Examples come from both plants and animals, and range from intraspecific to interordinal taxonomic ranges. The use of mutationally defined candidate genes to predict evolutionary mechanisms has so far been most fruitful in explaining intraspecific variants, where it has been effective in both plants and animals. In several cases these efforts were facilitated or supported by parallel results from quantitative trait loci studies, in which natural alleles controlling continuous variation in developmental model organisms were mapped to mutationally defined genes. However, despite these successes the approach's utility seems to rapidly decay as a function of phylogenetic distance. This suggests that the divergence of developmental genetic systems is great even in closely related organisms and may become intractable at larger distances. We discuss this result in the context of what it teaches us about development, the future prospects of the candidate gene approach, and the historical debate over process in micro- and macroevolution.

Key words.—Development, evolution, mutation, phenocopy, quantitative trait loci, saltation.

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Developmental geneticists study the processes that create organismal form by generating mutations that disrupt that form. It has been noted by many that these same developmental processes must at some level be the same ones that are modified during evolution (e.g., De Beer 1958; Gould 1977; Raff and Kaufman 1983). However, the degree to which the specific genes identified by developmental mutants are identical to those upon which evolution depends has been a subject of great controversy since the integration of evolution and genetics. The purpose of this paper is to evaluate new data from studies that have directly tested, by intention or not, the connection between developmental genetics and the evolutionary diversification of form. In particular, we assess the extent to which mutations causing one taxon to mimic another, which we dub phylomimicking mutations, define evolutionarily relevant loci.

The relevance of laboratory mutants to evolution has generally been discussed as part of the larger debate over whether the evolution of new species is slow and gradual or erratic and saltational. For an excellent summary of the place of mutants in this debate, we direct the reader to the recent Perspectives piece by Stern (2000). In short, the ''modern synthesis'' endorsed the idea of speciation occurring via the accumulation of many small changes, adding up over time to eventual species-level distinctions (Mayr and Provine 1980). The most stalwart opponent to this idea was Richard Goldschmidt, who saw in both environmental and genetic perturbations of development the essential means to to span otherwise ''bridgeless gaps'' between species (Goldschmidt 1940). As Goldschmidt himself pointed out (1940, p. 310), he was not the first to propose that the evolution of development could be saltational, and like his predecessors he believed that radical mutations like those demonstrated in genetics laboratories pointed to a necessary alternative to slow allopatric speciation. Conversely, his gradualist opponents saw the accumulating evidence of small-effect muta-

tions and populational differentiation as being directly contrary to the notion of rapid developmental evolution through mutations of large effect, and thus rejected any relevance of extreme mutants to the differentiation of species (Mayr and Provine 1980, pp. 20, 131, 420–421). The insistence of both camps in regarding the problem in dichotomous terms reinforced the gulf between evolutionary theorists and developmental geneticists. In hindsight, however, the mutual exclusivity of these ideas is far from apparent. Indeed, a recent model of adaptation (Orr 1998) suggests that the initial steps of an adaptive shift involve selection on alleles with large effects, followed by more gradual optimization via alleles of smaller effect. The studies we discuss below lend support to the idea that phylomimicking mutations can identify loci that respond to selection during adaptation at either phase without insisting that they enabled the creation of a new species in a single generation.

In the 1980s, interest in the evolutionary significance of mutationally defined genes exploded as their molecular identities, mechanisms of regulation, and conservation across taxa were revealed. Being unfamiliar or unimpressed with the carefully wrought evolutionary theory, empirically minded developmental biologists began testing a simplistic but irresistible hypothesis: that phylomimicking mutations define loci with key roles in the evolutionary differentiation of the taxa in question—despite the fact that the mainstream of evolutionary genetic thinkers had decades earlier succeeded in discrediting the relevance of such mutations to evolution as part of the defeat of Goldschmidtian saltationalism. Arguing the case for the special role of development in macroevolution, Gilbert et al. (1996) state in their abstract that, ''In this nascent synthesis, macroevolutionary questions are not seen as being soluble by population genetics, and the developmental actions of genes involved with growth and cell specification are seen as being critical for the formation of higher taxa.''

Similar to Waddington (1942, 1956) and Goldschmidt (1940), Stebbins and Basile (1986) argued that manipulation of the physiology of a species through either genetic mutations or nongenetic manipulations can reveal a potential plasticity of form that can bridge the gaps between differentiated species. The nongenetic approach, which they call ''phyletic phenocopy,'' pertains to experimental manipulation of a developing organism (e.g., via temperature, hormones, medium composition) that leads to phenocopies of other taxa. The significance of such plasticity is its revelation of the potential for developmental systems to produce novel outcomes that may fall under direct, hard-wired genetic control if selection for such an outcome exists. More recently, DeSalle and Carew (1992) and Stark et al. (1999) invoked the phyletic phenocopy notion to refer to mutations that mimic related taxa. (We suggest that to avoid confusion ''phyletic phenocopy'' be reserved for the nongenetic sense in which it was originally intended.) It is such phylomimicking mutations that strike many (the authors included) as excellent starting points for the investigation of the evolution of development. We note that of course this is not a new idea, only one made more amenable to experimentation with the advent of molecular genetics.

As the comparative work by developmental biologists gathered momentum, quantitative geneticists were empirically determining the number and (recently) the identities of genes that control a selected or naturally occurring difference in form using quantitative trait loci (QTL) approaches. Remarkably, these two intellectually distinct approaches, which can be seen to descend from the two opposing camps discussed above, have converged on a surprisingly harmonious result. Numerous traits have been shown to be largely determined by a relatively small number of QTL (usually a few to a dozen or so), which when precisely mapped in model organisms often correspond to genes familiar to developmental geneticists. Thus, either path can implicate the same loci in evolution. Below we review studies that have intentionally used mutationally defined candidate genes to probe the mechanisms of evolutionary variation, and also discuss QTL data that have implicated mutationally defined genes. Finally, we discuss what these first few studies have revealed about potential differences in how development evolves within and between species.

The Domestication of Maize

Perhaps the most spectacular case in which laboratory mutants have been used to identify major loci responsible for a morphological species difference is the domesticated crop maize and its wild ancestor teosinte. The pioneering work of Doebley and colleagues has over the last decade confirmed a model proposed by Beadle (1939), stating that the differences between maize and teosinte are caused by changes in five genes that have taken place during the domestication of maize (reviewed by White and Doebley 1998). Two of the trait differences between maize and teosinte have each been found to be caused in large part by alleles at single major loci, *teosinte branched 1* (Doebley et al. 1995) and *teosinte glume architecture 1* (Dorweiler et al. 1993). These loci were identified by a two-step process. The first step involved QTL mapping, which identified regions of the genome where major genetic factors underlying maize-teosinte differences resided (Doebley and Stec 1991). Then known maize candidate mutants that mapped to those regions having effects similar to the differences between maize and teosinte (i.e., which made maize plants look more like teosinte) were confirmed to be specifically involved by marker-assisted backcrossing. The maize *tb1* locus encodes a putative transcriptional regulatory protein expressed in the small axial organs of the developing maize stalk. In *tb1* mutants of maize apical dominance is lost and instead many long axial branches form, as in teosinte (Doebley et al. 1997). This led to the hypothesis that *tb1* functions as a repressor of apical growth in maize. Because no fixed amino acid substitutions differentiate maize and teosinte *tb1* alleles, the difference appears to be at the level of regulation of expression. Consistent with this, Doebley et al. (1997) found that the maize *tb1* allele is expressed at about twice the level of the teosinte allele. Interestingly, *tb1* is a homologue of the *Antirrhinum majus* (snapdragon) gene *CYCLOIDEA*, which is involved in repression of axial growth in flower development and has been implicated in the evolution of floral morphology (see below).

The success of the candidate gene approach in the maize-teosinte story is undoubtedly due in part to two special factors

behind the evolution of maize. First, maize was derived from teosinte extremely recently, during the last 10,000 years (White and Doebley 1998). In fact, maize and teosinte show virtually complete interfertility and are therefore members of the same biological species. Thus, there has been very little time for a great number of substitutions to become fixed, other than those selected to confer agriculturally important qualities to domestic maize (although QTL mapping studies suggest that substitutions of modifiers have occurred; Doebley and Stec 1991). This means that atavistic mutants that revert maize morphology may be more common and more easily identified at the molecular level than if the two taxa had been isolated for a longer period, in which many modifiers could have arisen throughout the genome (perhaps involving epistatic effects) and larger numbers of phenotypically significant substitutions had been fixed at each developmentally important locus.

Second, the derivation of maize by artificial selection probably means that particular types of mutant alleles were favored that may not be favorable in cases of natural selection in the wild. Such dominant, large-effect mutants (Darwin's ''sports'') are seen in many species grown by humans but because they usually confer fitness reductions are found only at low frequencies, if at all, in nature. Therefore, although morphological mutants of the sort favored in maize during its domestication have been shown to occur in natural populations of other organisms (e.g., *Drosophila;* Spencer 1957), their deleterious effects in most cases probably outweigh their ability to be favored by most forms of natural selection. Nevertheless, until more data are obtained on differences among wild species, the maize-teosinte divergence provides an excellent model and example for geneticists interested in the evolution of form.

Aspects of Flowering

The evolution of flowers to meet a wide range of life-history and pollination strategies is one of the most splendid adaptive triumphs (Darwin 1877, 1885). The stunning diversity of angiosperms has evolved recently relative to the Cambrian explosion of metazoan bauplans, making key events of developmental evolution potentially more accessible to genetic analysis in plants than in animals. Recent studies have sought to link the genetic analysis of plant form in model species such as *Arabidopsis thaliana, A. majus,* and tobacco with intra- and interspecies diversity, and have been remarkably successful in the former. Two major aspects of angiosperm development have been explored comparatively—the morphology of the individual flower and the control of the timing and architecture of the inflorescence as a whole.

Floral Symmetry

Dicot flowers are either radially symmetrical (actinomorphic) or bilaterally symmetrical (zygomorphic), and typically whole families of plants are characterized by having one or the other arrangement (Bhattacharyya and Johri 1998). Radial symmetry (actinomorphy) is the primitive angiosperm condition (Iwatsuki and Raven 1997), but zygomorphy has evolved multiple times and also been lost during floral evolution (Theissen 2000). The familiar ornamental *Antirrhinum* has strongly zygomorphic flowers, but their bilateral symmetry can be disrupted by mutations in the *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*) genes. Either mutation alone partially radializes flowers, but double mutants produce perfectly actinomorphic flowers (Carpenter and Coen 1990; Almeida et al. 1997). In such radialized flowers all petals assume a ventral identity because of the absence of the dorsally expressed *CYC* (and perhaps *DICH*) gene product (Luo et al. 1996). Mutants disrupting a set of ventrally expressed genes also radialize *Antirrhinum* flowers (Almeida et al. 1997).

Radializing mutants of zygomorpic species allow one to ask if the genes disrupted in them were key innovations in the evolution of bilateral symmetry. *CYC* homologues exist in both monocots (Doebley et al. 1997) and actinomorphic dicots (Cubas et al. 1999a), suggesting that their origin as genes predates the evolution of zygomorphy. Therefore, their regulation or biochemical role may have changed in zygomorphic or secondarily actinomorphic taxa. A recent success in using *CYC* to explain floral diversity relates to a naturally occurring actinomorphic population of the otherwise zygomorphic toadflax *Linaria vulgaris* (Cubas et al. 1999b). Hypothesizing that this population resembles *CYC* mutants of *Antirrhinum* because of a similar loss-of-function allele, they demonstrated that this is the case. Interestingly, the nature of the allele is not a sequence mutation, but rather a heritable methylation of the entire locus (Cubas et al. 1999b). Thus, although developmental genetics provided an invaluable clue as to which gene might explain natural variation, the nature of the allele itself was not predictable. It remains unknown whether *CYC* can also explain variation in floral symmetry at higher taxonomic levels, although Thiessen (2000) has expressed doubts.

Timing and Determinacy of Flowering

Unlike floral morphology, which is diagnostic for large families of angiosperms, the timing and determinacy of the inflorescence as a whole is highly variable among closely related species, and even within species (Bhattacharyya and Johri 1998; Kole et al. 2001). Both mutational genetic and QTL approaches have been used to identify loci important for controlling these key characteristics. These loci have also been shown recently to be important in distinguishing different inflorescence architectures.

The *Antirrhinum* gene *CENTRORADIALIS* (*CEN:* Bradley et al. 1996 and references therein) and its *Arabidopsis* homologue *TERMINAL FLOWER 1* (*TFL1;* Shannon and Meeks-Wagner 1991; Bradley et al. 1997; Ohshima et al. 1997) are both required for the indeterminate flowering seen in these species and are thought to directly antagonize expression of the floral meristem identity genes *FLO* and *LFY,* respectively, in the apical meristem (Ma 1998). This raises the possibility that if species producing terminal flowers have *CEN/TFL1* homologues, they may express them differently. Amaya et al. (1999) tested this hypothesis in tobacco, a determinately flowering solonaceous species. They find that expression of *CET2* and *CET4,* the endogenous *CEN/TFL1*-like genes of tobacco, is also complementary to that of *NFL,* its *FLO/LFY* homologue. But unlike the indeterminate models,

CET2 and *CET4* are expressed in the axillary, but not apical, meristems. Their role in tobacco may therefore be to allow some vegetative growth in axial shoots prior to their undergoing terminal flower development. The lack of apical expression of *CET2/CET4* correlates with early activation of *NFL* in this region, which by analogy to other species may repress them and allow terminal flower development at the shoot apex.

If the absence of *CET2/CET4* in the apical meristem is responsible for determinate growth, then their ectopic overexpression should convert flowering to an indeterminate mode. As an approximation of this, Amaya et al. (1999) ectopically expressed both *CEN* and *TFL1* in tobacco. Only the former produced an effect, which was a lengthened vegetative phase of development eventually followed in most cases by terminal flowering. One reasonable interpretation of this result is that in tobacco the role of *CEN* homologues is to promote vegetative growth, rather than inflorescence growth. If this is correct, then the broader role for all *CEN* homologues may be to extend development of a meristem, be it vegetative or inflorescent. Thus, the work of Amaya et al. (1999) suggests that although *CEN*-like genes may underlie a key difference in architecture between tobacco and *Antirrhinum,* it is not floral determinacy per se. This interpretation is supported by the finding that *SELF PRUNING* (*SP*), another *CEN* homologue from tomato, is required for normal vegetative growth (Pnueli et al. 1998). Another lesson emerging from these studies is that comparisons between species with different architectures can allow a fuller understanding of gene function than is possible in a single model species.

Two other studies have connected floral architecture mutations to natural variation indirectly via a QTL approach. Swarup et al. (1999) used different ecotypes of *Aribodopsis* to identify six QTL that work additively to control variation in circadian rhythms. One QTL, *ANDANTE,* was subsequently identified as allelic to *FLOWERING LOCUS C* (*FLC*), a MADS-box transcription factor gene already cloned (Michaels and Amasino 1999) via mutations that primarily affect flowering timing. Analysis of *FLC* mutants revealed that they do, in fact, have a circadian defect (Swarup et al. 1999). Two other QTL also map very near other flowering time genes, supporting models proposing a general requirement for an intact circadian system in flowering regulation (e.g., Millar 1999). In this case the use of natural genetic variation prompted the reexamination of severe mutagen-induced alleles, revealing previously unknown processes in which a gene participates. QTL approaches have also been successful in linking mutations in model systems to natural variation in floral timing in different species. Kole et al. 2001, working in the crucifer *Brassica rapa,* identified QTL for flowering time that distinguish annual and biennial strains. One of these mapped precisely to the *B. rapa* homologue of *FLC.* Whether species that are exclusively annual or biennial also are distinguished by variation in *FLC* homologues remains to be seen, however.

New Uses for Old Genes: The Hox Complex and the Notch Pathway

Many of our insights into the evolution of animal form have come from studying conserved transcriptional regulatory proteins. By far the most celebrated case has been the Hox complex genes, a family of transcription factors that regulate anterior posterior segment identity and that exist in an evolutionarily conserved tandem array in most bilaterians (de Rosa et al. 1999). These genes were first characterized by Lewis and colleagues as homeotic mutants in *Drosophila,* whose gain and loss of function phenotypes caused spectacular transformations of entire segments toward the identity of other segments, such as an antenna to a leg. In his very influential review, Lewis (1978) extrapolated from his studies of homeotic mutations in *Drosophila* Hox genes to hypothesize that evolution of these genes may have played a key role in the morphological evolution of insect body plans. In particular, genes that suppressed leg formation had evolved in the myriapod ancestor of insects to cause the loss of legs in abdominal segments. Further, genes that promoted the reduction of hindwings toward the tiny dipteran halteres must have arisen in the four-winged ancestor of flies (reviewed by Carroll 1995). These ideas formed the basis of the first models for the role of Hox genes in the evolution of animal body patterns, a research program that is still thriving today.

Warren et al. (1994) tested Lewis's hypotheses by examining the role of Hox genes in two body-plan differences between dipteran and lepidopteran insects: the absence of abdominal prolegs in dipteran larva and the reduction of hindwings to dipteran halteres. Warren et al. asked if the differences between dipterans and lepidopterans could be due to simple changes in the domains of Hox gene expression and obtained different answers for the two different traits. In the case of larval abdominal prolegs, expression of the Hox genes *Ubx* and *Abd-a* appears to have changed to suppress these appendages in dipterans. This probably takes place through direct Hox repression of the limb selector gene *Distal-less* and other genes in the limb program (Vachon et al. 1992). In lepidopterans, Warren et al. (1994) showed that *Ubx* and *Abd-a* expression is suppressed in the abdominal limb primordia before and during the formation of these primordia, as indicated by *Distal-less* expression. Subsequently, many other comparative studies of gene expression patterns have also shown that changes in domains of Hox gene expression are correlated with diversification in segmental architecture throughout arthropods (e.g., Averof and Akam 1995; Averof and Patel 1997).

The case of dipteran reduction of hindwings to halteres is not as simple, however. Studies of *Ubx* mutants in *Drosophila* indicate that *Ubx* expression is needed to suppress development of wings in the third thoracic segment (T3), where halteres form instead. This led to the hypothesis that insects bearing the ancestral two pairs of similar or identical wings may not express *Ubx* in T3, thus allowing the T3 flight appendages to develop under the same program as the T2 wings. However, Warren et al. (1994) showed that butterflies express *Ubx* throughout T3, just as *Drosophila* do. Therefore, the evolution in Diptera of reduced T3 wings did not take place by simply changing Hox gene expression. Instead, changes must have arisen in genes regulated by *Ubx,* which are involved in T3 flight appendage morphology (see Weatherbee et al. 1998).

Although the phenotypes of Hox gene mutations are often radical, there is now mounting evidence that these ''master

control genes'' have subtle and more pervasive roles in morphological variation between and even within species. A recent study by Stern (1998) shows that *Ubx* expression differences between closely related species of *Drosophila* cause a species difference in leg trichome (cell hair) patterns. Also, in an artificial selection experiment inspired by Waddington's (1956) experiments on genetic assimilation of phenocopies, Gibson and Hogness (1996) showed that naturally occurring regulatory polymorphisms in the *Ubx* locus underlie heritable differences in predisposition to produce a *Ubx* phenocopy in *Drosophila* exposed to ether vapor during development.

Many other genes with mutationally defined roles in development also show phenotypically significant variation in natural populations. The most expansive body of work on this subject has been by Mackay, Long, and their colleagues studying the quantitative genetics of bristle number, a continuously varying trait in *D. melanogaster* (reviewed by Mackay 1995). Mackay's group has undertaken artificial selection experiments beginning with highly genetically variable populations consisting of naturally occurring alleles. When high- and low-bristle-number lines were analyzed after several or many generations of selection, a finite number of QTLs, many with large effects (accounting for greater than 5% of the trait variance), were found. A majority of these QTLs map to positions in the genome where loci with roles in bristle development reside (Long et al., 1995, 1996). Further analyses of particular QTLs and other candidate genes have confirmed that alleles of several *Notch* pathway components indeed harbor polymorphisms that contribute significantly to naturally occurring variation in bristle number, including *Delta* (the signalling ligand for the *Notch* receptor), *Hairless* (a transcriptional corepressor), and *scabrous* (a likely secreted antagonist of *Notch* signaling; Lai et al. 1994; Long et al. 1998; Lyman and Mackay 1998; Lee et al. 2000). Many other candidate loci involved in aspects of bristle development other than the *Notch* pathway components (e.g., *extramacrochaetae*, *hairy*, and the *achaete-scute* complex) have also been implicated in these studies, and it is likely that similar naturally occurring variants in these genes also contribute to variation in the wild.

These experiments on naturally occurring *Drosophila* alleles suggest that polymorphisms at developmental regulatory loci have a significant capacity to contribute to morphological evolution when individuals and populations experience environmental perturbations or novel selective regimes. The presence of this type of variation at many loci can also be uncovered in experiments where laboratory-generated Mendelian mutants are crossed into different naturally occurring genetic backgrounds. Strikingly, Gibson and colleagues have demonstrated that the effects of *Drosophila* eye and homeotic mutants can vary tremendously, ranging from complete suppression of the mutant phenotype to effects even more extreme than null mutants (Polaczyk et al. 1998; Gibson et al. 1999). These modifying factors, which are highly epistatic and vary greatly in the magnitude of their effects, contribute little to phenotypic variance under normal conditions but constitute a mechanism by which phenotypic changes of large effect may occur in evolution. Although more experiments of this type are needed, it is very possible that a microevolutionary process at the genetic level, that is the accumulation and maintenance of molecular polymorphism throughout the genome, may underlie more macrolevel phenotypic changes, such as those seen in the fossil record.

The Evolution of Behavior and Sex in Nematodes

Caenorhabditis elegans, like *Drosophila*, is an animal model of development for which mutations have been isolated that affect nearly all tissues and organs (Wood 1988; Riddle et al. 1997). Nematodes have only recently become a system for studying the evolution of development, but significant progress already has been made. At the intraspecific level, a recent success was achieved in explaining natural intraspecific variation of aggregation behavior (de Bono and Bargmann 1998; Thomas 1998). Different wild isolates of *C. elegans* are either clumping or solitary in their foraging behavior (Hodgkin and Doniach 1997). The N2 wild-type strain, normally solitary, can be rendered clumping by induction of recessive mutations at the *npr-1* locus. Cloning of *npr-1* by transformation rescue revealed it to be a transmembrane protein of the neuropeptide Y receptor family (de Bono and Bargmann 1998). When *npr-1* gene sequences from clumping or solitary populations were compared, amino acid position 215 varied consistently between either valine (in solitary species) or phenylalanine (in clumping species). This substitution alone can determine whether an *npr-1* transgene is able to rescue *npr-1* mutants. Thus, it is likely that *npr-1* encodes a receptor that when maximally active represses clumping behavior, and thus the allele responsible for clumping behavior in some wild isolates represents a less active form.

The most striking feature that varies between species of *Caenorhabditis* is mating system. All species produce two sexes, one of which is male. But the egg-producing sex is either an obligately outcrossing female (the ancestral condition) or a self-fertile protandrous hermaphrodite, a derived state that has evolved multiple times in the Rhabditidae (Fitch and Thomas 1997). One hermaphrodite cannot inseminate another and is essentially a somatic female that transiently makes sperm to fertilize the subsequently produced eggs in the absence of males. The evolution of hermaphroditism therefore required acquisition of mechanisms to independently regulate germline and somatic sex. Candidates for these mechanisms are suggested by numerous mutations that disrupt the sperm-oocyte switch in the *C. elegans* hermaphrodite (Puoti et al. 1997). Three classes of mutations cause hermaphrodites to resemble females of related species by preventing spermatogenesis in hermaphrodites but not in males. All three are thought to cause misregulation of *tra-2*, a key regulator of both somatic and germline sex. One class, the dominant *tra-2*(*gf*) alleles (Doniach 1986), disrupts a translational control element in the 3′ untranslated region (Goodwin et al. 1993). Another, the *tra-2*(*mx*) alleles, is defined by missense mutations in the cytoplasmic C-terminus of the TRA-2 protein (Kuwabara et al. 1998). The third, the recessive *fog-2* mutants, is thought to remove a factor that participates in the repressor complex that binds the 3′ UTR site defined by the *tra-2*(*gf*) alleles (Schedl and Kimble 1988; Clifford et al. 2000).

Recently, Haag and Kimble (2000) looked for the regu-

latory sites defined by the *tra-2*(*gf*) and *tra-2*(*mx*) mutations in the *tra-2* homologue of *Caenorhabditis remanei*, the closest male-female relative of *C. elegans*. They found that, despite extensive sequence divergence of the homologues, the two sites are conserved. They suggest that *tra-2* posttranscriptional regulatory elements were not key targets of selection during the evolution of mating system seen in these species or that, if they were, the outcome was a subtle quantitative or qualitative change rather than a wholly novel mechanism. An excellent candidate for a qualitative difference in *tra-2* regulation in hermaphrodites and females is *fog-2*, which appears to have recently evolved via genomic amplification of a large gene family (Clifford et al., 2000). It is still unclear whether any of the phylomimicking mutations identified in *C. elegans* will turn out to reveal key players in mating system evolution. As discussed by Haag and Kimble (2000), it is certainly possible that although they identify mechanisms necessary for hermaphrodite development, they are not sufficient to explain its evolution from a female ancestor.

Conclusions

In their review, Palopoli and Patel (1996) challenged population geneticists and developmental biologists to undertake studies that would link their disciplines into a new, more empirical evolutionary genetics. Many of the studies discussed above have accomplished this by investigating the role of mutationally defined developmental genes in the evolution of both discrete and continuously varying traits. These studies have frequently succeeded in associating variation in these genes with intra- and interspecific morphological change, indicating that they indeed play roles in evolution, but in many cases not those originally inferred from their (typically null) laboratory mutant phenotypes. In the continuously varying trait of bristle number in *Drosophila*, alleles harboring minor genetic changes in noncoding regions contribute significant but subtle effects that accumulate as populations and species diverge. Slight molecular changes, such as single nucleotide substitutions and small indels, at these loci can have measurable effects in isogenic backgrounds in the laboratory. But the loci studied so far also show evidence of multiple polymorphisms, which may contribute independent effects (Mackay and Langley 1990; Long et al. 1998, 2000; Lyman et al. 1999). Furthermore, although there is not an infinite number of genes involved, many more than a few genes can potentially contribute to selection for quantitative variation. Nuzhdin et al. (1999), have mapped no less than 26 bristle number QTLs on two of the three major *Drosophila* chromosomes. Thus, the picture of how variation in developmentally important genes contributes to continuous phenotypic evolution is far more complicated than originally envisaged from laboratory mutant phenotypes. Many genes may be involved in causing even subtle trait differences, and the alleles at those genes do not typically encode large changes in gene function, such as nulls or neomorphs, but instead involve minute changes, probably frequently in regulatory DNA. Add to this picture the observation that these types of alleles frequently exhibit strong genotype-by-environment, sex-limited, and epistatic interactions (Gurganus et al. 1999; Vieira et al. 2000), and our view of the genetic mechanisms underlying phenotypic change becomes even a further cry from Goldschmidt's hopeful monsters.

One might reasonably suppose, however, that the genetic architecture of changes in discrete characters might be simpler. In these cases, how much explanatory power has the incorporation of developmental genetics actually provided? The studies of *Linaria* floral shape, maize domestication, *Brassica* flowering time, and *C. elegans* foraging discussed above suggest that for predicting the mechanisms distinguishing intraspecific variants, it works quite well. But sibling-species comparisons have yielded mixed results, and at greater taxonomic levels the mechanisms distinguishing forms have generally been opaque. Why does the success of the candidate gene approach fall off with phylogenetic distance? For one, even supposedly conservative genes like those encoding actins can undergo rapid evolution in number and tissue-specificity in taxa that are morphologically very similar (Kissinger et al. 1997; Kissinger and Raff 1998). Second, a naive analysis of mutant phenotype in a single model species can lead one to be overly optimistic. For example, the radialized mutants of *Antirrhinum* discussed above appear to disrupt the balance between opposing dorsal and ventral morphogenetic systems, both of which may be unique in a particular zygomorphic lineage. Thus, these mutations may confer only coincidental similarity to other taxa. Finally, we also know that the genetic specification of highly similar homologous traits in different species can vary significantly (Sternberg and Felix 1997; Meise et al. 1998; Sommer et al. 1998; Kramer and Irish 1999). This suggests that once species are reproductively isolated, their developmental genetic systems diverge in ways that may or may not affect phenotype, analogous to macromolecular sequence divergence. Given this, we should instead expect that the architecture of interspecies differentiation, even of discrete characters, will be quite complex because change occurs in pathways via both adaptive and neutral processes. Sorting out the fraction of divergence that is causal of phenotypic variation will thus become increasingly difficult as the time since the last common ancestor increases.

It is also worth noting that even for characters whose evolution is genetically tractable, the initial choice of model taxon can greatly bias the evolutionarily variable characters that can be revealed through mutational genetics. For example, had a naturally clumping strain been chosen as the standard wild-type strain of *C. elegans* instead of the solitary N2, *npr-1* mutants could not have been isolated because the starting strain would already possess the phenotype produced by gene knockout. Likewise, if a gonochoristic species had been chosen instead of the androdioecious *C. elegans* in screening for sex determination mutants, the elegant genetics that revealed the architecture of the sperm-oocyte switch could not have been performed. In general, the polarity of transformations that can be mimicked by loss-of-function mutants is fixed, and thus only a limited set of phyletic mimics are possible. Perhaps even more importantly, transformations that require as few as two loci to mimic will be exponentially more difficult to discover through mutational analysis.

Despite the above challenges, the emerging harmony between mutational and population genetic approaches indicates that the two once disparate fields of developmental genetics and evolutionary genetics are merging, a possibility

not forseen by the builders and opponents of the modern synthesis. Such a merger shows us a way out of the gradual versus saltational dichotomy by allowing for developmental control genes to have both massive and subtle effects on phenotype. And it should also be clear that even when interspecies comparisons based on candidate genes fail to explain how they differ, they do generally reveal something fundamental about the developmental system of the model organism. Thus, although this review has stressed the ability of a developmental perspective to illumine evolution, clearly the converse is also true.

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Literature Cited

Almeida, J., M. Rocheta, and L. Galego. 1997. Genetic control of flower shape in *Antirrhinum majus.* Development 124: 1387–1392.

Amaya, I., O. J. Ratcliffe, and D. J. Bradley. 1999. Expression of CENTRORADIALIS (CEN) and CEN-like genes in tobacco reveals a conserved mechanism controlling phase change in diverse species. Plant Cell 11:1405–1418.

Averof, M., and M. Akam. 1995. Hox genes and the diversification of insect and crustacean body plans. Nature 376:420–423.

Averof, M., and N. Patel. 1997. Changes in Hox gene regulation associated with the evolution of specialised crustacean appendages. Nature 382:682–686.

Beadle, G. W. 1939. Teosinte and the origin of maize. J. Hered. 30:245–247.

Bhattacharyya, B., and B. M. Johri. 1998. Flowering plants: taxonomy and phylogeny. Narosa Publishing House/Springer-Verlag, New Dehli.

Bradley, D., R. Carpenter, L. Copsey, C. Vincent, S. Rothstein, and E. Coen. 1996. Control of inflorescence architecture in *Antirrhinum.* Nature 379:791–797.

Bradley, D., O. Ratcliffe, C. Vincent, R. Carpenter, and E. Coen. 1997. Inflorescence commitment and architecture in *Arabidopsis.* Science 275:80–83.

Carpenter, R., and E. S. Coen. 1990. Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus.* Genes Dev. 4:1483–1493.

Carroll, S. B. 1995. Homeotic genes and the evolution of arthropods and chordates. Nature. 376:479–485.

Clifford, R., M. H. Lee, S. Nayak, M. Ohmachi, F. Giorgini, and T. Schedl. 2000. FOG2, a novel F-box containing protein, associates with the GLD-1 RNA binding protein to direct male sex determination in the *C. elegans* hermaphrodite germline. Development 127:5265–5276.

Cubas, P., N. Lauter, J. Doebley, and E. Coen. 1999a. The TCP domain: a motif found in proteins regulating plant growth and development. Plant J. 18:215–222.

Cubas, P., C. Vincent, and E. Coen. 1999b. An epigenetic mutation responsible for natural variation in floral symmetry. Nature 401: 157–161.

Darwin, C. 1877. The different forms of flowers on plants of the same species. John Murray, London.

———. 1885. The various contrivances by which orchids are fertilised by insects. John Murray, London.

De Beer, G. 1958. Embryos and ancestors. Clarendon Press, Oxford, UK.

de Bono, M., and C. I. Bargmann. 1998. Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans.* Cell 94:679–689.

de Rosa, R., J. K. Grenier, T. Andreeva, C. E. Cook, A. Adoutte, M. Akam, S. B. Carroll, and G. Balavoinne. 1999. Hox genes in brachiopods and protostome evolution. Nature 399:772–776.

DeSalle, R., and E. Carew. 1992. Phyletic phenocopy and the role of developmental genes in morphological evolution in the Drosophilidae. J. Evol. Biol. 5:363–374.

Doebley, J., and A. Stec. 1991. Genetic analysis of the morphological differences between maize and teosinte. Genetics 129: 285–295.

Doebley, J., A. Stec, and C. Gustus. 1995. *teosinte branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. Genetics 141:333–346.

Doebley, J., A. Stec, and L. Hubbard. 1997. The evolution of apical dominance in maize. Nature 386:485–488.

Doniach, T. 1986. Activity of the sex-determining gene *tra-2* is modulated to allow spermatogenesis in the *C. elegans* hermaphrodite. Genetics 114:53–76.

Dorweiler, J., A. Stec, J. Kermicle, and J. Doebley. 1993. *Teosinte glume architecture 1*: a genetic locus controlling a key step in maize evolution. Science 262:233–235.

Fitch, D. H. A., and W. K. Thomas. 1997. Evolution. Pp. 815–850 *in* D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Priess, eds. *Caenorhabditis elegans.* Vol. II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Gibson, G., and D. S. Hogness. 1996. Effect of polymorphism in the *Drosophila* regulatory gene *Ultrabithorax* on homeotic stability. Science 271:200–203.

Gibson, G., M. Wemple, and S. van Helden. 1999. Potential variance affecting homeotic *Ultrabithorax* and *Antennapedia* phenotypes in *Drosophila melanogaster.* Genetics 151:1081–1091.

Gilbert, S. F., J. M. Opitz, and R. A. Raff. 1996. Resynthesizing evolutionary and developmental biology. Dev. Biol. 173: 357–372.

Goldschmidt, R. B. 1940. The material basis of evolution. Yale Univ. Press, New Haven, CT.

Goodwin, E. B., P. G. Okkema, T. C. Evans, and J. Kimble. 1993. Translational regulation of *tra-2* by its 3′ untranslated region controls sexual identity in *C. elegans.* Cell 75:329–339.

Gould, S. J. 1977. Ontogeny and phylogeny. Belknap/Harvard Univ. Press, Cambridge, MA.

Gurganus, M. C., S. V. Lyman, J. W. Leips, and T. F. Mackay. 1999. High-resolution mapping of quantitative trait loci for sternopleural bristle number in *Drosophila melanogaster.* Genetics 152:1585–1604.

Haag, E. S., and J. Kimble. 2000. Regulatory elements required for development of *Caenorhabditis elegans* hermaphrodites are conserved in the *tra-2* homologue of *C. remanei,* a male/female sister species. Genetics 155:105–116.

Hodgkin, J., and T. Doniach. 1997. Natural variation and copulatory plug formation in *Caenorhabditis elegans.* Genetics 146: 149–164.

Iwatsuki, K., and P. H. Raven. 1997. Evolution and diversification of land plants. Springer-Verlag, Tokyo.

Kissinger, J. C., and R. A. Raff. 1998. Evolutionary changes in sites and timing of actin gene expression in embryos of the direct-and indirect-developing sea urchins, *Heliocidaris erythrogramma* and *H. tuberculata.* Dev. Genes Evol. 208:82–93.

Kissinger, J. C., J. Hahn, and R. A. Raff. 1997. Rapid evolution in a conserved gene family: evolution of the actin gene family in the sea urchin *Heliocidaris* and related genera. Mol. Biol. Evol. 14:654–665.

Kole, C., P. Quijada, S. D. Michaels, R. M. Amasino, and T. C. Osborn. 2001. Evidence for homology of flowering-time genes *VFR2* from *Brassica rapa* and *FLC* from *Arabidopsis thaliana.* Theor. Appl. Genet. *In press.*

Kramer, E. M., and V. F. Irish. 1999. Evolution of genetic mechanisms controlling petal development. Nature 399:144–148.

Kuwabara, P. E., P. G. Okkema, and J. Kimble. 1998. Germ-line

regulation of the *Caenorhabditis elegans* sex-determining gene *tra-2*. Dev. Biol. 204:251–262.
Lai, C., R. F. Lyman, A. D. Long, C. H. Langley, and T. F. C. Mackay. 1994. Naturally occurring variation in bristle number and DNA polymorphisms at the scabrous locus of *Drosophila melanogaster*. Science 266:1697–1702.
Lee, E. C., S. Y. Yu, and N. E. Baker. 2000. The *scabrous* protein can act as an extracellular antagonist of *Notch* signalling in the *Drosophila* wing. Curr. Biol. 10:931–934.
Lewis, E. B. 1978. A gene complex controlling segmentation in *Drosophila*. Nature 276:565–570.
Long, A. D., S. L. Mullaney, L. A. Reid, J. D. Fry, C. H. Langley, and T. F. C. Mackay. 1995. High resolution mapping of genetic factors affecting abdominal bristle number in *Drosophila melanogaster*. Genetics 139:1273–1291.
Long, A. D., S. L. Mullaney, T. F. Mackay, and C. H. Langley. 1996. Genetic interactions between naturally occurring alleles at quantitative trait loci and mutant alleles at candidate loci affecting bristle number in *Drosophila melanogaster*. Genetics 144:1497–1510.
Long, A. D., R. F. Lyman, C. H. Langley, and T. F. Mackay. 1998. Two sites in the delta gene region contribute to naturally occurring variation in bristle number in *Drosophila melanogaster*. Genetics 149:999–1017.
Long, A. D., R. F. Lyman, A. H. Morgan, C. H. Langley, and T. F. C. Mackay. 2000. Both naturally occurring insertions of transposable elements and intermediate frequency polymorphisms at the *achaete-scute* complex are associated with variation in bristle number in *Drosophila melanogaster*. Genetics 154:1255–1269.
Luo, D., R. Carpenter, C. Vincent, L. Copsey, and E. Coen. 1996. Origin of floral asymmetry in *Antirrhinum*. Nature 383:794–799.
Lyman, R. F., and T. F. Mackay. 1998. Candidate quaitiative trait loci and naturally occurring phenotypic variation for bristle number in *Drosophila melanogaster*: the *Delta-Hairless* gene region. Genetics 1998:983–998.
Lyman, R. F., C. Lai, and T. F. Mackay. 1999. Linkage disequilibrium mapping of molecular polymorphisms at the scabrous locus associated with naturally occurring variation in bristle number in *Drosophila melanogaster*. Genet. Res. 74:303–311.
Ma, H. 1998. To be, or not to be, a flower—control of floral meristem identity. Trends Genet 14:26–32.
Mackay, T. F. C. 1995. The genetic basis of quantitative variation: numbers of sensory bristles of *Drosophila melanogaster* as a model system. Trends Genet. 11:464–470.
Mackay, T. F. C. and C. H. Langley. 1990. Molecular and phenotypic variation in the *achaete-scute* region of *Drosophila melanogaster*. Nature 348:64–66.
Mayr, E., and W. B. Provine. 1980. The evolutionary synthesis. Harvard Univ. Press, Cambridge, MA.
Meise, M., D. Hilfiker-Kleiner, A. Dubendorfer, C. Brunner, R. Nothiger, and D. Bopp. 1998. *Sex-lethal*, the master sex-determining gene in *Drosophila*, is not sex-specifically regulated in *Musca domestica*. Development 125:1487–1494.
Michaels, S. D., and R. M. Amasino. 1999. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11:949–956.
Millar, A. J. 1999. Biological clocks in *Arabidopsis thaliana*. New Phytol. 141:175–197.
Nuzhdin, S. V., C. L. Dilda, and T. F. Mackay. 1999. The genetic architecture of selection response: inferences from fine-scale mapping of bristle number quantitative trait loci in *Drosophila melanogaster*. Genetics 153:1317–1331.
Ohshima, S., M. Murata, W. Sakamoto, Y. Ogura, and F. Motoyoshi. 1997. Cloning and molecular analysis of the *Arabidopsis* gene *Terminal Flower 1*. Mol. Gen. Genet. 254:186–194.
Orr, H. A. 1998. The population genetics of adaptation: the distribution of factors fixed during adaptive radiation. Evolution 52: 935–949.
Palopoli, M. F., and N. H. Patel. 1996. Neo-Darwinian developmental evolution: can we bridge the gap between pattern and process? Curr. Opin. Gen. Dev. 6:502–508.
Pnueli, L., L. Carmel-Goren, D. Hareven, T. Gutfinger, J. Alvarez, M. Ganal, D. Zamir, and E. Lifschitz. 1998. The *SELF-PRUNING* gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. Development 125:1979–1989.
Polaczyk, P. J., R. Gasperini, and G. Gibson. 1998. Naturally occurring genetic variation affects *Drosophila* photoreceptor determination. Dev. Genes Evol. 207:462–470.
Puoti, A., M. Gallegos, B. Zhang, M. P. Wickens, and J. Kimble. 1997. Controls of cell fate and pattern by 3′UTRs: the *C. elegans* sperm/oocyte decision. Pp. 19–24 *in* Cold Spring Harbor Symposia on Quantitative Biology no. 62.
Raff, R. A., and T. C. Kaufman. 1983. Embryos, genes, and evolution. Indiana Univ. Press, Bloomington.
Riddle, D. L., T. Blumenthal, B. J. Meyer, and J. R. Priess. 1997. *Caenorhabditis elegans*. Vol. II. Cold Spring Harbor Press, Cold Spring Harbor, NY.
Schedl, T., and J. Kimble. 1988. *fog-2*, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics 119:43–61.
Shannon, S., and D. R. Meeks-Wagner. 1991. A mutation in the *Arabidopsis TFL1* gene affects inflorescence meristem development. Plant Cell 3:877–892.
Sommer, R. J., A. Eizinger, K. Z. Lee, B. Jungblut, A. Bubeck, and I. Schlak. 1998. The *Pristionchus* HOX gene *Ppa-lin-39* inhibits programmed cell death to specify the vulva equivalence group and is not required during vulval induction. Development 125: 3865–3873.
Spencer, W. P. 1957. Genetic studies on *Drosophila mulleri*. I. The genetic analysis of a population. Univ. Texas Publ. 572:186–205.
Stark, J., J. Bonacum, J. Remsen, and R. DeSalle. 1999. The evolution and development of dipteran wing veins: a systematic approach. Annu. Rev. Entomol. 44:97–129.
Stebbins, G. L., and D. V. Basile. 1986. Phyletic phenocopies: a useful technique for probing the genetic and developmental basis of evolutionary change. Evolution 40:422–425.
Stern, D. L. 1998. A role of *Ultrabithorax* in morphological differences between *Drosophila* species. Nature 396:463–466.
———. 2000. Evolutionary developmental biology and the problem of variation. Evolution 54:1079–1091.
Sternberg, P. W., and M. A. Felix. 1997. Evolution of cell lineage. Curr. Opin. Gen. Dev. 7:543–550.
Swarup, K., C. Alonso-Blanco, J. R. Lynn, S. D. Michaels, R. M. Amasino, M. Koornneef, and A. J. Millar. 1999. Natural allelic variation identifies new genes in the *Arabidopsis* circadian system. Plant J. 20:67–77.
Theissen, G. 2000. Evolutionary developmental genetics of floral symmetry: the revealing power of Linnaeus' monstrous flower. BioEssays 22:209–213.
Thomas, J. H. 1998. Social life and the single nucleotide: foraging behavior in *C. elegans*. Cell 94:549–550.
Vachon, G., B. Cohen, C. Pfeifle, M. E. McGuffin, J. Botas, and S. M. Cohen. 1992. Homeotic genes of the *Bithorax* complex repress limb development in the abdomen of the *Drosophila* embryo through the target gene *Distal-less*. Cell 71:437–450.
Vieira, C., E. G. Pasyukova, Z. B. Zeng, J. B. Hackett, R. F. Lyman, and T. F. C. Mackay. 2000. Genotype-environment interaction for quantitative trait loci affecting life span in *Drosophila melanogaster*. Genetics 154:213–227.
Waddington, C. H. 1942. Canalization of development and the inheritance of acquired characteristics. Nature 150:563–565.
———. 1956. Genetic assimilation of the bithorax phenotype. Evolution 10:1–13.
Warren, R. W., L. Nagy, J. Selegue, J. Gates, and S. B. Carroll. 1994. Evolution of homeotic gene regulation and function in flies and butterflies. Nature 372:458–461.
Weatherbee, S. D., G. Halder, J. Kim, A. Hudson, and S. Carroll. 1998. *Ultrabithorax* regulates genes at several levels of the wing-patterning hierarchy to shape the development of the *Drosophila* haltere. Genes Devel. 12:1474–1482.
White, S., and J. Doebley. 1998. Of genes and genomes and the origin of maize. Trends Genet. 14:327–332
Wood, W. B. 1988. The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

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