Wing patterning gene redefines the mimetic history of Heliconius butterflies

Heather M. Hines\textsuperscript{a},\textsuperscript{1} Brian A. Counterma\textsuperscript{b}, Riccardo Papa\textsuperscript{c}, Priscila Albuquerque de Moura\textsuperscript{d}, Marcio Z. Cardoso\textsuperscript{d}, Mauricio Linares\textsuperscript{e}, James Mallet\textsuperscript{f},\textsuperscript{g}, Robert D. Reed\textsuperscript{d}, Chris D. Jiggins\textsuperscript{c}, Marcus R. Kronforst\textsuperscript{c}, and W. Owen McMillan\textsuperscript{a,\textsuperscript{k}}

\textsuperscript{a}Department of Genetics, North Carolina State University, Raleigh, NC 27695; \textsuperscript{b}Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762; \textsuperscript{c}Department of Ecology and Center for Applied Tropical Ecology and Conservation, University of Puerto Rico-Rio Piedras, Rio Piedras, Puerto Rico PR 00931; \textsuperscript{d}Departamento de Botánica, Ecología e Zoológia, Universidade Federal do Rio Grande do Norte, Natal RN 59072-970, Brazil; \textsuperscript{e}Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Carrera 24 No. 63C-69, Bogotá Colombia; \textsuperscript{f}Department of Genetics, Evolution and Environment, University College London, London, W12 1BT United Kingdom; \textsuperscript{g}Department of Organismal and Evolutionary Biology, and Faculty of Arts and Sciences Center for Systems Biology, Harvard University, Cambridge MA 02138; \textsuperscript{h}Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697; \textsuperscript{i}Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, United Kingdom; and \textsuperscript{j}Smithsonian Tropical Research Institute, Apartado Postal 2072, Balboa, Panama

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The mimetic butterflies Heliconius erato and Heliconius melpomene have undergone parallel radiations to form a near-identical patchwork of over 20 different wing-pattern races across the Neotropics. Previous molecular phylogenetic work on these radiations has suggested that similar but geographically disjunct color patterns arose multiple times independently in each species. The neutral markers used in these studies, however, can move freely across color-pattern boundaries, and therefore might not represent the history of the adaptive traits as accurately as markers linked to color pattern genes. To assess the evolutionary histories across different loci, we compared relationships among races within H. erato and within H. melpomene using a series of unlinked genes, genes linked to color pattern loci, and optix, a gene recently shown to control red color-pattern variation. We found that although unlinked genes partition populations by geographic region, optix had a different history, structuring lineages by red color patterns and supporting a single origin of red-rayed patterns within each species. Genes closely linked (80–250 kb) to optix exhibited only weak associations with color pattern. This study empirically demonstrates the necessity of examining phenotype-determining genomic regions to understand the history of adaptive change in rapidly radiating lineages. With these refined relationships, we resolve a long-standing debate about the origins of the races within each species, supporting the hypothesis that the red-rayed Amazonian pattern evolved recently and expanded, causing disjunctions of more ancestral patterns.

Müllerian mimicry | population genetics | phylogeography

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esearchers typically rely on neutrally evolving loci to generate a phylogenetic and population genetic history of adaptive divergence. The rationale is that these markers provide an unbiased view of the relationships among divergent phenotypes and a better understanding of the evolutionary processes generating variation. However, the genome is a complicated mosaic shaped by an interplay of mutation, drift, selection, and recombination. Recombination allows different regions of the genome to experience alternative restrictions to gene flow, and thus develop different evolutionary trajectories. The closer a genetic marker is to the alleles responsible for adaptive differences, the more likely that it will trace the history of phenotypic change.

Understanding how phenotypic variation is generated in nature is greatly enhanced by studying groups that are actively undergoing diversification. By deciphering the history of such diverse phenotypes we gain a clearer understanding of the evolutionary process, including the tempo and mode of phenotypic change. Heliconius butterflies present one of the most striking examples of a recent phenotypic radiation. The 40 species in the genus exhibit hundreds of wing patterns that are involved in Müllerian mimicry complexes, where distasteful species converge on a shared warning signal to avoid predation. This convergence is particularly remarkable in two species, Heliconius erato and Heliconius melpomene. These species are phylogenetically distant and do not hybridize (1), yet they have converged to share over 20 different mimetic color patterns across the Neotropics (Fig. 1) (2, 3). Most of the color-pattern diversity in these species can be partitioned into two major groups: “rayed” patterns, involving orange-red rayed hindwing patterns with orange-red basal forewings, and “red-banded” patterns, involving crimson-banded forewings and hindwings that are black and may have a yellow bar (Fig. 1). The rayed phenotypes are comimetic with several other Heliconius species across a broad contiguous Amazonian distribution. In contrast, the red-banded phenotypes are mostly restricted to just the two comimics H. erato and H. melpomene, and are found in multiple disjunct regions around the periphery of the Amazon.

A long history of research has been devoted to understanding the historical processes generating the wing pattern diversity within H. erato and H. melpomene. Earlier investigators proposed an allopatry-based Plastocene refugium hypothesis to explain these patterns, whereby the identical patchwork of color patterns that characterize H. erato and H. melpomene arose simultaneously when populations of the two species became isolated together in forest refugia during Pleistocene cooling (2–4). Mallet proposed an alternative parapatric-based hypothesis, where color patterns evolved through a process similar to Wright’s shifting balance (5). Under his hypothesis, novel color patterns we see today may have had a single origin and been created when a rayed pattern originated and spread from the Amazon, displacing and fragmenting a previously contiguous red-banded population (8).

As a test of these hypotheses, molecular markers unlinked to color pattern, including mtDNA (9–11), nuclear sequences (12), and amplified fragment length polymorphism (AFLPs) (11), have been used to more carefully dissect the timing of the two parallel radiations and the relationships among color pattern races within the two comimics. These studies inferred an older (10–12) and different phylogenetic history for H. erato than


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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. JN897400 and JN899803) and in the Dryad database (doi:10.5061/dryad.8h15f46).

1To whom correspondence should be addressed. E-mail: heather_hines@ncsu.edu.

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Understanding how phenotypic variation is generated in nature is greatly enhanced by studying groups that are actively undergoing diversification. By deciphering the history of such diverse phenotypes we gain a clearer understanding of the evolutionary process, including the tempo and mode of phenotypic change. Heliconius butterflies present one of the most striking examples of a recent phenotypic radiation. The 40 species in the genus exhibit hundreds of wing patterns that are involved in Müllerian mimicry complexes, where distasteful species converge on a shared warning signal to avoid predation. This convergence is particularly remarkable in two species, Heliconius erato and Heliconius melpomene. These
**H. melpomene**, supporting earlier hypotheses (13) that the more abundant *H. erato* was the “model,” and the rarer *H. melpomene* diverged more recently to mimic it. This theory refuted a simple refugium hypothesis, as both species did not evolve simultaneously. Furthermore, rather than clustering by color pattern, phylogenies from these studies cluster individuals within both species by geographic proximity, with the major lineages dividing the Amazon from the Central American/West Andean region (9–12). Given the geographically disjunct nature of the phenotypes, these data led to the intriguing suggestion that similar color-pattern types within each species were acquired through multiple independent origins. However, even a large sample of color-pattern-independent markers may not be adequate to refute the possibility of a single origin within each comimic. Turner et al. (14) argued, using allozyme data, that markers unlinked to color pattern exhibit a different history to color-pattern loci and are poor indicators of the evolutionary history of these phenotypic races. This notion is supported by recent association data showing that only loci tightly linked to the adaptive color patterns have restricted gene flow at racial hybrid zones within both *H. erato* and *H. melpomene* (15–17). Phylogenetic data from the genomic regions near color-pattern loci should provide a more accurate picture of the history of these phenotypes.

The color-pattern radiation of *Heliconius* butterflies is one of only a handful of cases (e.g., refs. 18–20), where it is now possible to examine the history of a radiation at the loci responsible for the adaptive changes. The genomic interval controlling red pattern variation (Fig. 1) has recently been localized to a homologous region in both comimics (16, 17, 21). Further population genetic and comparative gene-expression work on this region has shown that only loci tightly linked to the adaptive color patterns have restricted gene flow at racial hybrid zones within both *H. erato* and *H. melpomene* (22). Although no other genes show the pattern-specific expression of *optix*, other genes in this interval have shown significant genotype-by-phenotype associations (16, 17, 23) and expression differences between divergent color-pattern races (16, 17, 24).

Here we examine sequence variation in *optix* and four other candidate genes linked to red pattern elements (*hves, kinesin, GPCR, and VanGogh*) across the mimetic races in both *H. erato* and *H. melpomene*. We compare these data to sequences from four unlinked nuclear genes (*SUMO, Suz12, 2654, and CAT*) and a mitochondrial COI-COI1 fragment, to address whether the color-pattern history inferred for *optix* and nearby genes is different from neutral genomic signal. We hypothesize that the phylogeny of a color-pattern locus, compared with unlinked markers, will show more population structure by red color-pattern phenotypes and demonstrate the history of adaptive phenotype evolution more clearly. Specifically, if shared red patterns have a common origin within each species, they should form a single cluster in phylogenetic analyses of *optix* alleles. If convergently derived, their *optix* alleles should cluster in multiple independent lineages. We use these data to examine the historical events leading to extant color-pattern complexes within each species and to compare the timing and patterns of these radiations between species.

### Results

To infer the history of the color-pattern radiations in *H. erato* and *H. melpomene*, we examined patterns of genetic diversity, phylogenetic relationships, and population structure in markers linked and unlinked to color pattern among individuals of 14 distinct color pattern races of *H. erato* (*n* = 73) and 11 comimetic races of *H. melpomene* (*n* = 61) (Fig. 1 and Table S1).

#### Genetic Diversity Within Comimics.

There were marked differences within species and among different phenotypes in standing levels of genetic variation. *H. erato* had over twofold higher estimates of nucleotide diversity (*θ*<sub>SNP</sub>) (Table S2) and recombination (*γ*) (Table S3) than *H. melpomene* across nuclear loci. Within species, patterns of overall genetic diversity (Table S2), recombination (Table S3), and phylogenetic signal (Table 1) did not differ consistently between color pattern-linked and -unlinked nuclear markers. However, color pattern-linked genes showed a reduction in diversity (Table S2) and recombination (Table S3) among rayed individuals of both species. This difference is particularly strong in *optix* for *H. erato*, which showed a fourfold reduction in diversity in rayed versus nonrayed individuals. This discrepancy can be visualized using mismatch plots (Fig. 2). At unlinked loci—and increasingly so for linked markers and *optix*—there were greater pairwise genetic distances among red-banded phenotypes than among rayed phenotypes for both species. These plots also highlight the greater pairwise genetic distances in *H. erato* than in *H. melpomene* and show that the difference between them is more pronounced for red-banded phenotypes, with rayed phenotypes having more similar genetic distances between species.

#### Population Structure of Unlinked Markers.

As previously observed (11, 12), nucleotide variation at markers unlinked to color pattern...
were structured mostly by geography. In *H. erato*, nucleotide variation at three (2654, *Suzy*, and *SUMO*) of the four unlinked nuclear markers was broadly distributed among populations with very little evidence of population structure among races, geographic regions, or color-pattern phenotypes in the analysis of molecular variance (AMOVA) (Table 1) or phylogenetic analyses (Fig. S1A–J). In contrast, the mitochondrial fragment and the unlinked nuclear marker *CAT* had clear population structure, largely reflecting geography (Figs. 1A, Table 1, and Fig. S1G). Both genes recognized distinct lineages that match closely to the Amazonian, Caribbean, and Chacoan/Paraná biotic domains of Morocco (25) (Fig. 1). In the AMOVA, a large amount of variation in both of these genes was explained by geographic divisions into Amazon + Chacoan and Caribbean domains (72.1% each). A fair amount of variation was also explained by color pattern (rayed vs. nonrayed patterns; 15.9–20.1%). However, this pattern was likely the result of regional differences in color-pattern phenotypes, as rayed phenotypes are found only in the Amazon region (Fig. 1). When we reduce the effect of geographic structure by examining color-pattern structure within the Amazonian region, none of the genetic variation was explained by color pattern (Table 1).

In *H. melpomene*, there was a strong geographic pattern to the distribution of variation across unlinked loci. The mitochondrial fragment demarcated four major populations, including the same three lineages for *H. erato* (Amazon, Caribbean, and Chacoan/Parana) but it differed in resolving a Guiana Shield lineage consisting of Trinidad and French Guianan specimens (Figs. 3A and Fig. S1F). The unlinked nuclear markers have less straightforward phylogenetic clustering of populations (Figs. 3A and Fig. S1 G–J). However, all of these genes demonstrated significant geographic signal with a fair amount of variation explained by the geographic division of the Amazon+Chacoan from the Caribbean region (12.9–35.2%) in the AMOVA. These same genes exhibited virtually no variation that could be explained by color pattern (Table 1).

**Table 1. Population structure inferred from AMOVA and phylogenetic signal in color pattern**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type (kb)</th>
<th>Color pattern</th>
<th>Geography</th>
<th>Color pattern: Amazon</th>
<th>Phylogeny</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. erato</em></td>
<td></td>
<td>% Var</td>
<td>P value</td>
<td>% Var</td>
<td>P value</td>
</tr>
<tr>
<td><strong>optix</strong></td>
<td>Target</td>
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<td>0.0010</td>
<td>21.70</td>
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<td><em>kinesin</em></td>
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<td>8.19</td>
<td>0.0323</td>
<td>7.84</td>
<td>0.0411</td>
</tr>
<tr>
<td><em>GPCR</em></td>
<td>Linked</td>
<td>8.24</td>
<td>0.0547</td>
<td>26.37</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>bves</em></td>
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<td>9.06</td>
<td>0.0147</td>
<td>2.53</td>
<td>0.1799</td>
</tr>
<tr>
<td><em>VanGogh</em></td>
<td>Linked</td>
<td>-0.94</td>
<td>0.4135</td>
<td>7.98</td>
<td>0.0147</td>
</tr>
<tr>
<td><em>SUMO</em></td>
<td>Unlinked</td>
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<td>0.5591</td>
<td>0.94</td>
<td>0.0391</td>
</tr>
<tr>
<td><em>Suzy</em></td>
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<td>0.15</td>
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<td><em>2654</em></td>
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<td>0.4230</td>
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<td>0.7146</td>
</tr>
<tr>
<td><em>CAT</em></td>
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<td>0.2102</td>
<td>72.14</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>mt</em></td>
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<td>0.0459</td>
<td>72.06</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>H. melpomene</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>optix</strong></td>
<td>Target</td>
<td>21.84</td>
<td>0.0147</td>
<td>31.67</td>
<td>0.0029</td>
</tr>
<tr>
<td><em>kinesin</em></td>
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<td>18.40</td>
<td>0.0538</td>
</tr>
<tr>
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<td>3.94</td>
<td>0.0997</td>
</tr>
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<td>34.80</td>
<td>0.0049</td>
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<td>0.1799</td>
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</tr>
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<td>0.2581</td>
<td>24.10</td>
<td>0.0020</td>
</tr>
<tr>
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<td>0.4438</td>
<td>13.38</td>
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</tr>
<tr>
<td><em>mt</em></td>
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<td>-0.51</td>
<td>0.3744</td>
<td>35.21</td>
<td>0.0078</td>
</tr>
</tbody>
</table>

CP Steps represents the number of color-pattern changes inferred on the neighbor-joining trees using parsimony, with ranges representing alternative reconstruction of polytomes; Var, variance. Gray shading represents color pattern linked genes. Unshaded rows represent unlinked genes.

*Because mt has only one haplotype for each individual, approximately half the number of steps are expected.

**optix Exhibits Population Structure by Color Pattern.** In contrast to unlinked markers, *optix* showed strong population structure based on color pattern. This structure was most apparent in *H. erato*, where the Bayesian phylogeny (Fig. S1A), neighbor-joining tree (Fig. 3A), and haplotype network (Fig. 3B) of the inferred haplotypes for *optix* place nearly all haplotypes of rayed races into a single derived lineage. Over half of the *optix* variation was explained by color pattern phenotype (55.2%, *P* = 0.00098), a much larger portion than any other gene (Table 1). This large and significant contribution of color pattern remained when the effect of geographic structure is removed (52.5%, *P* = 0.01466).

There was less intraspecific variation in *optix* in *H. melpomene*, making phylogenetic and network inferences more difficult. Nonetheless, individuals clustered by color-pattern phenotype. In the Amazonian tree, most of the rayed phenotypes formed a basal polytomy (Fig. S1A). In contrast, the neighbor-joining tree clustered most of the rayed alleles together, but the alleles fell on a derived lineage. In the haplotype network (Fig. 3B) the rayed haplotypes clustered together near the origin of *H. melpomene*. AMOVA similarly supported color-pattern clustering in *optix* for *H. melpomene*. Using all populations, *optix* had the highest variation of any locus explained by color pattern (21.8%, *P* = 0.01466). When removing the effect of geographic structure, the variation explained by color pattern structure for *optix* is reduced, but nearly significant (14.7%, *P* = 0.0557). The inferred patterns of population structure for unlinked markers and *optix* were further supported by STRUCTURE analyses (Fig. SI Text and Fig. S2). Similar to every locus thus far examined, a neighbor-joining tree combining *optix* sequences for both *H. melpomene* and *H. erato* resulted in two distinct clades, with no sharing of alleles between the comimics (Fig. S1A).

There were a few exceptions to the complete clustering by color pattern in both the *H. erato* and *H. melpomene* *optix* data (Fig. 3B and Fig. S1A). In many of these exceptions, an individual possessed both a rayed and nonrayed haplotype. For example, three of the five *H. erato microleia* individuals possessed...
haplotypes that grouped with both rayed and nonrayed clusters. Similarly, in H. melpomene, a single haplotype of a rayed H. melpomene aegaope individual was resolved in a nonrayed clade and a single haplotype in a nonrayed H. melpomene amaryllis individual fell within a rayed clade. Furthermore, we observed evidence for recombination between the rayed and nonrayed haplotypes in an H. erato hydara individual from Trinidad (TT05_hyd_b) (Fig. S14), which possessed one haplotype that matched other nonrayed haplotypes and another that appeared to be a recombinant between nonrayed and rayed haplotypes.

Decay of Color-Pattern Signal in Other Linked Genes. Relationships between allelic variation and color pattern for the other loci linked to color pattern were less pronounced. Phylogenetic trees of these linked markers had considerable admixture of rayed and nonrayed patterns among lineages in both H. erato and H. melpomene (Fig. 3A and Fig. S1 B–E). This admixture resulted in many more phylogenetically inferred evolutionary shifts in color pattern for these linked markers than for optix in H. erato, and a similar but less extreme difference in H. melpomene (Table 1). Despite such reduced signal, unlinked markers generally had more inferred evolutionary steps in color pattern than linked markers, and less variation explained by color pattern after correcting for geographic signal for both comimics (Table 1). Among these linked genes, kinesin for H. melpomene was the most notable color-pattern clustering. It had the highest levels of variation explained by color pattern after correcting for geography (15.2%) and the fewest evolutionary transitions in pattern of all other optix-linked markers (Table 1).

Discussion

Contrasted Modes of Evolution in the Same Genome. In the two comimics, H. erato and H. melpomene, distinctly different pictures of the history of adaptive change emerge depending on where variation is sampled in the genome. Variation at markers broadly scattered across the genome and unlinked to loci underlying phenotypic differences consistently support the hypothesis that similar color-pattern phenotypes have evolved multiple times within both radiations (10–12). In contrast, genetic variation in the red-determining transcription factor optix structures lineages primarily by color pattern and supports a common origin for similar patterns within each species. This was previously observed in H. melpomene evolution. The origin of the rayed phenotype in H. melpomene is more difficult, in part because lower allelic variation limits phylogenetic resolution. In contrast to H. erato, the genetic evidence suggests that the rayed pattern was present early in H. melpomene evolution. The origin of the rayed phenotype in H. melpomene is reconstructed near the ancestral node in the haplotype network of optix, which divides the Amazonian rayed and nonrayed lineages. The haplotype network thus suggests that
the *H. melpomene* radiation may have originated and spread from the Amazon and colonized other regions of Central and South America, a finding consistent with results from large-scale AFLP data (10). Support for this hypothesis, however, is weak, and there is more variation within nonrayed phenotypes than rayed phenotypes. Furthermore, although patterns are not transferred between *H. erato* and *H. melpomene*, *H. melpomene* may have acquired different red pattern phenotypes through hybridization with its closest relatives. *H. melpomene* is a member of a larger complex of species that are known to hybridize in nature, a number of species in the Amazon region with rayed phenotypes (1, 27). The acquisition of new color patterns by hybridization is thought to play an important role in the evolution of pattern variation in *Heliconius* (24, 28, 29). Additional sequence data around *optix* across the *H. melpomene* species-complex should help resolve the origins of red patterns within the *melpomene* group.

**Advergence and Convergence and the Origins of Mimicry Between *H. erato* and *H. melpomene*.** These data allow us to reassess hypotheses about the timing of the parallel coradiations and the origins of the mimetic relationship between *H. erato* and *H. melpomene*. Our data provide additional evidence against simultaneous diversification of *H. erato* and *H. melpomene*, but do not rule out the idea that the rayed patterns may have diverged more recently in parallel in the Amazon. Overall, *H. erato* harbors substantially more variation and has deeper intraspecific genealogies than *H. melpomene*. The two species are also inferred to have different phylogeographic history, with eastward spread of color pattern in *H. erato* and westward spread in *H. melpomene*. Although exact dating is difficult given that selection will impact rates of genetic divergence (16, 17), the discrepancies in timing and geographic pattern of the radiations appear to be driven mostly by the considerably older origins of the nonrayed *H. erato* patterns. Extant levels of variation in *optix* and unlinked markers in rayed phenotypes are more similar between comimics, suggesting that rayed patterns diverged at about the same time in the two species. The parallel radiation can thus be explained by *H. erato* establishing the red-banded populations first, *H. melpomene* adorning on these patterns, and both species acquiring the Amazonian rayed mimetic pattern around the same time, with subsequent spread of this pattern fragmenting established red-banded populations. It is also possible that both *H. erato* and *H. melpomene* converged together on the rayed pattern to mimic other Amazonian heliconines. Unlike the nonrayed forms of *H. erato* and *H. melpomene*, the rayed phenotypes are part of a larger mimicry complex composed of over a dozen species (mostly *Heliconius* but also other butterfly genera, and even day-flying moths) that share the same pattern (4, 13, 30). The deeper phylogenetic origins of this convergence can now be more thoroughly explored using the genes underlying the phenotypic change.

**Conclusions and Future Directions.** Nucleotide variation at regions tightly linked to the functional sites driving adaptive change provide unique insights into the origins of the patchwork of mimetic color pattern races in *H. erato* and *H. melpomene* that has intrigued biologists for over 150 y. Contrary to the history reflected in the majority of the genome, data around the functional sites driving phenotypic variation suggest that similar wing-pattern phenotypes share a common origin within each of the two parallel radiations. These data also suggest that the rayed Amazonian phenotype evolved recently and around the same time in *H. erato* and *H. melpomene*, and spread rapidly, replacing the ancestral red-banded phenotype. Although variation in red is a major aspect of the complex story of mimicry between *H. erato* and *H. melpomene*, a number of other loci interact with the red locus to generate the phenotypic variation that characterizes the
two radiations. Current research is identifying these loci, including loci that modulate the shape of the forewing band and the presence of a yellow hindwing bar (21). A combination of sequence information across the different color-pattern loci promises an even more complete picture of the history of these coadaptations and a deeper understanding about how novel variation arises and spreads during adaptive change.

Materials and Methods

Taxonomic Sampling and DNA Data Collection. We sequenced 137 individuals of *H. erato* (including three *Heliconius himera* and *H. melpomene*, and two outgroups for each species (Fig. 1 and Table S1). Specimens were sequenced for 10 gene fragments, including four unlinked nuclear markers (CAT [1081 bp], SUMO [805 bp], Suz12 [520 bp], Gene 2654 [872 bp]), a mitochondrial region COI- TRANS-LEU-COI (1,510 bp), and five genes within the red color-pattern interval. These genes included an 800-bp optic transcript (432 bp of coding sequence and 361–370 bp of 3′ UTR) and coding regions of four genes within 250 kb of optic (Table 1), including *kin'eo* (501 bp), *GPCR* (522 bp), *VanGogh* (715 bp), and *bves* (385 bp). Further gene information is available in Table 1 and Table S4.

DNA was extracted from thoracic tissue using the Qiagen DNA Plant Kit, PCR-amplified (Table S4), and purified using ExoSAP-IT (USB, Affymetrix). We sequenced both forward and reverse strands using ABI Big Dye Terminator v3.1 reactions and PCR primers, and called SNPs based on consistent double peaks in chromatograms. For optic, we cloned (TOPO TA Cloning kit; Invitrogen) a few individuals (Table S1) to facilitate phasing. Haplotypes were inferred from polymorphic sequences using PHASEv2.1 (31), allowing recombination (32) with a recombination rate prior of 0.04, and declaring known phases from cloned individuals.

Reconstructing Phylogenetic Trees and Haplotype Networks. We constructed neighbor-joining trees of phased haplotypes for each gene and species in PAUP* 4.0b10 (33). We also performed a combined neighbor-joining analysis on optic for the two species to test for allele sharing. For mtDNA COI and mtDNA cyt b sequences we used Bayesian phylogenies of haplotypes using MrBayes v3.1.2.3 (34).

Tests for Population Structure. We inferred population structure for each gene and species using an AMOVA implemented in Arlequin 3.1 (36). Population structure was assessed relative to geographic region (Caribbean, Amazon +Chaco) (Fig. 1 and Table S1), color pattern (rayed, nonrayed), and race within these groups by comparing variance in uncorrected genetic distances of phased haplotypes. Outgroups, *H. himera* and *H. erato* chestertoni were excluded, as these lineages show strong reproductive isolation from other races. We assigned individuals to populations based on Hardy-Weinberg equilibrium assumptions using STRUCTURE 2.2 (37) (SI Text). As a phylogenetic measure of color-pattern signal for each gene, we inferred the number of evolutionary steps between rayed and nonrayed color patterns using parsimony-based character reconstruction on neighbor-joining trees in MacClade (38).

Genetic Diversity and Recombination Estimates. Methods for estimating genetic diversity and recombination are outlined in Tables S2 and S3. Matches (nucleotide differences) between pairs of haplotypes were calculated within species and between rayed and nonrayed bands (Table S1) of individuals using concatenated sequences of the nuclear unlinked genes for each individual in Arlequin 3.1.4.

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