DOI: 10.1534/genetics.106.059527

Note

Parallel Genetic Architecture of Parallel Adaptive Radiations in Mimetic Heliconius Butterflies

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Manuscript received April 15, 2006
Accepted for publication June 15, 2006

ABSTRACT

It is unknown whether homologous loci underlie the independent and parallel wing pattern radiations of Heliconius butterflies. By comparing the locations of color patterning genes on linkage maps we show that three loci that act similarly in the two radiations are in similar positions on homologous chromosomes.

WHEN different taxa independently evolve the same trait, do they use the same genes to do it? Available data suggest that the answer is "sometimes." Multiple studies have found that the same gene plays a role in the evolution of convergent morphological characters. The independent evolution of albinism in multiple populations of the Mexican tetra (*Astyanax mexicanus*) has resulted from mutations in the gene *Oca2* (Protas *et al.* 2006); repeated loss of the pelvic skeleton and reductions in armor plates in threespine sticklebacks (*Gasterosteus aculeatus*) have been linked to *Pitx1* (Shapiro *et al.* 2004) and *Eda* (Colosimo *et al.* 2005), respectively; loss of larval trichomes and convergent evolution of abdominal pigmentation in different species of Drosophila result from regulatory changes of the genes *svb/ovo* (Sucena *et al.* 2003) and *bab2* (Gompel and Carroll 2003), respectively; and melanism in a wide variety of animals is associated with the gene *MC1R* (Mundy 2005). However, convergent evolution does not necessarily require the same genetic mechanisms. For instance, different genes are responsible for the evolution of melanism in different populations of rock pocket mice (Hoekstra and Nachman 2003) and while expression of the gene *yellow* is sometimes correlated with Drosophila pigmentation (Wittkopp *et al.* 2002a,b), other times it is not (Wittkopp *et al.* 2003). In theory, an ideal system to study the genetic basis of convergent evolution would contain multiple independent instances of convergence across multiple distinct traits. The Neotropical butterfly genus Heliconius is such a system.

Heliconius butterflies are distasteful, warningly colored, and mimetic. As a classic example of Müllerian mimicry, different Heliconius species, while all distasteful, gain protection by resembling one another and thereby distributing the cost of educating naïve predators. In Heliconius, intrageneric wing pattern mimicry has resulted from two parallel radiations: the genus consists of two major clades, and each mimetic wing pattern is shared by at least one species from each of these two lineages (Figure 1). Average pairwise mtDNA divergence between species of the two Heliconius clades is 9.148% (SD = 0.521%), which suggests that the two lineages separated ~4 million years ago, assuming an evolutionary rate of 1.1–1.2% per lineage per million years (Brower 1994). Hence, since sharing a common ancestor as recently as 4 million years ago, the two Heliconius groups have radiated onto the same myriad of wing pattern phenotypes at both the racial and the species levels. Decades of crossing experiments have identified the phenotypic effects of discrete color patterning loci and have verified the homology of many of these loci among species and races of the same clade (Turner and Crane 1962; Turner 1971; Sheppard *et al.* 1985; Nijhout and Wray 1988; Mallet 1989; Nijhout *et al.* 1990; Linares 1996; Jiggins and McMillan 1997; Gilbert 2003; Naisbit *et al.* 2003). However, virtually nothing is known about color pattern homology between the clades because they cannot be interbred. Comparative genetic mapping provides our first insight into the potential homology of wing patterning loci in the two lineages.

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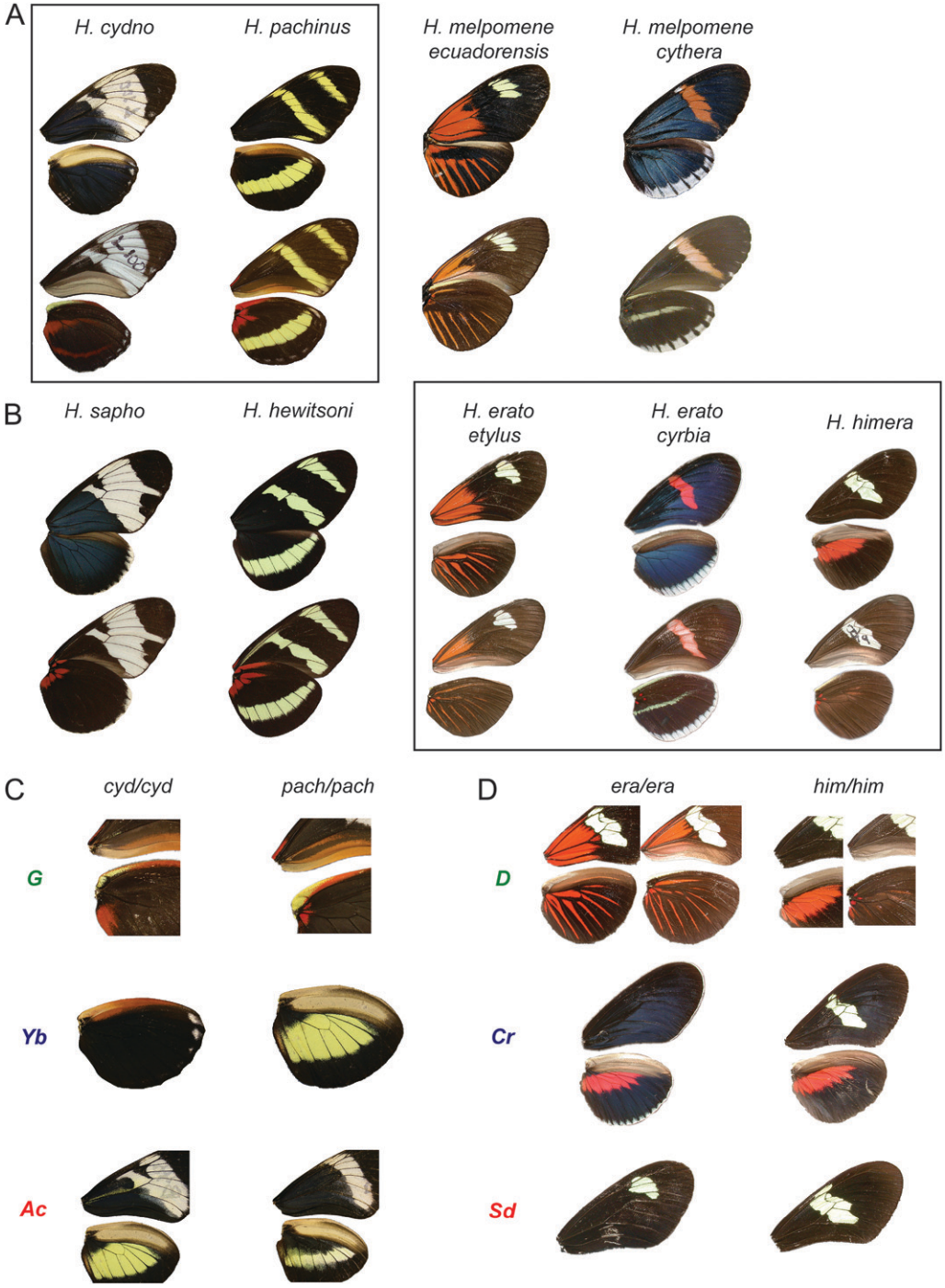

Figure 1.—Mimicry and mimicry genes in Heliconius butterflies. (A) Four phenotypes (dorsal, top; ventral, bottom) from three species belonging to one major Heliconius clade. *H. cydno* and *H. pachinus* (shown in a box) were crossed to study color pattern genetics from this clade. (B) Five phenotypes from four species belonging to the second Heliconius clade are shown below their respective comimics; *H. himera* does not have a corresponding comimic. Crosses between *H. himera* and each of the two *H. erato* races (shown in a box) were used to study color pattern genetics in this clade (Tobler *et al.* 2005; Kapan *et al.* 2006). (C) Effects of alternative alleles at three color-patterning loci that distinguish *H. cydno* and *H. pachinus*: *G* controls whether the ventral base of the fore and hind wings is red or brown, *Yb* controls the presence or absence of melanic scales on the hind wing, and *Ac* controls the presence or absence of melanic scales on the proximal portions of both the fore and the hind wings. (D) Effects of alternative alleles at three color-patterning loci that distinguish *H. erato* and *H. himera*: *D* controls the distribution of red/orange scales on the wings ($D^{e}D^{e}$ *vs.* $D^{hi}D^{hi}$ shown), *Cr* controls the placement of melanic scales on the fore and hind wings ($Cr^{cy}Cr^{cy}$ *vs.* $Cr^{hi}Cr^{hi}$ shown), and *Sd* controls the portion of the forewing that is covered in melanic scales ($Sd^{e}Sd^{e}$ *vs.* $Sd^{hi}Sd^{hi}$ shown).

To address this issue, we compared the genomic positions of three color-patterning loci that act similarly in the two radiations. Using two hybrid crosses between different *Heliconius erato* races and the closely related species *H. himera*, Tobler *et al.* (2005) and Kapan *et al.* (2006) localized the positions of three large-effect color-patterning loci. The *D* locus controls the distribution of red/orange scales on the wings, the *Cr* locus controls the placement of melanic scales on the fore and hind wings that "shutter" or limit the distribution of yellow and white scales on the wings, and the *Sd* locus controls the portion of the forewing that is covered in melanic scales (Figure 1; Sheppard *et al.* 1985; Mallet 1989; Jiggins and McMillan 1997; Kapan *et al.* 2006). To evaluate the potential for homology between the two radiations, we identified the positions of three color-patterning loci that act similarly to those mapped in *H. erato*, on a map derived from a hybrid cross between two species from the other Heliconius radiation, *H. cydno* and *H. pachinus*. The *G* locus controls the distribution of

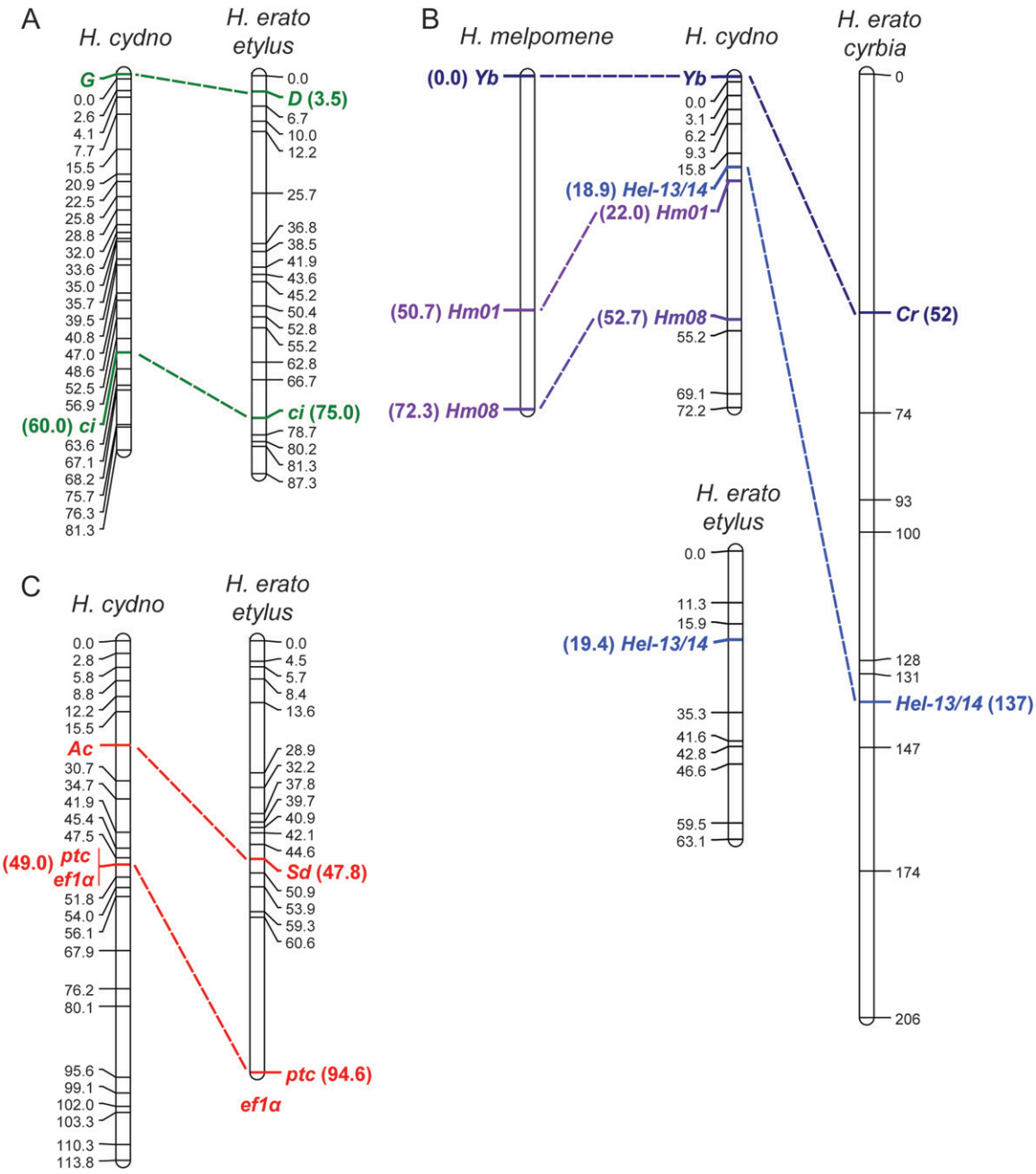

Figure 2.—Comparative genetic mapping of mimicry genes in Heliconius. (A) *H. cydno* color-patterning gene *G* and *H. erato D* are both syntenic with anchor locus *ci*. (B) *H. cydno* color-patterning gene *Yb* and *H. erato Cr* are both syntenic with anchor locus *Hel-13/14*. The *H. erato cyrbia* linkage group containing *Hel-13/14* (Tobler *et al.* 2005) is substantially longer than that of *H. erato etylus* (Kapan *et al.* 2006), suggesting that distances may be artificially inflated in the former. *H. cydno Yb* is also syntenic with microsatellite loci *Hm01* and *Hm08*, which are linked to *Yb* in crosses between races of sister species *H. melpomene* (Jiggins *et al.* 2005; Joron *et al.* 2006). (C) *H. cydno* color-patterning gene *Ac* and *H. erato Sd* are both syntenic with anchor loci *ptc* and *ef1*α. Although *ef1*α is syntenic with *ptc* and *Sd* in *H. erato etylus*, its exact position is unknown (Kapan *et al.* 2006). *H. erato* maps were estimated using backcross or F_2 broods derived from crosses between *H. himera* and either *H. erato cyrbia* (for the linkage group with *Cr*) as described in Tobler *et al.* (2005) or *H. erato etylus* (for linkage groups with *D* and *Sd*) as described in Kapan *et al.* (2006). The *H. melpomene Yb* linkage group was mapped using F_2 broods derived from crosses between *H. m. cythera* and *H. m. melpomene* as described in Jiggins *et al.* (2005) and Joron *et al.* (2006). For *H. cydno*, we used segregation data from 33 *H. pachinus* (female) × *H. cydno* (male) F_1 hybrids (pseudotestcross design) to map amplified fragment length polymorphism (AFLP) loci, microsatellite loci, and single-copy nuclear loci that were heterozygous in the *H. cydno* male parent (Kronforst *et al.* 2006). We typed *ci*, *Hel-13/14*, *Hm01*, and *Hm08* using PCR product length variation and *ptc* and *ef1*α using single-nucleotide polymorphisms. We then scored 65 F_2 offspring for allelic variation at color-patterning loci and a subset of the mapped markers and used these data to locate the positions of color-patterning loci *G*, *Yb*, and *Ac*. Completely linked AFLP loci in opposite phases were used to score each F_2 individual as marker present (homozygous or heterozygous for the *H. cydno* allele) or marker absent (homozygous for the

(*continued*)

red scales on the wings, which in *H. pachinus* is limited to small basal spots on the ventral side (Figure 1). In other crosses this locus has been shown to segregate with the presence *vs.* the absence of much larger regions of red, such as the red forewing band of *H. melpomene rosina* (Naisbit *et al.* 2003). The *Yb* locus [also know as *Cs* (Nijhout *et al.* 1990)] controls the presence or absence of melanic scales on the hind wing, which prevent or allow the expression of underlying yellow or white scales (Figure 1; Gilbert 2003; Jiggins *et al.* 2005). Finally, the *Ac* locus [also known as *Ps* (Nijhout *et al.* 1990)] controls the presence or absence of melanic scales on the proximal portions of both the fore and the hind wings (Figure 1). The hind wing action of *Ac* is unique to *H. pachinus* (Nijhout *et al.* 1990; Gilbert 2003).

Comparing the actions of the loci between the two groups reveals that in a broad sense they act similarly. Both *D* and *G* influence the distribution of red, both *Cr* and *Yb* control the placement of melanic scales that influence the distribution of yellow and white, and both *Sd* and *Ac* influence the size of the nonmelanic region revealed on the forewing (although the *H. pachinus Ac* allele has a similar effect on the hind wing). Our comparison of their genomic locations revealed that all three loci map to homologous chromosomes in the two lineages. Both *D* and *G* are syntenic with the locus *cubitus interruptous* (*ci*), both *Cr* and *Yb* are syntenic with the microsatellite locus *Hel-13/14*, and both *Sd* and *Ac* are syntenic with the loci *patched* (*ptc*) and *elongation factor 1* α (*ef1*α) (Figure 2). Furthermore, *Yb* in *H. cydno* is syntenic with microsatellite loci *Hm01* and *Hm08*, which are linked to the *Yb* locus in closely related *H. melpomene* (Jiggins *et al.* 2005; Joron *et al.* 2006). Assuming that the polarity of the aligned chromosomes is correct (Figure 2), the ~95% confidence intervals for the placement of potentially homologous loci, relative to the anchor loci, overlap for all comparisons.

These results provide tentative support for the positional homology of wing patterning loci in the two Heliconius radiations. All species studied here have $n = 21$ chromosomes (Brown *et al.* 1992), so the probability of randomly assigning all three *H. cydno* loci to homologous chromosomes identified in *H. erato* is very small ($1/21^3 = 0.0001$). However, aside from the relationship between *G* and *D* the positional correspondence between potentially homologous loci does not appear to be strong. There are several possible explanations for this. First, given that we have identified homologous chromosomes on the basis of one or two shared markers in each case, the possibility remains that the chromosomes, and the color-patterning loci, are not homologous at all. Second, the chromosomes may be homologous but the color-patterning loci may not be. Third, the overall amount of recombination differs between species in the two lineages: *H. erato*'s total map length is 1430 cM (Kapan *et al.* 2006) while that of *H. cydno*'s sister species, *H. melpomene*, is 1616 cM (Jiggins *et al.* 2005). Thus, for any given comparison we expect different amounts of recombination and different distances, between linked, homologous loci in the two radiations. Finally, there is undoubtedly error associated with estimating recombination frequencies from the relatively small data sets that have been analyzed so far, which may contribute to the lack of strong positional correspondence between potentially homologous loci. Current and future research that incorporates larger sample sizes and more homologous anchor loci will better refine the positions of major color-patterning genes in the two radiations and will help identify the extent to which synteny has been conserved. Indeed, Joron *et al.* (2006) recently utilized this approach to show that the *Yb* locus of *H. melpomene*, the *Cr* locus of *H. erato*, and the *P* locus of *H. numata* all lie within ~1 cM of one another.

Studies of wing pattern development in other butterflies suggest that future, fine-scale association studies, physical mapping, and comparative analyses of gene expression will likely verify the homology of color-patterning loci between the two Heliconius clades. Brunetti *et al.* (2001) compared the expression patterns of three transcription factors (Engrailed/Invected, Distal-less, and Spalt) during development of eyespot wing patterns across four butterfly species representing two different families and three genera within the family Nymphalidae. They found that the expression patterns differed among species but often correlated with adult wing pattern. Similarly, Reed and Serfas (2004) surveyed Notch and Distal-less expression among eight moth and butterfly species and found that both genes were generally associated with intervein midline/eyespot development in butterflies. While there is no evidence that Heliconius wing patterning is homologous to these eyespot determination systems (Reed and Gilbert 2004), these studies do suggest that wing-patterning genes are likely to be conserved over evolutionary time.

We thank Joan Strassmann and Dave Queller for use of lab facilities and reviewers for comments on the manuscript. This work was supported by National Science Foundation grant DEB 0415718.

H. pachinus allele) while AFLP loci without a linked marker in the opposite phase were scored as marker present or missing data. Using likelihood we assigned each *H. cydno* color-patterning locus to the interval with the highest probability, but we did not estimate approximate positions within intervals. We also used likelihood to estimate placement support limits (~95% confidence intervals) for each of the loci in both studies (Kapan *et al.* 2006). Mapping was performed with Joinmap 3.0 (Van Ooijen and Voorrips 2001) and Mapmaker/Exp 3.0 (Lincoln *et al.* 1993) using the Haldane mapping function and maps were drawn with MapChart 2.1 (Voorrips 2002). Markers are labeled with their position, in centimorgans, and homologous loci are highlighted.

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Communicating editor: A. D. Long