

## Chapter Five

### The Flexibility of Butterfly Wing Color Patterns and Evolution in Morphospace

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**B**iologists do not yet fully understand how the various potential processes of evolutionary change actually integrate over time to yield the patterns in which species occupy morphospace. Dramatic examples of convergent or parallel evolution appear, at least superficially, to support an all-powerful natural selection that orchestrates evolution to mold functional forms in all available ecological environments. However, natural selection can only operate in a fully unconstrained manner when there are no limitations on generating the fuel for evolution as provided by phenotypic variation in all those traits with the potential to contribute to adaptation. Perhaps, because of the properties of genetics and development, evolution tends to be biased to occur along certain trajectories rather than others (e.g., Maynard Smith et al. 1985; Schluter 1996; Brakefield 2006). History and contingency may also play a major role such that at any one time the paths traced by evolution are to some degree biased by what has gone before (Blount et al. 2008). Lynch (2007) argues persuasively, in comparing evolution in lower and higher organisms, that the more complex genomes of the latter with substantial non-coding DNA are a by-product of smaller effective population sizes with stronger stochastic influences and weaker natural selection. If future work can quantify how a radiation of related species has explored morphospace through evolutionary time (see for impressively documented examples from the fossil record, McGhee

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Figure 5.1. Two fanciful morphologies in finch morphospace. Cartoons of Henry Horn drawn at the meeting.

2006), and at the same time explain why the density of species varies through this space, we will more fully understand how evolution works. Here, we describe how work on two butterfly systems can contribute to such an approach.

Darwin's finches on the Galápagos archipelago are the paradigm for adaptive radiation. The agencies of natural selection that have led to the matching of functional morphologies in these finches to their diverse ecological environments have been revealed in an exhilarating way by Peter and Rosemary Grant (e.g., Grant and Grant 2008). Numerous distinctive morphologies of finch have evolved on the Galápagos (although not a chicken finch or a shrike finch, fig. 5.1; and cf. Grant and Grant 1989). Research on Darwin's finches has also quantified genetic variances and covariances underlying phenotypic variation in target traits for selection, and is beginning to identify developmental mechanisms that map ecologically relevant phenotypes on to genotype. However, even in this case study, the extent to which the processes are

understood in sufficient detail to fully explain the pattern of occupancy in morphospace is unclear. Is this pattern primarily the result of how natural selection screens phenotypic variation, or are there important compromises established by historical contingencies and the processes that generate the phenotypic variation?

In this chapter we compare work on two systems involving butterfly wing color patterns: the marginal eyespots on the wings of *Bicyclus* butterflies and the warning colors associated with mimicry in *Heliconius* butterflies (see reviews: Beldade and Brakefield 2002; Joron et al. 2006a; Baxter, Johnston, and Jiggins 2008). Work with *B. anynana* has used a series of eyespot elements with shared development and genetics to examine the flexibility of such repeated pattern elements to follow their own individual paths in evolution and, thus, become different to each other. This issue has been explored by applying artificial selection to the eyespot pattern in different directions of morphospace together with “evo-devo” studies of morphogenesis. Knowledge of the “tool-kit” pathways of wing development in *Drosophila* flies has proved invaluable for exploring the developmental genetics of butterfly wings (see Carroll et al. 2005). The observations on the model species can now be compared with patterns of morphological diversity within radiations of related species to reveal whether the evolvability of the pattern in laboratory selection experiments may be reflected in patterns of occupancy in morphospace. We know in broad terms why eyespots matter to adult *Bicyclus* butterflies, but we have little notion about how natural selection influences the details of eyespot patterns in species in different environments. In contrast for *Heliconius*, the dynamics of mimicry in terms of both models of the evolution of the phenomenon and the action of natural selection arising through bird predation and mate choice are better understood. This broad understanding of how natural selection influences wing patterning in *Heliconius* can now be examined in the context of an ever expanding knowledge about the Mendelian loci that can switch among mimetic patterns. Thus, we can begin to develop ideas about the extent to which the genetic architecture of mimicry could bias the evolution of mimetic color patterns, even in the face of the powerful natural selection. Here, we will highlight opportunities for the future to more fully understand the balance of processes which underlie the evolution of patterns of occupancy in morphospace.

*BICYCLUS* EYESPOTS: EVO-DEVO AND DIVERGENCE  
OF SERIAL REPEATS

Research has begun to show how components of the tool-kit pathways of wing development in insects have been recruited and elaborated to yield novel structures, including the pigmented scale cells (from bristles and the *Achete-scute* pathway; Galant et al. 1998) and the eyespot pattern elements with their nested color rings (recruitment of genes such as *Distal-less*, *engrailed*, and *Spalt*; Carroll et al. 1994; Brunetti et al. 2001; Saenko et al. 2008). Butterfly wings are covered by sheets of scale cells that form a sort of colored mosaic like tiles on a roof. This provides the basis for the process of painting patterns on the wings in development. Eyespots and other pattern elements on butterfly wings are functional forms that contribute to variation in fitness in the wild both via interactions with predators and in mate choice (Robertson and Monteiro 2005; Stevens 2005).

DEVELOPMENT OF EYESPOTS AND ARTIFICIAL SELECTION  
EXPERIMENTS IN SIZE MORPHOSPACE

A reconstruction of an evolutionary “groundplan” for Nymphalid butterflies shows series or modules of different pattern elements, including bands, chevrons, and the marginal eyespots, that are arranged in anterior-posterior columns over the wings (Nijhout 1991). Both dorsal and ventral wing surfaces are subdivided by veins. In the groundplan, each section of the wing between a pair of marginal veins shows its own combination of single copies of the pattern elements, whereas in extant species some or all of these elements are often absent (e.g., the eyespots in *Heliconius*). Morphogenesis of one module, the marginal eyespots, is becoming clearer both in terms of cell-cell signaling mechanisms and candidate genetic pathways (Beldade and Brakefield 2002). Understanding pattern formation involves discovering how the epithelial cells—the scale cells to be—gain information during wing development to become fated to synthesize different color pigments shortly before adult eclosion.

The eyespots of *B. anynana* are all formed by the same developmental process (Brunetti et al. 2001; Beldade and Brakefield 2002; Reed and Serfas 2004; Saenko et al. 2008). Transplantation experiments performed in early pupae reveal that each eyespot forms around a group of organizing cells called a focus; transplanting a focus to a novel wing location yields an ectopic eyespot around the grafted tissue. Establishment of the foci occurs in late larval development, following which, in the early pupa, each focus establishes an information gradient in the surrounding epithelial cells, presumably via diffusible morphogens. These epithelial cells then respond to the gradient of the signal and, thus, become fated to subsequently synthesize a particular pigment in the late pupa. We also know from additional transplantation experiments with divergent selection lines that eyespot size depends largely on signal strength; a focus from a line with large eyespots yields larger ectopic patterns than one from a line with small eyespots irrespective of the host epithelial tissue into which it is grafted (Monteiro et al. 1994). The eyespots in *B. anynana* express the same genes in development (Brunetti et al. 2001; Reed and Serfas 2004). Also, mutant alleles typically affect all eyespots, and artificial selection on a single eyespot yields highly correlated responses for the target trait in other eyespots (Beldade et al. 2003). Shared morphogenesis led us to design artificial selection experiments in this model system to examine the potential for independent evolution of the different eyespots (fig. 5.2).

The first experiments explored whether a change toward a pair of eyespots in which one became smaller and the other larger could occur as readily as one in which both eyespots were either larger or smaller. We targeted the wild type pattern for the forewing of *B. anynana* which shows a small anterior eyespot and a larger posterior one. Replicated lines were established and then selected over twenty-five generations toward each corner of the morphospace for this pattern; that is, along both the “coupled” axis for eyespot size, and an “uncoupled” axis orthogonal to a proposed genetic line of least resistance or plane of developmental drive (fig. 5.2b).

As expected, artificial selection either “up” or “down” the “coupled” axis of shared morphogenesis produced rapid responses and highly novel phenotypes, with butterflies eventually having either no eyespots

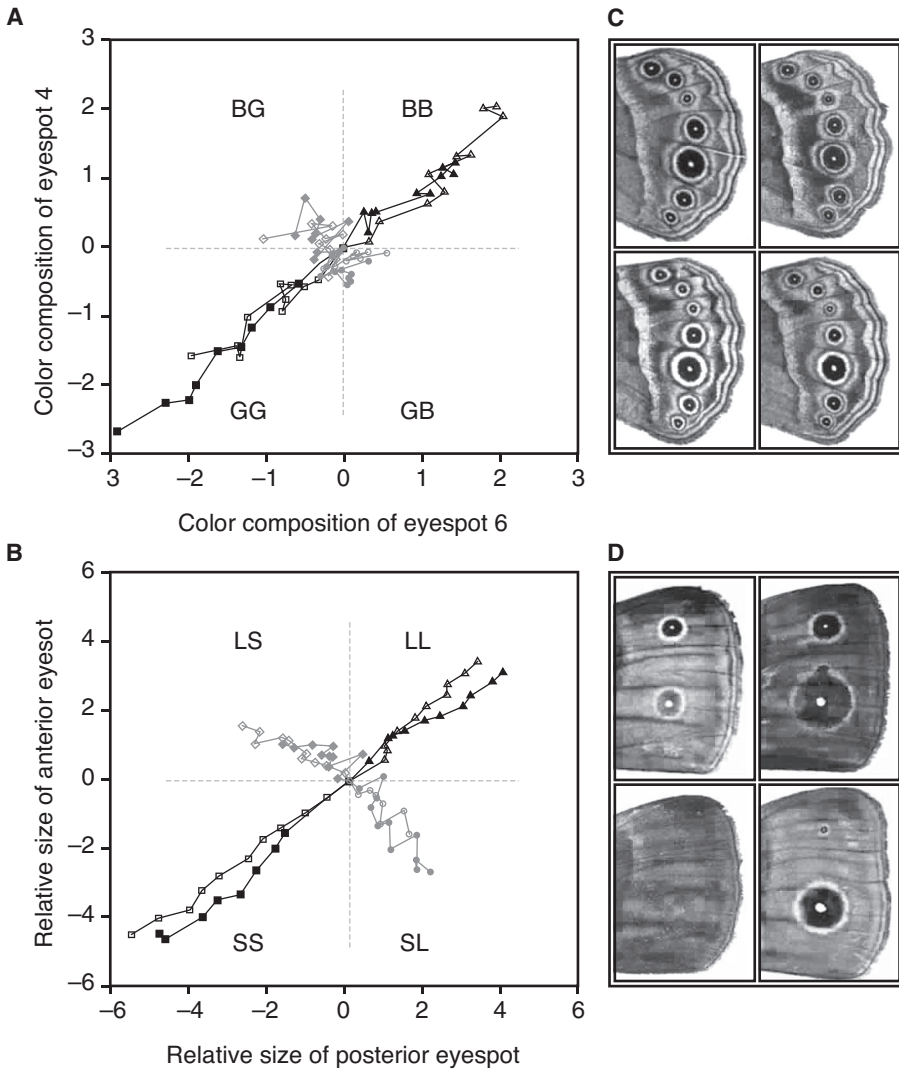


Figure 5.2. Response to artificial selection in *Bicychus anynana* for changes through morphospace in eyespot color composition and size. (a) and (b) Responses in each generation relative to unselected control values, plotted from the starting population mean plotted at the origin. Both characters were selected in replicated populations for concerted (black points and lines) and antagonistic (gray) change in two eyespots. (a) Selection for color composition of the fourth and sixth ventral hindwing eyespots (E4 and E6): 'BB' ('Black-Black') and 'GG' ('Gold-Gold') are concerted directions; 'BG' ('Black-Gold') and 'GB' ('Gold-Black') are antagonistic directions. (b) Selection for size (relative to

at all, or two very large ones (Beldade et al. 2002). However, populations along the uncoupled axis also responded well to selection (fig. 5.2b), eventually producing highly novel phenotypes, but here with one eyespot very large, and the other absent or very small. The phenotypes resulting from this experiment were, thus, situated toward all four corners of the two-dimensional morphospace, demonstrating an overall high evolvability (Beldade et al. 2002). We suggested that this capacity for independent evolution was the product of a long legacy of natural selection and evolutionary tinkering in favor of a diversity of eyespot sizes across the wings (Beldade et al. 2003).

*Experiments with Eyespots in Color Morphospace*

More recent experiments on *B. anynana* have focused on making a comparison between the dynamics of the pattern of eyespot size with that of eyespot color composition (Allen et al. 2008). Like size, there is some variability in whether a particular eyespot has a comparatively narrow outer gold ring or a broader one (black or gold, respectively). Early experiments on this trait which targeted the large forewing eyespot also demonstrated a high heritability with positive genetic correlations among eyespots (Monteiro et al. 1997a). There was, however, only a very low genetic correlation between eyespot color and eyespot size indicating differing genetic architectures. Again, artificial selection along the coupled axis for a particular pair of eyespots, this time from the hindwing series, rapidly yielded novel morphologies in which both targeted eyespots have narrower or broader gold rings (as well as any flanking eyespots). However, in marked contrast to the results for eyespot size, morphological change was much more strongly limited along the uncoupling axis in which the two eyespots were selected in opposite directions in color morphospace (fig. 5.2a). This bias was emphasized by the finding that no novel “antagonistic” color phenotypes—for example,

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Figure 5.2. (*Continued*) wing size) of the anterior and posterior eyespots on the dorsal forewing: ‘LL’ (‘Large-Large’) and ‘SS’ (‘Small-Small’) are concerted directions; ‘LS’ (‘Large-Small’) and ‘SL’ (‘Small-Large’) are antagonistic directions. (c) and (d) Representative phenotypes for each selected direction in generation 10. (c) Ventral hind wings shown for color composition lines; (d) Dorsal forewings shown for eyespot size lines with the wings arranged according to axes in (a) and (b). From Allen et al. (2008).

in gold-black or black-gold combinations—had resulted after eleven generations of selection (Allen et al. 2008).

Previous transplantation experiments among selected lines with gold or black eyespots had shown that color composition, unlike size, is determined by the threshold responses (to the signal) of the cells surrounding the focal organizer; thus, gold epithelial host tissue always yields a gold ectopic eyespot irrespective of the donor source of the signal (Monteiro et al. 1997a). This has led to a working hypothesis that whereas the pattern of relative size can readily evolve because each eyespot is determined largely by its own specific signaling organizer, the color composition over the set of eyespots on a wing is dependent on the properties of the single sheet of epithelial cells and is, therefore, much less flexible. In other words, it is possible to account for different patterns of evolvability of these eyespot traits under artificial selection in morphospace through an underlying difference in how development works. It remains unclear whether these differences are the consequence of hardwired differences in development or whether strong stabilizing selection over eyespots for color composition has resulted in little evolution of individuality among eyespots for this trait (unlike size).

Is the property of high flexibility for eyespot size reflected in the pattern of morphological disparity among extant species of *Bicyclus* and related genera? We have used museum collections to collect preliminary data on variability in the size of the two dorsal forewing eyespots among species of *Bicyclus* and *Mycalesis*, a very closely related genus in Asia within the same tribe of Mycalesina (Brakefield and Roskam 2006). When plotted together, the mean values showed a rather complete occupancy of morphospace for the pattern of relative size of the two eyespots (comparable disparity in pattern is also evident on the hind wing). In contrast, the eyespots in each of these species appear to vary little in color composition, especially when on the same wing surface; each species is characterized by eyespots of similar color, although those of different species may show comparatively narrow, intermediate, or broad gold rings (Brakefield and Roskam 2006; Allen et al. 2008). Thus, the differences in evolvability of the two eyespot traits in a single model species, as revealed in their responses to synergistic and antagonistic modes of artificial selection, appear to be reflected in patterns of disparity in morphospace that have evolved among related



species. We can now move on to examining whether the generation of phenotypic variation could also influence the evolution in morphospace for *Heliconius* butterflies in which the evolution of mimicry and the ecology clearly play a dominant role.

*HELICONIUS* WARNING PATTERNS: COULD GENETICS OR ECOLOGY  
BIAS THE EVOLUTION OF MIMICRY?

*Heliconius* butterflies show dramatic color pattern variation among species and between geographic locations. The wing patterns of *Heliconius* are warning signals that advertise their unpalatability to predators. The variation in wing patterns is almost always linked to mimicry involving other species of *Heliconius* and/or other local butterflies and moths with which sharing the warning patterns is beneficial. The evolution of novelty, and indeed diversity, in mimetic wing patterns is somewhat unexpected since species convergence should gradually erode phenotypic diversity. Therefore, mimetic patterns are a good example of traits where both the genetics and the ecology of selection could influence the exploration of morphospace (plate XX).

*Ecological Constraints on the Distribution of Mimetic  
Patterns in Morphospace*

Natural selection on mimetic wing patterns is frequency-dependent since rare or new variants are strongly selected against because they have a low chance of being encountered by predators which have previously learned through experience to avoid them. This density and frequency-dependent mode of selection produces a rugged adaptive landscape in which abundant patterns correspond to sharp peaks of fitness, while rare and/or intermediate patterns correspond to fitness valleys. As mimicry rings often involve numerous species (Beccaloni 1997), the topography of the fitness landscape depends not only on intrinsic qualities of the warning patterns (e.g., how memorable they are) but, more importantly, on the wing patterns present locally in the species' preferred habitat (Mallet and Gilbert 1995; Joron et al. 1999; Estrada and Jiggins 2002; Joron 2005). The exploration of morphospace through this rugged fitness landscape is therefore heavily dependent on the ecological structure of the community of coexisting species.

Indeed, mimetic mutualistic relationships are sufficiently pervasive to directly influence niche structure in forest butterfly communities (Elias et al. 2008). Therefore, the height of fitness peaks for any specific wing morphology is strongly affected by the ecological preferences of co-mimics, and varies widely across microhabitats. Although this might appear to reflect an ecological limitation on the exploration of new wing morphologies, the distribution of diversity within mimetic butterfly clades suggests otherwise. Widespread genera, such as *Heliconius*, *Eueides* (Heliconiinae), or *Napeogenes* (Ithomiinae), have representatives in most available mimicry rings in their size class (Beccaloni 1997; Jiggins et al. 2006; Elias et al. 2009), suggesting that the ecological conditions to invade new mimicry rings are rather permissive. Rampant fluctuations of selection pressures through evolutionary time via the interplay of mimicry and habitat preferences could facilitate an efficient exploration of locally available mimetic morphologies. However, the question remains as to whether the available mimetic patterns and the areas of the morphospace actually used for mimicry are themselves in part determined by the specific details of the underlying gene-phenotype mapping, or whether these patterns can occupy any position in the morphospace and depend solely on ecological contingency of communities and predator cognition.

#### *Qualitative Exploration of Morphospace in Mimicry*

As deviant mimetic patterns are selected against (Benson 1972; Langham 2004), and gradual changes in warning signals are supported neither by theory nor by experiments (Turner 1984; Lindström et al. 1999) (although they do shift geographically—see below), the exploration of morphospace for mimicry involves shifting between distinct mimetic patterns by crossing adaptive valleys in a single step via sudden, large phenotypic changes. Major mimicry switches that provide a rough resemblance to a new model could be favored if the new mimicry ring provides improved numerical or unpalatability protection. A second phase would then involve refinement and fixation in the population by classic Fisherian selection for increased resemblance to the new mimetic pattern (Turner 1977; Baxter, Papa, et al. 2008). This form of selection is likely to sieve out specific types of genetic architectures to control wing pattern variation, a prediction which most species of

*Heliconius* studied to date seem to conform to. One of the striking features in *Heliconius* genetics is the action of Mendelian loci that switch on or off large patches of color (phase 1 loci; Brown and Benson 1974; Sheppard et al. 1985; Gilbert 2003). In most species, such as the classic examples *H. melpomene* or *H. erato* which mimic each other nearly everywhere, a small number of such large-effect loci appears to control most aspects of geographic variation in wing pattern (Mallet 1989; Jiggins and McMillan 1997).

Perhaps the most spectacular case of large-effect locus in *Heliconius* is found in the highly polymorphic *H. numata*, whose multiple “tiger-patterned” forms fly together and mimic different coexisting species of *Melinaea* Ithomiines (Brown and Benson 1974). *H. numata* polymorphism is entirely controlled by a single supergene locus (called P), with hierarchical dominance between mimetic alleles (Brown and Benson 1974; Joron, Papa, et al. 2006). This architecture enables *H. numata* simultaneously to occupy the tops of multiple, well-separated fitness peaks in morphospace, while avoiding the non-mimetic intermediate forms that might be formed by recombination between loci, or by co-expression of alleles in heterozygous genotypes. Tight linkage locks together allelic combinations and limits the occurrence of phenotypic recombinants (Joron, Papa, et al. 2006; M. Joron unpublished data), which can be considered a form of genetic bias on the phenotypic diversity available for selection in any single locality. However, color pattern recombinants are occasionally found, albeit rarely, in polymorphic populations (Brown and Benson 1974; M. Joron unpublished data), showing that other wing patterns can be formed.

#### *Quantitative Exploration of Morphospace in Mimicry*

Examination of population samples reveals substantial levels of phenotypic variation around each mimetic pattern (Brown 1976), suggesting that despite strong selection, considerable standing genetic variation is maintained that can affect wing patterns in minor ways. Genetically, quantitative variation in *Heliconius* has been classically interpreted as the result of the segregation at so-called modifier loci, which epistatically refine the resemblance provided by alleles of large-effect loci, and might have arisen through directional selection during phase 2 of mimicry evolution (Sheppard et al. 1985). In *H. melpomene*, numerous

racings have a prominent red forewing patch, varying in size and shape. The presence of this patch is controlled by alleles of locus HmB, which does not influence its shape. Crosses between different red-forewing races of *H. melpomene* have recently shown that red patch shape is influenced by a QTL (quantitative trait locus) unlinked to HmB or to any other major-effect locus (Baxter, Papa, et al. 2008), acting as a modifier locus tuning the forewing appearance for optimal mimicry, epistatically with HmB.

Similarly, although coexisting mimetic patterns in a given locality are usually well separated from each other in the morphospace, variation can be gradual across geographic distances, as can be found in *H. numata* and *Melinaea* tiger pattern themes across the South American continent, or in the shape and size of yellow forewing patches and red hindwing rays among Amazonian races of *H. melpomene* or *H. erato* (Brown 1976, 1979). This is in fact common in many other widespread mimetic genera (e.g., *Napeogenes*, *Ithomia*; Jiggins et al. 2006; Elias et al. 2009). It is intriguing how local multi-species communities can gradually explore the morphospace. This may be due to some random drift in the local models, or their response to ecological contingency, in combination with an ability in the other mimetic species to quickly match those small variations (Flanagan et al. 2004). It will be of great interest to quantify the occupancy of morphospace in widespread mimetic taxa across their ranges to start describing the coverage attained via such “community drift.” This however demonstrates that although mimetic selection appears to be strongly constraining in terms of how populations can shift mimetic patterns locally, their actual morphospace positions do not appear to be determined by strong constraints in pattern formation, and that there is in most cases sufficient standing genetic variation to allow for a gradual exploration of the morphospace by several species simultaneously (plate XX).

#### *Imperfect Mimicry: Evidence for Constraints?*

Exceptions to this observation of near-perfect mimicry are, however, not uncommon. For example, east-Ecuadorean co-mimics *H. erato notabilis* and *H. melpomene plesseni* show an inversed placement of pink and white areas in the proximal forewing patch (Brown 1979; Papa et al. 2008). Given the very precise mimicry of the two species nearly

everywhere else, it could be considered that *H. m. plesseni* has attained a local maximum of resemblance, as if those populations were occupying a suboptimal fitness peak. This would in turn suggest that further improvement is constrained, perhaps because it requires some developmental novelty to arise or a rare favorable mutation. It is hard, however, to distinguish whether such examples of imperfect mimicry are a consequence of constraints in the machinery of wing pattern formation, or due to relaxed selection on specific pattern elements, or to population contingency and drift.

*Homology of Major Loci: Evidence for Genetic Constraints?*

The ecological circumstances which favor the evolution of mimicry, together with the requirement of large-effect variation to allow populations to cross adaptive valleys, might imply that the identity of the loci recruited in different species is to some degree a product of genomic contingency, that is, species-specific. Alternatively, there may be few ways of switching patterns, and genetic architectures might bias which genes must be recruited to switch to an appropriate new pattern. The rich comparative genetics data now available for *Heliconius* may help in tackling this question. The major pattern observed is the strong positional conservation of the genomic regions controlling both convergent and divergent color pattern variation in most *Heliconius* (Jiggins et al. 2005; Joron, Papa, et al. 2006; Kapan et al. 2006; Kronforst, Kapan, et al. 2006; Baxter et al. 2008b; Papa et al. 2008). For instance, the parallel geographic radiation in *H. erato* and *H. melpomene* (which are not very closely related species) involves orthologous genomic regions, on three chromosomes, in the two species. Since those species mimic each other nearly everywhere, this conserved “toolbox” of pattern genes (Gilbert 2003) might be considered evidence for biases in the patterns which can readily be evolved. However, the data suggest otherwise. Mimetic polymorphism in *H. numata*, closely related to *H. melpomene* but with very different color pattern diversity, involves a single switch locus which is a positional homologue of a cluster of loci controlling the presence of yellow patches in *H. melpomene*. The *H. numata* whole-wing variation is entirely controlled by only one of the tools in the *Heliconius* toolbox of color pattern loci (Joron, Papa, et al. 2006; Baxter, Papa, et al. 2008). The disjunct arrays of mimetic patterns in

*H. melpomene* and *H. numata* suggest the diversity of phenotypes controlled by this switch locus is highly flexible with few, if any, limitations. Genetic variation appears to be always available in the genome repeatedly to evolve the control of mimetic novelty by the same switch loci.

## DISCUSSION AND PERSPECTIVES

Artificial selection experiments on the pattern of repeated eyespots in *B. anynana* have attempted to assay the potential role of developmental flexibility in generating phenotypic variation in the context of the evolution of occupancy of morphospace by related species. For one of the two traits examined in this way, there is evidence that it does indeed matter how development works. Thus, the mechanisms underlying variation in eyespot color composition appear to introduce a strong developmental bias that appears to be reflected in patterns of disparity among species of *Bicyclus* in Africa, and of *Mycalesis* in Asia. In contrast, eyespot size behaves in a highly flexible manner both for the response to artificial selection in the model species and for morphospace across the two lineages. Although there is a match between these experimental results and earlier interpretations of the developmental genetics of the two traits, we cannot at this stage distinguish between explanations in terms of a tight, long-term, developmental constraint, and a difference in the way in which natural selection has favored the evolution of evolvability for color and size. A combination of a wing-level developmental process and a history of strong stabilizing selection on eyespot color composition within each species in its particular environment could account for the observed absence of evolvability in this trait. This would then result in the observed lack of individuality for color composition in an eyespot module and a coupled lack of potential for independent evolution.

Another trait of interest for a related reason is eyespot shape. Here there was little evolvability for “fat” or “thin” eyespots in *B. anynana*, and what response there was to artificial selection occurred via changes in wing shape and the arrangement of scale cells across the wing (Monteiro et al. 1997b, 1997c). It is intriguing to speculate that some developmental novelty may be required to convert a circular, cone-shaped,

signal to a non-circular one (although ellipsoidal eyespots occur in some butterflies, e.g., Nijhout 1991). However, using a similar reasoning to that for eyespot color, there may be valid functional explanations in terms of predation and stabilizing selection for eyespots being typically circular, and for the low evolvabilities observed for this trait.

Although these ideas are expressed here in terms of underlying developmental mechanisms and bias, they can also be set out from a genetic perspective. More data sets of this type are necessary to resolve the most appropriate terminology. Further progress will be made through the application of high throughput genetic tools, fine genetic mapping of different traits, and more detailed developmental analyses in the model species (Beldade et al. 2009), in combination with examining patterns of occupancy in morphospace in different lineages using a comparative phylogenetic framework.

The experiments and observations on eyespot patterns in the mycalesine butterflies do not directly examine evolvability in the context of the functional performance of the different forms. Although it is known that eyespot size in *Bicyclus* is influenced by natural selection and sexual selection, much more work is needed in this area. In common with many evo-devo systems, research on eyespot patterns is revealing details about the generation of phenotypic variation, but it will be much more challenging to measure fitness curves and understand exactly how natural selection works in the wild. In *B. anynana*, an attempt has been begun for patterns of allometric growth (wing:body, and forewing:hindwing size) to combine artificial selection experiments with subsequent analyses of the relative fitness of the resulting phenotypes (Frankino et al. 2005, 2007). However, *Heliconius* butterflies hold a particularly rich promise for understanding how phenotypic variation is generated in the context of a detailed picture of how natural selection influences different phenotypes in morphospace.

Unlike *Bicyclus*, knowledge about the evolvability of wing patterns in *Heliconius* is not coming from developmental genetics and artificial selection, but from genetic dissection of extant wing pattern diversity from natural populations set into the context of a rich history of theoretical and empirical studies of mimetic evolution (Turner 1977, 1984; Mallet et al. 1990). This does not directly reveal the option set of patterns that are potentially evolvable in morphospace, but rather an

understanding of how the observed, somewhat discontinuous coverage of morphospace is controlled by the variation at key loci shared among all members of the clade. The overall results demonstrate an astonishing flexibility in the phenotypic action of those loci, co-opted to control a high pattern diversity as well as convergence among distantly related species (Joron, Papa, et al. 2006; Kronforst, Kapan, et al. 2006; Baxter, Papa, et al. 2008).

As was noted early on by Turner (1977), there are reasons why the ecological conditions governing the exploration of morphospace might select for specific types of genetic architectures, which in turn could limit or channel adaptation along particular directions. However, the dominant pattern is that of a lack of any obvious limitations on the evolution of new mimicry associations (although mimicry itself could be considered an inherently limited way of exploring morphospace). As the evolution of new patterns in a population must involve a significant pattern shift, it may be necessary for genetic loci of major effect to be maintained in the population as a transient polymorphism under disruptive selection (Naisbit et al. 2003). Therefore, the ecology of mimicry evolution may restrict pattern switches to be in functionally integrated regions in the genome, to be rarely disrupted by recombination, and to exhibit relatively little pleiotropy and epistasis with the rest of the genome. Switch loci can then be the prime targets of selection for large-effect variation, which could explain why the same loci are repeatedly recruited to control pattern evolution, even in locally monomorphic species. This view predicts that mimicry switch loci are eminently evolvable *units* which facilitate the exploration of morphospace. The fact that the *Heliconius* colour pattern “toolbox” is largely shared across species and composed of “plug-and-play” transferable units is further supported by the fact that foreign alleles can be introgressed into host genomes, either through natural hybridization (Kronforst, Young, et al. 2006; Mavárez et al. 2006; Dasmahapatra et al. 2007), or via artificial crosses (Gilbert 2003). They then tend to retain the same phenotypic effects as in the source species, sometimes to the point of causing hybrid speciation in certain documented cases (Mavárez et al. 2006). It appears that the switch loci recruited during early mimicry evolution could be interpreted as the evolutionary novelty



allowing mimetic taxa to quickly explore and invade available mimetic niches; thus, there appears to be minimal constraint in transgressing a rugged fitness landscape. This may explain why butterfly taxa involved in color pattern mimicry (Heliconiines, Ithomiines, dismorphiine Pierids, some riodinid genera, and so on), often show spectacular adaptive radiations into numerous mimetic morphologies.

This mode of morphospace exploration does not, however, preclude quantitative variation over long distances, and one aspect that is often neglected is the richness of quantitative geographical variations in mimicry, even in species which also show highly differentiated color-pattern races. This in itself can be seen as strong support for the notion that the exact position of mimicry patterns in *Heliconius* morphospace is largely accounted for by the biology of signal-receiver interactions and ecological contingency rather than by any tight constraints introduced via developmental mechanisms. Contingency may be considered a form of constraint on morphospace exploration if the path initially taken to evolve a novel morphology diminishes the array of possible morphologies available thereafter. In this sense, given the spectacular diversity present today, the evolution of novel switch loci in the early stages of mimicry evolution could reflect the opposite of a constraint.

However, this interpretation awaits a quantitative validation through a more precise description of morphospace occupancy with the level of detail that is available for *Bicyclus*. Although *Heliconius* are not as amenable to artificial selection as *Bicyclus*, the molecular dissection of variation in their color patterns is making rapid progress toward characterizing such integrated switch loci and their regulation (Joron, Papa, et al. 2006; Baxter, Papa, et al. 2008; Papa et al. 2008). It will then become fascinating to integrate knowledge of these mechanisms of genotype-phenotype mapping with a quantitative analysis of the occupancy of morphospace for all the pattern elements modulated by such loci. Overall, the prospect for work on butterflies is to be able to examine how the developmental pathways regulating scale morphology and color patterns have been recruited in evolution to respond to the ecology of diversification in different elements of butterfly wing patterns.

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