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Evolution

Contrasted modes of evolution in the same genome: Allozymes and adaptive change in *Heliconius*

(Lepidoptera/mimicry/heterozygosity/control genes/neutral evolution)

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ABSTRACT **Butterflies in the South American genus *Heliconius* have undergone a spectacular adaptive radiation (with convergent evolution between some lines) in their color patterns; this has been produced by natural selection for muellerian mimicry. The genetic basis of this radiation, shown by crossing highly differentiated races within two of the species, is homozygosity for alternative alleles at some half dozen loci. In complete contrast, allozyme loci in these butterflies are strongly heterozygous and show only frequency differences (never amounting to homozygosity of alternative alleles) between races; the amount of allozyme divergence is the same between races of *H. erato* and *H. sara*, although in color pattern the first forms marked races and the other does not. For the allozymes, there is a strong correlation over loci for rate of divergence between species and average heterozygosity. This is not true of the genes controlling color pattern. Heterozygosity of the enzymes is correlated with subunit molecular weight. Thus, different parts of the genome can evolve in different ways simultaneously; genes controlling color pattern in the "classical" mode, and allozymes in a different mode in which the rate of evolution is related to their heterozygosity (a "balance" or "neutral" mode).**

What is the link between the processes of adaptive evolution studied by population geneticists in the field and long-term evolution of the kind revealed by molecular biology? Allozymes, molecular variants that can be studied in natural populations, despite providing a better sample of the genome than anything available previously, have not provided a clear answer, for their adaptive significance remains controversial and their relationship to adaptive evolution in doubt (1). Indeed, several recent studies have shown a high degree of independence of molecular evolution and morphological evolution (e.g., see refs. 2–6) and of molecular evolution and speciation (7–9).

In none of these studies do we know both the adaptive significance of the morphological evolution and its genetic basis: this makes it difficult to compare directly with evolution at the molecular level. Only by knowing the genetic architecture of a proven adaptive radiation can we find the connection, if any, between this process and the evolution of allozymes.

Such information is provided by the evolution of mimicry in butterflies. The muellerian mimicry complexes in the neotropical genus *Heliconius* provide one of the most thoroughly understood of adaptive radiations. Within subdivisions of the genus (defined by structural morphology and probably corresponding with phyletic lines) there has been an extensive and spectacular radiation of the color patterns of the butterflies so that, superficially, two closely related species may bear very little resemblance to one another; at the same time two butterflies in different subdivisions, with very different morphologies and life histories, may be so similar in color pattern that even a specialist has difficulty discriminating them (compare the top two rows of Fig. 1). Thus the radiation within lines is accompanied, as are the classical adaptive radiations familiar to all zoologists, by convergent evolution between the radiating lines (11, 12).

We are fortunate in understanding something both of the function and genetics of this adaptive radiation. Its central feature, the extreme convergence of unrelated forms, has been produced by selection for muellerian mimicry (that is, mimicry between species which are all protected by bright colors and nauseous qualities) (13). Laboratory experiments have shown that the butterflies are distasteful to insectivorous and omnivorous birds (14, 15) and that, not surprisingly in view of the difficulty it causes entomologists, the mimicry is effective against the birds, training with one species of butterfly causing the birds to avoid a mimic (14). The butterflies are attacked by birds in the wild (12, 16).

Because divergence of the pattern can occur (sometimes to a very extreme degree) between the races of a single species, the genetic basis of the adaptive radiation can be found: races of the two thoroughly investigated species, *H. erato* and *H. melpomene* (Fig. 1), differ by up to seven genetic loci with large detectable effects on the color pattern (Table 1) (refs. 17–19; P. M. Sheppard, J. R. G. Turner, K. S. Brown, W. Benson, and M. C. Singer, unpublished data). As is to be expected from the prediction that a muellerian mimic is strongly selected to repeat an identical signal to its predators, the races are homozygous for alternative alleles at these loci. Thus, the butterflies show an adaptive radiation of known function, which has been produced by evolution in what is now known as the "classical" mode (1), switching from homozygosity for one set of alleles to homozygosity for another (12).

The striking adaptive radiation of races in *Heliconius* raises several questions regarding genomic evolution: (*i*) Is the sharp phenotypic differentiation of the geographical races a reflection of similar sharp racial differences in the genome as a whole? (*ii*) Is the whole genome of these butterflies undergoing classical evolution? (*iii*) Do species of *Heliconius* that do not form mimetic races differ from those that do, in their pattern of allozyme variation and divergence? To answer these questions, we have studied allozyme variation in several species of *Heliconius*, including species that do form mimetic races and species with only slightly differentiated races.

The cause of the extreme divergence between the races of some species is in itself a question of great interest, but beyond the scope of the present paper; the matter will be fully discussed elsewhere and has been reviewed by Turner (12, 20, 21).

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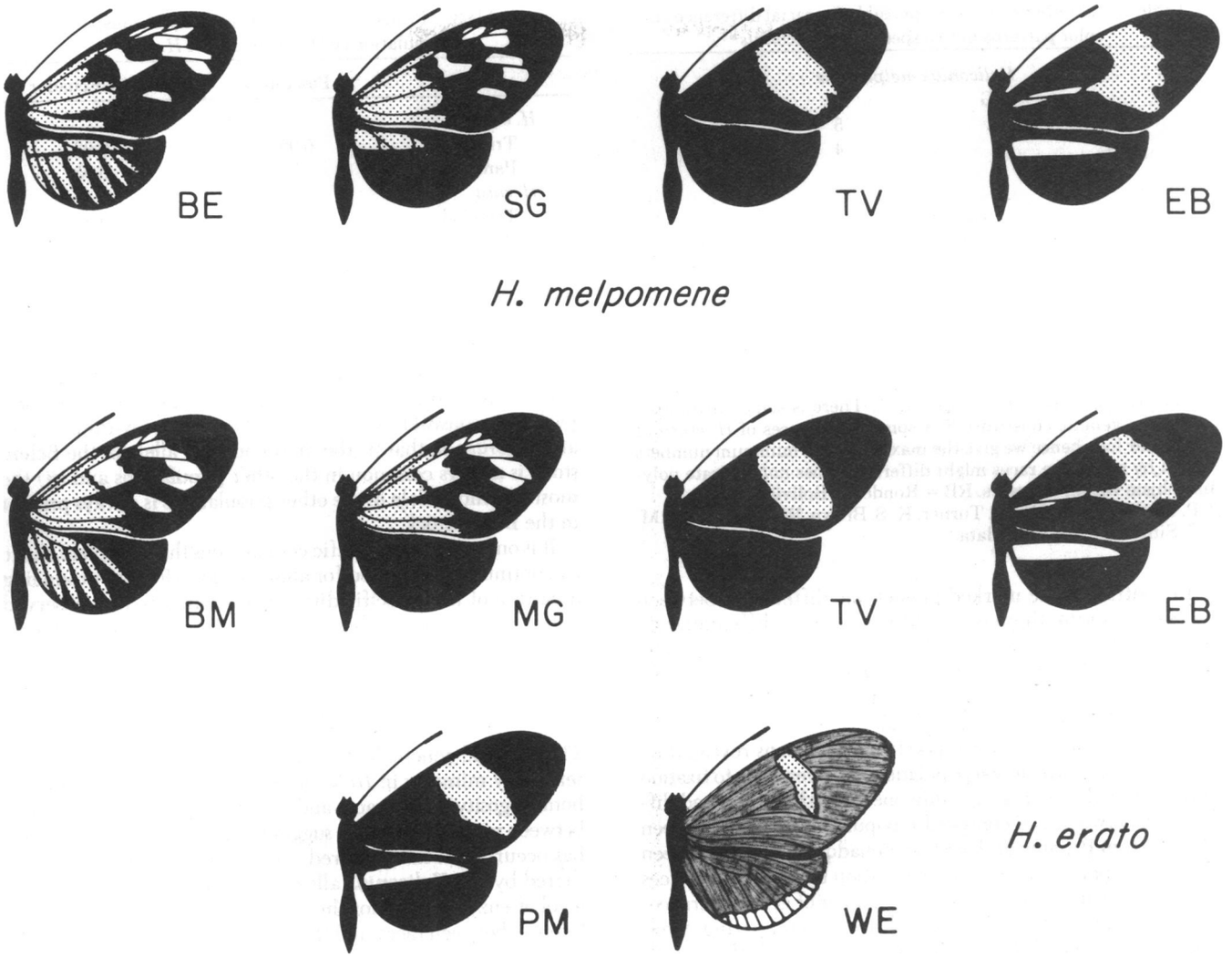

FIG. 1. Some of the races of *H. melpomene* (*Upper*) and *H. erato* (*Middle* and *Lower*). Differentiation between races within *H. erato* and within *H. melpomene* is so extreme that all but the most recent systematic works divide them into several species. Conspecificity of the races is proved not only by hybridization in the insectary, but by the occurrence of natural hybrid populations at all the known meeting places of the races. They are *H. melpomene:* BE, Belém; SG, Suriname (Guiana); TV, Trinidad/Venezuela; EB, East Brazil; *H. erato:* BM, Belém/Mato Grosso; MG, Manaus/Guiana; TV, Trinidad/Venezuela; EB, East Brazil; PM, Panama/Mexico; WE, West Ecuador. Color code: black = black; stippled = red; white = white (W. Ecuador) or yellow (others); striated = iridescent blue. For a color illustration see ref. 10.

MATERIALS AND METHODS

Samples of eight species of *Heliconius* and the related genus *Dryas* were assayed for genetic variation at 17 allozyme loci by means of horizontal electrophoresis in gels of 12% Electrostarch (Otto Hiller, Madison, WI). The enzymes assayed and the buffer systems used are listed in Table 2. Assay conditions were essentially those of Brewer (24) and Nichols *et al.* (25).

Variation within populations was expressed as the proportion of heterozygotes observed. Because *6Pgd* is sex linked, only males (the homogametic sex in these butterflies) were used to estimate heterozygosity for this locus. Comparisons between populations were expressed as values of *I*, the normalized probability of genetic identity (26). *Est* was excluded from the interpopulation comparisons because we had insufficient material to allow verification of shared electrophoretic mobility among the large number of allozymes at this locus.

The species sampled and the geographic source of the samples were *H. aliphera* (Panama and eastern Ecuador); *H. atthis* (western Ecuador); *H. erato* (Panama, Trinidad, and western Ecuador); *H. melpomene* (Panama, Trinidad, and western and eastern Ecuador); *H. numata* (Venezuela and eastern Ecuador); *H. sara* (Panama, Trinidad, and western Ecuador); *H. clysonymus* (western Ecuador); and *Dryas iulia* (Panama, Trinidad, and western and eastern Ecuador). These butterflies are seldom abundant, and some are hard to catch; to obtain a minimum sample of 20 genomes per locus, all conspecific specimens were treated as a single sample, with the exception of *H. erato* and *H. sara*. The parallel samples of these two species from Panama, Trinidad, and western Ecuador provide a direct comparison of a species that forms strongly differentiated races of adult mimics (*H. erato*) and a species (*H. sara*) in which there is only slight racial divergence (minor changes in the shape of the yellow mark, addition of a thin white border to the hindwings).

RESULTS

Heliconiine allozymes are highly variable: within species, 47–76% of the loci studied are polymorphic, and the average heterozygosity per individual ranges from 9–24%. Over all 17 loci, the average heterozygosities ±SEM are: *H. erato*, 0.24 ± 0.05; *H. melpomene*, 0.15 ± 0.04; *H. aliphera*, 0.11 ± 0.04; *H. atthis*, 0.17 ± 0.04; *H. clysonymus*, 0.20 ± 0.06; *H. numata*, 0.12 ± 0.03; *H. sara*, 0.09 ± 0.03; and *D. iulia*, 0.14 ± 0.03.

Table 1. Number of genes responsible for racial differences in color patterns in two species of *Heliconius**

	Heliconius melpomene			
	SG	TV	EB	
BE	2	5	5	
SG		4	4	
TV			2	
	Heliconius erato			
	MG	RB	PM	TV	EB
BM	1–4	1–2	3–7	2–6	4–6
MG		3–4	4–6	3–5	5–7
RB			4–6	2–6	3–7
PM				1–2	3
TV					4

For designations of races, see Fig. 1. There is some uncertainty about the genetic constitution of some of the races of *H. erato* at particular loci; hence we give the maximum and minimum numbers of loci by which the races might differ; this does *not* indicate polymorphism within the races. RB = Rondônia/Bolivia.

* P. M. Sheppard, J. R. G. Turner, K. S. Brown, W. Benson, and M. C. Singer, unpublished data.

In contrast to the marked phenotypic differences between races of *H. erato*, there is very little allozymic differentiation between the three races of this species represented in our samples. In addition, the amount of interpopulation differentiation in *H. sara*, which does not form marked adult races, is similar to that in *H. erato* (Table 3).

More strikingly, the intraspecific comparisons revealed no allozyme loci at which two populations are even close to fixation of different alleles (Fig. 2). Only one locus shows marked differences between two conspecific populations: *Pep-1* between *H. sara* from Panama and western Ecuador. We have not been able to compare allozyme differentiation between those races of *H. erato* which show the greatest number of gene differences for their color patterns: this would involve comparing Amazonian races (BM, MG, and RB in Table 1) with those from outside the Amazon Basin (PM, TV, and EB). However, we have examined the standard laboratory stock of the Belém race (BE) of *H. melpomene*, which, despite passing through a severe foundation bottleneck and several other bottlenecks since, is still heterozygous for many of the loci examined. The comparison is consistent with those between races within *H. sara* and *H. erato* in that (*i*) the most common allele in the Belém stock is always common in the other populations and (*ii*) the most common allele in the other populations is always present in the Belém stock.

Table 2. Enzymes examined in *Heliconius* butterflies

Enzyme	Locus	Buffer*	Heterozygosity†
Aldolase	*Ald-1*	TC	0.03
Esterase	*Est*	LiOH	0.28
Glutamate oxaloacetate transaminase-1	*Got-1*	TEB	0.17
Glutamate oxaloacetate transaminase-2	*Got-2*	TEB	0.04
α-Glycerophosphate dehydrogenase	*αGpd*	TM	0.05
Isocitrate dehydrogenase	*Idh*	TM	0.10
Malic dehydrogenase-1	*Mdh-1*	TC	0.04
Malic dehydrogenase-2	*Mdh-2*	TC	0.001
Malic enzyme	*Me*	TEB	0.14
Mannose-phosphate isomerase	*Mpi*	TEB	0.37
Peptidase-1	*Pep-1*	LiOH	0.27
Peptidase-2	*Pep-2*	LiOH	0.20
6-Phosphogluconate dehydrogenase	*6Pgd*	TC	0.12
Phosphoglucose isomerase	*Pgi*	TM	0.27
Phosphoglucomutase	*Pgm*	TM	0.39
Tetrazolium oxidase-1	*To-1*	TEB	0.05
Tetrazolium oxidase-2	*To-2*	TEB	0.03

* LiOH = buffer 2, TEB = buffer 6, and TM = buffer 9 of Selander *et al.* (22). TC = buffer described by Whitt (23).

† Average of eight species.

Table 3. Probability of genetic identity of conspecific populations of *H. erato* and of *H. sara*

	Panama	West Ecuador
H. erato		
Trinidad	0.99	0.93
Panama		0.95
H. sara		
Trinidad	0.97	0.98
Panama		0.96

It is only with interspecific comparisons that complete allelic discontinuity is observed for allozyme loci (Fig. 2), producing a pattern of interspecific differences similar to that observed in several previous studies (e.g., refs. 27 and 28) and a pattern of genetic distances (Table 4) that corresponds closely with the conventional taxonomic arrangement of this group (11).

DISCUSSION

The essential genetic features of the adaptive radiation represented by mimicry in *Heliconius* are (*i*) monomorphism and homozygosity within races and (*ii*) sharp genetic discontinuity between races. This pattern suggests that when gene substitution has occurred, it has occurred rapidly. These features are not shared by the *Heliconius* allozymes, as indicated by the high level of enzyme polymorphism, the low level of allozyme differences between races, and the absence of genetic discontinuity between races.

The allozymes and the genes underlying mimicry also differ in the relationship between variability within populations and divergence between populations. A repeatedly made observation is that those characters that are highly variable within populations tend also to be highly divergent between populations (29–32). This correlation between evolutionary rate and intrapopulation variability has recently been shown to hold for allozymes (32).

The same relationship can be seen for Heliconiine allozymes. At each locus, the amount of divergent evolution among the species studied is measured by the range of allelic frequencies observed at that locus: $R = \Sigma(P_{i\max} - P_{i\min})$, in which $P_{i\max}$ is the maximum frequency of allele i observed in any popula-

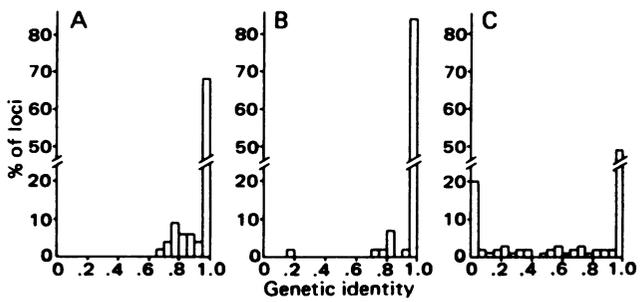

FIG. 2. Frequency distribution of genetic identity of loci in comparisons between (*A*) populations of *H. erato*, (*B*) populations of *H. sara*, and (*C*) species of *Heliconius*.

Table 4. Probability of genetic identity between species of *Heliconius* and *Dryas*, based on comparisons for 16 enzymes

	Heliconius						
	att-his	*num-ata*	*sara*	*era-to*	*cly-son-ymus*	*ali-phe-ra*	*Dryas iulia*
H. melpomene	0.82	0.84	0.72	0.62	0.71	0.61	0.53
H. atthis		0.87	0.69	0.69	0.74	0.61	0.53
H. numata			0.78	0.63	0.65	0.62	0.59
H. sara				0.74	0.55	0.64	0.49
H. erato					0.75	0.55	0.55
H. clysonymus						0.61	0.56
H. aliphera							0.64

tion, and $P_{i\min}$ is the minimum observed frequency of allele i (32). R is the minimal amount of change in allelic frequencies that must have occurred at a locus in the course of evolution within the group of species studied. For the Heliconiine allozymes, evolutionary rate, as measured by R, is highly associated with average heterozygosity (Fig. 3).

This association of evolutionary rate with variability may reflect a process of continuous divergence, the rate of which is correlated with the rate of mutation at each locus. In a study of *Drosophila* enzymes, a positive correlation was found between heterozygosity and subunit molecular weight (33, 34). Although we do not have data on subunit molecular weight of Heliconiine enzymes, such data are available for several of the same enzymes in humans (35). Given the likely conservatism of subunit molecular weight for a given enzyme in comparison with the large differences in subunit molecular weight between enzymes, the values from humans are probably reasonable indicators of the values for the Heliconiine butterflies. Using these values, there is a clear correlation between subunit molecular weight and the heterozygosity of the butterfly allozymes (Fig. 4). Since the larger the molecule the greater the number of mutable sites, it is a reasonable hypothesis that mutation rate is a major determinant of both variability and evolutionary rates of allozymes.

This hypothesis is strengthened by the fact that the two

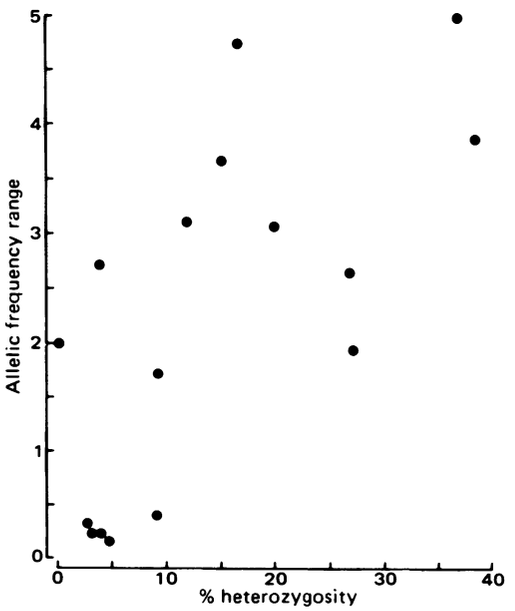

FIG. 3. Association of evolutionary divergence (allele frequency range) and average heterozygosity for 16 loci in *Heliconius* and *Dryas*. The Spearman's rank correlation, r_s, is 0.70.

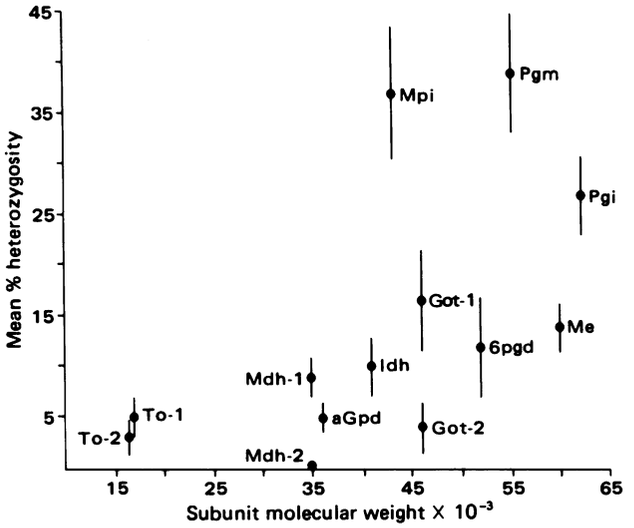

FIG. 4. Association of average heterozygosity (in *Heliconius* and *Dryas*) and subunit molecular weight (from human enzymes) (35) for 13 enzymes. Homology of Heliconiine and human enzymes cannot be assured for *Est* and *Pep* because of nonspecificity of enzyme assay, nor for *Ald* because of questions of quaternary structure. Vertical bars are SEM; $r_s = 0.71$.

monomeric enzymes (*Mpi* and *Pgm*) are more variable and the one tetrameric enzyme (*Me*) less variable than predicted from subunit molecular weight alone (Fig. 4). Interactions between subunits of multimeric enzymes should impose constraints on amino acid substitutions, thereby reducing the proportion of permissible mutations. Consequently, monomeric enzymes, free from such constraints, tend to be more variable than multimeric enzymes (36). The proportion of amino acid residues tied up in surface interactions has been estimated as 14 and 28% in dimers and tetramers, respectively (34). If the rate of selectively acceptable mutation is the prime determinant of variability, reduction of subunit weights by 14% for dimers and 28% for tetramers should improve the correlation of heterozygosity with molecular weight. For the Heliconiine enzymes, such adjustment increases the Spearman rank correlation from 0.71 to 0.83, removing the apparent discrepancy between monomers and dimers.

This close association of variability, evolutionary rate, and mutability indicates that Heliconiine allozymes are diverging continuously and gradually enough for heterozygosity to be maintained. This is not the mode of evolution for the genes affecting mimicry. These genes clearly do not conform with the general association of variability and evolutionary rate: they have diverged considerably, but are generally homozygous within populations.

In short, the mimicry genes and the allozymes represent entirely different modes of evolution within the same genome: we can see clearly the co-occurrence in one genome of classical evolution with evolution of the neutral or balance type (1).

There are two ways in which such contrasted modes of evolution could coexist within the genome: the contrast might be between different classes of alleles or between different kinds of loci. First, all loci could be evolving in the same way, and the mode of evolution might depend on our methods for detecting genetic variation or on the functional level at which we examine it. Thus, we may perceive the color pattern genes as homozygous only because we cannot see molecular variation by looking at the wings; the neutral hypothesis would lead us to expect high levels of heterozygosity within the major allelic classes defined by the effects of the gene on the color pattern. Some versions of the balance hypothesis might do so as well. However, even

if molecular studies of the color pattern loci did reveal such cryptic heterozygosity, these loci would still differ radically from the allozyme loci in that the races would still be seen to be sharply differentiated, with no alleles from one race occurring in the other. On the other hand, it might be that the allozyme loci, if examined with more sophisticated methods (37), would turn out to have cryptic alleles within the mobility classes that we have defined, some or most of which were confined to particular races. If both these conditions were fulfilled, then there could be one mode of evolution at the genetic level, with a combination of high heterozygosity and marked racial differentiation at all loci. In this case, some classes of alleles would be evolving in the classical mode and other allelic classes in the balanced or neutral mode.

Second, it may be that the loci coding for allozymic variation and the control of color pattern really are evolving in quite different ways, possibly being fundamentally different kinds of genetic variation. The allozymes are apparently mutants of structural loci; in view of the fact that the changes in color pattern involve alterations in the distribution of pigment but never the total absence of any pigment, it may well be that these are mediated by control genes, such as have been shown by other workers to produce important adaptive changes in both the laboratory (38) and the field (39, 40), or by genes controlling developmental rates (which may or may not be control genes in the stricter biochemical sense), as has been postulated as an explanation for the high morphological divergence between people and chimpanzees (41).

A few loci have sufficed to produce a visually striking adaptive radiation between the races of two of our *Heliconius* species, and this divergence, as has been suggested by other studies (3–5, 9, 42), does not reflect pervasive genetic change in the genome. Although in some instances sets of genes are known to vary concordantly (e.g., ref. 43), some of the spectacular "events" of evolution may have involved only a small portion of the genome. While one set of genes is evolving in one mode, another set may be evolving in another: we cannot necessarily generalize about evolution at the genetic level from studying a sample of only one kind of gene.

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