CHAPTER TEN

Rapid speciation, hybridization and adaptive radiation in the *Heliconius melpomene* group

JAMES MALLET

In 1998 it seemed clear that a pair of 'sister species' of tropical butterflies, *Heliconius melpomene* and *Heliconius cydno* persisted in sympatry in spite of occasional although regular hybridization. They speciated and today can coexist as a result of ecological divergence. An important mechanism in their speciation was the switch in colour pattern between different Müllerian mimicry rings, together with microhabitat and host-plant shifts, and assortative mating produced as a side effect of the colour pattern differences. An international consortium of *Heliconius* geneticists has recently been investigating members of the *cydno* superspecies, which are in a sense the 'sisters' of one of the original 'sister species', *cydno*. Several of these locally endemic forms are now recognized as separate species in the eastern slopes of the Andes. These forms are probably most closely related to *cydno*, but in several cases bear virtually identical colour patterns to the local race of *melpomene*, very likely resulting from gene transfer from that species; they therefore can and sometimes do join the local mimicry ring with *melpomene* and its more distantly related co-mimic *Heliconius erato*. I detail how recent genetic studies, together with ecological and behavioural observations, suggest that the shared colour patterns are indeed due to hybridization and transfer of mimicry adaptations between *Heliconius* species. These findings may have general applicability: rapidly diversifying lineages of both plants and animals may frequently share and exchange adaptive genetic variation.

Introduction

Speciation is increasingly recognized to be associated with ecological divergence, and may indeed occur in the presence of gene flow (see other contributions in this volume). But the extent to which species formation is driven by ecology and the possible role of hybridization in adaptive radiation are still debated (Schluter 2000; Coyne & Orr 2004; Mallet 2007). Here I analyse speciation in *Heliconius* butterflies, which has provided a somewhat classical story of ecology-driven speciation. There are some surprising recent discoveries which cast doubt on the generality of some of our earlier ideas on diversification in this group.

Speciation and Patterns of Diversity, ed. Roger K. Butlin, Jon R. Bridle and Dolph Schluter. Published by Cambridge University Press.

Ten years ago, we argued on somewhat slender evidence that speciation in *Heliconius* was largely due to ecological divergence, particularly in mimicry as well as in host and microhabitat choice, and that this was coupled with the evolution of assortative mating (Mallet *et al.* 1998b). Since then, evidence for ecological, especially mimicry-mediated, speciation has become firmer, but in the course of this work additional modes of speciation have been revealed. I begin by assessing *Heliconius* speciation work since the 1990s. I next review evidence on hybridization and introgression between species. Finally, I discuss the possibility that adaptive genes, particularly for mimicry, are transferred among members of rapidly radiating lineages.

Speciation driven by mimicry

Müllerian mimicry among unpalatable *Heliconius* species and between *Heliconius* and another unpalatable butterfly group, the Ithomiinae, has been known since the work of Henry Walter Bates (Bates 1862). Fritz Müller (1879) provided the first theoretical analysis of Müllerian mimicry, but Bates was the first to explain mimicry in Darwinian terms. Bates was also the first to recognize the existence of mimicry now designated 'Müllerian' between pairs of unpalatable 'co-mimic' species, as well as 'Batesian' mimicry between palatable and unpalatable 'model' species. Bates' most convincing evidence of mimicry was provided by parallel geographic variation in colour patterns of different lineages. He argued that colour-pattern mimicry acted as an important means of local protection for rarer unpalatable *Heliconius*, and it is thus clear he had an inkling that frequency-dependent selection was involved. He argued that divergence in mimicry was a likely means whereby natural selection led to speciation (Bates 1862).

For most of the 20th century, evolutionists saw mimicry as an interesting visual demonstration of natural selection, but no longer related it to speciation (Fisher 1930; Futuyma 1998). Natural selection, such as that for mimicry, was seen mainly as a source of 'microevolution' within populations and species, whereas geographic isolation, and incidental hybrid inviability and sterility were more heavily emphasized as important for speciation. Yet by the 1990s, evidence was accumulating for divergent ecological selection as causes of speciation of a variety of organisms (Mallet 2001b).

By 1998, the majority of our work on contacts between the species pair *Heliconius himera* and *H. erato* had already been published. *Heliconius himera* and *H. erato* differ in colour pattern, tolerance of dry versus wet forest and mate choice (McMillan *et al.* 1997; Mallet *et al.* 1998b). Hybridization rates in narrow ~5 km hybrid zones are low (6%) (Mallet *et al.* 1998a). The two species lack larval food-plant and microhabitat differences (Jiggins *et al.* 1997b), and competition thus probably explains the narrowness of the overlap. All hybrids, backcrosses and further crosses, when formed, are viable and fertile (McMillan *et al.* 1997), but for poorly understood ecological reasons, probably in part due to non-mimetic colour pattern combinations, do not go on to establish successful hybrid swarms in the overlap zone. Furthermore, mtDNA and allozyme profiles of the two species remain distinct (Jiggins *et al.* 1997a). The two forms are considered species on the basis of this coexistence of separate genotypic clusters within the zone of overlap, even though ~10% of the population in the centre of the zone are F1 and backcross hybrids. Divergently patterned geographic races of *erato* also form narrow hybrid zones where they meet, but, in these contact zones, hybrids are always abundant, while the pure forms do not differ at mitochondrial or nuclear markers, and so are considered members of the same species. In conclusion, the sister species *himera* and *erato* have diverged in colour pattern and mate choice, but have no genomic incompatibilities (Mallet *et al.* 1998b).

Work on the *H. cydno* and *H. melpomene* 'sister species' pair was embryonic in 1998, but 10 years later has become a major area of investigation. The two species are sympatric throughout Central America and in the Western Andes. They hybridize regularly, albeit at a very low rate of one in a thousand or less (Mallet *et al.* 2007). This hybridization explains shared polymorphisms at (only some) nuclear genes (Bull *et al.* 2006; Kronforst *et al.* 2006b).

Each of these species mimics different, divergent members of an unrelated *Heliconius* lineage: *cydno* is black with white or yellow bands, and normally mimics *Heliconius sapho* or *Heliconius eleuchia*; in West Ecuador and parts of Colombia it can also be polymorphic and mimic both (Kapan 2001). *H. melpomene* instead has forms with monomorphic 'postman' patterns (black with broad red forewing band, sometimes with a yellow hindwing bar) that mimic postman-patterned *erato*. Other races of *melpomene* also have various 'dennis-ray' patterns (black with a forewing yellow band, a basal forewing orange patch known as 'dennis', and hindwing rays) that mimic 'dennis-ray' *erato* races and those of a number of other species in the Amazon basin. In only the Cauca Valley of Colombia, which lacks *H. melpomene* altogether, does *Heliconius cydno weymeri* have a morph 'gustavi' that mimics the local *Heliconius erato chestertonii* (Linares 1997). Probably, it is not a coincidence that *chestertonii* is also the only race of *erato* that lacks red markings: it has a postman-like yellow hindwing bar, but has entirely blueish-black iridescent forewings. However, whether the other *cydno* races are congenitally unable to match red-marked *erato*, or *H. e. chestertonii* has converged to a pattern like that of *cydno* only in this one valley, is not known.

The colour pattern differences between *cydno* and *melpomene* are controlled by many of the same loci involved in geographic mimicry differences within each species (Linares 1996; Naisbit *et al.* 2003; Joron *et al.* 2006; Kronforst *et al.* 2006a). Because *cydno* and *melpomene* are considerably closer genetically to one another than *sapho* or *eleuchia* are to *erato*, *cydno* and *melpomene* diverged more recently, probably as mimics of *erato*, *sapho* and *eleuchia* (the already divergent 'models'), rather than vice versa (Jiggins *et al.* 2001; Flanagan *et al.* 2004). In addition, comparative evidence strongly implicates *melpomene* and *cydno* as mimics of *erato* and allies, rather than vice versa (Eltringham 1917; Mallet 2001a).

Colour pattern divergence between the two species leads to a number of interesting evolutionary consequences: (1) the rare hybrids that are produced are non-mimetic, and are likely to suffer strongly reduced survival due to bird attacks (Mallet *et al.* 1998b; Jiggins *et al.* 2001); (2) mate location is partly visual and has apparently co-evolved with mimicry, leading to strong assortative mating that can be demonstrated with paper models as well as with natural wing patterns (Jiggins *et al.* 2001; Naisbit *et al.* 2001); (3) Panama *melpomene* are sympatric with *cydno* and mate more assortatively than Guianan *melpomene*, which do not overlap with *cydno* (Jiggins *et al.* 2001). This is consistent with 'reinforcement', i.e. adaptive evolution of more discriminating mate choice to avoid production of low-fitness hybrids with another species in sympatry; (4) the two mimetic pairs also differ in microhabitat – co-mimics *cydno* and *sapho* inhabit forest understory, whereas co-mimics *melpomene* and *erato* are normally found in more open areas (Estrada & Jiggins 2002). Nocturnal gregarious roosts are also stratified by mimicry (Mallet & Gilbert 1995). It is probable that the mimicry switch took place as a response to a habitat shift, but the reverse is possible. In any case, the mimicry shift also provides an additional ecological dimension which further enhances assortative mating due to habitat segregation; (5) hybrids between *cydno* and *melpomene* of both sexes are discriminated against in mate choice experiments, a form of disruptive sexual selection against hybrids (Naisbit *et al.* 2001). Thus, mimicry has important direct and indirect effects which result in manifold, pleiotropic effects on reproductive isolation (Mallet *et al.* 1998b; Jiggins *et al.* 2005).

Not all speciation-related differences between *cydno* and *melpomene* can be attributed to mimicry: hybrid females are usually sterile, an example of Haldane's Rule, because in butterflies it is the females that are heterozygous for sex-determining chromosomes (Naisbit *et al.* 2002, 2003). Like other 'Dobzhansky–Muller incompatibilities', Haldane's Rule sterility is thought to be caused by divergence at sex-linked loci with pleiotropic effects on gamete production (Coyne & Orr 2004). Female hybrid sterility is a major form of 'post-mating isolation' between these two species (~70%), but it is only about as strong as mimicry selection against hybrids (~64%), and host-plant and microhabitat differences are additional selective effects that have not been estimated. However, all these are weaker barriers than the 'pre-mating isolation' due to assortative mating (> 99.9%) (Naisbit *et al.* 2002; Mallet 2006). Thus we became convinced of what might be termed the classical story: that ecological divergence, especially mimicry and its co-evolved effects on mate choice and microhabitat use, was the major cause of *Heliconius* speciation.

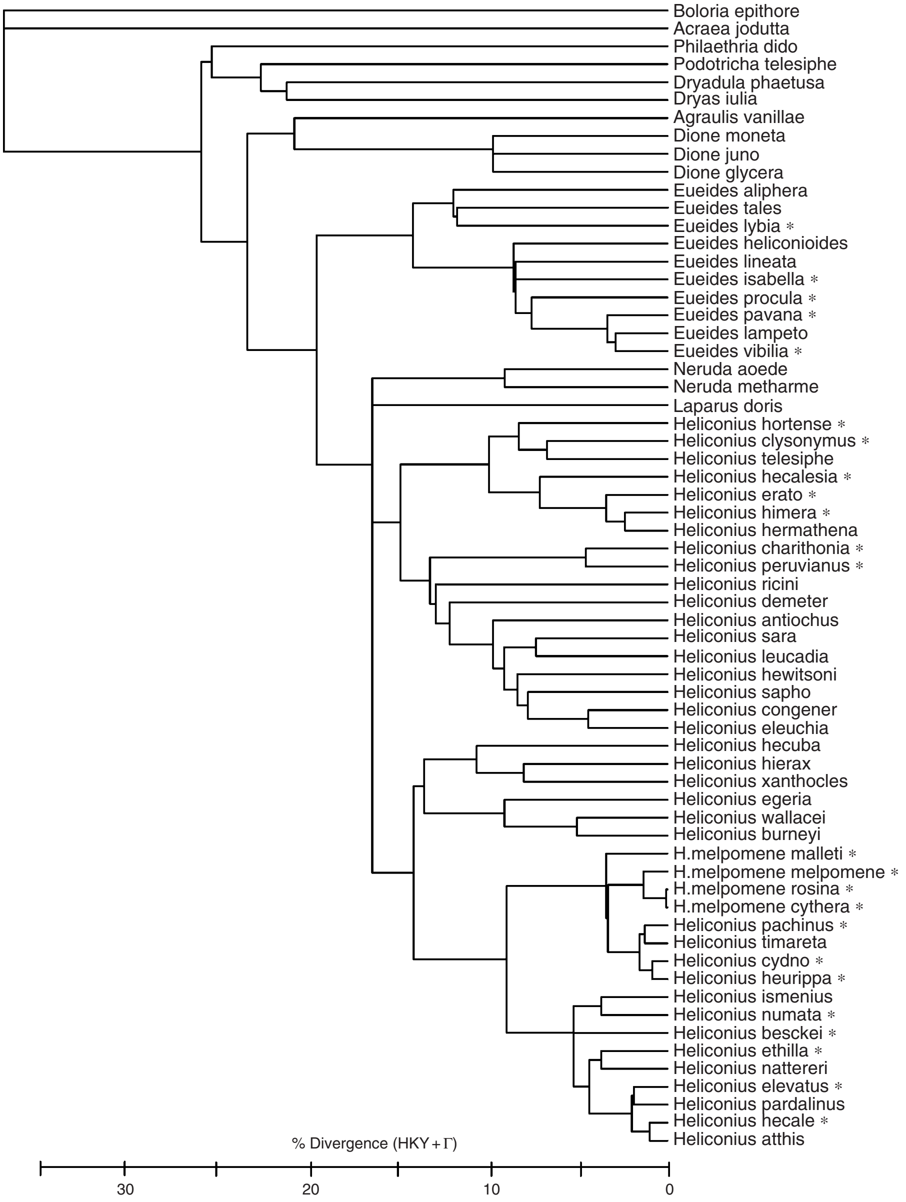

Figure 10.1 Phylogenetic relationships of the Heliconiini. The diagram shows an mtDNA-based Yang rate-smoothed estimate of phylogenetic relationships among species of the Heliconiini. Species that hybridize in nature (starred) tend to be most closely related, and are in the most recent radiations of *Heliconius* and *Eueides*, rather than from older species in the 'basal genera' (Beltrán *et al.* 2007; after Mallet *et al.* 2007). (see color plate)

Speciation not driven by mimicry

In *Heliconius*, closely related species often belong to different mimicry rings, and co-mimics in the same ring usually belong to different lineages, as though species in each lineage were adaptively radiating onto different mimicry rings (Turner 1976; Mallet *et al.* 1998b). However, recent evidence suggests that this classical story of mimicry-driven speciation holds for by no means all speciation events among Müllerian mimics. Dasmahapatra *et al.* (in preparation) have recently discovered via DNA sequencing a cryptic species most closely related to *Heliconius demeter*, with which it shares a nearly identical rayed mimicry pattern. Not only are these two scarce sister species difficult to distinguish from one other using morphology, but are also hard to distinguish from the related sympatric rayed *H. erato* throughout the Amazon basin. As another example, in *Ithomia*, from a different subfamily, the Ithomiinae, sister species are frequently Müllerian co-mimics (Jiggins *et al.* 2006), suggesting that speciation in this group can only sometimes be attributed to mimetic divergence, if at all.

Furthermore, although our group were using the term 'sister species' to refer to the *cydno* and *melpomene* species pair (Mallet *et al.* 1998b; Jiggins *et al.* 2001), the actual situation is more complex: one of the 'sister species' itself has sisters. A number of local endemic segregate taxa, such as *Heliconius pachinus*, *Heliconius heurippa*, *Heliconius tristero* and *Heliconius timareta*, thought to be more closely related to *cydno* than to *melpomene*, were interpreted 10 years ago as subspecies or 'semi-species' of *cydno* (Mallet *et al.* 1998b). In all cases, these local endemics overlap with *melpomene*, but occur outside the range of *H. cydno* (*sensu stricto*). DNA sequence data have mostly confirmed closer relationships of these segregate taxa to *cydno* (Brower 1996a,b; Salazar *et al.* 2005; Mavárez *et al.* 2006; Kronforst *et al.* 2006b; Beltrán *et al.* 2007), although the picture is clouded by possible paraphyly of *melpomene* (Fig. 10.1) as well as ongoing allele sharing and autosomal introgression between *cydno* (including its segregates) and *melpomene* (Bull *et al.* 2006; Kronforst *et al.* 2006b).

Whether these disjunct segregate taxa are regarded as full species or geographic races of *cydno* is to some extent a matter of taste. Early systematists, lacking detailed distribution data or large series from zones of sympatry, tended to confuse the red, yellow and black colour patterns of *heurippa*, *timareta* and *tristero* with those found in hybrid zones between mimetic races, and interpreted the segregate taxa as forms of *melpomene* (Eltringham 1917; Emsley 1964). However, it is now becoming usual to treat *heurippa*, *tristero* and *timareta* as separate species, due to their sympatry with *melpomene*; their pronounced morphological divergence from the black and white or yellow mimetic patterns typical of *cydno*; their often prominent red markings; and their assortative mating in tests with both *cydno* and *melpomene* (Brown 1981; Brower 1996a; Mavárez *et al.* 2006; Kronforst *et al.* 2006c). In the most recent checklist, the

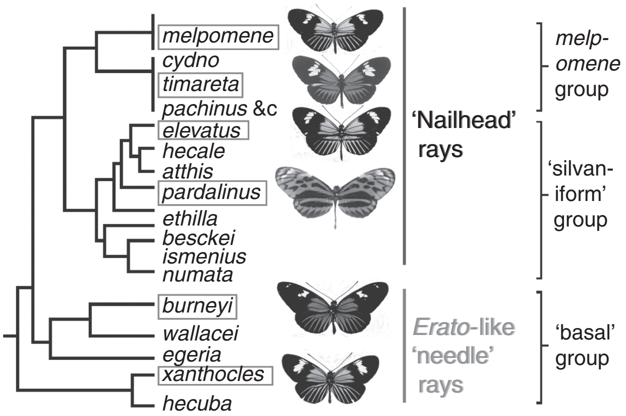

Figure 10.2 Homoplasy of colour patterns in the *melpomene* group. 'Nailhead' rays, as in mimetic Amazonian races of *H. melpomene*, appear to provide a novel method of producing rays, and are characteristic only of the most recent radiation of the *melpomene*/*cydno*/ silvaniform group of *Heliconius* (upper four 'boxed' species). Plesiomorphic 'needle' rays are the norm in unrelated, rayed co-mimics such as *erato*, *Neruda* and *Laparus*, and even in related *H. burneyi* and *H. xanthocles* (lower two 'boxed' species). The species having 'nailhead rays', are not, however, necessarily each other's closest relatives. Furthermore, red forewing band 'postman' patterns in *besckei*, the *cydno*/*timareta* group, and *melpomene* also show a patchy distribution across the phylogeny (not shown). Unless the ancestral *melpomene*/silvaniform species was polymorphic for both rays and postman patterns (which is extremely unlikely), either postman or nailhead ray patterns must be homoplasious. A likely source of such homoplasy is hybridization and introgression, as virtually all species in the *melpomene* and silvaniform clades can hybridize and backcross in nature.

entirely yellow and black *pachinus* is treated as a subspecies of *cydno*, while red-marked *heurippa*, *tristero* and *timareta* are regarded as full species (Lamas 2004).

Renewed interest in the *melpomene*–*cydno* group has been triggered by the suggestion that hybridization is important in the evolution of mimetic and non-mimetic colour patterns (Gilbert 2003; Salazar *et al.* 2005; Mavárez *et al.* 2006), as well as further discoveries of new segregate forms related to *cydno* and *melpomene* in the Eastern Andes.

Hybridization, mimicry and speciation

My former PhD supervisor, Prof. Lawrence E. Gilbert Jr. proposed that *Heliconius* may gain much of their mimicry adaptability because they have available via hybridization, a 'shared genetic toolbox' allowing acquisition of mimicry colour patterns (Gilbert 2003). Often 1–3 genetic loci ('supergenes') can cause major mimetic switches in colour pattern (Sheppard *et al.* 1985; Joron *et al.* 2006). Adaptive introgression could explain the repeated re-evolution of the same apomorphic mimicry traits, such as 'nailhead rays' or the 'postman' pattern (see Fig. 10.2). This was the first time the proposal for the importance of

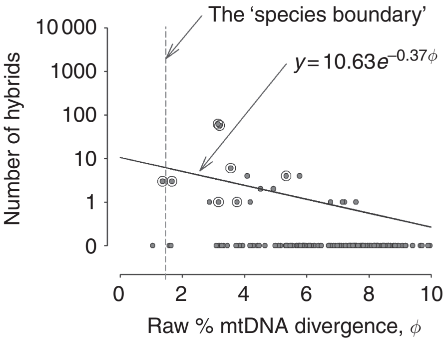

Figure 10.3 Hybridization between species of *Heliconius*. The graph shows the numbers of wild-caught interspecific hybrids in *Heliconius* and *Eueides* from a survey of world museum collections, plotted against genetic distance (mitochondrial DNA sequence data, based on *CoI* and *CoII*). Each point represents a different pair of species; points ringed with a halo represent pairs of species for which backcrosses, as well as F1 hybrids, are known in nature. The regression line of best fit is also shown. Genetic distances for the same loci tend to be less than ~1.8% within species; this distance therefore marks the approximate 'species boundary'. The numbers of hybrids, and of hybrids that backcross, as well as the fraction of species pairs that hybridize all tend to be higher for species nearest the species boundary (after Mallet *et al.* 2007).

hybridization and introgression had appeared in print, although another Gilbert PhD student, Mauricio Linares, had advocated similar ideas in his PhD thesis (Linares 1989). While I recognized that introgression could explain some *Heliconius* colour pattern evolution, at that time I felt that the idea had limited applicability. In my view, a more pressing problem was the evolution of novel *Heliconius* mimicry patterns, which could not be generated readily using existing adaptations. However, this skepticism about the evolution of true novelty via introgression underestimated the degree to which recombination of existing patterns can generate a much larger array of colour patterns than exists today. For example, many of Gilbert's lab-reared hybrids show how novel patterns can be created via hybridization and recombination in the *melpomene* group to produce convincing mimics of existing unpalatable model species, even some never mimicked by *Heliconius* in nature (Gilbert 2003). Hybridization might be an important source of variation for rapid adaptation to novel mimetic opportunities, even though the components needed to create the new mimicry pattern may be old.

Hybridization between species of *Heliconius* in nature is rare (< 0.1% of specimens), but regular and ongoing, as well as widespread (Figs. 10.1 and 10.2). It results in documented cases of introgression between species such as *melpomene* and *cydno* (Mallet *et al.* 1998b, 2007; Bull *et al.* 2006; Dasmahapatra *et al.* 2007). It might be imagined that only sister species hybridize, but F1 hybrids and in some cases backcrosses are known to occur regularly between non-sister species (e.g. *melpomene* and silvaniforms such as *Heliconius ethilla* and *Heliconius numata*, Figs. 10.1 and 10.3) (Dasmahapatra *et al.* 2007; Mallet *et al.* 2007). In captivity, the 'silvaniforms' *H. ethilla*, *hecale*, *ismenius*, *atthis* and *numata* can be mated with *cydno*, *pachinus* and *melpomene* to produce viable male and female offspring. Females are usually sterile (see above), again according to Haldane's Rule, but backcrosses of hybrid males can be used to transmit colour pattern genes between species (Gilbert 2003; Mavárez *et al.* 2006; Mallet *et al.* 2007). Thus hybridization is a likely conduit for flow of mimetic adaptations among species of *Heliconius*, provided that initial disadvantages of hybrid sterility and non-mimetic patterns can be overcome.

H. heurippa and *H. pachinus*

H. heurippa is found in the foothills of the Andes near Villavicencio, Colombia, up to about 1800 m, where it overlaps but rarely hybridizes with *Heliconius melpomene melpomene* (Fig. 10.4). *H. heurippa* itself is not obviously mimetic, though it is somewhat reminiscent of the local black, yellow and orange forms of *Melinaea marsaeus*, *Melinaea isocomma*, *H. numata* and *Heliconius hecale*. Its pattern is curiously like those obtained by backcrossing postman-patterned *m. melpomene* × *cydno cordula* F1 hybrids to *Heliconius cydno cordula*. The latter still occurs on the same eastern flanks of the Andes a few tens of kilometres to the North, while *melpomene* is sympatric with *heurippa* near Villavicencio. Mitochondrial and some nuclear sequence data suggest a closer relationship to *cydno* than to *melpomene*. However, microsatellite data suggest that *heurippa* forms a genotypic cluster separate from *cydno* as well as from *melpomene*, even though many alleles are shared among all three species. *H. heurippa* mates assortatively in the laboratory, and males of *cydno* and *melpomene* shun *heurippa* (Mavárez *et al.* 2006) – see also similar work with *H. pachinus* versus *cydno* and *melpomene* (Kronforst *et al.* 2007). This assortative mating is shown via painted models to be due to differences in colour pattern. Thus the hybrid colour pattern phenotype is itself partially reproductively isolated. These strands of evidence suggest that *heurippa* evolved by hybridization – a case of hybrid speciation (Mavárez *et al.* 2006). Furthermore, in one locality further to the North (San Cristobal, Venezuela) the same parental races, *H. m. melpomene* and *H. cydno cordula*, are in a somewhat intermediate phase, with abundant polymorphic colour pattern variants, presumably caused by recent introgression (Mavárez *et al.* 2006). This gives an example of a population where colour patterns have yet to be purified by selection for mimicry (as is normally expected, see p. 180). Such populations are the expected precursors that could start a shift to a novel, monomorphic warning colour pattern, such as that in *heurippa*.

The suggestion that *heurippa* evolved via hybrid speciation has been contentious (a discussion of these critiques can be found online at www.mailinglists.ucl.ac.uk/pipermail/heliconius/2006-June.txt). Meanwhile, recent studies have suggested that *H. pachinus* is not a hybrid species (Kronforst *et al.* 2007), in spite of earlier suggestions (Gilbert 2003). If hybridization does not involve chromosome doubling, as here, speciation followed by introgression may be almost

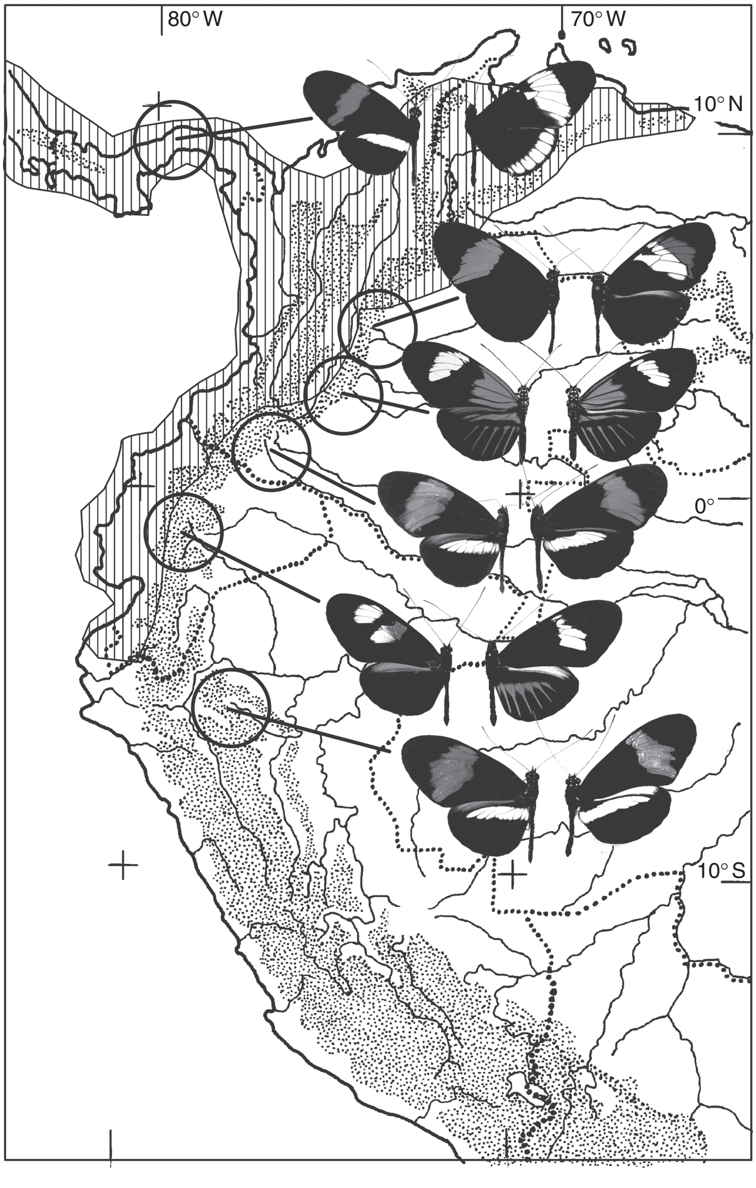

Figure 10.4 Distribution of endemic species allied to *Heliconius cydno* and *H. timareta* along the eastern slopes of the Andes (wings to the right), compared with all the local *melpomene* races (wings to the left). *Heliconius melpomene* occurs essentially thoughout the whole area shown below about 1800 m altitude (above 1800 m shown stippled), and the mainly Western Andean distribution of *cydno* sensu stricto is shown hatched. Note, however, that some *cydno* populations are found on the eastern slopes of the Andes in northern Colombia and Venezuela just north of the distribution of *heurippa*. Geographic races of *melpomene* (lefthand wings, all of which mimic *H. erato*) are, top to bottom: *m. rosina*, *m. melpomene*, *m. malleti*, *m. bellula* and *m. plesseni* and *m. amaryllis*. *Heliconius cydno* and related species (righthand wings), top to bottom: *H. cydno chioneus* (Panama), *H. heurippa* (Villavicencio, Colombia), unnamed species (Florencia, Colombia), *H. tristero* (Mocoa, Colombia), *H. timareta* (Eastern Ecuador) and unnamed species (Upper Mayo Valley, Peru). (see color plate)

indistinguishable from 'true' hybrid speciation. The only clear distinction is that hybridization should be shown to be a major cause of the changes leading to the formation of the hybrid species (Mallet 2007). *H. cydno* and *H. melpomene* hybridize with introgression wherever the two overlap (Bull *et al.* 2006; Kronforst *et al.* 2006b; Mallet *et al.* 2007), and if a few colour pattern genes leaked across they could readily lead to a new stable form, like *H.* (*cydno*) *heurippa*. This is a situation where the genome will not necessarily end up an approximate 50% mosaic of the two species (Kronforst *et al.* 2007; M. Turelli & J. Coyne pers. comm.); instead, the proportion of one of the species might be very low. Backcrosses between almost any other pair of randomly picked geographic races of *cydno* and *melpomene* would fail to give the *heurippa* pattern. So this particular hybrid pattern seems to be rather convincing evidence of hybrid origin only from these particular two local forms of *melpomene* and *cydno*. And the finding that the hybrid colour pattern itself causes reproductive isolation with both parent species (Mavárez *et al.* 2006) seems a reasonable argument for calling *heurippa* a 'good' hybrid species, even though it is not known to overlap with *cydno*. Of course, there remains some doubt as to whether the nuclear genes determining the *heurippa* colour pattern were really inherited from both parents, but we will only know this with certainty when the genetic regions responsible for colour pattern changes are finally characterized and sequenced (Joron *et al.* 2006).

H. cydno *and its relatives further south*

In Ecuador, *H. timareta* has been recognized as a separate species since 1867 (Lamas 2004). This non-mimetic form is peculiar for *Heliconius* in that it is apparently polymorphic throughout its narrow range for *melpomene*-like 'dennis' and 'nail-head ray', as well as for yellow forewing band patterns. It occurs only at rather high altitudes (for *Heliconius*) in the eastern slopes of the Andes (approx. 1000–1800 m above sea level) and co-exists with *Heliconius erato notabilis* and *Heliconius melpomene plesseni* (Fig. 10.4). The latter have co-mimetic 'twin-spot' pink and white patterns that bear no resemblance to 'dennis-ray' patterns, even though further down the Pastaza valley, these two upland races form a hybrid zone with 'dennis-ray' Amazonian races *Heliconius erato lativitta* and *Heliconius melpomene malleti*, respectively. Polymorphisms and lack of suitable co-mimics show that *timareta* is clearly non-mimetic. Most *Heliconius* are widely distributed, but, like *heurippa*, *timareta* is restricted to a small strip of submontane forest along the eastern slopes of the Andes and appears to be most closely related to *cydno*, although likelihood and other analyses of DNA sequence data do not entirely rule out *melpomene* as its closest relative. One likely possibility is that *timareta* is another form that has stabilized after hybridization between Amazonian rayed *melpomene* and an unidentified, maybe extinct form of *cydno*. Recently, a new subspecies, *Heliconius timareta timoratus*, was described (Lamas 1998). The specimen came from above 1000 m altitude in the Cordillera del Condor, on the formerly disputed border of Ecuador and Peru. The new subspecies is known from a single specimen, but in this case it is a near-perfect mimic of the local *melpomene* race *H. m. malleti*.

Another recently discovered taxon in this group is *H. tristero*. This form is found at middle elevations, >800 m altitude above Mocoa, in the Southeastern Andes of Putumayo, Colombia. The species description was based on only two males, both of which had *cydno*-like mtDNA, but were extremely similar in appearance to the local *Heliconius melpomene bellula* (Brower 1996a). *H. tristero* was therefore apparently an Eastern Andes form of *cydno* that mimicked *melpomene* and *erato* (Fig. 10.4). Perhaps understandably, several of us were sceptical that *tristero* was a separate species in its own right: could it not be an aberration of cydno mtDNA introgression into melpomene? Most morphological characters suggested by Brower to characterize *H. tristero* appeared labile, and so only the mtDNA evidence was at all convincing. 'It is straightforward to produce wing pattern/mtDNA combinations closely resembling Brower's new taxa, … Thus, in the case of (*cydno*) *tristero* and *melpomene*, we probably have a case of *melpomene* red patterns shared by common descent from F1 fathers in an area of natural hybridization, rather than mimicry between *cydno* and *melpomene* (which is not known to exist)' (Gilbert 2003). *H. tristero* did not appear likely to be a stable form, firstly because so few specimens had been collected, so that ongoing hybridization could not be ruled out, and secondly because *cydno* and *melpomene* were unknown previously to mimic one another. As we have seen, the classical story was that *cydno* and *melpomene* had diverged as a result of a shift in mimicry ring.

Today, nuclear DNA data from Brower's original specimens (Mavárez, in preparation), as well as more recent collections from Putumayo (Arias & Huertas 2001), suggest that *tristero* does constitute a separate species from *melpomene*. As still more new *cydno*-like lineages are discovered that are also mimics of other *melpomene* races in the Eastern Andes, Brower's early insight is increasingly supported.

The first such additional example discovered was in the upper Mayo valley near the Río Serranoyacu, San Martín, Peru (Fig. 10.4). Mathieu Joron, Gerardo Lamas and I had long recognized what we thought of as a distinct 'upland' postman-patterned form of *melpomene* from above 1000 m altitude of the upper Río Mayo valley. This form occurred adjacent to and sometimes overlapping with a well-studied, but generally lower elevation form, the postman-patterned *Heliconius melpomene amaryllis* (Mallet *et al.* 1990). Although the overriding impression is of great similarity of the two forms, the 'upland' form is characterized by larger size, narrower and more whitish hindwing bar, more sinuous and narrower forewing band, and strongly expressed basal red spots on the undersides of fore- and hind-wings. All these colour pattern characteristics tend to enhance the otherwise *amaryllis*-like 'postman' pattern's weak mimetic alliance with another abundant upland form, *Heliconius telesiphe*, which has a twin-barred 'postman' pattern and occurs in Ecuador, Peru and Bolivia (Vane-Wright *et al.*

1975), and often replaces *erato* ecologically in steep submontane forests above about 800 m altitude (Benson 1978; Mallet 1993). In the 1980s, I ran allozyme electrophoresis gels on 'upland *melpomene*', and found no major differences from *H. m. amaryllis*. However, we now know that polymorphic nuclear genetic markers are frequently shared between *cydno*, *melpomene* and other relatives, in part because of introgression between all the species (Beltrán 1999; Bull *et al.* 2006; Kronforst *et al.* 2006b). Recently, Jesús Mavárez *et al.* (in preparation) have shown that mtDNA and some nuclear sequences of these 'upland *melpomene*' match *timareta* more closely than those of local *H. m. amaryllis*, and that morphology differences correlate perfectly with DNA sequence differences. Again we have a form with *cydno*-like DNA profile and a colour pattern whose most notable feature is near-identity with that of a local race of *melpomene*.

A second cryptic taxon is now being investigated in Colombia in Mauricio Linares' laboratory (in preparation). In upland regions near Florencia, Colombia, a 'dennis-ray' *Heliconius* with *cydno*-like mtDNA again co-occurs with the local *melpomene* form, the Amazonian 'dennis-ray' *H. m. malleti* (Fig. 10.4). The Linares group have made remarkably complete genetic, morphological, ecological and behavioural studies of these cryptic species. They have demonstrated that host-plant usage and assortative mating correlate closely with nuclear and mitochondrial DNA markers, and with slight diagnostic colour pattern differences between the two mimetic forms (Giraldo 2005). Here again is a cryptic species that shares a mimetic colour pattern with the local form of *melpomene*.

Introgression and hybridization as a source of mimetic adaptations

Much biological work remains to be done on these new taxa. However, recent discoveries of multiple *cydno*-like or *timareta*-like *Heliconius* taxa are no longer in doubt (Fig. 10.4). These all overlap in extensive sympatry with local *melpomene*. In some cases (*heurippa*, *timareta*), the forms are non-mimetic, although their colour patterns strongly suggest evidence of past hybridization with *melpomene* (and in the case of *heurippa*, with *H. cydno cordula* as well). In other cases, the patterns are nearly identical to the local *melpomene*, and are best interpreted as co-mimetic with that species and with *erato*. It is striking that these include both Amazonian 'dennis-ray' (e.g. *timareta*) and also Andean 'postman' patterns (e.g. *tristero*) found in *melpomene* (Fig. 10.4) as well as in the latter's usual co-mimic *erato*. It would now not be surprising to find multiple other *cydno*-like *melpomene*-mimicking cryptic taxa at similar elevations in many other East Andean valleys in Ecuador, Peru and Bolivia.

The detailed morphology of these *cydno*-like taxa (Fig. 10.4), together with knowledge of the inheritance of colour pattern in *cydno*, *melpomene* and their hybrids (Naisbit *et al.* 2003), suggests that the object of mimicry itself, *melpomene*, is a likely source of the mimicry adaptation via introgression. Alternatively, mimicry-determining genes might be extremely labile, allowing repeated, homoplasious re-evolution of nearly identical mimetic patterns, including two re-inventions of nailhead rays, by *H. timareta* and the related taxon from Florencia. However, such homoplasy fails to explain why even the *non*-mimetic forms *heurippa* and *timareta* have colour patterns derivable from sympatric or closely adjacent forms of *melpomene*. In the case of *heurippa*, the pattern is clearly derived only from local *cydno* as well. These hypotheses will be testable as soon as genes for colour pattern mimicry are fully characterized and sequenced (Joron *et al.* 2006; Kronforst *et al.* 2006a). For the moment, parsimony and existing evidence suggest that *timareta*-like and *cydno*-like Andean forms have hybrid origins with local *melpomene*.

Generality of adaptive introgression

It could be argued that mimicry is a freak adaptation for which hybridization is particularly likely to be adaptive. Relatively few loci need to be introgressed to encode major switches in mimicry (Naisbit *et al.* 2003), and the colour patterns needed are exactly those already found in other local *Heliconius* species. But I don't believe that mimicry is unusual in this respect. Adaptive radiations often occur when a dispersal event or a novel general adaptation allows a species to colonize an enhanced range of pre-existing specialized ecological opportunities. In many cases, the same range of niches may have been colonized already by the same lineage in an earlier radiation, so that hybridization between the earlier lineage and the new species could enhance the possibility of re-acquiring many of the same species-specific niche-adaptations to allow a new radiation. There are possible examples of such traits in *Heliconius* that have nothing to do with mimicry. For instance, the larvae of most heliconiine species are oligophagous within sub-groups of the plant genus *Passiflora* (Passifloraceae). However, distinct butterfly lineages from 'basal genera', *Eueides* and *Heliconius* (Fig. 10.1) have repeatedly recolonized Passifloraceae of the same large subgenera *Granadilla*, *Plectostemma*, *Distephana* and *Astrophea* (Benson *et al.* 1976). Suppose a member of a radiating lineage acquired a new key adaptation, say adult pollen-feeding, or the extraordinary pupal mating habit (Beltrán *et al.* 2007). Then hybridization and recombination with members of the earlier radiation would permit a new radiation having both the key novel adaptation as well as the earlier-developed array of species-specific host-plant chemical adaptations.

Beak shape in Darwin's finches is another characteristic which affects not only trophic niche (the range of seeds that can be handled in competition with other species), but also song characteristics that partially determine mating behaviour (Grant & Grant 1998; Podos 2001). High variance in beak shape, in part due to occasional hybridization, will enhance evolutionary flexibility (Grant 1993; Grant & Grant 1998, 2008; Podos 2001) and at the same time allow repeated associated evolution of reproductive isolation via its effect on song characteristics, as is the case with *Heliconius* mimicry. Similar cases are also evident in cichlid fish, where ecologically relevant trophic or spawning habit traits, such as water depth, may affect colouration perception during courtship, and so lead to changes in optimal male sexual colouration and reproductive isolation (Seehausen 2004).

Conclusion

Hybridization and introgression could thus play a much greater role in adaptive radiation and speciation than traditionally assumed likely, in animals as well as in plants (Arnold 1997; Mallet 2005, 2007; Grant & Grant 2008). Species in many rapidly radiating groups are incompletely reproductively isolated, even though this hardly affects their ability to remain distinct when in sympatry with congeners. Leaky reproductive barriers are now generally acknowledged to be common, given extensive evidence from sequencing and genotyping studies (Coyne & Orr 2004; Seehausen 2004; Mallet 2005; Grant & Grant 2008); meanwhile, genes useful in adaptation should show enhanced success of transfer compared with introgression of the anonymous molecular markers normally documented. It no longer seems unlikely that rapidly diversifying lineages owe some of their evolutionary flexibility and success to genetic variance shared via occasional hybridization, providing introgression with which they can build new and useful combinations of adaptations. It is still unknown just how important hybridization is in influencing adaptive radiation, but studies on tractable groups such as *Heliconius* butterflies are likely to provide some of the answers.

Acknowledgements

I am very grateful for discussions of many of the many exciting new discoveries on these taxa among members of the *Heliconius* Consortium. Here, I am particularly grateful for unpublished information from Kanchon Dasmahapatra on the *H. demeter* cryptic species work; from Natalia Giraldo, Adriana Tapia and Mauricio Linares on the work in Florencia, Colombia; from Jesús Mavárez and Mathieu Joron on the work in the Upper Río Mayo Valley of Peru; and from Blanca Huertas about *H. tristero* in Churumbelos, Colombia. I am also grateful to Mathieu Joron, Mauricio Linares, Jesús Mavárez, Jon Bridle and two anonymous reviewers for critically reading the manuscript. This work was supported by NERC and DEFRA Darwin Initiative.

References

Arias, J. J. and Huertas, B. (2001) Mariposas diurnas de la Serranía de los Churumbelos, Cauca. Distribución altitudinal y diversidad de especies (Lepidoptera: Rhopalocera: Papilionoidea). *Revista Colombiana de Entomologia* **27**, 169–176.

Arnold, M. L. (1997) *Natural Hybridization and Evolution*. Oxford University Press, Oxford.

Bates, H. W. (1862) Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. *Transactions of the Linnean Society of London* **23**, 495–566.

Beltrán, M. (1999) Evidencia genética (alozimas) para evaluar el posible orígen híbrido de *Heliconius heurippa* (Lepidoptera: Nymphalidae). Universidad de los Andes, Bogotá.

Beltrán, M., Jiggins, C. D., Brower, A. V. Z., Bermingham, E. and Mallet, J. (2007) Do pollen feeding and pupal-mating have a single origin in *Heliconius*? Inferences from multilocus sequence data. *Biological Journal of the Linnean Society* **92**, 221–239.

Benson, W. W. (1978) Resource partitioning in passion vine butterflies. *Evolution* **32**, 493–518.

Benson, W. W., Brown, K. S. and Gilbert, L. E. (1976) Coevolution of plants and herbivores: passion flower butterflies. *Evolution* **29**, 659–680.

Brower, A. V. Z. (1996a) A new mimetic species of *Heliconius* (Lepidoptera: Nymphalidae), from southeastern Colombia, revealed by cladistic analysis of mitochondrial DNA sequences. *Zoological Journal of the Linnean Society* **116**, 317–332.

Brower, A. V. Z. (1996b) Parallel race formation and the evolution of mimicry in *Heliconius* butterflies: a phylogenetic hypothesis from mitochondrial DNA sequences. *Evolution* **50**, 195–221.

Brown, K. S. (1981) The biology of *Heliconius* and related genera. *Annual Review of Entomology* **26**, 427–456.

Bull, V., Beltrán, M., Jiggins, C. D., *et al.* (2006) Polyphyly and gene flow between non-sibling *Heliconius* species. *BMC Biology* **4**, 11.

Coyne, J. A. and Orr, H. A. (2004) *Speciation*. Sinauer Associates, Sunderland, Mass.

Dasmahapatra, K. K., Silva, A., Chung, J.-W. and Mallet, J. (2007) Genetic analysis of a wild-caught hybrid between non-sister *Heliconius* butterfly species. *Biology Letters* **3**, 660–663.

Eltringham, H. (1917) On specific and mimetic relationships in the genus *Heliconius*. *Transactions of the Entomological Society of London* **1916**, 101–148.

Emsley, M. G. (1964) The geographical distribution of the color-pattern components of *Heliconius erato* and *Heliconius melpomene* with genetical evidence for the systematic relationship between the two species. *Zoologica; Scientific Contributions of the New York Zoological Society* **49**, 245–286.

Estrada, C. and Jiggins, C. D. (2002) Patterns of pollen feeding and habitat preference among *Heliconius* species. *Ecological Entomology* **27**, 448–456.

Fisher, R. A. (1930) *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

Flanagan, N. S., Tobler, A., Davison, A., *et al.* (2004) The historical demography of Müllerian mimicry in the neotropical *Heliconius* butterflies. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 9704–9709.

Futuyma, D. J. (1998) *Evolutionary Biology*, 3rd edn. Sinauer, Sunderland, Mass.

Gilbert, L. E. (2003) Adaptive novelty through introgression in *Heliconius* wing patterns: evidence for a shared genetic 'toolbox' from synthetic hybrid zones and a theory of diversification. In: *Ecology and Evolution Taking Flight: Butterflies as Model Systems* (ed. C. L. Boggs), pp. 281–318. University of Chicago Press, Chicago.

Giraldo, N. (2005) Posible convergencia mimética entre las especies *Heliconius melpomene* y *H. cydno* (Lepidoptera: Nymphalidae) en Florencia (Caquetá): aproximación morfológica y genética. Universidad de Los Andes, Bogotá.

Grant, P. R. (1993) Hybridization of Darwin's finches on Isla Daphne Major, Galápagos. *Philosophical Transactions of the Royal Societies of London B* **340**, 127–139.

Grant, B. R. and Grant, P. R. (1998) Hybridization and speciation in Darwin's finches. The role of sexual imprinting on a culturally transmitted trait. In: *Endless Forms. Species and Speciation* (ed. D. J. Howard), pp. 404–422. Oxford University Press, New York.

Grant, P. R. and Grant, B. R. (2008) *How and Why Species Multiply. The Radiation of Darwin's Finches*. Princeton University Press, Princeton, NJ.

Jiggins, C. D., Emelianov, I. and Mallet, J. (2005) Assortative mating and speciation as pleiotropic effects of ecological adaptation: examples in moths and butterflies. In: *Insect Evolutionary Ecology* (ed. M. Fellowes), pp. 451–473. Royal Entomological Society, London.

Jiggins, C. D., Mallarino, R., Willmott, K. R. and Bermingham, E. (2006) The phylogenetic pattern of speciation and wing pattern change in Neotropical *Ithomia* butterflies (Lepidoptera: Nymphalidae). *Evolution* **60**, 1454–1466.

Jiggins, C. D., McMillan, W. O., King, P. and Mallet, J. (1997a) The maintenance of species differences across a *Heliconius* hybrid zone. *Heredity* **79**, 495–505.

Jiggins, C. D., McMillan, W. O. and Mallet, J. L. B. (1997b) Host plant adaptation has not played a role in the recent speciation of *Heliconius himera* and *Heliconius erato* (Lepidoptera: Nymphalidae). *Ecological Entomology* **22**, 361–365.

Jiggins, C. D., Naisbit, R. E., Coe, R. L. and Mallet, J. (2001) Reproductive isolation caused by colour pattern mimicry. *Nature* **411**, 302–305.

Joron, M., Papa, R., Beltrán, M., *et al.* (2006) A conserved supergene locus controls colour pattern diversity in *Heliconius* butterflies. *PLoS Biology* **4**, e303.

Kapan, D. D. (2001) Three-butterfly system provides a field test of Müllerian mimicry. *Nature* **409**, 338–340.

Kronforst, M. R., Kapan, D. D. and Gilbert, L. E. (2006a) Parallel genetic architecture of parallel adaptive radiations in mimetic *Heliconius* butterflies. *Genetics* **174**, 535–539.

Kronforst, M. R., Salazar, C., Linares, M. and Gilbert, L. E. (2007) No genomic mosaicism in a putative hybrid butterfly species. *Proceedings of the Royal Society B* **274**, 1255–1264.

Kronforst, M. R., Young, L. G., Blume, L. M. and Gilbert, L. E. (2006b) Multilocus analysis of admixture and introgression among hybridizing *Heliconius* butterflies. *Evolution* **60**, 1254–1268.

Kronforst, M. R., Young, L. G., Kapan, D. D., *et al.* (2006c) Linkage of butterfly mate preference and wing color preference cue at the genomic location of *wingless*. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 6575–6580.

Lamas, G. (1998) Comentarios taxonómicos y nomenclaturales sobre Heliconiini neotropicales, con designación de lectotipos y descripción de cuatro subespecies nuevas (Lepidoptera: Nymphalidae: Heliconiinae). *Revista Peruana de Entomologia* **40** (1997), 111–125.

Lamas, G. (2004) Heliconiinae. In: *Hesperioidea – Papilionoidea* (ed. G. Lamas), pp. 261–274. Association for Tropical Lepidoptera. Scientific Publishers, Gainesville, FL.

Linares, M. (1989) Adaptive microevolution through hybridization and biotic destruction in the neotropics. University of Texas at Austin.

Linares, M. (1996) The genetics of the mimetic coloration in the butterfly *Heliconius cydno weymeri*. *Journal of Heredity* **87**, 142–149.

Linares, M. (1997) The ghost of mimicry past: laboratory reconstitution of an extinct butterfly ‘race’. *Heredity* **78**, 628–635.

Mallet, J. (1993) Speciation, raciation, and color pattern evolution in *Heliconius* butterflies: evidence from hybrid zones. In: *Hybrid Zones and the Evolutionary Process* (ed. R. G. Harrison), pp. 226–260. Oxford University Press, New York.

Mallet, J. (2001a) Causes and consequences of a lack of coevolution in Müllerian mimicry. *Evolutionary Ecology* **13**, 777–806.

Mallet, J. (2001b) The speciation revolution. *Journal of Evolutionary Biology* **14**, 887–888.

Mallet, J. (2005) Hybridization as an invasion of the genome. *Trends in Ecology & Evolution* **20**, 229–237.

Mallet, J. (2006) What has *Drosophila* genetics revealed about speciation? *Trends in Ecology & Evolution* **21**, 386–393.

Mallet, J. (2007) Hybrid speciation. *Nature (London)* **446**, 279–283.

Mallet, J., Barton, N., Lamas, G., *et al.* (1990) Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in *Heliconius* hybrid zones. *Genetics* **124**, 921–936.

Mallet, J., Beltrán, M., Neukirchen, W. and Linares, M. (2007) Natural hybridization in heliconiine butterflies: the species boundary as a continuum. *BMC Evolutionary Biology* **7**, 28.

Mallet, J. and Gilbert, L. E. (1995) Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biological Journal of the Linnean Society* **55**, 159–180.

Mallet, J., McMillan, W. O. and Jiggins, C. D. (1998a) Estimating the mating behavior of a pair of hybridizing *Heliconius* species in the wild. *Evolution* **52**, 503–510.

Mallet, J., McMillan, W. O. and Jiggins, C. D. (1998b) Mimicry and warning color at the boundary between races and species. In: *Endless Forms: Species and Speciation* (ed. D. J. Howard), pp. 390–403. Oxford University Press, New York.

Mavárez, J., Salazar, C., Bermingham, E., *et al.* (2006) Speciation by hybridization in *Heliconius* butterflies. *Nature* **441**, 868–871.

McMillan, W. O., Jiggins, C. D. and Mallet, J. (1997) What initiates speciation in passion-vine butterflies? *Proceedings of the National Academy of Sciences of the United States of America* **94**, 8628–8633.

Müller, F. (1879) *Ituna* and *Thyridia*; a remarkable case of mimicry in butterflies. *Transactions of the Entomological Society of London* **1879**, xx–xxix.

Naisbit, R. E., Jiggins, C. D., Linares, M. and Mallet, J. (2002) Hybrid sterility, Haldane's rule, and speciation in *Heliconius cydno* and *H. melpomene*. *Genetics* **161**, 1517–1526.

Naisbit, R. E., Jiggins, C. D. and Mallet, J. (2001) Disruptive sexual selection against hybrids contributes to speciation between Heliconius cydno and H. melpomene. *Proceedings of the Royal Society London B* **268**, 1849–1854.

Naisbit, R. E., Jiggins, C. D. and Mallet, J. (2003) Mimicry: developmental genes that contribute to speciation. *Evolution & Development* **5**, 269–280.

Podos, J. (2001) Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**, 185–188.

Salazar, C. A., Jiggins, C. D., Arias, C. F., *et al.* (2005) Hybrid incompatibility is consistent with a hybrid origin of *Heliconius heurippa* Hewitson from its close relatives, *Heliconius cydno* Doubleday and *Heliconius melpomene* Linnaeus. *Journal of Evolutionary Biology* **18**, 247–256.

Schluter, D. (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, New York.

Seehausen, O. (2004) Hybridization and adaptive radiation. *Trends in Ecology & Evolution* **19**, 198–207.

Sheppard, P. M., Turner, J. R. G., Brown, K. S., Benson, W. W. and Singer, M. C. (1985) Genetics and the evolution of muellerian mimicry in *Heliconius* butterflies. *Philosophical Transactions of the Royal Society of London B* **308**, 433–613.

Turner, J. R. G. (1976) Adaptive radiation and convergence in subdivisions of the butterfly genus *Heliconius* (Lepidoptera: Nymphalidae). *Zoological Journal of the Linnean Society* **58**, 297–308.

Vane-Wright, R. I., Ackery, P. R. and Smiles, R. L. (1975) The distribution, polymorphism and mimicry of *Heliconius telesiphe* (Doubleday) and the species of *Podotricha* Michener (Lepidoptera: Heliconiinae). *Transactions of the Royal Entomological Society of London* **126**, 611–636.