Introgression of wing pattern alleles and speciation via homoploid hybridization in Heliconius butterflies: a review of evidence from the genome

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The diverse Müllerian mimetic wing patterns of neotropical Heliconius (Nymphalidae) have been proposed to be not only aposematic signals to potential predators, but also intra- and interspecific recognition signals that allow the butterflies to maintain their specific identities, and which perhaps drive the process of speciation, as well. Adaptive features under differential selection that also serve as cues for assortative mating have been referred to as ‘magic traits’, which can drive ecological speciation. Such traits are expected to exhibit allelic differentiation between closely related species with ongoing gene flow, whereas unlinked neutral traits are expected to be homogenized to a greater degree by introgression. However, recent evidence suggests that interspecific hybridization among Heliconius butterflies may have resulted in adaptive introgression of these very same traits across species boundaries, and in the evolution of new species by homoploid hybrid speciation. The theory and data supporting various aspects of the apparent paradox of ‘magic trait’ introgression are reviewed, with emphasis on population genomic comparisons of Heliconius melpomene and its close relatives.

1. Introduction

Neotropical Heliconius butterflies, renowned for their diverse warning coloration, Müllerian mimicry and intraspecific geographical variation [1–5], have become an important model system in a wide range of evolutionary and ecological disciplines. According to a keyword search in Web of Knowledge (http://wokinfo.com), nearly 300 articles addressing aspects of Heliconius biology have been published in the past five years. A major area of research has been ecological and genetic aspects of speciation, with some authors arguing that the genus exemplifies Darwin’s [6] notion that the species boundary is only an arbitrary point on a continuum [7]. Lately, Heliconius has become a focus for comparative evolutionary genomics as well [8–14], providing a vast new source of data to address longstanding questions about the diversification of aposematic wing patterns within and among members of the genus. This study reviews recent literature as it bears upon a specific controversy: the hypothesis that one or more Heliconius species in the H. melpomene–sylvaniform clade have arisen as the result of homoploid hybrid speciation (HHS). (The sylvaniform clade, sister to the H. melpomene + H. cydno clade [15–17], includes 10 species, most of which exhibit yellow, orange and black ‘tiger’ patterns that are Müllerian mimics of one another and of ithomiine butterflies, especially Melinaea spp. Two sylvaniform species, H. besckei and H. elevatus, are mimics of H. erato and H. melpomene forms.)

A homoploid hybrid species is a distinct evolutionary lineage resulting from the admixture of genetic material from two (or more) hybridizing parental species. Unlike polyploid hybrid taxa, which are chromosomally incompatible with their progenitors, homoploid hybrid species have the same number and arrangement of chromosomes as their parental species and so are, at least initially, reproductively compatible. To become established as distinct, true-breeding entities, the
Authors advocating the HHS model in *Heliconius* have promoted their hypothesis effectively [8,22–28], and although the evidence has received little critical scrutiny, the idea now seems to be generally accepted. For example, a recent Nature letter [11] announcing a complete sequence of the *H. melpomene* genome, asserted the following: ‘Most species in [the *H. melpomene*–siltaniform] clade occasionally hybridize in the wild with other clade members. Gene genealogies at a small number of loci indicate introgression between species, and one non-mimetic species, *H. heurippa*, has a hybrid origin (pp. 94–95).’ These three statements, presented as established facts, provide the conceptual framework for the interpretation of new data as further supporting and extending the hybrid speciation hypothesis to additional species such as *H. timarctea*, and perhaps even the silvaniform *H. elevatus*. However, as discussed below, there is reason to question each of these claims. HHS is theoretically unlikely even under ideal circumstances [29,30], so if it has occurred multiple times just within this charismatic genus of butterflies, then speciation theory would seem to be headed for a paradigm shift.

This study briefly reviews the evolution of aposematic wing patterns and mimicry in *Heliconius*, as well as mechanisms for maintaining specific identity, interspecific hybridization, introgression and HHS, and concludes with an alternative hypothesis to explain the origin of *H. heurippa* and other Amazonian *H. cydno* cognates (figure 1). Since Eltringham [1], diverse *Heliconius* forms that interbreed and produce hybrids have been viewed as conspecific geographical races, and, despite debated alternatives [35–37], the biological species concept (BSC) [38] continues to serve as the primary criterion by which *Heliconius* nomenclature is organized. From a BSC perspective, it is arguable that all the taxa illustrated in figure 1 should be considered geographical races of *H. cydno*.

2. Primer on selection for Müllerian mimicry among *Heliconius* species

Müllerian mimicry among aposematic, distasteful prey is maintained by positive frequency-dependent stabilizing selection [39–42]: if predators learn to avoid unpalatable, brightly coloured butterflies by remembering an association between an unpleasant predation event and a colour pattern, then the more common that colour pattern is, the lower the risk of predator attack for each individual exhibiting it. Individuals exhibiting a rare or unique colour pattern are at a selective disadvantage because their patterns are unfamiliar to potential predators and therefore fail to serve as aposematic signals to deter predation. For example, a mark–release–recapture study of *Heliconius erato* [43] reported a cumulative selection coefficient of 52 per cent against novel wing pattern phenotypes relative to endemic phenotypes on opposite sides of an interracial hybrid zone.

Given this strong selection against novelty, one might expect that all Müllerian mimics in a given area, and all individuals of each species, would exhibit the same aposematic pattern. The great paradox of *Heliconius* evolution [44,45] is that across tropical America, multiple dissimilar *Heliconius* mimicry complexes occur in sympathy, while multiple species exhibit dramatic geographical polymorphism with numerous, different-looking allopatric or parapatric races, most spectacularly demonstrated by the co-mimics *H. melpomene* and *H. erato*. Unlike the more genetically integrated wing patterns of most other butterflies [46], the various elements of *Heliconius* wing patterns are encoded by genes in multiple independently segregating linkage groups [4]. While usually monomorphic and presumably homozygous at wing pattern loci throughout most of their ranges, different-looking geographical races freely interbreed where they abut in narrow hybrid zones, producing viable and fertile offspring with no obvious intrinsic pre- or post-zygotic barriers to gene flow [4,42,43]. F₂ interzonal hybrids (heterozygous at wing pattern loci) often appear similar to one of the parental races owing to dominance, but independent assortment and recombination in F₂ and backcross generations result in a broad range of novel phenotypes that are selectively disadvantageous because they do not share the common aposematic pattern of either of their parental forms. By contrast, loci not associated with wing patterns do not exhibit differentiation across these intraspecific hybrid zones [5,20,47–49].

How did the diversity of wing patterns within and among *Heliconius* species evolve, given the aforementioned selection against novelty? The traditional ‘parsimonious’ explanation has been allopatry, often invoking isolation of local populations in Pleistocene forest refugia [3,4,50], which would have allowed colour patterns of local mimicry complexes to diverge by differential selection, genetic drift, or both [51]. While vicariance remains a viable explanation for neotropical speciation events, species-level divergence of many taxa has been argued to be too old to be explained by Pleistocene events alone [52–55]. Brower [5,56] inferred that ages of divergence (at least among the more closely related races within *H. erato*, *H. melpomene* and *H. cydno*) were consistent with a Pleistocene origin (i.e. less than two Myr old), and those age estimates have been corroborated by more recent studies [9,57,58]. Mallet [42,49,59] argued that evidence for the existence of Pleistocene refugia is tenuous, and that, in any event, allopatry is unnecessary for divergence, presaging more recent discussions of ecological speciation [34]. Recent phylogenetic analysis of optix, a gene associated with red-pigment expression in *H. erato* and *H. melpomene* [60], suggests that the red-rayed wing patterns have recently evolved and expanded in Amazonia, relegating races with ancestral colour patterns to the periphery of the species’ ranges in upper Amazonian river valleys and west of the Andes [10], a pattern predicted by Sheppard et al. [4] and Mallet [42,49]. Again, recent large-scale molecular analyses imply that regions of the genome not linked to colour pattern loci show either little variation among races of *H. melpomene* and *H. erato*, or variation correlated with geography and not wing patterns [10,61,62].

3. Natural hybridization and introgression between *H. cydno* and *H. melpomene*

*Heliconius cydno* and *H. melpomene* are broadly sympatric, geographically variable sister species in Central America.
and trans-Andean South America [13,36]. The two species belong to different Müllerian mimicry complexes: H. cydno is usually bluish black with white or yellow bands on fore- and hindwings (figure 1) and mimics sympatric H. sapho and H. eleuchia, whereas H. melpomene is usually brownish black with either red forewing bands or radiating red basal streaks and yellow forewing bands, and mimics H. erato.

Heliconius melpomene’s range extends throughout Amazonia, whereas H. cydno is largely replaced along the eastern slope of the Andes by related forms that mimic either H. erato and H. melpomene (e.g. H. timareta florencia, H. tristero) or exhibit ‘non-mimetic’ wing patterns with some red pattern elements (e.g. H. heurippa, H. timareta forms).

The different wing patterns of H. melpomene and H. cydno are thought to play a key role in maintaining their specific identities in sympatry. Heliconius melpomene males are stimulated to court conspecific females by their red wing pattern elements, whereas H. cydno males are stimulated by white or yellow pattern elements and deterred by red [63–66]. The loci responsible for these wing pattern elements and mate choice are linked, and the respective alleles of the two species are thought to exhibit pleiotropy that promotes discriminatory mate choice [67,68].

from Costa Rica, and developed a model explaining the evolution of phenotypic diversity among these species. Gilbert argued that because wing pattern genes are homologous among Heliconius species [70] (corroborated at the molecular level by [61,62,71–74]), evolution of wing pattern diversity occurred in two phases. Initially, mutations produced differentiated prototypical phenotypes, but subsequently, most of the phenotypic diversity of geographical races in *H. melpomene* and *H. cydno* and its offshoots evolved not by further mutation, but instead via interspecific exchange of wing pattern alleles. Thus, for example, Gilbert hypothesized that the presence of a marginal white band on the hindwing of *H. melpomene cythera*, a feature not found in other *H. melpomene* races, originated by introgression of alleles from *H. cydno alitha*. In the opposite direction, he suggested that red pattern elements in *H. cydno* relatives *H. heurippa*, *H. timareta* and *H. tristera* originated by introgression of alleles from various *H. melpomene* forms.

Although Gilbert [69] pointed out that populations of *H. melpomene* and *H. cydno* can coexist in a greenhouse ‘for years without occurrence of interspecific courtship or mating’ (p. 309), he argued that interspecific hybridization occurs, rarely, in nature, citing as evidence a then-incipient database of putative interspecific *Heliconius* hybrid specimens [75]. Mallet *et al.* [7] compiled a more extensive list of 161 specimens in public or private collections that they believed to be wild-caught interspecific hybrids. More than one-third of these are hybrids between *H. himena* and *H. erato*, and not of concern here, except to note that until Mallet ([42], p. 245) opined that *H. himena* is a ‘good species’, it had been considered a geographical race or ‘semispecies’ of *H. erato* [3,76,77] based on the fact that the two hybridize in nature. Mallet [35] subsequently proposed an alternative species concept to the BSC that permits such hybridizing taxa to be viewed as specifically distinct.

Because hybridization between the parental species in nature is a prerequisite for hybrid speciation, the dataset of Mallet *et al.* [7] has become the empirical cornerstone for the *Heliconius* hybrid speciation story, justifying the claim that species boundaries within the genus form a ‘continuum’, supporting assertions such as that ‘most species in [the *melpomene*-silvaniform] clade occasionally hybridize in the wild with other clade members’ ([11], p. 94) and ‘surveys of wild-caught specimens have revealed many instances of natural hybridization in *Heliconius*’ ([28], p. 1; see also [13,78]), and fuelling more general discussions of ecological speciation [79,80]. The keystone status these data have taken on in subsequent work demands a detailed re-examination of their evidentiary plausibility.

For a museum specimen to provide evidence of interspecific hybridization, we must be confident (i) of its identity (that it is indeed an interspecific hybrid) and (ii) of its provenance (that its label data are bona fide, ideally with a direct chain of custody between the collector in the field and an institutional repository). Because unusual *Heliconius* specimens have long been sought after by collectors and are objects of commerce [81], the burden of proof for their acceptance as scientific evidence must be high. Most of the 93 putative hybrid specimens representing the *H. melpomene*-silvaniform clade that reside in public collections are old and of uncertain provenance. Mallet *et al.* viewed antiquity itself as evidence of authenticity, arguing that old specimens must have been wild-caught, because captive breeding of *Heliconius* is a relatively recent enterprise, but this is unconvincing in terms of either (i) or (ii) shown above: vague locality labels such as ‘Kolumbien’ or ‘Bogotá’ leave much open to interpretation, and historical collectors often had motives other than the advancement of scientific knowledge. For example, Anton Fassl (1876–1922), a prolific collector of Colombian Lepidoptera (including putative *Heliconius* interspecific hybrids) and curator at the Vienna Natural History Museum, confessed in 1906 to stealing butterflies valued at more than $7500 (some $275 000 in 2012) from the museum to establish his own business [82].

The rarity of wild *H. cydno × H. melpomene* hybrids is attested by the fact that almost none have been found by modern *Heliconius* researchers: of the 66 post-1960 records, 42 are from the personal holdings of private collectors who obtained the specimens by purchase or trade from intermediaries. For example, 28 *H. cydno × H. melpomene* hybrids in the database originated with either C. Farrell, L. Denhez or J. Urbina, and wound up in the cabinets of five different amateurs. In 1998, one of these (Mallet *et al.* [7] co-worker Walter Neukirchen) advertised his personal collection of *Heliconius* butterflies, including 24 interspecific hybrids included in [7], at the asking price of US $580 000 (in litt. to A.V.Z.B.). It is not unreasonable to suspect that many of these oddities could have been captive-reared for the butterfly trade, despite assurances to the contrary [7].

In sum, so many of the Mallet *et al.* [7] records are dubious, at least for *H. cydno × H. melpomene* ‘hybrids’, that this dataset must be discounted as convincing evidence of widespread natural hybridization between those species, or more generally for ‘the species boundary as a continuum’.

Of course, even if phenotypically intermediate specimens did imply that interspecific hybridization has occasionally occurred among *Heliconius* species, that would not constitute evidence that introgressive gene flow has established foreign alleles across species boundaries [18]. To support the hypothesis of interspecific gene exchange, several studies [22,83–85] have invoked as evidence of hybridization and introgression similar alleles shared among *Heliconius* species in gene genealogies of various nuclear protein-coding loci (mannose phosphate isomerase, triose phosphate isomerase, distal, invected, white and scalloped). A limitation of these studies is that each compared variability among species only at one or a few geographical localities. Brower [20] combined data for each of these genes in global analyses encompassing multiple geographical regions, and found that many of the ‘introgressed’ alleles are distributed widely among members of the *H. cydno–H. melpomene* clade from throughout their geographical ranges, a pattern explained at least as well by retention of ancestral polymorphism as by recent introgressive hybridization [86,87]. Thus, oft-cited claims of ongoing, evolutionarily significant gene flow between *H. cydno* and *H. melpomene* [83,84,88] should be viewed with circumspection (as suggested by Kronforst *et al.* [89]).

### 4. Heliconius heurippa: homoploid hybrid species?

A highly publicized *Nature* letter [22] invoked Gilbert’s model [69] to develop a scenario for the hybrid origin of *H. heurippa*, a narrowly endemic form from the eastern slopes of the Andes in Colombia, where it is sympatric with *H. melpomene melpomene* but not with any *H. cydno* form. *Heliconius heurippa* has an *H. cydno*-like yellow forewing
band with a distal red border, a phenotype reproduced in the laboratory by successive generations of selective backcrossing between *H. cydno cordula* and *H. melpomene melpomene* [22]. The authors asserted that *H. heurippa* exhibits novel prezygotic barriers that prevent backcrossing to either of its putative parental species, and, based on DNA sequence and microsatellite data, that it has a 'hybrid genome'.

Brower [20] re-evaluated the evidence supporting the *H. heurippa* HHS hypothesis, and pointed out several difficulties in addition to the weak 'support' from the ambiguous genealogies mentioned earlier. To briefly summarize, the prezygotic barriers separating *H. heurippa* from *H. melpomene* and *H. cydno* are not novel, but rather are ancestral features stemming from the mate recognition cues that allow the two 'parental' species to avoid interspecific mating where they occur in sympatry [27,63,66,78]. *Heliconius heurippa* males will mate with *H. cydno* females [65], suggesting that the only barrier to gene flow between those species is allopatry. Likewise, there is no intrinsic postzygotic loss of fitness in *H. heurippa* × *H. cydno* crosses. By contrast, *H. melpomene* is prezygotically and partially postzygotically isolated from *H. heurippa* and *H. cydno*. The preponderance of the genetic markers, including mtDNA [20,36], nuclear SNPs [8] and the weight of evidence from recent genomic comparisons [11,13,14], show that *H. heurippa* is more closely related to *H. cydno* than it is to *H. melpomene*. Because *H. cydno* × *H. melpomene* hybrid F₁ females are sterile, the HHS scenario requires F₁ males to repeatedly backcross to *H. cydno* in order to obtain this homogeneous genetic background, while somehow retaining the *H. melpomene* alleles responsible for the red forewing band and unmarked ventral hindwing.

‘Classic’ homoploid hybrids are expected to exhibit mosaic genomes composed of blocks of DNA from the two parental species [90,91]. Mallet [23] and Jiggins et al. [26], recognizing that the *H. heurippa*’s widespread genomic affinity to *H. cydno* does not fit that model, have relaxed their concepts of HHS by suggesting that only a few ‘magic traits’ (i.e. alternative alleles subject to divergent selection that are pleiotropically associated with alleles promoting reproductive isolation [79,92,93]) are necessary. Wing patterns, under selection for both mimicry and mate recognition [63], are the obvious phenotypic traits that might lead to such selection-driven ecological speciation [94], and so the genomic search is on for chromosomal segments correlated with (and, ideally, causally regulating) differential expression of those characters and corresponding mate choice. Salazar et al. [8] suggested that a region at the 3’ end of the *kinesin* gene exhibits the pattern of differentiation expected of such a feature, but Brower [20] argued that while shared SNPs in the gene region are consistent with introgression between *H. heurippa* and *H. melpomene*, they are mostly silent substitutions invisible to selection, and so do not represent the ‘smoking gun’. It is interesting to contemplate how, under the Mavárez et al. [22] model, selection could favour *H. heurippa*’s ‘non-mimetic’ phenotype, particularly in the initial stages when it would be rare, given that more common combinations of alleles (including the all-heterozygous F₁ phenotype) that would cause it to bear a closer resemblance to its sympatric ‘parent’ (*H. melpomene*) would seem to be favoured by selection for mimetic resemblance. The HHS scenario seems fundamentally at odds with the frequency-dependent selective basis of Mullerian mimicry described earlier.

Nadeau et al. [13] suggested that *H. heurippa* may instead be the homoploid hybrid offspring of *H. melpomene* and *H. timarcta*, but that hypothesis obviates the need for an interspecific hybrid origin, because various *H. timarcta* forms already exhibit the red forewing pattern elements needed to produce the *H. heurippa* phenotype (figure 1). Further, this begs the question of the origin(s) of the red wing pattern elements in *H. timarcta*, which were themselves hypothesized to be the results of interspecific gene flow [11,25].

5. New phylogenomic evidence of introgression

Theory predicts that closely related species that have arisen in allopatry will exhibit genetic divergence across broad regions of the genome, whereas species that evolve in parapatry or sympatry with ongoing gene flow will tend to exhibit divergence only in ‘islands’ surrounding those loci that establish and maintain specific differences by selection (and regions closely linked to them) [95–98]. Nadeau et al. [12] have argued that *Heliconius* of the *H. melpomene*–silvaniform clade exhibit the second type of pattern, based on sliding-window estimates of *F*ₘₛ, with relatively narrow peaks of differentiation representing genomic regions associated with wing patterns. However, genomic genealogies of the same *Heliconius* species appear to exhibit the first pattern, yielding trees that consistently reflect the traditional ‘species phylogeny’, except for specific gene regions associated with expression of mimetic wing patterns that cluster according to those patterns and are incongruent with the ‘species phylogeny’ [11,13,14]. These interpretations of the data appear to contradict one another: if large portions of the genomes of these species are not genetically differentiated from one another, then what characters consistently support the standard groups in the various phylogenetic analyses? The phylogenetic evidence for wing pattern allelic introgression in the absence of more widespread gene flow of ostensibly ‘neutral’ parts of the genome seems highly counterintuitive, given that it is precisely those phenotypes that are under strong selection and have been implicated in the establishment and maintenance of species boundaries [12,34,63,64,89]. Rather than being the only gene regions that flow between hybridizing species, wing pattern alleles should be highly resistant to introgression, as the putative ‘magic traits’ that establish species boundaries in the first place. How might this be explained?

There are three means by which an adaptive phenotype can be shared among species. The most parsimonious of these is common ancestry. As discussed earlier, however, the rampant geographical variation within and among *Heliconius* species indicates that the diversity of wing patterns cannot be explained parsimoniously as the simple result of descent without abundant homoplasy [5]. The classic alternative, recognized by Bates [99], is independent evolution of similar features via convergence: for example, wing patterns shared between ithomiine and disphormine butterflies. Subtly different from convergence is parallelism, in which phenotypes arise independently in related lineages using homologous genetic and developmental architectures, termed ‘homoiology’ by Plate [100]. As understanding of the developmental mechanisms regulating butterfly wing patterns has grown [46], patterns once considered convergent have come to be seen as parallelisms, to a greater or lesser degree. Within *Heliconius*, the genetic architecture responsible
for various discrete wing pattern components is homologous at the molecular level across the genus [61,62,71,72,101], and phenotypic differences result from differential expression controlled by as-yet-unidentified regulatory factors.

At a finer scale, phenotypes shared within and among Heliconius species may have arisen ‘independently’ from this underlying architecture, may have been preserved as ancestral polymorphisms by selection, or may be the result of horizontal transfer via hybridization (Gilbert’s ‘toolbox’ [69]). There is no doubt that some instances of common mimetic patterns have evolved intrinsically without hybridization. For example, if it is not the ancestral pattern of the genus (cf. [4]), then the red-rayed phenotype must have evolved at least four times among species or clades that are reproductively isolated from one another (H. xanthocles clade; H. melpomene–silvaniform clade; H. erato; H. demeter; plus additional times in related heliconiine genera Neruda and Eueides). Within the H. melpomene–silvaniform clade, the red-rayed pattern occurs as an apparently derived state in H. melpomene, H. timanu and H. elevatus, and recent phylogenomic analyses have revealed shared groups of DNA polymorphisms that imply horizontal transfer of these alleles from H. melpomene to the others [11,14]. These trees ostensibly refute the independent origin hypothesis proposed by Brower [20].

It is easy to be intimidated by the overwhelming quantity of data and elaborate analyses presented in genomics publications, and to accept the authors’ interpretations of their results at face value. However, third-generation sequencing and comparative population/phyllogenomics are still in their infancy, and there are issues both of data quality and analytical rigour that raise concerns [102,103]. The H. melpomene genome paper [11] includes comparative sequence data for chromosomal regions of interest for nine H. melpomene–silvaniform clade species, comprising alignments more than 1.7 million bp long (K. Dasmahapatra 2012, unpublished data). Unfortunately, these data matrices contain an enormous amount of missing data. For instance, one of the 10 kb blocks of the N/Yb region providing phylogenetic support for the introgression hypothesis contained an average of more than 53 per cent N’s per taxon, with some taxa missing more than 70 per cent. To be sure, 30 per cent of 1.7 million bp is a lot of data, and phylogenetic analyses of various walk segments do yield the published topologies. However, eyeball scrutiny of the nucleotide sites that provide support for grouping of the taxa by wing pattern reveals that they are rife with ambiguity: there is not a single fixed character state difference (sensu [104,105]) supporting the tidy assortment of individuals by wing pattern in the 480–490 kb segment of the N/Yb region, and only one in the 580–590 kb segment. Published trees [11,13,14] do not make clear how many or what kind of characters support these patterns, nor what models were used to produce the trees.

The statistic Dasmahapatra et al. [11] used to infer introgression is Patterson’s D, a measure of bias from an expected equal distribution of homoplasy in a four-taxon comparison. They observed that D levels appear significantly high in chromosomes 15 and 18, where wing pattern-regulating genes B/D and N/Yb, respectively, reside. However, the test assumes neutrality, and its authors [106] noted that ‘natural selection could potentially bias D statistics’ (p. 2290). It therefore seems that this evidence does not unequivocally support the introgression hypothesis, and could also support the traditional, alternative hypothesis of convergent natural selection for shared wing patterns among species. A similar problem occurs with use of the statistical program IM [107] and linkage-disequilibrium tests [108] to infer interspecific gene flow [14]. These methods also assume neutrality of tested loci, and empirical and simulation studies [109–111] have shown that violations of the model assumptions can result in spurious estimates of population parameters such as rates of introgression. The bottom line is that when the traits of interest are under selection, as genes responsible for wing patterns manifestly are, then inferences drawn from coalescent-based methods for inferring gene flow that assume neutrality may be unreliable.

6. Paradigms and paradoxes

Recent publications raise a number of new conundrums regarding the evolution and diversification of Heliconius butterflies. How can wing mimetic pattern alleles flow from one species to another (and apparently be the only gene regions that do so), when it has been shown that wing patterns are perhaps the key adaptions responsible for intrinsic maintenance of species boundaries by mate choice? How can wing pattern alleles spread from one species to another when such introgression does not occur across intraspecific hybrid zones in geographically differentiated species in which there are no barriers to interrassial hybridization? How can ‘non-mimetic’ phenotypes arise and become fixed as a result of interspecific gene flow when there is a strong selective advantage to phenotypic conformity due to Müllerian mimicry? And, once again, if wing patterns are promiscuously shared across species boundaries, then why has this not led to fixation of a single, shared aposematic pattern, which would represent a stable, selectively advantageous global optimum for all Heliconius butterflies?

With particular regard to the H. cydno cognates on the eastern slopes of the Andes (figure 1), Brower [20] hypothesized that mutations producing red wing pattern elements in peripheral H. cydno populations that have dispersed over the Andes where their otherwise sympatric co-mimics do not occur might be at a selective advantage. Given the underlying homology of the genetic architecture and the pleiomorphic occurrence of red pigment production in the clade, such mutations seem like a plausible mechanism, and would explain the existence of ‘non-mimetic’ forms (as evolving towards, rather than away from, locally dominant mimicry rings). Allowing that evolutionarily significant interspecific hybridization could have taken place, shared red wing pattern elements could also be explained by simple introgression and selection, if the introgressing alleles were uniformly advantageous in the novel genetic background [112–114]. Perhaps rare H. cydno immigrants with no co-mimics could interbreed with more common endemic H. melpomene. But this does not explain why the preponderance of genetic markers in these ‘hybrids’ imply H. cydno ancestry—one would expect backcrosses to the more common parental form [20]. Nor are non-mimetic phenotypes explained by the introgression hypothesis: if selection for mimicry drives the process of introgression, then phenotypes resulting from introgressed alleles should be identical to those of the species from which they came. In addition, the absence of introgressed neutral loci (e.g. microsatellites
between *H. heurippa* and *H. melpomene* does not fit the pattern of shared genetic material expected if significant hybridization had taken place between those two species or the 'parental' *H. cydno* and *H. melpomene* populations [115]. Further, the biogeographical pattern, with six or seven different *H. cydno* cognates east of the Andes exhibiting at least three different *H. melpomene*-like mimetic phenotypes (figure 1), implies that the extremely unusual genetic phenomena proposed to produce them must have occurred independently in multiple populations.

For 150 years, *Heliconius* butterflies have yielded insights into the evolutionary process, and the ability to plumb the genome now provides a vast frontier for extending this knowledge. The criticisms presented this review are intended to provoke further, more detailed and extensive studies of both comparative genomics and natural history in this outstanding system. Undoubtedly, surprises remain to be revealed.

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