*Biological Journal of the Linnean Society* (1996), *59:* 221–242. With 4 figures

# What can hybrid zones tell us about speciation? The case of *Heliconius erato* and *H. himera* (Lepidoptera: Nymphalidae)

CHRIS D. JIGGINS[1], W, OWEN McMILLAN[1], WALTER NEUKIRCHEN[2]
AND JAMES MALLET[1]

[1] *The Galton Laboratory, Department of Biology, University College London, 4 Stephenson Way, London NW1 2HE*

[2] *Fuchsiensweg 23, 12357 Berlin, Germany*

*Received 25 October 1995, accepted for publication 19 January 1996*

---

To understand speciation we need to study the genetics and ecology of intermediate cases where interspecific hybridization still occurs. Two closely related species of *Heliconius* butterflies meet this criterion: *Heliconius himera* is endemic to dry forest and thorn scrub in southern Ecuador and northern Peru, while its sister species, *H. erato*, is ubiquitous in wet forest throughout south and central America. In three known zones of contact, the two species remain distinct, while hybrids are found at low frequency. Collections in southern Ecuador show that the contact zone is about 5 km wide, half the width of the narrowest clines between colour pattern races of *H. erato*. The narrowness of this cline argues that very strong selection ($s \approx 1$) is maintaining the parapatric distributions of these two species. The zone is closely related with a habitat transition from wet to dry forest, which suggests that the narrow zone of parapatry is maintained primarily by ecological adaptation. Selection on colour pattern loci, assortative mating and hybrid inviability may also be important. The genetics of hybrids between the two species shows that the major gene control of pattern elements is similar to that found in previous studies of *H. erato* races, and some of the loci are homologous. This suggests that similar genetic processes are involved in the morphological divergence of species and races. Evidence from related *Heliconius* supports a hypothesis that ecological adaptation is the driving force for speciation in the group.

ADDITIONAL KEY WORDS:—parapatry – *Passiflora* – refugium biogeography – hybridization.

## CONTENTS

Introduction . . . . . . . . . . . . . . . . . . . . . . . . . . . . 222
Methods and material . . . . . . . . . . . . . . . . . . . . . . . . . 224
Results . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 225
  Geography of the hybrid zones . . . . . . . . . . . . . . . . . . . 225
  Ecology of the hybrid zones . . . . . . . . . . . . . . . . . . . . 225
  Genetics of hybrids . . . . . . . . . . . . . . . . . . . . . . . . 230
Discussion . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 233
  Taxonomic status of *H. himera* . . . . . . . . . . . . . . . . . . 233
  Structure and maintenance of the hybrid zone . . . . . . . . . . . 234

Correspondence to Chris Jiggins. email: c.jiggins@ucl.ac.uk

0024–4066/96/011221 + 22 $25.00/0

Genetics of species differences . . . . . . . . . . . . . . . . . . . . . 237
Mode of speciation . . . . . . . . . . . . . . . . . . . . . 238
Geographic context of speciation . . . . . . . . . . . . . . . . . . . . 238
Conclusion . . . . . . . . . . . . . . . . . . . . . . . . . 239
Acknowledgements . . . . . . . . . . . . . . . . . . . . . . . . 239
References . . . . . . . . . . . . . . . . . . . . . . . . . 239
Appendix . . . . . . . . . . . . . . . . . . . . . . . . . 242

## INTRODUCTION

Speciation links evolution within populations to the generation of biological diversity on a wider scale, but the genetic processes involved are still poorly understood. Perhaps the best way to understand the genetics and ecology of speciation is to study intermediate cases. The majority of such studies have been carried out in hybrid zones where hybrids are abundant. Many of these cases have been stable over huge periods of evolutionary time, although abundant hybrids are still produced (Endler, 1977; Barton & Hewitt, 1989; Hewitt, 1989; Harrison, 1993). These studies have been extremely useful for understanding the interactions of gene flow and selection. However, inferences about speciation from these studies have been limited, because, under most definitions of species, speciation has not happened. For example hybrid zones in *Bombina* separate forms differing in mating call, warning coloration, life history, preferred habitat, enzymes and mtDNA, and are maintained by hybrid inviability at many loci (Szymura & Barton, 1986; 1991). However hybrids are in Hardy-Weinberg equilibrium and the zone itself has been stable, probably since secondary contact about ten thousand years ago. Moreover, genetic differences between forms suggests that divergence has been continuing for 3–4 million years without leading to speciation.

To understand the genetics and ecology of speciation it will be valuable to study examples where hybrids are produced, but are rarer than expected under random mating. In these situations, the two parental forms have reached that stage in speciation where they can maintain their genetic integrity in the face of gene flow, but have diverged sufficiently recently that genetic and ecological differences between them are likely to be involved both in current species maintenance, and in the initial divergence which led to speciation. Whether such forms are considered to be ‘good’ species depends on the species definition which one chooses to invoke. Under a genotypic cluster definition (Mallet, 1995), maintenance of the genetic integrity of taxa in sympatry is used as the criterion for species. Under the biological species concept the occurrence of any hybrids may be taken to imply that speciation is incomplete (Dobzhansky, 1937; Mayr, 1940). Whatever definition one uses, taxa which hybridize occasionally in sympatry are clearly important for the study of speciation as they have begun to acquire some, if not all, of the characteristics of species. Furthermore the existence of hybrids allows genetic analysis of species differences through the study of naturally occurring and laboratory produced crosses.

Although taxa meeting this criteria have been studied in the laboratory (Coyne & Orr, 1989), there has been little work done in the wild. This is unfortunate for two reasons. First, interspecific hybridization in animals is much commoner than has been thought: approximately 9% of bird species are known to hybridize (Grant & Grant, 1992) and the proportion is similar or even higher in other groups, for example European butterflies and coral reef fishes (Guillaumin & Descimon, 1976;

Pyle & Randel, 1994). Second, it is important to study interspecific hybridization in the wild, in order to identify factors maintaining the genetic integrity of hybridizing forms in a natural context. Interspecific hybrid zones are particularly useful as many of the factors which are important in maintaining such zones are also important in speciation; for example assortative mating, ecological adaptation, and hybrid sterility or inviability.

Since Dobzhansky (1937) most genetic work on reproductive isolation between species has concentrated on sibling species of *Drosophila*. Speciation in butterflies of the genus *Heliconius* provides an interesting contrast to *Drosophila*. In many continental *Drosophila* speciation has occurred with little ecological radiation, producing groups of reproductively isolated sibling species which are almost identical morphologically (Dobzhansky, 1937). In contrast *Heliconius* species show a remarkable diversity of colour pattern, which is evident both within and between species. Together with its sister genera, *Eueides* (12 spp.) and *Neruda* (3 spp.), *Heliconius* (40 species) appears to have undergone a recent radiation, compared to more basal genera of heliconiines with far fewer species, for example *Dryas* (1 sp.), *Agraulis* (1 sp.), *Dione* (3 spp.) and *Podotricha* (2 spp.) (Brown, 1981). In the *Heliconius* radiation this may be associated with the unusual ecological adaptations of the genus, such as pollen feeding, traplining of food and oviposition resources and longevity of adults (Gilbert, 1975; Brown, 1981).

Moreover, within *Heliconius* there is a range of intermediate steps on the road to speciation. Closely related species pairs such as *Heliconius clysonymus* and *H. erato* overlap in parapatry without hybridizing (Benson, 1978). At the other end of the spectrum, *Heliconius* species often consist of a large number of colour pattern races, which are stable over wide geographic areas, but hybridize freely where they abut. Some are separated by broad clines at a few colour pattern loci, others by narrow hybrid zones where more loci and greater pattern differences are involved (Turner, 1971; Benson, 1982; Mallet, 1986; 1993; Mallet et al., 1990). These hybrid zones are probably maintained by frequency-dependent mimetic selection against rare colour patterns (Brown, Sheppard & Turner, 1974; Mallet & Barton, 1989b). The extraordinary diversity of mimetic geographic races, and the fact that closely related species pairs nearly always show divergent wing colour patterns suggests that adaptive radiation of colour patterns preceded, or at least accompanied speciation in this group (Turner, 1976).

Between these extremes of geographic races and good, non-hybridizing species, are forms which produce hybrids, but which are stable to introgression when in contact. Hybridization in sympatry is regularly found, especially among the species closely related to *H. melpomene* (e.g. *H. melpomene, H. cydno* and the 'silvaniforms' *H. numata, H. ethilla, H. hecale*; Ackery & Smiles, 1976; Brown, 1976; Holzinger & Holzinger, 1994). Parapatric hybridization between *Heliconius* species is known only between *H. erato* and *H. himera* (Descimon & Mast de Maeght, 1984; Konig, 1986; Mallet, 1993). Descimon and Mast de Maeght suggested a more complete study of hybridization between these forms would be of great interest, although "one may cast doubts about the observational and experimental facilities offered by populations flying in remote parts of southern Ecuador". We attempt such a study here.

In this paper the biogeography, ecology and genetics of hybridization are investigated in the wild. Distributions and contact zones of *H. erato* and *H. himera* are mapped in detail. The study concentrates on one of these regions of contact in southern Ecuador where the habitat and food plant preferences of the two species are investigated for evidence of ecological divergence. The width and structure of the hybrid zone are determined, yielding clues to the levels and types of selection. All wild-caught hybrids known from the zone are recorded and the commonest phenotypes figured in colour. The genetic basis of colour pattern differences deduced from the phenotypes of these hybrids is used to infer relationships between *H. himera* and other races of *H. erato*. Descimon & Mast de Maeght (1984) proposed that there was a deficit of hybrids, especially females (Haldane's [1922] Rule), compared with that expected under Hardy-Weinberg; this is tested more fully with the larger collections now available. Levels of hybridization seen in nature are used to assess the taxonomic status of the forms and the extent of gene flow between them. This paper concerns only the field data; further papers in preparation deal with mate choice, hybrid inviability and sterility, and introgression of molecular markers across the contact zone between the two species.

## METHODS AND MATERIAL

Southwest Ecuador and northern Peru were visited at various times between 1984 and 1995. The distribution of *Heliconius* species was investigated over a wide area and especially in the provinces of Loja, El Oro, Zamora-Chinchipe (Ecuador), and the Departments of Amazonas, Cajamarca, Piura and Lambayeque (Peru). The study concentrated on the zone of contact found between *H. himera* and *H. erato* in southern Ecuador described by Descimon & Mast de Maeght (1984). A number of sites were visited on the Guayquichuma to Zambi road, which runs along the Río Yaguachi valley, and in the vicinity of Buenavista and Chaguarpamba in the adjacent valley. Collections were made during both dry and wet seasons.

A 'species index' was calculated for each site as an indirect indicator of gene frequencies across the zone, using colour pattern markers to identify parental and hybrid genotypes. Each individual collected was scored as follows *H. himera* = 0, *H. erato* = 1, putative F1 hybrid = 0.5 and putative backcrosses 0.25 and 0.75 respectively. The hybrid genetics were inferred from field collections, but have since been confirmed in laboratory hybridizations and allozyme studies of field-caught hybrids. The mean species index value for each site was calculated. A line was drawn along the Río Yaguachi valley to form the Guayquichuma transect line. This line was chosen as the forest remnants in this valley form a naturally linear habitat. Perpendiculars were dropped from each site along the valley onto the line, and a graph of species index against transect distance was then plotted. The width (w) of the cline was determined by drawing a tangent to the steepest section of the cline and measuring the distance which this line projects onto the x-axis.

Ecological effects on the contact zone were assessed by records of larval food plants and a general plant survey. Passifloraceae are virtually the sole host plants for *Heliconius* (Gilbert, 1975; Brown, 1981). To determine which hosts are utilized, a search was made at all sites visited for *Passiflora* and associated larvae or eggs, which were reared to determine species and sex. The distributions of all *Passiflora* and *Heliconius* species were also recorded. Five common tree species were recorded across the area to give general information on vegetation. Plant specimens were collected and deposited in the Herbario Nacional in Quito and in the herbarium of the Universidad Nacional, Loja. Vouchers of the butterflies were deposited in the Museo de Ciencias Naturales in Quito.

## RESULTS

### *Geography of the hybrid zones*

*H. erato* is a widespread species occurring from south Texas to southeastern Brazil and Argentina where it is generally found in gaps and disturbed areas of wet or gallery forest. *H. himera* replaces *H. erato* in a restricted area centred on the western slopes of the Andes in southern Ecuador and northern Peru and the Marañon valley in north eastern Peru (Fig. 1). There are three known contact zones between *H. himera* and different races of *H. erato* and hybrids have been found, albeit rarely, in all of these (Fig. 1; Descimon & Mast de Maeght, 1984; Konig, 1986; Mallet, 1993). The contact zone studied here is in the Río Puyango drainage, Loja and El Oro provinces, Ecuador (Fig. 2). The east-west extent of the contact zone is restricted by the Andes to the east, and mountains and coastal desert to the west. Across this region there is an abrupt transition from *H. erato cyrbia* to *H. himera*. The majority of collections are from the Guayquichuma transect, which follows a linear forest habitat running along the Río Yaguachi valley floor and in tributary valleys (Table 1A). Across the transition there is a smooth, monotonic cline with no evidence of a mosaic pattern. The width of this cline is approximately 5 km (Fig. 3). Collections in the Chaguarpamba region are less complete but show a similar pattern (Table 1B). The only inconsistency is at site 17 which is a pure *himera* site that lies between two mixed sites (Fig. 2). However, this is probably a sampling problem, as only 14 individuals were collected here; otherwise the transition occurs over approximately the same distance as in the Guayquichuma transect, and a comprehensive sampling of the whole region would almost certainly show a simple cline across the whole zone of contact.

### *Ecology of the hybrid zones*

Collections of *Heliconius* larvae and eggs in the hybrid zone area show that the primary host plants for *H. himera* and *H. e. cyrbia* are *Passiflora rubra* and *P. punctata* (Table 2). These plants are both relatively common throughout the hybrid zone (Table 3). There is no evidence that either of the two primary host plant species is preferred by either butterfly species. In cage experiments *H. himera* and *H. erato* females lay eggs freely on *P. rubra*, *P. punctata* and a third species, *P. auriculata*, and the larvae of both species survive well on all three (Jiggins, McMillan & Mallet, 1996). *Passiflora auriculata* is known to be an important host of *H. erato* in other areas (Brown, 1981) and although there are currently no records of larvae or eggs on this species from this area, it is likely that *H. erato* does utilize it here as well. *Passiflora auriculata* only occurs in the wetter areas alongside *H. erato* and so is largely unavailable to *H. himera*.

*H. himera* and *H. erato* have divergent altitudinal ranges. Museum specimens and field experience indicate that *H. himera* is abundant up to 2000 m, with a lower limit of around 400 m where contact with *H. erato lativitta* occurs in the Marañon valley (Mallet, pers. obs.), although larval host plants occur both higher and lower. On the pacific slopes the lower limit of *H. himera* is restricted by contact with *H. erato* in Ecuador, and by desert in Peru. In contrast, *H. erato* rarely occurs above 1500 m but descends to sea level where suitable habitat occurs (Brown, 1979; pers. obs.).

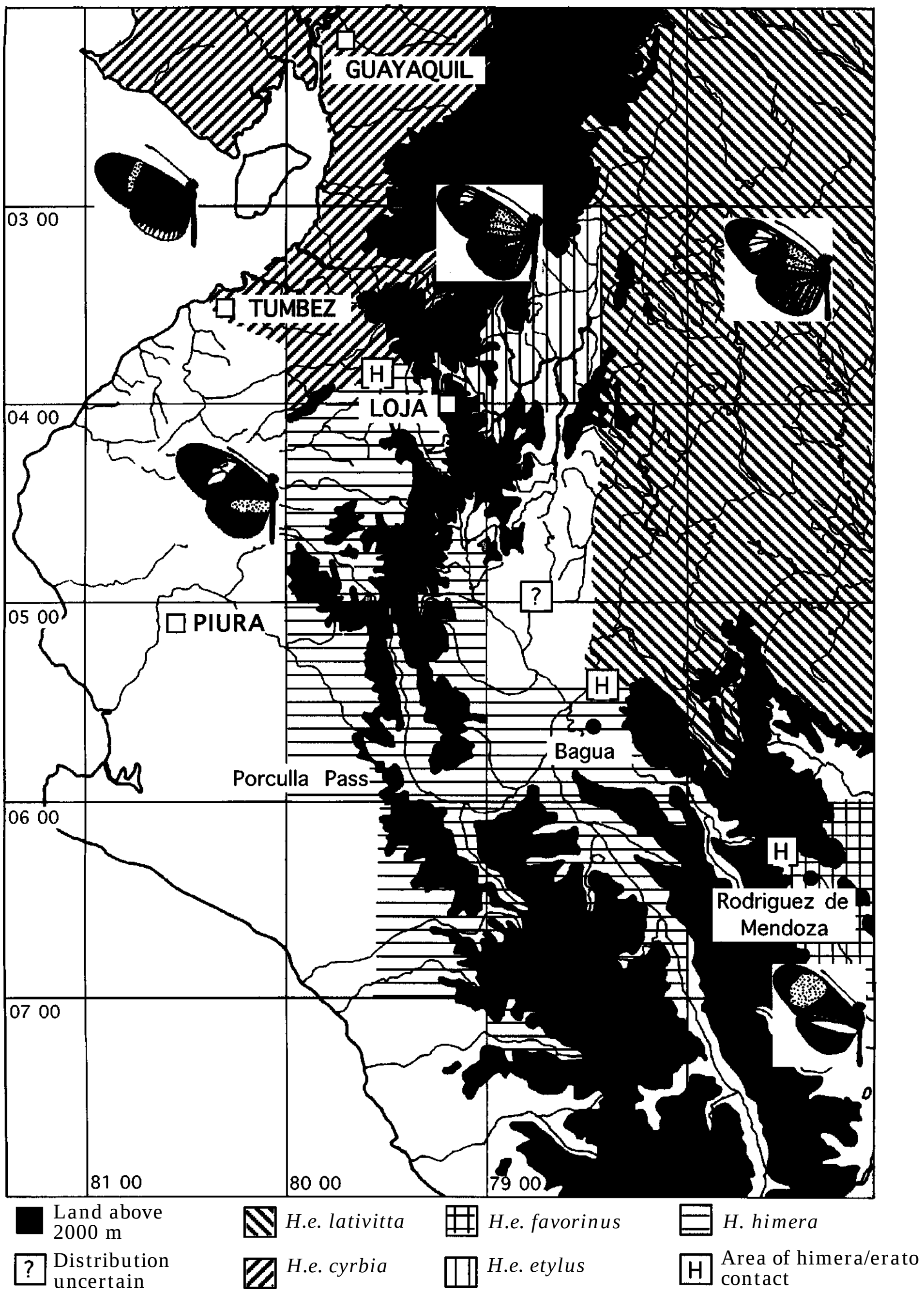

Figure 1. Distribution of *Heliconius himera* and surrounding races of *Heliconius erato*. Data from collections by the authors and quarter degree grid square data from Brown (1979). On the butterflies, stippled shading represents red/orange and white is yellow/white.

The two species are associated with very different habitats (Table 3). North of the contact zone, where *H. erato* is common, the forest vegetation is dominated by species such as *Cecropia sp.* and *Ochroma pyramidale*. These are large leaved, 'light demanding' species typical of secondary wet forest. At the other extreme, in areas where *H. himera* is common, the vegetation is thorn scrub dominated by *Acacia macracantha*, a small-leaved xerophytic species. In the area of contact there is a patchwork of secondary and disturbed primary forest remnants surrounded by open pasture grazed by cattle, which is devoid of *Heliconius*. Associated with this transition are changes in other *Heliconius* and their host plants. *Heliconius melpomene cythera*, which mimics *H. e. cyrbia*, and *H. sara* drop out as the habitat becomes dryer, the latter presumably because its primary host-plant *P. auriculata* is not found in dry forest (see above). We have no information on the host plants of *H. melpomene* as it is extremely rare in this region compared to *H. erato*. The *himera/erato* hybrid zone correlates well with the centre of this transition, but the habitat change appears to be considerably broader than the cline between the butterflies.

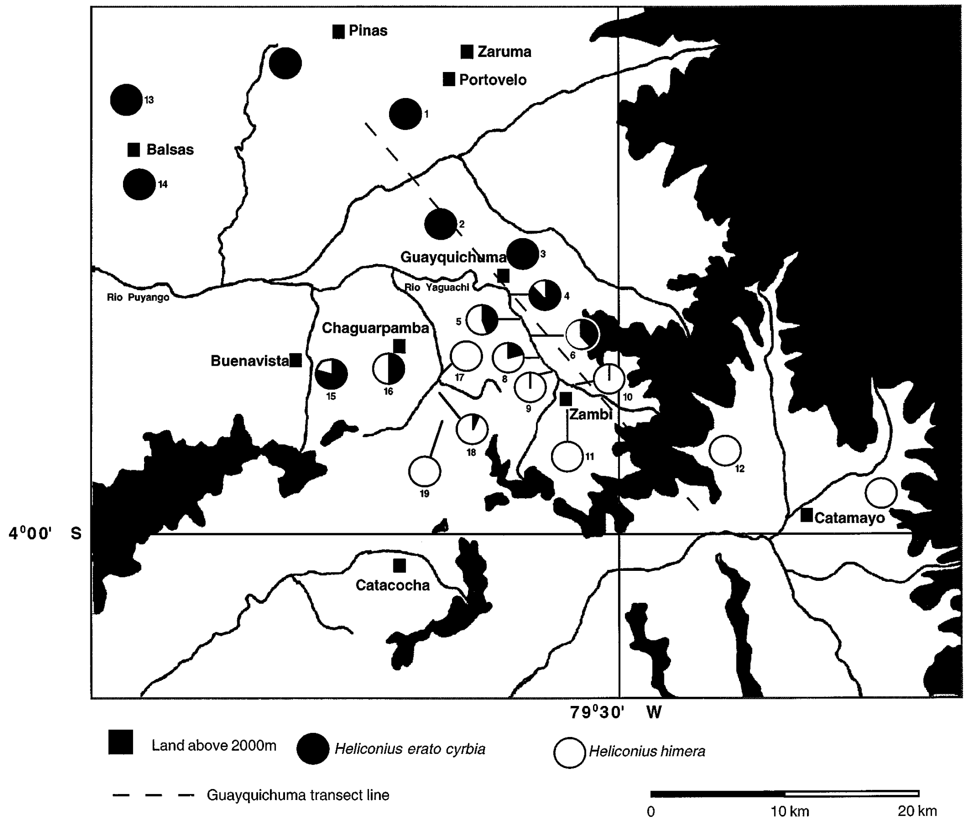

Figure 2. Distribution of phenotypes in the contact zone between *Heliconius himera* and *H. erato cyrbia* in southern Ecuador. Pie diagrams represent 'species index' values for each site (Tables 1A, B).

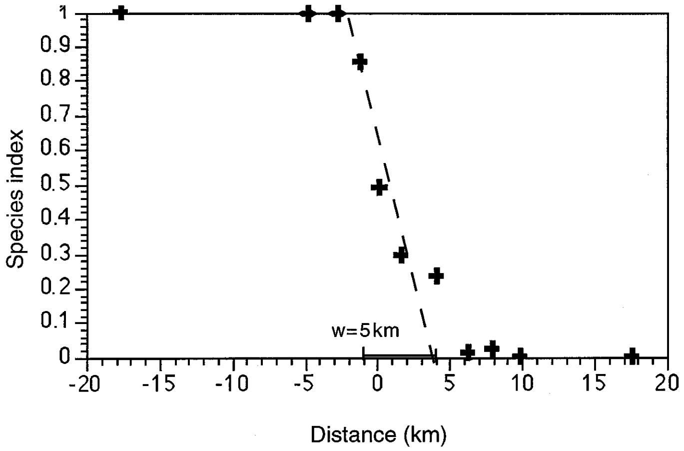

Figure 3. The cline between *H. himera* and *H. erato* along the Guayquichuma transect in southern Ecuador (Table 1A; Fig 2). The width (w) of the cline is determined by drawing a tangent to the steepest section of the cline and measuring the distance which this line projects onto the x-axis.

TABLE 1(A). Guayquichuma transect. Data collected along the road between Portovelo (site 1) and Zambi (site 11). Transect distance refers to the distance along a straight line drawn through the zone when a perpendicular is dropped from the site onto this line. Distances are measured towards Zambi (positive) and towards Portovelo (negative) with zero at the site closest to a 0.5 species index value. This line and all sites are marked on Fig. 2. Descimon & Mast de Maeght (1984) collected at site 4, which is 8–10 km south of Guayquichuma. Numbers indicate individuals collected or marked. Hybrid classifications are according to colour pattern phenotype; BC = backcross to *H. e. cyrbia* and BH = backcross to *H. himera*. See text for details of 'species index' calculations. The individuals of unknown sex are those collected by Descimon & Mast de Maeght (1984)

| Transect distance (km) | | –17.7 | –4.9 | –2.9 | –1.3 | 0 | 1.5 | 2.5 | 3.95 | 6.2 | 7.8 | 9.8 | 17.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site number | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Altitude (m) | | 1150 | 800 | 1000 | 950 | 1000 | 1050 | 1100 | 1100 | 1150 | 1250 | 1250 | 1800 |
| Latitude | South 3° | 42.7 | 48.0 | 48.8 | 50.0 | 50.7 | 51.5 | 52.0 | 52.6 | 53.6 | 54.2 | 55.7 | 56.5 |
| Longitude | West 79° | 39.6 | 34.5 | 33.8 | 34.2 | 34.3 | 33.9 | 33.7 | 33.2 | 32.5 | 31.7 | 31.6 | 26.8 |
| *H. himera* | males | | | | 15 | 13 | 13 | 1 | 6 | 25 | 48 | 14 | 7 |
| | females | | | | | 1 | 2 | | 2 | 8 | 3 | 12 | 15 |
| | sex unknown | | | | 11 | | | | | | | | |
| Hybrids | | | | | | | | | | | | | |
| F1 | males | | | | 7 | 4 | 2 | | 1 | 1 | | | |
| | females | | | | | | | | | | | | |
| BC | males | | 1 | | 4 | 1 | 1 | 1 | | | | | |
| | females | | | 1 | 1 | | | | | | | | |
| BH | males | | | | 2 | 1 | 1 | 1 | | | | | |
| | feales | | | | 1 | 1 | | | | | | | |
| *H.e.cyrbia* | males | 16 | 16 | 15 | 104 | 11 | 2 | | 1 | | 1 | | |
| | females | 7 | 14 | 1 | 38 | 3 | 3 | | 1 | | | | |
| | sex unknown | | | | 31 | | | | | | | | |
| Species index | | 1 | 0.99 | 0.99 | 0.85 | 0.49 | 0.29 | 0.33 | 0.23 | 0.01 | 0.02 | 0 | 0 |

TABLE 1(B). Chaguarpamba transect. Data collected in the vicinity of Chaguarpamba (site 16), Buenavista (near sites 15) and Balsas (sites 13 & 14). Details as in (A)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Site number | | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| Altitude (m) | | 1000 | 900 | 1100 | 1100 | 1350 | 1275 | 1500 |
| Latitude | South 3° | 43.6 | 46.5 | 53.5 | 52.0 | 53.6 | 54.4 | 56.1 |
| Longitude | West 79° | 50.4 | 48.0 | 41.8 | 39.8 | 37.7 | 38.5 | 38.2 |
| *H. himera* | males | | | 10 | 8 | 8 | 16 | 16 |
| | females | | | 1 | | 6 | 6 | 8 |
| Hybrids | | | | | | | | |
| F1 | males | | | | | | | |
| | females | | | 1 | | | | |
| BC | males | | | 1 | | | | |
| | females | | | | | | | |
| BH | males | | | | | | | |
| | females | | | | | | | |
| *H.e.cyrbia* | males | 13 | 10 | 37 | 5 | | 1 | |
| | females | 3 | 2 | 7 | 3 | | 1 | |
| Species index | | 1 | 1 | 0.79 | 0.50 | 0 | 0.08 | 0 |

TABLE 2. Host plant records from the hybrid zone and surrounding areas. This tables shows the number of wild collected larvae and eggs reared to emergece from each host plant species (letters represent the phenotype of the adult raised from the collected egg or larva; h = *Heliconius himera*, c = *H. erato cyrbia*, F1 = F1 hybrid, BH = backcross to *H. himera* and BC = backcross to *H. e. cyrbia*). Site numbers correspond to those in Fig. 2. The 'X' marks the fact that *P. punctata* was not found in Vilcabamba, although cultivated plants of both species were readily used by wild *H. himera* there. *P. sanguinolenta* is a closely related form to *P. rubra* and the two are difficult to distinguish when sterile, so records from these species have been combined. Vilcabamba lies 25 km to the south of Loja and Alluriquín is on the road from Quito to Santo Domingo. All of the *Passiflora* species shown are in the subgenus Plectostemma

| Site | *P. rubra* | *P. punctata* |
|---|---|---|
| Celica | | 1 h |
| Vilcabamba | 9 h | X |
| 19 | 1 h | |
| 11 | 1 h | 1 h |
| 10 | 2 h | |
| 9 | | 1 h |
| 8 | | 1 h |
| 7 | | 1F1, 1BC |
| 6 | 2 h | |
| 5 | 1BH | |
| 4 | | 4c |
| 2 | 1c | |
| Balsas | 1c | 4c |
| Alluriquin | 3c | 2c |

### *Genetics of hybrids*

A total of 43 field-caught hybrids are now known from the S. Ecuador contact zone (Appendix), the most known from any of the three *himera/erato* contact zones. The commonest hybrid phenotype is intermediate to the two parental species (Fig. 4A); it has the *himera* red hindwing bar and *cyrbia* red forewing band on a black background with a faint bluish iridescence (*cyrbia* has strong blue iridescence, *himera* has none). The shape of the forewing band is intermediate to the parental types and there is sometimes a trace of the *himera* yellow band in the forewing. These are interpreted as F1 types; this implies that the red *cyrbia* forewing band and red *himera* hindwing bar are both dominant, and the yellow *himera* forewing band is recessive. All other cyrbia traits (blue iridescence, white hindwing fringe, yellow underside hindwing bar) are largely recessive. The remaining hybrid phenotypes are interpreted as backcrosses (Fig. 4 B–H), and can be used to test deductions made from the F1s, as well as demonstrating whether loci act independently or show linkage. The interpretations of hybrid phenotypes made here are confirmed by allozyme studies of hybrids and the results of laboratory crosses (in prep.).

The inheritance of the colour pattern appears surprisingly simple, consisting of six major loci, each of which controls a simple pattern element with nearly complete dominance. Using the nomenclature of Sheppard *et al.* (1985), two putative loci are: *R* controlling the *cyrbia* red forewing band (present-dominant); and *Cr* controlling the presence of *cyrbia* yellow underside hindwing bar (absent, or shadow-dominant). At least four further loci can be assigned to explain the other pattern elements: these are

TABLE 3. Habitat data from the Guayquichuma hybrid zone. Data is shown from six hybrid zone sites across the transition. For comparison there are a further two sites where each of the two parental species is common (*H. himera*: Catamayo and Vilcamba, *H. erato cyrbia*: Balsas and Piñas). Vilcabamba lies 25 km to the south of Loja. The presence/absence of five common tree species shown. Also shown are collections of other *Heliconius* species and host plant (*Passiflora*) species. Only one of three *Heliconius* species, two of four *Passiflora* species and none of the tree species, are shared by the sites at either end of the transition

| | Balsas | Piñas | | | | | | | Catamayo | Vilcabamba |
|---|---|---|---|---|---|---|---|---|---|---|
| Site number | | | 2 | 4 | 5 | 7/8 | 9 | 10 | 12 | |
| Altitude (m) | 950 | 1000 | 800 | 950 | 1000 | 1100 | 1150 | 1250 | 1800 | 1600 |
| species index | 1 | 1 | 0.99 | 0.85 | 0.49 | 0.24 | 0.02 | 0 | 0 | 0 |
| Tree species | | | | | | | | | | |
| *Ochroma sp.* | + | + | | + | | | | | | |
| *Inga sp.* | + | + | + | + | + | + | | | | |
| *Cecropia sp.* | + | + | + | + | + | + | | | | |
| *Ficus cf. maxima* | | | | + | + | + | + | + | + | + |
| *Acacia macracantha* | | | | | | | | + | + | + |
| Other *Heliconius* species | | | | | | | | | | |
| *H. charithonia peruviana* | + | + | + | + | + | + | + | + | + | + |
| *H. sara* | + | + | + | + | + | | | | | |
| *H. melpomene cythera* | + | | | + | | | | | | |
| *Passiflora* species | | | | | | | | | | |
| *P. rubra* | + | + | + | + | + | + | + | + | + | + |
| *P. punctata* | + | + | + | + | + | + | + | + | + | |
| *P. adenopoda* | | + | + | | | | | | | |
| *P. auriculata* | + | + | + | + | + | | | | | |

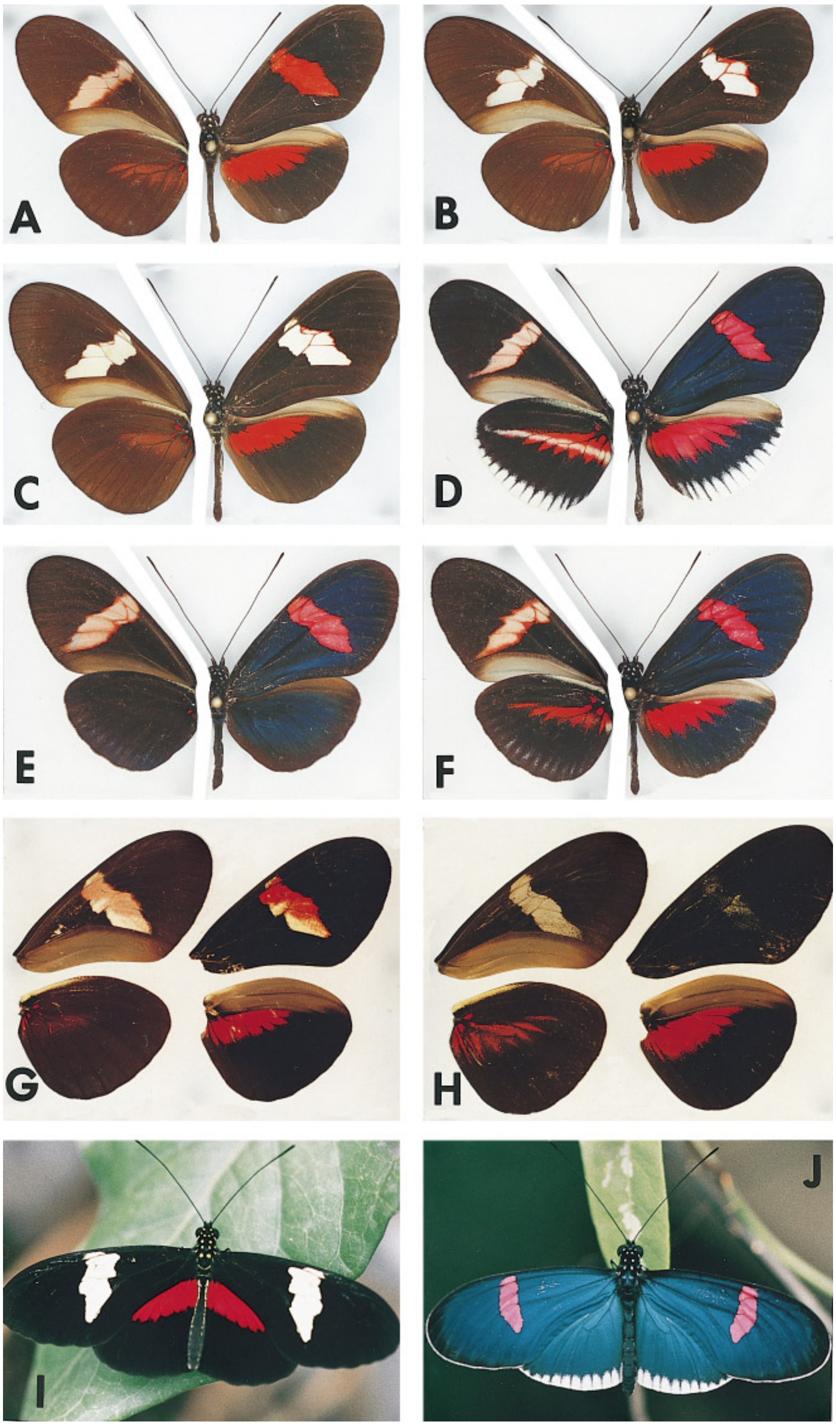

Figure 4. Hybrid and parental phenotypes. All hybrids shown were collected in the wild and full details are listed in the appendix (see ref. nos.). Proposed hybrid categories: F1, BH = backcross to *himera* and BC = backcross to *cyrbia*. Proposed colour pattern loci (see results) *cyrbia* red forewing band, *R*, (present-dominant); *cyrbia* yellow underside hindwing bar, *Cr*, (absent-dominant); blue *cyrbia* iridescence, *Ir*, (absent-dominant); *cyrbia* white hindwing margin, *We*, (absent, or trace-dominant); *himera* red hindwing bar, *Rb* (present-dominant); and *himera* yellow forewing band, *Y* (absent-dominant). A, F1, ref. no. 11; Rr, Crcr, Irir, Wewe, Rbrb, Yy; B, BH, ref. no. 10; R-, Cr-, Ir-, We-, Rb-, yy; C, BH, ref. no. 15, R-, Cr-, Ir-, We-, Rb-, yy; D, BC, ref. no. 17, R-, crcr, irir, wewe, Rb-, Y-; E, BC, ref. no. 14, R-, Cr-, irir, We-, rbrb, Y-; F, BC, ref. no. 12, R-, Cr-, irir, We-, Rb-, Y-; G, BH, ref. no. 41, R-, Cr-, Ir-, We-, Rb-, yy; H, BH, ref. no. 25, rr, Cr-, Ir-, We-, Rb-, Y-; I, *Heliconius himera*, rr, CrCr, IrIr, WeWe, RbRb, yy. J, *Heliconius erato cyrbia*, Rr, crcr, irir, wewe, rbrb, YY.

blue *cyrbia* iridescence controlled by *Ir* (absent, or trace-dominant); *cyrbia* white hindwing margin, controlled by *We* (absent, or trace-dominant); *himera* red hindwing bar, *Rb* (present-dominant); and *himera* yellow forewing band, *Y* (absent, or trace-dominant). The necessity for two loci controlling the forewing band is shown by two hybrids with almost completely black forewings (Appendix Nos. 25 & 31; Fig. 4h). This is impossible under a simple single locus or tightly linked supergene system, such as that seen in other races of *H. erato* (Sheppard et al., 1985; Mallet, 1989), where no recombinants are known.

Although the genetics is simple, hybrids show some disruption of pattern elements suggesting additional polygenic effects. When the forewing band is yellow in hybrids, some red scales are often present, especially around the edge of the band (Fig. 4B,C), presumably indicating the expression of homozygous *yy* on an *R*- background. Both the hindwing margin (Fig. 4E,F) and the yellow underside bar can be present as a faint shadow, although for the purposes of this analysis they are considered absent. Hybrids also show a slight 'raying' of the *himera* red hindwing bar (Fig. 4D,F), which could represent the effect of the genetic background of *erato*, in which there are some races with red-rayed hindwings. The red hindwing bar also shows through on the underside of the hindwing in hybrids to a far greater extent than in *H. himera* (Fig. 4D,F.H). Iridescence also seems more continuously variable than expected under a single locus hypothesis.

Almost every possible F1 and backcross phenotypic combination is seen in the wild (Table 4). This suggests independence of action, as well as a lack of strong linkage. The combinations we have not seen in the field are mostly those combining recessive phenotypes from both species, which can only be produced by F2 or other hybrid × hybrid crosses. These are very unlikely to be seen given that even F1

TABLE 4. Combinations of colour pattern traits seen in wild caught hybrids. There are more data points than hybrids as any one individual can feature a number of times. Dominant/dominant combinations are produced in F1, backcross and F2 broods; dominant/recessive combinations can be produced in backcross and F2 type broods whilst recessive/recessive combinations could only be produced by F2 or other interhybrid crosses. Recessive-recessive combinations are not seen and are likely to be extremely rare due to the low frequency of hybrids in the contact zone. Also absent are recombinants between *cyrbia* yellow bar and *cyrbia* white edge which may imply linkage between loci affecting these traits

| **himera traits** | *dominant* red fw; present **R -** | *dominant* yellow fw; absent **Y -** | *recessive* red hw bar; absent **rbrb** | *recessive* yellow hw bar; present **crcr** | *recessive* blue iridescence; present **irir** | *recessive* white hw edge; present **wewe** | |
|---|---|---|---|---|---|---|---|
| *recessive* **rr**<br>red fw band; absent | XX | 2 | 0 | 0 | 0 | 0 | Recessive /recessive combinations |
| *recessive* **yy**<br>yellow fw band; present | 7 | XX | 0 | 0 | 0 | 0 | |
| *dominant* **Rb-**<br>red hw bar; present | 15 | 10 | XX | 6 | 6 | 6 | |
| *dominant* **Cr-**<br>yellow hw bar; absent | 16 | 11 | 7 | XX | 9 | 0 | |
| *dominant* **Ir-**<br>iridescence; absent | 10 | 5 | 1 | 3 | XX | 3 | |
| *dominant* **We-**<br>white margin; absent | 16 | 11 | 7 | 0 | 9 | XX | |

Dominant/dominant combinations

hybrids are rare. Other combinations not found are recombinants between *cyrbia* yellow hindwing bar and *cyrbia* white hindwing margin, which suggests these two loci are tightly linked. Rare recombinants are known from our laboratory crosses (in prep.), so two loci have been assigned rather than one. In *H. erato* the allele that determines hindwing marginal 'cream rectangles' in S.E. Brazil also determines yellow bar, hence the name *Cr* for the locus we have assigned for the yellow hindwing bar (Sheppard *et al.*, 1985; Mallet, 1989).

Descimon & Mast de Maeght (1984) have suggested that there are fewer hybrids in this *himera* × *erato* zone than expected given random mating and full viability of hybrids. This holds for the more extensive data now available. Random collections from the km –1.3 site total 26 *H. himera*, 173 *H. e. cyrbia* and 15 hybrids of which 7 can be interpreted as F1, 3 as backcrosses to *himera* and 5 as backcrosses to *cybria* (Table 3). Frequencies of *cyrbia* alleles estimated assuming Hardy-Weinberg from this site give $q_R = 0.6383$, $q_{cr} = 0.9069$, $q_{ir} = 0.9069$, $q_{we} = 0.9069$, $q_{rb} = 0.9069, q_Y = 0.9056$; so the average *cyrbia* allele frequency can be estimated as $q = 0.8619$. Hybrids are deficit, so Hardy-Weinberg estimates of dominant allele frequencies will be incorrect — this is why $q_R$ is estimated to be so much lower than the other loci. However, the estimate obtained is very similar to the average frequency based on the proportions of pure types, which gives $q = 173/(173 + 26) = 0.8693$. If all five loci are in linkage equilibrium, we expect 0.004 pure *himera* phenotypes, 69.8 pure *cyrbia* phenotypes, and 134.6 intermediates. We actually find 26:173:14, giving $G_1 = 709.1$, $P < 0.001$. Of course, this result is in part due to gametic correlations (linkage disequilibrium), rather than merely an absence of heterozygotes. Linkage disequilibrium is expected in hybrid zones as a consequence of gene flow (Barton & Gale, 1993), even between unlinked loci. However, this cannot be the whole story in this case: even if the whole pattern were inherited at a single gene, we still expect many more intermediates (50.9, all of which are expected to be $F_1$ in phenotype), together with fewer pure phenotypes, 4.1 *himera* and 159.0 *cyrbia*. This has $G_1 = 88.59$, $P < 0.001$, showing that there is a shortage of hybrid phenotypes even if there is strong linkage disequilibrium.

It is common in cases of hybrid incompatibility to observe sex ratio biases. This is often greater inviability or sterility in the heterogametic sex, an effect known as Haldane's rule (Haldane, 1922; Coyne & Corr, 1989). Descimon & Mast de Maeght (1984) caught only male hybrids and suggested that Haldane's rule may apply in this case. A total of 7 female and 36 male hybrids are now known in all hybrid classes. This is not significantly different from the ratio of 81 females to 269 males among parental types at the hybrid sites ($G_1 = 1.109$, NS). Similarly there is no significant excess of males when only F1s are considered (1 female: 18 males; $G_1 = 2.193$, NS).

## DISCUSSION

### *Taxonomic status of H. himera*

For many years *Heliconius himera* has been considered a race of *H. erato* (Eltringham, 1916; Lamas, 1976; Brown, 1979; but see Kaye, 1916 and Emsley, 1965). However, our exhaustive collections in southern Ecuador make it clear that despite some hybridization, *H. himera* and *H. erato* maintain their genetic integrity in contact zone populations. In the centre of the hybrid zone parental phenotypes are common and hybrids rare. Moreover studies of mtDNA and allozyme loci show a similar genetic break in the hybrid zone, with little evidence for introgression of genetic markers between *H. erato* and *H. himera* (in prep.). This pattern contrasts with collections from the centres of interracial hybrid zones which are composed mainly of hybrids, with colour pattern loci approximately in Hardy-Weinberg equilibrium (Turner, 1971; Benson, 1982; Mallet, 1986; Mallet *et al*., 1990). This pattern of random mating is reflected in the lack of genetic differentiation across interracial hybrid zones at mtDNA (Brower, 1994) and allozyme loci (Turner, Johnson & Eanes, 1979).

We consider *himera* and *erato* to be separate species because of the strong hybrid deficit (see also Descimon & Mast de Maeght, 1984). Nonetheless, the two forms are geographic replacements and, with the exception of wing color pattern, are virtually identical morphologically, with only minor differences in genitalic structure (Emsley, 1965) and wing shape (Fig. 4). This species pair represents an important intermediate stage in the evolution of parapatric geographic races into a pair of species that overlap in broad sympatry. The remainder of this discussion focuses on the conclusions that can be drawn from our data on the maintenance of the extremely narrow cline between these two forms and, more generally, about speciation in *Heliconius*.

### *Structure and maintenance of the hybrid zone*

Perhaps the most striking feature of the *erato/himera* hybrid zone is its narrowness (Fig. 3). At only 5 km, it is considerably narrower than interracial zones in *Heliconius erato*, which vary between 10 km (Mallet *et al*., 1990), where colour pattern differences are greatest, to several hundred kilometres where pattern differences are small (Brown & Mielke, 1972; Mallet, 1986). Most clines and hybrid zones can be explained as a dynamic balance between selection and migration. In such cases the width of the hybrid zone should be approximately proportional to $\sigma/\sqrt{s}$ ($\sigma$ is dispersal distance, s is selection pressure). Although we have no direct estimate of dispersal distance across our study area, it seems reasonable to assume that it is similar to that observed in other *H. erato* hybrid zones. Thus, a reduction in zone width from 9.6 km, the mean width over three loci in Mallet *et al*. (1990), to 5 km represents a four-fold increase in selection. As $s \approx 0.5$ in the interracial hybrid zone this means that s must be close to 1, the maximum possible, in this case. This calculation assumes that the *himera/erato* zone is maintained via warning colour selection, as in the case of *H. e. lativitta* and *H. e. favorinus* (Mallet & Barton, 1989b). This may not be the case, but other selection models yield similar results (Mallet & Barton, 1989a; Barton & Gale, 1993); selection must be intense, however caused.

#### *Selection on colour pattern*

The narrow hybrid zones between colour pattern races of *Heliconius* can be explained by purifying frequency dependent selection on mimetic colour patterns. This type of selection in hybrid zones can be very strong ($s \approx 0.5$ as shown in a release-recapture experiment [Mallet & Barton, 1989b]; $s > 0.01$ per locus from linkage disequilibria [Mallet *et al*., 1990]). The strength of frequency dependent selection depends critically on colour pattern differences between forms (as perceived by predators) and levels of predation. The pattern differences between *himera* and *erato cyrbia* on the one hand, and *erato favorinus* and *erato lativitta* on the other both appear similarly great to our eyes, although *H. erato cyrbia* is somewhat unique in its iridescent blue background colour, very distinct from the black, yellow and red colours of *himera*. This could contribute to the narrower width of this zone if predators could more easily distinguish *himera* from *erato cyrbia* than *favorinus* from *lativitta*. However, there is reason to suppose that levels of predation may be lower in S. Ecuador, as jacamars (Galbulidae) have not been sighted in the contact zones. Jacamars are probably a major selective agent acting on *Heliconius* wing patterns, although other potential predators such as motmots (Motmotidae) and flycatchers (Tyrranidae) could also be important, and are present near Guayquichuma. Selection on colour patterns could be important in this hybrid zone, but it is hard to believe that it explains a four-fold increase in selection over that found in interracial hybrid zones.

Frequency dependent selection will also be critically dependent on the local abundance of other species in the same mimicry ring. However the only known mimic of either *H. erato cyrbia* or *H. himera* in the contact zone area is the *cyrbia* mimic, *H. melpomene cythera*. This species is extremely rare in the area. During the entire study only four individuals of *H. melpomene* were collected, two at site 4 and two in Balsas. This suggests that mimicry does not play a major role in this hybrid zone.

### *Assortative mating and hybrid inviability*

Other selection pressures, not found in interracial zones, are almost certainly important in this hybrid zone. Neither the deficit of hybrids nor the correlation of the zone with ecological variables can be explained by predator selection on colour patterns alone. The hybrid deficit must be caused either by assortative mating (premating isolation) or selection against hybrids (postmating isolation). Behavioural and breeding experiments suggest that this is mainly a result of mating preferences rather than hybrid inviability (in prep.). The relative abundance of backcross hybrids (Table 1A, B) also suggests a lack of hybrid inviability or sterility, at least in the F1 generation. Assortative mating can explain the rarity of hybrids, but on its own cannot maintain a stable hybrid zone unless there is a “rare mate disadvantage” (Sanderson, 1989). Assortative mating could explain the hybrid deficit when coupled with some other form of selection that maintains the position and narrowness of the hybrid zone.

### *Habitat-dependent selection*

The *himera/erato* hybrid zone studied here shows a clear association with the ecotone between wet and dry forest. Similar associations are found in the other *himera/erato* contact zones known. To the east of Bagua in the Río Marañon valley, *H. e. lativitta* replaces *H. himera* at a similar transition from dry thorn forest to wet forest (Konig, unpubl.; Mallet, 1993; Fig. 1). Also in northeastern Peru, *himera/erato* hybrids are known from the wet forest around Rodriguez de Mendoza where *H. e. favorinus* is common (Konig, 1986; Fig. 1); here, hybrids presumably result when *H. himera* flies over a mountain pass from Río Marañon valley dry forest to the wet forest habitat of *H.e. favorinus*.

The evidence for ecological association of the hybrid zones is supported by the wider scale biogeography of the two species. The range of *H. himera* (Fig. 1) has a dry climate and distinctive cactus/thorn scrub or gallery forest vegetation, in marked contrast to the tropical wet forest found further to the north and on the eastern slopes of the Andes, where *H. erato* occurs. This dry forest region is a centre of endemism for both flora and fauna and has been described as a Pleistocene refugium where speciation occurred (Brown, 1979, 1981, 1982, Lamas, 1982). It is actually unlikely that this was a rainforest refuge as is claimed for other centres of endemism in the neotropics: it is more likely that the endemism is driven by adaptation to the distinctive habitat. Other butterfly species/races characteristic of the area include *Heliconius charithonia peruviana, H. clysonymus tabaconas*, the ithomiines *Hyalyris coeno latilimbata, Mechanitis mazaeus* ssp. nov., *Elzunia pavonii* and *Scada kusa* and the papilionid *Parides erlaces chinchipensis* (Brown, 1979; Lamas, 1982).

Distribution patterns across a hybrid zone can give clues as to the importance of habitat in structuring the cline. In some cases the association is so strong that a mosaic distribution pattern is found. A good example of this is between the crickets *Gryllus pennsylvanicus* and *G. firmus* (Rand & Harrison, 1989) in which an interlocking patchwork distribution of the two forms is strongly related to soil type. Such a pattern might be expected where forms with different ecological adaptations colonize habitat types distributed in a mosaic, and where each patch is sufficiently large relative to the dispersal of the organism (Harrison, 1990). In S. Ecuador, there is little evidence that such a pattern exists for *Heliconius*, and the clines appear to form simple monotonic transitions. This is probably due to the relative high per-generation dispersal rate in *Heliconius* which is sufficient to overcome the effect of habitat patchiness. Habitat patches would presumably have to be on the order of two hybrid zone widths (≥ 10 km wide) to allow significant local adaptation. The *Heliconius* pattern is more like that in high-dispersal animals such as birds, where mosaic patterns are not found and hybrid zones are monotonic; these also commonly show correlations with habitat, but on a broader scale. Moore & Price (1993) found several bird hybrid zones in the Great Plains of N. America that correlated well with the forest/savannah boundary, including that between the northern flicker races (*Colaptes*).

Interracial *Heliconius* hybrid zones are also monotonic and in general show little association with habitat. Benson (1982) presents some evidence for a relationship between colour pattern races and aridity in *H. erato*. Rayed races tend to be found in the dense amazonian forests, while forms with red forewing and yellow hindwing bands (known as ‘postman races’) are often found in more open habitats of S. Brazil and the llanos of Colombia and Venezuela. Despite these broad associations there are plenty of exceptions to the pattern such as *H. e. favorinus*, a postman race which occurs in dense wet forest in eastern Peru (Mallet, 1993). There is not much evidence that hybrid zones so far studied between *H. erato* races show much correlation with habitat variables (Mallet, 1986, 1993; but see Benson, 1982). The ecological correlates of the *himera/erato* hybrid zones contrast strongly, and highlight the ecological differences between *H. himera* and the other races of *H. erato*.

Given the strong and consistent association of *H. himera* with dry regions, it seems likely that both the position and width of the transition zones between the two species are largely determined by ecological selection. What features of the habitat might cause this selection? There is no host plant specialization between the species and the primary host plants occur right across the hybrid zone (Table 2; Jiggins, McMillan & Mallet, 1996). This is good evidence that host plant adaptations have not diverged. More likely are adaptations to altitude or water stress. *H. himera* reaches some 400–500 m higher than *H. erato*, and this may be associated with physiological adaptations to altitude and in particular to lower temperatures. *H. himera* may be able to reach higher altitudes than *H. erato* simply because of the greater daytime temperatures in the arid habitat. While altitude may be important, there is relatively little altitudinal variation across the hybrid zone (Table 1A, B), which is much more clearly associated with aridity and vegetation changes (Table 3). This suggests that selection associated with the wet/dry transition is more important. A possible candidate might be desiccation tolerance.

### *Competitive interactions*

Interspecific competition is known to be important in determining species boundaries (Bull, 1991; Hoffmann & Blows, 1994). The classic studies where this has been demonstrated experimentally are in sessile organisms such as plants (Santelmann, 1991) and intertidal organisms (Connell, 1961); however there are also examples in more dispersive organisms such as between red and arctic foxes, *Vulpes vulpes* and *Alopex lagopus*. The fox species boundary is believed to be an interaction between body size and interspecific competition for food (Hersteinsson & Macdonald, 1992). If, as argued above, the *Heliconius* contact zone is determined by ecological adaptations to different habitats, competitive exclusion of the two ecologically similar species should take place where their respective habitats meet along an ecotone. This is perhaps a result of competition for the primary host plant species, *P. rubra* and *P. punctata* (Table 2). A switch of competitive advantage, from one species to the other, should occur at some point across the ecotone. This might give rise to the observed pattern even if the ecotone itself were considerably wider than the contact zone.

### *Multiple selection pressures*

It seems probable that all five proposed selective forces, i.e. habitat, competitive exclusion, frequency-dependent selection on colour patterns, assortative mating and hybrid inviability (in approximately that order), may act together to maintain the narrowness and position of this contact zone. Although it will be hard to separate all of these effects, the geographic correlations discussed above give clear evidence that ecology plays a major role. Whatever the exact nature of selection, it must be very strong to explain the abrupt transition from *H. erato* to *H. himera*.

## *Genetics of species differences*

To understand the evolutionary origins of the two species we need to compare the genetic basis of differences between them with those between races of *H. erato*. This study shows that there are at least six major loci, each of which codes for a different colour pattern element. Genetic studies of a number of races of both *H. erato* and *H. melpomene* have also shown major locus inheritance of colour patterns (Sheppard *et al.*, 1985). For example hybrid zones near Tarapoto in Peru involve three colour pattern loci in *H. erato* and four in *H. melpomene* (Mallet, 1989). Although the loci found here are similar to those known in *H. erato*, not all are homologous. In particular the loci controlling the yellow *himera* forewing band and red *erato* forewing band are independent; in *H. erato* races, no recombinants have been produced (Sheppard *et al.*, 1985; Mallet, 1989). The divergence in colour pattern between *H. himera* and *H. erato* is similar to, but more extensive than that between geographic races of *H. erato*. This suggests that the divergence of colour pattern between species and races involves the same genetic processes.

### *Mode of speciation*

While the colour pattern differences between *H. himera* and *H. erato* are like those between geographic races, the strong ecological differences between the two species are not. The importance of ecological divergence in speciation is supported by evidence from other species closely allied to *H. erato*, which almost invariably show strong habitat differences. *Heliconius clysonymus* and *H. telesiphe* both occasionally overlap with *H. erato*, but are found at higher altitudes (800–2000 m as opposed to 0–1500 m). There is evidence for competitive exclusion between *H. clysonymus* and *H. erato* (Benson, 1978). Another close relative, *H. charithonia*, is found in similar forest edge and secondary growth habitats to those of *H. erato* (Table 3). Although *H. charithonia* feeds on most host plants used by *H. erato*, it also commonly feeds on *Passiflora adenopoda* whose hooked trichomes kill *H. erato* larvae placed on the plant (Gilbert, 1971). *Heliconius charithonia* lays larger clutches of smaller eggs, and is also more mobile, being the only member of the genus that regularly colonizes Caribbean Islands, and as far north as central Texas and Florida. In Panama and Costa Rica it is commonest in seasonally suitable cloud forest and savannah habitats; but is rare in habitats that are permanently suitable such as the Osa Peninsula, Costa Rica (Gilbert, 1991). This suggests that *H. charithonia* relies more on colonization of seasonally available habitats than the other species, in addition to its host plant divergence. Similarly, *H. hermathena*, a close relative of *H. charithonia*, is found in patches of savannah in the central Amazon, very different habitats to the surrounding rainforest where *H. erato* is found. According to Brown & Benson (1977), *H. hermathena* may have diverged in dry habitats surrounding Pleistocene forest refugia, so that its range has contracted into a few small pockets now that the rainforest has expanded. There is therefore substantial evidence among the species most closely related to *H. erato* that habitat-dependence is important in speciation, but is involved much less, if at all, in the divergence of races (see above under *Habitat-dependent selection*).

Within other clades in the genus, different ecological factors may be important. For example within the *H. melpomene* group, related species are often sympatric and apparently avoid competition by means of strong differences in host plant specificity and microhabitat (Smiley, 1978; Mallet & Gilbert, 1995). In conclusion, whereas geographic race formation does not involve much adaptation to habitat or host plants, speciation in *Heliconius* is strongly associated with such ecological changes.

### *Geographic context of speciation*

The limited geographic extent of contact between *himera* and *erato* might at first sight suggest that divergence and speciation occurred in allopatry. However this seems somewhat unlikely as the wet and dry forest habitats of the two species would always have been in contact. Ecological traits can easily diverge in parapatry, providing habitat patches are sufficiently large relative to dispersal (Haldane, 1948).

Clines for a wide variety of different characters often occur together; for example the northern flicker hybrid zone is correlated with a habitat boundary, but was initially identified in a plumage trait, which is presumably sexually selected and not itself directly related to ecology (Moore, 1987; Moore & Price, 1993). In the *himera/*

*erato* hybrid zone, clines of colour pattern, ecological adaptation, mtDNA, allozymes, and mate choice all occur together. This has been used as *prima facie* evidence for secondary contact (Barton & Hewitt, 1985). However, a genetic barrier to gene flow is likely to accumulate further differences on each side, just as with a geographic barrier (Hewitt, 1989; Mallet, 1993). In *Heliconius*, strong adaptation to an ecological gradient could have prevented other traits, such as colour patterns, mate choice, and genetic markers spreading across the zone. As each selected trait became fixed, the genetic barrier would become more intense, and further accumulation of differences would become possible. Parapatric differentiation is at least as likely as allopatric on the available evidence.

## CONCLUSION

The *himera/erato* hybrid zone is a good example of an intermediate stage in speciation. We propose that these two forms have already speciated because, although they hybridize, hybrids are rare in the contact zone, and gene flow does not result in homogenization. The contact zone is extremely narrow compared with interracial hybrid zones of *H. erato*, suggesting that selection is intense, $s \approx 1$. Although the hybrid zone between *himera* and *erato* conforms to a classical idea of a zone of secondary contact, being limited in extent and consistent at many genetic clines, it is clear that ecological adaptation in parapatry could have produced similar results. The strong habitat differences between *H. erato, H. himera* and other species in the clade, coupled with little ecological differentiation between geographic races, implicates ecological adaptation as a prime mover of speciation in *erato* group *Heliconius*. This study shows that considerable genetic, ecological, and behavioural information relevant to understanding speciation can be deduced directly from field data collected in an interspecific hybrid zone. Examples such as this are clearly more useful than allopatric species, where it is unclear whether, given sympatry, genetic integrity would be maintained or dissolve.

## ACKNOWLEDGEMENTS

We would like to thank all those in Ecuador who have helped the project, in particular INEFAN for granting permission to carrying out research in the country; the Museo de Ciencias Naturales and Peter Wilson in Quito and Fundación Arcoiris in Loja for their support. Angus Davison, Ashleigh Griffin, Pablo Losano, Bolivar Medino and Pablo Andrade helped with fieldwork and plant identification. We are also grateful to Henrí Descimon, Andrew Brower, Keith Brown and Gerardo Lamas for advice and comments. This project is funded by a BBSRC research grant and studentship.

## REFERENCES

**Ackery PR, Smiles RL. 1976.** An illustrated list of the type-specimens of the Heliconiinae (Lepidoptera: Nymphalidae) in the British Museum (Natural History). *Bulletin of the British Museum (Nat. Hist.), Entomology* **32:** 171–214.

**Barton NH, Gale KS. 1993.** Genetic analysis of hybrid zones. In: Harrison RG, ed. *Hybrid Zones and the Evolutionary Process*. New York: Oxford University Press, 13–45.

**Barton NH, Hewitt GM. 1985.** Analysis of hybrid zones. *Annual Review of Ecology and Systematics* **16:** 113–148.

**Barton NH, Hewitt GM. 1989.** Adaptation, speciation and hybrid zones. *Nature* **341:** 497–503.

**Benson WW. 1978.** Resource partitioning in passion vine butterflies. *Evolution* **32:** 493–518.

**Benson, WW. 1982.** Alternative models of infrageneric diversification in the humid tropics: tests with passion vine bufferflies. In: Prance GT ed. *Biological Diversification in the Tropics*. New York: Columbia University Press, 608–640.

**Brower AVZ. 1994.** Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Science*, USA **91:** 6491–6495.

**Brown KS, Mielke OHH. 1972.** The heliconians of Brazil (Lepidoptera: Nymphalidae). Part II. Introduction and general comments, with a supplementary revision of the tribe. *Zoologica, New York*. **57:** 1–40.

**Brown KS. 1976.** An illustrated key to the silvaniform *Heliconius* (Lepidoptera: Nymphalidae) with descriptions of new subspecies. Transactions of the American Entomological Society **102:** 373–484.

**Brown KS. 1979.** *Ecologia Geográfica e Evolução nas Florestas Neotropicais*. 2 vols. (Livre de Docencia) Universidade Estadual de Campinas, Campinas, Brazil.

**Brown KS. 1981.** The biology of *Heliconius* and related genera. *Annual Review of Entomology* **26:** 427–456.

**Brown KS. 1982.** Paleoecology and regional patterns of evolution in neotropical forest butterflies. In: Prance GT, ed. *Biological Diversification in the Tropics*. New York: Columbia University Press, 255–308.

**Brown KS, Benson WW. 1977.** Evolution in modern Amazonian non-forest islands: *Heliconius hermathena. Biotropica* **9:** 95–117.

**Brown KS, Sheppard PM, Turner JRG. 1974.** Quaternary refugia in tropical America: evidence from race formation in *Heliconius* butterflies. *Proceedings of the Royal Society, London (B)* **187:** 369–378.

**Bull CM. 1991.** Ecology of parapatric distributions. *Annual Review of Ecology and Systematics* **22:** 19–36.

**Connell JH. 1961.** The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus. Ecology* **42:** 710–723.

**Coyne JA, Orr HA. 1989.** Two rules of speciation. In: Otte D, Endler JA, eds. *Speciation and its Consequences*. Sunderland, MA: Sinauer Associates. 180–207.

**Descimon H, Mast De Maeght J. 1984.** Semispecies relationships between *Heliconius erato cyrbia* Godt. and *H. himera* Hew. in southwestern Ecuador. *The Journal of Research on the Lepidoptera* **22:** 229–239.

**Dobzhansky T (ed.). 1937.** *Genetics and the origin of Species.* Columbia University Press.

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

**Emsley MG. 1965.** Speciation in *Heliconius* (Lep., Nymphalidae): morphology and geographic distribution. *Zoologica, N.Y.* **50:** 191–254.

**Endler JA. 1977.** *Geographic Variation, Speciation and Clines*. Princeton, N.J: Princeton University Press.

**Gilbert LE. 1971.** Butterfly-plant coevolution: has *Passiflora adenopoda* won the selectional race with heliconiine butterflies? *Science* **172:** 585–586.

**Gilbert LE. 1975.** Ecological consequences of a coevolved mutualism between butterflies and plants. In: Gilbert LE, Raven PR. eds. *Coevolution of Animals and Plants*. Austin, TX: University of Texas Press, 210–240.

**Gilbert LE. 1991.** Biodiversity of a Central American *Heliconius* community: pattern, process, and problems. In: Price PW, Lewinsohn TM, Fernandes TW, Benson WW eds. *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions*. New York: John Wiley & Sons. 403–427.

**Grant PR, Grant BR. 1992.** Hybridization of bird species. *Science* **256:** 193–197.

**Guillaumin M, Descimon H. 1976.** La notion d'espèce chez les Lépidoptères. In: Bocquet C, Génermont J, Lamotte M, eds. *Les Problèmes de l'Espèce dans le Règne Animal*, Vol. 1. Paris: Société zoologique de France, 129–201.

**Haldane JBS. 1922.** Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics* **12:** 101–109.

**Haldane JBS. 1948.** The theory of a cline. *Journal of Genetics* **48:** 277–284.

**Harrison RG. 1990.** Hybrid zones: windows on evolutionary processes. In: Futuyma D, Antonovics J, eds. *Oxford surveys in Evolutionary Biology. Vol. 7*. Oxford: Oxford University Press, **7:** 69–128.

**Harrison RG. 1993.** Hybrids and hybrid zones: historical perspective. In: Harrison RG, ed. *Hybrid Zones and the Evolutionary Process*. New York: Oxford University Press, 3–12.

**Hersteinsson P, Macdonald DW. 1992.** Interspecific competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus. Oikos* **64:** 505–515.

**Hewitt GM. 1989.** Divergence and speciation as viewed from an insect hybrid zone. *Canadian Journal of Zoology* **68:** 1701–1715.

**Hewitt GM. 1989.** The subdivision of species by hybrid zones. In: Otte D, Endler JA, eds. *Speciation and its Consequences*. Sunderland, MA: Sinauer Associates, 85–110.

**Hoffmann AA, Blows MW. 1994.** Species borders: ecological and evolutionary perspectives. *Trends in Ecology and Evolution* **9:** 223–227.

**Holzinger H, Holzinger R. 1994.** *Heliconius* and related Genera. Lepidoptera: Nymphalidae. The Genera *Eueides, Neruda* and *Heliconius*. Sciences Nat., Vennette, France, 328 pages.

**Jiggins CD, McMillan WO, Mallet J. 1996.** Host plant adaptation has not played a role in the recent speciation of *Heliconius himera* and *Heliconius erato* (Lepidoptera: Nymphalidae). *Evolution*. submitted.

**Kaye WJ. 1916.** A reply to Dr. Eltringham's paper on the genus *Heliconius. Transactions of the Entomological Society, London* **1916:** 149–155.

**König F. 1986.** Ein *Heliconius erato himera* — Hybrid aus Nord-Peru (Lepidoptera, Heliconiiae). *Zeitschrift der Arbeitsgemeinschaft Österreichischen Entomologen* **38:** 49–50.

**Lamas G. 1976.** Notes on Peruvian butterflies (Lepidoptera). II. New *Heliconius* from Cusco and Madre de Dios. *Revista Peruana Entomologia* **19:** 1–7.

**Lamas G. 1982.** A preliminary zoogeographical division of Peru based on butterfly distributions (Lepidoptera, Papilionoidea). In: Prance GT ed. *Biological Diversification in the Tropics*. New York: Columbia University Press. 336–357.

**Mallet J. 1986.** Hybrid zones in *Heliconius* butterflies in Panama, and the stability and movement of warning colour clines. *Heredity* **56:** 191–202.

**Mallet J. 1989.** The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *H. melpomene. Proceedings of the Royal Society, London (B)* **236:** 163–185.

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

**Mallet J. 1995.** A species definition for the Modern Synthesis. *Trends in Ecology and Evolution* **10:** 294–299.

**Mallet J, Barton NH. 1989a.** Inference from clines stabilized by frequency-dependent selection. *Genetics* **122:** 967–976.

**Mallet J, Barton NH. 1989b.** Strong natural selection in a warning colour hybrid zone. *Evolution* **43:** 421–431.

**Mallet J, Barton NH, Lamas G, Santisteband J, Muedas M, Eeley H. 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, Gilbert LE. 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.

**Mayr E. 1940.** Speciation phenomena in birds. *American Naturalist* **74:** 249–278.

**Moore WS. 1987.** Random mating in the Northern flicker hybrid zone: implications for the evolution of bright and contrasting plumage patterns in birds. *Evolution* **41:** 539–546.

**Moore WS, Price JT. 1993.** Nature of selection in the northern flicker hybrid zone and its implications for speciation theory. In: Harrison, RG ed. *Hybrid Zones and the Evolutionary Process*. New York: Oxford University Press, 196–225.

**Pyle RL, Randel JE. 1994.** A review of hybridization in marine angelfishes (Perciformes, Pomacanthidae). *Environmental Biology of Fishes* **41:** 127–145.

**Rand DM, Harrison RG. 1989.** Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution* **43:** 432–449.

**Sanderson N. 1989.** Can gene flow prevent reinforcement? *Evolution* **43:** 1223–1235.

**Santelmann MV. 1991.** Influences on the distribution of *Carex exilis*: an experimental approach. *Ecology* **72:** 2025–2037.

**Sheppard PM, Turner JRG, Brown KS, Benson WW, Singer MC. 1985.** Genetics and the evolution of muellerian mimicry in *Heliconius* butterflies. *Philosophical Transactions of the Royal Society, London (B)* **308:** 433–613.

**Smiley JT. 1978.** Plant chemistry and the evolution of host specificity: new evidence from *Heliconius* and *Passiflora. Science* **201:** 745–747.

**Szymura JM, Barton NH. 1986.** Genetic analysis of hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata* near Cracow in Southern Poland. *Evolution* **40:** 1141–1159.

**Szymura JM, Barton NH. 1991.** The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. *Evolution* **45:** 237–261.

**Turner JRG. 1971.** Two thousand generations of hybridization in a *Heliconius* butterfly. *Evolution* **25:** 471–482.

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

**Turner JRG, Johnson MS, Eanes WF. 1979.** Contrasted modes of evolution in the same genome: allozymes and adaptive change in *Heliconius. Proceedings of the National Academy of Science*. USA **76:** 1924–1928.

## APPENDIX

Complete list of wild caught hybrids and their phenotypes

| Ref. no. | Proposed hybrid class | Sex | Site no. | Collection | Fw band | Yellow hw bar cyrbia | Red hw bar himera | Blue iridescence | White hw edge |
|---|---|---|---|---|---|---|---|---|---|
| Parental phenotypes | | | | | | | | | |
| *Heliconius erato cyrbia* | | | | | red | + | – | + | + |
| *Heliconius himera* | | | | | ye | – | + | – | – |
| 1 | F1 | m | 4 | Descimon | red(wh) | – | + | (+) | – |
| 2 | F1 | m | 4 | Descimon | red(wh) | – | + | (+) | – |
| 3 | F1 | m | 4 | Descimon | red(wh) | – | + | (+) | – |
| 4 | BC | m | 4 | Descimon | red | + | + | + | + |
| 5 | BC | m | 4 | Descimon | red | + | + | (+) | + |
| 6 | BH | m | 4 | Descimon | wh/red | – | + | – | – |
| 7 | F1 | m | 4 | Mallet | red(wh) | – | + | (+) | – |
| 8 | F1 | m | 4 | Mallet | red | – | + | (+) | – |
| 9 | BC | m | 4 | Mallet | red | + | – | – | + |
| 10 | BH | m | 4 | Neukirchen | wh/red | – | + | – | – |
| 11 | F1 | m | 4 | Neukirchen | red | – | + | (+) | – |
| 12 | BC | m | 4 | Neukirchen | red | – | + | + | (+) |
| 13 | BC | m | 4 | Neukirchen | red | – | + | + | (+) |
| 14 | BC | f | 4 | Neukirchen | red | (+) | – | + | (+) |
| 15 | BH | m | 4 | Neukirchen | ye/red | – | + | – | – |
| 16 | F1 | m | 4 | Neukirchen | red(wh) | – | + | (+) | – |
| 17 | BC | m | 4 | Neukirchen | red | + | + | + | + |
| 18 | BC | f | 4 | Neukirchen | red | + | + | – | + |
| 19 | F1 | m | 5 | J/M/M | red | (+) | + | – | – |
| 20 | F1 | m | 9 | J/M/M | red | (+) | + | – | – |
| 21 | F1 | m | 6 | J/M/M | red | (+) | + | – | – |
| 22 | F1 | m | 6 | J/M/M | red | (+) | + | – | – |
| 23 | BH | m | 5 | J/M/M | wh/red | – | + | – | – |
| 24 | F1 | m | 4 | J/M/M | red(wh) | – | + | (+) | – |
| 25 | BH | m | 4 | J/M/M | none(ye) | – | + | – | – |
| 26 | BC | m | 4 | J/M/M | red | (+) | – | + | (+) |
| 27 | BC | f | 4 | J/M/M | red | (+) | – | + | (+) |
| 28 | BC | f | 3 | J/M/M | red | + | + | + | + |
| 29 | BC | m | 2 | J/M/M | red | (+) | – | + | (+) |
| 30 | BC | m | 6 | J/M/M | red | (+) | – | + | (+) |
| 31 | BH | M | 6 | J/M/M | none(ye) | – | + | – | – |
| 32 | F1 | M | 5 | J/M/M | red | – | + | (+) | – |
| 33 | F1 | M | 5 | J/M/M | red(wh) | – | + | (+) | – |
| 34 | F1 | m | 5 | J/M/M | red(wh) | – | + | (+) | – |
| 35 | BC | m | 5 | J/M/M | red | (+) | – | + | – |
| 36 | F1 | m | 8 | J/M/M | red | – | + | (+) | – |
| 37 | F1 | f | 15 | J/M/M | red | – | + | (+) | – |
| 38 | BC | m | 15 | J/M/M | red | (+) | – | + | (+) |
| 39 | BH | f | 4 | J/M/M | red(ye) | – | + | – | – |
| 40 | F1 | m | 4 | J/M/M | red | – | + | (+) | – |
| 41 | BH | m | 7 | J/M/M | red/ye | – | + | – | – |
| 42 | BH | f | 5 | J/M/M | red/ye | – | + | (+) | – |
| 43 | BC | m | 7 | J/M/M | red | + | + | + | + |

Site numbers are as shown in Figure 2. Nos. 1–6 were illustrated by Descimon & Mast de Maeght (1984). Nos. 10–18 are held in the collection of W. Neukirchen. Nos. 19–43 were collected by Jiggens, Mallet and McMillan in 1994–5 and are held by the authors. The ‘proposed hybrid class’ is based upon our interpretation of the phenotypes where BC = backcross to *H. e. cybria* and BH = backcross to *H. himera*. Where pattern elements are shown in brackets they were present as a trace; for the purposes of our analysis (Table 4) they were considered absent. Forewing band colours are wh = white, ye = yellow.