

The population genetics of mimetic diversity in *Heliconius* butterflies

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Theory predicts strong stabilizing selection on warning patterns within species and convergent evolution among species in Müllerian mimicry systems yet *Heliconius* butterflies exhibit extreme wing pattern diversity. One potential explanation for the evolution of this diversity is that genetic drift occasionally allows novel warning patterns to reach the frequency threshold at which they gain protection. This idea is controversial, however, because *Heliconius* butterflies are unlikely to experience pronounced population subdivision and local genetic drift. To examine the fine-scale population genetic structure of *Heliconius* butterflies we genotyped 316 individuals from eight Costa Rican *Heliconius* species with 1428 AFLP markers. Six species exhibited evidence of population subdivision and/or isolation by distance indicating genetic differentiation among populations. Across species, variation in the extent of local genetic drift correlated with the roles different species have played in generating pattern diversity: species that originally generated the diversity of warning patterns exhibited striking population subdivision while species that later radiated onto these patterns had intermediate levels of genetic diversity and less genetic differentiation among populations. These data reveal that *Heliconius* butterflies possess the coarse population genetic structure necessary for local populations to experience pronounced genetic drift which, in turn, could explain the origin of mimetic diversity.

Keywords: genetic differentiation; genetic drift; Müllerian mimicry; population structure

1. INTRODUCTION

Biological organisms are incredibly diverse but some aspects of this diversity are puzzling. For instance, why is there a multitude of distinct mimetic wing patterns among distasteful butterflies when theory predicts strong stabilizing selection on warning patterns within species and Müllerian mimicry theory predicts pattern convergence among species? This paradox is particularly acute in the Neotropical butterfly genus *Heliconius*, which has radiated into hundreds of distinct mimetic wing patterns at both the population and species level (Turner & Mallet 1996; Joron & Mallet 1998; Mallet & Joron 1999). A classic example of the mimetic diversity present in the genus *Heliconius* is the co-mimetic species pair *Heliconius erato* and *Heliconius melpomene*. These two distantly related species exhibit nearly identical wing patterns throughout their sympatric range of Central and South America but across this range they have radiated, in parallel, into approximately 30 distinct wing pattern phenotypes (Brown *et al.* 1974; Sheppard *et al.* 1985; Turner & Mallet 1996). This example highlights a larger pattern of mimetic convergence and divergence within *Heliconius*. The genus consists of two major clades (one clade includes *H. erato* while the other includes *H. melpomene*) and the vast majority of mimetic wing pattern phenotypes are shared by at least one species from each of these two clades (Turner 1971*a*, 1976*a*; Brown 1981; Sheppard *et al.* 1985; Brower 1994*a*; Brower & Egan 1997). The coincident mosaic of wing pattern phenotypes in *H. erato* and *H. melpomene*, and the larger parallel radiations of *Heliconius* in general, present a major evolutionary enigma:

sampling by predators should result in stabilizing selection on warning signals within species and it should also generate natural selection for signal convergence among defended species; yet *Heliconius* butterflies exhibit striking wing pattern diversity. How do novel wing patterns arise in the face of selection to minimize variation?

Historically, Pleistocene rainforest refugia played a prominent role in explaining the intra-clade divergence and inter-clade convergence of *Heliconius* wing patterns (Brown *et al.* 1974; Turner 1976*b*; Sheppard *et al.* 1985; Turner & Mallet 1996). Under this ‘refugium/biotic drift’ model, it was hypothesized that *Heliconius* populations were reduced to isolated subpopulations during periods of drying and cooling associated with Pleistocene glacial advances. During these periods of isolation, random extinction of mimicry models in subpopulations generated natural selection for different warning patterns in different locations, thus leading to the geographical patchwork of mimetic patterns seen today. An appealing feature of this hypothesis is that it explains both the within-clade divergence and the between-clade convergence seen in the genus but a major shortcoming is that it cannot account for genuine novelty; the hypothesis explains switching among previously established warning patterns but it does not explain the origin of new patterns (Mallet 1993; Turner & Mallet 1996). In addition, today there is serious doubt as to the importance of Pleistocene refugia in shaping the evolution of tropical biota (Knapp & Mallet 2003) and recent molecular genetic data have shown that major predictions of this hypothesis do not hold for *Heliconius* butterflies. For instance, divergence times among *Heliconius* races and species are considerably older than the

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last glacial advance and co-mimetic taxa have experienced very different demographic and evolutionary histories (Brower 1994*b*, 1996; Flanagan *et al.* 2004).

As a whole, the available data are most consistent not with a hypothesis of bilateral convergence fuelled by random extinction in isolated Pleistocene refugia, but rather with a hypothesis of mimetic 'advergence' in which one clade, that which contains *H. erato*, radiated first and thus established the diversity of mimetic wing patterns while the second within-*Heliconius* clade, that which includes *H. melpomene*, subsequently radiated to co-occupy the mimetic niche space generated by the first clade (Mallet *et al.* 1996; Mallet 1999; Gilbert 2003; Flanagan *et al.* 2004). This hypothesis, which was initially proposed almost a century ago by Eltringham (1916), is consistent with several lines of evidence (Mallet 1999; Gilbert 2003). First, recent research has shown that DNA sequence divergence among *erato* clade species is approximately twice that among *melpomene* clade species (Brower 1996; Brower & Egan 1997) and inferences of historical demography for both *H. erato* and *H. melpomene* based on multilocus DNA sequence data suggest that populations of *H. erato* expanded earlier than those of *H. melpomene* (Flanagan *et al.* 2004). Second, various chemical, behavioural and ecological attributes predispose species of the *erato* clade to initiate novelty and serve as mimicry models. For example, members of the *erato*-related subclade that includes *Heliconius sara*, *Heliconius sapho* and *Heliconius hewitsoni* sequester specific cyanogens from their *Passiflora* hostplants and thus eclose more highly cyanogenic than most *Heliconius*, which manufacture cyanogens *de novo* as adults (Engler-Chauat & Gilbert 2007). These species also lay egg clusters and mate gregariously around large host vines (Gilbert 1991; Deinert *et al.* 1994; Reed 2003), behaviours that make them more conspicuous and thus enhance the efficiency of predator education. These behaviours also introduce pulses of siblings into local populations, which increase the likelihood of inbreeding and thus maximize the opportunity for fixation of novel patterning alleles (Gilbert 2003). Species of the *erato* clade also tend to be more abundant and more widely distributed than their *melpomene* clade counterparts (Eltringham 1916; Mallet 1999). Third, wing pattern is less constrained by mate choice in species of the *erato* clade because males typically locate pupae using chemical cues and then mate with females as they eclose (Gilbert 1976; Deinert *et al.* 1994). In this clade, visual cues are less of a factor in mate choice since, typically, neither sex sees the wings of its mate before copulation and females rarely remate after pupal mating. In contrast, wing pattern cues play a major role in mate selection and courtship among species of the *melpomene* clade (Jiggins *et al.* 2001, 2004; Kronforst *et al.* 2006*a*). Therefore, novel colour patterns are more freely established in the clade that is hypothesized to be driving colour pattern evolution.

While the advergence hypothesis provides a powerful explanation for the observed phenotypic concordance between the two *Heliconius* lineages, it does not address the fundamental evolutionary enigma of the *Heliconius* radiation—how do entirely novel warning patterns emerge in the first place? One potential answer to this question is simply random genetic drift. Under this model, a novel warning pattern that emerges in an isolated population may occasionally, as a result of drift, increase in frequency

until it reaches the threshold at which it is sufficiently common to be protected (Mallet & Singer 1987; Turner & Mallet 1996). While not necessary, this process would be aided by a reduction in purifying selection, something that would result from a temporary reduction in the local predator community (Gilbert 2003). Once protected, the new pattern could then go on to dominate the local population and eventually spread out to neighbouring populations. If the novel pattern provided protection equal to that of the ancestral pattern (i.e. it was just as noticeable and memorable) then there is a certain probability that the new pattern would fix in the local population and spread out simply as a result of drift. Alternatively, if the novel pattern offered superior protection then its spread would be favoured once it passed the frequency threshold necessary for protection.

The potential for genetic drift to aid in the early establishment of novel, adaptive traits is often discussed in terms of Sewall Wright's shifting balance theory (Wright 1932, 1977). Indeed, Mallet (1986*a*, 1993; Mallet & Singer 1987; Turner & Mallet 1996; Joron & Mallet 1998; Mallet & Joron 1999) has suggested that *Heliconius* wing pattern diversification may provide an empirical example of the shifting balance process in action, arguing that novel patterns could initially become established as a result of genetic drift and then spread out to neighbouring populations as a result of clinal movement. Recent evidence of hybrid zone movement in *H. erato* demonstrates that the later stage of this process does occur (Blum 2002*a*). While Wright's idea of an adaptive landscape is appealing and has been embraced broadly, the general importance of the three phase shifting balance in driving adaptive evolution has been strongly criticized (Coyne *et al.* 1997, 2000). The argument that genetic drift may influence the evolution of mimetic novelty in *Heliconius* has been particularly controversial because as long-lived and potentially highly vagile insects, *Heliconius* butterflies are unlikely to experience the population subdivision necessary for genetic drift to overcome the strong purifying selection acting on mimetic warning patterns. While this expectation is intuitive, it has never been adequately tested. Here we use large-scale multilocus genotype data to explicitly measure the local population genetic structure of eight *Heliconius* butterfly species from Costa Rica. The results indicate that contrary to expectation, *Heliconius* butterflies generally exhibit pronounced population subdivision and local genetic drift over very limited spatial scales.

2. MATERIAL AND METHODS

(a) *Sample collection and AFLP genotyping*

We collected samples of eight *Heliconius* species from various locations throughout Costa Rica. Our analysis included 81 *H. erato* samples from 11 collecting locations, 56 *Heliconius cydno* from 9 locations, 44 *Heliconius pachimus* from 6 locations, 27 *H. melpomene* from 7 locations, 44 *Heliconius hecale* from 10 locations, 48 *H. hewitsoni* from 5 locations, 8 *H. sara* from 6 locations and 8 *H. sapho* from 3 locations. All individuals were sampled over a limited geographical range (distances between collecting locations ranged from 6 to 221 km) within which each species, except *H. sara*, was phenotypically monomorphic. *Heliconius sara* occurs as two racial phenotypes in Costa Rica; one form, *Heliconius sara theudela*, has a yellow hindwing marginal band and occurs on Costa Rica's Pacific drainage

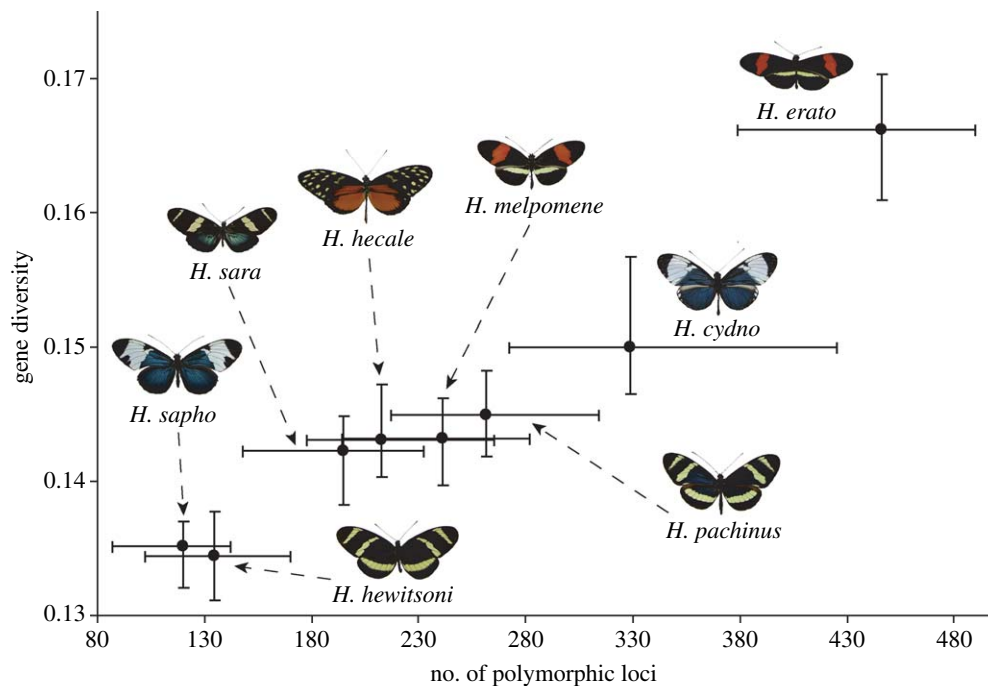


Figure 1. Variation in gene diversity and number of polymorphic AFLP loci among eight *Heliconius* butterfly species from Costa Rica. To account for sample size differences on our estimates of genetic diversity, we resampled species datasets 1000 times in groups of eight individuals from which we estimated means and 95% CIs (error bars).

while the second form, *Heliconius sara fulgidus*, lacks this yellow band and occurs on the Caribbean drainage. Our analysis included specimens of both *H. sara* forms. Sampling geographically proximate populations allowed us to address the local structure of populations. Furthermore, sampling individuals of each species from only a single racial phenotype allowed us to infer the influence of local genetic drift without our analyses being confounded by the effects of natural selection between distinct warning pattern phenotypes, which will drive greater genetic differentiation between populations. Most previous population genetic work in *Heliconius* has focused on analysing the distribution of genetic variation over large geographical regions (Turner *et al.* 1979; Davies & Bermingham 2002; Flanagan *et al.* 2004) or estimating differentiation and gene flow among closely related species (Jiggins *et al.* 1997; Jiggins & Davies 1998; Beltrán *et al.* 2002; Bull *et al.* 2006; Kronforst *et al.* 2006b; Mavárez *et al.* 2006) and thus has not addressed the issue of local genetic differentiation and drift.

All specimens were collected in the field as adults between June and August 2000 or 2002. Tissue was preserved in 95% ethanol and total genomic DNA was extracted using a DNeasy Tissue Kit (Qiagen, Valencia, CA). We genotyped each individual with amplified fragment length polymorphisms (Vos *et al.* 1995) using the PE Applied Biosystems AFLP plant mapping kit (PE Applied Biosystems, Foster City, CA.) and we separated fragments with an ABI Prism 3100 genetic analyzer. Four selective primer combinations were used to generate fragments; EcoRI-ACT/MseI-CAT, EcoRI-ACT/MseI-CTG, EcoRI-ACA/MseI-CAT and EcoRI-ACA/MseI-CTG. We sized and scored AFLP fragments using ABI GENEMAPPER software v. 3.7.

(b) Data analyses

We calculated gene diversity for each species using a Bayesian method designed specifically for dominant markers like AFLPs (Zhivotovsky 1999). To account for sample size

differences on our estimates of genetic diversity (number of polymorphic loci and gene diversity) we resampled, with replacement, each species dataset 1000 times in groups of eight individuals (the size of the smallest dataset) from which we estimated means and 95% CIs. Fixation indexes (F_{ST}) were estimated using ARLEQUIN (Schneider *et al.* 2000). For estimation of F_{ST} values, individuals from neighbouring collecting locations were pooled when sample sizes were small. Multidimensional scaling (MDS) was performed using the software NCSS (Hintz 2001), based on pairwise Euclidean square genetic distances which were calculated with ARLEQUIN. Mantel tests, implemented with the software ZT (Bonnet & Van de Peer 2002), were used to test each species for isolation by distance (IBD), or a correlation between pairwise genetic and straight-line geographical distances.

3. RESULTS

(a) Intraspecific genetic variation differs substantially across species

To examine the fine-scale population genetic structure of *Heliconius* butterflies, we genotyped a total of 316 individuals from eight Costa Rican *Heliconius* species with 1428 AFLP markers. The eight surveyed *Heliconius* species varied widely in the amount of standing genetic variation, from *H. erato*, which was polymorphic at 990 of the AFLP markers (69%) and fixed for the presence of a fragment at only four loci (0.3%), to *H. sapho*, which was polymorphic at only 149 markers (10%) and fixed for the presence of a fragment at 60 loci (4%). Since these estimates are sensitive to variation in sample size and our sample sizes varied substantially among species (from 8 to 81 samples per species), we resampled each species dataset in groups of eight individuals from which we estimated means and 95% CIs for our polymorphism statistics. Even after controlling for sample size differences, the amount of genetic variation varied considerably across the eight *Heliconius* species (figure 1).

Heliconius erato had the most genetic variation, followed by *H. cydno*, *H. pachinus*, *H. melpomene*, *H. hecale* and *H. sara* all had similar, moderate levels of genetic variation while both *H. hewitsoni* and *H. sapho* harboured relatively little genetic variation.

(b) Genetic differentiation among populations is common in *Heliconius*

We found that six out of the eight surveyed *Heliconius* species exhibited evidence of population subdivision and/or IBD indicating genetic differentiation among populations (figures 2 and 3). *Heliconius erato*, a species with extreme racial diversity throughout the Neotropics, exhibited the most genetic diversity and population subdivision of the eight species. All *H. erato* populations were genetically differentiated from one another with pairwise F_{ST} estimates as high as 0.216. Even *H. erato* populations that were geographically close to one another, such as Colon and Santiago (separated by approx. 20 km), exhibited pronounced genetic differences (figure 2a). *Heliconius melpomene*, which has diversified in parallel with *H. erato*, also exhibited genetic differentiation across Costa Rica, as did *H. cydno*, *H. hewitsoni*, *H. sara* and *H. sapho*. Only *H. pachinus* and *H. hecale* showed no evidence of genetic differentiation among populations.

Across species, variation in the extent of population subdivision correlated with the roles different species have played in generating pattern diversity. According to the advergence hypothesis, mimicry within the genus *Heliconius* has resulted from two parallel radiations that occurred at different times: the within-*Heliconius* clade that includes *H. erato*, *H. hewitsoni*, *H. sara* and *H. sapho* radiated first and thus established the diversity of mimetic wing patterns while a second within-*Heliconius* clade, which includes *H. melpomene*, *H. cydno*, *H. pachinus* and *H. hecale*, subsequently radiated to match the protected patterns established by the first clade. Our population genetic data show that members of the clade that originally generated the pattern diversity exhibit striking population subdivision and some have minimal genetic variation overall (*H. hewitsoni* and *H. sapho*), all of which is consistent with pronounced genetic drift. In contrast, species from the second clade have intermediate levels of genetic diversity and less genetic differentiation among populations.

4. DISCUSSION

The mechanisms by which diversity originates in mimicry systems are poorly understood. For novel forms to originate and then assume a place in a population or community, both genetic/developmental mechanisms that generate variation and population/environmental conditions that allow new forms to rise to dominance against selective gradients must interact in an unlikely way. Random genetic drift could allow novel mimetic warning patterns to occasionally pass the frequency threshold necessary for protection. Hence, this process could provide a mechanism for local populations to overcome stabilizing selection on warning patterns and thereby explore the adaptive landscape of warning patterns. The major dilemma in applying this explanation to *Heliconius* wing pattern diversification has been the expectation that natural *Heliconius* populations are unlikely to be subdivided or to be influenced strongly by genetic drift. Our

population genetic data reveal that this expectation is not true; *Heliconius* butterflies often exhibit pronounced population subdivision and local genetic drift over very limited spatial scales.

For some species the amount of population subdivision is striking. For instance, over the limited geographical range from which we collected specimens, differences among populations accounted for 12.5% of the genetic variation in *H. erato*. Similarly, differences among collecting locations accounted for 9.5% of the genetic variation in *H. erato*'s co-mimic, *H. melpomene*. Furthermore, while we analysed too few samples of *H. sara* and *H. sapho* to estimate F_{ST} s, there was a very strong association between genetic and geographical distance in *H. sara* (figure 3d) and a weak but significant association in *H. sapho* even though the most distant collecting locations for this species were separated by only 23 km (figure 3b). Three of the species that exhibited population subdivision, *H. erato*, *H. melpomene* and *H. sara*, are distributed throughout Costa Rica; so some portion of this differentiation could result from the geographical separation of Costa Rica's coastal drainages, separation caused by a mountain range that runs the length of the country. Indeed, the *H. erato* MDS analysis revealed that individuals cluster, in part, by drainage (figure 2a) and using a hierarchical AMOVA we found that of the genetic variation that is due to differences among populations, 60% is due to differences between the two drainages. MDS of *H. melpomene* (figure 2e) and *H. sara* (figure 2d) revealed a similar pattern whereby individuals from the two drainages were largely separated along the first dimension. However, there is a substantial amount of genetic differentiation that is independent of this central mountain range. For instance, in *H. erato* and *H. melpomene*, a significant portion ($p < 0.001$) of the genetic variation distributed among populations is a result of differences within drainages and some species only occur on one drainage and still exhibit evidence of population subdivision (*H. cydno*, *H. sapho* and *H. hewitsoni*). Interestingly, two species, *H. hecale* and *H. pachinus*, show no evidence of population subdivision and *H. hecale* is distributed across Costa Rica, suggesting that the central mountain range is not itself sufficient to generate genetic differentiation between populations.

There are a variety of biological factors that may account for the widespread genetic differentiation among *Heliconius* populations and the variation we observe among species in terms of overall genetic diversity and the extent of population subdivision. One major factor that probably promotes population subdivision in *Heliconius* butterflies is home-range behaviour. Various mark-recapture studies have shown that individual *Heliconius* butterflies return daily to the same larval hostplants, adult resources and nocturnal roosts indicating that they travel over a limited range throughout their lifetime (Turner 1971b; Ehrlich & Gilbert 1973; Cook *et al.* 1976; Mallet & Jackson 1980; Mallet 1986b; Quintero 1988). Mallet (1986c) found that newly emerged *H. erato* individuals do disperse some distance before they establish their home range but this dispersal was on average only 296 m. However, estimates of dispersal based on analyses of hybrid zones suggest that *Heliconius* butterflies may move greater distances than mark-recapture studies indicate.

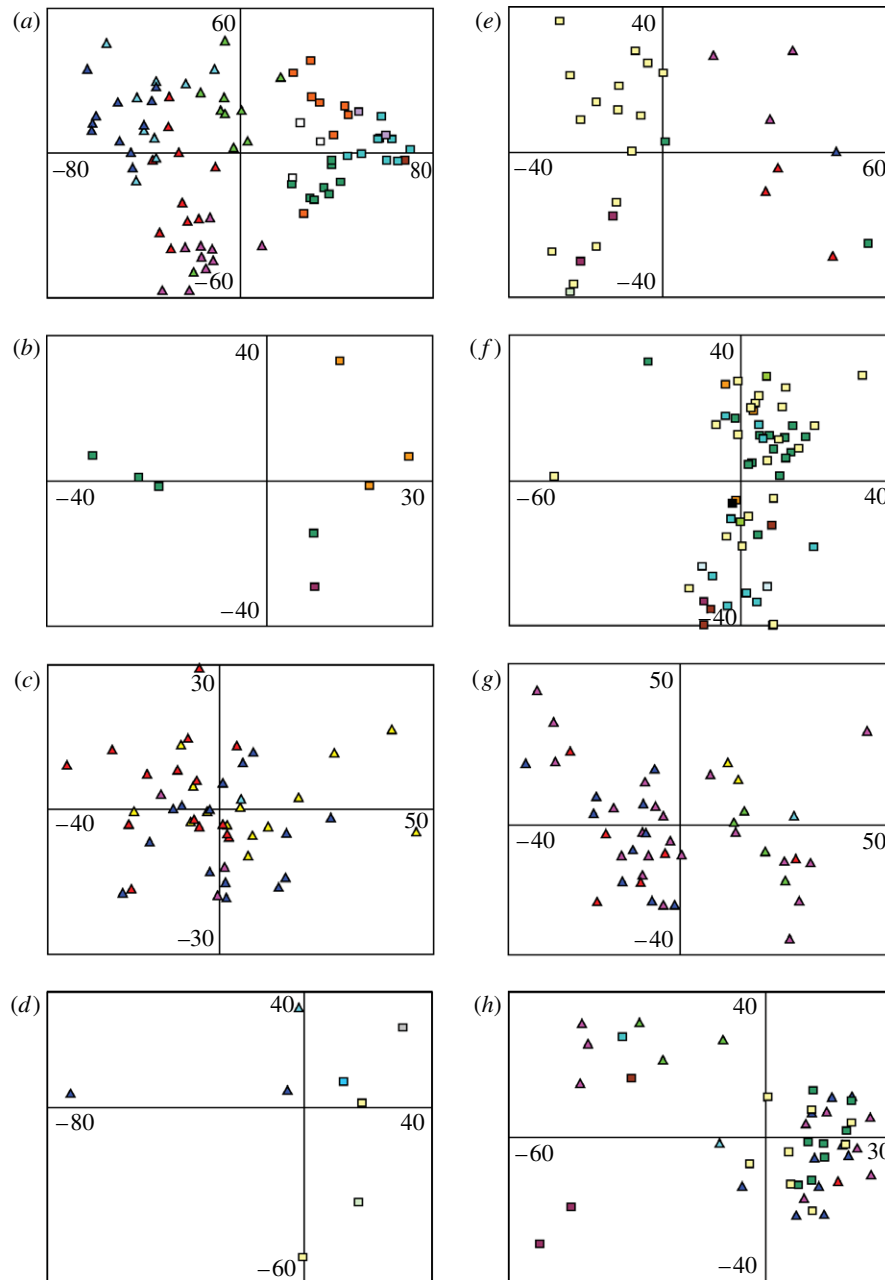


Figure 2. Examining the genetic structure of *Heliconius* populations. For each species ((a) *H. erato*: $F_{ST}=0.125$, $p=0.000$, pairwise $F_{ST}=0.029-0.216$; (b) *H. sapho*; (c) *H. hewitsoni*: $F_{ST}=0.038$, $p=0.000$, pairwise $F_{ST}=0.023-0.047$; (d) *H. sara*; (e) *H. melpomene*: $F_{ST}=0.095$, $p=0.000$, pairwise $F_{ST}=0.037-0.136$; (f) *H. cydno*: $F_{ST}=0.009$, $p=0.012$, pairwise $F_{ST}=0.002-0.018$; (g) *H. pachinus*: $F_{ST}=0.007$, $p=0.129$, pairwise $F_{ST}=-0.004-0.016$; (h) *H. hecale*: $F_{ST}=0.008$, $p=0.134$, pairwise $F_{ST}=-0.006-0.026$) we performed MDS based on pairwise genetic distances. Individuals were then plotted using the two dimensions that encompassed the most inter-individual variation. Fixation indexes (F_{ST}) were estimated for each species as a whole and pairwise between all populations using an AMOVA framework. The p -values for F_{ST} estimates were estimated by shuffling genotypes among populations 1000 times. Only eight individuals were analysed for (b) *H. sapho* and (d) *H. sara* so it was not possible to test for genetic differentiation among populations of these two species. Of the remaining six species, four exhibited significant genetic differentiation among populations; (a) *H. erato*, (c) *H. hewitsoni*, (e) *H. melpomene*, and (f) *H. cydno* (*Location legend*. Pacific drainage (triangles): dark blue, Sirena station; light blue, Dominical; red, PN Manuel Antonio; yellow, PN Carara; pink, Santiago; green, Colon. Caribbean drainage (squares): black, PN Tapanti; white, Orosi; dark orange, Cachi; light green, Cariblanco; yellow, OTS La Selva; light blue, Horquetas; maroon, Guapiles; dark blue, Guacimo; grey, Barbilla; pink, Bananito; light orange, PN Hitoy Cerrere; dark green, Vesta; violet, Cahuita).

Using measures of cline widths for *H. erato* and *H. melpomene* in Peru, Mallet *et al.* (1990) estimated that the average dispersal distance for both species was in the range of 3–4 km. Similarly, Blum (2002b) estimated that *H. erato* dispersal was on the order of 3–10 km based on analyses of hybrid zones in Panama and French Guiana.

Mirroring the discrepancy among dispersal estimates, previous population genetic studies of *Heliconius* have come to different conclusions regarding the extent of population subdivision. In general, most previous work has found little evidence for genetic differentiation among *Heliconius* populations. For instance, Kronforst & Fleming (2001) examined the population genetic structure of

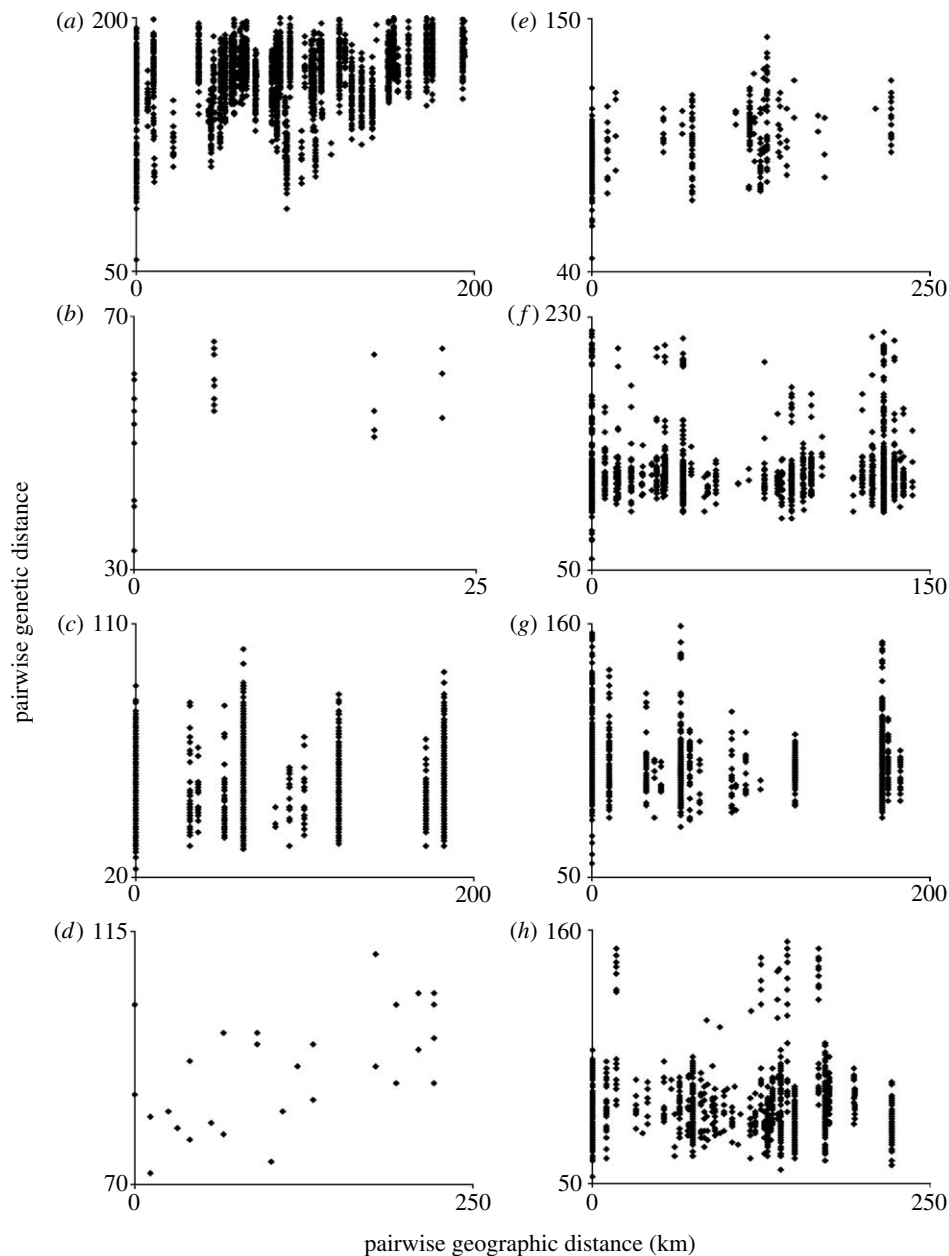


Figure 3. Examining IBD in *Heliconius* butterflies. For each species ((a) *H. erato*: Mantel $r=0.274$, $p=0.000$; (b) *H. sapho*: Mantel $r=0.301$, $p=0.042$; (c) *H. hewitsoni*: Mantel $r=0.056$, $p=0.054$; (d) *H. sara*: Mantel $r=0.553$, $p=0.004$; (e) *H. melpomene*: Mantel $r=0.406$, $p=0.000$; (f) *H. cydno*: Mantel $r=0.011$, $p=0.369$; (g) *H. pachinus*: Mantel $r=-0.019$, $p=0.315$; (h) *H. hecale*: Mantel $r=0.008$, $p=0.407$) we plotted pairwise geographical and pairwise genetic distance among all individuals and tested the association between these two variables using a Mantel test. Four species exhibited significant IBD; (a) *H. erato*, (b) *H. sapho*, (d) *H. sara* and (e) *H. melpomene*.

Heliconius charithonia (a member of the *erato*-clade) in southeast Florida using allozymes and found very low genetic diversity throughout the area and no evidence of genetic subdivision. The lack of genetic differentiation found in that study is in general agreement with allozyme data for other *Heliconius* species (Turner *et al.* 1979; Jiggins *et al.* 1997; Jiggins & Davies 1998; Mallet *et al.* 1998). However, one allozyme study did find evidence of significant differentiation among *Heliconius* populations. Mauricio-da-Silva & Araújo (1994) surveyed five populations of *H. erato* in Brazil that were separated by less than 30 km and found that pooling samples from adjacent locations resulted in an F_{ST} estimate of 0.08. Similarly, analyses of population structure based on microsatellites have found pairwise F_{ST} s as high as 0.04 in *H. erato* from French Guiana, 0.08 in *H. erato* from Panama and 0.23 in

H. melpomene from Panama (Blum 2002b). Our results, which are based on hundreds of molecular markers that span the genome, indicate that *Heliconius* butterflies may often be quite sedentary, which in turn allows genetic differentiation to accumulate among neighbouring populations as a result of genetic drift.

Differences among species in the amount of standing genetic variation are probably strongly influenced by differences in population size. For instance, extensive collection data have revealed that *H. erato* is widespread and relatively abundant but *H. erato*'s co-mimic, *H. melpomene*, tends to be less widely distributed and less abundant (Eltringham 1916; Gilbert 1984, 1991; Mallet 1999); the consequence of which we see reflected in the amount of genetic variation harboured by each species. Furthermore, the gregarious behaviour of

H. sapho, *H. hewitsoni* and *H. sara* is expected to increase the opportunity for inbreeding and indeed we see evidence of reduced genetic variation in these species.

Regardless of the cause, the fact that multiple species (particularly those that originally established the diversity of warning patterns) exhibit evidence of population subdivision indicates that genetic drift may have played a role in generating *Heliconius* warning pattern diversity. Combined with the advergence hypothesis, this model explains the origin of novel warning patterns, the mosaic distribution of racial phenotypes seen in many *Heliconius* species and the mimetic convergence between the two independent *Heliconius* radiations. Whether genetic drift generally plays a significant role in the origin of biological diversity remains an open question, however, our population genetic data lend support to the hypothesis that drift has played an important role in driving one classic and enigmatic example of morphological diversification.

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