Developmental system drift and flexibility in evolutionary trajectories

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SUMMARY The comparative analysis of homologous characters is a staple of evolutionary developmental biology and often involves extrapolating from experimental data in model organisms to infer developmental events in non-model organisms. In order to determine the general importance of data obtained in model organisms, it is critical to know how often and to what degree similar phenotypes expressed in different taxa are formed by divergent developmental processes. Both comparative studies of distantly related species and genetic analysis of closely related species indicate that many characters known to be homologous between taxa have diverged in their morphogenetic or gene regulatory underpinnings. This process, which we call "developmental system drift" (DSD), is apparently ubiquitous and has significant implications for the flexibility of developmental evolution of both conserved and evolving characters. Current data on the population genetics and molecular mechanisms of DSD illustrate how the details of developmental processes are constantly changing within evolutionary lineages, indicating that developmental systems may possess a great deal of plasticity in their responses to natural selection.

INTRODUCTION: WHEN HOMOLOGY IS ONLY SKIN DEEP

The study of homology has been the major theme behind the current re-synthesis of developmental and evolutionary biology (Bolker and Raff 1996; Abouheif 1997; Wagner 1999). It becomes important then to know whether homology in the cells, molecules, and pathways that underlie development typically involves conservation of function, and to what degree homology in the final phenotype indicates that the machinery producing it in different lineages has remained the same. It is reasonable to suppose that pathways underlying homologous characters are largely static, given that conserving a complex solution to an adaptive problem is simpler than reinventing the solution repeatedly (the "if it ain't broke, don't fix it" maxim). However, numerous recent studies have revealed the surprising result that developmental pathways do in fact diverge through time, even with no accompanying change in the phenotypic outcome. This process, which we call "developmental system drift" (DSD) is illustrated in Figure 1A. Here, the term "drift" is clearly distinct from genetic drift, but nevertheless is appropriate because, as with genetic drift, chance and not selection determines the details of how developmental systems change under DSD.

Here, we review recent studies in both developmental and evolutionary biology, which together suggest that over brief or long evolutionary periods, DSD can be routinely observed. Further, the relationship between time of divergence and the extent of DSD is complex and seems to depend on the sensitivity of various developmental pathways to genetic change. Pathways related to reproduction show constant and rapid divergence, as revealed by genetic analysis of speciation. On the other hand, developmental analyses of homologous traits in distantly related taxa and genetic studies of closely related species indicate that pathways underlying morphogenesis diverge more slowly and stochastically. Data on the molecular mechanisms of DSD, in combination with information from cases of convergent evolution, provide a new view of the importance and generality of flexibility and diversity that will need to be integrated with the current, well-established emphasis on conservation and constraint in evolutionary developmental studies.

DEVELOPMENTAL SYSTEM DRIFT

Hypotheses on the homology of morphological features usually begin with straightforward observations of similarity, and may be further bolstered by embryological or gene expression studies of their ontogeny. Because these analyses utilize gross phenotypic similarities and, typically, data from relatively few genes (e.g., major developmental regulatory proteins), they understandably do not emphasize the subtle differences in developmental mechanisms that are indicative of DSD. This may be because such features are either not visible, due to incomplete gene expression profiles, or not of direct interest, as in phylogenetic analyses at macroevolutionary levels. Moreover, features that are identical in closely related species are tacitly assumed to have identical
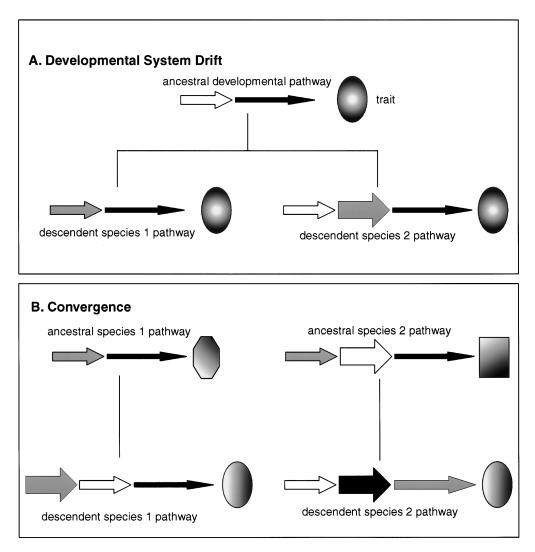
Fig. 1. Flexibility in the development of similar traits can occur by two different modes. (A) By developmental system drift (DSD), an ancestral trait (oval) is conserved but the developmental mechanisms (arrows) by which that trait is produced diverge. In the left-hand lineage, the first step has been altered (indicated by an arrow with different color). In the right-hand lineage, an additional pathway step has been recruited. (B) By convergence, distinct lineages can independently evolve a similar trait (ovals) from non-identical original traits (different shapes) in their respective ancestors. The developmental pathways involved in convergence can be similar or different.

developmental underpinnings. However, recent studies of developmental and evolutionary genetics, concentrating on both subtle and major details of phenotype and ontogeny at both small and great phylogenetic distances, are now revealing that DSD is a very general phenomenon.

At the short end of the time scale, there is a great deal of evidence of DSD between recently diverged species. Many studies indicate that divergence of developmental homeostasis leads to an increased fluctuating asymmetry (e.g., between meristic traits on the left and right sides of the body) in species hybrids (Felley 1980; Graham and Felley 1985; Leary et al. 1985). In addition to indicating DSD in homeostatic properties, interspecific genetic studies have found that

system drift can affect very specific morphological traits, which are identical in the parental species but show aberrations in species hybrids. In hybrids between *Drosophila melanogaster* and *D. simulans*, thoracic bristles with conserved patterns in the parental species are often missing (Fig. 2). Takano (1998) has found that these bristle losses are not seen in hybrids between *D. simulans* and a closer relative, *D. mauritiana*. This suggests that the appearance of morphological anomalies may be a function of the time of divergence of the two species. Recently, Takano-Shimizu (2000) reported that one X-linked locus accounted for greater than half of the variation between *D. simulans* strains showing high and low bristle number loss, and that this locus showed
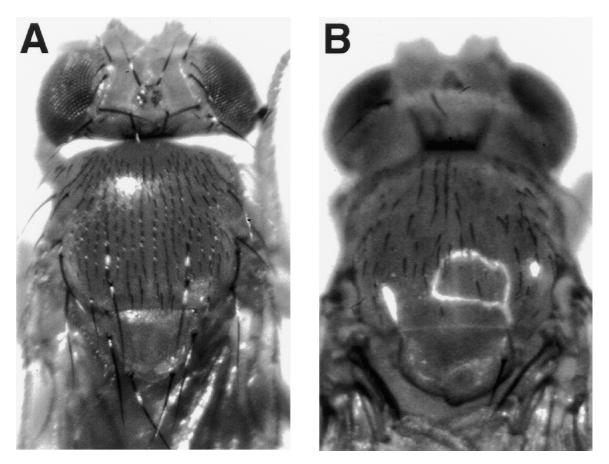
Fig. 2. Bristle loss in hybrids of *Drosophila melanogaster* and *D. simulans.* (A) *D. melanogaster* male showing the wild type thoracic bristle pattern, which is identical in *D. simulans* (not shown). (B) F1 male hybrid between *D. simulans* and *D. melanogaster* showing loss of many thoracic bristles. Images courtesy of T. Takano.

significant epistatic interactions with autosomal loci. Another example of the failure of developmental systems in hybrids to produce wild-type morphology that is identical in parental species occurs in the species pair *Drosophila subobscura/madierensis*, whose hybrids show high frequencies of partial transformation of second (T2) and third thoracic (T3) segments toward the first thoracic segment (T1). These are particularly noticeable due to the presence of male-specific bristle patterns in T2 and T3 legs of ectopic sex combs, which are normally only found on T1 legs (Khadem and Krimbas 1991; Papaceit et al. 1991). A genetic analysis of this hybrid anomaly indicated that at least five loci contribute to the loss of sex comb suppression in T2 and T3, and maternal effects may also be involved (Papaceit et al. 1991). These studies in *Drosophila* indicate that morphological anomalies in hybrids can have quite different genetic architectures, and the traits most strongly affected by DSD can differ markedly between taxa.

DSD has also been revealed by developmental analyses of highly conserved structures fundamental to animal body plans. In the nematode genera *Caenorhabditis* and *Pristionchus*, the vulva is produced by a group of ventral epidermal cells that express the homeotic gene *lin-39*. In *C. elegans*, nonvulval cells fuse with the epidermis, but in *P. pacificus* the homologous cells undergo programmed cell death. Eizinger and Sommer (1997) found that although in both species *lin-39* mutants are vulvaless, in *C. elegans* the cells that would normally give rise to the vulva instead adopt the cell fusion fate of nonvulval cells; however, in *P. pacificus* the vulvaless phenotype is caused by apoptosis of these cells. Thus, although *lin-39* promotes nonvulval fate in both species, and in this respect has not undergone functional divergence, the intrinsic fate of ventral epidermal cells in these two species has diverged since the time of their common ancestor.

Another example in which a fundamental, conserved feature of an animal body plan has apparently diverged in its developmental pathway is chordate neurulation, the process in which dorsal ectoderm is specified to produce the neural tube. In all vertebrates studied thus far, a signaling center in

the mesoderm (the Spemann-Mangold organizer in amphibians, the node in amniotes, and the dorsal shield in teleost fish) is required to induce the neural plate from the surrounding non-neural ectoderm. Such a fundamental process would seem to be rigidly constrained, yet surprising distinctions have emerged between anamniote (fish and frog) and amniote (chick and mouse) model organisms (reviewed by LaBonne and Bronner-Fraser 1999). In anamniotes, overexpression studies in *Xenopus* (reviewed by Weinstein and Hemmati-Brivanlou 1997) and mutations in zebrafish (Hammerschmidt et al. 1996; Nguyen et al. 1998; Dick et al. 2000) support a model in which inhibition of BMP family signaling molecules is necessary and sufficient to induce neural fates. However, although BMP antagonists are expressed in the node of amniotes (Connolly et al. 1997), misexpression experiments in chick (Streit et al. 1998) and knockouts in mouse of either BMP antagonists or BMPs themselves (McMahon et al. 1998; Zhang and Bradley 1996; Dudley et al. 1995; Winnier et al. 1995) cause no obvious defects in neural specification. This suggests that in amniotes other factors have evolved roles in the organizer that either replace or function redundantly with BMP signaling to specify the neural plate.

Limb development also shows hints of underlying diversity among vertebrate lineages in what is thought to be a highly conserved developmental genetic program. One case involves Radical-fringe (R-fng), one of a family of extracellular proteins that bind Notch during cell signaling (reviewed by Wu and Rao 1999). R-fng is expressed at the dorsal-ventral boundary of the limb bud of both mouse and chick, where it is thought to direct the formation of the apical ectodermal ridge (AER) (Johnston et al. 1997). Genetic manipulations in different taxa, however, suggest that the role of R-fng may have diverged during amniote evolution. R-fng has been shown to be sufficient to induce AER formation in chickens (Rodriguez-Esteban et al. 1997; Laufer et al 1997), but mouse null *R-fng⁻* mutants show no defects in limb development (Moran et al. 1999). Although more experiments are needed to resolve this unexpected finding, one possible explanation is that functional redundancy of fringe family members may be utilized differently in the chick and mouse, resulting in one family member bearing most or all of a particular task in one species and another member performing that task in the other.

Another case of DSD in vertebrate limbs involves heterochrony of events in the development of autopods (hands and feet). The autopods of urodele salamanders, like all tetrapods, show a conserved posterior-to-anterior patterning polarity of the *sonic hedgehog*, *BMP*, and *Hoxd* genes during development (Torok et al. 1998), but their digits differentiate in the opposite order (anterior to posterior) from other known tetrapods (reviewed by Gardiner et al. 1998). Although the mechanism and importance of this reversal in the timing of digit formation is not known, this type of ontogenetic shift may also be involved in many cases of convergent evolution seen in salamander limbs. For example, in plethodontid salamanders, increased foot webbing has evolved multiple independent times, using at least two different developmental mechanisms (paedomorphic arrest of digit growth and late growth of cutaneous webbing; reviewed by Wake 1991).

Perhaps the most striking examples of diversity in a conserved regulatory process are found in animal sex determination. Although primary sex determining signals vary widely among animals, from environmental to chromosomal or genotypic, the hierarchy of regulatory molecules points to striking cases of both conservation and functional divergence (reviewed by Marin and Baker 1998; Schutt and Nothiger 2000). One level at which a great degree of evolutionary divergence has taken place occurs very early in the sex determination regulatory cascade. In *Drosophila*, the splicing factor *Sex-lethal* (*Sxl*) is activated in chromosomal (XX) females and specifies female development by directing the female-specific splicing of *Sxl* itself as well as its downstream target *transformer*. In chromosomal (XY) males, *Sxl* remains inactive and the default pathway of male development takes place (reviewed by MacDougall et al. 1995). In the housefly *Musca domestica*, genetic studies have indicated that an analogous sex determination switch gene, called *F*, exists, which is active in females and inactive in males (McDonald et al. 1978; Schmidt et al. 1997). *F* and *Sxl*, thus, seem to have parallel functions. In order to determine if *F* and *Sxl* are homologous genes, Meise et al. (1998) isolated the homolog of *Sxl* from *M. domestica*. They found that the protein sequence was highly conserved (83% identical between *Drosophila* and *Musca*), but does not show sex-specific regulation and is expressed apparently identically in both sexes throughout development. Further, when ectopically expressed in *D. melanogaster*, *M. domestica Sxl* had no effect on expression of genes known to be regulated by *D. melanogaster Sxl*. In a similar experiment, the *Sxl* homolog from the Mediterranean fruitfly *Ceratitis capitata* also had no effect on *Sxl* target genes when expressed in *D. melanogaster* (Saccone et al. 1998). Thus, it appears that sex determination pathways have evolved substantially since these dipteran flies diverged over the last 150 Myr. The failure of *Sxl* homologues to produce sexual transformation in *Drosophila* suggests that they cannot recognize or act upon the appropriate RNA targets because of sequence evolution. Rapid sequence change is a hallmark of the molecular evolution of genes associated with sex determination and sexual differentiation in many animal species (e.g., Civetta and Singh 1998; Wyckoff et al. 2000), and therefore this is not surprising. But the lack of sex-specific regulation of *Sxl* in *Musca* suggests that it has no sex determination function at all in *Musca*. However, the high level of conservation of *Sxl*, especially in

the two RNA binding domains, strongly suggests that it must still function in mRNA splicing in *Musca* and *Ceratitis*. Thus, in addition to the accumulation of sequence change, DSD can also alter the connectivity of genes in a developmental pathway. Indeed, even though upstream sex determination genes like *Sxl* show a high degree of evolutionary divergence, the transcription factor *doublesex/MAB-3/DM* appears to have retained a highly conserved role as a readout of the sex determination hierarchy in invertebrates and vertebrates (Zhu et al. 2000; see reviews by Marin and Baker 1998 and Schutt and Nothiger 2000).

GENES, POPULATIONS, AND DSD

How do differences in the development of conserved phenotypes among different species evolve? One genetically well-studied set of diverging developmental processes underlies the speciation process itself. In sexually reproducing organisms, biological species are often defined by inviability and infertility occurring in hybrids. In fact, these syndromes are manifestations of DSD in embryogenesis and gametogenesis. Recent research on the genetics of how biological species originate has shown that two factors dictate the dynamics of DSD as it relates to speciation. The first is a characteristic of the developmental pathways underlying zygote viability and gamete production. These are both absolutely essential and extremely polygenic processes, so it is not surprising that interspecies hybrids often fail due to incompatibilities among the many genes controlling them (reviewed by Wu and Palopoli 1994; Coyne and Orr 1998). Some systems, such as fertility in the heterogametic sex of animals (Haldane's rule), appear particularly susceptible to DSD. Various workers have suggested that this is because their components are subject to strong sexual selection (Wu et al. 1996), sexually antagonistic pleiotropy (Rice 1992, 1996; Price 1997), or selection involving meiotic drive systems (Frank 1991; Hurst and Pomiankowski 1991). Other traits, involving embryogenic, morphogenetic, and metabolic pathways, appear to evolve DSD more slowly. Perhaps this is because they are subject to weaker and/or stabilizing selection or because they are canalized against genetic and environmental fluctuation (Gibson and Wagner 2000), and thus better able to withstand the molecular deviations brought together in species hybrids. However, although differing selection between species may often drive the appearance and degree of DSD, this need not always be the case. The direct causes of DSD may also include processes distinct from selection but causing correlated effects, such as linkage and pleiotropy, as well as genetic drift.

The second characteristic influencing the appearance of DSD involves the structure of populations. Panmictic populations tend to remain as intact species, without divergence in developmental systems, but isolated populations and nascent species inevitably develop incompatibilities, even if exposed to the same selection regimes. Biological speciation always involves the isolation of gene pools (Mayr 1963; Higashi et al. 1999; Dieckmann and Doebeli 1999). The classic genetic model of speciation (Dobzhansky 1936; Muller 1940), most recently updated by Orr (1995), describes how these isolated gene pools, gradually fixing novel alleles at many loci, come to possess incompatibilities. Each population's set of new alleles has not been tested against those of other populations, and the number of these differential fixations is expected to be approximately proportional to the time of isolation. There is no reason to believe that DSD stops once reproductive incompatibility is complete, however, and thus long-separated species will exhibit DSD in many traits as they evolve new ways to construct these traits.

How the genetic factors underlying DSD arise and are maintained in populations is not well studied, but there is abundant evidence of intraspecific variation in these genes. Some of the best evidence comes from populations of *Drosophila simulans* and *melanogaster* polymorphic for alleles that "rescue" the hybrid inviability and sterility usually seen in *simulans* × *melanogaster* crosses (Sawamura et al. 1993a,b; Davis et al. 1996; Barbash et al. 2000). Similarly, different populations of the oyster mushroom *Pleurotus djamor* have been shown to harbor distinct alleles at different loci, contributing to the failure to form dikaryons with a sister species, *P. calyptratus* (Liou 2000). Within-species variation for factors underlying DSD in morphology can also be found. Takano-Shimizu (2000) reported a high degree of variation for susceptibility to hybrid bristle loss among *D. simulans* strains. Also, Wade and colleagues have demonstrated that different strains of the flour beetle *Tribolium castaneum* have different propensities to produce abnormalities in antennal morphology in F1 hybrids with closely related species (Wade and Johnson 1994; Wade et al. 1994; Wade et al. 1997). In all of these cases, crosses within populations produce no abnormal progeny, but more distant outcrosses uncover polymorphic genetic factors that may eventually become fixed barriers to hybrid development.

MOLECULAR MECHANISMS OF DSD

What molecules are involved in the evolution of DSD? Developmental pathways consist largely of interactions of gene products with each other and with regulatory elements of genes themselves. These interactions depend on specific sequences and structural motifs in proteins, DNA, and RNA. Although many proteins and nucleic acid binding sites have been highly conserved during evolution, until recently it has been unclear how the functions and interactions of these factors undergo conservation and evolutionary change.

As described above for sex determination evolution, functional roles of proteins in conserved developmental pathways can diverge among different lineages. Homeobox transcription factors provide another example of how the protein functions that underlie conserved embryonic developmental pathways can fundamentally change. The proteins *fushi tarazu* (*ftz*) and *bicoid* (*bcd*) both have undergone functional divergence in insects over the past 100 million years (reviewed by Gibson 2000). The *ftz* gene, which is physically located within the Hox tandem array of anteroposterior (AP) selector genes, appears to have undergone a fundamental change in its expression pattern since the common ancestor of insects and chelicerates. In the relatively primitive mites, centipedes, and onychophorans, *ftz* is expressed in an AP modulated pattern, similar to its Hox complex neighbors (Telford 2000), and is thus presumed to have a homeotic function. However, in relatively derived insects such as flies and beetles, embryonic *ftz* expression has evolved into a pair-rule (every other segment) pattern. Consistent with this pattern, insect *ftz* mutants do not have homeotic phenotypes, but instead indicate a requirement during patterning of each segment (Wakimoto et al. 1984). On an even shorter evolutionary time scale, during the evolution of dipteran insects, *bcd* appears to have arisen and adopted a new function. In *Drosophila*, maternal *bcd* is expressed in an AP gradient in the oocyte and is required in the zygote to activate the gap gene *hunchback* (*Hb*). However, orthologs of *bcd* have not been found in non-dipterans, suggesting that some other protein serves to regulate zygotic *Hb* expression in other insects. Recently, Wimmer et al. (2000) performed genetic manipulations in *Drosophila* to show that the requirement for *bcd* could be obviated by maternally expressing *Hb*, which is able to activate zygotic *Hb* via an autoregulatory circuit at the *Hb* locus. In the absence of *bcd*, this expression is able to rescue aspects of segmentation that are defective in *bcd* mutants. Thus, Wimmer et al. (2000) have proposed that the ancestral mode of embryonic *Hb* regulation may have been autoregulatory.

Many instances of evolutionary change involve alterations in patterns of gene expression, rather than gene product function. Transcription patterns are mediated by regulatory regions of genes, which are often organized into discrete modules called enhancers. Ludwig and colleagues have elucidated how enhancers change during interspecific divergence while mediating the same developmental function. This work concentrated on the "stripe 2" enhancer of the *Drosophila even-skipped* (*eve*) gene, which encodes a transcription factor required for embryonic segmentation. The *eve stripe 2* element in *D. melanogaster* is a 480 bp DNA sequence that regulates expression of the gene in one of seven stripes in the *Drosophila* blastoderm prior to cellularization. In *D. melanogaster*, 12 transcription factor binding sites have been identified by mutagenesis studies, six for the activators Bicoid and Hunchback, and six for the repressors Giant and Kruppel. Ludwig et al. (1998) sequenced the *eve stripe 2* region from five other *Drosophila* species that have diverged in the last 60 Myr and were surprised to find many changes in the sequences and spacing of these binding sites, as well as in the total length of the enhancer. Even more surprising was the absence of one Bicoid site from two species and one Hunchback site from three species. Strikingly though, when *eve stripe 2* enhancers from other species were tested in reporter assays in transgenic *D. melanogaster*, all of the elements were expressed in the normal *eve stripe 2* spatial and temporal pattern. Thus, although this enhancer has undergone a rate of sequence substitution equivalent to other non-coding regions, its function has nevertheless been strictly maintained. Ludwig et al. (1998) concluded that because the heterologous enhancers functioned in *D. melanogaster*, stabilizing selection on the enhancer itself must be responsible for the functional conservation because the alternative, species-specific compensatory changes between regulatory elements and transcription factors, would have prevented the heterologous enhancers from functioning in *D. melanogaster*. In a further experiment, Ludwig et al. (2000) found that chimeric enhancers constructed between *D. melanogaster* and *D. pseudoobscura* were not able to drive reporter expression in the proper *eve-stripe 2* pattern, further supporting the stabilizing selection hypothesis.

Another recent study in *Drosophila* has also provided a view of the involvement of enhancer sequences in the evolution of DSD. Skaer and Simpson (2000) undertook an extensive developmental genetic analysis of notum bristle loss in *D. melanogaster/simulans* hybrids. They found that hybrid bristle loss showed temperature sensitivity that differed between the sexes. A heat-sensitive early stage, affecting both sexes, corresponds to the time of expression of the X-linked proneural genes of the *Achaete-scute* complex (*ASC*) in bristle prepatterns. A later cold-sensitive stage, affecting females only, corresponds to the time when bristle precursor cells are born and require high-level expression of *Achaete* and *scute*. Skaer and Simpson implicated *cis*-regulatory *ASC* sequences in hybrid bristle loss because when hybrids were made using *D. melanogaster* chromosomes containing deletions of *ASC* enhancers needed for establishment of particular bristles, these bristles were affected significantly more than hybrids with wild-type chromosomes. They further proposed that sex differences in heat sensitivity might arise because male hybrids possess only the *D. simulans* X chromosome and hence only one *ASC*, whereas females possess an *ASC* from both species. The early activation of the *ASC* is dependent on binding of autosomal transregulators whose function may be temperature sensitive. If this is the case, then both sexes should be affected because both have autosomal factors from both species that must bind the *ASC* of the other species. On the other hand, the subsequent mainte-

nance of *ASC* expression depends on autoregulation by high levels of *Achaete* and *scute*. This process may also be temperature sensitive, which would affect females more than males. This is because in female hybrids, *Achaete* and *scute* from both species would need to bind the *ASC* from the other species, whereas in males the *ASC* and its gene products are from the same species, and thus their interaction may be less sensitive to temperature. This study thus provides a detailed model of how interspecific divergence at *cis*-regulatory sites of the proneural *ASC* genes has resulted in an inability of transcription factors from one species to properly regulate transcription at the *cis*-regulatory sites of the other species.

It is still unclear what mechanisms drive these alterations in protein and enhancer function. Conventional mutational processes, such as nucleotide substitutions and small insertion/deletion events, probably underlie many functionally important changes of amino acid sequences, as well as transcription factor binding sites like those in *eve stripe 2* and the *ASC*. On a more saltational level, the process of gene duplication followed by functional divergence has occurred in the evolution of many developmentally crucial multigene families, most notably the animal *Hox* complex genes (reviewed by Holland and Garcia-Hernandez 1996; Akam 1998). The most common result of a gene duplication event is thought to be rapid degeneration of one copy by lesions that lead to transcriptional or translational silencing (Nei and Roychoudhury 1973). However, at some frequency, which has not yet been measured, duplicated genes evolve changes in coding sequence such that one copy retains the original function and the other is able to adopt a new function. Enhancer regions may also undergo changes following duplication events, resulting in novel expression patterns of one or both gene duplicates. One recent model of this process by Lynch and Force (2000) predicts that gene duplications can often be preserved by "subfunctionalization," whereby different enhancers are inactivated in members of a duplicate gene pair, such that the "sum" of their expression patterns still covers the original pattern of the ancestral gene. This process may act to extend the time during which both gene duplicates remain functional, allowing more time for the evolution of novel functions.

FLEXIBILITY AND CONVERGENCE

If developmental pathways underlying conserved phenotypes can display divergence, then can we expect a similar amount of diversity in the mechanisms by which distinct species and populations evolve the same traits during convergence (Fig. 1B), or are constraints important in delimiting these possibilities? Just as DSD becomes more likely with increasing numbers of divergent genes, one might expect that the more complex the organism—in terms of the number of genes, biochemical pathways, or cell types involved in de-

velopment—the more distinct ways that organism might be able to evolve. Many cases of convergence are well known in both simple and complex organisms. Current data suggests a mixture of flexibility and constraint operating in microbial systems, but many fascinating cases of convergence in higher organisms still await characterization.

Studies of antibiotic resistance mechanisms in bacteria indicate that there is often more than one type of genetic alteration that bacteria have deployed against each of seven classes of chemical antibiotics, but these appear to be quite finite and have occurred repeatedly in different species (Baquero and Blazquez 1997; Walsh 2000). Moreover, horizontal gene transfer, by means of plasmids and tranposons, has been found to be a key mechanism in the spread of most clinically important forms of antibiotic resistance (Maiden 1998). This indicates that resistance genes typically evolve only infrequently and are "found" and utilized by multiple populations and species, thus eliminating the need for microbes to reinvent genes. In addition to these typically oligogenic responses to strong environmental selection, experimental evolutionary studies in phage and bacteria have also provided an excellent means to observe evolutionary trajectories. In one such experiment (Wichman et al. 1999), independent replicate lines of bacteriophage ϕX174, whose normal host is *E. coli*, were exposed to higher temperatures while infecting a novel host, *Salmonella typhimurium*, and allowed to adapt for 1000 generations. After this regime, phage growth rates had improved between 4000 to 18000 fold. In two lines analyzed by genomic sequencing, one had experienced 14 nucleotide substitutions and the other had experienced 13. Constraint in evolutionary trajectories was apparent in that six of these substitutions and one 27 base pair deletion occurred in both lines. Further experiments by Wichman et al. (1999) found that such parallel evolution seems to be quite common in these phage; only five substitutions were unique to particular lines and, furthermore, four of the substitutions that occurred in selected ϕX174 lines were also seen as fixed changes in the closely related phage S13, whose natural host is *Salmonella.* Extensive studies of experimental evolution in *E. coli* by Lenski and colleagues have also demonstrated a mixture of flexibility and convergence or parallelism, depending on the trait studied. Distinct evolutionary responses to starvation conditions (Vasi and Lenski 1997) and fluctuating nutrition (Vasi et al. 1994; Vasi and Lenski 1997), as well as divergence in cell size (Travisano et al. 1995), showed evidence for distinct trajectories and dependence on previous evolutionary history in independent strains. However, traits that are directly related to fitness, such as overall competitive growth ability, showed evidence of convergence and tended to be independent of previous evolutionary history, suggesting limitations in the number and kinds of trajectories (Vasi et al. 1994; Travisano et al. 1995).

Multicellular organisms utilize a greater number of biochemical and developmental pathways and components of these pathways are often used during multiple stages and in multiple tissues. This conceivably provides more possible ways for developmental systems to respond to selection. Indeed, intuitively obvious examples of unique solutions to a common problem in deeply divergent lineages have been recognized for many years, such as the distinct skeletal details underlying the three independently evolved instances of flight in vertebrates (i.e., pterosaurs, bats, and birds) or the different tail axes in fish versus whales. However, the current revolution in molecular systematics is revealing many cases of convergent and parallel evolution over much shorter time scales that could provide detailed insights into the flexibility of evolutionary trajectories in complex organisms. For example, discoveries of convergent radiations in Caribbean *Anolis* lizards (Losos et al. 1998) and North American stickleback fish (Taylor and McPhail 1999) have been celebrated as instances where the great power of natural selection has overcome contingent factors to produce quite predictable outcomes in independent episodes (Harvey and Partridge 1998). While the developmental processes underlying these striking convergent radiations in vertebrates have yet to be investigated, recent studies of convergence and parallelism in insects provide significant evidence of flexibility in evolutionary trajectories.

One of the most famous convergent phenomena, butterfly mimicry, gives tantalizing indications of diversity during evolution of similar phenotypes in distinct species. For example, in the closely related species pair *Heliconius melpomene*/*erato*, populations from different geographic regions are involved in several Mullerian mimicry complexes, each with a specific pattern. Within each complex, the patterns appear similar enough to fool a predator, but upon close scrutiny many subtle differences become apparent. This is

shown in Figure 3, which illustrates co-mimics from western Brazil. On their forewings, both the *melpomene* (Fig. 3A) and *erato* (Fig. 3B) co-mimics show a conspicuous pale yellow patch (black arrows) with a proximal black indentation, and on their hindwings both species have a red-orange proximal stripe (blue arrowheads). However, the black forewing indentation appears quite different between the two species. In *melpomene*, the indentation appears in a more anterior wing cell (intervein area) than in *erato*. Also, in the hindwing, the proximal stripe is much wider in *melpomene* than *erato*. These types of differences occur between all *melpomene* and *erato* co-mimic pairs (see Nijhout 1991, Plate 5), suggesting that although these closely related species probably utilize the same ancestral patterning mechanisms, at least slightly different developmental alterations have been fixed in independent lineages evolving toward the same pattern. The phylogenetic relationships of morphs within these species are still controversial, but could ultimately shed light on the likelihood of different modes of pattern change. In particular, extensive co-evolution between *melpomene* and *erato*, which has been traditionally argued (Sheppard et al. 1985), might favor identical evolutionary trajectories. This view is supported by the similar Mendelian genetic architectures for certain pattern motifs in the two species (reviewed by Nijhout 1991; Mallet et al. 1996). Surprisingly though, molecular phylogenetic analysis by Brower (1994, 1996) indicates that relationships among color pattern morphs within the two species are not congruent and suggests very different time estimates for the radiations of morphs within each species (less than one Ma for *melpomene* and 1.5–2 Ma for *erato*). Thus, quite different paths of evolutionary change might be envisioned for the co-mimics of these two species.

Striking instances of diverse but parallel trajectories of adaptation can even be found between populations of the same species. A recently discovered example occurs in *Dro-*
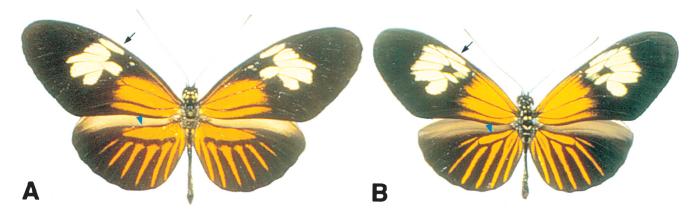

Fig. 3. Fine scale differences in pattern elements in Mullerian mimics suggest diversity in developmental mechanisms. Western Brazil morphs of *Heliconius melpomene* (A) and *H. erato* (B). Subtly different forewing (black arrows) and hindwing (blue arrowheads) pattern elements are indicated (see text for description). Images courtesy of H. F. Nijhout.

sophila wing size clines. In native European populations of *D. subobscura*, wing size increases with increasing latitude. In North America, which was colonized only in the last few decades, *D. subobscura* populations appear to have rapidly evolved a similar latitudinal cline. Interestingly, Huey et al. (2000) found a subtle but distinct difference in the developmental mechanism by which the wing length changes have been achieved. In European populations, greater wing length is caused by lengthening a basal part of one wing vein, whereas in North America, increased size is caused by lengthening a more distal portion of this wing vein. Molecular genetic and developmental characterization of these species- and population-level differences in patterning and morphogenesis would greatly aid in determining the relative importance of constraint and flexibility in phenotypic evolution.

CONCLUSION: GHOSTS IN THE EVO-DEVO MACHINE

One of the major issues that arose in the wake of the Neo-Darwinian synthesis was the Classical versus Balance debate (reviewed by Dobzhansky 1970; Lewontin 1974). Advocates of the Classical view, following T. H. Morgan, emphasized the relative paucity of morphological variation within species, and argued that genes in populations were largely invariable and that the fittest "wild type" alleles dominated. Balance advocates, on the other hand, led by S. Chetverikov, predicted a large amount of hidden variation in populations. The development of molecular population genetics, starting in the 1960s, revealed that natural populations abound with molecular genetic variation, largely proving that the intuitions of the Balance advocates were correct (Powell 1997). This debate has been largely abandoned, but perhaps its most glaring unsolved problem now haunts evolutionary developmental biologists (e.g., Stern 2000): how much and what kinds of variability in developmental systems are meaningful and available for natural selection to act upon? Views based on the widely held neutral theory posit that much of the molecular variation in natural populations must be neutral or nearly neutral with respect to phenotype (Kimura 1983). Nevertheless, although difficult to quantify, significant phenotypic effects have been routinely demonstrated for non-protein-coding molecular polymorphisms (e.g., Laurie et al. 1991; Gibson and Hogness 1996; Long et al. 1998). However, by necessity, these studies rely on examining effects in isogenic backgrounds, whereas in nature, it is probable that no two genetic backgrounds in which an allele occurs are identical. Moreover, recent work in both developmental and quantitative genetics has found epistatic interactions among genes to be of paramount importance in determining phenotypes. Indeed, studies of major-effect *Drosophila* mutants in multiple strains have shown that the effects of single genes

depend heavily on the genetic background, which can completely suppress the mutant phenotype, highly enhance it, or result in intermediate states between these extremes (e.g., Gibson et al. 1999; Polaczyk et al. 1998). Thus, in light of the importance of genetic context in which mutants arise, current views of the importance of molecular variation may need to be substantially revised.

Developmental evolutionary biology must ultimately address and model how natural selection acts upon variation in components of developmental pathways to cause evolutionary change. Evolutionary genetics has been advancing toward this goal for some time, but developmental genetics has yet to assimilate its lessons in a meaningful way. We have aimed in this review to illustrate the great complexity and taxon-specificity of development, and to provide some examples of how it came to be. This picture of divergence, based to a large degree on contingency and chance, greatly complicates our notions of the process of adaptation and increasingly makes developmental evolution a series of case studies with few overarching laws. The widespread occurrence of DSD suggests that within species, developmental systems may be constantly struggling to suppress or accommodate the entropic and context-specific effects of genetic variation. Through fixation of many epistatic alleles and the constant shuffling of genetic backgrounds, initially identical developmental systems become species-specific Rube Goldberg contraptions of a sort, with each independent lineage developing a unique set of expedient measures to deal with current adaptational needs. These independently evolving contraptions are thus constantly diverging, and species are produced with many superficially similar, but ultimately unique and non-interchangeable, solutions to selection's ever-present conundrums.

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