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Genomic hotspots of adaptation in butterfly wing pattern evolution

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What is the genetic architecture of morphological evolution? Is there uniform potential for novelty across a genome or, on the contrary, can a small number of large-effect genes explain the phenotypic variation observed within and between species? Here we highlight recent work on butterfly wing pattern genetics showing that a small set of loci can be repeatedly involved in the evolution of complex traits. These loci behave as genomic hotspots for diversification because they underlie adaptive variation within and between species with both convergent and highly divergent wing patterns. These findings suggest that certain loci may be more likely than others to facilitate rapid evolutionary change.

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Introduction

The last few years have seen remarkable advances in understanding the molecular basis of morphological evolution. Widespread access to new technologies has made it possible to identify the genomic targets of selection that control adaptive phenotypes, including in species not normally considered to be genetic model systems. This has now set the stage to ask more general questions about the genetic architecture of evolving phenotypes [1,2]. One of these questions is whether certain genes tend to underlie phenotypic adaptation more than others. Are there certain loci — genomic hotspots of adaptation — that make a disproportionate contribution to evolutionary change across a phylogenetic spectrum?

The recent literature regarding the genetic basis of convergent and parallel evolution in animals is of particular interest because it reveals some potential examples of adaptive hotspots — instances where the same gene underlies adaptation in multiple lineages. There are now numerous cases known of specific genes underlying parallel and convergent evolution [3]. For example, regulatory elements of *yellow* underlie multiple cases of convergent evolution of both abdomen and wing pigmentation in *Drosophila* species [4,5], independent events of albinism in cavefish were linked to mutations in *Oca2*, a determinant of pigmentation in human populations [6,7], and derived pigmentation in several stickleback populations is linked to a regulatory allele of *Kitlg*, a gene associated with skin color in humans [8]. Repeated fixation of an allele of the *Eda* gene is responsible for the reduction of armor plates in sticklebacks [9–11], while its receptor *Edar* matches a quantitative trait locus (QTL) for hair thickness in humans [12]. Modulation of *Pitx1* expression explains repeated pelvic reduction in two different species of sticklebacks, and is also thought to be responsible for pelvic reduction in manatee, a marine mammal [13,14]. Also, *Bmp4* has been proposed as a hotspot for the evolution of feeding strategies since it acts as a QTL influencing the mechanical properties of cichlid fish mandibles [15] and presumably the beaks of Darwin's finches [16]. These are all examples of loci repeatedly exploited by evolution to produce similar adaptive traits in different lineages.

In retrospect it is not surprising that the evolution of similar phenotypes often occurs through changes in the same genes. At this point there are enough examples of specific genes underlying parallel and convergent evolution that it might appear to be a general evolutionary trend. It has been noted, however, that there are a similar number of examples of convergence occurring through changes in different genes [3]. Accordingly, to identify potential adaptive hotspots it may be more useful to ask if some loci tend to underlie the evolution of *different* morphologies across multiple lineages. This is a phenomenon for which there are fewer examples. One case of this is the *Mc1r* gene, in which coding mutations trigger divergent patterns of melanization in mammals, birds, and lizards, including at the population level [17,18]. Another possible example is that regulatory evolution of *svb/ovo* explains multiple cases of convergence in the larval bristle patterns of species of *Drosophila* [19,20], while the nematode ortholog of *svb/ovo* underlies the evolution of excretory duct morphology in *Caenorhabditis elegans* [21]. In this latter example, however, it is questionable whether comparing only two taxa that are so phylogenetically distant represents a trend rather than a coincidence.

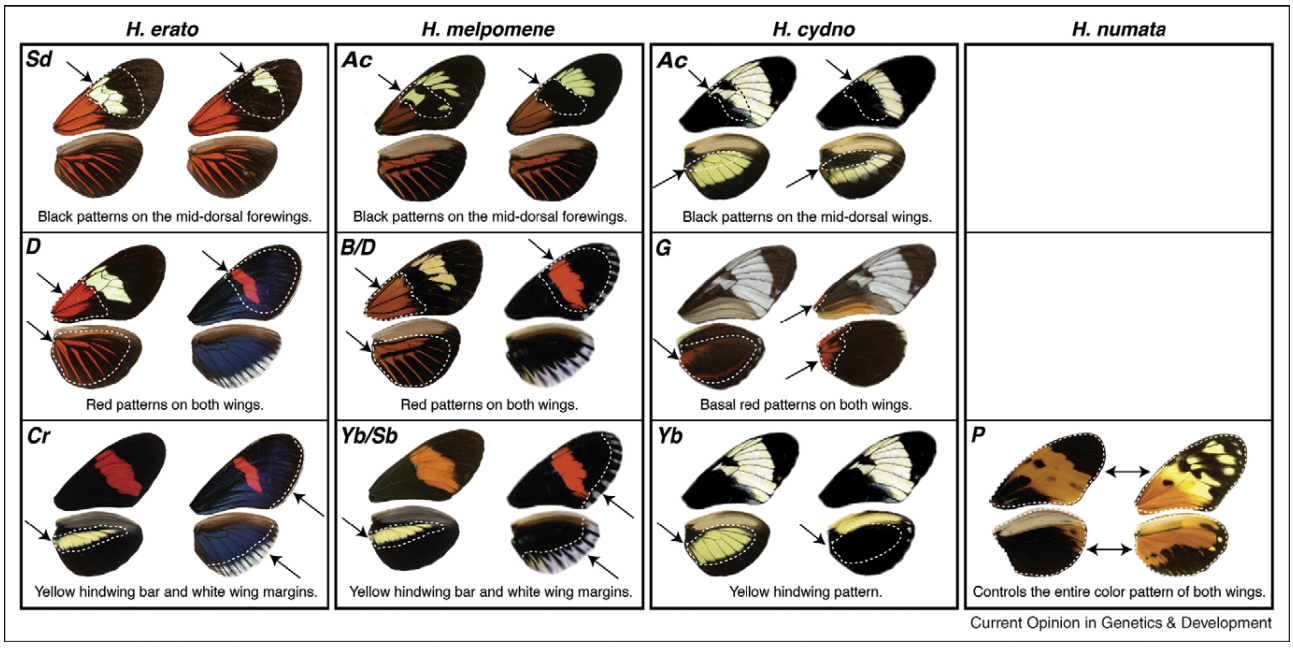
Despite the case studies described above, one can argue that the adaptive hotspot hypothesis is premature, or the result of a 'street light syndrome', where inferences are biased by expectations. This is especially a concern in studies relying on a candidate gene approach (i.e. focusing on gene in one system because its function is known in another system), which many of the above examples do. In this respect, we argue that butterfly wing patterns are a uniquely powerful system for assessing potential adaptive hotspots. Genetic mapping efforts are underway in several different butterfly species [22,23], allowing completely independent discoveries of loci controlling wing pattern variation and divergence in nature. This approach precludes biases resulting from candidate genes. Furthermore, the links between morphological variation and adaptive evolution are particularly well known in butterflies. Indeed, butterfly wing pattern genes are frequently known to be under selection because they play a clear role in adaptation — particularly in situations involving mimicry, crypsis, and mate choice. For these reasons, loci controlling natural variation in wing patterns are exceptionally useful as 'model genes' for comparative studies of adaptation.

Color pattern genetics of *Heliconius* butterflies

While color pattern related linkage mapping is being done for several butterfly species, most published work is from the neotropical nymphalid butterfly genus *Heliconius* — the group we focus on here. The most recent phylogenetic work places 38 species in this genus [24], most of which are highly polymorphic and participate in mimicry [25]. *Heliconius* is most famous for the spectacular convergent radiations between the two distantly related comimics *Heliconius erato* and *Heliconius melpomene* [26,27]. In this textbook example of Müllerian mimicry (i.e. mimicry where all participants are distasteful) each species consists of at least 20 named geographic races, most of which mimic co-occurring races of the other *Heliconius* species, as well as other species of butterflies and day-flying moths. Population genetic work suggests that *H. erato* represents an older radiation and that *H. melpomene* evolved to resemble *H. erato*'s wing patterns [28].

Over the last 50 years intraspecific crossing experiments using different wing pattern races of *H. erato* and *H. melpomene* have led to the description of a toolkit of more than a dozen Mendelian genes controlling natural wing pattern variation in each species [29•,30•,31•,32–35]. Even though many genes affecting color patterns have been named, much of the color pattern variation in each species can be attributed to only a few loci of major effect (Figure 1). Efforts to map these color patterns genes have focused on four species: *H. erato*, *H. melpomene*, *Heliconius*

Figure 1



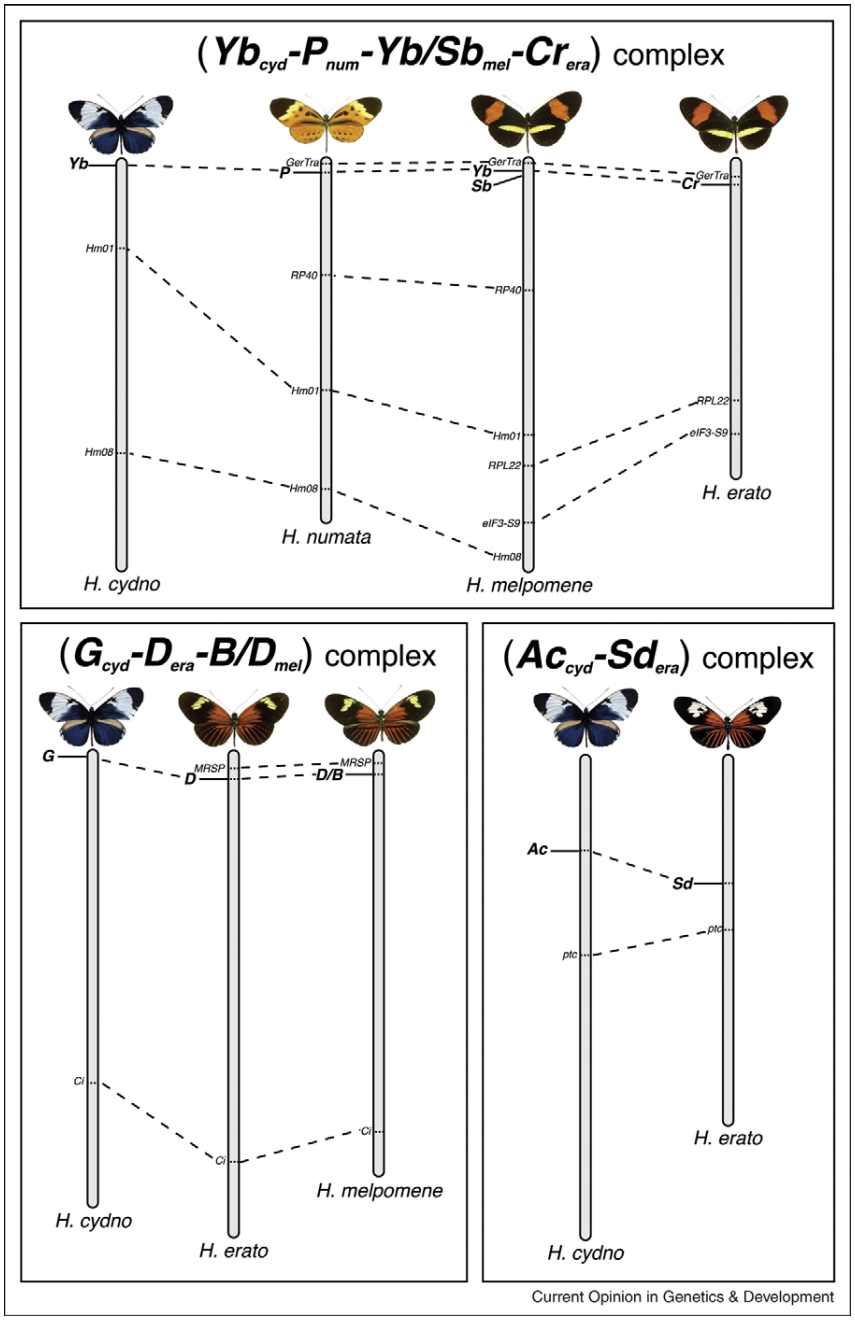
A brief outline of the effects of three major color pattern loci across four different *Heliconius* species. Each column represents a different species, while each row represents a homologous color pattern locus. Names of loci are in upper left corners of panels, and arrows and dashed lines specify the regions of effect. All wings are presented by their dorsal surfaces with the exception of the *H. cydno G* locus panel, for which the ventral surface is shown. Note that in *H. melpomene B* and *D* are tightly linked loci, where *B* controls the color of the forewing band, and *D* controls the hindwing rays and red at the base of the forewing. Similarly, in *H. melpomene Yb* and *Sb* are tightly linked loci, where *Yb* controls the hindwing yellow bar, and *Sb* controls the white wing margins. In *H. numata* the entire color pattern variation is controlled by a single Mendelian locus, *P*.

cydno, and *Heliconius numata*. Of these, the *H. erato* lineage is distantly related to the lineage containing the three closely related species *H. melpomene*, *H. cydno*, and *H. numata*. This sampling of species represents an intentional strategy to compare distantly related species with convergent phenotypes (*H. erato* and *H. melpomene*), as well as closely related species with highly divergent phenotypes (*H. melpomene*, *H. cydno*, and *H. numata*) [27,36••,37••].

Homologous loci control convergent and divergent wing patterns across species

The most remarkable finding to emerge from mapping efforts in *Heliconius* is that the same few loci appear to

Figure 2



Comparative linkage mapping in different species of *Heliconius* suggests that a small set of homologous loci control both convergent and divergent color patterns across species. The *Yb–P–Yb/Sb–Cr* locus controls multiple pattern elements in a complex way across four species, the *G–D–B/D* locus controls various basal red patterns in at least three species, and the *Ac–Sd* locus controls forewing melanin patterns in a complex way in at least two species.

underlie the majority of natural variation across all of the species studied so far (Figure 2). This is perhaps best exemplified by Joron *et al.* who showed that the *Cr* locus of *H. erato* maps to the same location as *Yb/Sb* in *H. melpomene* [36••]. This in itself may not be completely surprising because these loci control similar color pattern elements in the two species, and there is a strong precedent for a conserved genetic basis of convergent evolution as we previously discussed. The most interesting discovery, however, was that the *P* locus, which controls wing pattern variation in *H. numata*, mapped to the very same position [36••]. *H. numata* is closely related to *H. melpomene*, but it looks completely different and its entire wing pattern is controlled by a single locus with many major-effect alleles, as opposed to a multi-gene toolkit as in *H. melpomene*.

This phenomenon extends to other loci and species as well. Kronforst *et al.* mapped three color pattern genes in *H. cydno*: *Yb*, *Ac*, and *G* [37••]. *H. cydno* is closely related to *H. melpomene* and *H. numata*, but has very different wing patterns. *Yb* in *H. cydno* has a similar effect to *Yb* in *H. melpomene* — it controls the presence and absence of a yellow hindwing bar. It was therefore unsurprising to find that *Yb* maps to the same locus in both species. More surprising was that *Ac* in *H. cydno* maps to the *H. erato Sd* locus. The effect of this locus is fairly different in the two species, except in both species it is involved in controlling the melanic pattern elements on the forewing. Another surprise was that the *G* locus maps to *H. erato*'s *D*, and by extension, *H. melpomene's B/D*. *G* phenotypes in *H. cydno* do not closely resemble *D* phenotypes, except that they both influence red patterns in the basal regions of wings.

These comparative mapping data are extremely interesting; however, the specific molecular identities of the *Heliconius* color pattern genes remain unknown. Accordingly, research in several labs is now focused on positionally cloning these loci. To date, researchers have characterized the ~300 kb sequence interval including *Cr–Yb* and the ~600 kb interval including *D–B/D* in both *H. erato* and *H. melpomene* [38•,39••]. Comparative sequence analysis of these regions shows almost perfect synteny in predicted genes between *H. erato*, *H. melpomene*, and the silkmoth *Bombyx mori*. This finding not only suggests that synteny is highly conserved across the Macrolepidoptera, but it also supports the hypothesis that the *Heliconius* color pattern loci are homologous between species at the DNA sequence level. The next challenge is to identify the specific recombining sequences underlying *Heliconius* color pattern variation. This will not only be informative as to what kind of genetic elements adaptive hotspots may tend to be (e.g. protein-coding regions, *cis*-regulatory regions, or regulatory RNAs), but it will also allow a better understanding regarding the age and diversity of the variation-causing alleles relative to the color pattern radiations themselves.

Portrait of a genomic hotspot for morphological evolution

Presumably it takes dozens or hundreds of genes to make a butterfly wing pattern. If this is so, why do such a small number of genes repeatedly underlie the evolution of wing patterns? One possible explanation is that developmental and genetic constraints channel evolutionary change in such a way that only a small number of loci have significant potential to produce novel phenotypes. One situation that would produce this effect would be if adaptive hotspots tended to be minimally pleiotropic downstream effector genes. Functional changes in structural genes could be relatively difficult to buffer against developmentally and would often have large effects in specific tissues. There is a strong precedent for this model in other proposed hotspots: *Mc1r* and *Oca2* are specific to the pigmentation process [18], and *Edar* is restricted to skin appendage development [40]. This model probably does not apply to *Heliconius*, however. Although regulation of the pigment gene *vermilion* appears to be controlled by *D* in *H. erato* [41], thus far there are no obvious pigmentation genes in the color pattern gene sequence intervals [38•,39••] or yet identified as being genetically linked to the color pattern loci [29•,30•,31•].

Another scenario that would focus evolutionary change on a few loci would be if key mutations occurred in highly pleiotropic genes that act through controlling the expression of downstream developmental modules. Large-effect tissue-specific changes could be achieved through changes in the regulatory regions of such upstream regulatory genes [4,19,21,42,43]. Indeed, modeling work has suggested that regulatory networks should tend to evolve an architecture where the majority of the network is robust to mutation, but mutations at specific hub genes in the network will have large effects tending to be relatively benign [44]. Work on the *Edar* network in sticklebacks has also led to the proposal that these hub genes should have larger and more modular *cis*-regulatory regions, and thus would be more likely to undergo regulatory evolution [45]. On the basis of these ideas one might predict that the *Heliconius* color pattern loci represent relatively upstream developmental regulatory genes with complex *cis*-regulatory regions.

Conclusion

Mapping and positional cloning work across the genus *Heliconius* has shown that a small set of homologous loci repeatedly underlie the evolution of both convergent and divergent wing patterns within and between species. It remains to be seen, however, exactly what the molecular elements underlying this variation are. Further fine-scale mapping, nucleotide polymorphism association studies, comparative sequencing, and gene expression studies will hopefully allow us to better understand the nature of these adaptive hotspots in the butterfly genome. Ongoing work will be greatly facilitated by the continuing de-

velopment of genomic resources like BAC libraries, EST databases, and expression microarrays [23], including the scheduled sequencing of two *Heliconius* genomes in 2009.

Future work on other butterfly species will give us a notion of how 'hot' these genomic hotspots of adaptation really are. Do these hotspots extend into other genera or families of butterflies? Many species of butterflies are polymorphic — is it possible that these *Heliconius* hotspots also underlie variation in other groups of butterflies? Comparative analysis of *Heliconius* color pattern polymorphism, modular control of eyespot size in *Bicyclus* butterflies [46,47], and swallowtail mimicry [48] could allow the first steps into gaining insight into this broader question. Indeed, the genetic mechanisms that lead to wing pattern diversity could be more directly reflective of an evolutionary ground plan [49] than previously thought.

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