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Speciation, Raciation, and Color Pattern Evolution in *Heliconius* Butterflies: Evidence from Hybrid Zones

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Hybrid zones often involve either morphological traits such as color patterns of vertebrates with poorly understood genetics or genetic traits such as chromosomes, allozymes, and mitochondrial DNA, which tell us little about selection. In neotropical *Heliconius* butterflies, hybrid zones for warning, mimetic wing patterns are known in which both genetics and selection can be comprehended. While learning to avoid unpalatable prey, predators cause frequency-dependent selection against rare color-pattern morphs. Good evidence for this evolutionary constraint on color pattern change comes from both sympatric Müllerian mimicry between *Heliconius* butterflies and narrow hybrid zones between color pattern races within *Heliconius* species. Given selection against rare morphs (which occurs even though a new morph might be advantageous if common), it is difficult to explain the rampant geographic variation we see in *Heliconius* color patterns (Fig. 9-1). Once divergence *has* occurred, it is preserved by local selective pressures, even in parapatry, but the explanation of the initial divergence remains elusive.

It has been generally accepted that *Heliconius* races differentiated in allopatric Pleistocene refugia and that differentiation was in response to divergent mimetic pressures within each refugium (Turner, 1965, 1971a; Brown et al., 1974; Sheppard et al., 1985; Brown, 1987a). In this chapter, it is argued that allopatry is not necessary for divergence, and that de novo warning color evolution must have been partially responsible for raciation in *Heliconius.* I develop hypotheses of divergence and test them against data from hybrid zones and from distribution patterns of the races and species of *Heliconius,* which they separate. Little evidence is found for the importance of refugia in divergence once one admits that a null hypothesis of allopatric divergence is invalid on current theoretical grounds. The implications of these studies for those of other systems of hybrid zones and refugia are discussed. Conservation strategies that employ refugium theory as a means of choosing conservation areas should be urgently reexamined; it would be better to conserve areas that house particularly endangered species or areas with high levels of species diversity than to trust a poorly supported evolutionary theory as a guide.

HELICONIINE BUTTERFLIES AND THEIR EVOLUTION

Heliconius butterflies are a characteristic element of the neotropical biota from Texas and Florida to Argentina. The color patterns of *Heliconius* are famous for their warning colors (aposematism) and mimicry, indeed the "Heliconiidae" formed part of the basis for Bates' (1862) original theory of mimicry[1]. Bates might have dismissed as a coincidence similarities of a few butterflies in any one area, but his extensive collections across many regions of the Amazon basin revealed a striking geographic pattern. First, there were often 10 or more species in each "mimicry ring" within any area. Second, these color patterns changed regionally (see, for example, Fig. 9-1): "In tropical South America a numerous series of gaily-colored butterflies and moths, of very different families, which occur in abundance in almost every locality a naturalist may visit, are found all to change their hues and markings together, as if by the touch of an enchanter's wand, at every few hundred miles" (Bates, 1879).

Bates argued that rare unprotected butterflies such as dismorphiines came to imitate protected Ithomiinae because of selection by predators against unprotected, rare species. Bates recognized that *Heliconius* were protected because they were themselves often the objects of mimicry (Bates, 1862, p. 510), but he also argued that rare protected heliconiines such as the "silvaniform" *Heliconius* (Eltringham, 1916; Brown, 1976a) and ithomiines such as *Napeogenes* might gain an advantage by mimicking commoner protected ithomiines such as *Melinaea* (Bates, 1862, pp. 507, 549–550). Essentially, Bates proposed an early version of "Müllerian" mimicry 17 years before Müller; Müller's (1879) major advance was to clarify and generalize the argument to any pair of protected species with arbitrary relative abundance. Bates (1879) was unimpressed by Müller's explanation of sympatric mimicry between protected species, perhaps in part because Müller's theory did not explain how mimicry rings diverged geographically. Few people now read Bates' original papers, and it is not generally realized that Darwin (1863), Wallace (1865), and Bates himself thought that the evolution of mimicry was a highly convincing case of geographic speciation by natural selection, rather than just another example of trivial microevolution. Following Wilson and Brown (1953) and Mayr (1963, 1970), many biologists view species as qualitatively different taxa from races and subspecies, and they dismiss racial variation such as that in *Heliconius* as unimportant for speciation (e.g., Grimaldi, 1984). In this chapter I use theory and data from *Heliconius* to revive the idea that mimetic races have much to say about the nature and biogeography of speciation.

Heliconius and allies (e.g., *Eueides, Dryas,* and *Agraulis*—hereafter heliconiines) are closely related to the holarctic fritillaries *Argynnis, Speyeria,* and *Boloria* according to detailed morphological work by Harvey (1991). The caterpillars of heliconiines are almost completely restricted to Passifloraceae (Benson et al., 1976; Brown, 1981). These plants have many secondary chemicals, but is is still not clear to what extent the

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[1] Bates (1862) included the Ithomiinae as "danaiform Heliconiidae" within his "Heliconiidae" and referred to the butterflies we now term Heliconiini (Brown, 1981) or Heliconiiti (Harvey, 1991) as "acraeiform Heliconiidae," although he clearly understood that these groups were unrelated. Bates also used the name "Leptalidae" for what we now term Dismorphiinae (Pieridae).

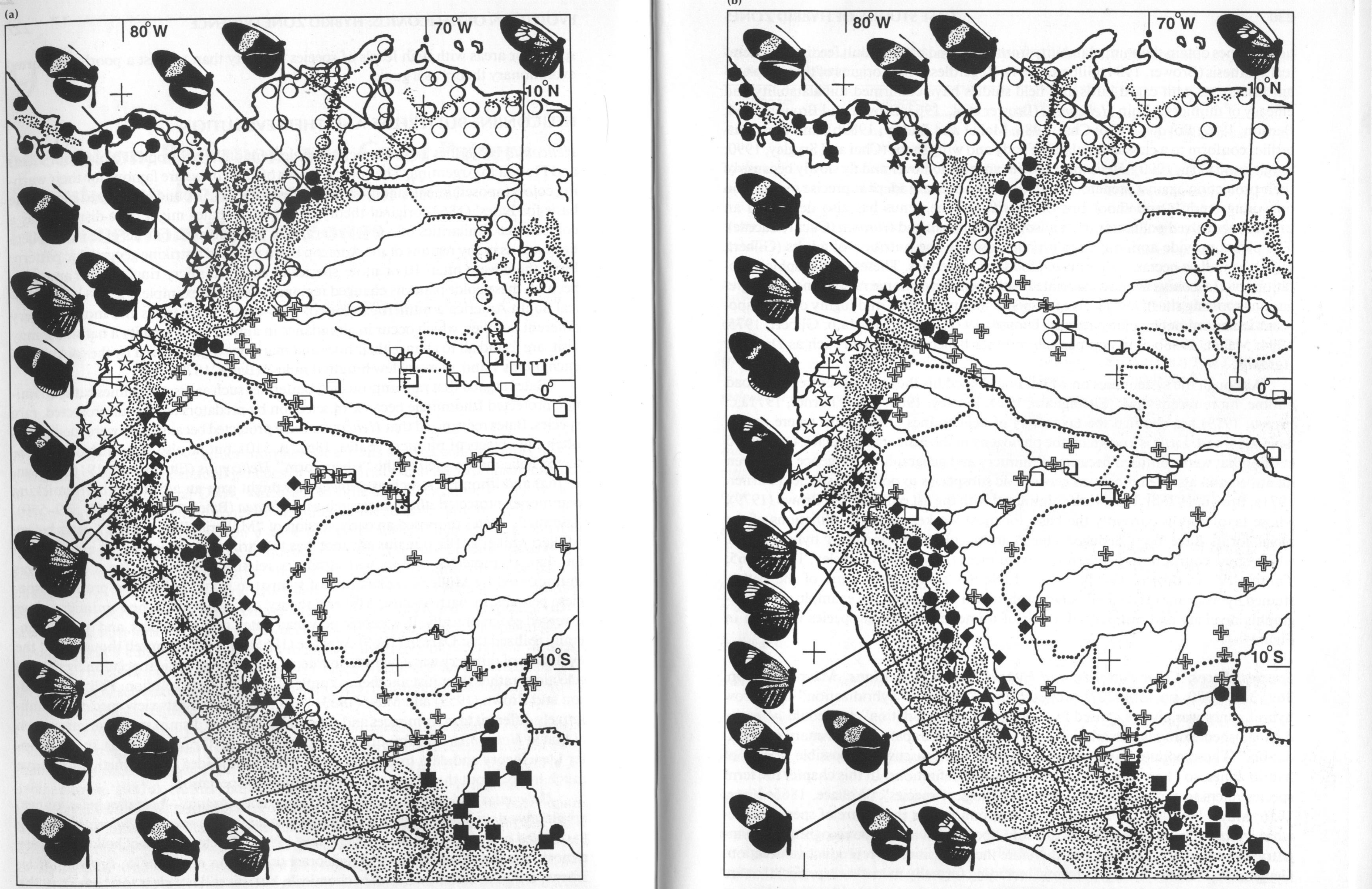

Figure 9-1. Distribution of mimetic color pattern races of *Heliconius* in Andean South America. (A) *Heliconius himera* (* symbols) and *H. erato* (all other symbols). (B) *Heliconius melpomene.* Land higher than about 1500 meters above sea level is stippled. Colors of butterflies: stippled = red; white = yellow; black = dark melanin. In *H. erato venus, H. melpomene vulcanus, H. e. cyrbia,* and *H. m. cythera* (filled and empty stars on west coast of Ecuador and Colombia) the dark area of the forewing has a strong blue iridescence, there is a yellow bar on the underside of the hindwing, and the pale fringe on the hindwing is white. Hybrid zones are known between most parapatric races, although hybrids are rare between *H. himera* and adjacent races of *H. erato* (see also Tables 9-1 and 9-3). (Redrawn from Brown, 1979, and Sheppard et al., 1985, with some modifications based on the author's work.)

heliconiines obtain their unpalatability from their food plants, adult feeding, or chemical synthesis (Brower, 1984; Gilbert, 1991). Regardless of the origins of their unpleasant taste, tests with caged birds and field studies have confirmed unpalatability and efficacy of mimicry within *Heliconius* (Brower et al., 1963; Brower and Brower, 1964; Benson, 1972; Boyden, 1976; Chai, 1986; Mallet and Barton, 1989b), and these butterflies conform to a classic unpalatable body and wing shape (Chai and Srygley, 1990; Srygley and Chai, 1990). *Heliconius* species are able to hover and fly slowly because of their protection against predators, and they have become adept at precise oviposition on young tendrils and shoot tips of *Passiflora.* The genus has also developed an unusual coevolved pollinator mutualism with *Psiguria* and *Gurania* (Cucurbitaceae). The plants provide amino-acid-rich pollen used in the nutrition of adults (Gilbert, 1975), as well as nectar, a more usual food for butterflies. These novel trophic adaptations of *Heliconius* are also associated with memorized home ranges (including gregarious roosting sites), low levels of dispersal and migration, and highly derived aposematic and mimetic color patterns (Benson, 1971; Turner, 1971a,b; Gilbert, 1975, 1984; Mallet, 1986b,c) in comparison to other heliconiine genera such as *Dryas* or *Agraulis.*

Although the systematics on which Bates based his theories was correct in broad outline, more recent work (Eltringham, 1916; Emsley, 1964, 1965; Turner, 1971a,c; Brown, 1979) has clarified the taxonomy of heliconiines to the point where names have stabilized. Little is known on the phylogeny of the heliconiine species, but named entities that were confused because of mimicry and geographic divergence have been identified and assigned mostly as geographic subspecies to polytypic species (Turner, 1971a; Brown, 1976b). This work culminated with the list published by Brown (1979), whose taxonomy is currently the basis for most workers. Species affiliations of the racial forms have been deduced chiefly from collections made in hybrid zones, which show connections between divergent races (Kaye, 1916; Emsley, 1964, 1965; Turner, 1971c; Brown, 1976b, 1979). Table 9-1 summarizes some of the better-studied hybrid zones and clinal polymorphisms in *Heliconius.* An example of the geographic diversity and mimicry of a pair of mimetic heliconiine species is shown in Figure 9-1.

Species, Races, Color Patterns, and Hybrid Zones: Definitions. Mayr (1963, pp. 369, 381; 1970, pp. 214–227) proposed that "allopatric hybridization" in narrow hybrid zones was always caused by "secondary intergradation" and could be easily distinguished from clinal variation that evolved in parapatry by "primary intergradation." These connotations make it impossible to discuss the possible origins of hybrid zones, so I here attempt to use theory-free definitions. In this chapter the term species refers to reproductively isolated "biological species" (Wallace, 1865; Kaye, 1916; Mayr, 1963, 1970). Though this definition makes the nature of species somewhat arbitrary in allopatry (Wallace, 1865), species can always be recognized in sympatry and parapatry. Speciation is therefore the acquisition of reproductive isolation. Geographic races are divergent forms (including formally named subspecies) that are not reproductively isolated but that are relatively constant over wide areas; they are connected to other races by hybrid zones or clines that are narrow relative to the distributions of the races they connect. Clines are more or less continuous geographic changes in the frequency of alternative forms of a single gene, chromosome, or char-

Table 9-1. Large-sample Field Studies of Hybrid Zones and Polymorphisms in *Heliconius* Known to the Author

Species[a]	Subspecies[a]	Place	Reference
doris	various (usually polymorphic)	Neotropics	Sheppard (1963); Turner (1971a)
ethilla	*ethilla* (polymorphism)	Trinidad	Sheppard (1963); Turner (1968)
numata	all (often polymorphic)	Amazon basin	Brown and Benson (1974); Brown (1976a)
cydno	*galanthus* × *pachinus*	Costa Rica	L. Gilbert (unpublished)
cydno	*weymeri* × *gustavi* (× others)	Colombia (Cauca valley)	M. Linares (unpublished)
telesiphe	*telesiphe* × *sotericus* × *cretacea*	Andes of Peru and Ecuador	Vane-Wright et al. (1975); J. Mallet (unpublished)
erato	*erato* × *hydara*	Surinam, French Guiana	Sheppard (1963); Benson (1982)
erato	*hydara* × *amalfreda*	Brazil (Amazon), S. Guyana	Benson (1982); P. & J. Mallet (unpublished)
melpomene	*melpomene* × *meriana* × *thelxiopeia*	Surinam, French Guiana, Guyana	Sheppard (1963); Turner (1971c); P. & J. Mallet (unpublished)
erato	various	Neotropics	Emsley (1964)
melpomene	various	Neotropics	Emsley (1964)
erato	*amazona* × *venustus*	Brazil (Riozinho/ Mato Grosso)	Brown and Mielke (1972)
erato	*notabilis* × *lativitta*	E. Ecuador	P. Brakefield (unpublished); K. Brown (unpublished); J. Mallet (unpublished)
melpomene	*plesseni* × *aglaope*	E. Ecuador	
erato	*petiverana* × *hydara* × *venus*	Panama	Mallet (1986a)
melpomene	*rosina* × *melpomene* × *vulcanus*	Panama	Mallet (1986a)
erato	*favorinus* × *emma*	N. Peru (Huallaga)	Lamas (1976); Mallet (1989); Mallet et al. (1990)
melpomene	*amaryllis* × *cognata*	N. Peru (Huallaga)	Lamas (1976); Mallet (1989); Mallet et al. (1990)
erato	*dignus* × *lativitta*	S. Colombia (Putumayo)	Mallet (this chapter)
melpomene	*bellula* × *aglaope*	S. Colombia (Putumayo)	Mallet (this chapter)
erato	*microclea* × *emma*	C. Peru (Río Palcazu)	J. Mallet (unpublished)
melpomene	*xenoclea* × *cognata*	C. Peru (Río Palcazu)	J. Mallet (unpublished)
erato	*phyllis* (polymorphism)	S.E. Brazil	Pansera and Araújo (1983)
erato	*phyllis* × various	S.E. Brazil	K. Brown (unpublished)

Table 9-1. Large-sample Field Studies of Hybrid Zones and Polymorphisms in *Heliconius* Known to the Author (*Continued*)

Species[a]	Subspecies[a]	Place	Reference
himera × *erato*	*cyrbia*	S. Ecuador	Descimon and Mast de Maeght (1984); J. Mallet (unpublished); Brown (unpublished)
himera × *erato*	*favorinus*	N. Peru (Mayo valley)	König (1986); J. Mallet (unpublished)
himera × *erato*	*lativitta*	N. Peru (Río Marañon)	Mallet (this chapter)
hecalesia	*hecalesia* × *ernestus* × *longarena*	W. Colombia	Brown and Benson (1975)
sapho	*sapho* × *chocoensis*	W. Colombia	Brown and Benson (1975)

[a] Nomenclature used is that of Brown (1979) rather than necessarily that of original authors.

acter; and hybrid zones are then narrow clines, or, more usually, clusters of narrow clines that connect races or incipient species together.

"Warning color," "aposematism," and "mimicry" also include evolutionary hypotheses within terms for phenomena, but in this case the hypotheses seem generally accepted. By warning color or aposematism, I mean colors that seem adapted for reminding predators of previous unpleasant experiences. By mimicry I mean a color pattern that is too similar to the pattern of an unrelated species to be explained by chance and that is not due to common ancestry; the pattern of the mimetic species must have converged on a warning pattern in the model species.

POPULATION STRUCTURE AND COLOR PATTERN EVOLUTION

Theory of Warning Colors in a Single Deme. Novel warningly colored morphs in cryptically colored unpalatable species are selected against for two reasons: (1) They are more visible to predators; and (2) predators lack experience with these new morphs (reviewed by Mallet and Singer, 1987). The first effect places the more conspicuous morph at a constantly greater risk of predation. The second effect is more interesting, as predator learning causes frequency-dependent selection against rarity, as shown below. The argument is framed in terms of discrete morphs because it is applied to *Heliconius* butterflies in which major genes control color pattern. Similar arguments can be made for continuous variation.

Any unpalatable species risks damage or death during an encounter with a naive predator because it always takes one or more encounters for the predator to learn the morphology of that species. A novel morph is not recognized even by predators experienced with the typical form, and the learning process begins again. (The two morphs can of course be confused by predators, but to the extent it occurs it is ignored here because it does not lead to selection.) Because the first individual of a novel morph is rare, a high rate of damage or death is expected on a per-individual basis relative to that of the wild type (approximately in the ratio $r_a{:}r_c/N_c$, where r_a, r_c = total risk or fitness reduction expressed as numbers killed by local predators during learning of the novel aposematic and the cryptic morphs respectively; N_c = local population size of the cryptic insect morph). It is difficult to imagine that warning color evolves by individual selection because the advantages of a new pattern (e.g., enhanced memorability) rarely reduce the risk of death by as much as $1/N_c$ compared with a cryptic morph: For aposematic coloration to evolve, we must have $N_c r_a < r_c$, with N_c assumed large in a panmictic population. It is even more difficult for a rare cryptic morph to invade an unpalatable, warningly colored species because we must have $N_a r_c < r_a$, where we expect from the definition of aposematism that $r_a < r_c$. The selection is thus density-dependent against the rarer morph (Harvey et al., 1982). In a population of approximately constant size (perhaps maintained by density-dependent regulation acting on larval stages), this density-dependent selection is also frequency-dependent because frequency is proportional to numbers.

Most discussions of the evolution of warning color consider warningly colored morphs in cryptic but unpalatable species. However, in *Heliconius* we are more concerned with novel warningly colored morphs in already warningly colored species. This problem has similar dynamics, differing only in parameter values from the situation in a cryptic species (Mallet and Singer, 1987). In the simplest case both patterns are equally conspicuous and do not differ in memorability or palatability ($r_a \approx r_c$), so only frequency-dependent selection is involved. Within a species, frequency-dependent selection favors commoner aposematic morphs, impedes the evolution of novel patterns, selects against rare immigrants from populations with different colors, and, in doing so, stabilizes hybrid zones between different races (Brown et al., 1974; Benson, 1982; Harvey et al., 1982; Turner, 1982; Sheppard et al., 1985; Mallet, 1986a; Mallet and Singer, 1987; Endler, 1988). Frequency-dependent selection is also the engine that drives Müllerian mimicry between two or more unpalatable species. Mimicry, which would not evolve unless there were selection against rare aposematic forms, in fact provides some of the best evidence for a frequency-dependent advantage of common warning patterns (Mallet and Singer, 1987; Mallet, 1990). Purifying selection therefore explains (1) stasis of color patterns and Müllerian mimicry within regions and (2) maintenance of an interregional diversity of warningly colored races within species (Brown et al., 1974; Turner, 1982; Mallet, 1986a; Mallet and Barton, 1989a). In contrast, the origin of new aposematic patterns is seemingly prohibited by this purifying selection, which makes the rampant color pattern diversity within genera and even species of butterflies such as *Heliconius* especially puzzling.

One model for the evolution of novel warning colors is usually known as "kin selection" (Harvey and Greenwood, 1978). However, important differences were recognized between warning color and other possibly kin-selected altruisms such as unpalatability and eusociality, so the process for the evolution of warning color has also been called a "family model" (Harvey et al., 1982), kin-based "green beard selection" (Guilford, 1985), and "indirect selection" (Wiklund and Sillén-Tullberg, 1985). To my mind, these alternative names and the terms "altruism" and "kin selection" in this context confuse the process that establishes the novel form. The underlying idea is that warning colors can evolve if there are only a few families of an unpalatable species in an area containing few predators. A novel morph can then increase suddenly in the progeny of one of these families, thereby exceeding a threshold frequency such that the novel pattern becomes locally favored by frequency-dependent selection (Harvey and Greenwood, 1978; Harvey et al., 1982). This model is similar to the process of establishment of a novel underdominant chromosomal morph by genetic drift (Wright, 1941); essentially, N_c is assumed small, so a reasonable memorability advantage can cause local evolution; $N_c r_a < r_c$. Although Harvey et al. (1982, p. 712) proposed that novel warning colors increase "deterministically," small numbers of families per predator territory are required; and thus the model involves genetic drift or something closely resembling it (Mallet and Singer, 1987). "Kin-founding," used by Hedrick and Levin (1984) to describe a possible mode of chromosomal evolution in plants, is perhaps a better name than "kin selection."

Kin-founding and drift, which require small local populations, can be contrasted with standard "mass" or "individual" selection. There are at least three possible major ways in which warning colors can evolve by mass selection despite the frequency dependence: preadaptation, evolutionary enhancement, and mimicry. First, many palatable butterflies have bright colors that are used for signaling to predators and potential mates. If such species became unpalatable, their colors would act as preadaptations for warning color. Second, the patterns could then become enhanced by mass selection if modified patterns acted as "supernormal stimuli" of the original pattern memorized by predators (Mallet and Singer, 1987).

Mimicry is a third way in which warning colors can evolve by mass selection; it includes the evolution of frightening stimuli such as eyespots and color flashes, which can be interpreted as generalized mimicry of dangerous attacks by enemies of the insect predator (Mallet and Singer, 1987). In *Heliconius,* most races of most species have clearly evolved novel warning colors by means of Müllerian mimicry because there are usually more than two species within any mimicry ring, although this finding still does not explain the initial geographic divergence of the patterns. An interesting feature of mimicry is that genes with a major effect on the color pattern may be needed to bridge the phenotypic gap between the original nonmimetic pattern and the new mimetic pattern, which might explain why *Heliconius* races have only a small number of major Mendelian factors involved in pattern expression (Turner, 1976, 1984; Sheppard et al., 1985).

Warning Colors in Multidemic or Continuous Populations: Shifting Balance. The evolution of warning color, unlike typical altruisms between relatives, does not require continued proximity of relatives. Therefore occasional kin-founding could cause the evolution of novel warning colors, which would then be conserved by ordinary natural selection within a deme. Because this occasional population structure depends on random factors influencing local population size, it is a form of genetic drift. The kin-founding process can lead in multidemic or continuous populations to Wright's "shifting balance" (Wright, 1977, pp. 454–455). In general, models of kin selection *(sensu lato),* originally suggested by Fisher (1958) in connection with aposematic insects, are closely related to Wright's ideas, despite the strong disagreements between these two authors about the shifting balance. Both theories depend on interactions between small numbers of individuals within groups.

The shifting balance consists of three phases (using warning color as an example): (1) genetic drift (or kin-founding, or both) within a deme or group of demes leading to local establishment of a superior color pattern at a critical frequency; (2) selection within the deme or demes to fix or stabilize the new pattern; and (3) spread of the new pattern by "interdemic selection" to other demes. The term "interdemic selection" was used because deterministic or at least biased competition between demes can quickly spread advantageous adaptive peaks to new areas, even though there is initial selection against the new pattern during its introduction to any deme. In the absence of interdemic selection, each deme would have to evolve the new peak separately by genetic drift, and the process would be greatly slowed.

The shifting balance envisioned by Wright is only one of a series of models that seem to encapsulate his original idea. Extensions to the model are as follows: (1) extension from multiple epistatic loci to any genetic systems that exhibit multiple equilibria; (2) extension from demic to continuous populations; (3) extension of interdemic selection (the third phase) to any asymmetry between adaptive peaks that move the peaks from one area to another; (4) extension of the concept of interdemic selection to include movements of clines or hybrid zones within continuous populations.

Wright usually referred to multiple epistatic genes, but the first extension to the shifting balance includes the simplest example of a genetic system with more than one equilibrium: a single gene or chromosome with heterozygous disadvantage (Wright, 1978; Barton, 1979; Lande, 1985; Barton and Rouhani, 1991). Frequency-dependent selection on a single warning color gene is similar to selection on an underdominant trait, such as a chromosomal rearrangement (Mallet and Barton, 1989a), and should also be included. It is of course true that warning colors themselves are usually determined by two or more epistatic genes (Sheppard et al., 1985; Mallet, 1989).

Second, Wright and some of his followers (Wright, 1977; Crow et al., 1990; Wade and Goodnight, 1991) usually phrased the shifting balance in terms of discrete demes, but as migration between demes increases populations become effectively continuous (Slatkin and Barton, 1989). For shifting balance in a continuous population, substitute "neighborhoods" for "demes" in the above description (see also Barton and Rouhani, 1991).

Spread of Warning Colors to New Areas by Habitat-Independent Moving Clines. The two other extensions to the shifting balance involve the third phase of the shifting balance: interdemic selection. Wright proposed that interdemic selection would occur because demes that had reached the new, more adaptive peak would produce more emigrants than demes stabilized at the less adaptive peak. When selection affects population numbers, as in this case, it is known as "hard selection" (Wallace, 1968). This migration asymmetry due to adaptation would preserve and spread the new adaptive peak. In the third extension to the shifting balance, selection asymmetries on their own might also aid the spread of a new peak, even if selection is "soft" (i.e., does not affect local population size) (Wallace, 1968). For example if a new warning color is more memorable (i.e., less selected against when rare) than the old pattern, it can spread even if parasites and predators of larvae determine population size, so there is no migration asymmetry (Mallet, 1986a; Mallet and Barton, 1989a).

In the final extension of the shifting balance, the preservation and spread of a new morph behind a moving cline or hybrid zone in a continuous population is considered equivalent to interdemic selection in a demic population (Barton, 1979; Mallet, 1986a; Barton and Hewitt, 1989). Contact between two areas differing at a single locus or chromosome, with underdominant or purifying frequency-dependent selection, causes a cline or "tension zone" (Key, 1981; Hewitt, 1988) to form. Migrants crossing the cline are selected against, and this selection stabilizes the cline to a width that is a multiple of $\sigma/\sqrt{s}$, where σ = the migration distance, and s = the selection pressure (Bazykin, 1969). This result can also be generalized to multilocus hybrid zones (Barton, 1983; Mallet and Barton, 1989a) or to any other kind of genetic system where more than one equilibrium occurs (e.g., Turelli and Hoffmann, 1991).

Hybrid zones or narrow clines between forms differing in habitat-independent traits can form anywhere and are free to move from place to place (Bazykin, 1969; Barton, 1979; Mallet, 1986a; Turelli and Hoffmann, 1991) because selection is independent of habitat, or "endogenous" *sensu* Moore and Price (see Ch. 8). By "habitat-independent" traits, I mean only those "general" adaptations (W. L. Brown, 1958) with more than one stability peak and with unstable intermediate equilibria. Such traits include warning color patterns (Mallet, 1986a), chromosomal rearrangements or other types of heterozygous disadvantage (Bazykin, 1969; Barton, 1979), some kinds of genes affecting assortative mating (e.g., chirality in snails of the genus *Partula*) (Johnson et al., 1987, 1990), and traits affected by multiple epistatic loci (Wright, 1977). In the case of warning color, cline movement could occur if one form warned predators more effectively, but anything causing asymmetry of selection or migration across the zone, such as dominance of a color pattern gene or differences in population density, can also cause cline movement (Barton, 1979; Mallet, 1986a; Mallet and Barton, 1989a). Moving clines become trapped at density troughs because a density gradient causes asymmetric migration, and clines move to the bottom of the trough where migration is minimized (Bazykin, 1969; Barton, 1979; Hewitt, 1988; Barton and Hewitt, 1989). Because of this effect, habitat-independent clines are often associated with habitat discontinuities. Intermediate stages of evolution by the shifting balance in a continuous population then consist of a patchwork of different races, each connected to others by stationary or moving tension zones, similar to those shown by Wright (1977, p. 459) or those in Figure 9-1.

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A good criticism of my view that moving clines can explain geographic patterns is that clines move slowly and often become trapped by slight barriers to dispersal or density troughs (Barton, 1979; Barton and Hewitt, 1989). Because cline movement is part of the third phase of the shifting balance, this amounts to a critique of Wright's model; if cline movement rarely occurs except by accidental effects of the environment, it is difficult to justify the shifting balance as having much importance in adaptive evolution. Clines should move at a speed proportional to (and have units of) $\sigma\sqrt{s}$ (Barton, 1979; Mallet and Barton, 1989a; Turelli and Hoffmann, 1991). In the three-locus color pattern hybrid zone in *Heliconius erato* studied by Mallet et al. (1990), dominance of two of the color pattern genes is theoretically expected to move the hybrid zone at about $0.07\sigma\sqrt{s}$ (Mallet and Barton, 1989a). Because $\sigma \approx 2.6$ km and $s \approx 0.23$ per locus (Mallet et al., 1990), we expect movement of about 0.42 km per generation. At four generations per year, this movement is about 1700 km in 10,000 years—sufficient to blur biogeographic patterns created during the Pleistocene. This analysis assumes that the pure patterns are equally able to warn away predators; if one pattern is more memorable, cline speeds may be considerable increased or reversed. With selective asymmetry (S) and heterozygote disadvantage ($\approx$ frequency-dependent selection) maintaining the cline (s), cline speed is proportional to $\sigma S/\sqrt{s}$ (Barton, 1979); thus any increase in selective asymmetry causes the speed of a moving cline to increase in direct proportion.

Although hybrid zone movement seems potentially rapid for *Heliconius*, the criticism that slight density gradients or weak dispersal barriers commonly trap such zones remains serious. The trapping effect depends on whether spatial variation in population density in the field is large relative to movement tendency ($\sigma S/\sqrt{s}$), but there is a problem with the models that predict local hybrid zone trapping: a constant value for σ may be unrealistic. On encountering an area of low resource density, individuals of many mobile species are likely to disperse further, thereby increasing σ locally. This dispersal can at least partially compensate for small density troughs that would trap clines if the species had the purely diffusive dispersal assumed in the models.

Neutral and Habitat-Dependent Clines. Wright (1977, 1978) did not claim that races such as those in Figure 9-1 were completely neutral, as some have assumed (Provine, 1986, pp. 287–291). Wright thought only that neutral genetic drift might cause the initial divergence between two races, thereby leading to subsequent divergent selected evolution. The problem is with the definition of the term "neutral" on a local and global scale. Racial differences could be neutral in the sense that they are not specially adapted for the environment in which they are found; any *Heliconius* pattern could probably teach many types of predator in a wide variety of habitats. On the other hand, such "neutral" races may be strongly selected toward the local adaptive peak that exists within their distribution range, as is clear from *Heliconius* warning color, which involves mimicry. There are of course other genetic differences, such as allozymes or third basepairs in codons, that might indeed be effectively neutral, both locally and globally. Clines of such elements could be caused by neutral secondary contact or local genetic drift, in which case the clines will slowly decay until their widths are much greater than the dispersal distance (Endler, 1977).

Biogeographic patterns such as those shown in Figure 9-1 might also be caused by habitat-dependent selection ("special" adaptations *sensu* W. L. Brown, 1958, or "exogenous" selection *sensu* Moore and Price, Ch. 8); that is, selection depends on the environment of the taxon under consideration, including geography, climate, vegetation, food sources, competitors, parasites, predators, and symbionts. Habitat-dependent traits include adaptation to sooty bark by melanics in the peppered moth *Biston betularia* (Bishop, 1972), plant adaptations to local soil or wind conditions (Jain and Bradshaw, 1966), physiological adaptations to climate (e.g., Hagen, 1990), and mimicry (Turner, 1971a, 1982). Clines between traits that are under habitat-dependent selection quickly move to areas of environmental or biotic change and thereafter remain stationary, unless the environment itself changes.

In the case of Müllerian mimicry rings, the whole ring evolves relatively independently of habitat, given the existence of predators that can learn, whereas rare species within the mimicry ring evolve as though their environment were determining the local selection. In addition, a particular color pattern might have a habitat-dependent advantage (e.g., melanism in a cool climate) but may be unable to evolve because of a habitat-independent disadvantage (e.g., frequency-dependent selection against rare morphs in an unpalatable species). Thus habitat-independent and habitat-dependent selection are not mutually exclusive. We can also imagine that either neutral or habitat-dependent divergence between parapatric or completely allopatric populations might eventually result in habitat-independent reproductive isolation. Pleiotropic habitat-independent incompatibilities in the mating system or in postmating barriers seem likely to cause hybrid breakdown, such as that in "Haldane's rule" (Charlesworth et al., 1987; Barton and Hewitt, 1989). Geographic variation leading to speciation is often a mix of neutral, habitat-dependent, and habitat-independent components (Hewitt, 1989).

Evidence from Heliconius _Population Ecology and Hybrid Zones._ Although mass selection for mimicry can occur in any population structure, the probability of kin-founding or the shifting balance requires small local population sizes (Wright, 1941, 1977; Harvey et al., 1982; Lande, 1985; Barton and Rouhani, 1991). Current population structure may be inferred from ecological or genetic studies, but it might not help in understanding the past population structures leading to divergence. However, because species of *Heliconius* have similar population biology (Gilbert, 1984), and most *Heliconius* species have evolved multiple novel color patterns (Brown, 1979), it seems likely that population studies will be useful for understanding raciation in the genus. The method is not likely to be so useful for elucidating ancient evolution, such as that of the first warning colors in the progenitor of the genus.

Heliconius individuals repeatedly visit feeding, mating, and nocturnal gregarious roosting sites within small home ranges about 100 meters wide (Turner, 1971a,b; Gilbert, 1975; Mallet, 1986b) and survive as adults for up to 6 months, more than six times the egg to adult development time, so that altruistic acts and kin selection between and within generations would be possible. These observations together suggested that *Heliconius* was a good candidate for kin selection and kin-founding in the evolution of unpalatability, warning colors, and knowledge-sharing within gregarious roosts (Benson, 1971; Turner, 1971a; Gilbert, 1975, 1977; Wilson, 1975; Harvey and Greenwood, 1978; Harvey et al., 1982).

Any genetic markers, such as allozymes, could give some information about population structure and the likelihood of kin-founding. In hybrid zones between heliconiine color pattern races, however, we can observe polymorphisms similar to those that must have existed when novel color patterns first arose. Sheppard et al. (1985) reviewed inheritance of warning color patterns in *Heliconius*. Color patterns in hybrid zones are therefore especially useful as genetic markers for testing hypotheses of kin selection and the shifting balance, as they are the very genes in whose evolution we are interested.

If population structure in *Heliconius* were suitable for "deterministic" kin-founding, we might expect that as we pass from the home range or territory of one predator to another within a hybrid zone there will be sharp changes of color pattern gene frequencies. It might occur in two ways. Dispersal might be as limited as measured in mark-recapture studies, and hybrid zones would be little wider than a single butterfly home range (~ 100 meters) because butterflies would rarely disperse, and the interface would be formed by adjacent predator home ranges containing divergent butterfly color patterns. Alternatively, dispersal might be greater, leading to a broad hybrid zone. Under these conditions, because most populations are founded by few individuals, a broad mosaic of mostly fixed populations with alternative color patterns should be present in the transition area. In this case the mosaic would consist of locally selected color patterns, based on the alleles present in initial founder populations; in contrast to other mosaic hybrid zones (Harrison and Rand, 1989), local genetic rather than environmental conditions would determine the selection.

Table 9-2. Allelic and Genotypic Frequencies for *H. erato* near Villa Garzón, Putumayo, Colombia

	No. of Alleles		No. of Genotypes		No. of Genotypes	
	D^{Ry}	d^{rY}	$Cr-$	$crcr$	$Sd-$	$sdsd$
N. of Río Mocoa and W. of Villa Garzón	15	25	11	9	15	5
N. of Río Mocoa and E. of Villa Garzón	13	29	13	8	15	6
S. of Río Mocoa and E. of Villa Garzón	22	36	12	17	25	4
S. of Río Mocoa and W. of Villa Garzón, (mark-recap. site);						
Total	68	132	52	48	63	37
Subsite A	4	4	2	2	2	2
Subsite B	10	26	9	9	8	10
Subsite C	10	26	12	6	9	9
Subsite D	10	20	8	7	13	2
Mobile individuals	34	56	21	24	31	14

Villa Garzón (formerly Villa Amazónica) is situated on the southern bank of the Río Mocoa, at 420 m above sea level; *H. e. dignus* from the upper Putumayo (Fig. 9-1A; ● symbols at about 2°N) here hybridizes with the Amazonian race *H. e. lativitta* (Fig. 9-1A; + symbols). Color pattern genetics are assumed similar to that near Tarapoto, Peru (Mallet, 1989; Mallet et al., 1990) on the basis of the appearances of hybrids, although the yellow bar of $Sd-$ $crcr$ genotypes is apparently more strongly expressed in Colombia than in Peru. Allelic frequencies are shown for the codominant locus D^{Ry}/d^{rY}, and phenotypic frequencies are shown for the two dominant loci Sd/sd and Cr/cr. All sites were within easy walking distance of Villa Garzón, about 5 km, though exact distances are not known. A site immediately to the west of Villa Garzón on the road to Mocoa was studied intensively using mark-recapture; individuals could be classified into those that had restricted home ranges (about 100–300 m across) and those that moved between subsites A, B, C, and D. Groups of gregarious roosts were discovered for subsites B, C, and D.

There is now good evidence from *Heliconius erato* and its Müllerian mimic *H. melpomene* that hybrid zones between races are relatively broad and do not consist of mosaics, suggesting that dispersal is greater than measured in mark-recapture experiments. For example, populations of *Heliconius erato* of the order of 1–5 km apart near Villa Garzón in Colombia are remarkably similar in gene frequency (Table 9-2): Homogeneity tests for $\mathrm{D^{Ry}/d^{rY}}$ (allelic frequencies), $G = 0.71$, 3df; for Cr/cr (genotypic frequencies), $G = 2.22$, 3 df; and for Sd/sd (genotypic frequencies), $G = 6.68$, 3 df—giving a sum of $G = 9.61$, 9 df overall ($p > 0.05$). This gene frequency heterogeneity is equivalent to an average (but nonsignificant) F_{st} of 0.02. Each population can be subdivided again and again, even down to individual gregarious roosting sites, and still only small gene frequency differences result. For instance, in the intensively studied S./W. site in Table 9-2, the overall heterogeneity was again insignificant (lumping adjacent subsites A and B, and including the mobile individuals as a separate group, summed $G = 12.40$, 9 df, $F_{st} \approx 0.06$). The Fisher-Ford estimates of population size for the S./W. sites A, B, and C summed (D was observed as roosts only) varied between 26 and 40, with average life expectancy of 41 days. The low population sizes, together with the low turnover rate and high recapture rate (86%) shows that virtually all individuals were captured in this site, so these estimates of F_{st} must be close to actual. Therefore it is possible that the measured F_{st} is entirely due to the small local population size, rather than because there is underlying gene frequency variation from population to population. More extensive data showing similarly small and insignificant gene frequency differences between adjacent sites has been collected from *H. erato* and *H. melpomene* hybrid zones in Panama and Peru (Mallet, 1986a; Mallet et al., 1990). The transitions from one color pattern to another in Panama and Peru are smooth gene frequency clines about $w \approx 10$–100 km wide with little "noise" attributable to strong gene frequency differences between adjacent populations (Mallet, 1986a; Mallet et al., 1990).

In addition, local hybrid zone populations have no significant deviations from Hardy-Weinberg at color pattern loci, suggesting that the populations are not subdivided. Near Villa Garzón, the lack of a heterozygote deficit at D^{Ry}/d^{rY}, $F \approx 0.14$, $G = 3.45$, 1 df ($p > 0.05$, using all individuals in Table 9-2) shows that there is no significant substructuring even after lumping the populations studied; again, this lack of heterozygote deficit is also true for Panama and Peru (Mallet, 1986a; Mallet et al., 1990) and for other hybrid zones (Turner, 1971c). Methods based on linkage disequilibria (see Ch. 2) and direct field experiments have indicated that selection coefficients required to stabilize some hybrid zones of *H. erato* and *H. melpomene* must be strong: $s \approx 0.23$–0.26 per locus against the foreign alleles in the narrowest (10 km) hybrid zones (Mallet and Barton, 1989b; Mallet et al., 1990). Indirect estimates of dispersal made from the *H. erato* hybrid zones in Peru show that gene flow, measured as the standard deviation in parent-offspring distances, is $\sigma \approx 2.6$ km gen$^{-1/2}$ (Mallet et al., 1990). Linkage disequilibria were not significant at Villa Garzón, perhaps because of the small total sample size, and so could not be used to estimate dispersal. This work has mostly been done with *H. erato,* but preliminary evidence from disequilibria in *H. melpomene* suggests an even greater tendency to movement ($\sigma \approx 3.7$ km gen$^{-1/2}$) (Mallet et al., 1990; see also Turner, 1971c).

Detailed mark-recapture studies in Colombia and Costa Rica show how this population structure is realized. Although some individuals have home ranges centered on faithfully attended gregarious roosts, other individuals are much more prone to change roosts. In the Villa Garzón mark-recapture site (Table 9-2), 45% of individuals moved between roosting groups or disappeared after one capture; the number moving between roosts within subsites was higher still. Further data showing movement between roosting sites in Costa Rica is given by Mallet (1986b). Home ranges of individuals attending different roosts overlap markedly, and there is no evidence for communal feeding behavior exclusively involving roostmates (Mallet, 1986b). Most dispersal of *H. erato* occurs before learned home ranges are set up, as is normal for mammals and birds; this point would not have been detectable in earlier mark-recapture studies. Movements of recently eclosed adults of *H. erato* suggest that gene flow, $\sigma \geq 0.3$ km gen.$^{-1/2}$ (Mallet, 1986c). Because the sampling area for this mark-recapture study was small, it seems likely that the indirect estimate of gene flow obtained from hybrid zones (above), $\sigma \approx 2.6$ km gen$^{-1/2}$, is more accurate.

The population structures of these two species are therefore difficult to reconcile with a simple-minded "deterministic" kin-founding model for the evolution of aposematic patterns in gregarious roosting families or with any of the other population-level kin selection models for the evolution of altruisms proposed for *Heliconius.* To sum up the evidence: individuals roost and fly together with unrelated individuals most of the time; home ranges overlap, allowing for gene flow between roosting groups; individuals on the same roost have different home ranges, suggesting a lack of communication about feeding, mating and oviposition sites, and further possibility for gene flow; newly eclosed individuals often leave their parental home range; gene flow is of the order of $\sigma \approx 2$–4 km gen$^{-1/2}$, in contrast to the 0.05 km gen$^{-1/2}$ observed in earlier mark-recapture studies; F_{st} is low and insignificant for warning color genes in hybrid zones, suggesting that the effective population size is larger than local population sizes centered on gregarious roosts; hybrid zones between color pattern races are broad in comparison with the butterflies' home ranges and form smooth clines, suggesting that local populations do not usually exhibit sudden frequency changes because of founder events. These results for *Heliconius* seem to reflect a general problem for kin selection in unpalatable butterflies, as *Heliconius* were thought to be one of the best examples of kin grouping. Distasteful ithomiines, danaines, and troidines appear to lack home ranges and are much more migratory than *Heliconius* (Gilbert, 1969; Brown and Benson, 1974; Brown and Neto, 1976; Eanes and Koehn, 1978; Brown et al., 1981) and so would be even less likely to evolve unpalatability or warning colors by kin selection or deterministic kin-founding.

Instead, population structural conditions for kin-founding and kin-selection must occur only periodically (if at all), as in classical genetic drift. Genetic drift and kin-founding could lead to habitat-independent establishment of a new warning color pattern in a local area or subpopulation. Although this initial change is likely to be very rare, once an area of reasonable size is fixed for an advantageous new pattern it is resistant to invasion by the old pattern because stable clines, maintained by frequency-dependent selection, form around it (see above; also Benson, 1982, p. 633). The new form is preserved and spread if it is selectively superior or has some other advantages, such as a high rate of emigration, or is dominant; clearly this mode of divergence would be an example of Wright's "shifting balance."

Some calculations (Rouhani and Barton, 1987; Barton and Rouhani, 1991) indicate that local shifting balances are fairly likely in continuous populations with restricted movement such as those in *Heliconius. Heliconius* population structure, however, is probably still too poorly known for a convincing estimate of the probability of this mode of divergence. In the rest of the chapter, I instead concentrate on whether the biogeography of *Heliconius* and allies gives evidence for either habitat-dependent selection or the shifting balance in color pattern evolution.

EVOLUTION OF NOVEL COLOR PATTERN RACES AS A MODEL FOR SPECIATION

As we have seen, geographic variation may be neutral, selected and habitat-dependent, or selected and habitat-independent. We normally think of the evolution of reproductive isolation, or "biological" speciation, as habitat-independent; we expect mating incompatibilities or hybrid breakdown to be maintained under most field or laboratory circumstances. How might habitat-independent reproductive isolation evolve? *Heliconius* color pattern races are partially reproductively incompatible because there is selection against hybrids (heterozygous color patterns are "fuzzier" and presumably less effective as warning signals), because of frequency-dependent selection against rare introgressing patterns, and because the pattern of any given race consists of a self-adapted set of alleles at epistatically interacting loci (Mallet, 1989; Mallet et al., 1990).

With *Heliconius,* both habitat-dependent and habitat-independent routes for the evolution of this kind of incompatibility seem likely.

Warningly colored species that are unpalatable and common cause strong habitat-dependent selection for Müllerian mimicry of local species with similar color patterns (Brown et al., 1974; Turner, 1982). Other traits related to habitat, such as host-plant survival or choice, or physiological adaptations to temperature or altitude, are also likely to diverge geographically under selection in continuous populations (Benson, 1982; Endler, 1982). There can be no doubt that mimicry is the major reason for color pattern divergence in most heliconiines and ithomiines with geographically differentiated color patterns (Turner, 1982): There are almost always more than two species in a local mimicry ring, implying that more species are mimics than models. However, it is unlikely that mimetic convergence alone can cause all this divergence: some independent divergence of color patterns in the major "model" species seems necessary to trigger novel color patterns in these butterflies. This initial diversification could be provided by the shifting balance. Indeed, the shifting balance is suggested by the existence of the alternative stable states demonstrated by Müllerian mimicry and strongly selected narrow hybrid zones.

An important feature of both mass selection and the shifting balance is that neither requires allopatry. It has been clear since the 1940s that habitat-dependent adaptation can take place in the absence of geographic barriers, provided the patches under selection for divergence are greater than about $\sqrt{(3\sigma^2/s)}$ across (Haldane, 1948; Fisher, 1950; Slatkin, 1973; Endler, 1977). Genetic drift can also cause divergence for habitat-independent or neutral traits in viscous but continuous populations; once again, the scale of divergence is some critical small multiple of σ (see Barton and Charlesworth, 1984; Barton and Hewitt, 1989; Slatkin and Barton, 1989; Barton and Rouhani, 1991).

Because it causes partial reproductive isolation, color pattern change in warningly colored species has some of the characteristics of speciation, but further change is required to complete the process. Any mass selection or habitat-dependent adaptation may produce pleiotropic effects causing pre- or postmating incompatibilities. Pleiotropic reproductive isolation may seem questionable if color pattern alone is involved. Nonetheless, mimicry is often limited to females, which suggests that mimicry often interferes with mating behavior and sexual selection, at least in Batesian mimics (Turner, 1978; Silberglied, 1984). This effect of color pattern evolution does not seem to be important in *Heliconius,* as there is no evidence for heterozygote deficits in hybrid zones (see above), and sexual dimorphism is rare. Color pattern divergence due to a shifting balance could potentially have similar pleiotropic effects on reproductive traits. However, it is perhaps more likely that habitat-dependent evolution or the shifting balance at noncolor traits would cause the pleiotropic effects that lead to speciation. In addition, population structures suitable for color pattern changes via the shifting balance are also likely to produce shifting balances for other habitat-independent traits such as chromosomal morphs and other underdominant loci, epistatic gene complexes, and sexually selected traits. Similarly, chromosomal evolution may be associated with rapid speciation, rather than itself being the prime cause of speciation (Bush et al., 1977; Barton and Charlesworth, 1984).

The heliconiines display a rich spectrum of geographic divergence: weakly differentiated forms separated by broad clines, e.g. within *Heliconius doris* or *H. hecale* (Turner, 1971a; Brown and Benson, 1974; Brown, 1976a) or among rayed races of *H. erato* and *H. melpomene* (Fig. 9-1) (Brown and Mielke, 1972), strongly differentiated forms separated by narrow hybrid zones with extensive hybridization, e.g., rayed and unrayed races of *H. erato, H. melpomene,* and *H. hecalesia* (Fig. 9-1, Table 9-1) (Emsley, 1964; Turner, 1971c; Brown and Benson, 1975; Brown, 1976b; Benson, 1982; Mallet, 1986a; Mallet et al., 1990), parapatric "good species" abutting at narrow contact zones with few or no hybrids produced, e.g., *H. erato* and *H. himera, H. erato* and *H. clysonymus* (Fig. 9-1A; Tables 9-1 and 9-3) (Benson, 1978, 1982; Descimon and Mast de Maeght, 1984; König, 1986; G. Lamas, unpublished), and finally sympatric closely related species that rarely if ever hybridize, e.g., *H. melpomene* and *H. cydno; H. eleuchia, H. antiochus,* and *H. sapho; H. sara* and *H. leucadia* (Kaye, 1916; Emsley, 1964, 1965; Brown and Mielke, 1972; Brown and Benson, 1975; Brown, 1976b). Even if we do not know exactly how speciation occurs, this spectrum of reproductive isolation implies that speciation is gradual (but not necessarily slow), and that usually many genetic changes are involved in the completion of speciation. Single genes or chromosomes rarely cause complete reproductive isolation, as suggested by White (1978) in his "stasipatric" speculation model.

Table 9-3. Captures of *Heliconius himera, H. erato lativitta,* and *H. melpomene aglaope* in the Marañon Valley of Peru: July 1984 and June 1986

	Captures of Heliconius at Certain Distances (km) North of Muyo													
Species	−10.2	−9.7	−8.0	−6.0	−1.5	1.0	3.0	9.0	9.5	20	30	60	130	160
H. himera	6	26	12	21	11	0	0	1	0	0	0	0	0	0
H. erato	0	0	0	0	0	0	0	1	5	1	5	1	2	2
H. melpomene	0	0	0	0	1	1	5	1	4	0	2	4	2	0

Distances are expressed as kilometers north of Muyo (near Aramango), Amazonas. Muyo is about 30 km NNE of Bagua and is the site of the abrupt change from thorn scrub upriver toward Bagua and moist forest downriver toward Chiriaco and Sarameriza. Aramango is at +1.0 km, Chiriaco is at about +20 km, and Sarameniza is at +160 km on the Rio Maranon/Amazonas. One of the specimens of *H. melpomene* from 3 km north of Muyo has reduced rays, presumably mimicking *H. himera,* and therefore corresponds to the form designated "ssp. nov." (Fig. 9-1B; ⋆ symbols) by Brown (1979).

BIOGEOGRAPHIC EVIDENCE FOR ALLOPATRIC AND PARAPATRIC DIVERGENCE IN *HELICONIUS*

The remainder of this chapter uses the preceding models of evolution as a basis for reviewing evidence for and against hypotheses of divergence in *Heliconius.* The argument focuses on the possible role that Pleistocene refugia have played in the evolution of *Heliconius* color pattern races and their hybrid zones. I am here interested less in whether Pleistocene refugia existed than in whether they contributed to morphological divergence and current geographic distributions. On the basis of the distribution of neotropical taxa and the hybrid zones between them, I argue not only that parapatric hypotheses for divergence in neotropical species have not been falsified (see also Connor, 1986) but also that parapatric hypotheses seem at least as likely (based on current evidence) as allopatric ones. Two sorts of allopatric hypotheses might be proposed: First, raciation or speciation could have occurred initially in allopatry (e.g., Mayr, 1963); and second, allopatric spread from refugia could be the most important factor that has determined current distributions of taxa and the positions of contact zones, even though the initial divergence might have been in parapatry (Barton and Hewitt, 1985; Hewitt, 1988). I shall argue for the plausibility of extreme parapatry—parapatric divergence as well subsequent parapatric maintenance—because this model must be effectively rejected before we can think about accepting one or both allopatric hypotheses. At present, I believe that none of these models can be accepted; we do not know the relative likelihoods of different modes of speciation, and so it does not seem sensible to accept either model as a null hypothesis (Turner, 1982).

Refugium Hypothesis. Ideas about Pleistocene refugia were developed to explain an apparent paradox. Evolutionary biologists were at one time largely convinced by the arguments of Mayr (1963) that speciation required allopatry, and many continue to hold this conviction (e.g., Futuyma and Mayer, 1980; Haffer, 1985). However, the rain forest on the neotropical mainland is one of the most speciose areas in the world for birds, insects, and flowering plants despite a pronounced lack of geographic barriers, especially in Amazonia. Jürgen Haffer (1967, 1969, 1987a) summarized geological evidence for previous drier neotropical climates associated with glaciation in the temperate zone, which may have limited the extent of the forest during the Pleistocene. Haffer (1967, 1969, 1974, 1985, 1987b) suggested that forest birds were able to persist and speciate in the remnant forest refugia, giving rise to forms that subsequently spread out and formed zones of secondary contact during the more humid interglacials. The huge species diversity observed today in many neotropical groups might have been produced via several such cycles (Haffer, 1969).

Similar arguments have been used to explain divergence in aposematic tropical Heliconiini and Ithomiinae (Turner 1965, 1971a, 1976; Brown, 1976b, 1979, 1981, 1982, 1987a,b,c; Brown et al., 1974; Lamas, 1973, 1982); but see also Turner's (1982) "faunal drift" refugium hypothesis, discussed under Mimicry in Refugia below.

Geological Evidence and the Supposed Necessity for Allopatry. Although some have emphasized geological evidence for drier Pleistocene climates, others argue that much of the Amazon remained wet throughout the glacial periods (Colinvaux, 1989). As to whether allopatry was necessary, I have already pointed out that parapatric divergence now seems much more likely on the basis of population genetic theory than seemed credible during the 1960s and 1970s (Endler, 1982; Connor, 1986). Mechanisms for divergence in allopatric refugia are rarely discussed, but the implication of Mayr's (1963) founder effect speciation and similar models is that genetic drift and habitat-dependent selection in small populations can combine to initiate speciation. It is difficult to imagine, however, how refugia would help the operation of genetic drift unless the refugia were extremely small (e.g., less than a few kilometers across). Such refugia are more likely to cause extinction than speciation (Barton and Charlesworth, 1984; Barton, 1989).

***Importance of Habitat for Speciation and Raciation in* Heliconius.** Some species of *Heliconius* are characteristic of particular ecological zones, rather than being centered on regions of high rainfall. *Heliconius himera* is restricted to the dry, scrubby vegetation of the upper Marañon drainage and western slopes of the Andes Mountains in Peru and Ecuador (Fig. 9-1A, ∗ symbols). This species has been treated on the basis of larval and adult morphology as a race of *H. erato* (Brown, 1979). Hybrids of *H. himera* with *H. erato* have been found where the two meet (Descimon and Mast de Maeght, 1984; König, 1986); however, the hybrids are rare in comparison with the parental species, so it is more sensible to regard *H. himera* as a good species. In my own collections at the contact zone between *H. himera* and *H. erato lativitta* of the Marañon, Peru, I found no hybrids (Table 9-3), though König has found a single *erato* × *emma* hybrid in this area (G. Lamas, pers. comm.). The position of the contact zone correlates perfectly with the change of vegetation from the acacia scrub and dry forest habitat of *H. himera* to moist tropical forest near Muyo and Aramango, Amazonas, Peru; the latter is typical habitat for *H. erato* (Table 9-3). The other contact zones between *H. himera* and *H. erato* are on similar ecotones (pers. obs.; Keith Brown, pers. comm.). The himera–erato contact zones are not by themselves proof of a biogeographic pattern, but essentially the same distributions are known for many of the other aposematic and nonaposematic butterflies of the Marañon (Brown, 1979; Lamas, 1982). In the case of the Marañon fauna, endemism is centered on a valley habitat that is dry owing to the rain shadow effect of surrounding mountains, rather than being a likely area for a moist forest Pleistocene refugium.

Another species that is closely related to *H. erato* is *H. clysonymus.* This species has a color pattern similar to that of *H. himera* (Fig. 9-1A, ∗ symbols), although it is unclear whether the patterns in the two species are homologous. *Heliconius clysonymus* is found only above about 800 meters in the Andean chain, as well as at similar altitudes in Central America. *Heliconius clysonymus* overlaps but does not hybridize with *H. erato* at intermediate elevations. There is some evidence for competitive exclusion between the two species (Benson, 1978). *H. himera* and *H. clysonymus,* both close relatives of *H. erato,* provide good evidence for the importance of habitat-dependent divergence in speciation: Both are found in habitats different from those usually occupied by *H. erato,* and both are less interfertile with *H. erato* than any of the races within *H. erato.* Other close relatives of *H. erato—H. charitonia, H. hermathena, H. hortense, H. telesiphe,* and *H. hecalesia*—are also found either in drier or more montane or marginal habitats than typical *H. erato.* Moreover, there is good evidence among the ithomiines for restriction of certain species with lowland rain forest relatives to montane or semiarid forest types (Brown, 1979; Lamas, 1982; Mallet and Lamas, in prep).

Some races of polytypic species, as well as sibling species of *Heliconius,* appear to be adapted to particular environments, but here the correlation is less clear. Andean races of species in *Melinaea, Mechanitis,* and *Hypothyris* (Ithomiinae), as well as *Heliconius,* among other genera, are often more melanic than their conspecifics in the Amazon basin (Brown 1977). In *Heliconius erato* and *H. melpomene* the large basal patch on the forewing and the greater degree of yellow scaling on the thorax of the Amazonian rayed races contrast with the blacker bodies and blacker distal portion of wings of Andean races (Fig. 9-1). Although experimental work has never been performed on *Heliconius,* this type of butterfly melanism is often associated with an ability to attain daytime operating temperature rapidly in cooler climates (Watt, 1968; Douglas and Grula, 1978; Roland, 1982), as might be expected in the higher altitudes of the Andes. For humid areas near the equator, an elevation increase of 100 meters corresponds approximately to a temperature decrease of 0.6°–1°C (MacArthur, 1972).

Although this general correlation between climate and color pattern exists and is undoubtedly important, it does not explain all of the differentiation patterns. For example, *H. erato emma* is an Amazonian rayed race that also invades the Andean valley of the Pozuzo river in Peru together with its *H. melpomene* mimic (Fig. 9-1; ♦ symbols at about 10°S). These co-mimics reach an altitude of 1200–1500 meters in this valley; similar neighboring valleys contain the melanic Andean races *H. erato microclea* (Fig. 9-1; ▲ symbols to the south) and *H. erato favorinus* (Fig. 9-1; ● symbols to the north, at about 6°–9°S) as well as their *H. melpomene* mimics, which reach altitudes as low as 200 meters, and exclude *H. e. emma* and similar rayed *H. melpomene.* Even within valleys, racial distributions are not clearly altitude-limited. *H. erato emma* hybridizes with the Andean *H. e. favorinus* at Pongo de Aguirre at 210 meters altitude in the Huallaga River Valley, whereas the same hybrid zone is at about 300–320 meters near Pongo de Cainarache on the eastern slopes of the Andes. There are large areas of the Mayo and Huallaga River Valleys upriver from Pongo de Aguirre that are less than 300 meters in elevation, but that have pure Andean *H. e. favorinus. Heliconius erato hydara* appears adapted to coastal and open areas, as well as to low-lying areas along the lower Amazon River, in Brazil, Suriname, and Cayenne; rayed races *H. e. erato* and *H. e. amalfreda* are found in denser inland forest (Benson, 1982). However, this correlation breaks down in Guyana, where *H. e. hydara* is found in tall inland rain forest, as well as savannah and more open areas. This race occurs from the Atlantic coast inland to Marudi Mountain, where there is a hybrid zone between *H. e. hydara* and *H. e. amalfreda.* Andean forms which are good species, such as *H. congener* and numerous ithomiines, are found only above about 500 meters in mountain chains ringing river valleys (Lamas, 1982; Mallet and Lamas, in prep.), but *Heliconius* races within species rarely follow climatic or habitat factors so exactly. Poor correlations between Amazonian hybrid zones and habitat change have been used as evidence for refugia (Haffer, 1982, 1985; Brown, 1987c) as a cause of divergence. An alternative explanation is parapatric, habitat-independent divergence, such as by the shifting balance.

The habitat-related biogeographic patterns that do exist might be explained by direct climatic selection on color patterns (above), by an influence of habitat type on the effectiveness of warning colors or mimicry (Benson, 1982; Endler, 1982) or, alternatively, by a buildup of associations between genes for mimicry and genes for host-plant or climatic adaptation and in which partial or complete reproductive isolation might maintain the association (Mallet and Lamas, in prep.). Whatever the causes, strong habitat adaptation to arid or montane habitats seems often to have led to speciation, as exemplified by *H. clysonymus* and *H. himera* versus *H. erato,* whereas the weaker differences between habitats occupied by the various lowland rain forest races of *H. erato* seem to have caused less reproductive isolation.

Correlations of Hybrid Zones with Rainfall Patterns. Some apparent centers of endemism correlate approximately with regions of high rainfall, especially on the slopes of the Andes. This evidence has been used to support the idea that such areas, which are purportedly more likely to have retained forest during a dry period, were indeed refugia during the Pleistocene (Haffer, 1969; Brown, 1979, 1987b). However, many of the proposed correlations may not stand up under close scrutiny. There is a need to map hybrid zones and races accurately in order to investigate this proposed correlation. The patterns mentioned above for Marañon Valley endemics show that, rather than always involving high rainfall, centers of endemism often occur in areas with unusually low levels of rainfall, as well as in areas of higher altitude, with hybrid zones being often found in ecotones between habitats (see also Benson, 1982).

There are also other patterns of correlation with rainfall within species. Rather than being centered in drier zones that are often considered to be interrefugial (Brown, 1979, 1987c), some hybrid zones in neotropical butterflies occur almost exactly at orogenic rainfall peaks at the base of the eastern slopes of the Andes. A number of hybrid zones for *Heliconius erato* and *H. melpomene* between Andean Valley forms and forms from the Amazonian lowlands are arranged in this way; at Pongo de Cainarache, in the centers of hybrid zones between races of *H. erato* and of *H. melpomene,* the rainfall peaks (3637 mm per year), compared with nonhybrid populations of the Andean Valley *H. e. favorinus* and *H. m. amaryllis* at Tarapoto (1004 mm) and Rioja (1728 mm) and the Amazonian *H. e. emma* and *H. m. aglaope* at Yurimaguas (2279 mm) on either side of the hybrid zone (Fig. 9-1; ● and ♦ symbols at about 6°–9° S in the eastern Andes) (Mallet and Barton, 1989a,b; Mallet et al., 1990; rainfall data were obtained from SENAMHI, Peru). Andean valley races of *H. erato* (and *H. melpomene* equivalents) hybridize with Amazonian races in other high-rainfall lowland areas east of the easternmost Andes. They include the Peruvian races *H. erato favorinus* near Tingo María, *microclea* near Iscozacín, and *euryades* on the Río Urubamba below Quillabamba, all of which hybridize with rayed races from the Amazon basin. All three of these races (and their *melpomene* equivalents) are centered on drier areas in the rain shadows of the easternmost Andes chain (Fig. 9-1; ●, ♦, and O symbols in the Andes between 6° and 14° S) (Benson, 1982). These distribution patterns, which are repeated many times in other heliconiines and ithomiines from the same areas (Mallet and Lamas, in prep.), again point to a lack of correlation of centers of endemism with regions that currently have high rainfall.

Why should hybrid zones be found in areas with high rainfall? A possible explanation of the anomaly is that moving clines might become trapped by rainfall peaks if the latter act as migration troughs. Although aposematic butterflies probably fly more than palatable butterflies during wet weather (Mallet and Singer, 1987; Chai and Srygley, 1990), cloudy weather and rainfall do considerably reduce the activity of heliconiines and ithomiines, which in turn could reduce their reproductive rate and survival. Butterflies must mate and find adult and larval host plants in order to reproduce; and these activities are reduced during rainy weather. In 1977 I observed at the Río Negro on the eastern slopes of the Andes near Caqueza, Colombia, *Heliconius* that had decomposing wing margins owing to long-term exposure to rain and humidity; local people reported rain had fallen every day for a month before my visit. Such anecdotes suggest the possibility that orogenic rainfall peaks might act as barriers to the free movement of butterfly clines, even though populations of the same species are distributed throughout the zone. In contrast, we have seen that the refugium hypothesis predicts the reverse: Centers of endemism, rather than hybrid zones, should correlate with rainfall peaks.

Correlations of Hybrid Zones with Rivers, Mountains, and Other Features. Under the allopatric model, it is rather puzzling that hybrid zones for mammals, birds, and butterflies are often associated with major rivers in Amazonia (Hershkovitz, 1968; Haffer, 1974, 1985, 1987a,b; Brown, 1979; Lamas, 1982; Beven et al., 1984), as the rich alluvial soils and the high probabilities for gallery forests remaining near rivers during dry periods would tend to cause refugia to be associated with the rivers themselves, rather than with higher ground between major rivers. Many probable "model" species for Müllerian mimicry rings can be currently found year-round in gallery forests, even near smaller, seasonally dry rivers, in the savannah and Llanos regions of Guyana and Venezuela (Brown and Fernandez, 1985; pers. obs.). They include *Lycorea* (Danainae), *Melinaea, Tithorea, Mechanitis,* and *Hypothyris* (Ithomiinae), as well as *Heliconius erato, H. melpomene,* and other heliconiini. It would probably take complete desertification to remove this "weedy" fauna (many species of which have among the highest rates of geographic divergence) from near major rivers. Partial barriers such as rivers should not in any case much delay advancing population fronts emerging from refugia: These fronts should spread until they contact another race in a location that is independent of partial barriers (*contra* Turner, 1982; Sheppard et al., 1985; Hewitt, 1989).

Butterfly hybrid zones in the neotropics often seem to occur at rather major discontinuities: along major rivers, associated with low mountain ridges (though not usually on the tops of these ridges; see above, under Correlations of Hybrid Zones with Rainfall Patterns), at passes through high mountain ranges, and at or near the Isthmus of Panama (Fig. 9-1) (Brown, 1979; Lamas, 1982). Because we do not especially expect secondary contact along partial barriers to dispersal, these distributions imply that hybrid zones can move to areas that act as major partial barriers and are not trapped as easily by very minor local population density troughs as theory leads us to expect.

Correlations Between Clines Within Taxa. Multiple genetic changes within a single pair of hybridizing taxa have been used as evidence for secondary contact at hybrid zones (e.g., Hewitt, 1988, 1989). This within-species pattern can also be understood under a parapatric hypothesis because linkage disequilibria can build up and cause clines to coalesce (Slatkin, 1974; Key, 1981; Barton, 1983; Mallet and Barton, 1989a). Clines are not attracted to each other from any great distance; but if two clines are moving at different rates, a collision results; and the clines tend subsequently to stay together. Both habitat-dependent and habitat-independent clines can accumulate other clines, so that moving habitat-independent clines can become trapped at ecotones where stationary habitat-dependent clines are found. Similarly, clines trapped at a partial dispersal barrier cause other clines to accumulate, in addition to the effect of the density trough itself. Any epistatic interactions that have built up further enhance the effect of multiple clines. Once started, this process of accumulation results in multilocus hybrid zones and causes an increasingly strong barrier to gene flow and further cline movement, even without reinforcement by the evolution of mating barriers (Barton and Hewitt, 1989). Concordance of gene clines within a taxon therefore gives only weak evidence for secondary contact.

Habitat-dependent clines are also likely to occur together if environmental changes are steep (Fig. 9-2). For example, *Papilio glaucus* hybridizes with *P. canadensis* in the northeastern United States and Canada; clines for host plant adaptation, voltinism, and mimetic color pattern all exist in this hybrid zone (Hagen, 1990).

Reality of Subspecies. As well as being important for the interpretation of multiple-locus hybrid zones, theory and data on clines can justify the category of race or subspecies. Hybrid zones that have accumulated many clines cause severe barriers to gene flow, even though production of heterozygotes may be near Hardy-Weinberg. At neutral or habitat-dependent loci, or at loci with multiple stable equilibria, such hybrid zones separate races, which can evolve virtually independently (Barton, 1983). Only alleles that are advantageous everywhere easily penetrate the hybrid zones. Even less "congealed" hybrid zones, such as those in *Heliconius,* often separate races differing at more than one character or gene (Mallet, 1986a; Mallet et al., 1990). Wilson and Brown (1953) argued that subspecies could not be defined because characters involved in subspecies definitions were uncorrelated. We now have theory predicting the buildup of multilocus hybrid zones, as well as theory predicting that such hybrid zones may become major barriers to gene exchange (Barton, 1983). We also have data showing multiple character or multilocus changes across hybrid zones (Barton and Hewitt, 1983, 1989). When defined properly, subspecies are "real."

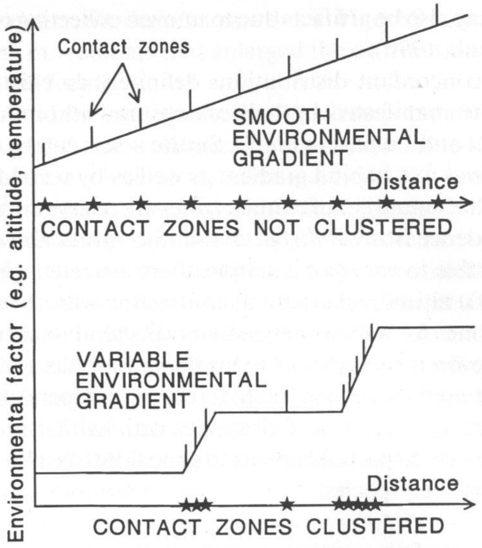

Figure 9-2. Reasons for independent contact zones becoming clumped. If differentiation is habitat-dependent, maps of contact zones in different taxa do not appear clumped, provided the habitat gradient is linear (upper portion of figure). More generally, there are geographically abrupt changes in habitat gradients, leading to an apparent clustering of contact zones on a map (lower portion of figure), even though each contact zone is associated with the same level of environmental parameter as before. For a similar reason, independent clines within a species also appear clumped.

Correlations of Hybrid Zones Between Taxa. Groups of hybrid zones forming a "suture zone" between two endemic regions have also been used as evidence for multiple secondary contact of distinct, allopatrically derived faunas (Remington, 1968). In some cases, evidence for suture zones is poor: For example, statistical tests failed to reveal concordant patterns of distribution in the birds studied by Haffer (1969), except that hybrid zones were often found along major rivers (Beven et al., 1984). Plant "centers of endemism" may also be artifacts due to uneven collecting coverage in the Amazon basin (Nelson et al., 1990).

In other cases, concordant distributions definitely do exist: Lamas and I have studied a suture zone manifested by heliconiines and ithomiines in the Huallaga region of Peru (Mallet and Lamas, in prep.). Suture zones can be explained by a common response of species to a habitat gradient as well as by secondary contact, and we have good evidence that a number of contact zones are responding to habitat gradients (for example, see evidence from *Heliconius* and ithomiines above). The steepness of habitat gradients is liable to vary (for example, there are relatively flat plains, valleys, and plateaus with little altitudinal gradient, contrasting with steep-sided mountains). Clines and contact zones for habitat-dependent traits tend to accumulate at the steep parts of an ecotone, even if each pair of hybridizing taxa has a different point on the gradient at which the contact zone is liable to occur, independently of all the others (Fig. 9-2). In addition, hybrid zones of all species with habitat-independent variation will accumulate on the same partial barriers to gene flow (see above), which can again cause concordance between species.

Patterns of Disjunction. One prediction of the shifting balance hypothesis for color pattern evolution is that new taxa should frequently originate in the center of the range of a species—a centrifugal pattern of speciation (W. L. Brown, 1957)—rather than a pattern that might be explained by peripheral isolation, with more derived taxa invading from the periphery (Mayr, 1963). K. S. Brown (1979, 1987b) has compiled examples of disjunct or nearly disjunct distributions that suggest "inundação" (inundation) of one race of ithomiine or heliconiine by another: He has interpreted them as movements of races occurring since refugia expanded and united. If racial redistributions were slightly more rapid than envisaged by Brown, however, distributions might become too blurred to make inferences about refugia. "Leapfrog" (i.e., disjunct) distribution patterns in parapatrically distributed neotropical birds (Remsen, 1984a) seem likely to be caused by derived taxa arising in the center of a distribution and displacing ancestral taxa to the peripheries. *Heliconius erato* (as well as its mimic *H. melpomene*) provides good examples of disjunct apparently primitive color patterns (Fig. 9-1) (see discussion by Sheppard et al., 1985). Phenotypes with red forewing bands and yellow hindwing bars are found in five disjunct locations: Central America and Mexico, the northern Magdalena Valley of Colombia, the upper Putumayo in Southern Colombia, the Mayo and upper Huallaga Valleys of Peru, and Bolivia to southeastern Brazil (Fig. 9-1, ● symbols). Western Colombia and western Ecuador also have red-banded races but with yellow hindwing bars expressed on the underside only (Fig. 9-1, filled and hollow ★ symbols). Forms with red bands but no yellow bar occur in northern South America, as well as in the Urubamba Valley of southern Peru (Fig. 9-1, ○ symbols). The races with yellow hindwing bars are separated from similar forms by other races, particularly by rayed phenotypes with yellow forewing bands. Hybrid zone phenotypes and some crosses suggest that the genetic basis of the color patterns in all races with red forewing bands and yellow hindwing bars is the same within each species (though different between species) (Sheppard et al., 1985; Mallet, 1989). The most parsimonious explanation for these disjunctions is that rayed races evolved in Amazonia and then expanded, isolating older unrayed patterns in the periphery of the distribution of *H. erato* (and *H. melpomene*). Although the initial evolution could have occurred in allopatry, the disjunct patterns in Figure 9-1 do at least suggest extensive past hybrid zone movements that enlarged the distributions of the rayed races at the expense of unrayed forms.

It seems possible to use cladistics to test between parapatric evolution involving moving clines and peripheral isolation. Under peripheral isolation, the peripheral morphologies would be evolutionarily advanced, and the central taxa would have more plesiomorphic morphologies. Unless parallel evolution is common, there should be few disjunctions between peripheral isolates. Under centrifugal evolution brought about by the shifting balance, the innovations would come from the center of the range, and the isolated valleys should have plesiomorphic patterns, which might very well show disjunction patterns. Unfortunately, mimetic color patterns are not ideal for testing between these hypotheses because of the tendency for color patterns to converge on those of other species, but the frequent disjunctions in the peripheries of *Heliconius* distributions tend to support the centrifugal pattern rather than peripheral isolation. Disjunctions in Andean birds (Remsen, 1984a) seem to provide similar evidence of centrifugal evolution.

Mimicry in Refugia. Turner (1982) is unusual among refugium biogeographers in agreeing that differentiation among taxa might easily occur in parapatry. Turner suggested instead that allopatric refugia would be important because of the effect of "faunal drift," where random extinctions affect biotic interactions in the remaining species. Different species of butterflies are expected to have gone extinct in each refugium, which would have caused frequencies of aposematic patterns to vary between refugia. Subsequently, mimicry rings in each refugium would diverge under the pressure of the differing model species.

One problem for this hypothesis is that there are now more racial mimetic patterns of butterflies than there are species mimicking each other (Benson, 1982). Some species, such as *H. erato* and *H. melpomene,* have more than 25 races, although they belong to local mimicry rings of only 2–10 heliconiine species (Brown, 1979). To explain the divergence of the current races of 10 species in the Amazon basin (*Eueides tales, Neruda aoede, H. burneyi, H. astraea, H. egeria, H. demeter, H. xanthocles, H. elevatus, H. erato,* and *H. melpomene*) by means of mimicry alone, we require about 10 refugia. In each of these refugia, one of the available 10 species must have become established; and, simultaneously, it must have gone extinct or become rare in the other refugia. Only with this unlikely scenario could about 10 patterns be produced with only 10 available species. This hypothesis would also entail that eight of these rayed species (all except *H. astraea* and *H. egeria*) secondarily recolonized and adapted to virtually every local mimicry ring formed in these 10 refugia.

Outside the Amazon basin, there are few extant model species to explain divergence in *H. erato* and *H. melpomene.* The model species that have been postulated for these extra-Amazonian races, such as *Pereute* (Pieridae) or *Altinote* (Acraeinae) (Sheppard et al., 1985) are unconvincing to me because the analogous mimetic similarity of the two *Heliconius* is so much greater than the resemblance of either *Heliconius* to any of the purported model species. In other cases, there are simply no available models.

How could so much divergence occur when there are so many fewer species than there are color patterns? One possibility is that many model species have gone extinct (Sheppard et al., 1985); although possible, this *ad hoc* hypothesis has no evidence.

Another possibility was suggested by Sheppard et al. (1985). New mimicry rings could form that combine components of older mimicry rings. However, this second explanation would still require separate mimicry rings to become more and more alike with every cycle of refugia. With no independent tendency to diverge, all mimicry rings would ultimately converge to a single pattern. The third possibility that must be considered is that an intraspecific process, such as the shifting balance, leading to truly novel color patterns, has caused geographic divergence of aposematic patterns in a common and unpalatable model. This divergence in a model species would then enforce divergence in the rest of the mimicry ring.

Mimicry has clearly been important in causing color pattern change of some mimic species, but it would be difficult to explain such rampant divergence on the basis of mimetic convergence alone. It seems likely that at least some model species must have diverged despite, rather than because of, Müllerian mimicry. As we have demonstrated, this kind of divergence would not require refugia at all.

One way to evaluate the effect of refugia on mimicry is to investiate the fauna of current refugia. Obervations in a tiny present-day wet forest refugium isolated by the Guajira desert in Colombia show that the butterfly fauna is indeed depauperate, but that *H. erato hydara* and the ithomiine species *Greta andromica* are little altered in their color patterns from populations in the forests hundreds of kilometers away (Knappett et al., 1976) despite the apparent extinction of all other heliconiines and ithomiines. Similar observations have been made by K. S. Brown on *H. erato phyllis* in the Serra Negra modern forest refuge in NE Brazil (Sheppard et al., 1985, p. 588). *H. erato* is a common species found in wet forest habitats as well as in scrubby vegetation such as that in the Guajira Mountains and gallery forests and other woodlands in currently dry savannahs (see above under Correlations Between Clines Within Taxa). The abundance of *H. erato* makes it a likely candidate to drive evolution in a noncoevolutionary mimicry ring (Bates, 1862; Müller, 1879, Eltringham, 1916; Gilbert, 1983), and the species apparently survives better than its co-mimics under poor conditions. Thus extinction is typically nonrandom (Benson, 1982, p. 632), and Turner's faunal drift does not occur. Once again, there seems to be evidence that divergence involved forces other than simple mimicry or the "faunal drift" refugium hypothesis. We need more observations from small refugia of this kind or from other areas of reduced species diversity such as the Guyana savannahs mentioned above. Under the faunal drift hypothesis, we would expect to see reduced numbers of species having divergent but mimetic color patterns in refugia; under parapatric, centrifugal divergence, there would be no reason to expect greater geographic divergence in modern refuges than in modern continuous rain forest.

Correlations Between Cladograms of Different Taxa. One proposed method for distinguishing parapatric from allopatric divergence is to compare the cladistics of groups of overlapping taxa in an area. Cladograms that are geographically concordant across groups would demand a common explanation (Cracraft and Prum, 1988); in Amazonian birds, it might indicate a common set of origins in Pleistocene refugia. Two criticisms of this viewpoint can be raised. First, we expect related forms to inhabit ecologically similar sites, provided some of the divergence is habitat-dependent: concordant cladograms might indicate similar environments rather than common patterns of vicariance. Second, testing for concordance between cladograms is still in its infancy. What few data we have seem to indicate rather little concordance. Cracraft and Prum (1988) chose the Amazonian bird taxa they analyzed because these taxa appeared to give similar cladograms, but they admitted that cladograms of excluded groups appeared to differ, suggesting that a variety of histories may have caused divergence in the Amazon basin. This fallacy of proposing a common historical explanation on the basis of selected concordant data has been pointed out by Page (1988).

The use of phylogenies of molecular markers within a species is rather more convincing evidence for secondary contact because the markers are likely to be nearly neutral (Rand and Harrison, 1989). However, once a hybrid zone containing a number of loci has formed, a partial barrier to gene flow is erected that excludes neutral, habitat-dependent, or habitat-independent genes much more effectively than genes advantageous everywhere (Barton, 1983). Thus these loci can evolve independently after hybrid zone formation. On the other hand, there seems to be strong evidence for some sort of secondary contact between patches when there is concordance across molecular markers in "mosaic hybrid zones" (Rand and Harrison, 1989), provided the markers are entirely neutral. However, these mosaics do not prove that the race originated in allopatry, only that isolated patches in the mosaic originated via dispersal to the appropriate area. For *Heliconius* or other neotropical organisms, molecular work remains to be done.

Cladistic Evidence for Secondary Contact. Hybrid zones between unrelated taxa can also be used to indicate "secondary contact" (Thorpe, 1984; Nixon and Wheeler, 1990). However, this type of secondary contact does not necessarily indicate that differences evolved in allopatry. Suppose a race of *Heliconius* in the western Amazon basin adapts to montane conditions. Its novel adaptations, evolved in parapatry, may then enable it to spread up and down the Andean chain, bringing it into "secondary contact" with other races of its species, even though there could have been continual parapatric contact via other races in such a "ring" species.

Spatial Scale of Differentiation. Different groups of forest organisms show different scales of divergence. For example, *Heliconius* races are more finely divided than the birds studied by Haffer, and races of small ithomiine butterflies much more finely divided than those of *Heliconius* (Brown, 1979). Presumed warning color patterns in some *Dendrobates* frogs have even narrower ranges than those of the ithomiines; Rainer Schulte (pers. comm.), a resident of Tarapoto, Peru, has found that *Dendrobates bassleri* patterns diverge on either side of the Río Mayo, and that there are strong local differences even within the mountains to the northeast of Tarapoto. *Heliconius erato* and *H. melpomene* are completely monomorphic throughout this area. Silverstone (1975) reported similar localized color pattern variation in *Dendrobates histrionicus* and *D. quinquevittatus.* The differences in the scales of divergence between major groups suggest that the characteristic dispersal distances of a species, rather than gross forest changes, are chiefly responsible for divergence between its forms or races.

Direct Historical Evidence. Some differentiation seems to have occurred more recently than the last glaciation. The Ilha de Marajó on the mouth of the Amazon was probably completely inundated about 5000 years ago, and yet unusual forms of *Eueides, Heliconius, Tithorea,* and *Napeogenes* now appear there (Brown, 1979). This phenomenon is especially surprising because the Marajó has a small land area compared with other lowland Amazonian areas containing endemic races of these butterflies.

There are one or two reports of rapid historically documented movement by butterfly contact zones or geographic races, suggesting that neotropical butterfly biogeography is dynamic even on a 100-year time scale. For example, during the building of the Panama Canal during the early twentieth century, *Heliconius hecale* in the canal zone was polymorphic and included mimetic forms with white-spotted forewings. This polymorphism has been interpreted as a hybrid zone between the Colombian race *H. hecale melicerta* and the Central American *H. h. zuleika* (Brown, 1976a). The canal zone is today monomorphic for *H. h. melicerta,* and Gordon B. Small (pers. comm.) has found a hybrid zone between *H. h. zuleika* and *H. h. melicerta,* including white-spotted forms, about 100 km to the west of the canal zone. There is a tantalizing possible example of the swamping of a race of *H. erato* in eastern Peru. Red-banded *H. erato amphitrite* and *H. melpomene euryades* (Fig. 9-1; O symbols) have been collected on the Ríos Urubamba, Cosñipata, and Inambari (Lamas, 1976) around the turn of the century. Today, only yellow-banded rayed *erato* and *melpomene* are found in the latter two valleys, and red-banded forms are present only in the Río Urubamba. Unfortunately, we cannot be sure of the accuracy of the label data of the earlier Peruvian specimens. It is well worth carefully mapping as many neotropical hybrid zones as possible, so that future generations can detect potential movement 50–100 years from now. Similar studies performed in birds seem to indicate similarly rapid evolution of races and species (e.g., Remsen, 1984b).

CONCLUSIONS AND IMPLICATIONS FOR CONSERVATION

I have attempted to show how knowledge of the traits under selection in hybrid zones enables one to make more detailed models of divergence, and how an understanding of divergence can provide information about the geographical circumstances, whether allopatric or parapatric, in which divergence occurs. Warning color is a particularly useful tool for this work because it provides a visual model of partial reproductive isolation due to the strong selection in hybrid zones. For neotropical Lepidoptera, warning color changes seem to be associated strongly with various levels of speciation, and it is not easy to exclude parapatric differentiation. For color patterns, habitat-dependent associations such as mimicry and climatic adaptation seem important; but there seems a clear possible role for the shifting balance in generating utterly new patterns. Neither of these modes of divergence requires allopatry.

Although I have dealt only with neotropical butterflies, similar arguments could be applied to other taxa, including taxa in the temperate zone. Some work in Europe seems definitely to implicate secondary contact as a reason for the current positions of hybrid zones. In *Bombina* toads genetic differences are great, implying divergence so old that known glaciations must have repeatedly separated currently hybridizing taxa (Szymura and Barton, 1986, 1991; see Ch. 10). For *Podisma,* a flightless grasshopper, low levels of dispersal ($\sigma \approx 20$ meters) imply slow potential cline movement and a great tendency for clines to become stuck on minor density troughs. Population restructuring caused by glaciation is thus likely to be faster than inherent tendencies of *Posidma* clines to move. The hypothesis of secondary contact is further enhanced by the position of the *Podisma* hybrid zone on the ridge of the Alpes Maritimes, where retreating ice would be expected to leave it (Nichols and Hewitt, 1988; Hewitt, 1989). It is unclear, however, whether these arguments apply to less well studied systems, such as bird hybrid zones in the Great Plains of North America (Rising, 1983; see Ch. 8). As with the neotropics, I suggest that much, perhaps most, of the divergence ascribed to refugia in the temperate zone should more be carefully tested against alternative hypotheses before a causative role of refugia can be contemplated, however likely the existence of actual refugia during Pleistocene glaciation.

Currently, refugium theory is being used by conservation authorities in Brazil, Peru, Venezuela, and other countries in the neotropics (Brown, 1979; Myers, 1982; Brown and Fernandez, 1985; Rylands, 1990). One of the aims of this chapter is to cast doubt on the role of refugia in causing divergence because I believe the hypothesis is weakly supported at best. In our current state of ignorance, a naive approach to conservation might be optimal: Conserve areas with interesting species or high species diversity, regardless of the possibility that these areas were or were not in Pleistocene refugia. Species are a far less renewable resource than theories about their evolution.

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10

Analysis of Hybrid Zones with *Bombina*

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The study of fire-bellied toads in Europe has a long history, and for much of it debate has centered around whether the two species, *Bombina bombina* and *B. variegata,* hybridize in nature. The existence of individuals with intermediate morphology in areas where the ranges of *B. bombina* and *B. variegata* meet (overlap) was explained by invoking either hybridization (Méhely, 1892; Karaman, 1922; Mertens, 1928) or response to local environmental conditions. Artificial hybrids could be obtained in the laboratory, and heterospecific pairs in amplexus were observed in the field (Michałowski, 1958), but fertility of hybrids was unknown and morphology of F_1 hybrids did not resemble most intermediate forms found in nature (Michałowski and Madej, 1969). Spotting pattern and coloration of the belly, although characteristic for each of the forms, are variable and difficult to describe, making simple morphological analysis of presumed hybridization inconclusive. Furthermore, the lowland and the mountain form of *Bombina* have distinct mating calls (Lörcher, 1969), which traditionally have been regarded as effective barriers to gene exchange in many amphibian species. Even more confusing, intermediate morphologies are also found far from the area of contact, and some association between habitat type and morphology has been observed within the species' overlap. Therefore until recently evidence for hybridization in the fire-bellied toads was ambiguous.

With the advent of molecular techniques, which allowed classification of individuals into clearly defined genotypic classes, the question of hybridization could be answered. Application of protein electrophoresis demonstrated that the fire-bellied toads *B. bombina* and *B. variegata* do interbreed west of Cracow (Szymura, 1976) in a morphological transition described earlier by Michałowski (1958). The same study revealed that there is a genuine transition from *bombina* to *variegata* alleles over just 6 km near Cracow rather than patchy, localized hybridization. All enzymes showed a similar pattern. Central populations consisted almost exclusively of individuals of mixed ancestry, but F_1's were rare, if present at all. This finding is not surprising if pure parental types have little opportunity to meet. Hybrids appeared to be fertile because a whole array of backcross and recombinant genotypes was revealed. Similarly, mating appeared to be random because the genotypic composition of each population could be derived from allelic frequencies. Location of the zone did not seem to be associated