Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 6491–6495, July 1994
Evolution

Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution

(molecular clock/Pleistocene refugia/biogeography/Müllerian mimicry)

ANDREW V. Z. BROWER*

Section of Ecology and Systematics, Cornell University, Ithaca, NY 14853-2701

Communicated by Paul R. Ehrlich, February 22, 1994 (received for review September 28, 1993)

ABSTRACT **The neotropical *Heliconius* butterflies are famous examples of Müllerian mimicry, due to the diverse array of shared, brightly colored wing patterns that advertise the butterflies' unpalatability. The parallel geographical variation in these patterns within several widespread species has been invoked to support the controversial Pleistocene refugium hypothesis of tropical diversification. However, in no *Heliconius* species have either evolutionary rates or relationships among geographical races been explicitly examined. I present a phylogenetic hypothesis based on mitochondrial DNA sequences for 14 divergent races of *Heliconius erato*, which reveals that similar wing patterns have evolved rapidly and convergently within the species. There is a basal split between groups of races from east and west of the Andes, reflecting a vicariant separation at the base of the Pleistocene. Within each of these clades, sequence divergence is very low, and some haplotypes are shared between allopatric races with radically different wing patterns. The topology implies a simultaneous radiation of races in these two areas within the last 200,000 years. Ages for the clades are estimated by comparing sequence divergence to a plot of mitochondrial divergence in several arthropod taxa with independently dated divergence times. This plot is linear and suggests that mitochondrial DNA in arthropods evolves in a clocklike manner, at least initially, when sequence divergence is low.**

The historical origins of neotropical biodiversity have been an increasingly important concern of biologists in recent years. While geographical heterogeneity of the neotropical biota was first noted more than a century ago (1), divergence times of and relationships among closely related, geographically differentiated taxa inhabiting various regions of South America remain poorly understood (2). Current geographical barriers, such as rivers and mountain ranges, have undoubtedly played a significant role in the historical subdivision of populations (3). An appealing but controversial additional explanation for geographical diversification is the fragmentation of the continuous moist Amazonian forest into small, isolated pockets (refugia) surrounded by savannah or grassland during cool phases of the Pleistocene (4). A repeated cycle of forest contraction and reexpansion may have contributed to the genetic differentiation of closely related taxa in regions not separated by currently recognizable barriers to dispersal, by enhancing genetic drift or by allowing differential local adaptation (5). Alternatively, natural selection may have played the dominant role in erecting the current boundaries between closely related but geographically distinct taxa, regardless of refugia or other extrinsic impediments to gene flow (6).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Distribution data from butterfly collections have provided the most complete picture of spatial patterns of variation in the neotropics. The geographical distributions of races in many species are concordant (7, 8) and appear also to match the distributions of differentiated populations or subspecies in other groups of plants and animals (4, 9). Such congruent spatial patterns of diversity among disparate neotropical taxa support the idea that all the taxa displaying those patterns have shared a single biogeographical history, which is best explained by vicariance (10).

If the finer details of this history can be revealed for an exemplary taxon that shares the common geographical pattern, the data offer a testable hypothesis for relationships within other covicariant clades. This gives researchers the opportunity to shift the Pleistocene refugium debate from the theoretical stalemate between vicariance biogeographers arguing for allopatric speciation (9, 11) and selectionists arguing for parapatric speciation (6) to the more immediate and answerable questions of which taxa (and areas) are most closely related to which, and when did they diverge? Once spatial and temporal patterns of diversification are clearer, hypothetical evolutionary processes may be more fruitfully distinguished from one another.†

THE STUDY SYSTEM

The widespread neotropical butterfly *Heliconius erato* displays remarkably diverse geographical variation in its aposematic wing patterns: >20 distinct geographical races have been described (Fig. 1). All races participate in Müllerian mimicry rings with at least one other species of *Heliconius* (usually *H. melpomene*, which has geographically concordant, mimetic races matching 20 of the 21 races mapped in Fig. 1). Within the range of a given *H. erato* race, there is very little phenotypic variation, due to normalizing selection on the aposematic wing color patterns. All races are potentially interfertile (7), yet adjacent races with different wing patterns intergrade only in restricted hybrid zones. The narrowness of these zones is maintained by migration-selection equilibrium, because interracial migrants and hybrid individuals with unusual recombinant phenotypes are preferentially attacked by predators (12, 13). The lack of variation within and the discrete boundaries between races allow their recognition as terminal taxa for phylogenetic analysis (14, 15).

Prior work on *H. erato* racial relationships has concentrated on the evolution of wing patterns. Sheppard, Turner, and colleagues (7, 16, 17) have developed a complex theory of geographical variation and mimicry in *Heliconius*, inte-

*Present address: Department of Entomology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024-5192.

†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U08512, U08530, U08543, U08545, U08558, and U08560–U08594).

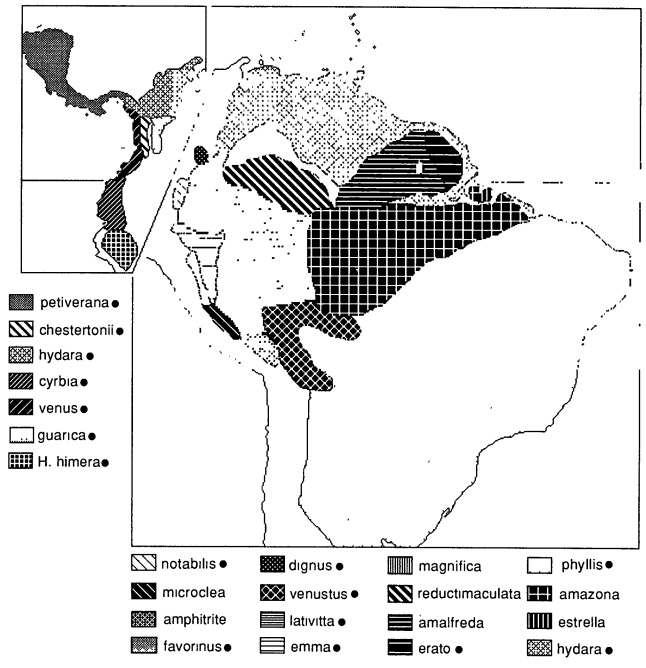

FIG. 1. Geographical distribution of the major races of *H. erato* and the close relative *H. himera*. Races occurring west of the Andes and in Central America are represented in the *Inset* and labeled in the left column. Races from east of the Andes are shown in the main map and labeled in the four columns beneath it. Areas unshaded on either map contain no *H. erato* or have not been adequately sampled. The 14 races of *H. erato* sampled are indicated with a black dot on the legend. The eastern and western populations are separated in this figure for clarity but are not contiguous, except possibly in northern Colombia. (Adapted from ref. 7 with permission; copyright 1985, the Royal Society.)

grating aspects of both the selectionist model and the Pleistocene vicariance model for neotropical diversification. They have argued that the occurrence of very similar wing patterns in widely separated populations is a historical consequence of selection for mimicry in central Amazonia, resulting in peripheral isolation of more ancestral wing patterns at the periphery of the range. Because *H. erato* wing patterns are currently under intense selection, however, it is difficult to draw robust historical conclusions based on that character system. Phylogenetic analysis of an independent data set unrelated to labile phenotypic characters allows an assessment of the roles of selection and history in producing the current diversity of wing patterns.

Mitochondrial DNA (mtDNA) is an ideal marker to examine phylogenetic relationships among *H. erato* races in several respects. It is maternally inherited and unlikely to recombine, thus permitting the recovery of an unambiguous phylogenetic hypothesis for its evolution. mtDNA evolves fairly rapidly, most of the changes that occur among closely related taxa are nucleotide substitutions at silent sites, and the molecule unlinked to nuclear genes is responsible for wing-pattern differentiation (18). mtDNA thus represents an unbiased, neutral marker for maternal ancestry and, arguably, the best single piece of evidence revealing historical relationships among populations as well. It is unlikely to reflect selective events related to mimicry that might have obscured the evolutionary history, such as independent convergence on similar phenotypes.

In addition, there is at least weak evidence to support a constant initial rate of evolution in mtDNA, allowing the estimation of provisional dates of origin for clades of interest. Studies of allozyme divergence in Amazonian birds (3, 19) have suggested that neotropical geographical diversification may have occurred substantially earlier than the mid-Pleistocene, prior to the proposed period of Pleistocene climatic cycles. No similar estimate of evolutionary rates or divergence dates has been made for any neotropical insect group. A mtDNA clock for arthropods presented here allows a provisional estimate to be made for the divergence dates of races within *H. erato*. The clock is corroborated by evidence from prior studies of mtDNA and from South American tectonic history. Thus, this study provides not only a cladistic hypothesis for the relationships among widespread, differentiated races within a paradigmatic neotropical species but also a first estimate of the large-scale biogeographical affinities and ages of South American biotic regions.

MATERIALS AND METHODS

I sequenced a region of mtDNA from 52 butterflies representing 14 races of *H. erato*, and four closely related species of *Heliconius* (Fig. 2). In most cases, at least two individuals from separate localities were sampled from each race. DNA was extracted from individual frozen or ethanol-preserved butterflies, amplified by symmetric and asymmetric PCR (21, 22), and sequenced by the dideoxy chain-termination method (23). Oligonucleotide primers were designed from consensus sequences for *Drosophila* (24) and *Apis* (25), and from other *Heliconius* sequences (26). Positions of oligonucleotide primers used for PCR and sequencing and full details of the protocols employed have been presented elsewhere (27).

A 950-bp mtDNA fragment was sequenced, including the 3′ end of the cytochrome oxidase subunit 1 gene from position 2783, the leucine tRNA, and the cytochrome oxidase subunit 2 to position 3772 (positions refer to the homologous site in the *Drosophila yakuba* sequence, ref. 24). This region, although not the most rapidly evolving in the mitochondrial genome, was selected over the A+T-rich region (control region), because A+T-rich sequences contained many insertions and deletions, even among populations within single races, and could not be reliably aligned (data not shown). In contrast, the sequences used in this study contain no intraspecific insertions or deletions.†

Sequences were aligned by eye. Mean and maximum pairwise distances within and among clades were calculated by hand from distance matrices produced by PAUP (28). Phylogenetic analysis of all haplotypes, excluding identical sequences, was conducted in PAUP, using the heuristic search option with 10 random-addition replications, in a heuristic search with 10 iterations (TBR and ACCTRAN options). All characters were equally weighted.

RESULTS

The phylogram shown (Fig. 2) is one of 2094 shortest, maximum-parsimony trees (272 steps) found by PAUP (28). In the strict consensus of all the most parsimonious trees (not shown), the eastern clade, western clade, *H. himera*, and the two *H. erato chestertonii* clades form an unresolved basal polytomy within the species. The analysis thus reveals two major mtDNA clades within *H. erato*, distributed on opposite sides of the Andean Cordillera. The western clade contains six races from Central America and the Pacific slope of South America, whereas the eastern clade contains nine races from Amazonia, southeastern Brazil, and Guiana. *H. erato chestertonii*, from the upper Cauca valley in Colombia, is paraphyletic with respect to all other races. Relationships among the five races in the western clade and among the nine races in the eastern clade are unresolved, due to their extreme similarity in DNA sequence. Within the eastern clade, a single haplotype is shared among four races from southeastern Brazil, eastern Ecuador, and southeastern Co-

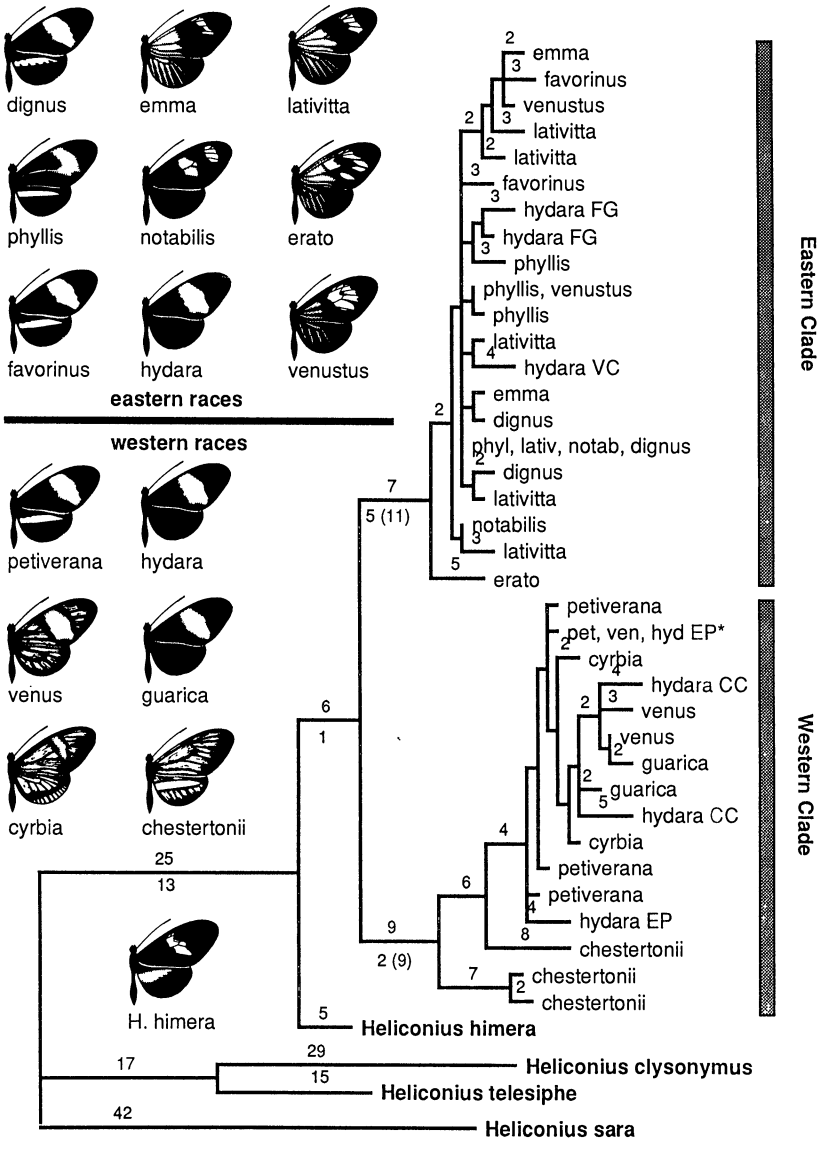

FIG. 2. Relationships among races of *H. erato*, inferred by maximum parsimony from mtDNA sequences (tree length, 272 steps; consistency index excluding uninformative characters, 0.532; retention index, 0.840) (20). Phenotypes of races studied and of the semi-specific sister taxon *H. himera* are illustrated. Gray shading denotes red; white denotes yellow; striations indicate iridescence. The phylogram is constructed on the basis of shared, derived characters only, but autapomorphies are used to reconstruct terminal branch lengths. Branch lengths are indicated above branches (zero- and one-step branches are not labeled). Numbers below branches indicate the number of steps longer the tree must be before the clade defined by that branch becomes paraphyletic with respect to its sister taxon, or polyphyletic, in the case of the two major clades (indicated in parentheses). Several terminal branches represent more than one individual sharing the same mitochondrial haplotype. The starred clade contains eight individuals from Costa Rica and Panama representing three races (four *petiverana*, two *hydara*, two *venus*). Localities of hydara specimens are indicated (FG, French Guiana; VC, Villavicencio, Colombia; CC, Cartagena, Colombia; EP, eastern Panama). (Illustrations adapted from ref. 7 with permission; copyright 1985, the Royal Society.)

lombia, while different specimens from the same race are sometimes widely distributed within the clade.

Mean pairwise sequence divergence (uncorrected) between the large eastern and western *H. erato* clades is 3.4%, whereas within each of these clades, divergence is under 0.6%. *H. erato chestertonii* is 1.5–2% divergent from other races in the western clade and also exhibits nearly 2% divergence between individuals from populations on opposite sides of the Cauca valley, suggesting that the race is much older than any other extant race. *H. erato chestertonii* (and *H. himera*) are the only *H. erato* races lacking a corresponding *H. melpomene* mimic. The mimetic association between the species may thus have evolved quite recently, after *H. erato chestertonii* and *H. himera* had already diverged from the ancestral *erato* stock.

One race, *H. erato hydara*, appears in both the eastern and western clades. *H. erato hydara* is continuously distributed from Panama along the Caribbean coast of South America to beyond the mouths of the Amazon (Fig. 1). Individuals examined in this study from eastern Panama and from Cartagena in Colombia fall in the western clade, whereas individuals from Villavicencio in Colombia and from French Guiana fall in the eastern clade, following the major biogeographical split produced by the Andes. The *hydara* phenotype undoubtedly represents two or more distinct populations bearing a similar wing pattern that have not been split into distinct named races because they have a continuous, if attenuated, distribution from Panama to Santarem, Brazil (7). This finding is consistent with the observation that numerous allopatric races with similar phenotypes but different names (e.g., *dignus, petiverana, favorinus*) are not sister taxa. Thus, the presence of *hydara* in two clades represents a nomenclatural rather than a biogeographical anomaly, and the east/west split between the two large clades is preserved.

DISCUSSION

Biogeographic Patterns Among *H. erato* Races. The east/west split between the two main groups of races corresponds to the most obvious biogeographical barrier in South America, the Andes. Basal clades (*H. himera* and *H. erato chestertonii*) fall on the western side of the mountain range, implying that the species may have begun to diversify on the Pacific slope and subsequently spread eastward into the Amazon basin. Although very similar wing patterns are shared by allopatric races in the eastern and western clades, the clear division in the mtDNA phylogeny between the clades indicates that these patterns are not explicable by trans-Andean migration or by the simple retention of ancestral character states. Given the mtDNA topology of relationships, there is no simple way to explain the distribution of wing patterns among races by vicariance or by the peripheral isolation model suggested by Sheppard, Turner, and cowork-

ers (7, 17), unless we also invoke long-term stabilizing selection to preserve ancestral patterns in widely separated and genetically divergent races. A more plausible interpretation is the independent gain of convergent patterns, possibly due to simple genetic changes in the genetic pathways governing wing pattern development (29).

Within the eastern and western clades, the lack of phylogenetic resolution and the short branch lengths separating individual haplotypes indicate very recent divergence of mitochondrial haplotypes. The shared presence of single haplotypes in multiple, widespread Amazonian races is probably due to retention of ancestrally polymorphic neutral mtDNA variants among populations of butterflies differentially selected for alternative wing patterns. Introgressive gene flow of mtDNA between established races undoubtedly occurs but cannot easily account for the distribution of haplotypes over thousands of miles and multiple races, given the low dispersal rate and apparently short time since divergence (30). Thus, allopatric races with similar wing patterns may have evolved simultaneously and convergently, perhaps even within the eastern and western clades. Alternatively, the similarly low levels of mtDNA divergence in the two clades could be the result of recent selective sweeps that occurred in the two regions, in parallel, although such a coincidence seems unlikely. The recent-origin explanation for low mtDNA diversity within clades would be supported by the discovery of similar spatial patterns and degrees of sequence divergence in additional taxa.

Estimating a Preliminary Molecular Clock for Arthropod mtDNA. Although various authors have speculated on the age of the intraspecific radiations of mimetic races in *Heliconius* (7, 16), there are no benchmark dates for the vicariant events separating the various clades of *H. erato*. As a first approximation to solving this problem, I have compiled data for other recently diverged arthropod taxa from various sources which contain both mitochondrial sequence divergence measures and dates of divergence for the taxa studied (Table 1). The data come from recent reports of mitochondrial divergence within species, between closely related species, or within genera of five arthropod orders. The estimates of divergence times for these taxa are based on dated geological events reported by the authors or are presented as mean rates for a group of taxa, also based on dates of divergence inferred from biogeography and paleoclimatology. All the date estimates are independent and represent geographically diverse regions: two from Hawaii (*Laupala, Drosophila silvestris*), one from Panama (*Alpheus*), one from Australia (*D. buzzatii*), two from North America (*Magicicada, Tetraopes*), and one from the Indian Ocean (*D. melanogaster* subgroup). It is important to note that all of these taxa are recently diverged: the oldest estimated divergence date in Table 1 is 3.25 million years.

These data points are derived from studies of different taxa, using different subsections of the mitochondrial genome, and were produced by a variety of different techniques. That the plot (Fig. 3) does not bend toward the *x* axis implies that the absolute amount of sequence divergence (even among *Alpheus* species) is so low that mutational saturation has probably not yet occurred and that most changes are synonymous. This probably also explains why there is so little scatter in the plot, even though the observations come from different regions of the mtDNA genome. As divergence increases, varying levels of selective constraint on different genes and in different lineages increasingly affect relative overall divergence rates (36), but the initial rate of divergence for all mitochondrial genes is similar (37). The slope of the line in Fig. 3 (2.3% pairwise sequence divergence per million years) thus approximates an underlying constant mutation rate of 1.1–1.2% per million years per lineage, for silent sites.

There is undoubtedly a large compounded error in the data due to uncertainty of timing of events, stochasticity in DNA evolution, sampling error, and methodological flaws. This error might be expected to overwhelm any signal in the data but not to err systematically in one direction and yield a highly significant yet spurious correlation. Variances in sequence divergence and date estimates for each point on the plot are difficult to gauge and, if large, would weaken the result. Nevertheless, the tight linearity of the plot (Fig. 3*A*) implies the existence of a predictable rate of mtDNA divergence in arthropods, at least among closely related taxa with low absolute amounts of sequence divergence. Encouragingly, this rate estimate is in quite close agreement with Brown *et al.*'s (38) original and oft-cited estimate for mtDNA divergence between primate lineages. The logarithmic transformation (Fig. 3*B*) is shown to emphasize the contribution of points with lower values and to allow finer resolution of estimates at the lower end of the scale.

Ages of Clades in *H. erato*. By assuming tentatively that the *H. erato* mtDNA is evolving neutrally, maximum uncorrected pairwise intra- and interclade sequence divergence can be plotted on Fig. 3. The corresponding age of the separation of the eastern and western clades is estimated to be 1.5–2 million years, coinciding with the beginning of the Pleistocene and with tectonic events in the northern Andes which may have formed the vicariant barriers observed today (33). Although the error associated with this estimate is unknown, its corroboration by the Andean orogeny (39) adds confidence in its accuracy. The maximum age of mitochondrial lineages within each clade is estimated to be 150,000–200,000 years (Fig. 3). Because overall sequence divergence within the eastern clade is so low, it is not possible to infer the existence of isolated forest refugia in the Amazon Basin from these data. The maximum apparent age of the eastern clade

Table 1. Seven independent observations of sequence divergence and age of splitting event (or mean rates based on such observations) among different groups of closely related arthropods

Taxon (order)	% sequence divergence	Estimated age, yr	Data type	Ref.
Drosophila buzzatii, Australian (Diptera)	0.00	300	Restriction sites	31
Magicicada septendecim A (Homoptera)	0.05	12,000	Restriction sites	32
Drosophila silvestris (Diptera)	0.3	<100,000	Restriction sites	33
Laupala spp. (Orthoptera)	1.4	700,000	12S and 16S rRNA sequence	*
Drosophila melanogaster subgroup (Diptera)	1.7 per 10^6 yr (rate estimate)		DNA·DNA hybridization	34
Tetraopes spp. (Coleoptera)	1.71 per 10^6 yr (rate estimate)		COI sequence	†
Alpheus spp. (Decapoda)	7.7	3,250,000	COI sequence	35

COI, cytochrome oxidase subunit I.
*K. Shaw, personal communication.
†B. Farrell, personal communication.

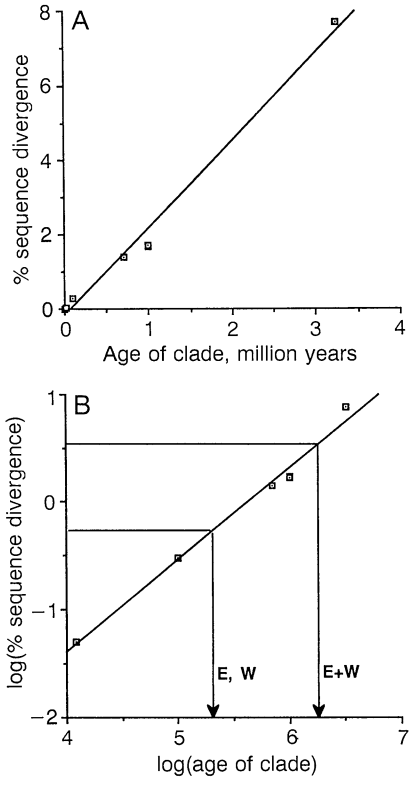

FIG. 3. Linear (*A*) and log–log (*B*) plots of divergence times and pairwise percent mtDNA sequence divergence for various arthropod taxa from data in Table 1. Slopes (y) and correlation coefficients (r^2) are as follows: for *A*, $y = -0.19241 + (2.3435 \times 10^{-6})x$ and $r^2 = 0.986$; for *B*, $y = -4.8129 + 0.85386x$ and $r^2 = 0.988$. Estimates of divergence times within (E, W) and between (E+W) the eastern and western *H. erato* clades are illustrated on the log–log plot (to enhance spread between lower values). Some points overlap one another.

implies that Amazonian populations were susceptible to vicariant events prior to the most recent glacial advance (21,000–13,000 years ago; ref. 39). However, the existence of shared mitochondrial haplotypes among phenotypically divergent allopatric races (Fig. 2) suggests that if isolation in refugia did occur during late Pleistocene cool periods, genetic bottlenecks were not extended or drastic enough to produce fixed genetic differences in neutral molecular markers. This inference is reinforced by data from allozymes in *Heliconius*, which also show a broad lack of geographical structure among races from Trinidad, Panama, and western Ecuador (40). Balancing selection could also explain widespread allozyme polymorphism but is an unlikely cause for the apparently neutral differences observed in the mtDNA data. These results strongly imply that the color pattern differences among races both east and west of the Andes (Fig. 2) have evolved very rapidly, in response to strong selection for mimicry or some other ecologically variable parameter.

Conclusion. The phenotypic diversity among *H. erato* races within Amazonia probably originated within the last 200,000 years, in the absence of prolonged genetic isolation between diverging populations. The mtDNA cladogram clearly shows that similar wing patterns have evolved independently several times within the species between the eastern and western clades (e.g., *petiverana–favorinus* and the two "*hydara*" races), and perhaps also among allopatric populations within the Amazonian clade (*favorinus–dignus*). Contrary to models hypothesizing parsimonious evolution of wing patterns (5, 7, 17), these data show that color patterns in *Heliconius* butterflies are labile and prone to intraspecific convergence. The data are consistent with, but do not directly support, the hypothesis of pattern evolution during short periods of allopatry produced by Pleistocene climate change (7, 16, 17).

I thank Su Borkin, Keith Brown, Jan Conn, Catherine Duckett, Lawrence Gilbert, Gerardo Lamas, James Mallet, Monica Medina, Carla Penz, and Robert Robbins for providing specimens and Charles Aquadro, Richard G. Harrison, James Liebherr, Amy McCune, Michael Nachman, Ben Normark, Kerry Shaw, Karen Sime, Carol Boggs, and an anonymous reviewer for helpful criticism and suggestions. This work was supported by the National Geographic Society and the National Science Foundation (Grant BSR-9111888 to A.V.Z.B. and R. G. Harrison) and by the Smithsonian Tropical Research Institute, Sigma Xi, the Harold E. Moore Fund, the Explorers' Club, and the Cornell Graduate School (to A.V.Z.B.).

1. Bates, H. W. (1862) *Trans. Linn. Soc.* **23,** 495–566.
2. Colinvaux, P. A. (1989) *Nature (London)* **340,** 188–189.
3. Capparella, A. P. (1990) *Proc. 20th Int. Ornithol. Congr.* 307–316.
4. Haffer, J. (1969) *Science* **165,** 131–137.
5. Turner, J. R. G. (1982) in *Biological Diversification in the Tropics*, ed. Prance, G. T. (Columbia Univ. Press, New York), pp. 309–335.
6. Endler, J. (1982) in *Biological Diversification in the Tropics*, ed. Prance, G. T. (Columbia Univ. Press, New York), pp. 641–657.
7. Sheppard, P. M., Turner, J. R. G., Brown, K. S., Benson, W. W. & Singer, M. C. (1985) *Philos. Trans. R. Soc. London B* **308,** 433–613.
8. Brown, K. S. (1982) *Am. Zool.* **22,** 453–471.
9. Prance, G. T., ed. (1982) *Biological Diversification in the Tropics* (Columbia Univ. Press, New York).
10. Cracraft, J. & Prum, R. O. (1988) *Evolution* **42,** 603–620.
11. Mayr, E. & O'Hara, R. J. (1986) *Evolution* **40,** 55–67.
12. Benson, W. W. (1972) *Science* **176,** 936–939.
13. Mallet, J. & Barton, N. H. (1989) *Evolution* **43,** 421–431.
14. Cracraft, J. (1989) in *Speciation and Its Consequences*, eds. Otte, D. & Endler, J. A. (Sinauer, Sunderland, MA), pp. 28–59.
15. Nixon, K. C. & Wheeler, Q. D. (1990) *Cladistics* **6,** 211–223.
16. Brown, K. S., Sheppard, P. M. & Turner, J. R. G. (1974) *Proc. R. Soc. London B* **187,** 369–378.
17. Turner, J. R. G. (1983) *Biol. J. Linn. Soc.* **20,** 277–300.
18. Harrison, R. G. (1989) *Trends Ecol. Evol.* **4,** 6–11.
19. Hackett, S. J. (1993) *Wilson Bull.* **105,** 301–315.
20. Farris, J. S. (1989) *Cladistics* **5,** 417–419.
21. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
22. Gyllenstein, U. B. & Erlich, H. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7652–7656.
23. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
24. Clary, D. O. & Wolstenholme, D. R. (1985) *J. Mol. Evol.* **22,** 252–271.
25. Crozier, R. H., Crozier, Y. C. & Mackinlay, A. G. (1989) *Mol. Biol. Evol.* **6,** 399–411.
26. Brower, A. V. Z. (1994) *Mol. Phylogen. Evol.* **3,** 159–174.
27. Brower, A. V. Z. (1994) Ph.D. dissertation (Cornell Univ., Ithaca, NY).
28. Swofford, D. L. (1989) *Phylogenetic Analysis Using Parsimony* (Ill. Nat. Hist. Surv., Champaign, IL), Version 3.0t.
29. Nijhout, H. F. (1991) *The Development and Evolution of Butterfly Wing Patterns* (Smithsonian Inst. Press, Washington, DC).
30. Mallet, J., Barton, N., Lamas M., G., Santisteban C., J., Muedas M., M. & Eeley, H. (1990) *Genetics* **124,** 921–936.
31. Halliburton, R. & Barker, J. S. F. (1993) *Mol. Biol. Evol.* **10,** 484–487.
32. Martin, A. & Simon, C. (1990) *Evolution* **44,** 1066–1080.
33. DeSalle, R. & Templeton, A. R. (1992) *J. Hered.* **83,** 211–216.
34. Caccone, A., Amato, G. D. & Powell, J. R. (1988) *Genetics* **118,** 671–683.
35. Knowlton, N., Weigt, L. A., Solórzano, L. A., Mills, D. K. & Bermingham, E. (1993) *Science* **260,** 1629–1632.
36. Lynch, M. & Jarrell, P. E. (1993) *Genetics* **135,** 1197–1208.
37. Kondo, R., Horai, S., Satta, Y. & Takahata, N. (1993) *J. Mol. Evol.* **36,** 517–531.
38. Brown, W. M., George, M. & Wilson, A. C. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1967–1971.
39. Van der Hammen, T. (1974) *J. Biogeogr.* **1,** 3–26.
40. Turner, J. R. G., Johnson, M. S. & Eanes, W. F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1924–1928.