The genetic basis of an adaptive radiation: warning colour in two *Heliconius* species

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SUMMARY

Mimetic colour pattern races of *Heliconius* butterflies provide a striking example of adaptive radiation and numerous crossing experiments have investigated the genetics of these racial differences. However, colour pattern differentiation between closely related *Heliconius* species has not been previously studied. Here we present data from crosses between *H. erato cyrbia* and its sister species, *H. himera*. The genetic architecture underlying colour pattern divergence between these species is identical to that observed between races of *H. erato*. As in inter-racial crosses, colour pattern differences resulted from segregation at a few major loci. Evidence from 1321 offspring in 4 F₁, 17 backcross, 7 F₂ and 21 further crosses showed that two major loci controlled most of the colour pattern differences between *H. erato* and *H. himera*. There were strong interactions between these loci in their patterns of expression and evidence for other loci with relatively minor phenotypic effects. More importantly, based on patterns of expression within broods and linkage with *Aconitase*, we conclude that these major loci were homologous with those known to be responsible for colour pattern differences within *H. erato*. Our crosses also permit a re-evaluation of the relationships between colour pattern races of *H. erato*. This suggests that *H. e. hydara*, which occurs across a major mtDNA break, is the ancestral phenotype from which other races have evolved. Based on this assumption, we find no evidence to support the recent suggestion that apparently homologous colour pattern alleles have arisen multiple times.

1. INTRODUCTION

The colour patterns of aposematic heliconiine butterflies are probably best known as a striking example of Müllerian mimicry. At any one site in the neotropical rainforest there are five or more easily distinguished mimetic assemblages. These groups of species, known as mimicry rings, consist of heliconiine and other lepidopteran species which share a common aposematic colour pattern. The common pattern in many of these mimicry rings changes abruptly every few hundred kilometres across the neotropics, producing concordant changes in the appearance of many species. Thus there are two dimensions to warning-colour diversity, distinct coexisting mimicry rings and abrupt changes in the dominant pattern across tropical America.

Perhaps the most extreme adaptive radiation of colour pattern has occurred in the Müllerian comimics, *Heliconius erato* and *H. melpomene*. These two species occur across the neotropics, where they have each evolved into nearly 30 parapatric colour pattern races (Brown 1979). Colour pattern radiation in both species is largely the result of divergence at just a few genetic loci. For example, although ten unlinked genes (or gene complexes) have been described

in previous analyses of inter-racial crosses within *H. erato*, most of the phenotypic variation observed in this species can be explained by changes at only four major loci (Sheppard *et al.* 1985). A similar number of loci are involved in colour pattern change in *H. melpomene* (Sheppard *et al.* 1985). This pattern, in which major phenotypic transitions involve changes at a few loci, is predicted by mimicry theory (Turner 1984), but could also result if the development of wing colour patterns is controlled by the expression of a few regulatory genes (Carroll *et al.* 1994; Nijhout 1994; Brakefield *et al.* 1996).

Previous attempts to unravel the history of colour pattern evolution in *H. erato* and *H. melpomene* have been based either on allelic changes at wing pattern loci or the pattern of variation within mitochondrial DNA (Turner 1983, 1984; Brower 1994*b*, 1996). These two approaches have yielded conflicting results. Under Turner's hypothesis of colour pattern parsimony similar geographically disjunct colour pattern races share a common ancestor. The mtDNA phylogeny appears to contradict this result and Brower speculated that colour pattern radiation within *H. erato* must have involved repeated evolution of identical patterns. Evaluating these competing hypotheses is made difficult because of the lack of information on the genetics of colour patterns in closely related out-group taxa. Morphological and molecular phylogenetic hypotheses show *H. erato* and *H. himera* to be

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sister taxa (Brown 1979; Brower 1994a; Jiggins *et al.* 1997). Furthermore, it is likely that the ancestor to both species resembled *H. himera* in coloration. The yellow forewing band and red hindwing bar pattern characteristic of *H. himera* also occurs in another closely related species, *H. clysonymus*. In addition, unlike *H. erato*, neither *H. himera* nor *H. clysonymus* are involved in mimicry or exhibit much geographic variation in colour pattern. Thus it is less likely that these species are subject to convergent evolutionary pressures that might lead to rapid colour pattern change.

Here we re-evaluate colour pattern evolution within *H. erato*, using inferences from crosses between *H. himera* (*himera*) and the west Ecuadorean race, *H. erato cyrbia* (*cyrbia*). We use these data, in the light of Brower's mitochondrial DNA phylogeny and past inter-racial crosses, to answer two specific questions about colour pattern evolution in this group. First, are the genetic loci responsible for colour pattern differences between *himera* and *cyrbia* homologous to those already observed within *H. erato*? Second, using the assumption that the *H. himera* pattern is ancestral, is it possible to reconcile the mtDNA phylogeny with a hypothesis of parsimonious colour pattern evolution?

2. METHODS

Heliconius himera and *H. e. cyrbia* hybridize in nature and are completely interfertile (Jiggins *et al.* 1996; McMillan *et al.* 1997). Crosses were carried out between stocks collected in Vilcabamba (*H. himera*), Piñas and Balsas (*H. e. cyrbia*) in southern Ecuador. Rearing methods are described elsewhere (McMillan *et al.* 1997). After eclosion, wings were removed and stored in glassine envelopes, whilst the bodies were immediately frozen in liquid nitrogen for genetic analysis.

Virgin females were obtained by rearing the offspring of wild-caught females. These were then mated in an outdoor cage with either wild-caught males or healthy reared males. In this manuscript 'F₁' and 'F₂' are *himera* × *cyrbia* and F₁ × F₁ crosses, respectively. 'BH' refers to a cross between an F₁ and a pure *himera*; 'BC' a backcross to *cyrbia*; 'BHH', a cross between offspring of a BH brood and a pure *himera*; 'BHF₂', a cross between a BH offspring and an F₂, etc.

We used goodness-of-fit and homogeneity *G*-tests to test hypotheses of wing pattern inheritance. All broods with the same expected pattern of segregation were combined for each test. Where significant heterogeneity was found between broods, this is discussed in the text.

We also examined linkage relationships with the cathodal *Aconitase* enzyme which is known to be linked to the *Cr* gene in *H. erato*, and shows a virtually fixed difference between *himera* and *cyrbia* (Mallet 1989; Jiggins *et al.* 1997; *Acon*¹⁰⁰ is the *cyrbia* allele, *Acon*¹⁵⁰ the *himera* allele). Eight backcross broods were analysed to investigate linkage between this marker and colour pattern genes.

3. RESULTS

We examined 1321 offspring from 4 F₁, 17 backcross, 7 F₂ and 21 further crosses (table 3). The

genetic basis of wing colour pattern differences between the two species proved to be remarkably simple and most of the phenotypic variation could be explained by segregation of alleles at two major loci. The red locus, *D* in the nomenclature of Sheppard *et al.* (1985), controlled placement of the forewing red band and hindwing red bar, whilst the yellow locus, *Cr*, controlled all yellow and white pattern elements (figure 1).

(a) The red locus, 'D'

The red *cyrbia* forewing band and red *himera* hindwing bar were controlled by codominant alleles at a single locus, *D*, here named *D*^{hi} in *himera* and *D*^c in *cyrbia* (figure 1). F₁ hybrids exhibited both elements, whilst backcrosses to *himera* all exhibited the red hindwing bar, and backcrosses to *cyrbia* all had the red forewing band. The presence and absence of red elements in backcross and F₂ broods followed the expected 1:1 and 1:2:1 ratios, respectively, (BC broods, hindwing red bar, +162:–150, *G*₁ = 0.46, n.s.; BH broods, forewing red, +127:–101, *G*₁ = 2.97, n.s.; F₂ broods, 48:86:52; *G*₂ = 0.70, n.s.). The red forewing in BH broods varied from a full red band to only 5% red scales on a yellow band. All 21 further crosses involving backcross or F₂ parents were consistent with the single locus hypothesis (table 3).

(b) The yellow locus, 'Cr'

The yellow and white colour pattern elements in both *himera* and *cyrbia* were similarly controlled by alternate alleles at a single locus (figure 1). This was confirmed by the association between yellow and white phenotypes and *Aconitase* genotypes (table 1). None of these yellow or white pattern elements were strongly expressed in F₁ broods implying that the alleles were largely recessive. Because of this recessivity, *himera* and *cyrbia* pattern elements do not segregate together in backcross broods and are initially considered separately. Similar patterns of expression and the linkage relationship with *Aconitase* imply homology of the 'yellow locus' described here and the *Cr* gene identified in previous *H. erato* crosses (Mallet 1989).

The *cyrbia* elements, yellow bar and white margin, segregated together in the expected 1:1 ratio in BC and further crosses (205:209; *G*₁ = 0.04, n.s.), and 1:3 ratio in F₂ broods (36:150, *G*₁ = 3.34, n.s.). There is no crossing over in female *Heliconius* (Turner & Sheppard 1975) and in two BC broods with female F₁ parents tested for allozyme linkage there was a complete association between presence of the *himera* *Acon*¹⁵⁰ allele and absence of the yellow bar and white margin (table 1). There was no significant association between the loci in broods with male F₁ parents (table 1) so the results imply that *Aconitase* and *Cr* are syntenic but not tightly linked.

Inheritance of the yellow forewing was best illustrated in three broods (108, 120 and 122) which showed segregation of yellow in the absence of red (i.e. parental genotypes are *Cr*^{hi}*Cr*^c, *D*^{hi}*D*^{hi} and

Table 1. *Linkage of Aconitase with yellow and white pattern elements*

(Combined results from BH broods 63, 68, 67, 126 and BC broods 51, 75, 72 and 127 are shown. In the BC broods, crosses with different parental genotypes are combined, so full allozyme genotypes of offspring are not shown. In broods with male F₁ parents there was no significant association between any of the markers (BH broods, $n = 61$, $G_1 = 2.72$, n.s.; BC broods, $n = 67$, $G_1 = 1.83$, n.s.). There is no crossing over in female *Heliconius* (Turner & Sheppard 1975) so the results imply that *Aconitase* and *Cr* are syntenic but not tightly linked.)

forewing band colour		allozyme genotype			
		BH broods with F ₁ mother		BH broods with F ₁ father	
		<i>Acon</i> ¹⁵⁰ <i>Acon</i> ¹⁵⁰	<i>Acon</i> ¹⁰⁰ <i>Acon</i> ¹⁵⁰	<i>Acon</i> ¹⁵⁰ <i>Acon</i> ¹⁵⁰	<i>Acon</i> ¹⁰⁰ <i>Acon</i> ¹⁵⁰
phenotype	genotype				
full yellow	<i>D^{hi}D^{hi}Cr^{hi}Cr^{hi}</i>	13	0	9	5
yellow/black	<i>D^{hi}D^{hi}Cr^{hi}Cr^c</i>	0	11	5	5
yellow with less than 50% red	<i>D^{hi}D^cCr^{hi}Cr^{hi}</i>	12	0	12	7
yellow with greater than 50% red	<i>D^{hi}D^cCr^{hi}Cr^c</i>	0	12	7	11

hindwing bar + white margin		BC broods with F ₁ mother		BC broods with F ₁ father	
		maternal marker		paternal marker	
		<i>Acon</i> ¹⁵⁰	<i>Acon</i> ¹⁰⁰	<i>Acon</i> ¹⁵⁰	<i>Acon</i> ¹⁰⁰
phenotype	genotype				
no bar and margin	<i>Cr^{hi}Cr^c</i>	18	0	15	13
full bar and margin	<i>Cr^cCr^c</i>	0	17	19	20

Cr^{hi}Cr^{hi}, *D^{hi}D^{hi}*). The offspring of these broods fell into two classes; one with an entirely yellow forewing band (full yellow) and the other a smudgy yellow forewing, which resulted from a mixing of black and yellow scales (yellow/black). These phenotypes segregated in the expected 1:1 ratio (28:26; $G_1 = 0.511$, n.s.). In the absence of red, the forewing band expression of *Cr^{hi}* is therefore partially recessive, although in more *cyrbia*-like genetic backgrounds *Cr^{hi}* may be completely recessive to *Cr^c*; e.g. male parent of brood 136 (table 3).

The *Cr* locus also controlled some of the variation in forewing band shape. In *H. himera* the forewing band was broader and more proximal than in *cyrbia*. F₁ broods were intermediate in position and shape. BH broods showed little variation and were similar to *himera*, suggesting that the *himera* allele(s) controlling this effect were largely dominant. BC broods showed considerable variation in phenotype, with *Cr^{hi}Cr^c* genotypes having significantly broader forewing bands than *Cr^cCr^c* genotypes (table 2).

Although the single gene hypothesis is the simplest explanation of the data, there was evidence from a single F₂ individual that the *Cr* locus may consist of two closely linked genes. This had a 30% yellow/70% black forewing band, and a hindwing white margin and yellow bar, a phenotype most easily interpreted as a heterozygote for the *himera* *Cr^{hi}* allele but a recessive homozygote for the *cyrbia* *Cr^c* allele. Unfortunately, identifying recombination is difficult in most broods because of phenotypic overlap between

genotypes (*Cr^{hi}Cr^cD^{hi}D^{hi}* with *Cr^cCr^cD^{hi}D^{hi}*, and *Cr^{hi}Cr^{hi}D^{hi}D^c* with *Cr^{hi}Cr^cD^{hi}D^c*). Only one brood (136) showed segregation of both yellow elements in the absence of the red forewing, allowing all *Cr* genotypes to be scored with confidence (table 3). This showed no evidence for recombination in 26 offspring.

The difficulty of identifying recombinant phenotypes was further highlighted by analysis of the *cyrbia* yellow bar and white margin traits. We observed six individuals (table 3) which expressed only one or other pattern element. However, three of these individuals occurred in first generation backcross broods with a female F₁ parent. As there is no recombination in females, these phenotypes could not have been generated by recombination. The most likely explanation was that hybrid combinations of modifier genes affect the expression of these elements. This must cast some doubt on the interpretation of unusual phenotypes as evidence for recombination within major loci.

(c) *Blue iridescence*

Levels of iridescence were difficult to score and more continuously variable than expected under a single locus hypothesis. F₁ broods showed intermediate levels of blue and BH broods mostly exhibited faint blue. BC broods segregated approximately into individuals with half versus full iridescence in a 1:1 ratio (143:1674; $G_1 = 1.86$, n.s.), which would imply that one partially recessive major gene was involved.

Figure 1. Summary of *H. himera* $\times$ *H. erato cyrbia* broods. The top left and bottom right phenotypes are *H. himera* and *H. e. cyrbia*, respectively. The two differ strikingly in appearance. The forewing band is yellow in *himera* and red in *cyrbia*. On the hindwing *himera* has a broad red bar on the upperside, whilst *cyrbia* has a narrow yellow underside bar and a white margin visible on both upper and undersides. Finally, the upperside background colour of *himera* is black, whilst that of *cyrbia* is iridescent blue. All hybrid broods were scored for the presence/absence of these colour pattern elements. The central phenotype is an F₁. The two boxes show the BH (dotted line) and BC (solid line) phenotypes. Codominant alleles at the *D* locus control positioning of the red elements. The *Cr* locus controls the yellow and white elements and both alleles are largely recessive. There is considerable variation in expression of certain genotypes. Overlap occurs between the phenotypes of $Cr^{hi}Cr^cD^{hi}D^{hi}$ and $Cr^cCr^cD^{hi}D^{hi}$ genotypes, which implies variation in the dominance of Cr^{hi} . In addition $Cr^{hi}Cr^{hi}D^{hi}D^c$ and $Cr^{hi}Cr^cD^{hi}D^c$ overlap in phenotype due to modifier loci affecting the interaction between red and yellow in the forewing (see text for details).

However, there was significant heterogeneity between broods ($G_{10} = 38.63$, $p < 0.0005$), suggesting that more loci are involved. There was no evidence for any association between expression of iridescence and the other colour pattern traits, implying that the genes involved are not linked to *Cr* or *D*.

(d) Modifier loci and interaction effects

Although most of the phenotypic variation in broods can be explained by segregation of alleles at two loci, there was evidence for additional loci of minor phenotypic effect and interactions between the major loci described above. The red *himera* hindwing bar was expressed on the underside of the wing in many hybrids, a phenotype never seen in 'pure' *himera*. This occurred to some degree in all F₁ individuals. In BH and further backcross broods there was stronger expression of red on the hindwing underside in those individuals which did not express red in the forewing (e.g. brood 147, 3 out of 16 D^cD^{hi} and 28 out of 28 $D^{hi}D^{hi}$ genotypes showed this trait). Hence, the homozygote *himera* genotype

shows stronger expression of the hybrid phenotype, which implies that other loci must interact with *D* in the underside expression of red bar.

There was also interaction between Cr^{hi} with D^c alleles in the expression of the forewing band colour. In BH broods, offspring with $D^{hi}D^c$ genotypes showed variation in expression of red in the forewing from 100% to 5% red scales, the remaining part of the band being yellow or white. Analysis of linkage with *Aconitase* implies that the relative expression of yellow and red reflects segregation of $Cr^{hi}Cr^{hi}D^cD^{hi}$ and $Cr^cCr^{hi}D^cD^{hi}$ genotypes (table 1). However, there was significant heterogeneity between BH broods ($G_{39} = 49.74$, $p < 0.05$) for the four forewing band phenotypes (full yellow:yellow/black:yellow with less than 50% red:yellow with greater than 50% red). This heterogeneity was due to variation in the proportions of yellow with less than 50% red:yellow with greater than 50% red phenotypes. Similar heterogeneity was seen in F₁ broods, which all have $D^{hi}D^c Cr^{hi}Cr^c$ genotypes. In most F₁ broods all individuals had full

Table 2. *Band position and width in backcross to cyrbia broods*

(The mean forewing band width and position are shown for *Cr* and *D* genotypes in BC broods only. Variation in the position and width of the forewing band was estimated by measuring the shortest distance from the distal and proximal edges of the band to the point at which the third medial vein joins the cell. The proportion of the band lying distal to this point was then calculated as an estimate of band position and the sum of the two measurements was used as a measure of band width. Mean values and standard errors are shown for each genotype and tested by a Mann–Whitney *U*-test. Values of *U* and *p* are shown, where n.s. signifies $p > 0.05$ and **, $p = 0.01$. These data imply that alleles at the *Cr* locus also affect the width of the forewing band.)

			<i>n</i>	band width (mm)	<i>U</i>	<i>p</i>	band position (mm)	<i>U</i>	<i>p</i>
<i>himera</i> red bar	$D^{hi}D^c$	+	88	3.08 ± 0.09	4337	n.s.	0.67 ± 0.016	4925	n.s.
	D^cD^c	–	112	3.28 ± 0.10			0.67 ± 0.013		
<i>cyrbia</i> yellow bar and white margin	Cr^cCr^c	+	98	3.02 ± 0.09	3966	**	0.66 ± 0.015	5377	n.s.
	Cr^cCr^{hi}	–	102	3.35 ± 0.09			0.68 ± 0.014		
iridescence		++	81	3.33 ± 0.10	4158	n.s.	0.65 ± 0.015	4234	n.s.
		+	119	3.10 ± 0.09			0.68 ± 0.014		

red bands, but others showed variation from pure red to 60% yellow scales in the band. Females were more likely to have a high proportion of yellow scales. For example, in brood 49, 16 males all showed greater than 80% red scales in the forewing, whilst 7 out of 15 females had greater than 80% red scales (the test for heterogeneity between males and females gives $G_1 = 14.7$, $p < 0.0005$). Therefore, modifier loci with sex-linked or sex-limited expression must affect the interaction of Cr^{hi} and D^c .

Another apparent interaction effect between *Cr* and *D* alleles was in the expression of white pigment in the forewing band, a phenotype not found in either parent. This seemed to result from disruption of yellow pigment production by the D^c allele. Hence white pigment was only observed where both Cr^{hi} and D^c alleles were present. For example, in F_2 broods 21 out of 23 $Cr^{hi}Cr^{hi}D^{hi}D^c$ genotypes, and 34 out of 46 $Cr^{hi}Cr^cD^{hi}D^c$ genotypes expressed some white scales in the yellow and red forewing band. In contrast none of the 42 $Cr^{hi}Cr^{hi}D^{hi}D^{hi}$ and $Cr^{hi}Cr^cD^{hi}D^{hi}$ genotypes expressed any white scales.

4. DISCUSSION

(a) *The genetics of mimicry*

The mimetic differences between *H. himera* and *H. erato cyrbia* are controlled by a very simple genetic system. Virtually all the variation observed in hybrid broods can be explained by the segregation of alleles at just two loci. The only major trait not influenced by these loci is the blue iridescent background colour of *cyrbia*.

The divergence of colour pattern between species and races seems to involve largely the same genetic machinery. The genetic loci described here are almost certainly homologous with those previously identified within *H. erato* (Sheppard *et al.* 1985; Mallet 1989). In races of *H. erato* recessive alleles at the *Cr* locus produce a yellow hindwing bar in a similar

manner to that found in *H. erato cyrbia*. Even more convincing, in both our *himera* $\times$ *cyrbia* crosses, and in inter-racial crosses, the *Cr* locus is syntenic with *Aconitase* (Mallet 1989). Given that there are 21 chromosomes in *H. erato* (Brown *et al.* 1992), the same linkage relationship is unlikely to occur by chance alone. Nonetheless, the phenotypic effects of *Cr* are somewhat different in *himera* $\times$ *cyrbia* crosses than in inter-racial crosses. Most notably, in previous crosses, the yellow forewing band phenotype is not controlled by *Cr*, but by a locus, *Y*, tightly linked to those controlling red pattern elements (Sheppard *et al.* 1985, see below). In our crosses, the red and yellow forewing phenotypes clearly represent segregation of two unlinked loci. This discrepancy may reflect non-homology of the forewing yellow band between *H. himera* and *H. erato*, or alternatively a different phenotypic expression of the *himera* *Cr* allele in the *cyrbia* genetic background. We have also shown that *Cr* influences the shape of the forewing band. This provides an interesting comparison with the previously identified *Yl*, *Sd* complex in *H. erato*, which similarly affects the hindwing yellow bar and forewing band shape (Mallet 1989). The fact that the unlinked loci, *Cr* and *Yl*, have similar effects on two apparently unrelated aspects of wing pattern phenotype may imply that they act on the same developmental pathway.

Similarly the *D* locus is probably homologous to the *D*, *R*, *Y* complex, which controls red and orange pattern elements in other races of *H. erato*. Sheppard *et al.* (1985) hypothesized that the red forewing and hindwing colour pattern elements were controlled by three tightly linked loci, named *D*, *R* and *Y*. However, given that in their crosses no recombinants are known between red forewing and red hindwing elements it seems more likely that *D*, *R* and *Y* represent the phenotypic effects of a single locus. Mallet (1989) caught a single apparent recombinant phenotype between *D* and *R*. This could be explained by the presence of two tightly linked genes, but might also represent a novel mutation at this or another locus.

Table 3. *Colour pattern phenotypes of all broods (except F₁)*

(Blue iridescence is scored separately from the other pattern elements, as no association was found between blue and any other element. In the table, '0' signifies a black *himera* background colour, '+' is an intermediate level of blue, and '++' is full *cyrbia* iridescence. Notation: *HIM*, *H. himera*; *CYR*, *H. e. cyrbia*; BH, a cross between an F₁ and a pure *himera*; BC, a backcross to *cyrbia*; BHH, a cross between offspring of a BH brood and a pure *himera*; BHF₂, a cross between a BH offspring and an F₂, etc. (M) and (P) denote the maternal and paternal phenotypes, respectively. Numbers in parentheses refer to the brood number of parental stock. Apparent recombinants within *Cr* are marked with an asterisk in the table notes. *Cr^{hi}Cr^c* genotypes occasionally showed a black shadow in the position of the yellow bar, and a scattering of white scales in the position of the white margin whilst *Cr^cCr^c* homozygotes always showed full expression of both elements. Full yellow bands may have fuzzy edges, but the centre of the band always consisted of pure yellow scales.)

colour pattern phenotypes													
hindwing <i>himera</i> bar	red	red	red	red	red	red	—	—	%				
hindwing <i>cyrbia</i> yellow bar + white margin	—	—	—	—	+	+	—	+	iridescence				
forewing band colour	full yellow	yellow/ black	yellow, less than 50% red	yellow, more than 50% red	black black	red red	red red	red red	0 0	+	++	++	
proposed genotypes	$C_r^{hi}C_r^{hi}$ $D^{hi}D^{hi}$	$C_r^{hi}C_r^c$ $D^{hi}D^{hi}$	$C_r^{hi}C_r^{hi}$ $D^{hi}D^c$	$C_r^{hi}C_r^c$ $D^{hi}D^c$	$C_r^cC_r^c$ $D^{hi}D^{hi}$	$C_r^cC_r^c$ $D^{hi}D^c$	C_r^{hi} D^cD^c	$C_r^cC_r^c$ D^cD^c					
brood no. mother father													
F ₂ crosses													
58	F ₁	F ₁	3	4 ^g	2	8	3	4	7	2	9	21	3
69	F ₁	F ₁	3	1	5	2	0	0	3	1	8	6	1
83	F ₁	F ₁	1	4 ⁱ	2	6	0	2	6 ^j	0	6	9	6
87	F ₁	F ₁	3	1	2	6	0	2 ^k	5	2	5	9	6
109	F ₁	F ₁	3	4	5	7	1	6 ^l	9	1	11	16	9
117	F ₁	F ₁	4 ^m	3 ⁿ	4	5	1	2 ^o	2	4	3	15	7
137	F ₁	F ₁	5	3	3	12	1 ^p	1	7	3	13	13	9
first generation backcrosses													
52	F ₁	<i>HIM</i>	6	10	18	2						36 ^a	
53	F ₁	<i>HIM</i>	3	3	4	1						11 ^a	
63	F ₁	<i>HIM</i>	6	9	5	9						29 ^a	
67	<i>HIM</i>	F ₁	7	8	11	11						34 ^a	
68	F ₁	<i>HIM</i>	8	4	9	5						23 ^a	
71	<i>HIM</i>	F ₁	4	3	7	5						19 ^a	
126	F ₁	<i>HIM</i>	7	4	9	8						27 ^a	
131	F ₁	<i>HIM</i>	0	1	0	0						1	
144	<i>HIM</i>	F ₁	7	2	4	5						18 ^a	
149	<i>HIM</i>	F ₁	0	3	4	4						11 ^a	
151	<i>HIM</i>	F ₁	1	5	1	5						12 ^a	
51	F ₁	<i>CYR</i>				9	4	6 ^b	8 ^b	17 10			
54	F ₁	<i>CYR</i>				4	2	8	3	10 7			
59	F ₁	<i>CYR</i>				0	2	1	0	2 1			
60	<i>CYR</i>	F ₁				7	6	6	7	12 14			
66	F ₁	<i>CYR</i>				1	3	1	0	0 5			
72	<i>CYR</i>	F ₁				9	13	11	7	14 25			
75	F ₁	<i>CYR</i>				16	13	8	13	22 27			
127	<i>CYR</i>	F ₁				7	16	8	4	24 11			
129	F ₁	<i>CYR</i>				12	15 ^b	14	20	31 30			
140	F ₁	<i>CYR</i>				8	10	12	7	5 32			
150	<i>CYR</i>	F ₁				2	3	5	1	6 5			

Table 3. (Cont.)

colour pattern phenotypes									
hindwing <i>himera</i> bar	red	red	red	red	red	red	—	—	%
hindwing <i>cyrbia</i> yellow bar + white margin	—	—	—	—	+	+	—	+	iridescence
forewing band colour	full yellow	yellow/ black	yellow, less than 50% red	yellow, more than 50% red	black	red	red	red	0 + ++
proposed genotypes	$C_{r^{hi}}C_{r^{hi}}$ $D^{hi}D^{hi}$	$C_{r^{hi}}C_{r^c}$ $D^{hi}D^{hi}$	$C_{r^{hi}}C_{r^{hi}}$ $D^{hi}D^c$	$C_{r^{hi}}C_{r^c}$ $D^{hi}D^c$	$C_{r^c}C_{r^c}$ $D^{hi}D^{hi}$	$C_{r^c}C_{r^c}$ $D^{hi}D^c$	$C_{r^{hi}}C_{r^c}$ D^cD^c	$C_{r^c}C_{r^c}$ D^cD^c	
brood no.	mother	father		further backcrosses					
85	BH(63)	<i>HIM</i>	3	8	2	4(M)			17
89	BH(63)	<i>CYR</i>		(M)		14	12		26
91	<i>CYR</i>	BH(63)		(P)		9 ^c	11 ^d		4 15
97	BC(60)	<i>CYR</i>					3(M)	1	4
98	<i>CYR</i>	BC(60)						7(P)	7
99	<i>HIM</i>	BH(63)	5	3	3	1(P)			12
107	BC	<i>CYR</i>					16(M)	12	28
108	<i>HIM</i>	BH(73)	1	4(P)					5
112	BH(68)	<i>HIM</i>	23		16(M)				39
113	BC(72)	<i>HIM</i>			6	6	(M)		6 6
114	<i>CYR</i>	BC(72)					2		2
116	<i>HIM</i>	BC(75)		28		23	(P)		52
120	<i>HIM</i>	BH(68)	16	13(P)					29
122	<i>HIM</i>	F ₂ (83)	11	9(P)					12 8
123	<i>HIM</i>	BH(71)	10	9	12	9(P)			29 11
136	BHH(85)	F ₂ (83)	9	10 ^e (M,P)		7			21 5
141	<i>HIM</i>	BH(68)	18		23(P)				32 9
142	F ₂ (109)	<i>HIM</i>			13	18	(M)		31 ^a
145	F ₂ (109)	<i>CYR</i>					15(M)	13	28
147	<i>HIM</i>	F ₂ (109)	28 ^f		16(P)				40 4

Notes: ^atrace of blue in most individuals; ^bno hindwing (hw) margin in one individual*; ^cone individual with weak white margin and yellow bar; ^dthree individuals with a weak hw yellow bar; ^emale parent and three offspring have an all black forewing (fw); ^fone individual with 10% black scales in fw; ^gtwo individuals with all black fw, trace of red and yellow on veins; ^hone individual with no yellow bar*; ⁱtwo individuals with all black fw band; ^jone individual with a 60% yellow and 40% red fw band; ^kone individual with no yellow bar*; ^lone individual with weak hw white margin and yellow bar; ^mone individual with half length fw band; ⁿone individual with all black fw band; ^oone individual with no yellow bar*; ^pone individual with a 30% yellow/70% black fw band*.

Our results broadly support Nijhout's (1991, 1994) model of *Heliconius* wing pattern development, which was based on inferred homologies with the 'nymphalid ground plan'. His model implies that changes in a few regulatory genes lead to large changes in pattern phenotype, as observed in our crosses. However, Nijhout's (1994) suggestion that red elements are 'pattern' whilst yellow is 'background' is not supported here. The mixed expression of red and yellow within the clearly defined forewing band suggests that these pigments are produced in a homologous manner.

(b) The history of the *H. erato* colour pattern radiation

Turner (1983, 1984) constructed a colour pattern network connecting races based on the assumption that similar wing pattern phenotypes share a common ancestor. He lacked information on the ancestral colour patterns, but used the assumption that dominant alleles are more likely to be derived to infer the direction of evolution (Turner 1983). Recently Brower (1994b, 1996) has produced a mtDNA tree of the races of *H. erato* which was very different to Turner's colour pattern parsimony tree. In

the mtDNA tree there was a phylogenetic break between races from the east and west of the Andes. Within these two major clades there was virtually no phylogenetic resolution. Races with very similar phenotypes occur on both sides of the Andes, and the break itself occurs within the race *H. e. hydana*, which stretches from Panama to Guyana. This discrepancy led Brower to conclude that identical colour patterns must have evolved independently.

Our crosses now provide information on the inheritance of colour pattern in the most likely ancestor to the *H. erato* radiation, *H. himera*. This removes the need to make assumptions about the direction of evolution from patterns of dominance. With these new data, we can reconstruct an evolutionary network which largely resolves conflict between the mtDNA tree and the assumption that similar colour patterns share a common ancestor (figure 2). Under our hypothesis the red forewing band race, *H. erato hydana* (*hydana*), which occurs across the mtDNA break, is the most ancestral pattern within *H. erato* and is derived by a single gene substitution from the *himera* ancestor. This genotype is actually produced in our F_2 broods, where $D^c D^c C_r^{hi} C_r^{hi}$ genotypes are very similar in appearance to *hydana* (figure 1). A *hydana* ancestor must have evolved before the mtDNA split, some 1.5 million years ago (Brower 1994b). All subsequent colour pattern evolution occurs in the two mtDNA clades, within which there is little phylogenetic resolution. Different wing colour phenotypes share the same mtDNA haplotype either because of recent colour pattern evolution or hybridization (Brower 1994b; Mallet *et al.* 1996). As a result, it is impossible to use the mtDNA tree to infer the direction of subsequent colour pattern change within each geographic region.

The allelic changes shown (figure 2) depend on a revised interpretation of previous crosses, in the light of our *himera* $\times$ *cyrba* broods. The hypothesis shows the Amazonian races as retaining the ancestral C_r^{hi} allele, giving rise to a yellow forewing phenotype. This is a slightly different interpretation to that of Sheppard *et al.* (1985) and implies that all races previously crossed are fixed for a homologue of C_r^{hi} , which gives a yellow forewing as the background phenotype, that is masked in the presence of the d^{ry} (red forewing) genotype. The alternative hypothesis, that the yellow forewing has arisen in the Amazon by a change at the *D* locus, would add another two steps to the tree (loss of C_r^{hi} followed by gain of D^{ry}) but would not alter the branching pattern. Crosses between *himera* and Amazonian races would now be interesting to test whether the yellow forewing is homologous in the two forms.

The only apparently non-parsimonious feature of the hypothesis is that the hindwing yellow bar has arisen twice, once on either side of the Andes, which somewhat contradicts previous interpretation of inter-racial crosses (Sheppard *et al.* 1985). However, a review of these data supports the contention that this phenotype is produced by independently derived alleles on either side of the Andes. Crosses between butterflies from west of the Andes (Panama)

Figure 2. Parsimony network for the evolution of mimetic patterns in *H. erato*. On the right are shown the four major clades within the mtDNA tree of Brower (1996). The mtDNA data provides little resolution within these clades. Positioning of the outgroups, *H. clysonimus*, *H. telesiphe* and *H. himera*, is also determined from mtDNA sequence data. All races which have been studied by means of crossing experiments are included. Allelic changes at the four major colour pattern loci are shown with bold vertical bars and genotypes listed, whilst phylogenetically uninformative changes at a further four loci are also shown with narrow bars, but genotypes for all races are not listed. Genotypes shown are deduced from Sheppard *et al.* (1985), Mallet (1989) and data from the present study. Ur and Or loci (Sheppard *et al.* 1985) are not included following Mallet (1989). The d^{ry} allele is assumed to be homologous to the D^c allele identified in our crosses. The race *venustus* is listed as having the Yl^t allele, although there is some evidence for homology with the yl^t allele. *H. e. chestertonii* is also included as this form has a distinct mitochondrial haplotype, although the exact position of this race is necessarily speculative as nothing is known about the genetic basis of the phenotype. This is not the only most-parsimonious tree based on colour pattern data alone. For example, deriving the rayed races from the ancestral *himera* pattern is equally parsimonious given only the colour pattern data, but less likely in the light of evidence from colour pattern biogeography and mtDNA phylogeography.

and those from east of the Andes (S. E. Brazil) produce F_1 offspring with a strongly disrupted yellow bar. This is not expected if the two genotypes are homologous. Indeed, the yellow bar in the east re-

sults from two independent loci, *Cr* and *yl*, in two races investigated (*phyllis* and *favorinus*) whilst a yellow bar phenotype in the west is produced by a single allele also at the *Cr* locus in both *cyrbia* and *petiverana* (Sheppard *et al.* 1985; Mallet 1989 and our crosses). Therefore, convergent evolution of the yellow bar phenotypes seems likely.

In conclusion, we find no evidence for multiple origins of similar colour patterns within *H. erato*, with the possible exception of the yellow hindwing bar. Furthermore, we find little support for Turner's hypothesis that dominant alleles are derived. In our tree, allelic changes occur in both directions, and in a number of cases one dominant allele is replaced by another, leading to codominant patterns of expression when such races are crossed. Although our hypothesis resolves the conflict between mtDNA and colour pattern data sets, there are a number of outstanding issues which remain to be answered about the evolution of colour pattern in *H. erato*. We know nothing about the colour pattern genetics of *H. e. chestertonii*. In our tree, this race is placed in a relatively derived position with the other west Andean yellow bar races. However, *H. e. chestertonii* has a distinct mtDNA haplotype that suggests it may be ancestral (Brower 1994b). It is also unclear how the present disjunct distribution of races with apparently homologous colour patterns arose. This pattern is particularly striking around the Amazon basin, where red and yellow banded patterns are found in a number of isolated Andean valleys (Sheppard *et al.* 1985; Turner & Mallet 1996). Furthermore, the mtDNA evidence seems to imply that *H. erato* and its comimic, *H. melpomene*, have not evolved in parallel (Brower 1996). This is difficult to explain if similar mimetic colour patterns are uniquely derived in both species. Ultimately, molecular phylogenies of the colour pattern loci themselves will provide the definitive test of these hypotheses of colour pattern evolution in *H. erato*.

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REFERENCES

- Brakefield, P. M., Gates, J., Keys, D., Kesbeke, F., Wijn-gaarden, P. J., Monteiro, A., French, V. & Carroll, S. B. 1996 Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**, 236–242.
- Brower, A. V. Z. 1994a Phylogeny of *Heliconius* butterflies inferred from mitochondrial DNA sequences (Lepidoptera: Nymphalidae). *Molec. Phylog. Evol.* **3**, 159–174.
- Brower, A. V. Z. 1994b Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natn. Acad. Sci. USA* **91**, 6491–6495.
- Brower, A. V. Z. 1996 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. 1979 *Ecologia geográfica e evolução nas florestas neotropicais*. (Livro de Docencia) Campinas, Brazil: Universidade Estadual de Campinas.
- Brown, K. S., Emmel, T. C., Eliazar, P. J. & Suomalainen, E. 1992 Evolutionary patterns in chromosome numbers in neotropical Lepidoptera. I. Chromosomes of the Heliconiini (Family Nymphalidae: Subfamily Nymphalinae). *Hereditas*, **117**, 109–125.
- Carroll, S. B., Gates, J., Keys, D. N., Paddock, S. W., Panganiban, G. E. F., Selegue, J. E. & Williams, J. A. 1994 Pattern formation and eyespot determination in butterfly wings. *Science* **265**, 109–114.
- Jiggins, C. D., McMillan, W. O., Neukirchen, W. & Mallet, J. 1996 What can hybrid zones tell us about speciation? The case of *Heliconius erato* and *H. himera* (Lepidoptera: Nymphalidae). *Biol. J. Linn. Soc.* **59**, 221–242.
- Jiggins, C. D., McMillan, W. O., King, P. & Mallet, J. 1997 The maintenance of species differences across a *Heliconius* hybrid zone. *Heredity* **78**. (In the press.)
- Mallet, J. 1989 The genetics of warning colour in Peruvian hybrid zones of *Heliconius erato* and *H. melpomene*. *Proc. R. Soc. Lond. B* **236**, 163–185.
- Mallet, J., Jiggins, C. D. & McMillan, W. O. 1996 Mimicry meets the mitochondrion. *Current Biol.* **6**, 937–940.
- McMillan, W. O., Jiggins, C. D. & Mallet, J. 1997 What initiates speciation in passion vine butterflies? *Proc. Natn. Acad. Sci. USA* **94**, 8628–8633.
- Nijhout, H. F. 1991 *The development and evolution of butterfly wing patterns*. Washington, DC: Smithsonian Institution.
- Nijhout, H. F. 1994 Developmental perspectives on evolution of butterfly mimicry. *Biosci.* **44**, 148–157.
- Sheppard, P. M., Turner, J. R. G., Brown, K. S., Benson, W. W. & Singer, M. C. 1985 Genetics and the evolution of muellerian mimicry in *Heliconius* butterflies. *Phil. Trans. R. Soc. Lond. B* **308**, 433–613.
- Turner, J. R. G. 1983 Mimetic butterflies and punctuated equilibria: some old light on a new paradigm. *Biol. J. Linn. Soc.* **20**, 277–300.
- Turner, J. R. G. 1984 Mimicry: the palatability spectrum and its consequences. In *The biology of butterflies* (ed. R. I. Vane-Wright & P. R. Ackery), Symp. of the Royal Entomological Society of London, vol. 11, pp. 141–161. London: Academic.
- Turner, J. R. G. & Mallet, J. 1996 Did forest islands drive the diversity of warningly coloured butterflies? Biotic drift and the shifting balance. *Phil. Trans. R. Soc. Lond. B* **351**, 835–846.
- Turner, J. R. G. & Sheppard, P. M. 1975 Absence of crossing-over in female butterflies (*Heliconius*). *Heredity* **34**, 265–269.

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