Evolution: **Mimicry meets the mitochondrion**

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A recent molecular study of the evolution of mimicry in tropical butterflies of the genus *Heliconius* proves that the mimics adapted to previously diverged 'model' species, but does not clearly distinguish between opposing views of how the model species diverged.

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Current Biology 1996, Vol 6 No 8:937–940

One hundred and thirty-five years ago, Henry Walter Bates noticed striking similarities between unrelated pierid, ithomiine and heliconiine butterflies collected from the same area [1]. Bates termed this phenomenon 'mimicry', and showed that rare or unprotected butterflies would gain by imitating the warning patterns of commoner, unpalatable forms. Later, Müller [2] showed that unpalatable species would also converge, because predators should take a lower proportion of individuals that share a warning signal. Today, mimicry is not merely accepted, it remains one of the clearest paradigms of natural selection, cited in almost all textbooks of evolution.

As Bates realized, however, mimicry theory poses more questions than it answers. Do Müllerian mimics coevolve [3]? Or does Müllerian mimicry, like Batesian mimicry, happen *via* one-sided evolution by a 'mimic' towards a highly protected 'model'? The real puzzler, however, is to explain the diversification of Müllerian mimicry patterns. Warning colour and mimicry are strongly selected, and mimics may survive twice as long as non-mimics of the same species [4]. There will therefore be strong selection against any novel coloration. The ultimate prediction of Müllerian mimicry is that butterflies of a similar size should all ultimately converge on the same colour pattern. And yet Müllerian mimics like *Heliconius erato* and *H. melpomene*, which mimic each other faithfully in almost every part of the neotropical rainforest, have evolved radically different colour-pattern races every few hundred miles (Fig. 1), separated only by narrow hybrid zones. These hybrid zones consist of a number of correlated narrow clines of different colour-pattern genes (a cline is a monotonic change of gene frequency with distance) [1,4,5]. Furthermore, closely related unpalatable species often belong to different locally sympatric mimicry 'rings' [5]. If Müllerian mimicry selects for convergence to a single adaptive peak, how could such extraordinary geographic and local diversity evolve?

Geographic divergence within vertebrate species complexes in Europe or North America is often explained by isolation in refuges during Pleistocene ice ages. Parapatric distributions — that is, where ranges abut — have been thought to result from 'secondary contact' after the ice melted, when the former refuges became contiguous. Climates in the tropics were certainly more equable than in the north temperate zone during glacial maxima,

Figure 1

Müllerian mimicry and geographic variation in *Heliconius erato*, *H. melpomene* and *H. himera* from Ecuador and northern Peru. *Heliconius erato* races are shown on the left; their Müllerian co-mimics within *H. melpomene* are shown on the right; and *H. himera*, which is closely related to *H. erato* but has no close Müllerian mimics, is shown in the centre. Geographic distribution: top, eastern Andes of Ecuador; second row, western Ecuador; third row, from near Zamora, south-east Ecuador; fourth row, from Andean valleys in southern Ecuador and northern Peru; fifth row, from the lower Huallaga, north-eastern Peru; bottom, from the upper Huallaga and Mayo valleys, north-eastern Peru. (Reproduced with permission from [15].)

but the rainforest has been postulated to have contracted to form Pleistocene refuges surrounded by savannah or semi-desert [6]. This refuge model was first applied to bird diversification in the Amazon, but was easily transferred to *Heliconius*. Haphazard species extinctions ('biotic drift') were hypothesized to cause different Müllerian models to persist in each refuge, leading to geographic divergence in mimics [5].

However, ecological adaptation without isolation is also a possible cause of divergence in the Neotropics, even under interglacial conditions like today's, and can account well for the patterns of distribution used in support of the refuge model [7,8]. Ecological adaptation is in fact close to Bates' own view that heliconiine and ithomiine butterflies diverged initially because of natural selection by "local, probably inorganic conditions" [1]. Another hypothesis, based on Sewall Wright's 'shifting balance' model of evolution, is that temporary relaxation of selection and genetic drift will occasionally allow a new warning pattern to evolve locally. If the new pattern is successful at warning away predators, it can spread to other areas behind a moving cline [9]. The three evolutionary hypotheses (Table 1) are hotly contested and hard to distinguish [10].

Recently, Andrew Brower [11] has used powerful modern tools in an attempt to understand the evolutionary history of mimicry independently of the strongly selected colour patterns. Brower sequenced a part of the mitochondrial (mt)DNA from a very large sample of *Heliconius* butterflies. Using these sequence data, he has produced an extremely useful phylogeny of the whole genus *Heliconius* [12], as well as genealogies of intraspecific variation in two of the most diverse co-mimics, *H. erato* and *H. melpomene* [11]. Brower hoped that, as in many other taxa, mitochondrial haplotypes would correlate with morphology, enabling phylogenies of *Heliconius* races to be constructed. Curiously, the mtDNA data settled fewer arguments than Brower expected.

In neither *H. erato* nor *H. melpomene* is the mtDNA genealogy closely associated with colour pattern. For example, a prominent phylogenetic feature of the mtDNA variation is that, in each species, a major genetic break occurs in north-east Colombia, corresponding approximately with the northern tip of the Andes (Fig. 2). In neither lineage, however, does the mitochondrial discontinuity correspond geographically with a contact zone between colour patterns; instead it occurs within the 'postman' race of each species — so-called because their coloration is similar to the uniform of South American postmen. This mimetic pair of races stretches from east Panama westwards to Venezuela, Trinidad, the Guianas and the mouth of the Amazon. Within eastern and western clades of each species, the mitochondrial phylogeny corresponds neither to geography nor to colour pattern. For the *H. melpomene* group, the mitochondrial phylogeny even crosses the species boundary; the *H. cydno* group of species (including *heurippa*, *besckei*, *pachinus* and *timareta*) appears in the genealogy to bud out from one of the branches within *H. melpomene*, making *H. melpomene* as a whole paraphyletic (Fig. 2). (In phylogenetic systematics, one attempts to classify biodiversity using only monophyletic groups, which include all descendents of a common ancestor; if one or more descendents, like *H. cydno*, is classified separately, the remaining group is said to be paraphyletic.)

Brower argues that the colour pattern variation of *H. erato* and *H. melpomene* did not coevolve — the lack of concordance of mtDNA genealogies between the co-mimics suggests they originated in different areas. The most basal branches in the *H. erato* group are in the Andes, with the semispecies *H. himera* and *H. erato chestertonii* each forming

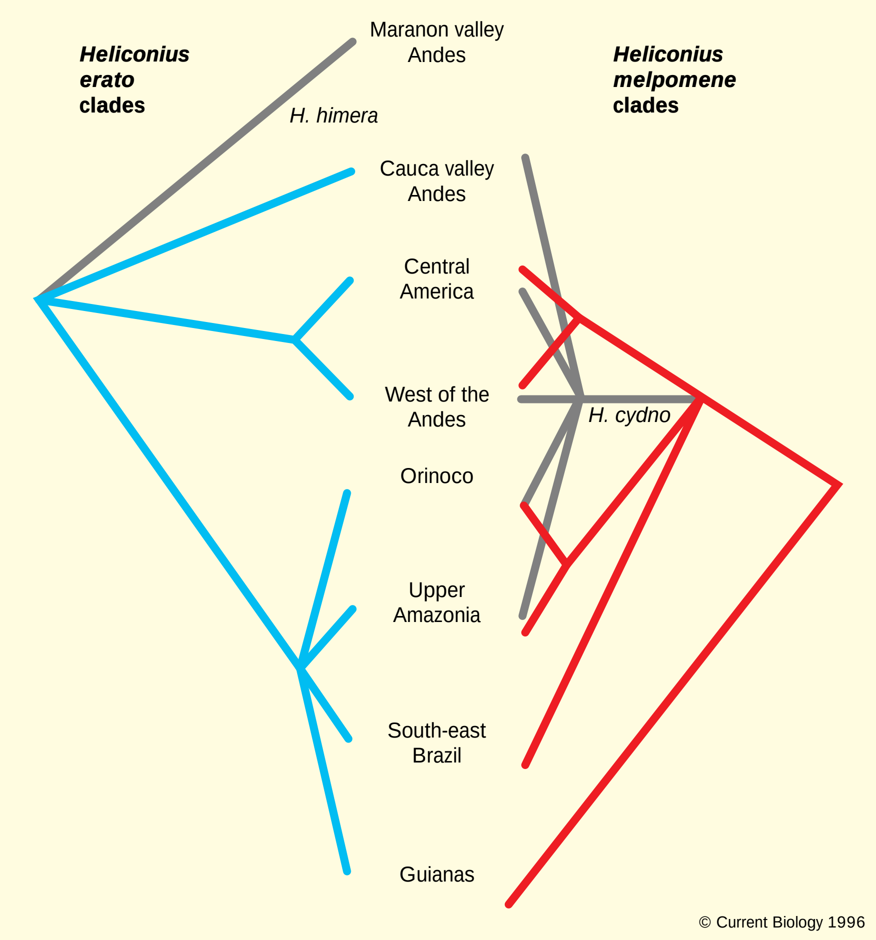
Table 1

Hypotheses for geographic diversification of Müllerian mimicry.

Name of model	Supporters	Geographic context	Initial force for local divergence in colour pattern	Generation of current geographic pattern	Origin of colour pattern variants	Prediction for neutral molecular markers
Pleistocene refuge	KS Brown PM Sheppard JRG Turner	Allopatry in refuges (geographic isolation)	Mimicry in response to 'biotic drift'	Spread from refuges during climatic optimum	Colour pattern parsimony: single origin per pattern	Geography similar to those of colour patterns, but more blurred; concordant phylogenies between unrelated species
Ecological adaptation	(HW Bates) WW Benson JA Endler	Parapatry (ranges abutting)	Local adaptation to non-mimetic environment	*In situ* spread	Unspecified ?	No association with pattern; no phylogenetic concordance between species
Shifting balance	J Mallet	Parapatry	Genetic drift	*In situ* spread by cline movement	Colour pattern parsimony: single origin per pattern	No association with pattern; no phylogenetic concordance between species

Figure 2

Simplified ‘area cladograms’ of the races of *Heliconius erato* and *H. melpomene*, based on Brower’s analysis of mtDNA sequences. (Adapted from [11].)



separate branches as distinct as those of eastern and western clades within *H. erato* (Fig. 2). In *H. melpomene*, a major fork leading to a Guianas radiation appears early; a later radiation leads to the *H. cydno* group species as well as the eastern and western clades of *H. melpomene* (Fig. 2). If the *H. erato* group evolved in the Andes, and the *H. melpomene* group evolved in the Guianas, they could not have coevolved in a mutual Müllerian mimetic radiation. One species (probably *H. melpomene*) must have ‘colonized’ the already-diversified warning patterns of the other. Molecular clock arguments suggest that the major Andean divergence in *H. erato* is about 1.5–2 million years old; in *H. melpomene*, the Andean split seems much younger [11].

In answer to the second question, about diversification, Brower avoids the obvious conclusion that the lack of concordance between colour pattern and mtDNA indicates parapatric differentiation (see Table 1). Instead, Brower holds that gene flow would have prevented colour pattern evolution; he feels allopatry (geographic isolation) must have been necessary, as in the refuge model. Brower puts the lack of concordance between mtDNA and colour pattern down to rapid bursts of warning pattern evolution not associated with mtDNA differentiation. This argument may be correct, but it is weak, because some mtDNA patterning is expected to remain from isolation in refuges (Table 1). Even more damning for the refuge model are the very different topologies of the trees for *H. erato* and *H. melpomene* (Fig. 2). Even if one of the species evolved in refuges, it seems likely that its co-mimic did not.

Refuge modellers used phylogenies based on the genetics of colour patterns to suggest that *H. erato* and *H. melpomene* radiated together [5]. Brower claims that this ‘colour pattern parsimony’ hypothesis is disproved by the molecular evidence. To Brower, deep forks in the molecular phylogeny indicate that disjunct but genetically similar patterns evolved independently. For instance, the central American race of *H. erato* (not shown), in the western mtDNA clade, is nearly identical to the Peruvian Huallaga race (Fig. 1, bottom right) in the eastern clade, both visually and in terms of colour pattern genetics. The same is true for the matching, co-mimetic *H. melpomene* patterns in each area.

Supporters of the refugium and shifting balance models regard the similarity in morphology and colour pattern genetics as good evidence that each pattern has arisen only once in each species, and that newly derived forms in intervening areas have caused the disjunctions. Instead, Brower now suggests that, because the central American and Peruvian forms belong to different mtDNA clades, similar patterns must have evolved independently (although at the same colour pattern loci) in each area. Brower even suggests that mitochondrial breaks within the postman races in north-east Colombia represent the stitching together of two independently derived, but identical, colour patterns that evolved allopatrically.

Brower's argument for independent evolution of similar colour pattern races either side of the Andes hinges on the mtDNA genealogy giving a reasonable estimate of the phylogeny of races. This requires the mtDNA to have remained associated with colour patterns, and seems hard to justify given the general lack of correspondence between mtDNA and colour patterns within major clades of each species. It is difficult to construct a genealogy of morphological features if the characters used in its construction dissociate themselves from morphology. There are two ways in which this dissociation may come about. Firstly, narrow clines for warning colour are not obviously rooted to any particular environmental feature; they will move if one form is advantageous [9], so that a colour pattern cline may move across a stationary mitochondrial contact zone. Secondly, lack of genetic recombination means that the whole mitochondrial genome will respond to any selection affecting even a point mutation [13]. If an advantageous (or selfish) mutation arises, the entire mitochondrial genome bearing it may spread throughout the population, even beyond a stationary contact between two colour pattern races.

These difficulties should only be seen as a temporary setback for the understanding of mimicry. It would now be interesting to know whether genealogies of nuclear loci are similar to those of mtDNA. Even better, if we could find and sequence the major control loci that determine the colour pattern switches, we would be able to investigate the genealogy of mimicry itself. Under the 'colour pattern parsimony' of the refugium and shifting balance models, the alleles determining disjunct colour patterns should be paraphyletic with respect to more recently evolved alleles found in intervening areas. If similar colour patterns evolved repeatedly, as under Brower's 'mtDNA parsimony' model, we would expect the alleles causing the parallel colour pattern switches to be completely unrelated. Finding and sequencing genes that specify the colour patterns of tropical butterflies may seem a tall order, but recent advances in understanding how butterfly colour patterns develop may place this within our reach in the next few years. Several groups are now racing towards this goal. The stimulus to this research is that genes homologous to those acting early in development in *Drosophila* are selectively activated in eyespots and other pattern elements of the developing wings of the butterfly *Precis coenia* [14].

Meanwhile, Brower's interesting mtDNA results need explaining in their own right. What can explain major geographic transitions of mtDNA within species lacking reproductive barriers? The *Heliconius* work apparently shows both that mtDNA ignores colour pattern genetic boundaries, and that colour patterns are oblivious to mtDNA boundaries. Until we achieve a better understanding of the spatial dynamics of both types of genetic change, opposing views of mimicry evolution (Table 1) will remain deadlocked. Nonetheless, a start has been made, and it may not be long before molecular phylogenetic approaches like Brower's solve one of the most visually appealing puzzles of evolutionary diversification.

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