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Mimicry and Warning Color at the Boundary between Races and Species

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Can Mimicry Cause Speciation?

Mimicry in butterflies is well known chiefly because it gives an easily understood visual example of adaptation. However, the major thrust of Bates's original paper "Contributions to an Insect Fauna of the Amazon Valley" (1862) concerned systematics and the origin of species, rather than merely mimicry. Bates felt that mimicry in ithomiine, heliconiine, and dismorphiine butterflies exemplified the continuum of geographic divergence and speciation by natural selection: "It is only by the study of variable species that we can obtain a clue to the explanation of the rest. But such species must be studied in nature, and with strict reference to the *geographic relations* of their varieties" (pp. 501–2). The geographic pattern Bates discovered was extraordinary: not only were there resemblances between species within any one area of the Amazon basin, but also the mimetic color patterns themselves changed every 100–200 miles. Repeated resemblances in different areas, using different color patterns, provided the highly convincing comparative evidence that clinched Bates's hypothesis of mimicry (see appendix 30.1 for discussion of Batesian vs. Müllerian mimicry). On top of this geographic divergence, closely related species within an area often belonged to different mimicry "rings" (see also Papageorgis 1975; Turner 1976; Mallet and Gilbert 1995). Bates's system had all the intermediate stages between local varieties, geographic races, and sympatric species.

Darwin (1863) wrote a glowing review of Bates's paper: "It is hardly an exaggeration to say, that whilst reading and reflecting on the various facts given in this memoir, we feel to be as near witnesses, as we can ever hope to be, of the creation of new species on this earth" (p. 92). Today, it may seem strange to propose that mimicry, normally viewed as an adaptation within species, should trigger speciation. On reflection, this idea is not so strange. "Isolating mechanisms" are divergently selected traits that must evolve initially within species, and reproductive isolation is usually, perhaps, a pleiotropic effect of environmental or genomic adaptation rather than directly selected. Selection for any ecological adaptation, such as mimicry, as well as any change directly promoting hybrid inviability, sterility, or mate choice, may instigate speciation.

Mimicry and warning color are in fact particularly good examples of traits that could maintain the separateness of species (i.e., "postmating isolating mechanisms"). Once a warning color pattern becomes abundant, the local predator community learns to avoid it. This favors the common pattern and causes frequency-dependent selection against rarer patterns. Rare hybrids and recombinants between divergent color patterns are not recognized as unpalatable and will form an adaptive trough between two adaptive peaks. A major difference between mimicry and traits more normally associated with speciation is of course that the agent of mimetic selection is ecological (predation) whereas postmating isolation is often genomic and independent of the environment. But, in terms of overall fitness, warning color selection and classical genomic incompatibility have much in common. Mimicry might therefore be an important cause of speciation in mimetic organisms.

We here explore selection at the boundary between geographic varieties and species in butterflies of the genus *Heliconius*. Armed with a criterion for species status, we ask which traits diverge before and after this criterion is reached. We use our data to test the idea put forward by Henry Walter Bates that mimicry and other ecological factors are important in speciation.

The Boundary between Races and Species

To study speciation, it helps to clarify what species are. Darwin and Wallace traveled widely and developed clear ideas of the continuum between geographic races and species: "independently of blending from intercrossing, the complete absence, in a well-investigated region, of varieties linking together any two closely-allied forms, is probably the most important of all the criterions of their specific distinctness" (Darwin 1871, pp. 214–5). Bates, like his traveling companion Wallace (1865), agreed with Darwin's view that species and geographic races formed a continuum, rather than conveniently delimiting themselves for us because of fundamental or essential differences: "distinct forms or species do not essentially differ from the undoubted varieties of the species cited" (Bates 1862, p. 501). A species differed from a race only in that it could coexist in sympatry without losing its integrity: "The new species cannot be proved to be established as such, unless it be found in company with a sister form which has had a similar origin, and maintaining itself perfectly distinct from it" (p. 530). By concentrating on the resultant distinctness rather than on mechanisms for its maintenance, Bates used a nonessentialist criterion of species (see appendix 30.2 for a discussion of essentialism in modern species concepts).

In practice, taxonomists still use something close to this operational criterion. This approach is often derided as the "morphological species concept" (e.g., Mayr 1982). But, after adding Mendelian genetics to this Darwinian cluster criterion, species can be seen as separate "genotypic clusters" that can coexist in sympatry (Mallet 1995a, 1996a,b; see also Feder, this volume). Genotypic clusters can be investigated in a local population sample (Darwin's "well-investigated region") characterized for a number of independent loci or independently inherited characters. If a single sample contains, at four recombining loci *A*, *B*, *C*, and *D*, one *AaBBCCDd*, 50 *AABBCCDD*, and 50 *aabbccdd* genotypes, it is clear that there are two major genotypic clusters, plus a single individual intermediate between these clusters. Darwin's criterion implies two separate species. On the other hand, if a single population with the same overall allele frequencies gives mostly *AABbCcDD*, *AaBBCcDd*, *AABbCcDd*, and other intermediate forms, the evidence points to a single polymorphic species. Geographic variation across a species range will also, of course, give rise to different genotypic clusters in different areas, but the forms are viewed as geographic races if intermediates predominate in areas of overlap. A genotypic cluster definition makes reference neither to past or future evolution, nor to inferred phylogeny, nor to processes by which clusters are maintained (while not denying their existence); these are left open as interesting areas for investigation, since they form material for the study of speciation. Speciation then simply becomes the evolution of the tendency for genotypic clusters to coexist as recognizably distinct forms where they overlap. Recognizability might involve morphology, behavior, physiology, or biochemistry, as long as these are multiple, independently inherited traits. Some readers may have philosophical grounds for disagreeing with this and may attempt to define species using the "underlying essence" of species that Darwin, Wallace, and Bates were so keen to avoid (appendix 30.2; see also chapters by de Queiroz, Harrison, Shaw, and Templeton, this volume). However, the explicitness and simplicity of a genotypic cluster criterion allow clear questions to be posed about the causes of speciation.

Bates believed (and Darwin agreed) that there was evidence for causes of speciation in the continuum between varieties and species of mimetic butterflies. In addition to mimicry, adaptation to abiotic conditions played a role in geographic divergence: "The selecting agent, which acts in each locality by destroying the variations unsuitable to the locality, would not in these cases be the same as in *Leptalis* [i.e. mimicry]; it may act, for anything we know, on the larvae; in other respects, however, the same law of nature appears, namely, the selection of one or two distinct varieties by the elimination of intermediate gradations" (Bates 1862, p. 514). Bates also hypothesized that mate preferences were important in speciation:

> The process of the creation of a new species I believe to be accelerated in the *Ithomiae* and allied genera by the strong tendency of the insects, when pairing, to select none but their exact counterparts: this also enables a number of very closely allied ones to exist together, or the representative forms to live side by side on the confines of their areas, without amalgamating. (p. 501)

Bates had only scattered field evidence (and the systematics of these butterflies was then very crude) for his assertion about the causes of speciation, but we show in this chapter that he was largely correct for the *Heliconius* we have studied. As Bates suggested, assortative mating, coupled with divergence in warning color and ecology, triggers speciation.

Geographic Diversity of *Heliconius* Races

Heliconius are renowned not only for mimicry, but also for geographic diversity of mimetic patterns within species. In *Heliconius erato* and its Müllerian comimic *H. melpomene* this "subspeciation" has reached a feverish pitch, with 28 subspecies recognized in *erato* and 29 in *melpomene* across Central and South America (Brown 1979). Subspecies in *Heliconius* are usually monomorphic across their range and are separated from other subspecies by more or less narrow hybrid zones. A few hybrid zones are up to 200 km wide between races that differ only slightly in color pattern (e.g., Brown and Mielke 1972), and some Amazonian forms of *erato* and *mel-*

pomene have such wide bands of polymorphism that the "pure" races form little more than the ends of broad clines (Brown et al. 1974; Brown 1979). More normally, hybrid zones between forms that differ at one or two major pattern elements are about 20–80 km wide (Turner 1971a; Benson 1982; Mallet 1986); those between subspecies that differ at three or more major color pattern elements may be as little as 10 km wide (figure 30.1; Brown and Mielke 1972; Mallet et al. 1990).

Heliconius subspecies are unusual, perhaps, in that they are what they seem: they differ chiefly at the very traits we use for identification, their color pattern. For example, subspecies differ very little at allozyme loci, and Nei's genetic distances between races vary between $D \approx 0.01$ and 0.05 (Turner et al. 1979; Jiggins et al. 1997a). Mitochondrial DNA (mtDNA) studies reveal two major clades within *H. erato* that diverged about 1.5–2 million years ago (Brower 1994a), but within each clade there is little patterning of the mtDNA genealogy with respect to color pattern races. Closely related mtDNA sequences are as likely to be found thousands of miles apart as in the same race (Brower 1994a, 1996a). Even major color pattern differences are inherited at a mere handful of loci (Sheppard et al. 1985; Mallet 1989; Jiggins et al. 1996; Jiggins and McMillan 1997). The ecologies of races are similar within each species; they are all denizens of sunny areas such as river edges, major tree falls, and man-made second growth. *Heliconius erato* lays eggs mainly on species of *Passiflora* in the subgenus *Plectostemma*, while its mimic *melpomene* specializes on subgenus *Granadilla* (Benson et al. 1976; Smiley 1978; Benson 1978). Some *erato* races do have minor differences in ecology or host plants (Benson 1978, 1982), but the differences are much greater between species.

It is not yet clear how geographic differentiation evolved in the face of stabilizing selection on warning color. One theory supposes that color patterns diverged in forest refuges formed during glacial maxima (Brown et al. 1974; Brown 1979, 1982, 1987; Turner 1971b, 1981, 1982), but geographic isolation would not be required if there were direct adaptation of particular color patterns to local mimetic (Turner 1982) or abiotic environments (Bates 1862; Benson 1982; Endler 1982) or if differentiation was initiated by genetic drift (Mallet 1986, 1993). These alternative geographic scenarios are the subject of much current argument (Mallet 1993; Brower 1996a; Turner and Mallet 1996; Mallet et al. 1996; Mallet and Turner 1998).

Selection in a Hybrid Zone between Races of *Heliconius erato*

More important for understanding the evolution of new species, hybrid zones between subspecies give information on the nature and strength of selection acting on geographically varying traits (Hewitt 1988; Barton and Hewitt 1989; Harrison 1990). The width, w, of a cline in allele frequency at a single locus (w = [maximum gradient of the cline]$^{-1}$) at equilibrium will be proportional to a ratio of migration and selection: $w \approx K\sigma/\sqrt{s}$, where σ is the dispersal distance, s is the selection acting on the cline, and K is a constant depending on the type of selection (Barton and Gale 1993). In *Heliconius*, frequency-dependent predation will remove rare color pattern variants on either side of the hybrid zone, giving $K \approx \sqrt{8}$ for a dominant gene and $K \approx \sqrt{12}$ for a codominant gene (Mallet and Barton 1989a).

To estimate selection, data were gathered from the hybrid zone between *Heliconius erato* races near Pongo de Cainarache, Peru. Across this zone, one codominant (D^{Ry}) and two dominant (Cr, Sd) unlinked loci determine the major color pattern differences, and we can therefore compare the theoretical prediction of cline width with field observations (figure 30.2; Mallet et al. 1990). The cline at

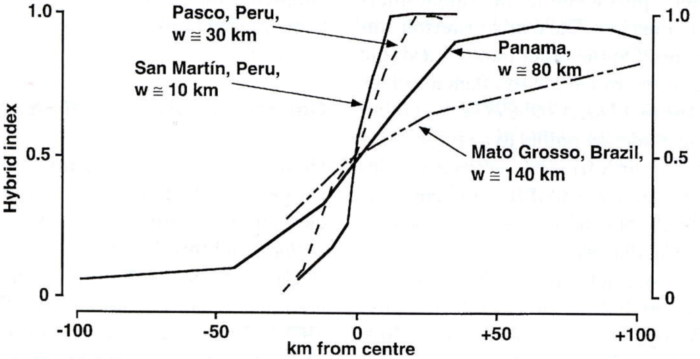

Figure 30.1. Hybrid zones between races of *Heliconius erato*. Each line represents an average "hybrid index" score based on one to four color pattern loci or characters between different races. Sources: Mato Grosso, Brazil (Brown and Mielke 1972); Panama (Mallet 1986); Pasco, Peru (Mallet, unpublished); and Pongo de Cainarache, San Martín, Peru (Mallet et al. 1990).

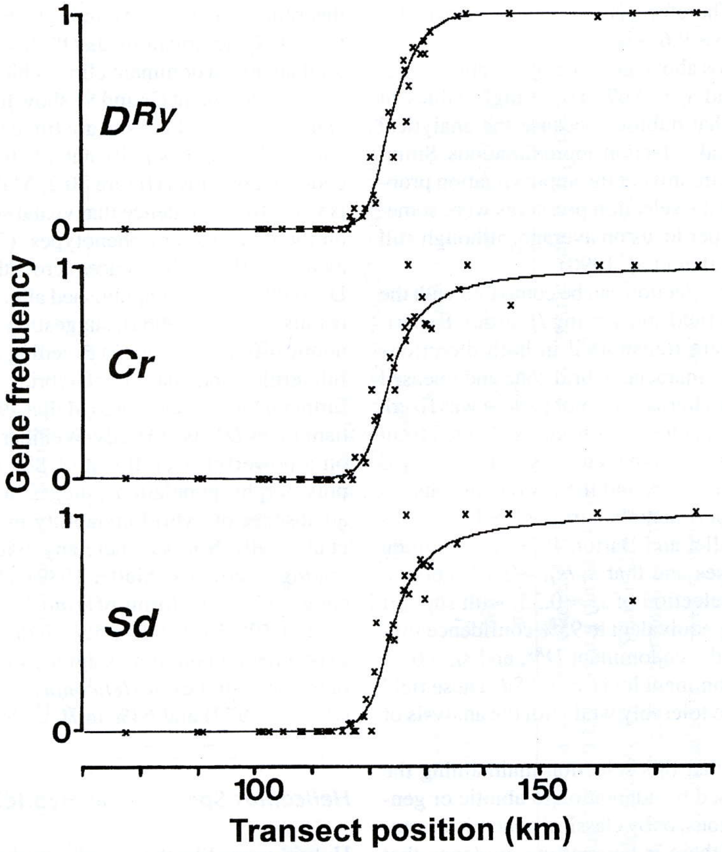

Figure 30.2. Clines making up the hybrid zone between *H. erato favorinus* (left) and *H. erato emma* (right) centered at Pongo de Cainarache, Peru. Top, symmetric cline at codominant locus D^{Ry}. The D^{Ry} allele adds rays and changes red band to yellow, and the cline shape fits the codominant model much better than the equivalent asymmetric recessive or dominant models ($P < 0.001$ in both cases). Middle, cline at dominant locus *Cr*, showing a long tail of introgression to the right of the hybrid zone, where the recessive allele escapes detection. In contrast, there is virtually no introgression of the highly visible dominant allele to the left of the hybrid zone. The recessive *cr* allele adds a yellow hindwing bar. Bottom, cline at dominant locus *Sd*. Again, there is a long tail of introgression to the right of the hybrid zone, and no tail to the left, as expected for a dominant gene. The *Sd* allele changes the shape of the forewing band from broad to narrow, and interacts with *Cr* to remove the full yellow hindwing bar. Both dominant loci fit asymmetric dominant clines significantly better than symmetric codominant clines ($P < 0.001$ and $P < 0.005$, respectively). The theoretically derived curves were fitted using likelihood to field data based on 1,572 individuals caught at 53 field sites (reprinted with permission from Mallet et al. 1990).

D^{Ry} is about 8.5 km wide, and the *Cr* and *Sd* clines are about 10.2 km wide (figure 30.2). This gives estimated ratios $\sigma/\sqrt{s_C} \approx 2.45$ km for the codominant D^{Ry}, and $\sigma/\sqrt{s_D} \approx 3.61$ km for the dominant *Cr* and *Sd*, so the ratio $s_C/s_D \approx 2.17$. In words, the codominant locus D^{Ry} is about twice as heavily selected (this is expected, since it has a greater phenotypic effect) as the two dominant loci *Cr* and *Sd*. If we knew the migration rate, σ, we would be able to estimate the absolute strength of selection, *s*, or vice versa.

The absolute strength of selection can be estimated indirectly, using gametic correlations (Barton and Gale 1993), or via direct experiments in the field. The first method is possible because migration across hybrid zones of a given width creates gametic correlations (also known as linkage disequilibria) between loci. Pairwise correlations between two genes *A* and *B* in the center of a hybrid zone are expected to be $R_{AB} \approx 4\sigma^2/c_{AB}w_Aw_B$ at equilibrium between selection and migration, where c_{AB} is the recombination fraction between loci (Barton and Gale 1993). In *H. erato* the correlations between color pattern loci ($R \approx 0.35$) and average cline width ($w \approx 9.6$ km) are known, and all three loci are unlinked ($c = 0.5$;

Mallet et al. 1990). Therefore, an estimate of dispersal is $\sigma \approx \sqrt{0.35 \times 0.5 \times 9.6 \times 9.6 \div 4} \approx 2.0$ km. Using the migration/dispersal ratios above gives per generation selection of $s_D \approx 0.31$, and $s_c \approx 0.67$. These high values of selection are somewhat dubious because the analytical theory depends on weak selection approximations. Simulations were used to circumvent the approximation problem, and suggest that the selection pressures were somewhat lower, $s \approx 0.23$ per locus on average, although still relatively intense (Mallet et al. 1990).

These estimates of selection can be compared with the results of a laborious field study using *H. erato*. Experimental butterflies were transported in both directions across the Pongo de Cainarache hybrid zone and released in four sites where the alternative color pattern was fixed. Controls with the local color pattern were collected from similar distances away from the release site and released alongside the experimentals, and their life expectancies were compared. Experimentals survived only 48% as long as controls (Mallet and Barton 1989b). Assuming multiplicative fitnesses and that $s_C/s_D \approx 2.17$ (above), we find a per locus selection of $s_C \approx 0.33$, with support limits (approximately equivalent to 95% confidence limits) of 0.14–0.51 for the codominant D^{Ry}, and $s_D \approx 0.15$ (0.07–0.23) for the dominant loci *Cr* and *Sd*. These field results therefore agree tolerably well with the analysis of gametic correlations.

It is conceivable that this selection maintaining the hybrid zone was caused by adaptation to abiotic or general ecological conditions, or by classical genomic incompatibility. However, there is compelling evidence that the strong selection was due mainly to warning color: (1) Beak marks of jacamars (*Galbula* spp., a bird that specializes on butterflies and other large insects) and other birds were found on the butterflies. More wing damage was found on experimentals than controls, showing that experimental butterflies were attacked, and suggesting (though not proving) a greater attack rate on foreign patterns. The strongest survival differences were found where jacamars were commonest (Mallet and Barton 1989b). (2) All three *erato* color pattern loci switch together in Pongo de Cainarache (figure 30.2). (3) The equivalent *melpomene* hybrid zone is also at an almost identical position, and disequilibrium-based estimates of selection ($s \approx 0.25$) are very similar to those in *erato*. The conjunction is expected since the two species have nearly identical patterns and should be under similar selection (Mallet et al. 1990). (4) Visual dominance of the pattern genes within *erato* correctly predicts the shapes of the clines. Genetic dominance should cause cline asymmetry because rare recessive alleles on the dominant side of the cline are mostly found as heterozygotes with the dominant phenotype, resulting in low selection and a long shallow "tail" of allele frequency on that side; rare dominants on the recessive side of the cline, on the other hand, are highly detectable, and will be strongly selected against, resulting in a steep gradient and no tail. As expected, a theoretical codominant cline, which is symmetric, fits the visually codominant locus D^{Ry} highly significantly better than does a dominant cline, which is asymmetric. The visually dominant *Cr* and *Sd* show long tails on the dominant side of the cline, and are fitted by asymmetric dominant clines highly significantly better than by symmetric codominant clines (figure 30.2; Mallet et al. 1990). This is very strong evidence that visual selection acts directly on the color pattern phenotypes. (5) There are no allozyme or mtDNA differences across the hybrid zone (Nei's D ≈ 0.001, Mallet, unpublished allozyme results; mtDNA results, Brower 1996a), suggesting a general lack of genomic differentiation. (6) Breeding experiments indicate full fertility and viability of hybrids, backcrosses, F2, and further hybrid generations (Mallet 1989). (7) The codominant locus D^{Ry} is at Hardy-Weinberg equilibrium, based on a powerful overall test of 883 individuals from 14 polymorphic populations, suggesting random mating and an absence of hybrid inviability in the field (see Mallet et al. 1990). Nor was there any evidence for assortative mating in crosses (Mallet 1989). (8) Ecology and host choice of the two forms of *erato* do not differ (Mallet and Barton 1989b). (9) The 52% reduction in longevity of experimental nonmimics agrees well with that found in other field studies of *Heliconius* mimicry: 22% in *erato* (Benson 1972) and 64% in *H. cydno* (Kapan 1998).

Heliconius Species That Hybridize

Hybrid zones like those within *erato* or *melpomene* have abundant hybrids, so the races have demonstrably not speciated. However, if hybrids are rare enough in areas of overlap, separate clusters of genotypes may be recognized. Using the existence of recognizable genotypic clusters as the criterion for the transition to species, comparisons between these two types of hybridization can lead to an understanding of speciation.

Natural interspecific hybrids are known between 25–28% of all *Heliconius* species (table 30.1). This is higher than the known fraction of hybridizing species among the world's birds (9% of species; see Grant and Grant 1992) or European butterflies (12%; see Guillaumin and Descimon 1976). However, American warblers (Parulinae) are known to hybridize almost as frequently (24% of species), perhaps because hybrids are readily noticed in this brightly colored subfamily (Curson et al. 1994). *Heliconius* are not only brightly colored; their hybrids are also highly prized by collectors.

Hybrid Zones between *H. erato* and *H. himera*

Although species that hybridize are common, hybrid individuals are very rare in comparison to their parent species. In *Heliconius*, hybrids between the sympatric

Table 30.1. Natural and laboratory hybridization between species of *Heliconius*.

Species 1	Species 2	Source[1]	Natural Hybrids[2]	Geographic Relationships	Laboratory Hybrids[2]	Assortative Mating[3]	Inviability or Sterility
numata	*melpomene*	2,6,9	+	Sympatric	–	(+)	?
ethilla	*melpomene*	2,9	+	Sympatric	–	(+)	?
ethilla	*numata*	1	+	Sympatric	–	(+)	?
ethilla	*heurippa*	1,9	+	Sympatric	–	(+)	?
ethilla	*besckei*	6	+	Sympatric	–	(+)	?
cydno	*melpomene*	2–6,9	+++	Sympatric	+++	++	Hybrid males are viable and fertile. F1 females are viable but sterile
heurippa	*melpomene*	4	+	Sympatric	–	(+)	?
pachinus	*cydno*	6,8	+	Parapatric	+++	+	All hybrids viable and fertile
ismenius	*cydno*	8	–	Sympatric	++	+++	Hybrids viable, F1 females sterile, F1 males have mechanical difficulties during mating
hecale	*melpomene*	8	–	Sympatric	+	+++	? (Only one laboratory hybrid known)
clysonymus	*hecalesia*	8,9	+	Sympatric	–	(+)	?
himera	*erato*	7,9	+++	Parapatric	+++	+	No detectable inviability/sterility; F1 males may have reduced mating propensity in one direction of cross

Hybrids between 11 species, or 28% of the 39 *Heliconius* species (*sensu* Brown 1979), have been collected. If we combine all allopatric *cydno* group species (including *pachinus*, *heurippa*, *timareta*, and *tristero*) into *cydno*, this reduces the number to 9 hybridizing out of 34 total species, or 25%.

[1] Sources: 1. Brown (1976); 2. Ackery and Smiles (1976); 3. Brown and Fernandez Yepez (1985); 4. Salazar (1993); 5. Posla-Fuentes (1993); 6. Holzinger and Holzinger (1994); 7. Jiggins et al. (1996); 8. L. E. Gilbert (personal communication); 9. W. Neukirchen (personal communicatiion).

[2] Frequency of hybrids: – = not known; + = 1–3 specimens known, ++ = 4–10 individuals known, +++ = over 10 individuals known.

[3] Assortive mating: +++ = extremely strongly assortative (<1% of trials when both sexes of both species kept together in laboratory); ++ = strongly assortive (~1% of trials), + = assortative (~10% of trials), (+) = assumed assortative; field hybrids are very rare.

species *H. cydno* and *H. melpomene* have been among the most regularly collected (table 30.1), but even they must occur at frequencies of ≤ 0.1% in nature compared with the pure forms. Hybrids between the parapatric *H. erato* and *H. himera* are much commoner, but contact zones are so narrow that they were discovered only within the last 20 years. Three *erato* races are known to abut with *himera*. In all three contacts, hybridization is infrequent but regular, and hybrids form about 10% of the population in an Ecuadorean overlap (figure 30.3; Descimon and Mast de Maeght 1984; Jiggins et al. 1996; Mallet et al. 1998). Because most butterflies in this hybrid zone population are parentals, even at cryptic allozyme loci (figure 30.3; Jiggins et al. 1997a), two genotypic clusters, species under our definition, are being maintained. All three contact zones correspond precisely with a transition from wet forest with *erato* to dry thorn scrub with *himera* (Mallet 1993; Jiggins et al. 1996). These hybrid zones are extremely narrow, $w \approx 5$ km, narrower than any known between *erato* races (figure 30.4), which implies very strong selection, $s \approx 1$ per locus (Jiggins et al. 1996).

Heliconius himera is similar to an ordinary geographic race of *erato* in many respects. It replaces *erato* in dry Andean valleys of southern Ecuador and northern Peru. Like *erato* races, *himera* differs from other *erato* in warning color pattern, and these pattern differences are controlled by genes homologous to those differing between races of *erato* (Jiggins et al. 1996; Jiggins and McMillan 1997). *Heliconius himera* feeds on the same *Passiflora* host plants as *erato* (Jiggins et al. 1996, 1997b), and differs in ecology only in its drier habitats and in lacking close mimics; *erato* is typically found in wetter forest and is usually accompanied by its Müllerian comimic *melpomene* (Jiggins et al. 1996). Although *himera* wing and body shape is similar to that of *erato*, *himera* differs strongly from *erato* at allozymes: Nei's D ≈ 0.28 due to nearly fixed or strong frequency differences at 11 of 30 allozyme loci studied (Jiggins et al. 1997a). Their mtDNA sequences are also very different, suggesting about 1.5–2 million years of separation, although this level of differentiation is similar to that found across the Andes within *erato* (Brower 1994a, 1996a; Jiggins et al. 1997a).

These hybrid zones provide an ideal opportunity to investigate what maintains the distinctness of newly formed *Heliconius* species. We have performed extensive laboratory tests to determine the causes of the hybrid deficit in the contact zone between *H. erato cyrbia* and *H. himera* near Loja in southern Ecuador. The rarity of hybrids is partly explained by assortative mating: interspecific matings are only 11% (5–22%) as probable as intraspecific matings in laboratory choice experiments (McMillan et al. 1997); in the field, a similar value of 6% (0.3–27%) was found (Mallet et al. 1998). However, our laboratory crosses, which produced a total of 4,570 eggs and nearly 2,500 adult butterflies, showed no evidence for inviability or sterility of hybrids compared with parentals in terms of numbers of eggs laid per day, egg hatch, larval survival, developmental time, or sex ratio (table 30.2).

The narrowness of the hybrid zone, and the near-complete association between allozymes, mtDNA, envi-

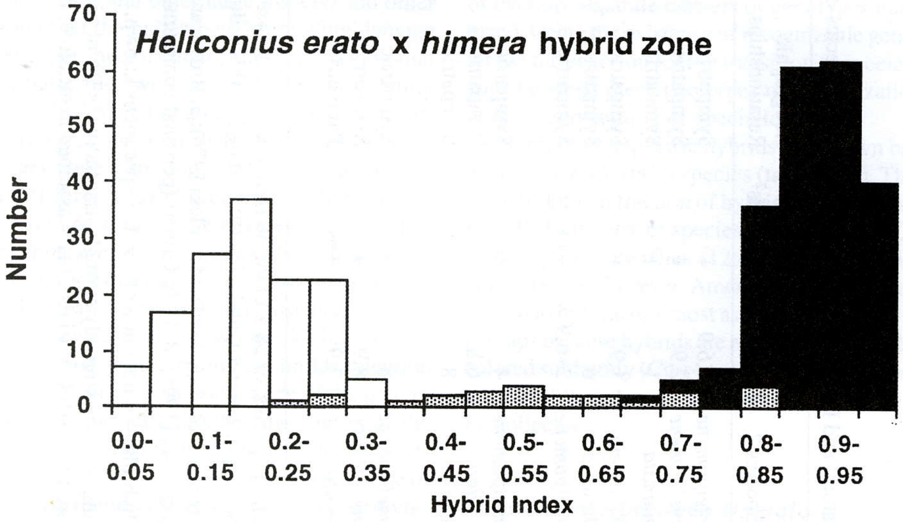

Figure 30.3. Distribution of scores of hybrid index of *Heliconius himera* × *H. erato cyrbia* in the centre of a hybrid zone near Guayquichuma, Ecuador. Individuals were classified using color pattern into three groups: *himera* (open), hybrids (stippled), and *erato* (solid). The hybrid index is calculated as the fraction of characteristic *erato* allozymes, based on 11 loci showing frequency differences between the species (Jiggins et al. 1997a).

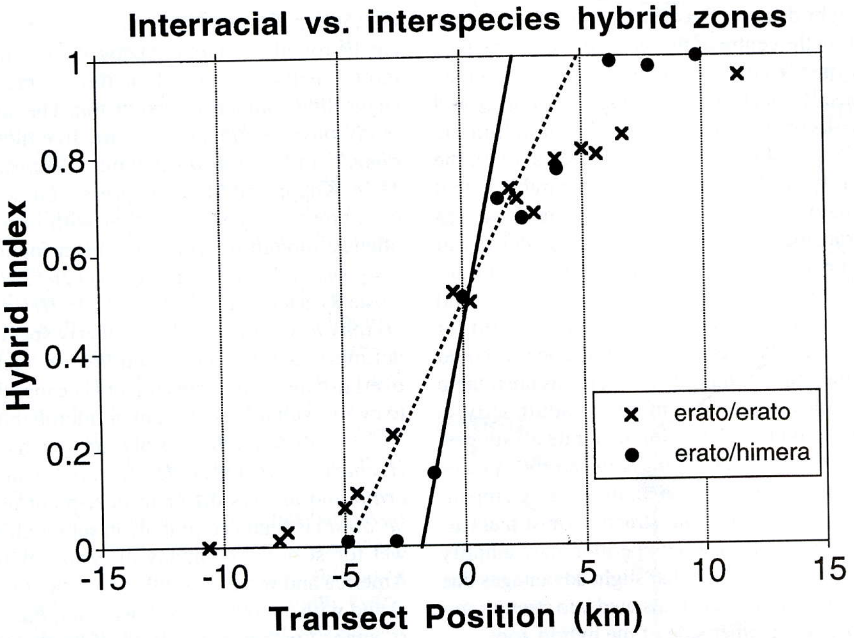

Figure 30.4. Comparison of the widths of an interracial hybrid zone within *H. erato*, and an interspecific hybrid zone between *H. erato* and *H. himera*. The interracial hybrid zone, near Pongo de Cainarache, Peru, is about 10 km wide (see also figure 30.2); the interspecific zone, near Guayquichuma, Ecuador, is about 5 km wide (Jiggins et al. 1996).

ronmental, and color pattern differences indicate that extremely strong selection maintains the hybrid zone and prevents amalgamation of the *himera* and *erato* genotypic clusters (figure 30.3). The supply of hybrids between *himera* and *erato* is undoubtedly limited by assortative mating, but 6% hybridization in each generation would quickly result in a hybrid swarm consisting largely of recombinant genotypes unless hybrids were removed by some form of selection, especially since pure forms mate as freely with F1 hybrids as with their own type (McMillan et al. 1997; Mallet et al. 1998). Classical hybrid inviability and sterility have been excluded, so this selection must be ecological.

There are two likely possibilities, and almost certainly both are involved. First, mimetic selection would take an interesting form in this hybrid zone compared with that

Table 30.2. Measures of viability of pure (E = *erato*, H = *himera*) and hybrid crosses (McMillan et al. 1997).

Cross Type	No. Broods	% Egg Hatch	% Larval Survival	Eggs/ Day	Development Time (d)	% Female
Pure						
E	12	90.3	57.5	1.9	29.8	48
H	14	92.7	66.7	2.6	28.5	49
F1 Hybrids						
E♀ × H♂	12	83.7	64.1	1.8	29.3	48
H♀ × E♂	8	89.2	53.8	2.4	29.2	45
Backcrosses						
F1 × E	7	90.0	62.3	2.2	28.9	53
F1 × H	10	85.7	53.8	2.0	28.1	44
Further crosses						
F2	7	92.0	84.5	1.5	30.2	51
F2 × E/H	6	99.3	83.4	2.5	28.1	50

in interracial hybrid zones. Because hybrids are rarer than the pure forms in the centre of the *himera*/*erato* zone, they should suffer more frequency-dependent predation than pure genotypes. If selection is as strong as that measured in interracial hybrid zones (survival of nonmimetic morphs ≈ 48% that of mimetic controls; see above), the hybrid inviability produced would be comparable to that caused by a major chromosomal rearrangement such as a reciprocal translocation, or by inviability or sterility of one sex of hybrids. Second, the abrupt ecological transition across all three hybrid zones between *erato* and *himera* suggests strong abiotic selection. If a hybrid (or pure form) were to fly across the hybrid zone, it could encounter temperature or humidity conditions unsuitable for survival or development. Studies of adult activity levels, fecundity and larval development rate all suggest differences in climatic adaptation between the species (McMillan et al. 1997; Davison et al. in press). Competition between sympatric *Heliconius* structures host-plant associations (Benson 1978), and competition may amplify the ecological differences such that slight advantages due to environmental adaptation are translated into strong competitive dominance on either side of the hybrid zone.

We are interested here more in the selective causes rather than the geographic context of speciation. However, correlations between allozymes, mtDNA, and morphology in hybrid zones have often been used as evidence for allopatric secondary contact. In this case, allozyme and mtDNA data show that the very strong genetic barrier produced by ecological selection must be as effective as a major geographic barrier in preventing the spread of approximately neutral alleles between the species (figure 30.3; Jiggins et al. 1997a). Strongly selected clines can accumulate other clines until extremely strong barriers result (Mallet 1993; Mallet and Turner 1998). Once a sufficiently strong barrier due to mimicry and ecological adaptation exists across a zone of this nature, novel genetic differences can accumulate faster than they are homogenized by gene flow. Thus, parapatric speciation remains difficult to rule out (Jiggins et al. 1996; see also Bush and Howard 1986; Bush 1993).

In conclusion, selection across this hybrid zone is strong. Its exact nature is obscure, but it must be mainly ecological rather than due to intrinsic hybrid inviability or sterility. Speciation between *himera* and *erato* has resulted from a combination of assortative mating and divergence of warning pattern and ecology.

Other *Heliconius* Species Pairs Related to *H. erato* and *H. himera*

Heliconius clysonymus and *H. telesiphe* are sister species native to the Andes (*clysonymus* is also found in the mountains of Central America), and are common in cloud forests at altitudes of between 800 and 2,000 m. Their closest relative, *erato*, is found in the lowlands at 0–1,500 m (Brown 1981; Brower 1994a). These species can be found overlapping between 800 and 1,500 m, but more usually only one of the three is present at any site, suggesting competitive exclusion. The larval host plants of *clysonymus* and *telesiphe* are, like those of *erato* and *himera*, in *Passiflora* subgenus *Plectostemma* (Benson 1978; Knapp and Mallet in press). These *Heliconius* are not known to hybridize either with *erato* or with each other, although hybrids have been found between *clysonymus* and another related species, *H. hecalesia*, in Costa Rica and Mexico (table 30.1). *Heliconius telesiphe*, *clysonymus*, and *erato* have clearly speciated under any definition but, like *erato* and *himera*, lack the ability to overlap extensively, presumably because they have failed to evolve suitable host-plant or microhabitat differences.

More distant relatives of *erato*, such as *H. hecalesia*, *H. charithonia*, and *H. demeter*, frequently coexist with *erato* and always differ in host-plant use. *Heliconius hecalesia* is found commonly in submontane and lowland wet forest in the company of *erato* relatives in central America and western South America; it is strongly associated with a cloud forest host plant, *Passiflora* (*Plectostemma*) *lancearia*, rarely used by the others (Benson et al. 1976; Mallet, personal observation). *H. charithonia* is found in a variety of lowland and submontane areas, where it often specializes on *Passiflora* ("*Tetrastylis*") *lobata* and *Passiflora* (*Granadilla*) *adenopoda*, both of which have hooked trichomes that kill other *Heliconius* larvae (Gilbert 1971); it also feeds on other *Passiflora* (*Plectostemma*) host plants more typical for the *erato* group (Benson et al. 1976; Jiggins et al. 1996; Jiggins and Davies 1998). Although its biology is poorly known, *demeter* is often sympatric with *erato* in lowland Amazonia, where it feeds especially on *Dilkea* and *Passiflora* (*Astrophea*) species (Brown and Benson 1975). Thus, closely related species in the *erato* group are often parapatric, but differ in aridity or altitudinal requirements. Where they become sympatric, they differ strongly in host-plant ecology (Jiggins et al. 1997b).

In the unrelated *melpomene* group of *Heliconius*, coexistence again seems to involve host-plant shifts. Related species in this group, such as *hecale*, *ismenius*, *melpomene*, *cydno*, *numata*, *elevatus*, *pardalinus*, and *ethilla*, partition species of *Passiflora* when they overlap (Benson et al. 1976; Benson 1978; Brown 1981; Gilbert and Smiley 1978; Gilbert 1991). The case of *H. cydno* and *H. melpomene* is particularly instructive. These are very closely related sister species: both mtDNA (Brower 1996a,b) and allozyme studies (Mallet, unpublished) suggest that *H. cydno* (together with semispecies *pachinus*, *heurippa*, *tristero*, and *timareta*) forms a monophyletic branch within a *melpomene-cydno* clade, making the species *melpomene* as a whole paraphyletic. *Heliconius melpomene* and *cydno* are sympatric throughout most of Central America and near the Andes (*melpomene* is also found east of the Andes in the Amazon basin, southern Brazil, and Argentina). *Heliconius melpomene* is typically red, black, and yellow and mimics *erato*, whereas *cydno* is typically blue-black and white or blue-black and yellow and usually mimics species in the *sapho/eleuchia* group of *Heliconius* (Linares 1996, 1997). (Three probable races or semispecies of *cydno* found on the eastern slopes of the Andes are exceptions to this rule and have some red coloration: *H. heurippa*, *H. tristero*, and *H. timareta*; see Brower 1996a,b). As already discussed, hybrids between *cydno* and *melpomene* are regularly found in the wild in Colombia, Venezuela, and Ecuador, although they are always exceedingly rare (table 30.1). Preliminary crosses show that female hybrids, although viable, are sterile, a "Haldane Rule" effect (Haldane 1922; Turelli and Orr 1995). Male hybrids between *cydno* and *melpomene*, on the other hand, are fully fertile and viable and can be used to transfer color pattern genes from one species to another in laboratory crosses (table 30.1; L. E. Gilbert, personal communication).

The ecology of *cydno* and *melpomene* has been well studied in Costa Rica. Whereas *melpomene* is a specialist on *Passiflora* (*Granadilla*) *menispermifolia* and *P.* (*G.*) *oerstedii* in open areas and young second growth, *cydno* oviposits and feeds on virtually every available species of *Passiflora* in Atlantic rainforest understory (Smiley 1978). This pattern of host and microhabitat partitioning is repeated in Pacific Costa Rica (Osa), where *pachinus* replaces *cydno* (Benson 1978; Gilbert 1991). However, the larvae of *melpomene* can use all the hosts of *cydno/pachinus*; host specialization in *melpomene* stems entirely from the oviposition behavior of the adult female (Smiley 1978).

The two species therefore differ in their mimetic allegiance and ecology (second growth specialist vs. forest understory generalist). F1 females are sterile, which is not found between *himera* and *erato*. These factors, together with extremely strong assortative mating, enable the coexistence, without fusing, of the two species. In this case it is not clear whether genomic incompatibilities (F1 female sterility) or ecological incompatibilities (host choice and mimicry) evolved first. But, although genomic selection against hybrids is strong (selection against F1s averaged across sexes is approximately 50%), selection against warning color intermediates (50% or more, judging from experiments with *erato*; see above) and on oviposition behavior (unmeasured, but presumably strong), is probably as or more intense. Even in this case, ecological selection and hybrid sterility are comparable in strength.

What Causes Speciation in *Heliconius*?

We can now summarize this information in the form of a sequence of events leading to speciation in *Heliconius* (figure 30.5), incorporating information from *erato* races, *himera/erato* hybrid zones, and the *cydno/melpomene* comparison. Warning color diverges most rapidly, in geographic races long before speciation. Related species usually differ in their mimicry rings (Turner 1976): there are nine pairs of sister *Heliconius* species inferred from a mtDNA phylogeny (Brower 1994b) and in only one pair, *H. sara* and *H. leucadia*, have the species not diverged strongly in their mimetic pattern. However, warning color evolution does not seem to trigger speciation on its own. Species such as *cydno* or *numata* may even become polymorphic within populations, as a result of mimicking several model species that vary in abundance in time and space (Brown and Benson 1974; Brown 1976; Linares 1997; Kapan 1998). More commonly, for Müllerian mimics, populations are monomorphic, and color patterns diverge geographically to form a diversity of color pattern races that show few signs of speciating.

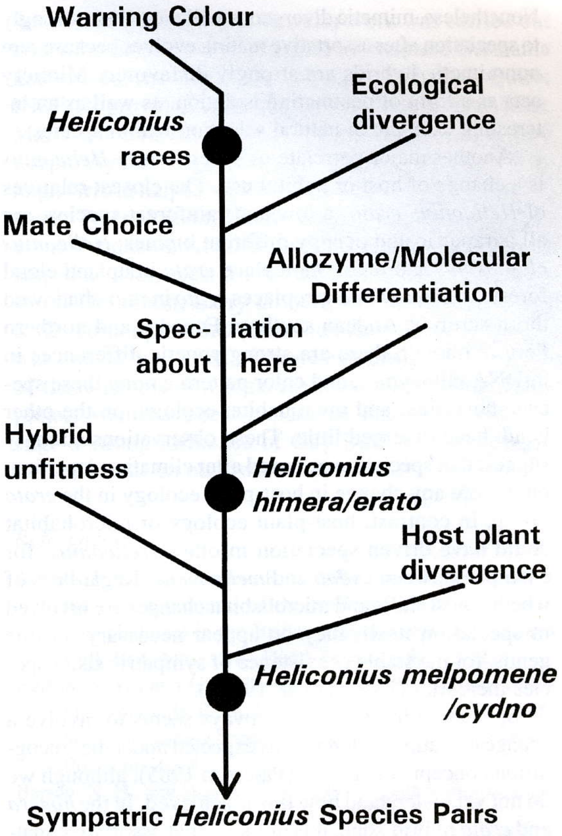

Figure 30.5. The sequence of divergence events leading from geographic race formation to speciation based on *Heliconius* butterflies. Events shown between studied divergent taxa (•) could have occurred in any order, and represent the approximate first occurrence of each event. For example, warning color evolution continues long after speciation but first occurs between races within species such as *erato*. Parapatric species pairs such as *erato* and *clysonymus* or *telesiphe* fall somewhere between *himera/erato* and *melpomene/cydno*, but hybrid compatibility status is unknown because of a lack of known hybrids.

Nonetheless, mimetic divergence will contribute strongly to speciation after assortative mating evolves because rare nonmimetic hybrids are strongly disfavored. Mimicry acts as a form of postmating isolation, as well as an interesting example of natural selection within species.

Another major correlate of speciation in *Heliconius* is a change of host or habitat use. The closest relatives of *Heliconius erato*, a lowland rainforest species, are all parapatric and occupy different biomes. *Heliconius clysonymus* and *telesiphe* replace *erato* in upland cloud forests, whereas *himera* replaces *erato* in rain-shadowed thorn scrub of Andean southern Ecuador and northern Peru. Although there are strong genetic differences in mtDNA, allozymes, and color pattern among these species, host-plant and microhabitat ecology, on the other hand, have diverged little. These observations strongly suggest that speciation occurred after climatic adaptation, but before any change in host-plant ecology in the *erato* group. In contrast, host-plant ecology or microhabitat could have driven speciation in other *Heliconius*, for example between *cydno* and *melpomene*. Regardless of whether host shifts and microhabitat changes are involved in speciation itself, they do appear necessary, in this genus, for the stable coexistence of sympatric sister species thereafter (Jiggins et al. 1997b).

Speciation in *Heliconius* always seems to involve a change in mating preference, as expected under the "recognition concept" of species (Paterson 1985), although we do not yet understand how this is achieved. In the *himera* and *erato* hybrid zone, it is unlikely that assortative mating is due to "reinforcement" (where selection against hybrids causes the evolution of mate choice; Dobzhansky 1940). Many experiments were done with *himera* collected >60 km from the hybrid zone, or *erato* collected >25 km from the hybrid zone, and these allopatric populations mated assortatively as strongly as those found in the area of overlap (McMillan et al. 1997). Of course, this is not strong evidence—conceivably, assortative mating evolved as a result of reinforcement inside the zone, and then spread to areas far away. However, for parapatric species such as *erato*, *himera*, *clysonymus*, and *telesiphe*, with narrow zones of overlap, reinforcement is unlikely on theoretical grounds (Butlin 1989). Mate choice is perhaps more likely a pleiotropic effect of some other evolutionary change. One candidate is a change of mimetic color pattern. Males, and sometimes females, of red, orange, and black species such as *erato* and *melpomene* can be attracted down from the forest canopy by means of red rags (Brown and Benson 1974; Mallet et al. 1990), but *cydno* are never attracted in this way. Black and white *cydno* can, however, be attracted by a white rag (Mallet and Kapan, unpublished). A profound mimetic switch may thus lead to a shift in long-range mating and social signals. However, color pattern change is not the only factor causing assortative mating: divergence in short-range pheromone signaling systems is almost certainly also involved. Whatever the signaling mode, coevolution between sexual traits and behavioral response may lead to rapid mating divergence (Lande 1982).

The compatibility of *himera* and *erato* demonstrates that hybrid unfitness induced by genomic, as opposed to ecological, incompatibilities may evolve some time after speciation. Even in *melpomene* and *cydno*, the fertility of male hybrids suggests that genes could flow relatively freely between the species. Pleiotropic changes leading to hybrid inviability and sterility, as in *cydno* and *melpomene*, may well evolve more readily after gene flow is effectively halted. Genomic incompatibility could therefore evolve as a consequence rather than a cause of speciation. Obviously, we cannot be sure that this is so in *cydno* and *melpomene* or more distantly related species pairs because hybrid incompatibilities and ecological differences are found together. The sequence of events may not always follow figure 30.5 exactly, and more comparative data are needed to test the generality of these ideas. Nonetheless, ecological factors—mimicry, and adaptation to particular biotopes—together with the evolution of assortative mating appear to be more important than genomic inviability and sterility in this group. Our results show that Henry Walter Bates's inferences were broadly correct. Mimicry, coupled with other ecological adaptations and the evolution of assortative mating, has caused speciation in heliconiine butterflies.

Appendix 30.1
Did Bates Discover Müllerian Mimicry?

Bates showed that palatable dismorphiines mimicked unpalatable ithomiine butterflies (Batesian mimicry). It is not as well known that Bates used exactly the same principle to explain why rare unpalatable forms such as *Napeogenes* and *Heliconius* mimic commoner, but also unpalatable ithomiine models. However, Bates did not fully understand why pairs of common unpalatable ithomiine species were also comimetic. He suggested "similar adaptation of all to the same local, probably inorganic, conditions." Mimicry between common unpalatable species was later explained by Müller (1879), who used one of the first explicit mathematical models in ecology to quantify the relative benefits to pairs of unpalatable species sharing a warning pattern. Mimicry where palatable species copy unpalatable species is now referred to as Batesian, whereas mimicry between any pair of unpalatable species is termed Müllerian.

Appendix 30.2
Essentialism in Modern Species Concepts

Reading Ernst Mayr (e.g., 1982), one obtains the impression that Darwin, Wallace, and other early evolutionists were confused about the nature of species. Our opinion is that the reverse is true (Mallet 1995a,b): Mayr himself confuses the causes of speciation (i.e., the way species are maintained) with species definitions. In our view, much of the current muddle over "species concepts" results from Mayr's adoption of an obviously essentialist view of species: that biological species, unlike lower or higher taxonomic categories, are "objectively real," defined by "isolating mechanisms" that "are a protective device for well-integrated genotypes"; that species are "evolutionary units" necessary for adaptive evolution, have "internal cohesion," and are "individuals," and so on. We fully realize, of course, that Mayr considers himself an enlightened *anti*-essentialist who frequently castigates other biologists, including Darwin, for adopting "typological" thought patterns (e.g., Mayr, 1982, p. 268). Mayr coined the derogatory term "typological" to imply a belief in the existence of a fixed "type," or Platonic "form" underlying a systematic category such as species; to Mayr, the "fixed type" was the essentialism he was fighting. Since it was Darwin's knowledge of natural variation that freed him from the essentialist view of species, it is odd that Mayr views the Darwinian "morphological species concept" as essentialist. The biological species concept can itself be seen as typological, in that Mayr, an admirer of of the great essentialist Hegel, seeks an underlying "essence" or "true meaning" for the term "species," rather than a useful and convenient criterion to delimit actual populations as species taxa; in fact, Mayr repeatedly emphasized that he was avoiding a taxonomically useful criterion in favor of a "concept" that embodied the biological meaning of species. Mayr decided that the true essence of species was reproductive isolation. Others reject this particular essence, but propose other essences which underlie "objectively real" species, for example ecology, mating behavior, "cohesion," or phylogeny/genealogy (see chapters by De Queiroz, Harrison, Shaw, and Templeton, this volume).

Darwin and Wallace both specifically rejected essentialist ideas of a "true meaning" of species. In Darwin's (1859) words:

> In short, we shall have to treat species in the same manner as those naturalists treat genera, who admit that genera are merely artificial combinations made for convenience. This may not be a cheering prospect; but we shall at least be freed from the vain search for the undiscovered and undiscoverable *essence* of the term species. (pp. 484–5, emphasis added).

Darwin and Wallace realized that species were often reproductively incompatible, but pointed out many difficulties with reproductive definitions of species (Mallet 1995a). Instead, they used a simple operational cluster criterion for species that was independent of the origin or maintenance of differences. The value of their species definition is manifest in the scientific revolution they achieved.

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