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Mimicry: developmental genes that contribute to speciation

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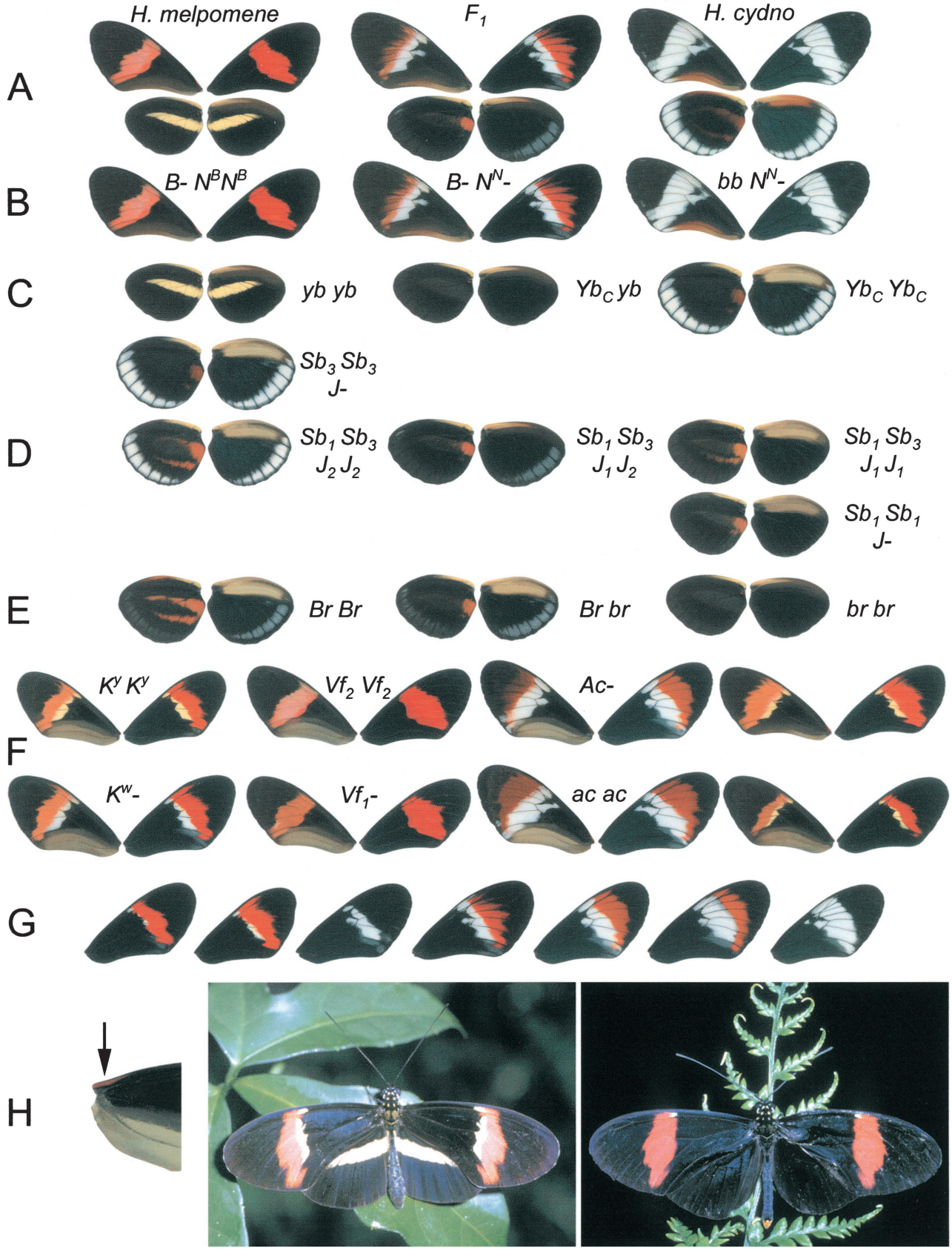
SUMMARY Despite renewed interest in the role of natural selection as a catalyst for the origin of species, the developmental and genetic basis of speciation remains poorly understood. Here we describe the genetics of Müllerian mimicry in *Heliconius cydno* and *H. melpomene* (Lepidoptera: Nymphalidae), sister species that recently diverged to mimic other *Heliconius.* This mimetic shift was a key step in their speciation, leading to pre- and postmating isolation. We identify 10 autosomal loci, half of which have major effects. At least eight appear to be homologous with genes known to control pattern differences within each species. Dominance has evolved under the influence of identifiable "modifier" loci rather than being a fixed characteristic of each locus. Epistasis is found at many levels: phenotypic interaction between specific pairs of genes, developmental canalization due to polygenic modifiers so that patterns are less sharply defined in hybrids, and overall fitness through ecological selection against nonmimetic hybrid genotypes. Most of the loci are clustered into two genomic regions or "supergenes," suggesting color pattern evolution is constrained by preexisting linked elements that may have arisen via tandem duplication rather than having been assembled by natural selection. Linkage, modifiers, and epistasis affect the strength of mimicry as a barrier to gene flow between these naturally hybridizing species and may permit introgression in genomic regions unlinked to those under disruptive selection. Müllerian mimics in *Heliconius* use different genetic architectures to achieve the same mimetic patterns, implying few developmental constraints. Therefore, although developmental and genomic constraints undoubtedly influence the evolutionary process, their effects are probably not strong in comparison with natural selection.

INTRODUCTION

The emerging field of evolutionary developmental biology seeks to explain changes in ontogeny that lead from altered genotype to altered phenotype, a process that has been called "developmental reprogramming" (Arthur 2000). It will provide a fundamental contribution to evolutionary theory if it yields answers to two major questions.

First, to what extent does development constrain or drive evolution? In other words, are there emergent properties of developmental reprogramming so that directionality in evolution is not the sole preserve of natural selection acting on random variation? Such "developmental drive" would act in conjunction with selection rather than in opposition, leading to beneficial but suboptimal evolution, so that detectable phylogenetic inertia should result (Arthur 2001). Does adaptive divergence typically proceed by the substitution of many genes of minor effect (Fisher 1930), or can genes of large effect contribute to adaptation and speciation (Orr and Coyne 1992; Coyne and Orr 1998)? Recent theory suggests that when natural selection optimizes quantitative traits, an exponential distribution of gene effects will become fixed, with many factors of small effect and a few of large effect (Orr 1998, 1999).

Second, how do development and selection interact in speciation and macroevolution, and can microevolution and macroevolution be explained by the same developmental processes? Most work has focused on major events in body plan diversification (Holland 2000; Shankland and Seaver 2000), with fewer studies of intraspecific or interspecies differences (Stern 1998; Beldade et al. 2002). It therefore remains unclear if there are fundamentally distinct levels of diversification, or if the higher levels can be extrapolated from microevolution (Leroi 2000). It has recently become apparent that speciation is often caused by normal processes of adaptive differentiation under divergent natural selection (Filchak et al. 2000; Rundle et al. 2000; Jiggins et al. 2001; Podos 2001), so that macroevolution might be simply extrapolated from microevolution. Although a few studies have identified both the key traits initiating reproductive isolation and their developmental genetic basis (Schemske and Bradshaw 1999; Hawthorne and Via 2001; Peichel et al. 2001), the field is still largely dominated by studies of hybrid sterility and inviability (Coyne and Orr 1998; Orr and Presgraves 2000).


A
H. melpomene
F1
H. cydno
B
B- NBNB
B- NN-
bb NN-
C
yb yb
Yb_C yb
Yb_C Yb_C
Sb3 Sb3
J-
D
Sb1 Sb3
J2 J2
Sb1 Sb3
J1 J2
Sb1 Sb3
J1 J1
Sb1 Sb1
J-
E
Br Br
Br br
br br
F
Ky Ky
Vf2 Vf2
Ac-
Kw-
Vf1-
ac ac
G
H

Genetic dissection of speciation may also provide answers to questions such as what roles do linkage, dominance, and epistasis play in adaptive speciation? Are these genetic constraints incidental properties of newly evolved genes, or do the constraints themselves evolve? For instance, tight linkage between functionally related loci may exist because of recent tandem duplication, or it may be adaptive, because clustering of loci into linked blocks can preserve associations between coadapted alleles (Noor et al. 2001; Rieseberg 2001). Epistasis in particular is fundamental to speciation, because it is the production of ecologically or intrinsically maladapted gene combinations in hybrids that causes reproductive isolation (Whitlock et al. 1995; Turelli and Orr 2000).

Mimicry in butterflies provides a useful and visually appealing example of adaptive evolution in which to answer these questions, where a link can be made between developmental genetics and fitness in the field (Fisher 1930; Goldschmidt 1945; Turner 1984; Mallet and Barton 1989; Kapan 2001). Mimicry is also implicated in speciation, because reproductive isolation can arise as an incidental by-product of adaptive divergence in color pattern (Bates 1862; Darwin 1863; Vane-Wright 1978; Turner 1981; Mallet et al. 1998). Goldschmidt (1940), who proposed that speciation occurred via major "systemic mutations" fundamentally different from normal quantitative variation, argued that major genes affecting mimicry were among the few examples of systemic mutations occurring within species. In 1945, Goldschmidt made the case for mimicry as an example of a wider phenomenon where "developmental constraints" were as important as natural selection in evolution. Despite being morphologically simple two-dimensional traits, butterfly wing color patterns are still poorly understood at the developmental level (Carroll et al. 1994; Koch et al. 1998; Brunetti et al. 2001; McMillan et al. 2002). One emerging generalization is that early developmental pathways are redeployed in wing pattern formation at a much later stage (Carroll et al. 1994; Brunetti et al. 2001; Beldade et al. 2002).

Mimicry in *Heliconius*

Heliconius butterflies are warningly colored and unpalatable and are often Müllerian mimics of other *Heliconius* or ithomiine butterflies (Turner 1984; Sheppard et al. 1985; Mallet et al. 1998). Extensive work has shown that major genes control color pattern differences between geographic races within species (Turner and Crane 1962; Sheppard et al. 1985; Mallet 1989; Linares 1996, 1997), but few studies involve more than one species (Nijhout 1991; Jiggins and McMillan 1997). Mimicry genes in *Heliconius* are developmental or regulatory rather than structural for two reasons. First, although changing pattern they do not alter the ability to produce pigment. For example, black (melanin), red or brown (xanthommatins), and yellow (3-hydroxykynurenine) are all usually present somewhere on the butterfly. Second, as with other butterfly color pattern genes (Goldschmidt 1945), there are correlations between the microstructure of wing scales and the pigments laid down (Gilbert et al. 1988; Janssen et al. 2001).

The genetics of mimicry is usually studied within an ecological genetics (microevolutionary) framework. However, divergence in mimicry of the butterflies *Heliconius cydno* and *H. melpomene* (Fig. 1A) has strongly affected mate preferences and has led to maladaptive nonmimetic hybrids (Mallet et al. 1998; Jiggins et al. 2001). The species also display female hybrid sterility (Naisbit et al. 2002) and reduced hybrid mating success (Naisbit et al. 2001), but these almost certainly evolved after initial divergence, whereas traits such as differences in microhabitat (Mallet and Gilbert 1995) and host plant use (Smiley 1978) provide only weak barriers to gene flow. Divergence in mimicry was therefore a key step

Fig. 1. (A) *Heliconius melpomene*, *H. cydno*, and their nonmimetic F_1 hybrid. All pairs of wings are shown at 60% life size, with the upper surface on the right and the lower surface on the left. (B) Interaction between the *N* and *B* loci in the forewing band. A dash indicates an allele undetermined because of dominance. (C) Control of the yellow hindwing bar of *H. melpomene* by the *Yb* locus. The effect of the *H. melpomene* allele is shown to the left and that of the *H. cydno* allele to the right. The white band in the wings on the right is added by the *Sb* locus. (D) Control of the white hindwing submarginal band of *H. cydno* by the *Sb* locus and modification of dominance by *J*. The *H. cydno* submarginal band phenotype is shown top left and that of *H. melpomene* bottom right. The center row shows modification of dominance in *Sb* heterozygotes by the *J* locus. (E) Control of the brown forceps-shaped marking of *H. cydno* by the *Br* locus. The effect of the *H. cydno* allele is shown to the left and that of the *H. melpomene* allele to the right. (F) Far left, control of forewing band color by the *K* locus. For this and the following two loci, the effect of the *H. melpomene* allele is shown in the top row, above that of the *H. cydno* allele. Center left, control of the color of the underside of the red forewing band by the *Vf* locus. Center right, control of the anterior half of the forewing white hourglass by the *Ac* locus. Far right, variation in the width of the red portion of the forewing band. (G) Variation in the width of the white portion of the forewing band. The wing in the center shows the phenotype of an F_1 hybrid. (H) Left, the red line (arrowed) of *H. melpomene* at the base of the forewing lower surface controlled by the *G* locus. Center and right, two hybrid phenotypes produced by recombination within the *N-Sb-Vf-Yb* linkage group. Both are the result of a cross-over between *N* and *Sb-Vf-Yb* in a backcross of F_1 male to *H. melpomene* female. Center, genotype $N^N N^B Sb_1 Sb_1 Vf_2 Vf_2 ybyb$, right, genotype $N^B N^B Sb_1 Sb_3 Vf_1 Vf_2 Yb_c yb$. Shown at 80% life size. A forewing of genotype $N^B N^B Vf_1 Vf_2$ is shown in F, center left lower wings. The only other cross-over seen within this linkage group was between *Yb* and *Vf-Sb-N*, producing the genotype $N^B N^B Sb_1 Sb_1 Vf_2 Vf_2 Ybyb$. The hindwing is shown in D, lower right, and the forewing was like that of *H. melpomene*.

in speciation of *H. cydno* and *H. melpomene*, and the developmental genes controlling color pattern differences are as much "speciation genes" as those for hybrid sterility in *Drosophila* (Orr and Presgraves 2000; Ting et al. 2000).

Here we investigate genes that determine differences in mimicry between *H. cydno* and *H. melpomene* to answer the following questions:

1. What is the genetic architecture of mimicry and does it suggest developmental constraints? Are individual gene effects major or minor? What roles do epistasis, linkage, and dominance play in the evolution of color pattern?
2. Are mimicry genes that contribute to speciation homologous to those used in mimetic shifts within each species?
3. Do partners in Müllerian mimicry use homologous genetic variation to achieve the same patterns? If so, developmental constraints could be much more important in the evolution of mimicry (Goldschmidt 1945; Nijhout 1991) than classically believed.

MATERIALS AND METHODS

Crosses were performed in Gamboa, Panama between September 1999 and March 2000 using *Heliconius cydno chioneus* and *H. melpomene rosina* collected from nearby forest in Soberanía National Park. To obtain crosses, we isolated virgin females with older males. After mating, females were kept individually in $1 \times 1 \times 2$-m outdoor insectaries and supplied with pollen sources (*Lantana* and *Psiguria*), artificial nectar (10% sugar solution), and *Passiflora* vines for oviposition. Eggs were collected daily, and caterpillars fed on new growth of *Passiflora biflora*.

We were able to obtain offspring only from crosses between male *H. melpomene* and female *H. cydno* due to strongly asymmetrical mate preferences. Sterility of F_1 females conformed to Haldane's rule and prevented F_2 crosses (Naisbit et al. 2002), so color pattern segregation was examined in backcrosses using fertile F_1 males. Single gene control was inferred when a 1:1 ratio of distinct phenotypes segregated in the backcross to the parental species bearing the recessive form of the trait, and unless indicated in the results, all tests are for deviation from this 1:1 ratio. Where a continuous distribution of intermediate phenotypes was produced for any single pattern element, control was judged polygenic. Homology was inferred if gene effects and linkage were identical with loci previously described from interracial crosses within either species. Summaries of individual genotypes are given in Tables 1–3, but segregation ratios and recombination frequencies include data from 19 additional individuals (14 from backcrosses to *H. cydno* and 5 from backcrosses to *H. melpomene*) that could not be scored at all loci due to wing damage or failure to eclose fully.

We investigated the clustering of color pattern loci into tight linkage groups by comparing the extent of linkage among the 10 color pattern loci with a null model assuming random distribution of loci across chromosomes to perform a test similar to that in Turner (1984, p. 158). The null distribution of loci per chromosome is approximately but not exactly Poisson distributed, where the dispersion = variance/mean = 1. Clustering was tested here numerically by assigning 10 loci randomly onto 21 chromosomes one million times. We used as a test statistic the log-likelihood ratio (G), where 10/21 loci are expected on average per chromosome. A more conservative test was also run using eight loci, because two pairs of putative loci that did not recombine (*B* and *G*, *Sb* and *Vf*) might each represent pleiotropic effects of a single gene.

Table 1. Genotypes produced without crossing-over in the backcross to *H. melpomene* (female *H. melpomene* × male F_1)

Genotype	Brood 345	Brood 341	?
$[BG_2br][-G_2br][ybSb_1Vf_2N^B][ybSb_1Vf_2N^B](K^yK^y)(J_1J_1)Ac-$ $[BG_2br][-G_2br][ybSb_1Vf_2N^B][ybSb_1Vf_2N^B](K^yK^y)(J_1J_2)Ac-$ $[BG_2br][-G_2br][ybSb_1Vf_2N^B][ybSb_1Vf_2N^B](K^yK^w)(J_1J_1)Ac-$ $[BG_2br][-G_2br][ybSb_1Vf_2N^B][ybSb_1Vf_2N^B](K^yK^w)(J_1J_2)Ac-$	8/9	5/4	
$[BG_2br][-G_2br][ybSb_1Vf_2N^B][Yb_cSb_3Vf_1N^N]K^yK^yJ_1J_1Ac-$	3/2	0/1	
$[BG_2br][-G_2br][ybSb_1Vf_2N^B][Yb_cSb_3Vf_1N^N]K^yK^yJ_1J_2Ac-$	1/4	0/0	
$[BG_2br][-G_2br][ybSb_1Vf_2N^B][Yb_cSb_3Vf_1N^N]K^yK^wJ_1J_1Ac-$	2/1	0/2	
$[BG_2br][-G_2br][ybSb_1Vf_2N^B][Yb_cSb_3Vf_1N^N]K^yK^wJ_1J_2Ac-$	3/1	0/1	
$[BG_2br][-G_1Br][ybSb_1Vf_2N^B][ybSb_1Vf_2N^B](K^yK^y)(J_1J_1)Ac-$ $[BG_2br][-G_1Br][ybSb_1Vf_2N^B][ybSb_1Vf_2N^B](K^yK^y)(J_1J_2)Ac-$ $[BG_2br][-G_1Br][ybSb_1Vf_2N^B][ybSb_1Vf_2N^B](K^yK^w)(J_1J_1)Ac-$ $[BG_2br][-G_1Br][ybSb_1Vf_2N^B][ybSb_1Vf_2N^B](K^yK^w)(J_1J_2)Ac-$	2/6	2/6	
$[BG_2br][-G_1Br][ybSb_1Vf_2N^B][Yb_cSb_3Vf_1N^N]K^yK^yJ_1J_1Ac-$	1/1	1/0	
$[BG_2br][-G_1Br][ybSb_1Vf_2N^B][Yb_cSb_3Vf_1N^N]K^yK^yJ_1J_2Ac-$	3/1	0/2	0/1
$[BG_2br][-G_1Br][ybSb_1Vf_2N^B][Yb_cSb_3Vf_1N^N]K^yK^wJ_1J_1Ac-$	3/3	0/1	
$[BG_2br][-G_1Br][ybSb_1Vf_2N^B][Yb_cSb_3Vf_1N^N]K^yK^wJ_1J_2Ac-$	2/0	3/3	

Genes within square brackets are linked, with maternal *H. melpomene* alleles given first. Genes in parentheses are not expressed on that genetic background (*K* on N^BN^B, and *J* on Sb_1Sb_1). A dash indicates an allele that cannot be determined due to dominance of the alternative allele. Counts are given as females/males.

Table 2. Genotypes produced by crossing-over in the backcross to *H. melpomene* (female *H. melpomene* mated to male F_1)

Genotype	Brood 345	Brood 341	?
[BG_2br][-G_2br][$ybSb_1Vf_2N^B$][$\mathbf{Yb_eSb_1Vf_2N^B}$](K^y-)(J_1-)*Ac*-	0/1		
[BG_2br][-G_1Br][$ybSb_1Vf_2N^B$][$ybSb_1Vf_2\mathbf{N^N}$]K^yK^w(J_1-)*Ac*-	0/1		
[BG_2br][-G_1Br][$ybSb_1Vf_2N^B$][$ybSb_1Vf_2\mathbf{N^N}$]K^yK^y(J_1-)*Ac*-	1/1		
[BG_2br][$\mathbf{-G_2Br}$][$ybSb_1Vf_2N^B$][$ybSb_1Vf_2\mathbf{N^N}$]K^yK^y(J_1-)*Ac*-	1/0		
[BG_2br][$\mathbf{-G_1br}$][$ybSb_1Vf_2N^B$][$Yb_eSb_3Vf_1\mathbf{N^B}$](K^y-)J_1J_1*Ac*-		0/1	
[BG_2br][$\mathbf{-G_1br}$][$ybSb_1Vf_2N^B$][$Yb_eSb_3Vf_1N^N$]$K^yK^w$$J_1J_1$*Ac*-	1/0	0/1	
[BG_2br][$\mathbf{-G_1br}$][$ybSb_1Vf_2N^B$][$Yb_eSb_3Vf_1N^N$]$K^yK^w$$J_1J_2$*Ac*-	1/0	1/0	1/0
[BG_2br][$\mathbf{-G_1br}$][$ybSb_1Vf_2N^B$][$Yb_eSb_3Vf_1N^N$]$K^yK^y$$J_1J_2$*Ac*-		0/1	
[BG_2br][$\mathbf{-G_1br}$][$ybSb_1Vf_2N^B$][$Yb_eSb_3Vf_1N^N$]$K^yK^y$$J_1J_1$*Ac*-	1/1		
[BG_2br][$\mathbf{-G_1br}$][$ybSb_1Vf_2N^B$][$ybSb_1Vf_2N^B$](K^y-)(J_1-)*Ac*-	2/0	2/1	
[BG_2br][$\mathbf{-G_2Br}$][$ybSb_1Vf_2N^B$][$Yb_eSb_3Vf_1N^N$]$K^yK^w$$J_1J_2$*Ac*-	0/2		
[BG_2br][$\mathbf{-G_2Br}$][$ybSb_1Vf_2N^B$][$Yb_eSb_3Vf_1N^N$]$K^yK^y$$J_1J_1$*Ac*-	1/1		
[BG_2br][$\mathbf{-G_2Br}$][$ybSb_1Vf_2N^B$][$Yb_eSb_3Vf_1N^N$]$K^yK^y$$J_1J_2$*Ac*-	0/1		
[BG_2br][$\mathbf{-G_2Br}$][$ybSb_1Vf_2N^B$][$ybSb_1Vf_2N^B$](K^y-)(J_1-)*Ac*-	2/1		

Conventions as in Table 1. Loci affected by crossing-over are shown in bold.

Table 3. Genotypes in the backcross to *H. cydno* (female *H. cydno* mated to male F_1)

Genotype	Brood 304	Brood 342	Brood 347	Brood 351
[bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][Yb_eSb_3--]K^w-J_2J_2*acac* [bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][Yb_eSb_3--]K^w-J_1J_2*acac* [bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][Yb_eSb_1--]K^w-J_2J_2*acac*	1/3	3/1	4/0	0/3
[bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][Yb_eSb_3--]K^w-J_2J_2*Acac* [bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][Yb_eSb_3--]K^w-J_1J_2*Acac* [bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][Yb_eSb_1--]K^w-J_2J_2*Acac*	1/2	1/5	1/6	3/0
[bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][$ybSb_3$--]K^w-J_2J_2*acac* [bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][$ybSb_3$--]K^w-J_1J_2*acac* [bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][$ybSb_1$--]K^w-J_2J_2*acac*	3/0	1/1	2/3	
[bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][$ybSb_3$--]K^w-J_2J_2*Acac* [bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][$ybSb_3$--]K^w-J_1J_2*Acac* [bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][$ybSb_1$--]K^w-J_2J_2*Acac*	0/0	4/3	1/1	
[bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][$ybSb_1$--]K^w-J_1J_2*acac*	3/1	1/3	1/1	
[bG_1Br][bG_1-][$Yb_eSb_3(Vf_1)N^N$][$ybSb_1$--]K^w-J_1J_2*Acac*	0/1	3/1	4/2	
[bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][Yb_eSb_3--]K^w-J_2J_2*acac* [bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][Yb_eSb_3--]K^w-J_1J_2*acac* [bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][Yb_eSb_1--]K^w-J_2J_2*acac*	1/3	4/4	4/2	0/1
[bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][Yb_eSb_3--]K^w-J_2J_2*Acac* [bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][Yb_eSb_3--]K^w-J_1J_2*Acac* [bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][Yb_eSb_1--]K^w-J_2J_2*Acac*	1/3	9/6	2/3	
[bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][$ybSb_3$--]K^w-J_2J_2*acac* [bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][$ybSb_3$--]K^w-J_1J_2*acac* [bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][$ybSb_1$--]K^w-J_2J_2*acac*	2/3	1/4	3/2	
[bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][$ybSb_3$--]K^w-J_2J_2*Acac* [bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][$ybSb_3$--]K^w-J_1J_2*Acac* [bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][$ybSb_1$--]K^w-J_2J_2*Acac*	3/1	2/4	0/1	
[bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][$ybSb_1$--]K^w-J_1J_2*acac*	1/1	3/0	1/0	
[bG_1Br][BG_2-][$Yb_eSb_3Vf_1N^N$][$ybSb_1$--]K^w-J_1J_2*Acac*	2/1	2/2	2/2	

Genes within square brackets are linked, with the maternal *H. cydno* alleles given first. Genes in parentheses are not expressed on that genetic background (*Vf* on *bb*). Certain genotypes cannot be distinguished due to epistasis involving *Sb* and *J*, so that full expression of white hindwing margin could be produced by Sb_3Sb_3 or Sb_1Sb_3 J_2J_2. Counts are given as females/males. Crossing-over was not observed in this backcross and would have been detectable between only two pairs of loci: *B* and *G* and *Yb* and *Sb* (in the latter case only in certain genotypes due to the interaction between *J* and *Sb*).

RESULTS

Crosses between *Heliconius cydno chioneus* and *H. melpomene rosina* revealed a number of loci with major effects on color pattern.

B locus

Alleles at the locus control the presence (*BB*, *Bb*) or absence (*bb*) of the red forewing band of *melpomene* (backcross to *cydno Bb*:*bb* 94:80 $G_1 = 1.13$, $P > 0.05$). There is epistatic interaction with the unlinked *N* locus, so that in *B*- N^N- individuals the red is moved distally in comparison with the position in *melpomene* (Fig. 1B). The pattern of gene action, epistasis with *N*, and linkage (see below) are very similar to those of the *B* locus in interracial crosses of *melpomene* (Turner 1972; Sheppard et al. 1985), confirming homology.

N locus

This locus controls the presence ($N^N N^N$, $N^N N^B$) or absence ($N^B N^B$) of an area of white or yellow in the forewing band seen in *cydno* (backcross to *melpomene* $N^B N^B$:$N^N N^B$ 54:65 $G_1 = 1.02$, $P > 0.05$). The mode of gene action and its linkage (see below) are identical to that of the *N* locus segregating in interracial crosses of *melpomene* (Sheppard et al. 1985). The locus is apparently distinct from the *L* locus controlling the forewing band in crosses between several Colombian races of *cydno* (Linares 1996, 1997). *L* lacks the linkage seen here of *N* to *Yb* and *Sb*, and absence of the band is almost completely dominant, whereas at *N* absence is recessive.

Yb locus

Alleles at the *Yb* locus control the presence (*ybyb*) or absence (Yb_cYb_c, Yb_cyb) of the hindwing yellow bar of *melpomene* (Fig. 1C). In heterozygotes the bar is usually visible as a shadow of melanic scales with altered reflectance, but occasionally very sparse yellow scales are present (backcross to *cydno* Yb_cYb_c:Yb_cyb 80:86 $G_1 = 0.22$, $P > 0.05$, backcross to *melpomene* Yb_cyb:*ybyb* 61:57 $G_1 = 0.14$, $P > 0.05$). On the basis of identical gene action and linkage, these crosses confirm the homology of this locus between species with that previously described from crosses within *melpomene* (Sheppard et al. 1985), and within *cydno* (Linares 1997).

Sb locus

This locus controls the presence (Sb_3) or absence (Sb_1) of the white submarginal band on the hindwing of *cydno* (Fig. 1D). The strength of expression in heterozygotes depends on at least one unlinked modifier (*J*; see below). Linkage and gene action are identical to that of *Sb* in interracial crosses of *cydno* (Linares 1996, 1997).

K locus

Forewing band color (Fig. 1F) is expressed as white (K^wK^w, K^wK^y) or yellow (K^yK^y) (backcross to *melpomene* in $N^N N^B$ individuals, K^wK^y:K^yK^y 35:30 $G_1 = 0.38$, $P > 0.05$). This is probably homologous with the *K* locus segregating in interracial crosses of *cydno* (Linares 1997). There is no obvious influence on the color of the yellow hindwing bar, but the locus can cause the inclusion of yellow scales in the normally white hindwing submarginal band. Fore- and hindwing band color is jointly controlled in polymorphic *cydno* populations in Ecuador (Kapan 1998). However, in some Colombian races a yellow forewing band is found with a white hindwing margin (Linares 1997).

In addition, several loci have less dramatic effects on mimicry.

Vf locus

Scale color on the ventral surface of the red forewing band (Fig. 1F) is either dark (Vf_1Vf_1, Vf_1Vf_2), or pale (Vf_2Vf_2) as in *melpomene* (backcross to *melpomene* Vf_1Vf_2:Vf_2Vf_2 62:56 $G_1 = 0.31$, $P > 0.05$). This locus has not been described in either species, although its action has been noted in interspecific crosses (Gilbert 2003). The pale ventral surface scales are white in *melpomene* but can be white or yellowish in backcross Vf_2Vf_2 individuals.

Ac locus

At the *Ac* locus, alleles control the presence (ac^cac^c) or absence ($AcAc$, $Acac^c$) of the anterior triangle of a white hourglass shape in the main forewing cell of *cydno* (Fig. 1F) (backcross to *cydno* $Acac^c$:ac^cac^c 97:79 $G_1 = 1.84$, $P > 0.05$). Gene action suggests homology with the *Ac* locus that segregates in crosses between a *melpomene* race from Trinidad with the red forewing band and Amazonian races in which the hourglass is present (Sheppard et al. 1985). Variation in the posterior half of the hourglass is more difficult to interpret: It is present in all of the backcross to *cydno* but is very variable in the backcross to *melpomene*.

Br locus

Alleles at the *Br* locus control the presence (*BrBr*, *Brbr*) or absence (*brbr*) of a forceps-shaped brown marking on the hindwing ventral surface seen in *cydno* (Fig. 1E) (backcross to *melpomene Brbr*:*brbr* 56:63 $G_1 = 0.41$, $P > 0.05$). Expression is variable in heterozygotes, which lack most of the distal part of either or both arms of the forceps, and is complicated by an epistatic interaction with the yellow bar, which occupies a similar position. The color is also variable, brown in *cydno*, but typically more orange in hybrids (compare Fig. 1, A and E). Linkage with *B* suggests that this locus is homologous with the *D* locus in *melpomene*, which controls orange "Dennis" and "ray" patterns on fore- and hindwing (Sheppard et al. 1985). There appear to be separable loci controlling the anterior and posterior components both of the *cydno* forceps (Gilbert 2003; M. Linares, personal communication) and of the rayed pattern in *melpomene* (Mallet 1989).

G locus

This locus controls the presence (G_2) or absence (G_1) of a short red line at the base of the costal vein on the forewing ventral surface of *melpomene* (Fig. 1H). Expression is intermediate and variable in heterozygotes (backcross to *cydno* G_1G_1:G_1G_2 82:95 $G_1 = 0.96$, $P > 0.05$, backcross to *melpomene* G_1G_2:G_2G_2 61:57 $G_1 = 0.14$, $P > 0.05$). This locus was first described from interracial crosses of Colombian *cydno* (Linares 1996).

Several of these loci are involved in epistatic interactions, in addition to that between *N* and *B* controlling forewing band shape and color. Some traits are expressed only in certain genotypes, for instance *K* only in N^N- individuals, and *Vf* only in the *B*-genotype. Modifier loci that adjust the strength and position of expression of other loci are discussed below.

J locus

This is a modifier of incompletely dominant *Sb* control of hindwing submarginal band (Fig. 1D). In Sb_1Sb_3 heterozygotes, J_1J_1 genotypes express the band as melanic scales with altered reflectance on the ventral surface only; J_1J_2 individuals show a mixture of white and melanic scales producing a gray band expressed most strongly on the dorsal surface; Sb_1Sb_3 J_2J_2 individuals are indistinguishable from the Sb_3Sb_3 phenotype with a white dorsal and ventral submarginal band like that of *cydno*. In the backcross to *melpomene*, this gives an expected 2:1:1 ratio of absent (Sb_1Sb_1 J_1J_1 and Sb_1Sb_1 J_1J_2) to altered reflectance (Sb_1Sb_3 J_1J_1) to scattered white scales (Sb_1Sb_3 J_1J_2) (57:28:32, $G_2 = 0.34$, $P > 0.05$). In the backcross to *cydno*, a 3:1 ratio of full expression (Sb_3Sb_3 J_1J_2, Sb_3Sb_3 J_2J_2 and Sb_1Sb_3 J_2J_2) to scattered white scales (Sb_1Sb_3 J_1J_2) is expected (137:40, $G_1 = 0.56$, $P > 0.05$).

Forewing band width

In both backcrosses, variation in the position of the distal edge of the white or yellow part of the N^N- forewing band is continuous, suggesting additive polygenic control (Fig. 1G). However, relatively few modifier loci must control this variation, because extreme phenotypes are common in backcrosses: Around 8% of individuals in the backcross to *melpomene* and 6% in the backcross to *cydno* have band widths similar to that in the F_1. In the background of these modifiers, there is evidence of a slight effect of *N* and *B* loci on the width of the pale part of the forewing band. On average, the band is slightly wider in N^NN^N than N^NN^B individuals (Turner 1972) (using linkage with *Yb* to distinguish heterozygous from homozygous *N*), and the band is increasingly wide in *BB*, *Bb*, and *bb* individuals (using evidence from linkage to *G* to distinguish *Bb* heterozygotes from *BB* homozygotes). The distal edge of the red part of the forewing band is also variable in position. Its boundary is much less sharply defined and often more distal in hybrids than in *melpomene* (Fig. 1F). In the backcross to *melpomene*, the distal boundary varies from a *melpomene*-like to an F_1-like position, independently of the effect of the *N* locus on the proximal boundary (Fig. 1G). In the backcross to *cydno*, the distal boundary is generally similar or slightly distal to that in F_1 hybrids.

Dorsal forewing band color

There is continuous variation of the red hue in the dorsal forewing. In the backcross to *melpomene* this ranges from the scarlet of *melpomene* to an orange-red (Fig. 1F, center left), whereas in the backcross to *cydno* it varies from orange-red to brownish (Fig. 1F, center right). This continuous variation suggests that control is not homologous with the *Or* locus controlling red versus orange coloration in *melpomene* (Sheppard et al. 1985).

Red spots

Melpomene has a variable number of red spots at the base of the hindwing ventral surface (Fig. 1A). There is often a single spot in the angle between the first anal vein and discal cell, but there may be up to three more, in the angles of the second anal vein and wing margin, the discal cell, and where the subcosta meets the discal cell. Penetrance is variable in hybrids, suggesting epistasis with modifier genes. Spots are absent from many F_1 offspring, but present in almost all offspring of backcrosses to *melpomene*. They are absent from almost all offspring of some backcrosses to *cydno* but are overrepresented in one brood (brood 342 present:absent 46:26, $G_1 = 5.63$, $P < 0.05$, compared with a 1:1 expectation).

Iridescence

The black areas of the wing are iridescent blue in *cydno* and matt black in *melpomene* (Fig. 1A). Iridescence is difficult to score but appears to be under polygenic control: Iridescence is strong in the backcross to *cydno*, intermediate in the F_1 and many from the backcross to *melpomene*, but absent in others.

Linkage

Seven of the 10 loci fall into two linkage groups, *Br-B-G* and *N-Sb-Vf-Yb*. Three loci, *K*, *Ac*, and *J*, are unlinked to any other, and none are sex linked. The recombination fraction between *Br* and *G* is 23/118 (19.5% with support limits 12.9%, 27.4%) in the backcross to *melpomene* (Table 2). *B* and *G* are very tightly linked or may be pleiotropic effects of the same locus, because no recombinants appear among 174 individuals in the backcrosses to *cydno*. The loci in the other linkage group can be ordered by assuming double recombinants are very rare. In the backcross to *melpomene*, heterozygotes can be distinguished from homozygotes at all four loci so that crossing-over between any pair of loci can be detected. Gene order is most likely *N-Sb-Vf-Yb*, with 4.3% recombination between *N* and *Sb-Vf-Yb* (5/115, support limits 1.5%, 9.2%) and 0.9% between *Yb* and *Vf-Sb-N* (1/115, support limits 0.05%, 3.9%). Further recombinants between *Yb* and *Sb* are

known from other crosses (Linares 1989), suggesting the two are indeed distinct loci. For recombinant phenotypes see Figure 1, H, F center left (lower Vf_1-wings), and D lower right. *Sb* and *Vf* are tightly linked or pleiotropic effects of the same locus, with no recombinants among 115 individuals. Because there are 21 chromosomes in *cydno* and *melpomene* (Brown et al. 1992), the clustering of 10 loci into linkage groups of three and four loci far exceeds that expected if color pattern genes were distributed randomly across chromosomes (dispersion = 2.44, $P < 0.001$). The level of clustering remains significant even under the assumption that pairs of genes that show no recombination (*Sb* with *Vf* and *B* with *G*) are in fact pleiotropic effects of the same gene, giving eight genes with largest linkage groups of three and two loci (dispersion = 1.7, $P = 0.015$).

Presumed genotypes for the two species are $[BrBrbbG_1G_1]$ $[N^NN^NSb_3Sb_3Vf_1Vf_1Yb_cYb_c]K^wK^wac^cac^cJ_2J_2$ for *Heliconius cydno chioneus* and $[brbrBBG_2G_2][N^BN^BSb_1Sb_1Vf_2Vf_2ybyb]$ $K^yK^yAcAcJ_1J_1$ for *H. melpomene rosina*. These loci produce almost perfect resemblance to respective co-mimics *H. sapho* and *H. erato*. This extends to such minor details as the lightening of the ventral forewing produced by Vf_2, the red line produced by G_2, and the red spots at the base of the hindwing, all of which are seen in *H. erato* and replicated in *H. melpomene*. The only exceptions that do not contribute to mimicry are the K^y allele (yellow) revealed on the pale forewing of hybrids, which cannot be expressed on the normal red forewing band of *H. melpomene rosina*, and the brown forceps-shaped mark on the hindwing of *H. cydno* produced by *Br*. *Heliconius sapho* differs from *H. cydno* in that it has large red patches near the base of the hindwing and has a red line in the forewing costa as in *H. erato* and *H. melpomene*.

DISCUSSION

Developmental drive and the role of major genes in mimicry

The 8–10 genes found here act together with several polygenic traits to control the mimicry difference between *H. cydno* and *H. melpomene*. Half of the loci are of major effect, and epistatic interactions and linkage affect most of them.

The gene effects are major in the sense that individual loci control a large fraction of the differences between the two species, affect large areas of the wing surface, and cause changes far beyond normal within-population variation (True et al. 1997; Orr 2001). Polymorphisms exist in just a few *Heliconius* populations, including *H. cydno* in Colombia and Ecuador (Linares 1996; Joron et al. 2001; Kapan 2001; Mallet 2001). Color pattern genes have major effects on pigmentation and scale morphology in specific areas of the wing (Gilbert et al. 1988) and are under very strong selection arising from mate choice (Jiggins et al. 2001) and mimicry (Mallet and Barton 1989; Kapan 2001). A similar distribution of mutational effects separates bee- and hummingbird-pollinated *Mimulus* flowers, recently interpreted as speciation due to floral mimicry (Bradshaw et al. 1998; Bleiweiss 2001). Mimetic adaptation apparently lacks selective constraints on fixation of developmental genes with major effects on fitness, even though Fisher (1930) argued that adaptation would typically proceed by fixation of many mutations of small effect. However, Fisher ignored the effect of selective advantage on the fixation probability of a new mutation (Kimura 1983), and the fact that adaptation involves sequential substitution of numerous alleles as the optimum is approached. When the entire process is considered, adaptation is expected to fix an exponential distribution of gene effects (Orr 1998, 1999).

Nonetheless, mimicry and perhaps many other traits involved in speciation do not fit the Fisher/Kimura/Orr model of adaptation. Mimicry is likely to evolve in two steps (Turner 1977; Sheppard et al. 1985; Mallet and Joron 1999). Only a major mutation yielding approximate resemblance can cross the adaptive valley between very distinct protected color patterns (Sheppard et al. 1985); this is followed by improvement of resemblance through natural selection on genes of more minor effect. If this model is correct, developmental mutations of major effect can in a sense be said to be "driving" adaptive evolution (Goldschmidt 1945; Turner 1984). The rugged adaptive landscape of mimicry contrasts with the smooth adaptive surface envisioned by Fisher and Orr, where gradual evolution occurs toward a single optimum. Despite these differences, a few large and several small mutations are expected under both theories, as in our empirical results (Turner 1984; Orr 1998). The distribution of gene effects therefore appears to reveal little about the adaptive landscape upon which evolution occurred.

Linkage and the evolution of Müllerian mimicry

Seven of the 10 loci described here fall into two linked groups. Tight linkage of color pattern loci like this is expected in polymorphic Batesian mimics but not in monomorphic Müllerian mimics (Turner 1984). Polymorphisms are expected in edible Batesian mimics of unpalatable models, because predators will learn to attack common mimetic forms more easily. In Batesian mimics such as *Papilio memnon*, polymorphisms are indeed found, with much of the pattern and wing shape changes inherited at a single "supergene." The supergene consists of multiple epistatic elements controlling traits whose separateness can be demonstrated via occasional recombinants (Clarke et al. 1968; Clarke and Sheppard 1971). Far from being evidence for "systemic mutations," as Goldschmidt (1940) proposed, it was now suggested that these supergenes had been constructed gradually from multiple unlinked genes that became more and more

tightly linked. Later, Charlesworth and Charlesworth (1975) demonstrated difficulties with the Clarke and Sheppard hypothesis, because unlinked elements in polymorphic populations would normally be selected against after producing abundant nonmimetic recombinants. Turner (1984) therefore proposed an alternative explanation: The supergene evolved because only already linked epistatic elements will survive selection.

In contrast, Müllerian mimics such as *H. cydno* and *H. melpomene* are under purifying frequency-dependent selection, and monomorphic populations are indeed generally observed. Although previously known mimicry genes from *H. erato* and *H. melpomene* were often linked, they were not significantly clustered (Turner 1984). The significant clustering of color pattern loci we have found in *H. cydno* and *H. melpomene* is therefore unexpected. There are two possible explanations. First, linkage in *Heliconius* might be due to color pattern divergence arising in sympatry. Hybridizing incipient species would in effect form a polymorphic population, into which new epistatic mutations must be linked as in Batesian mimicry (Turner 1984) to become established. However, parapatric divergence, perhaps along a habitat or altitudinal gradient, is more probable in *H. cydno* and *H. melpomene* (Mallet 1993). Adaptive evolution of linkage is therefore possible but less likely than for Batesian sympatry. Second, clustering of mimicry genes might result from developmental and genomic constraints on the number of chromosomal regions that affect pattern (Mallet 1989) rather than a selective constraint on the location of substitutions that can become established. The tightly linked genetic architecture we observe could have arisen by duplication of regulatory genes, so that color pattern evolution proceeds within limited linked blocks (Mallet 1989; Force et al. 1999). Some process of gene duplication followed by the acquisition of new functions seems especially likely in *Heliconius*, where linked genes sometimes promote development of similar pattern elements; for example, red markings are determined by loci in the *B* linkage group and white/yellow markings by the *N* group. Similar associations are observed in *H. erato* and *H. himera* (Jiggins and McMillan 1997). In *Papilio*, supergenes include more diverse tightly linked loci controlling wing shape (tails on the hindwing) and body color as well as wing color (Clarke and Sheppard 1971; Turner 1984). However, development of butterfly color pattern, scale morphology, and wing shape (the latter produced by cell death at the wing imaginal disc margin) can occur in response to a single signaling pathway (Carroll et al. 1994). A complete explanation of linkage in both systems must await molecular characterization of the loci involved, but it is tempting to predict that supergene inheritance in Müllerian mimics implies developmental and genomic constraints on color pattern control, instead of their construction by natural selection alone.

Role of epistasis in adaptive speciation

Epistasis plays a profound role in speciation: It is the source of genomic or ecological incompatibilities in hybrids. Here epistasis affects several levels. There is epistasis between specific pairs of loci, for example, the lack of *K* expression on an $N^B N^B$ background, the interaction between *N* and *B* in positioning the forewing band, and that between *Sb* and *J* in the strength of expression of the hindwing submarginal band. More general epistasis is dependent on genetic background, so that in hybrids the color pattern elements are less sharply defined. This suggests that canalization of pattern development breaks down in the absence of coadapted modifier genes (Clarke and Sheppard 1960; Mallet 1989). Finally, epistasis at the fitness level selects against nonmimetic pattern combinations, as in classic hybrid inviability and sterility (Turelli and Orr 2000). Quantitative genetic analyses of morphological differences between species typically find little evidence of epistasis (Orr 2001), but disruptive selection will generate epistasis for fitness even where the underlying genetic basis of the ecologically important trait is additive (Whitlock et al. 1995). In mimicry, as well as in classic postmating isolation, epistatic hybrid dysfunction should be a common incidental by-product of adaptive divergence.

Genetic architecture of intra- and interspecific divergence

Color pattern is strikingly diverse within *Heliconius*, involving convergence between the major clades of the genus, racial differentiation, and speciation (Turner 1976; Jiggins and McMillan 1997; Mallet et al. 1998; Gilbert 2003). Both *H. cydno* and *H. melpomene* have diversified into color pattern races across Central and South America, matching those of respective co-mimics *H. sapho* + *H. eleuchia* or *H. erato* and co-mimics (Brown 1979). Most of the loci encountered here have been described previously from interracial crosses within *H. melpomene* (*N*, *B*, *Yb*, *Ac*) (Sheppard et al. 1985; Mallet 1989) or within *H. cydno* (*Sb*, *Yb*, *K*, *G*) (Linares 1996, 1997). Linkage relationships are also similar to those previously described: *N* with *Yb* in *H. melpomene* (Sheppard et al. 1985) and *Sb* with *Yb* in *H. cydno* (Linares 1997). The linkage between *B* and *Br* suggests homology of *Br* (found here to control the brown forceps shape on the hindwing of *H. cydno*) with the *D* locus linked to *B* in *H. melpomene* (*D* controls the "Dennis" pattern of orange on the proximal part of the fore- and hindwings in Amazonian races; Sheppard et al. 1985; Mallet 1989). Homology also exists between the two major loci responsible for color pattern differences between another pair of sister species, *H. erato* and *H. himera*, and loci controlling pattern variation within *H. erato* (Jiggins and McMillan 1997). There is therefore no obvious distinction between the genetic control of inter- and intraspecific pattern differences: Divergence at both taxonomic levels is effected by many of the same loci. The participation of genes

of large effect in both cases also suggests that the "evolution by jerks" involved in mimetic shifts (Turner 1983) can promote rapid speciation.

Although *Heliconius* sister species typically belong to different mimicry rings (Turner 1976), mimetic shift does not always lead to speciation. Geographic variation in color pattern within species, particularly within *H. erato*, *H. melpomene*, and *H. cydno*, is often as dramatic as that between *H. cydno* and *H. melpomene* (Brown 1979). Yet contact zones between geographic races are characterized by rampant hybridization despite strong selection against nonmimetic hybrids (Mallet and Barton 1989; Mallet 1993). Initiation of speciation depends on the mimetic shift leading to a change in the predominant color that would normally act as a courtship releaser (Crane 1955). In most races of *H. melpomene*, red is the predominant color, in comparison with white or yellow in *H. cydno*, and this major shift may have driven male preference to coevolve, reducing male courtship toward females with the ancestral color pattern (Jiggins et al. 2001). Any initial reduction in gene flow due to pleiotropy with mate choice and selection against nonmimetic hybrids will facilitate further adaptive divergence and completion of speciation (Rice and Hostert 1993).

Evolution of dominance

In our crosses, dominance and penetrance are variable and are not simply intrinsic properties of alleles. Dominance is influenced by genetic background (Doebley et al. 1995), and a specific dominance modifier, *J*, can be identified that affects the hindwing margin. In heterozygotes at *Sb*, the *J* locus controls penetrance, from complete dominance of the melanistic allele, through to strong expression of white. Such variation casts doubt on the argument that the recessive phenotype comprises the ancestral color pattern in *H. melpomene* or *H. erato* (Turner 1984; Sheppard et al. 1985). If dominance normally evolves to this extent, it will be of little use in determining the ancestral phenotype (Mallet 1989).

Genetic architecture and introgression

Clustering of genes for the major pattern differences into just three chromosomal locations, near the *B*, *N*, and *K* loci, may prohibit gene flow between species at adjacent loci but leave other regions to introgress relatively freely (Barton 1979). Also, due to linkage of *N*, *Sb*, *Vf*, and *Yb* and dominance modifiers such as *J*, parental genes cosegregate so that almost half the backcross offspring are similar to *H. cydno* or *H. melpomene.* These phenotypes form passable Müllerian mimics, so that backcrosses may occur more commonly in nature than inferred from collections of aberrant hybrids (Mallet et al. 2001). Cryptic hybrids would largely escape the strong selection due to predation on nonmimetic patterns. Thus, although mimicry may drive speciation, the clustered architecture of color pattern directly reduces its efficiency as a barrier to gene flow, creating a semipermeable species boundary.

Homology of genes across *Heliconius* and developmental constraints in macroevolution

The mimetic pair *H. melpomene* and *H. erato* are congeneric species that have diversified in parallel across South and Central America. Goldschmidt (1945) suggested that Müllerian co-mimics like these might often exploit the same developmental pathways to achieve identical color patterns but that the specific genes involved would probably differ. More recently, the even more extreme "Goldschmidtian" argument has been made that mimicry genes are homologous between Müllerian mimics *H. erato* and *H. melpomene* (Turner 1984; Nijhout 1991). Given that genes acting late in butterfly color pattern determination are the same as those acting early in embryonic development of *Drosophila* (McMillan et al. 2002), at first sight the suggestion is not implausible. If these ideas are correct, the construction of the mimetic color pattern would depend far more on constraints imposed by the developmental system than envisaged in traditional mimicry theory (e.g., Fisher 1930).

Linkage patterns suggest some homologies between *Heliconius* mimics; for example, the orange Dennis and ray patterns in *melpomene* and *erato* are both inherited as dominant supergenes (Turner 1984; Sheppard et al. 1985; Mallet 1989) and the hindwing yellow bar is tightly linked to white margin in *cydno*, *melpomene*, and *erato* (Turner and Sheppard 1975; Jiggins and McMillan 1997). However, in most cases mimetic patterns show key differences in genetic control. The Dennis allele of *melpomene*, for example, expresses an orange hindwing bar for which there is no homolog in *erato*. In *H. erato*, forewing band color (red or yellow) is controlled together with Dennis and ray pattern elements by a supergene (D^{Ry}), unlinked to the *Cr* locus controlling yellow hindwing bar (Sheppard et al. 1985; Mallet 1989). In heterozygotes for band color, the forewing band is red but sometimes "overprinted" with faint yellow pigment (Sheppard et al. 1985; Mallet 1989; Jiggins and McMillan 1997). This contrasts with gene action and linkage in the *melpomene* group, where red and yellow/white forewing band elements are controlled by separate loci (*B* and *N*); only the former is linked to Dennis and ray, whereas the latter is linked to yellow hindwing bar (*Yb*). Forewing bands with both red and yellow elements in *melpomene* and *cydno* show distal displacement of red rather than overprinting as in *erato*. Some details of genetic control also differ between *H. erato* and its sister species *H. himera*. In *himera* × *erato* crosses, red forewing band and red hindwing bar are both controlled by the D^R supergene, whereas yellow forewing band is unlinked and controlled by the *Cr* locus affecting yellow hindwing bar (Jiggins and McMillan 1997); in *erato*, yellow forewing band is

tightly linked to D^R. There is even geographic variation in the genetic control of similar patterns within *H. erato*: The hindwing yellow bar has a disjunct distribution among races and is under the control of two loci in Peru (Mallet 1989) and Brazil (Sheppard et al. 1985) but only a single locus in Central America (Sheppard et al. 1985; Mallet 1986) and Ecuador (Jiggins and McMillan 1997).

Overall, these results suggest some role for developmental constraints in the evolution of Müllerian mimetic patterns. Yet linkage and gene action are evolutionarily labile between the *erato* and *melpomene* groups and even within the *erato* group. A variety of mutable loci therefore appears to be able to affect a single pattern element. Developmental mechanisms may thus only weakly interfere with the evolution of mimicry, whereas the major work of pattern construction is apparently achieved by natural selection. However, the final elucidation of these possibilities awaits mapping and molecular developmental characterization of the genes discovered here and in previous studies.

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