GENETICS AND THE EVOLUTION OF MUELLERIAN MIMICRY IN *HELICONIUS* BUTTERFLIES

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A protected and warningly coloured butterfly can become a muellerian mimic of another species in two steps: (i) a major mutation converts the pattern of the less protected species to an approximate resemblance of the better protected (one-way convergence); (ii) after the spread of this mutant, the species, which now resemble each other sufficiently to be mistaken one for the other by predators, undergo mutual convergence, using whatever major or minor genetic variation is available to them. Although sometimes one or other step might occur alone, in general early theorists were mistaken in attributing muellerian mimicry to only one of these processes. By hybridizing races of *Heliconius melpomene* and races of *H. erato* (a pair of parallel mimetic species from the neotropics, held in mutual muellerian mimicry across wide inter-racial variations in colour pattern) we have shown that, as expected from the two-step theory, the races differ at a number (two to nine) of genetic loci, usually unlinked or loosely linked, including at least one mutant of major effect in each case. We describe the genetic constitution of eight races of *H. melpomene* (for 11 loci affecting colour pattern) and of eight races of *H. erato* (for up to 15 loci), and have started to identify the linkage groups. Map distances for those loci that are linked range from around 0.3 to zero in males, with no recombination in females.

Muellerian mimicry is expected to produce total uniformity of pattern: universal exceptions to this are the existence of distinct mimicry rings flying within the same

habitat, geographical variation within nearly all the more widespread species (divergence in the face of normalizing selection), and, in a few species, polymorphism or sexual dimorphism. Sympatric mimicry rings will, according to the two-step model of evolution, persist indefinitely if their patterns are so distinct that under no circumstances do predators mistake one for the other. Gradual mutual convergence is then impossible, although members of a weakly protected mimicry ring that can produce a mutation giving sufficient initial resemblance to a better protected ring can still be captured by it. Batesian mimics promote this by lowering the protection of the ring that they belong to, but their models can escape only in this way as normalizing selection prevents their gradual evolution away from the batesian mimic. If the rings are too distinct in pattern even this capture of species becomes impossible as no single mutant is able to bridge the gap between the two patterns, and the necessary two mutations will be extremely unlikely to occur together. The five principal sympatric mimicry rings of the mature neotropical rain forests are very distinct in their appearance.

. The capture of a species by another ring can produce geographical variation both in the species captured and in the capturing ring, whose pattern is somewhat altered by mutual convergence with the captured species in the second step of the evolution of the muellerian resemblance. We suggest that the striking differences between the races within *H. melpomene*, *H. erato* and other *Heliconius* species resulted from these effects of inter-ring capture. Distributional evidence suggests that this chiefly occurred in refuges formed by the contraction of the neotropical rain forests during the cool dry periods in the Quaternary; these, by differential extinction of elements of the flora and fauna of different refuges, could have produced long-term changes in the relative abundances of the mimicry rings, and hence (as the protection given to a ring is proportional to its abundance) somewhat different capture events in each refuge.

Several existing species confirm that this mode of evolution occurs, by retaining a distinctive pattern in the absence of any other remotely similar species, but becoming mimetic in areas where they encounter a pattern somewhat like their own. The isolated populations of *Heliconius hermathena* show this particularly clearly; the effect can be discerned also in *H. melpomene* and *H. erato*.

Although polymorphism in muellerian mimics is largely unexplained, in two species of *Heliconius* it may result from the existence of two or more similar but slightly differing 'sub-rings' among their comimics in the family Ithomiidae, which show both spatial and temporal heterogeneity in their local distribution, which apparently is able to maintain a polymorphic equilibrium in the more uniformly distributed *Heliconius*.

We have tentatively reconstructed the ancestral patterns of *H. melpomene* and *erato* by two independent methods: first, as dominant genes are much more likely to be incorporated than recessive ones during changes of pattern, the phenotype produced by the recessive alleles at all the known loci will be close to the ancestral pattern; secondly, species that are becoming mimics evolve more than those that are not, so that non-mimetic relatives of *melpomene* and *erato* will have a pattern close to ancestral. Both methods suggest, for *both* species, that the ancestor was a black butterfly with yellow (or possibly white) bars, and it may be that *melpomene* and *erato* have been comimics for a very long time.

Previous climatic cycles in the Quaternary have apparently caused full speciation within two mutually mimetic evolving lineages, producing pairs of parallel mimetic species within the genus, of which *melpomene* and *erato* constitute one pair.

1. INTRODUCTION

Evolution is, in a large part, the result of ecological diversification and of the adaptive radiation of successful groups of organisms into a variety of niches. This radiation depends ultimately on the mutual genetic isolation of the diverging lines and hence on speciation. In most situations allopatric race formation is the necessary beginning of this process. Sokal (1974) has argued that, after geographical isolation, the critical event initiating speciation is not genetic
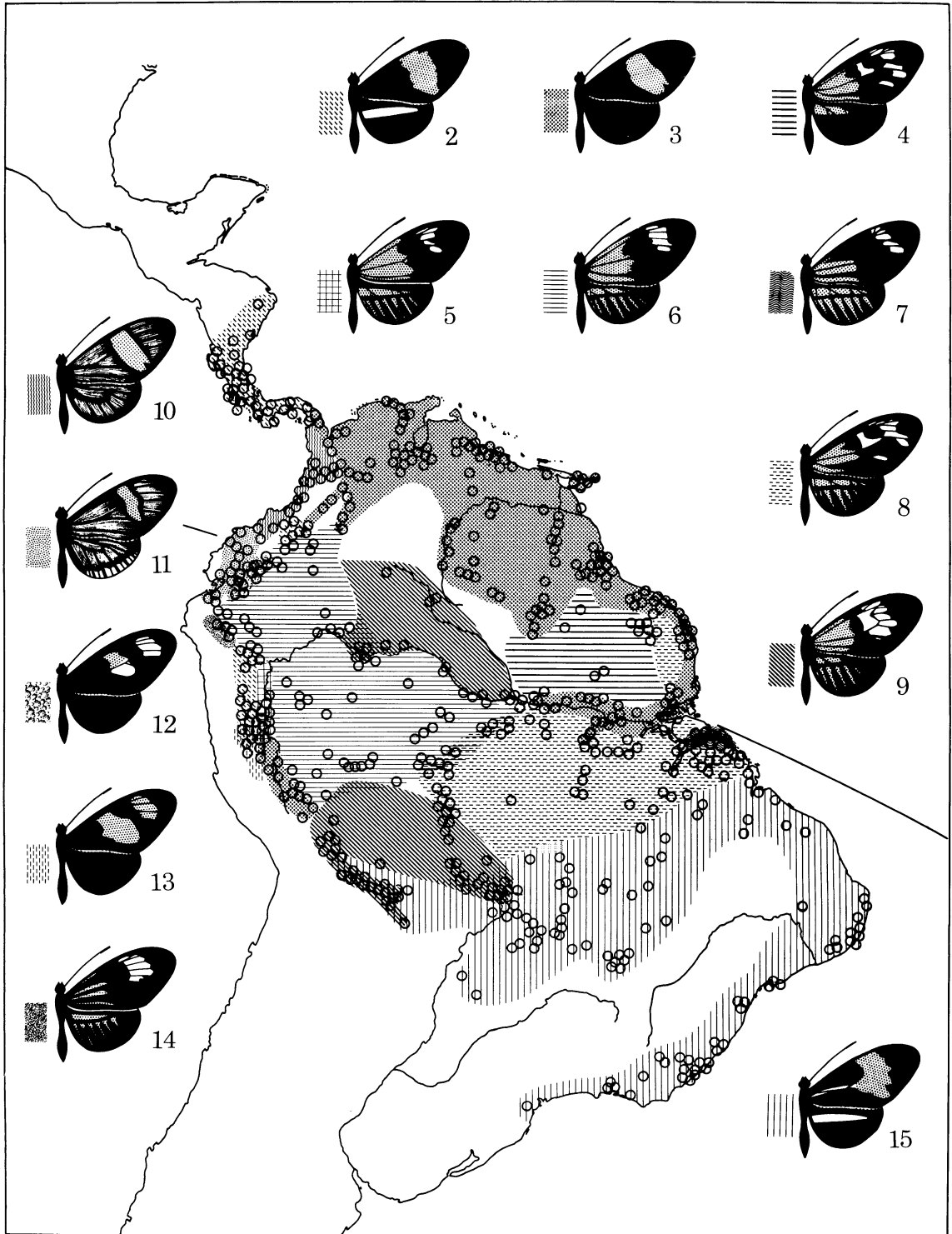
FIGURE 1. Geographical variation of *Heliconius melpomene*. Circles are collection points, grouped into quadrants of 30 min by 30 min of latitude and longitude; overlapping shading indicates a variable population; hybrid zones between races (see for instance Turner 1971 *b*, Benson 1982) are narrow and are not shown; some races showing only minor differences are not distinguished.
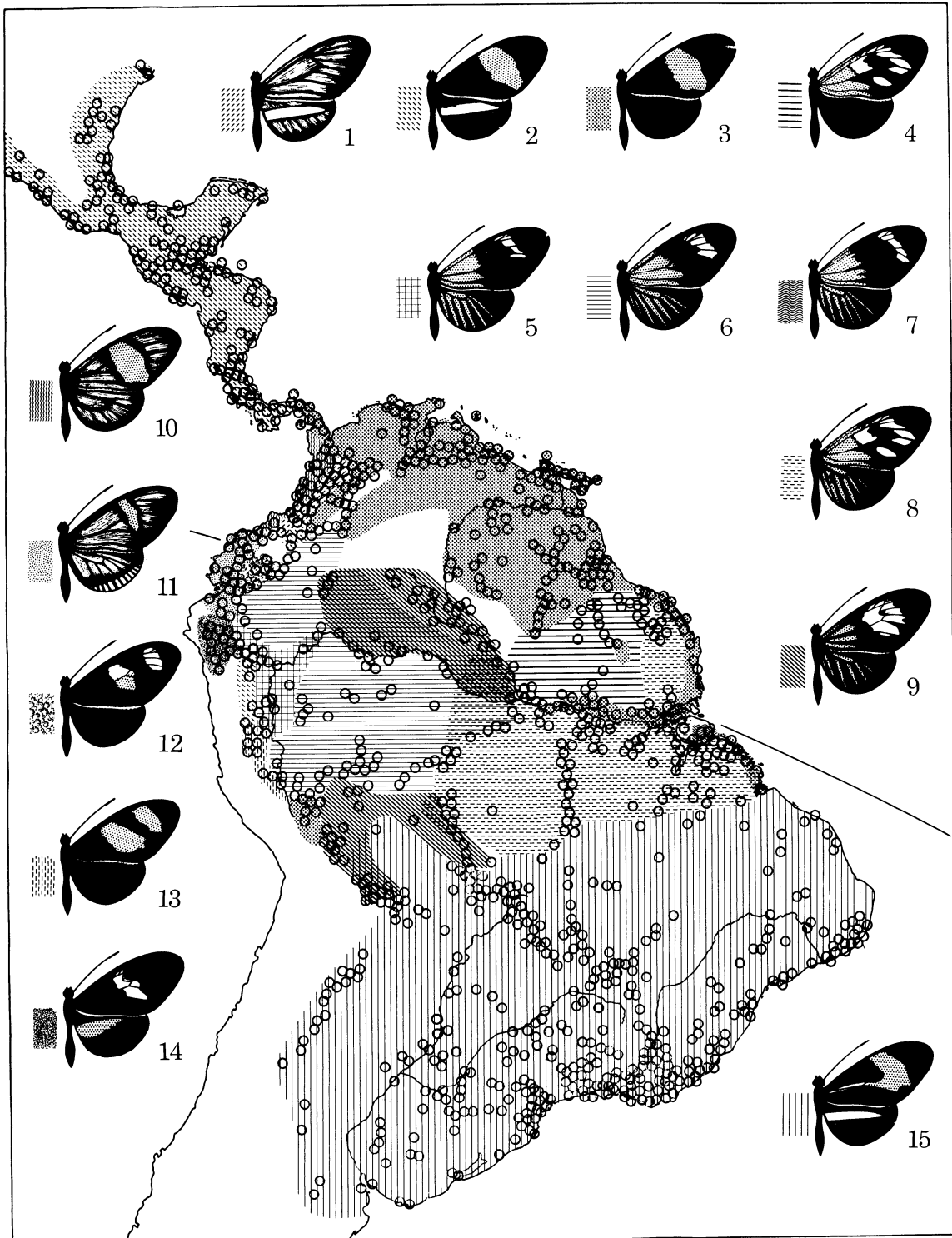
FIGURE 2. Geographical variation of *Heliconius erato*, the parallel comimic of *Heliconius melpomene*. Symbols as in figure 1; similar shading indicates a similar mimetic form. For sources of data for these figures, see acknowledgements; for nomenclature, see appendix 1. Colour versions of figures 1 and 2 can be found in Turner (1975). Shading is used here to represent colours: solid black, black; unshaded, yellow or, in Ecuador, white; stippled, red or orange; striated, blue iridescence.

isolation and divergence in itself, but a change in the niche of the isolated populations; this in turn will result in the evolution of genetical isolating mechanisms should the populations become sympatric.

We have been studying the genetics of this crucially important process, the change in ecological niche in mutually isolated populations, by working with an adaptive system concerned with only part of the total niche, but with most of its salient features. Muellerian mimicry is the mutual resemblance of a number of distasteful or otherwise protected species. This resemblance confers added protection on all members of the muellerian mimicry ring because it reduces the total number of trials that an uneducated predator must make before it learns to avoid all the protected species. Because its essence is the production of a simple warning pattern that a predator, after a number of unpleasant experiences, will remember and avoid, muellerian mimicry implies strong stabilizing selection on the appearance of all the members of the mimicry ring: predators will more often fail to recognize a phenotypic deviant as distasteful, and it will therefore stand a greater chance of being tried as food (Fisher 1930). This effect has been demonstrated experimentally in the field with *Heliconius erato*†, one of the species used in the present study (Benson 1972).

The muellerian mimics investigated in this study are two butterflies in the genus *Heliconius*: *H. melpomene* and *H. erato*. Their geographical variation, illustrated in figures 1 and 2, brings out three important features of muellerian mimicry. First, stabilizing selection on this pair of species keeps them in strict mutual mimicry throughout their range; with some apparent minor exceptions, the pattern exhibited by *melpomene* at any point in Latin America is closely similar to that of the local *erato*. The similarity in all cases is good enough to confuse a casual observer (plate 1), and in some races is so good that even an experienced worker may have to sniff the butterfly (the defensive odours of the species being quite distinct), or if it is dead or a virgin female (which is odourless), make a close examination of minor pattern elements such as the basal spots on the underneath of the hindwing, by which the species can normally be distinguished. This resemblance is the more impressive when one realizes that, within their genus, *melpomene* and *erato* belong to different species groups, as determined by the internal anatomy of the adults and the morphology of the pupae (Emsley 1965a; Turner 1968a; 1976a; Brown 1981). Within the Amazon basin this parallel variation is shared by six other *Heliconius* species, and in a less exact way by three others; in southern Brasil one further species shares the pattern common to *melpomene* and *erato*. There is thus a large mimicry ring, involving up to 11 species (or 12 if one includes the red form of the exceptional polymorphic species *H. doris*) in the Amazon basin, which reduces to a ring of two or three species outside that area. (For distribution maps, see for example Brown (1979, 1982) and Turner (1971a).)

Second, in accord with the hypothesis that muellerian mimics are subject to stabilizing selection, neither *H. melpomene* nor *H. erato*, nor any of the other species, is to any notable degree polymorphic for colour pattern. Monomorphic populations are the rule. Exceptions are *H. doris*, which has forms belonging to this ring and to two others, the slight sexual dimorphism of some races of one of the species in the ring (*H. demeter*), and the rampant polymorphisms of *melpomene* and *erato* at the places where two or more races meet. The polymorphism of *erato* and *melpomene* is clearly the result of hybridization (probably arising from secondary contact between the previously isolated races), for the patterns found in the zones of polymorphism are among those to be expected from the hydridization of the races on either side. Thus the polymorphic

† Authors of Latin names of all *Heliconius* species discussed in this paper are in Appendix 2.

zone of *melpomene* in the Guianas, which is the best studied, is comparatively narrow (certainly less than 50 km wide and probably less than 25 km), and has persisted for at least 200 years; the polymorphic forms in it are the same as the ones produced by hybridizing the putative parents in the laboratory, and apparently occur at roughly the frequencies that one would expect from random mating within the hybrid zone (Turner 1971 *b*; Brown *et al.* 1974). The same is true of *H. erato* in this region (Benson 1982).

Third, despite the absence of polymorphism and the presumed strong stabilizing selection, the races within each species have diverged considerably in their appearance, so much so that until two decades ago both *melpomene* and *erato* were divided by systematists into several distinct species (Neustetter 1929). That is to say that, at some stage in the past, a butterfly population occupying a strongly stabilized niche or adaptive peak has been able to evolve in such a way that it now occupies several different adaptive peaks.

The evolution of mimicry in these butterflies can thus be investigated by experimentally hybridizing races with different patterns; the populations used in the crosses reported here are indicated by filled symbols in figure 3, open symbols indicating other races used in earlier studies; all studies so far have used the Trinidad population (*) (Turner & Crane 1962; Sheppard 1963; Emsley 1965 *b*; Turner 1972).

For convenience in discussion we shall divide the patterns of both species into the two rather distinct groups that can be seen in the figures: the Amazonian 'radiate' pattern, with red bases to the wings and some kind of yellow band in the outer part of the forewing, and the extra-Amazonian 'postman' pattern, lacking the red over the wing bases, having a red (sometimes red and white) forewing band (absent in one race only), and often a yellow bar across the hind-wing. The radiate patterns are continuously distributed in the Amazon Basin, with the postman patterns almost entirely surrounding them in a broad 'C' from the northwest to the east, reduced to a narrow strip on the northeast; this slightly odd distribution is of some importance (see Discussion).

2. MATERIALS AND METHODS

Stocks were collected in the wild at the localities shown in figure 3: near São Paulo (Brasil), at Linhares in Espírito Santo (Brasil), in the region of Rio de Janeiro (Brasil), in the Ducke reserve near Manaus (Brasil), on the lower Rio Trombetas in Pará (Brasil), near Belém do Pará (Brasil), near Georgetown (Guyana), in Trinidad, near Quebrada Grande in Apure (southwestern Venezuela), in the Canal Zone area of Panamá, near Gomez Farias in Tamaulipas (northern México), near Riozinho in Rondônia (Brasil), at Areia Branca in western Mato Grosso do Norte (Brasil), and on the eastern slopes of the Andes in Ecuador near Palora and Puyo (Pastaza valley).

The broods were reared by three techniques: in fine mesh cages with semi-natural vegetation in protected courtyards in Brasil, and in the temperate zone either in similar cages within a heated greenhouse or flying free in the greenhouse itself. During the winter free flying butterflies were prevented from becoming chilled on the glass by lining the greenhouse with polyethylene sheeting. Mass-bred pure stocks were mostly kept flying free in this way. For single-pair matings, each pair requires a minimum space of around 2 m in each dimension to mate, and each female around the same volume to survive happily and to lay (the dimensions vary from race to race, and we have evidence that it may be possible to breed *melpomene* or even *erato* in a much smaller volume). Accordingly, each brood was placed in a separate greenhouse or in a cage
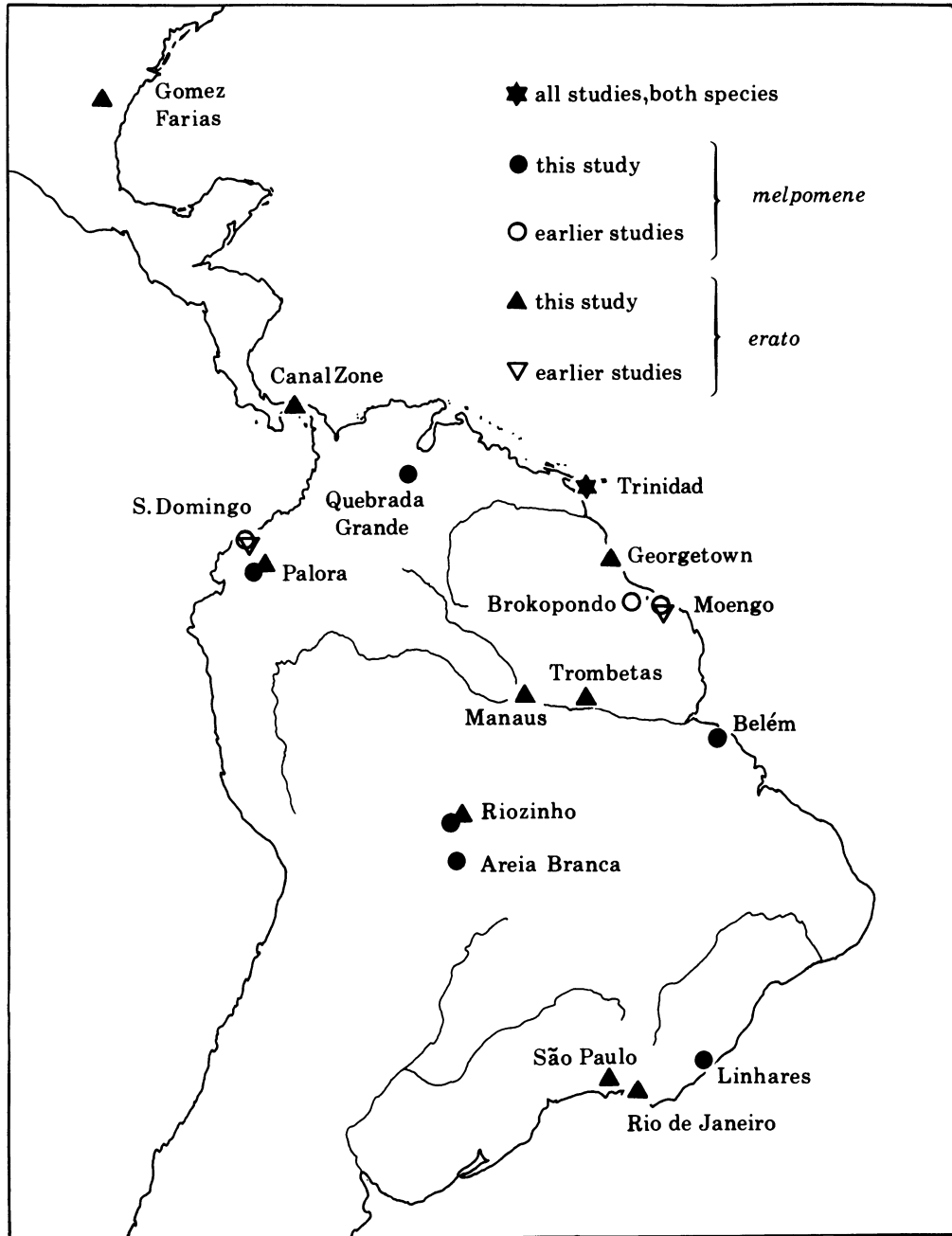
FIGURE 3. Points of origin of stocks used in the present experiments (closed symbols) and in previous studies (open symbols). Trinidad stocks have been used in all studies (star).

compartment. To increase the number of offspring in some broods, particularly F_2 broods, we sometimes placed several females of the same mating together in one cage. Eggs were laid and larvae were reared on growing potted *Passiflora* plants, chiefly *P. caerulea*, *P. laurifolia*, *P.* × *allardi*, *P. serratodigitata*, *P. alata*, *P. sidaefolia*, *P. actinia* and *P. antioquiensis* for *melpomene*, and *P. caerulea*, *P. allardi*, *P. auriculata*, *P. sidaefolia*, *P. organensis*, *P. punctata*, *P. capsularis* and *P. biflora* for *erato*, growing in the greenhouse or cage with the mother butterfly, or collected in the wild at Rio de Janeiro. Those offspring that were allowed to emerge freely in the cage

or greenhouse were collected every 1 or 2 days. Nutrition of adult butterflies was often critical for satisfactory egg production; favoured nectar and pollen-producing flowers were provided in abundance, and supplemented with honey water (1:10) in special feeders. A detailed description of the culture techniques is given elsewhere (Turner 1974; Brown & Benson 1974).

Natural matings were usually quite easy to obtain between vigorous older males and virgin females of *melpomene*, but younger or cage-reared males often refused to mate well. Matings were much more difficult to obtain in *erato*, the female usually accepting only very vigorous or field-captured males, and only in the first few hours after her emergence. This greatly limited the number of successful broods obtained in the latter species.

Offspring were killed by pinching or by being put in a translucent paper envelope and placed in a freezer. After death the butterflies were placed in the envelope in a spread position, with the brood data written on the outside.

Data for the distribution maps have been compiled from the collections listed in the acknowledgements, from fieldwork, from personal communications from other workers (see also the acknowledgements), and from a number of sources in the literature, which are listed in Brown (1979).

Unless otherwise stated, all χ^2 values quoted have been calculated with Yates' continuity correction. Probabilities quoted without χ^2 values have been computed direct from the binomial distribution, two-tailed. Tests for linkage use the orthogonal design of Bailey (1961) (testing coupling and repulsion phenotypes for a 1:1 segregation) unless a χ_c^2 or Fisher exact probability is given, in which case a 2×2 contingency table has been used.

Each butterfly bred has been individually numbered and scored for all characters of interest. Copies of the complete score sheets, showing the phenotype of each individual, have been deposited in the archives of the Royal Society and the British Library, Lending Division.[†] Appendixes 4 and 5 present an abstract.

3. GENETICS OF *HELICONIUS MELPOMENE*

3.1. *Races and phenotypes*

The races of *H. melpomene* used in the present experiments are illustrated in colour in plate 1 *a–d, i*. For quicker reference, see figure 4 (distributions are shown in figure 1). Authors of the Latin subspecific names appear in Appendix 1.

The Venezuela/Trinidad race (*H. melpomene melpomene*) (figure 1, no. 3; figure 4*a*; plate 1*b*) which has been used in all previous genetic experiments, and which occurs with minor variations across most of northern Colombia, Venezuela and into the Guianas, narrowing to a thin strip along the lower Amazonas, is a plain black butterfly with a *wide* red *band* across the forewing; this band is an intense, pinkish red. Our stocks came from Waller Field, Trinidad, and from Quebrada Grande, a small hamlet on the border of Táchira and Apure in extreme south-western Venezuela.

The race from East Brasil (*H. melpomene nanna*) (figure 1, no. 15; figure 4*b*; plate 1*c*) occupies most of the coast of Brasil between Rio Grande do Norte and Rio de Janeiro, and sparsely to Santa Catarina and Rio Grande do Sul; a variant form (*H. m. burchelli*) is found in a strip across the interior of Brasil from Maranhão to Mato Grosso. These forms have an intense red

band on the forewing, about the same width as in the race from Trinidad, but more concave on its inner edge and having a distinct *tooth* (much reduced in *burchelli*) on the outside toward the posterior angle of the wing. Along the centre of the forewing there is a bright *yellow line*, and across the base of the hindwing a *yellow bar* of the same colour. Our stock came from Linhares in Espírito Santo.

The race (*H. melpomene plesseni*) (figure 1, no. 12; figure 4c, plate 1d) from the slopes of the Andes in East Ecuador (above 1100 m appoximately) (Descimon & Mast de Maeght 1971) is like the race from Trinidad, except that the forewing band consists of two distinct coloured areas, *split* by a black region, is predominantly *white* (with some red colour in most specimens), and is of a different overall shape from the Trinidad band, being *shorter*, in not extending toward the posterior angle of the wing, and *round*, in extending much more toward the wing apex and being strongly convex on this outer edge (i.e. the outer edge of the outermost white mark). Our stock came from south of Puyo in the Pastaza valley. This race must not be confused with that from *West* Ecuador, not bred in the present experiments but briefly investigated by Emsley (1965 b), which has a very different pattern (see figure 1, no. 11).

In the race from Belém (*H. melpomene thelxiope*) (figure 1, no. 8; figure 4d, left insect; plate 1a), which occupies much of the southeast Amazon Basin, the red or white band of the other three races is replaced by a *yellow band, broken* up into a series of spots as shown in the illustrations. There are extensive areas of bright orange at the base of the forewing and hindwing, and a series of orange rays on the hindwing; these orange marks are collectively known as the *radiate* pattern. Our stock came from near the city of Belém.

A few of the butterflies from Belém deviate from this pattern. In some (sometimes apparently a majority) the orange marks are brilliant red; others lack the dumb-bell shaped yellow spot (the cell spot) that forms the innermost part of the band, and yet others have a yellow bar, like

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DESCRIPTION OF PLATE 1

(A) Races of *Heliconius melpomene* used in the present experiments (top row and *i*): (*a*) Belém (*thelxiope*), (*b*) Venezuela/Trinidad (*melpomene*), (*c*) East Brasil (*nanna*), (*d*) East Ecuador (*plesseni*) and (*i*) Bolívia (*penelope*).

(B) Races of *Heliconius erato* used in the present experiments (middle row and *j–l*): (*e*) Mato Grosso/Belém (*amazona*), (*f*) Venezuela/Trinidad (*hydara*), (*g*) East Brasil (*phyllis*), (*h*) East Ecuador (*notabilis*), (*j*) Bolívia (variable population from Rondônia), (*k*) Manaus (Guiana) (*amalfreda*) and (*l*) Panamá/Mexico (*petiverana*). *Notes*: *a*, *c*, *g*, *l* are living butterflies, photographed in captivity, *j* are living butterflies roosting in the wild, others are of stunned or freshly killed butterflies posed in the field at the place of capture; *b*, *f* are from the populations of the Venezuela/Trinidad races on the lower Amazonas (see figures 1 and 2).

Photographs by J. R. G. T. (*a*, *g*), L. E. Gilbert (*l*) and K. S. B. (all others).

DESCRIPTION OF PLATE 2

Heliconius melpomene. Hybrid phenotypes, to illustrate various segregating characters: (A) similarity of F_1 hybrids between Belém × Trinidad (*a*) and Belém × East Brasil (*b*); (B) types of forewing band TS (*a*, *b*, *c*, *d*), TY (*e*), S (*f*), O (*g*), W (*h*), Y (*k*), red and white (*i*); (C) broken band (*c*) versus fused band (*d*); (D) orange (*f*) versus red (*g*); (E) red responding to broken band gene (*l*), compared with fused (not responding) red band (*h*), and broken yellow (*k*); (F) yellow hindwing bar and forewing line (*h*), yellow hindwing bar in male of Belém stock (*k*) and yellow basal spot (*j*, right half); (G) plain (*c*, *d*) versus radiate (all others); (H) cell spot versus no cell spot (*j*, both halves); (I) yellow + thin red versus yellow (*j*, both halves); (J) short split band (*i*) versus long entire band (*j*, both halves); and (K) Belém spot (trace in left half of *j*, well developed in *k* and *l*) versus absence (*j*, right half).

All are living butterflies, photographed in captivity; *a* and *c–g* are Belém × Trinidad hybrids; *b* and *h* are Belém × East Brasil hybrids; *i*, *j* and *l* are Belém × East Ecuador hybrids; *k* is a pure Belém butterfly; *j* is a somatic and sexual mosaic.
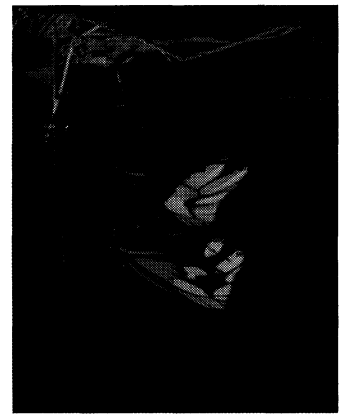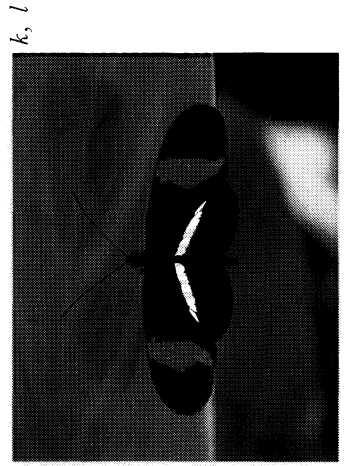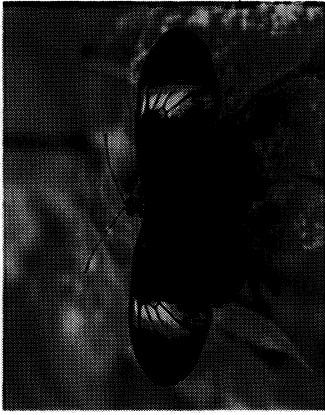
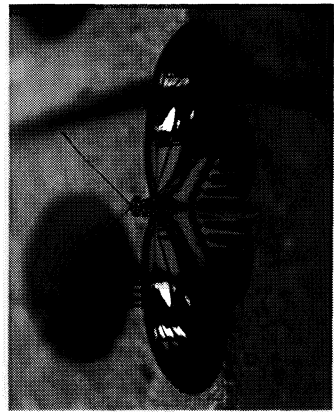Photographs by J. R. G. T.

PLATE 1. For description see opposite.
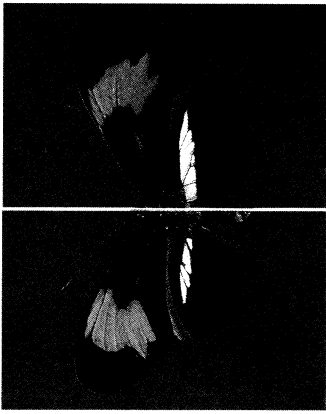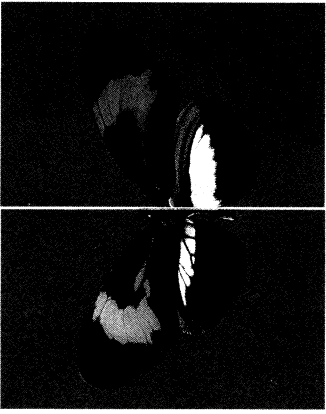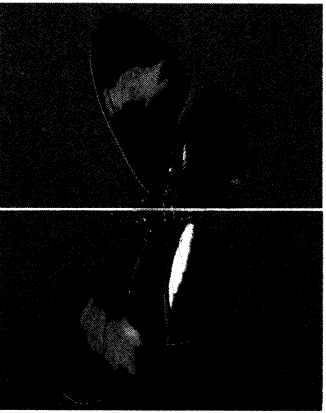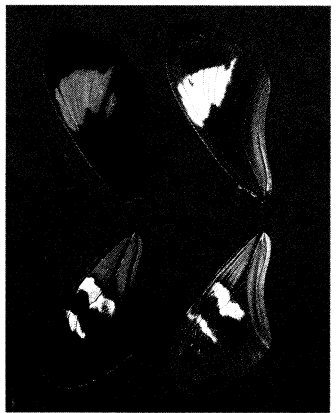
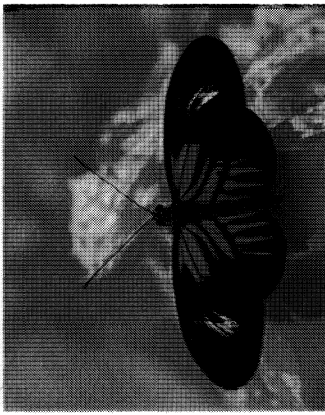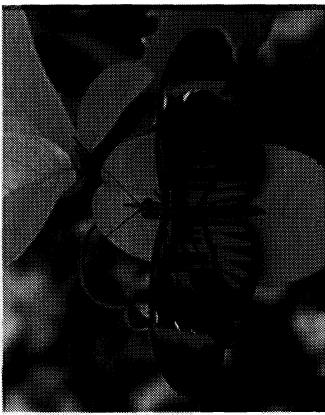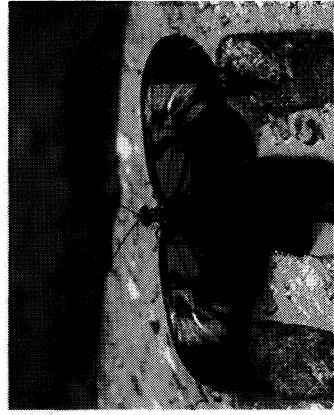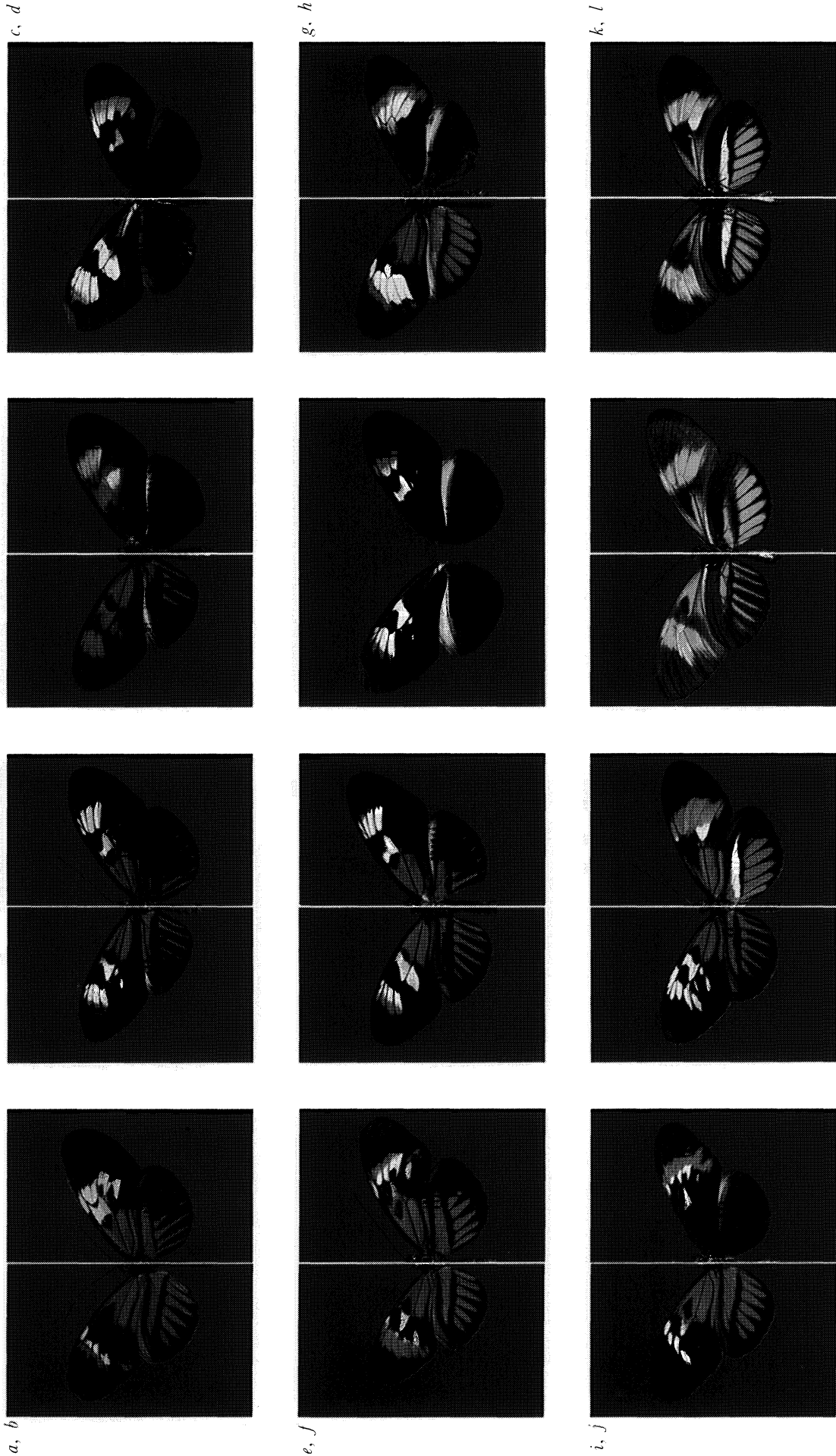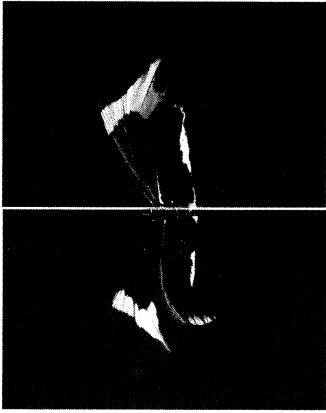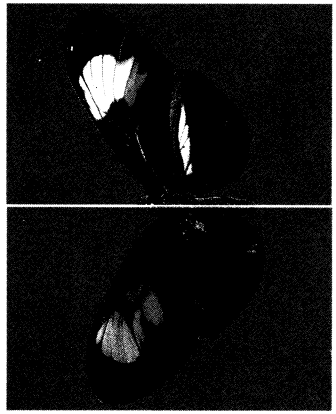PLATE **2**. For description see opposite plate **1**.
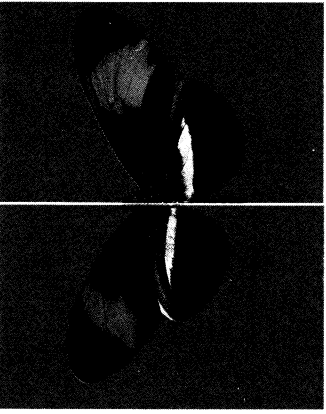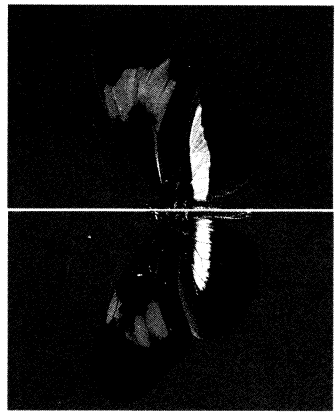
DESCRIPTION OF PLATE 3

Heliconius melpomene: hybrid phenotypes, arranged chiefly to show contrasting phenotypes. Left half described first.

(*a*) Belém × Venezuela F_1 (compare with plate 2*a*); Bolívia × Rio Madeira F_1, partly fused phenotype with concave distal margin to band (compare with fused (*ff*) in plate 3*h*, and Belém-style broken band with convex margin in plate 2*k*).

(*b*) Belém × East Ecuador F_1: *left* with red in band and partly developed Ecuador triangle; *right* with both red in band and Ecuador triangle barely developed.

(*c*) Split Ecuador bands ($N^B N^B B-$, with cell spot and Ecuador triangle): *left* entirely in red with red responding to *Split*; *right* in red and white, with the white but not the red responding to *Split*.

(*d*) To show placing of white colour between red and yellow in an $N^N N^N B-$ butterfly of the East Ecuador cross; an exceptional $N^B N^B B-$ phenotype in the Belém × East Ecuador F_2 in which the red band (with white added) is split but not short (§3.7*f*).

(*e*) The exceptional Belém × Trinidad F_1 (§3.5*b*): *left* with wide red; *right* with narrower red.

(*f*) Ecuador triangle (fully developed); no Ecuador triangle.

(*g*) $N^N N^N B-$ father of brood 19B; apparent $N^N N^B B-$ butterfly in brood 19B having appearance of $N^B N^B B-$ homozygote (with white added) (see §3.7*g*) (N^B almost dominant to N^N: compare with *h*).

(*h*) $N^N N^N B-$ heterozygotes with strong yellow like that normal in $N^N N^N$ homozygotes: *left* in the line descended from the F_1 shown in *e* (see §3.2*b*); *right* in the backcross to the Trinidad-like stock §3.2*c*) (both are fused (*ff*)). *Left* also has red scales mixed with yellow bar.

(*i*) Exceptional S phenotype in the Belém backcross of the exceptional F_1 shown in (*e*); East Brasilian tooth on band, manifested in hybrid brood CYHW. In both left and right halves the cell spot is represented by its basal half only.

(*j*) Ecuador triangle appearing in two separate halves (§3.8*d*); Ecuador triangle appearing in Belém × East Brasil F_2 (also *left* is red with narrow rays; *right* is orange with wide rays).

(*k*) Rays on underside: *left* narrow; *right* wide and almost fused (see also *j*).

(*l*) Red band with white scaling on underside; same, with yellow scaling on underside (also with Ecuador triangle).

(*c*, *h*, *i*) Radiate; plain.

Photographs by University of Leeds Audio-visual Service.

DESCRIPTION OF PLATE 4

Heliconius erato: major phenotypes. The plate is arranged chiefly to show antithetical phenotypes in the two halves of each picture. Left half described first. Where two pairs of forewings are shown, they are the upperside (above) and underside of the same butterfly.

(A) *Yellow lines.* (*a*) Full line (red tip); medial line (red dot). (*b*) Weak line with red tip; basal line. (*c*) Wedge-shaped line in brood E10; full line (mostly red).

(B) *Hindwing bars* (see figure 7 for full range). (*a*) Broad sharp (bs); thin fuzzy (tf). (*b*) Broad fuzzy (bf); eaten (ea). (*c*) Broad with black veins (bv); broad sharp combined with full Panamá bar ('superbar'). (*d*) Both eaten (ea), underside, Mexican cross. (*e*) Broken (br); no bar (nb). (*j*) No bar (nb); broad with black veins (bv).

(C) *Tips of hindwing bars* (underside). (*d*) Backward turn and cream rectangles (also *left* part of *l*); forward turn (Panamá type) and no rectangles.

(D) *Forewing bands.* (*b*) Concave; intermediate. (*c*) No tooth; tooth. (*e*) Long and convex; shortened and concave (pure Upper Amazon phenotype). (*f*) Split on underside (upperside short but not split); split on both surfaces. The 'entire' phenotype (not split on either surface) is shown by *e* and *g* (also *a–d* and *l*). (*g*) Round tip; flat tip: also *f* shows flat tip; round tip in company with split. (*h*) Broken; invaded. (*i*) Yellow, split both surfaces; red, shortened on under surface but not on upper (compare with right half of *e*). (*j*) Split and shortened; entire and shortened, shortened being exhibited on the upper surface by the white but not by the red marks.

(E) *Colour, radiate.* (*e*) Red; orange. (*h*) Radiate; plain.

(F) *Exceptional phenotypes.* (*k*) Synthetic 'mimic' of *Heliconius telesiphe* (for which see Vane-Wright *et al.* (1975)) from brood B; red tipped hindwing bar (p. 547). (*l*) Extra red rays on forewing tips (underside) and pale eye mutant (after death, see p. 553); extra black and red forewing 'rays' from brood 5A.

(G) *Other phenotypes* (scattered in the plate). Red forewing band (*a*, *b*, *c*, *d*, *f*, *g* etc.) versus white (*j*, right), versus yellow (*e*, *i*, left, *l* etc.). Costal spot (*f*, right, underside).

(The dark red spot placed distally on the underside of the left half of *i*, is a spot of red paint used for identification in the greenhouse.)

Photographs by University of Leeds Audio-visual Service.
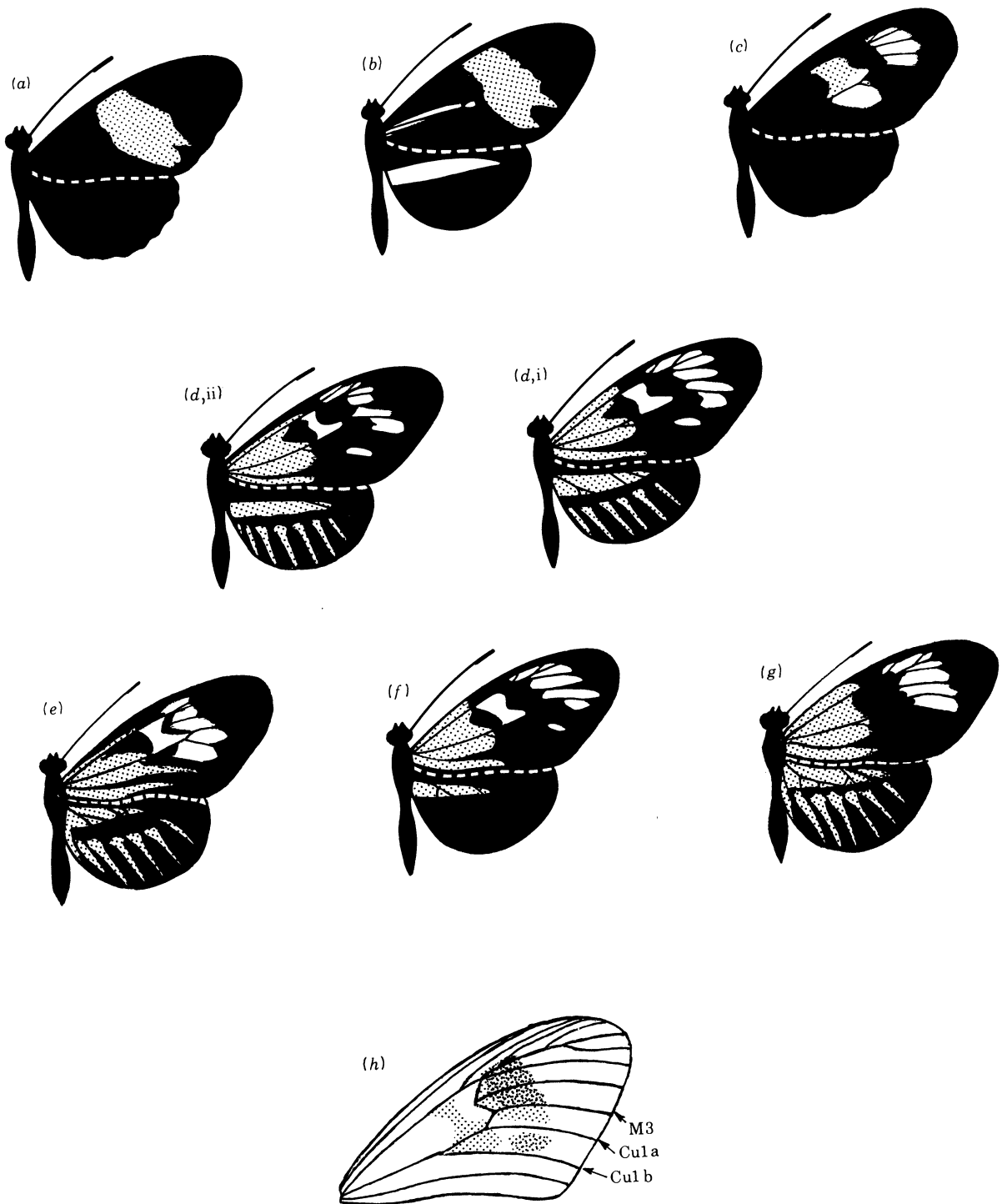
FIGURE 4. (a–g) Races of *Heliconius melpomene* hybridized in this and previous work (Turner 1972) (see also plate 1): (a) Venezuela/Trinidad, (b) East Brasil, (c) East Ecuador, (d) Belém and Rio Madeira, (e) Bolívia, (f) Suriname (Guiana), (g) Upper Amazon. (h) Veination, with parts of the forewing band: cell spot and Ecuador triangle (proximal, lightly stippled), long band (distal, lightly stippled), short band (heavy stippling, overlaid), and Belém spot (heavy stippling).

that of the East Brasilian race, mixed with the orange (or red) marks at the base of the hindwing (plate 2*k*). All these deviant forms appeared in the mass-reared Belém stock in our greenhouses; all can be found in museum specimens caught at Belém and at Chapeu Virado (state of Pará) as long ago as the early 1890s, except for the red colour, which cannot be detected because the pigment is unstable and fades to orange over a few years. In addition, one of us captured at Belém a specimen with a thin red band to the outside of the yellow marks; as far as we can ascertain this phenotype is not represented in museum specimens from the Belém area, though it can be found farther east near Bragança.

Populations of a similar phenotype, differing in minor quantitative characters (figure 4*d*, right insect) and recognized by some as a separate subspecies (*H. melpomene madeira*), occur on the south Amazonian tributary, the Rio Madeira. Our stock came from Riozinho (at km 587, west of Pimenta Bueno on the Porto Velho–Vilhena road) in the state of Rondônia (formerly Guaporé).

The Bolivian race of *melpomene* (*H. melpomene penelope*) (figure 1, no. 9; figure 4*e*; plate 1*i*) is distinguished from the Belém and Rio Madeira populations chiefly by having the yellow band on the forewing fused into a more or less solid patch, instead of broken into a series of dots; the radiate pattern is reduced and deep red. Although our stock came from Areia Branca (km 575 of the Cuiabá–Vilhena highway) in the Brasilian state of Mato Grosso do Norte, we shall refer to this as the Bolivian race.

Another Amazonian race (*H. melpomene aglaope*), occupying the upper part of the Amazon Basin, is similar to the Belém, Madeira and Bolivian populations, with which it intergrades extensively, but has the yellow band of the forewing very much reduced, lacking the posterior spot (the *Belém spot*) and the large yellow spot within the cell (the *cell spot*) (figure 1, no. 6; plate 2*j*, right half; figure 4*g*). We have not hybridized this Upper Amazonian race directly, but one of our East Ecuadorian butterflies, and an East Ecuadorian butterfly to be described by Turner *et al.* (1985) were carrying genes from this race introduced by natural hybridization.

The unit characters used in scoring the broods can be most readily described by referring to what is already known of their genetics from previous work with the Suriname (Guiana) race (*H. melpomene meriana*), which occupies the little-explored area from the interior of the Guianas south to the Rio Amazonas and which differs from the Belém race only in lacking the red rays on the hindwing (figure 1, no. 4; figure 4*f*) and in being red instead of orange (Turner 1972). The characters are (see plate 2):

Radiate versus plain. The red or orange marks of the Belém race, versus their absence as in Trinidad and East Brasil (plate 2, compare *a*, *b* with *c*, *d*; plate 3*c*, *h*, *i*). The stock of the Suriname race obtained from Brokopono in a previous experiment (Turner 1972) was polymorphic for the radiate pattern and for the reduced form of it, lacking the rays on the hindwing, described by the code word 'dennis', which is typical of the Suriname race (figure 4*f*). The radiate pattern was found to be produced by a single not recessive allele D^R; the reduced 'dennis' form without the rays by an allele D, recessive to D^R but dominant to *d*, which produced the plain phenotype in which all these red marks were absent.

Red versus orange (plate 2, compare *g* with *f*). The brilliant red of the East Brasilian and Trinidad races versus the orange colour typical of the Belém or Madeira race. In some of our broods reddish orange individuals appear that are difficult to assign to one category or the other; scoring this difference can be as hard as scoring *Drosophila* eye colour mutants. The red pigment is unstable and fades to orange in museum specimens within the course of 2 or 3 years. Most

individuals in this study have been scored within 2 years of emergence, and fortunately keeping a butterfly in a paper envelope inside an airtight box appears to slow down the fading considerably: material from the Suriname crosses of 1964–5 preserved in this way still had a strong red colour in the 1970s, and there was little difficulty in scoring most of the Belém × Trinidad cross, the only one in the present study scored after more than 2 years.

Colours of forewing bands. The difference between the broken yellow band of the Suriname race (identical in phenotype with that of the Belém race) and the wide red band of the Trinidad race is produced by two major loci and an undetermined number of other minor factors (Turner 1972); interaction of these loci produces four phenotypes not found in the pure races (plate 2 *a–g*). The interaction is most readily understood by reference to the chequerboard diagram in figure 5. The dominant gene B produces the wide red Trinidad band (W, bottom right); its recessive allele b from the Suriname race removes almost all the red pigment from this part of the wing, leaving a black forewing tip (O, bottom left). The N locus influences the amount of yellow. The genotype $N^B N^B$ from Trinidad (bottom row) has no yellow marks, even when all the red is removed by making the individual homozygous bb (bottom left). Addition of one N^N allele from Suriname, giving the genotype $N^N N^B bb$, adds a faint yellow band, in which the yellow scales are mixed with black; this weakened effect of the yellow tends very much to occur in the dumb-bell shaped spot within the cell (the innermost mark of the band) which in this rather variable genotype tends to be weak even when the rest of the yellow is strong (S, middle left). Addition of a further N^N allele completes the conversion to the Suriname genotype, $N^N N^N bb$, and phenotype (Y, top left). If red marks are present, as in the genotypes BB and Bb, the N locus influences the amount of red as well, the substitution of one N^N allele in the Trinidad genotype reducing the red band from wide to thin (TS, centre) and the substitution of a second to give the genotype $N^N N^N B-$ making the red band even thinner (TY, top). The interaction of the two loci thus produces the phenotypes of the two pure races (top left and bottom right), and the other four patterns, as shown; for convenience in tables the six phenotypes have been given the code letters shown in figure 5.

Shapes of forewing bands. This pattern of inheritance, with the red band pushing away the yellow band from the outside, but not itself being influenced by the loci that affect the shape of the yellow band, will be found again in the present crosses between Belém and Trinidad. In other crosses the inheritance becomes more complicated, the red and yellow bands may both respond to the same factors influencing their shape, and it becomes necessary to distinguish further the various parts of the forewing band (figure 4*h*). First, the band may be *long* and *entire*, that is, extending to the posterior of vein M3 (see figure 4*h* for veination), and almost touching the posterior corner of the cell, or it may be *short* (stopping at vein M3) and *split* (not approaching the cell by less than 2 mm). These characters are highly correlated, and short and split come from the East Ecuador race only (plate 2*i*; figure 4*c*). If the band is long, it may stop around vein Cu1a, as in the Upper Amazon race (plate 2*j*; right half; figure 4*g*), or it may be extended, in the form of a detached spot, into the next intervenular space; as this spot is characteristic of the Belém race, we have called it the *Belém spot* (figure 4*h*; plate 2*k–l*). To the interior, the band may be augmented by one or both of two marks, the *cell spot*, a dumb-bell mark lying within the cell of the forewing (found in Suriname, Belém and East Ecuador; absent in the Upper Amazon and the red-banded races) (plate 2*j*, left half), and a coloured triangle lying in the angle of the cubital vein and its branch Cu1b, which we have called the *Ecuador triangle* (plate 3*f*; figure 4*h*).

The Belém race has the yellow band *broken* up into a group of spots; most notably, the Belém spot and cell spot are separated from the rest of the band (plate 2c). In some segregant butterflies these are all *fused* together into an almost solid yellow patch, traversed by the black wing veins (as in plate 2d). In the Suriname × Trinidad cross, the fused phenotype was produced by a single recessive gene designated f. The Suriname race was homozygous FF, and Trinidad homozygous ff; as the locus had no effect on red markings, its effects were dificult to detect in Trinidad phenotypes (wide red band), but most individuals could be scored because ff produces a smooth spread of white scales on the underside of the band, whereas in $F-$ genotypes these are confined to a few patches. In other crosses, the red band also may become broken into dots (plate 2l).

Tooth on red forewing band. The two obvious ways in which the band of the East Brasilian race differs from Trinidad, are the concavity of the anterior proximal edge of the band (which we have not studied), and the *tooth* on the posterior distal edge. The tooth appears also in segregants with a narrow red band (plates 1c, 3i right).

Shadow of yellow band. In some individuals apparently of the genotype $N^N N^B$ the yellow marks in the band are only weakly developed; the extreme form of this occurs in the O phenotype (black forewing tip, $N^B N^B bb$) (plate 2g). It is still possible to score most such individuals for fused or broken yellow, because on the underside of the forewing the area that would be occupied by the yellow scales, although of the same black–brown colour as the rest of the wing, is shinier, so that the shape of the band can still be seen by looking at the wing in strong light at the correct angle.

White in the forewing band. Large parts of the forewing band of the East Ecuadorian race are white (plate 2i); in a few individuals the red colour almost vanishes, leaving a black and white pattern. The white colour can be discerned, sometimes only as a thin strip between the red and yellow colour of a TY band in many of the hybrids derived from this race (plate 3d, left).

Yellow line or yellow spot. The yellow line along the forewing of the East Brasilian race (plates 1g, 2h). A similar mark is also present in the Belém and Suriname races, but is mostly obliterated by the red marks on the forewing, appearing only as a small yellow spot at the very base of the wing (plate 2j, right half) (Emsley 1965b). In Suriname × Trinidad F_2 butterflies, a yellow line appeared in plain butterflies, where the red scales did not obliterate it. It was weakly developed and variable and, although it did not segregate clearly, was controlled in part by the N locus.

Yellow bar. The yellow bar across the hindwing occurs in several forms, which may or may not be produced by the same genes. First there is the strong yellow bar on both the upper and undersides of the hindwing of the East Brasil race (plate 2h). Secondly, there is a *shadow* of this pattern, on the underside only, detectable as a slight change in the shininess of the black–brown scales in the region where the bar would appear. In the cross Trinidad × West Ecuador, this phenotype was the heterozygote for a yellow bar gene that in West Ecuador and West Colombia, as in no other races, is expressed only on the underside even when homozygous (Emsley 1965b). Thirdly, in some individuals with the shadow, the yellow bar appears on the upperside as a thin sprinkling of yellow scales. Fourthly, some individuals of the Belém stock have a yellow bar in this same position (plate 2k). This last yellow bar may be strong and solid (no sprinkling of black scales), but is always more or less mixed with the red bar of the radiate pattern, which occupies the same position (see, for example, plate 3h, left); the mixing may involve separate areas of red and yellow scales, or in a few individuals an apparent mixture

of the two pigments within the scales, producing salmon pink. Among the Belém butterflies this yellow bar is much more strongly expressed in males than females, in whom it is frequently reduced to a trace of yellow at the distal end of the bar. Fifthly, in the Suriname × Trinidad F_2, some broods contained butterflies with faint dustings of yellow on the underside in the proximal and distal parts of the yellow bar, but with no 'shadow' of it; these were produced in part by the allele N^N. Finally, a similar yellow bar, dusted both on the upper and undersides but not producing a shadow, appeared in some of our F_2 butterflies from the cross East Ecuador × Belém.

Width of rays. In the Belém race the rays on the hindwing are narrow, with something of a 'nail-head' at their proximal end. In some segregants from the cross with Trinidad, and even more so from the cross with East Brasil, the rays become very broad for their full length, and may touch one another at their upper ends. On the underside this effect is very marked, and is accompanied by a thickening of the normally insignificant red bar across the base of the hind-wing; the total effect is not so much of a black wing with red rays as of a large red oval patch filling most of the wing, but invaded by black marks (plate 3k). A less extreme form of this widening was noted in some Suriname × Trinidad hybrids, but its genetics was not determined (Turner 1972).

3.2. *The cross Belém × Trinidad*

The Belém and Trinidad races differ in the shape and colour of their forewing bands and in the presence of radiate marks (figure 4a, d; plate 1a, b). The Trinidad race is always red; the Belém stock is variably red or orange. We obtained the F_1 (plate 2a), both backcrosses, the F_2 and a small number of further test crosses; as the backcross to Trinidad was small and the pure Trinidad stock weak and rapidly extinguished, one of these additional test crosses was of the F_1 to a Trinidad-like phenotype reconstituted from the F_2. The broods are fully tabulated in table A1 (appendix 4); a few are multiparental. The results show that inheritance of the differences is substantially the same as that in the Suriname × Trinidad cross (above), with the addition of the red/orange difference. Some further crosses will be discussed in §3.5.

(a) *Inheritance of radiate versus plain*

This character is inherited in exactly the same way as in the Suriname cross: the single dominant gene D^R produces the red or orange radiate marks of the Belém race, so that the F_1 and Belém backcrosses are radiate, the backcross to Trinidad (TBFT and B1TN) and the cross to Trinidad-like stock (YM43) segregate 1:1 (35 radiate:22 plain, $P = 0.11$) and the F_2 in a 3:1 ratio (149 radiate:53 plain; expected $151\frac{1}{2}:50\frac{1}{2}$) (plate 2). In the crosses with Suriname, the full dominance of the D^R allele was not confirmed, as the $D^R D^R$ homozygote was not formed for certain in those broods (Turner 1972); it is obvious from the uniformity of the large Belém × Trinidad F_1 (broods YM2 and YM6) that the Belém parent was homozygous D^R, and that this allele is completely dominant to its partner (d) which produces the plain phenotype of the Trinidad race. The allele D, which produces the reduced pattern of the Suriname race (figure 4b), is recessive to D^R and dominant to d; it is not found at Belém and did not segregate in the present broods.

(b) *Inheritance of red and yellow bands*

The two-locus hypothesis explaining the difference between the broken yellow band of the Suriname race and the wide red band of Trinidad is illustrated in figure 5 (plate 2 for colour),

and was explored rather thoroughly by Turner (1972). We started the present experiments with the hypothesis that the broken yellow band from Belém, being identical with the one from Suriname, would be inherited in the same way. The confirmation of this is most easily visualized by noting that the segregation of any kind of cross can be quickly predicted from figure 5: crossing butterflies from opposite corners will give the one in the centre, crossing the centre with a corner will give the four butterflies at and nearest the corner (ratio 1:1:1:1), crossing the centre butterflies in opposite edges will give the whole centre row (ratio 1:2:1) and so on. Of the possible crosses, we have performed the following, all in accord with the hypothesis (all in table A1, appendix 4):
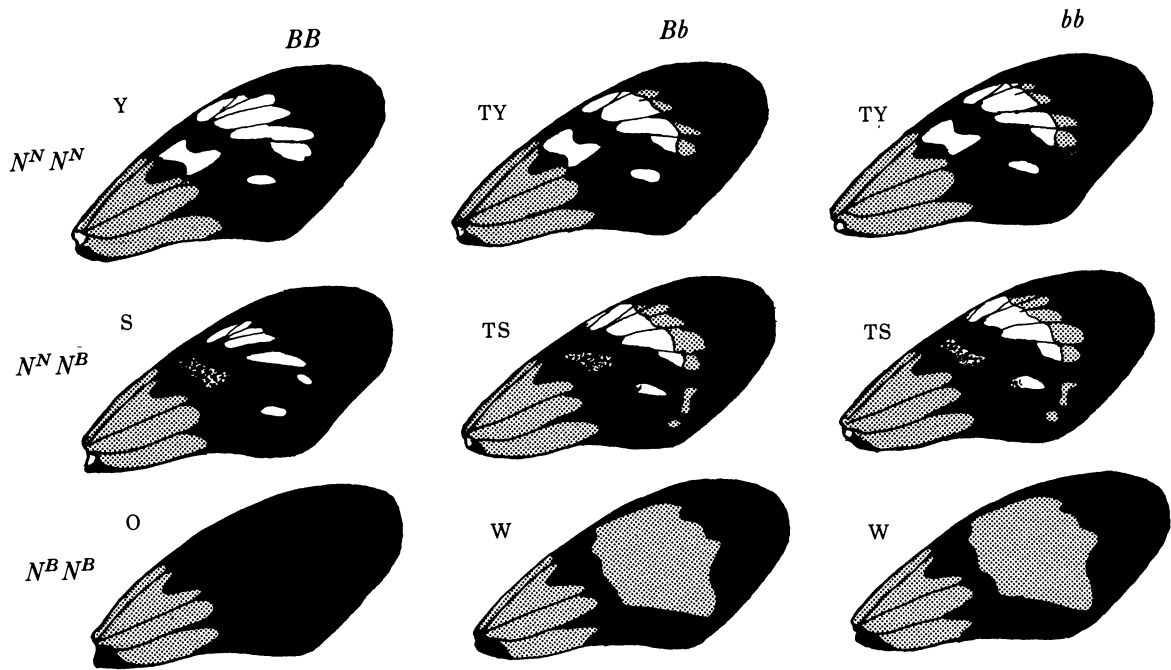
FIGURE 5. Interaction of the B and N loci in converting the forewing band of the Suriname race of *Heliconius melpomene* (top left) to that of the Trinidad race (bottom right). This method of genetic control is found also in the Belém × Trinidad cross. Genes are in italics; roman letters are codes for the phenotypes. From Turner (1972).

Y × W (corner), giving entirely TS – the F_1 (plate 2a, broods YM2 and YM6, total 91);

TS (centre) × TS (centre), giving the complete square, with Y, TY, S, TS, O and W in the ratio 1:3:2:6:1:3 – F_2 (broods YM15, YM21), numbers 12:32:23:77:15:43 compared with expected numbers (rounded off) 13:38:25:76:13:38 ($\chi_5^2 = 1.74$, $P = 0.88$);

TS (centre) × Y, giving Y, TY, S and TS in the ratio 1:1:1:1 – the backcrosses to Belém (broods YM27, YM34, YM39, YM36), giving total numbers 23:33:27:19 ($\chi_3^2 = 3.53$, $P = 0.68$);

TS (centre) × W (corner), giving TS and W in the ratio 1:1 – the backcross to Trinidad and to the Trinidad-like stock (broods YM43, BTFG, TBFT), with totals 26:31 ($P = 0.60$);

S × S, giving Y, S and O in the ratio 1:2:1 – brood YM51 in numbers 14:25:8 ($\chi_2^2 = 1.20$, $P = 0.55$); there was a slight difficulty in distinguishing between the S and O phenotypes;

S × TY (top centre), giving Y, TY, S and TS equally – the small brood YM52, giving 1Y, 3TY and 1TS, which is not a significant departure;

O × TS, giving TS and W equally if the TS parent is homozygous BB – brood YM65, segregating 17 TS:23 W ($P = 0.43$);

Y × Y, giving entirely Y – the pure Belém stock, in particular brood YM10 (39 butterflies) and untabulated broods (YM3, YM8, YM40) totalling 134 butterflies;

W × W (both corner), giving entirely W – brood YM44 (34 butterflies);

O × O, giving entirely O – brood YM64, in which most butterflies were obviously of the O phenotype (black forewing tip), but two or three had traces of yellow marks almost as strong as in some undoubted bbN^NN^B individuals (S phenotype), indicating that factors other than N can influence the amount of yellow in this part of the wing (see also S × S above).

(c) Enhancement of yellow

The action of these other factors is further seen in broods YM49 and YM57 (table A1, appendix 4). A mating between two O butterflies (both from the F_2, presumed genotypes bbN^BB^B) produced a brood of two individuals: a plain TY butterfly (apparent genotype BbN^NN^N), which is presumed to be a contaminant, and a radiate S female (apparent genotype bbN^NN^B). Whether this second butterfly is also a contaminant cannot be established but its genotype is quite clear from its offspring. It was mated to a TY (BbN^NN^N) male from the Belém backcross. From the apparent genotypes this mating should have produced equal numbers of Y, TY, S and TS butterflies; in fact it produced 29 S and 24 TS individuals, which is to say that the offspring segregated 1:1 for the B locus, but were uniformly N^NN^B heterozygotes. Only a cross between the top centre and bottom left corner of figure 5 will give this segregation (or a cross between the top left, Y, and the bottom centre, W, which is completely ruled out by the phenotypes of both parents). That is, the father was BbN^NN^N as he appeared to be, but the mother, although having a phenotype that would normally be regarded as indicating that she was bbN^NN^B, was in fact an N^BN^B homozygote. As this particular set of crosses could not be continued, and as the parentage of the mother is uncertain, we cannot tell whether the factors that enhance the amount of yellow so as to convert a black-tipped butterfly to an apparent N^NN^B heterozygote are environmental or genetic, nor how many genetic factors may be involved.

In the backcross to the Trinidad-like stock (brood YM43), which is known to involve equal segregation of N^NN^B and N^BN^B butterflies (all of them B–), around three of the N heterozygotes, although having red bands of the width normal for this genotype, had the yellow strong and firm as in N^NN^N homozygotes (plate 3 h). There is no clear segregation of this strong yellow phenotype, and it would therefore appear to be produced by several factors. These factors, in so far as they are genetic, can be presumed to derive from the Belém race, via the F_2 individuals that were mated to produce the Trinidad-like stock; the factors are clearly lacking in the Trinidad race, first because it has no yellow marks, and secondly because such enhanced yellow phenotypes have not appeared in any of the backcrosses, either of Belém, Suriname or East Brasil to pure Trinidad stock. We shall discuss these factors again, after we have dealt with the inheritance of the yellow hindwing bar (§3.5b).

(d) Additional test crosses

We were not able to make all the possible crosses that test the two locus hypothesis of figure 5; but as the yellow forewing bands of the Suriname and Belém races appear to be genetically identical, some of the test crosses reported with the Suriname × Trinidad cross can be used as additional confirmation. These were (Turner 1972):

Y × W (bottom centre), giving S and TS equally (numbers 4:5);

Y × S, giving Y and S equally (numbers 8:8);

S × W (corner), giving TS and W equally (numbers 6:13, $P = 0.17$);

S × TS (centre right), giving TY, TS and W in the ratio 1:2:1 (numbers 4:12:5; $\chi_2^2 = 0.21$, $P = 0.90$).

A further group of broods (Sheppard 1963), homozygous BB (therefore entirely in the column on the right of figure 5) segregated:

TS × W, giving TS and W equally (numbers from 17 matings, 388:354, $\chi_1^2 = 1.47$, $P = 0.23$);

TY × TS, giving TY and TS equally (numbers 24:26);

TS × TS, giving TY, TS and W in the ratio 1:2:1 (numbers 13:15:11, $\chi_2^2 = 1.65$, $P = 0.44$).

In the Suriname × Trinidad F_2 (Turner 1972) there was a statistically significant deficiency of the O phenotype. This is probably explained by the action of the yellow-enhancing factors described above, so that bbN^BN^B genotypes were scored as S. A much less likely explanation is the existence of more than one 'b' locus in the same chromosome.

Therefore our broods are fully consistent with the system of control of the forewing band (figure 5) which has already been shown in the Suriname × Trinidad cross (Turner 1972): the B locus adds and subtracts the red band, and the N locus adds and subtracts the yellow band, having also some effect on the extent of the red. Dominance is complete at B; N has an intermediate heterozygote. The Belém race is homozygous N^NN^Nbb; Trinidad is homozygous N^BN^BBB. The strength of yellow colouring can however be altered by other factors, to the point of making phenotype classification unreliable in a few broods.

(e) Inheritance of fused yellow

The F locus known from the Suriname × Trinidad cross segregates as expected, the recessive f allele from Trinidad causing one quarter of the F_2 brood to have a fused yellow forewing band (plate 2d; 153 broken bands:42 fused, $P = 0.30$). The Belém backcross is entirely broken banded, and the expected one half of the backcrosses to Trinidad and the Trinidad-like stock are fused (numbers, 24 broken:30 fused). The character can be scored on all those phenotypes with yellow marks in the band, and on the black forewing tip (O phenotype) as well, as a result of the slight traces of yellow or the occurrence of the potential yellow marks as a shadow pattern. The cross between two O phenotypes (table A1 (appendix 4), brood YM64), both of them apparently broken, can with some difficulty be scored as containing 12 broken and five fused phenotypes, which is an excellent fit to the expected 3:1 ratio ($P = 0.85$). Brood YM65 is clearly a backcross (22 broken:18 fused; one parent of each phenotype).

Except in a very few individuals, the F locus has no effect on red marks; two butterflies only in the very large F_2, plus one more probable F_2 individual that has strayed into brood YM44, show signs of the red marks forming into a broken band, and these effects are slight indeed. However, in butterflies with wide red bands, the locus influences the distribution of the white

scales which cover the band on the underside. These are smoothly spread all over the underside of the band in ff butterflies, but split into patches roughly corresponding to the shape of a broken band in $F-$ insects. It was reported that this characteristic was difficult to score on W (wide red band) butterflies in the Suriname cross (Turner 1972). This appears not to be the case here: a 2×2 classification of the present F_2 shows fused bands segregating in the same ratio (9:32) in the W butterflies as they do in the rest of the F_2 (32:121) (seven butterflies not scorable; $P \approx 1.00$, two-tailed). In the combined Trinidad backcross plus the backcross to the Trinidad phenotype (YM43), the same ratios are 17:9 and 13:13, which is not a significant association ($P = 0.40$; seven butterflies not scored or suspected of being contaminants). There is a significant association *within* YM43 ($P = 0.016$), but, in view of the overall result, this would appear to be a matter of chance.

For the probable function of the white scales, see p. 500.

(f) Inheritance of basal line or spot

The yellow spot (plate $2j$, right half) that appears at the base of the forewing in Belém butterflies was shown in the similar Suriname race to be produced mainly by the N^N allele (Turner 1972). Although we have not studied this mark exhaustively in the present cross, this seems also to be true here. The character is most easily scored in plain butterflies, where it is expressed as a short yellow line. In the F_2, the $N^N N^B$ and $N^B N^B$ insects (that is TS and W phenotypes) lack this yellow line, which is present in all but one of the $N^N N^N$ (TY) phenotypes. The same relation holds in Belém backcross YM34, in which all the butterflies in the S and TS ($N^N N^B$) classes lack the basal spot, and all but three TY butterflies in the Y and TY ($N^N N^B$) classes have it.

Thus the basal spot is largely produced by the N^N allele when homozygous; as band expression is affected by other factors (§ (c) above), it is an open question whether apparent $N^N N^N$ butterflies without the spot are showing lack of expression, or are misclassified $N^N N^B$ insects.

(g) Variation in intensity of yellow

As in the East Brasilian cross (§3.4 below) the amount of yellow in the F_1 is somewhat variable; the variation appears to be continuous, and we have not studied it.

However, one very clear effect, noted also in certain Suriname broods, is that the dusty yellow bands of $N^N N^B$ heterozygotes may be much stronger in females than in males. This is seen in both the S and TS classes in brood YM57, and was noted in some of the Suriname × Trinidad broods (Turner 1972; the effect was then reported only for S phenotypes, but a check reveals that it is just as clear in TS phenotypes). There is no sexual dimorphism in the yellow band of the pure Belém stock, nor in the F_1 nor in most other broods. For further examples of sex influencing expression see §§ 3.2 i, 3.5 a, 3.4 h, i and 4.10 g.

(h) The cell spot

The presence (Belém) or absence (Trinidad) of the big dumb-bell shaped yellow spot in the main forewing cell (plate $2j$, compare left and right halves) is not inherited in this cross as it is in crosses involving East Brasil and the Upper Amazon; there we shall show that a single major gene is involved. In the present broods the spot is variable, generally strong in $N^N N^N$ butterflies, weak in $N^N N^B$ and absent in $N^B N^B$. The salient pattern of inheritance is of con-

tinuous variation, with the major effect in suppressing the spot coming from the Trinidadian forewing band allele N^B. However, several other factors clearly influence this mark. N^B, even in company with $B-$ (wide red band), cannot necessarily suppress the spot entirely. Most of the wide red banded butterflies in YM43 have a smudgy yellow mark representing the posterior portion of the spot (it may help to think of the complete cell spot as two triangles joined apex to apex, in which case these insects have the lower triangle only); all the wide banded butterflies in YM65 have this mark. It is likely that the mark is produced by a single recessive gene, as in the F_2 (YM21), 12 of the 42 wide banded butterflies have this smudgy half spot ($P = 0.70$ for $1:3$; four more have the spot represented by a shadow only).

Conversely, homozygosity for N^N (even with bb) will not necessarily produce a full cell spot. Only one insect (with a TY band) in the whole F_2 of 202 individuals, which includes 44 $N^N N^N$, has a full spot; in the others there is only the posterior half. In some circumstances a single gene appears able to add the anterior half of the spot. The yellow (Y) banded butterflies in brood YM51 segregate 6 whole cell spots:8 posterior halves, and, as both parents have half cell spots (the character is harder, but not impossible to score on these S butterflies), it seems that the gene restoring the anterior half is recessive (for $1:3$, $P = 0.22$). This gene appears also to influence the most posterior mark on the forewing band (the Belém spot). If the Y butterflies are classified both ways, we obtain

Belém spot:	present	minute	absent
cell spot: whole	0	1	5
half	5	1	2

which condensed into a 2×2 table gives either $P = 0.033$ or $P = 0.056$.

There are therefore at least three genes responsible for suppressing the cell spot in the Trinidad race: (i) N^B; (ii) an apparent dominant allele which suppresses the anterior half (and which affects the Belém spot), and which may perhaps be a modifier of (iii) a dominant allele which suppresses either the whole spot or its posterior half. It would appear that, if all the suppressors are removed, then cell spots can appear even in $N^B N^B$ butterflies (wide bands), for such phenotypes are known in the Guiana hybrid zone between the Belém, Suriname and Trinidad/Venezuela races (illustrated in Turner 1971 b).

The two Trinidadian alleles will be provisionally designated Ac (anterior cell spot suppressor) and Tc (Trinidad cell spot repressor).

(*i*) *Inheritance of red versus orange*

Although many of the specimens from these crosses were finally scored for red or orange colour (plate 2) when 8 years old, the tight envelopes in which they were kept have prevented fading (which results from exposure to air rather than light) in most of them, and orange can be seen to behave chiefly as a single recessive gene. Most of the Belém butterflies used as parents were orange, although red does occur as a variety in that stock. The F_1 is entirely red, the F_2 segregates 141:48 red:orange (13 not scorable, most as a result of faulty eclosion), an almost perfect fit to $3:1$, and the backcross to Belém is a perfect fit to $1:1$ (47:47 in YM27, YM34 and YM39). Apart from two possible contaminants, the small backcrosses to Trinidad are entirely red. We designate the allele for orange or and the dominant red allele Or.

The inheritance of orange may be more complicated than this, but it is not possible to be certain how many of the anomalies are simply due to fading (which may occur during life if a

butterfly is kept for some months for breeding), or perhaps to genetically determined variations in the speed of fading. In brood YM65, which may be a red × orange backcross, there appears to be a considerable deficiency of red butterflies (10:30, $P = 0.0022$) especially in the TS class, and in the W (wide banded) class the orange colour is strongly associated with a change of the customary white scaling on the underside of the band to yellow (plate 3l) (all but one of the eight red butterflies having white scales, and all the 15 of the orange having yellow, $P = 6.5 \times 10^{-5}$). The colour is also strongly associated with sex; over the whole brood there were ten red males, no red females, five orange males, 25 red females ($P = 7.1 \times 10^{-6}$).

This association is present also in the F_2; although it is not obvious by inspection it is highly significant (77 red males, 64 females, 14 orange males, 34 females; $P = 0.0036$, two-tailed). In the backcross to Belém the association is absent when the F_1 parent is the father (YM34: numbers in same order, 9, 11, 5, 6; $P = 0.63$, one-tailed), but present, although not quite significant in the reciprocal cross with an F_1 mother (YM27 and YM39: numbers 29, 18, 24, 23; $P = 0.071$, one-tailed). There is thus a strong suggestion of a sex-linked or sex-limited gene, in addition to Or, controlling orange (see also §3.4h). The odd segregation in brood YM44 (6 orange:22 red, in a cross between a pale red–orange and a red butterfly) may be, in part at least, the result of fading.

(j) Linkage groups

In the studies of the Suriname × Trinidad cross already referred to, only B and D were found to be linked; the present broods confirm this. None of the loci (except for the problematical additional orange gene) gives any indication of being sex-linked.

If Turner & Sheppard (1975) are correct in believing that there is no recombination in female *H. melpomene* (originally suggested by cytological evidence (Suomalainen *et al.* 1973)), the linkage groups can easily be detected. This means that for genes carried on the same chromosome, no matter how far apart, there will be no recombinant phenotypes in backcrosses with a heterozygous female parent, nor in F_2 broods in which the alleles are introduced in repulsion. The presence of recombinant phenotypes in any of these broods, provided that they are too numerous to be contaminants, shows that the loci are unlinked, regardless of the results of any statistical tests for association. In this way, independent assortment is a hypothesis subject to direct testing, and the technique is more powerful than the use of contingency tests, in which independent assortment is assumed from failure to reject it as a null hypothesis. All possible pairs of loci are accessible, those in repulsion through the F_2, those in coupling by the appropriate backcross.

The loci B and N are shown to be unlinked by the Belém backcrosses with an F_1 female parent (table A1 (appendix 4): broods YM27, YM39, YM46, YM47, YM48); the backcross with a male F_1 parent (YM34) shows the same independent segregation. Similarly B and N are shown to be unlinked to Or. In the F_2, the doubly recombinant genotype bbN^BN^B (that is the black forewing tip or O phenotype) could not appear if the loci were linked as one of the bN^B arrangements has to come from the mother. The appearance of this phenotype in some numbers confirms the independence of B and N.

Similarly, the doubly recombinant genotypes ddN^NN^N, ffN^NN^N, and N^BN^B $oror$ in the F_2 (that is to say, plain TY, Y or TY with fused yellow, and O or W with orange marks), show that N is not linked to D, F or Or (numbers are 10, 10 and 7).

The backcross to Trinidad is too small to provide information on linkage. The backcross to

the Trinidad-like phenotype (table A 1 (appendix 4), brood YM43), in which the F_1 parent was a female, shows no linkage between N, D, Or or F, the only loci segregating.

The F_2 produces also the recombinant homozygous genotypes *bbff*, *ffor*or and *ddoror* (numbers 9, 10, and 8); as the genes enter the F_2 in repulsion, so that independent assortment in the female parent is necessary for the production of these genotypes, it follows that there is no linkage of B with F, or F with Or, or D with Or.

The recombinant genotype *bbdd*, that is to say a plain butterfly with a Y, S or O band, is entirely missing from the present F_2, and from the similar Suriname × Trinidad F_2 reported by Turner (1972). In the present F_2 the numbers are

	bb	*B–*
D^R–	50	99
dd	0	53

which can hardly result from a chance failure in the appearance of the recombinant (from the formula given by Bailey (1961) without Yates' correction, $\chi_1^2 = 24$, $P = 4 \times 10^{-7}$, one-tailed). The loci B and D are therefore in the same linkage group, which gives us strong grounds for believing that they are identical with the similar pair in the Suriname cross.

This last fact establishes the independence of the remaining two pairs of loci, B and Or and D and F, which are in coupling, and which cannot be tested in this way, for it follows that if Or is not linked to D it cannot be linked to B and that if F is independent of B it must also be independent of D. This confirms the finding of the backcrosses.

As orange colour can be produced by fading, and may be under the influence of additional loci, the strong test for independence used above may be misleading. We have therefore performed a series of contingency tests in the F_2, the Belém backcrosses and the backcross to the Trinidad phenotype (YM43). With use of one-tailed probabilities, no test if recombinants exceed parentals, the associations are as follows.

D^R: no association in YM43 ($P = 0.43$); a just significant association in the F_2 ($P = 0.044$).

F: no association in the F_2 (no test); a significant association in YM43 ($P = 0.0023$).

B: no association in the F_2 or Belém backcross (no test in either).

N: no association in the Belém backcross ($P = 0.34$) but an almost significant association in YM43 ($P = 0.057$) and a very strong one in the F_2 ($\chi_2^2 = 18.3$, uncorrected, $P = 5.4 \times 10^{-5}$); this would be even stronger if the seven 'red' radiate TY butterflies, which are only slightly redder than the orange individuals in their class, are regarded as orange.

There are clearly some oddities about the expression of orange colour, but there is no good case for regarding any of the associations as due to the linkage of Or with any of the other major loci, as they are not consistent between broods. The only possible candidate is the linkage of Or and N (the 'recombinants' from female parents being produced by fading or interference from other loci), but as the association does not turn up in the East Brasilian cross (§ 3.4), nor in the present Belém backcross, even this is unlikely. It seems more probable that the expression of red colour can be affected by a number of loci, including N or something linked to it. We shall say more of this in § 3.4*h*.

Turner (1972) reported an association between D and F which could not result from linkage as it appeared in the offspring of doubly heterozygous females. There is no indication of any such association in the present F_2 ($P = 0.11$) nor in YM43 ($P = 0.30$), and it seems likely that it was a matter of chance.

A further anomaly of the Suriname × Trinidad F_2 was a statistically significant absence of the O phenotype, attributable to the genotype bbN^BN^B. The absence of this class could be produced by the linkage of B and N, but this would entail the linkage of D and N, and hence absence of the genotype ddN^NN^N (plain-TY), of which there were ten. B and N are therefore unlinked in the cross of Trinidad both with Belém and Suriname.

In summary, B and D are in the same linkage group, and N, F and Or are independent. However, the expression of Or is affected by a number of other factors, which can produce apparent associations with the other loci, as well as with sex.

(k) Crossover rate between B and D

A repulsion F_2 with tight linkage provides little information about the recombination fraction; when there is zero recombination in females, as here, there is no information at all: recombinant phenotypes are absent even with 50 % recombination in males. As B and D are found naturally in repulsion (bD^R and bD in Belém and Suriname, Bd in Trinidad), back-crossing to the pure races produces no information either.

The only simple cross that will estimate the crossover rate is of a Belém (or radiate Suriname) × Trinidad F_1 to a Suriname butterfly: a cross of this type, made in 1965 before it was known that females lacked chiasmata, with use of an F_1 female carrying the D^R allele (derived from Suriname), showed as expected no recombination, although the brood is small (Turner 1972).

Using a stock derived from Moengo and Albina in northern Suriname (figure 3) (Sheppard 1963) where a Trinidad-type population contains about 6 % of Suriname and Belém genes, apparently introduced across the ecological barrier separating the races (see p. 589), Turner & Crane (1962) detected a recessive allele, reducing the amount of red in the forewing band, and linked to D. This has to be regarded, in the absence of contrary evidence, as the b allele, although the genotype bbN^BN^B, instead of having the black forewing tip as in the present broods and in the Suriname × Trinidad broods reported by Turner (1972), had a thin red band closely similar to that of the Belém × Trinidad F_1. This probably resulted from other loci enhancing the amount of red in this area. It must be remembered that the genetic background in the Moengo population is around 94 % from the Trinidad/Venezuelan race (Sheppard 1963); in our F_2 crosses in which homozygous bb appeared, such a high percentage of Trinidad genetic background is unlikely to have been found, particularly if many modifiers of the b allele are active.

A selection of Miss Crane's broods is shown in table 1. None is set up to give a direct estimate of the crossover rate, but we can obtain some information, as the combined use of the pedigree and the phenotype allows us to deduce the genotype of most of the parent butterflies. The two broods (nos. 22 and 24) with doubly heterozygous mothers contained no recombinants indicating an absence of crossing over in females. The wild male designated 'Dennis the Menace' is known from the pedigree to have been a repulsion heterozygote, bD/Bd. Among those of his descendants that were progeny-tested, one was carrying the recombinant BD and two others the recombinant bd, definitely not derived from another parental butterfly; in addition at least two of the seven wild Suriname butterflies used as parents were carrying the recombinant bd chromosome. Therefore linkage is not absolute. Because of the population structure of *Heliconius*, in which home ranges are quite limited (Turner 1971c; Ehrlich & Gilbert 1973; Benson 1982) it is quite likely that the two wild bd individuals were sibs collected as eggs from the same plant.

The detection of the three recombinant chromosomes by progeny testing allows an estimate

of the crossover rate. The repulsion heterozygote 'Dennis the Menace' is shown by his offspring in brood 14 to be a *Dd* heterozygote; *bb* homozygotes appeared among his grandchildren from this mating, and as no self-consistent scheme of genotypes allows his mate to be *Bb*, he must also be heterozygous *Bb*. The loci are in repulsion in this butterfly, as if he were *BD/bd* it would follow that *both* of his offspring mated to give brood 17 carry recombinant chromosomes, since both are clearly repulsion heterozygotes. It would also follow that at least two out of the four

TABLE 1. SELECTED BROODS OF *HELICONIUS MELPOMENE* BRED BY
.JOCELYN CRANE IN 1960, REANALYSED FROM TURNER & CRANE (1962)

(Recombinant chromosomes in **bold** type. All the named butterflies, except Zenobia, are wild individuals from Moengo, or from the neighbouring town of Albina, in Suriname. To simplify interpretation, the notation for phenotypes has been slightly changed: D, Dennis; d, plain; B, wide band (W in other tables); b, thin red band. The *N* locus did not segregate; all insects seem to have been $N^B N^B$. F segregated, but has not been scored.)

brood no.	female parent			male parent			offspring			
	origin	pheno-type	genotype	origin	pheno-type	genotype	B–D–	B–dd	bbD–	bbdd
1	Bertha	?	—	Sam	Bd	*Bd/–d*	0	4	0	0
2	brood 1	Bd	*Bd/–d*	Dennis the Menace	BD	*bD/Bd*	2	6	0	0
3	brood 2	BD	**BD**/*Bd*[1]	brood 32	BD	*bD/Bd*[1]	49	18	0	0
4	brood 3	BD	*bD/Bd*	brood 17[4]	BD	*bD/Bd*	5	2	2	0
8	Eve	Bd	*Bd/Bd*	Maharajah	Bd	*Bd/bd*	0	42	0	0
9	brood 8	Bd	*Bd/bd*	brood 8	Bd	*Bd/bd*	0	3	0	1
10	Hedi	Bd	*Bd/bd*	Maharajah	Bd	*Bd/bd*	0	20	0	3
14	Pamela	Bd	*Bd/Bd*	Dennis the Menace	BD	*bD/Bd*	25	33	0	0
17	brood 14	BD	*bD/Bd*	brood 14	BD	*bD/Bd*	4	1	5	0
19	brood 14	Bd	*Bd/Bd*[1]	brood 14	Bd	*Bd/–d*[1]	0	8	0	0
21	brood 14	Bd	*Bd/Bd*[1]	brood 14	Bd	*Bd/–d*[1]	0	71	0	0
22	brood 14	BD	**bD**/*Bd*	brood 8	Bd	*Bd/bd*	16	27	11	0
23	brood 17	bD	*bD/bD*	brood 8	Bd	*Bd/bd*	7	0	4	0
24	brood 17	BD[3]	*bD/Bd*	brood 9	Bd	*Bd/bd*	23	45	26	0
25	brood 17	bD	*bD/***bd**	brood 19	Bd	*Bd/Bd*	2	4	0	0
26	brood 17	bD	*bD/***bd**	brood 9	bd	*bd/bd*	0	0	27	17
27	brood 17	BD	*bD/Bd*	brood 17[4]	BD	*bD/Bd*	5	6	2	0
32	Zenobia	Bd	*Bd/Bd*[2]	Dennis the Menace	BD	*bD/Bd*	4	4	0	0

[1] The genotypes of male and female could equally well be reversed.
[2] Trinidad butterfly, known to be homozygous because from monomorphic population.
[3] Misprinted 'bD' in Turner & Crane (1962); the insect has been checked and is definitely a BD phenotype although the red band is somewhat invaded by black scales.
[4] The same male in both broods.

of his offspring forming the parents of broods 19 and 21 carry a recombinant chromosome (making them *Bd/Bd* homozygotes, instead of *Bd/bd*, where the *Bd* comes from their mother), *and*, what is more, that these two are distributed one per mating, *and* that the female parent of brood 22, also his daughter, carried *bD* as a recombinant chromosome. We can safely reject the idea that 'Dennis the Menace' was a coupling homozygote.

This wild repulsion heterozygote was mated to three females, giving broods 2, 14 and 32. Three insects from brood 14 can be unambiguously typed: the female parent of brood 22 is clearly a repulsion heterozygote, and so are both parents of brood 17 (even if the *B–dd* individual is a contaminant, both parents must be *Bb* from their offspring and *Dd* from their own phenotypes and that of their mother, in which case their mother's genotype prohibits them from having the loci in coupling). This gives no recombinant chromosomes out of three tested.

Brood 3 is a mating between two offspring of 'Dennis the Menace' (one from brood 2, the other from brood 32); both these butterflies carry Bd from their mothers, and the fact that one of their offspring (the parent of brood 4) is heterozygous Bd shows that at least one of them carries the chromosome bD. But if this were a mating between two such repulsion heterozygotes, the homozygote bb would appear in the offspring. This class is completely absent in the brood of 67 individuals. Therefore one of the two parents of bood 3 carries the parental combination bD/Bd, and the other, being homozygous BB, must have the recombinant chromosome BD. This gives one recombinant chromosome out of two tested.

Brood 17 has been shown to be a $bD/Bd \times bD/Bd$ mating. All six butterflies that have been mated, out of ten in the brood, have a fully ascertained genotype (broods 4 and 23–27); those proved by their offspring to be doubly heterozygous must be repulsion types, as they cannot inherit a coupling arrangement from their mother. In these butterflies, two out of the six carry the recombinant chromosome bd.

Brood 17 gives an unbiased estimate of the crossover value in males, as, although four of the brood were not tested, the recombinants occur with equal probability in each phenotype class, so that selection of butterflies for crossing introduces no bias. This gives a crossover rate of 2/6, or 33 %, with a standard error of ± 19 %; the upper 95 % confidence limit includes independent assortment. Inclusion of the five directly tested offspring of 'Dennis the Menace' (from broods 2, 14 and 32) increases the information but introduces a slight upward bias, as some mated individuals from brood 14 (the parents of broods 19 and 21) cannot be shown *not* to carry a recombinant chromosome, but could have been shown to carry bd in the rather unlikely event that both the mated butterflies carried this recombinant. However, the bias is extremely slight compared with the error variance. This estimate gives a total of $0 + 1 + 2 = 3$ recombinants out of $3 + 2 + 6 = 11$ tested, with a crossover rate of 27 %; the standard error is 13 %. The deficiency of recombinants compared with the null hypothesis of independent assortment is bordering on significance at the 5 % level.

A little more information is obtained by including the parents of broods 19 and 21, all of which are offspring of 'Dennis the Menace'. It is *certain* that two of these four inherit the chromosome Bd from their father. Adding this information gives a minimum for the crossover rate of $3/13 = 23 \pm 12$ %. It is *possible* that the other two of the four inherited the recombinant bd from their father; adding these gives a maximum estimate of $5/15 = 33 \pm 12$ %.

The best estimate of the recombination fraction between these loci is therefore zero in females and around 27 % in males, with a rather high standard error. The upper 95 % confidence limit for females given by Turner (1972) is now superseded because of the known absence of chiasmata in that sex.

It is difficult to be certain whether or not bd/bd recombinant genotypes have been found in the natural hybrid zone between the Trinidad/Venezuelan, Belém and Suriname races in (French) Guyane and Suriname; these phenotypes would be rare even if the genes were unlinked (Turner 1971 b), and some of them may not be recognizable because of the segregation in natural hybrid populations of genes affecting the amount both of red and yellow (§3.7 g) so that plain bb individuals may occasionally be mistaken for B^-. Among the many hundreds of specimens exported commercially from a hybrid population in western Guyane, and the smaller numbers obtained by collectors in Suriname, there are a few in the British Museum that appear to be $bbddN^N N^N$ and $bbddN^N N^B$ (that is, plain-Y and plain-S phenotypes); the theoretically possible all black *Heliconius melpomene*, the genotype $bbddN^B N^B$, is known to us

from only two specimens, although between us we have examined all the major collections in Europe and in the Americas. The type specimen of form "karschi" (Riffarth 1900) now in the main collection of the Berlin (D.D.R.) museum, has only a poorly defined smudge of yellow and red in the place where the band should be; it appears to have been captured somewhere on the lower Amazonas. A similar specimen, from Itacoatiara (rather less than 200 km downstream from Manaus), is in the collection of Sr Ricardo Diringshofen in São Paulo. Both of these specimens came, not from the Guiana† hybrid zone, but apparently from the similar hybrid populations along the lower Amazonas, where the Amazonian races meet the 'postman' race found along the river, from which the total number of collected specimens may be an order of magnitude, or even two, less than the number from the Guianas. It is possible therefore that these plain-O phenotypes are not in fact *bd* crossovers, but the result of some independent genetic modification. Similarly, the few existing plain-S and plain-Y butterflies might be crossovers or simply extreme TS and TY phenotypes with very reduced red marks (see § 3.7 for certain broods where this reduction is marked). It is interesting to note that the apparent recombinant phenotypes (plain-S, plain-Y) are quite frequent in the hybrid zone between the 'postman' race and northwestern Amazonian races in eastern Colombia (serranía La Macarena, and south of San José del Guaviare); they may be mimics of several local common black-and-yellow heliconians.

(*l*) Summary

The Belém and Trinidad races (plate 1*a*, *b*) differ at five major loci. *D* controls the radiate marks of Belém (dominant allele D^R) versus the plain wings of Trinidad (recessive allele *d*). *N* and *B* jointly replace the yellow forewing band of the Belém race with the wide red Trinidad band. Dominance is complete at the *B* locus; *N* has an intermediate heterozygote, and the loci interact in the way shown in figure 5. The orange colour of Belém is converted to the intense red of Trinidad chiefly by the dominant gene *Or*. The races differ also at the *F* locus: the recessive allele *f*, which converts the broken band of the Belém race into a fused yellow patch (plate 2*c*, *d*), functions in the Trinidad race, which has no yellow marks in the band, to spread white scales evenly in the underside of the band. *B* is linked to *D*, possibly rather loosely; all the other loci are independent. (Phenotypes are shown in plate 2*a*, *c–g*.)

This pattern of inheritance is closely similar to that already shown for the Suriname × Trinidad cross (Turner 1972), except that the Suriname race carries the 'middle' allele *D* instead of D^R (dennis marks instead of radiate) and has a red colour similar to that of Trinidad.

Several less well studied or 'minor' genes have been detected: (i) enhancers of yellow, which increase the yellow markings of $N^B N^B$ or $N^N N^B$ genotypes; (ii) an effect that converts the white underside colouring of red bands to yellow (plate 3*l*), tends to change red to orange, and is associated with the female sex; (iii) two dominant genes (*Ac* and *Tc*) in the Trinidad race which, in company with the N^B allele, repress the forewing yellow cell spot of the Belém race. In one brood the yellow band of $N^N N^B$ heterozygotes is much stronger in females than in males.

† See footnote on p. 541 for the distinction between Guiana, Guyana and Guyane.

3.3. *The cross Belém × Venezuela*

Apart from some minor differences in size and the intensity of the red colouring which have led to the Trinidad populations receiving a separate name (not recognized in this paper), the populations of *melpomene* on the Venezuelan mainland are identical in appearance with those on the offshore island of Trinidad. It is a reasonable hypothesis that the major genes controlling the colour pattern will be the same in both places.

(a) The hybrids

To test this proposal, we crossed a single female from Quebrada Grande in southwestern Venezuela with a male of our Belém stock (table A 1, appendix 4). The F_1 hybrids, 13 in all (plate 3 a, left), were identical in appearance with the F_1 hybrids of the Belém × Trinidad cross, showing some slight variation within the F_1 in the strength of the red and yellow marks in the forewing band. For reasons not ascertained, attempts to breed from this F_1 failed, the females either declining to lay, or laying sterile eggs. However, an F_1 male mated to a Belém stock female produced a single adult male offspring, of the phenotype radiate-S, broken, which, it will be recalled, is one of those produced in the similar backcross from Trinidad (plate 2f).

(b) Conclusion

Although these results are not extensive enough to prove that the Trinidadian and Venezuelan populations of *melpomene* are genetically identical for colour pattern, they do give reasonable ground for supposing that this is so. In particular the dominance relations in the F_1 with Belém are identical in both cases, and the Venezuelan population clearly contains an allele that behaves exactly like that designed N^B in Trinidad.

3.4. *The cross Belém × East Brasil*

The races found at Belém and in East Brasil (Espírito Santo) differ widely in their patterns (figure 4b, d; plate 1a, c); in addition the red marks are intense red in East Brasil, but orange in most Belém butterflies. The genetics was investigated by forming the F_1 (plate 2b), F_2 (broods CF2, NF2, YM59 and YM63) and backcross to Belém (brood F1BM); as the backcross to East Brasil could not be formed because an Espírito Santo stock could not be established, the F_1 was crossed to Trinidad, which differs from East Brasil in lacking the yellow marks and the toothed edge to the band (brood F1TM). The broods are described in table A 2, appendix 4; several are multiparental.

As only two further test crosses (YM 66 and CYHW) have been made, we cannot finally confirm the genetic hypotheses that we put forward to explain the results of these crosses. We give the most parsimonious explanations, but because these tally so well with what is known of the genetics of other crosses it is extremely likely that these are also the correct explanations. The F_2 numbers 299 butterflies, and the expected number of a recessive homozygote for a single factor is $74\frac{3}{4}$.

Major phenotypes are shown in plates 2 and 3.

(a) Inheritance of radiate versus plain

As in other crosses the radiate pattern from Belém is unifactorial and controlled by the alleles D^R and d, giving 223 radiate : 76 plain in the F_2 and a ratio of 61 : 81 in the backcross to

the plain recessive Trinidad genotype ($P = 0.11$); the F_1 and backcross to Belém are entirely radiate. The locus is the same as that segregating in the Belém × Trinidad cross, or at least tightly linked to it, for, if there were two 'D' loci of similar effect, the backcross to Trinidad would not give a $1:1$ segregation.

(b) Inheritance of red forewing band

In the backcross to Belém, forewing bands containing red segregate in equal numbers to those in which red is absent ($59:68$, $P = 0.48$), and, in the F_2, in the ratio $3:1$ (223 bands with red : 76 bands that are entirely yellow or absent); the character does not segregate in the backcross to Trinidad, this and the F_1 having red bands. The removal of the red colour in the band is clearly produced by a recessive gene from the Belém race; this is shown to be the b allele which segregates in the Belém × Trinidad cross, by brood YM66 (table A 2, appendix 4), in which an F_2 Belém × East Brasil male was mated to a black forewing tip female known to be bb, derived from the Belém × Trinidad cross; the $1:1$ segregation ($9:13$; $P = 0.52$) of phenotypes with and without red bands shows that only one locus is segregating, which suggests that the F_2 male was heterozygous Bb. On the basis of this hypothesis, the Belém race would be bb, and both Trinidad and East Brasil would be BB. Alternatively it is possible in this case to draw up a pedigree with two loci producing the red bar, Belém and the black tipped female being homozygous recessive for both of them (say $bbzz$), with Trinidad being $BBzz$ and East Brasil $bbZZ$; the F_2 male would then have been $bbZz$, and it would be Z, not B, that segregated in his offspring. However, this is unlikely , as the B locus of the Trinidad cross is linked to the D locus (§3.2j above), and so is the locus that segregates in the Belém × East Brasil F_2 (§ (o) below; this makes it very probable that we are dealing with the same locus. The B alleles from Trinidad and East Brasil are, if not identical, closely similar in their effects.

(c) Inheritance of amount of yellow in the forewing band

This character segregates in a similar way to the Suriname × Trinidad and the Belém × Trinidad crosses described above. The locus involved is shown to be the same as the N locus which segregates in the Belém × Trinidad cross, by the backcross of the Belém × East Brasil F_1 to the Trinidad stock. The character segregates equal numbers of wide red bands and yellow bands with a thin red edge (W and TS in figure 5) (brood F1TM, table A 2, in appendix 4; numbers $77:65$, $P = 0.36$); if two 'N' loci were involved the ratio would be $3:1$. The cross of a Belém × East Brasil F_2 male with the Belém × Trinidad female of genotype bbN^BN^B (brood YM66, table A 2) likewise shows only one 'N' locus segregating. The simplest hypothesis, given the established fact that Belém is homozygous N^NN^N and that the wide banded Trinidad butterflies are N^BN^B (figure 5), is that the wide banded East Brasil butterflies are likewise N^BN^B. The phenotypes observed are in reasonable accord with this hypothesis. The F_1 butterflies (N^NN^B) have a narrow red band with yellow marks like the TS phenotype of the Trinidad crosses (plate 2a, b) and in the F_2 the phenotypes which almost or entirely lack yellow in the band (O and W in figure 5), and which *ex hypothese* should be N^BN^B homozygotes, are 82 in number, which is not significantly different from expected ($\chi_1^2 = 0.81$, $P = 0.37$).

From the hypothesis we would in addition expect the phenotypes with well developed yellow (that is Y and TY in figure 5) to be the other homozygote, N^NN^N, and the heterozygotes N^NN^B to constitute the butterflies with only moderately developed yellow (S and TS in figure 5), as in the F_1. In fact the supposed N^NN^N homozygotes number only 26 (expected $74\frac{3}{4}$), and it is

clear that, if these genotypes can be distinguished at all, then more than half of the $N^N N^N$ homozygotes are indistinguishable from heterozygotes. Although the backcross to Belém segregates the supposed $N^N N^N$ and $N^N N^B$ classes (Y and TY versus S and TS in figure 5) in a quite good approximation to 1:1 (58:69, $P = 0.37$), it is clearly not possible in general to distinguish the two genotypes, and in tabulating the broods (in table A 2) we have classified the forewing bands merely as yellow (Y+S), yellow+thin red (TY and TS), no band (O) and wide red (W).

Thus the control of the forewing band colour is essentially the same as in the cross between Belém and Trinidad, with the red band of East Brasil produced by homozygosity for B and N^B and the yellow band of Belém by homozygosity for b and N^N. We will now see that the difficulty in distinguishing $N^N N^N$ from $N^N N^B$ (or if one likes the largely dominant effect of N^N) appears mainly to result from the segregation of further genes affecting the cell spot and the strength of the yellow marks, both of which help to distinguish the genotypes in the cross with Trinidad.

(d) Inheritance of cell spot

In the F_2 the butterflies with no band (O phenotype) and those with the wide red band (W phenotype) are clearly divided in the ratio 1:3 into those with and without the brilliant yellow dumb-bell shaped spot in the cell of the forewing (plate 2j, compare left and right halves); the numbers are 24:58, with expected numbers $20\frac{1}{2}:61\frac{1}{2}$ ($P = 0.44$). This is consistent with the presence of the spot being controlled by a single recessive allele, which we shall designate c; we shall call its dominant allele (absence of spot) C. This gene can be seen segregating in the remaining phenotypes, but with some problems of scoring. The yellow and yellow+thin red phenotypes in the F_2 divide into 146 without:71 with the spot (for 3:1, $\chi_1^2 = 6.49$, $P = 0.011$), the overall segregation in the F_2 being 204:95 ($\chi_1^2 = 6.96$, $P = 0.0083$). In the backcross to Belém, there are 79 with the spot:48 without, which is significantly wide of 1:1 ($P = 0.0075$). But the backcross includes 18 individuals with only part of the cell spot developed: if these are classified as lacking the spot, the segregation is a satisfactory 61:66.

As the segregation of C is not subject to scoring difficulties in $N^B N^B$ butterflies, the simplest explanation is that some $N^N N^N$ individuals can produce a weakly developed cell spot even if they carry C. If all $N^N N^N$ butterflies produced a cell spot, spots versus their absence would segregate 3:1 in the backcross; if only some of them do then the ratio will lie between 3:1 and 1:1. The scoring of the F_2 suggests that two-thirds of the $N^N N^N$ homozygotes are misclassified as $N^N N^B$; a contributing cause is undoubtedly the lack of a cell spot. If exactly two-thirds of $N^N N^N$, Cc butterflies lack the cell spot, then the overall segregation in the backcross will be 7:5, which is close to what is obtained ($P = 0.43$).

The control of yellow in the forewing band is therefore largely explained by the segregation of two loci, C controlling the cell spot, and N the rest of the band ($C-$ and $N^B N^B$ removing the yellow); in contrast to the Trinidad cross, $N^N N^N$ and $N^N N^B$ cannot be distinguished for certain, but it is likely that some $N^N N^N$ individuals produce a weak or partial cell spot even in the absence of cc.

In the backcross to Trinidad, the cell spot can be seen in about half the butterflies, appearing either as a thick sprinkling of yellow scales on the upperside and underside, or sometimes only on the underside; sometimes it appears only as a shadow pattern on the underside. Scoring all of these phenotypes as having the cell spot gives a segregation of 56 with cell spots:86 without them (table A 2 (appendix 4), brood F1TM). As the alleles C and c can be seen segregating in this cross, it would appear that Trinidad is either (i) homozygous cc, (ii) homozygous CC, but

with additional genes producing partial dominance reversal, (iii) homozygous for a third allele, say c^t, which partly suppresses the expression of c (Cc^t, no cell spot; cc^t, weak cell spot). Hypotheses (ii) and (iii) would produce segregation of full cell spots in the cross Trinidad × Belém; as such a segregation is not observed, we favour the first hypothesis, that Trinidad is cc. In this case, as Trinidad lacks the cell spot, and as full cell spots do not appear in the Trinidad × Belém F_1, it follows that there must be other dominant genes suppressing the cell spot in Trinidad. We have confirmed this hypothesis by demonstrating the segregation of dominant genes, suppressing different parts of the cell spot, in the Belém × Trinidad cross (§2h).

A curious reverse effect of the N^N allele, or of something in the same linkage group, is seen in brood CYHW (table A2, appendix 4), which may have a part Trinidad background, in which all the N^Byb/N^Byb butterflies have full cell spots, and all but one of the N^NYb/N^Byb butterflies have only the posterior part of the spot. This may be connected with the effect of Yb in suppressing the yellow forewing line in this brood (§3.4g below).

(e) Inheritance of strength of yellow

Scoring the yellow bands in the above way explains the segregation of the yellow cell spot, but leaves open the question of the genetics of the differences in the intensity of the yellow forewing band, which may be firm, clear yellow (strong) or contain a mixture of black scales (weak). The F_1 contains both strong and weak phenotypes, and as the F_2 segregates roughly in the ratio 189 strong : 94 weak (an almost perfect 2 : 1 ratio) it is clear that there is too much genetic heterogeneity between the parents of the F_2 (nine females in total), and probably too many loci segregating, for the inheritance of this character to be determined. It is possible that one or both of the parental races is heterogeneous.

(f) Inheritance of yellow hindwing bar

The yellow bar on the hindwing of the East Brasilian race is inherited in this cross as a single factor, almost recessive in effect, which we shall call yb. Absent in the F_1 and largely absent in the backcross to Belém, the bar appears in 74 of the F_2 butterflies (expected number for a recessive, $74\frac{3}{4}$) (plate 2h). Among the plain butterflies (that is, not radiate) the presence of the shadow pattern for the yellow bar can be scored readily on the underside. It sometimes appears also on the upperside. The segregation is no yellow bar, 18 : shadow pattern only, 38 : yellow bar, 20, confirming that as in the Belém cross (§3.5 below) the shadow pattern is the $Ybyb$ heterozygote, with a close fit to the expected 1 : 2 : 1 (19 : 38 : 19).

In the backcross to Belém, five individuals have a moderately developed yellow bar, and a further 29 have small traces of yellow pigment in this position, almost all of them on the underside. This suggests that there is some penetrance of yb in about half of the heterozygotes.

We should expect the Trinidad race to be $YbYb$, and the backcross to this race to segregate equal numbers of shadows ($Ybyb$) and absent bars ($YbYb$). Among the plain butterflies, which alone can be unambiguously scored, the number are 51 shadows : 29 absent ($P = 0.018$); the significant deviation from 1 : 1 appears to be merely a matter of chance, as the thin red bands and wide red bands segregate among the plain butterflies in a similarly distorted ratio (N being linked to Yb, see §(o) below), although their overall segregation in the brood is roughly the expected 1 : 1 (65 : 77, $P = 0.36$).

About half of those with the shadow of the bar on the underside show a light sprinkle of yellow scales in this region on the upperside (31 with yellow scales : 20 without, $P = 0.16$); the

yellow scales can also be seen, mixed into the red hindwing bar in ten of the radiate butterflies with wide red bands, leaving 15 in this category (most of them showing what can reasonably be interpreted as a shadow on the underside) without the sprinkling of scales. The clear 1:1 segregation of this character strongly suggests that partial expression of the bar on the upperside in heterozygotes is under the control of a single locus, which we provisionally designate Ub. As the F_1 butterflies lack the yellow scales, it is likely that expression is recessive, and that both East Brasil and Trinidad are $ubub$.

(g) Inheritance of yellow line

The segregation of this character is somewhat obscured by the fact that both of the races hybridized possess it in some degree, the East Brasilian as a thin yellow line right along the cubital vein (plates $1c$, $2h$), and the Belém race in the form of a yellow dot at the base of the wing (plate $2j$, right half), which can sometimes be seen, usually in plain butterflies, as the base of a short yellow line extending along the cubital vein. This basal line has been shown in other crosses (Belém with Trinidad and with East Ecuador, also Suriname with Trinidad (Turner 1972)) to be mainly the product of the $N^N N^N$ genotype.

One brood, probably of East Brasilian × Belém provenance, but possibly including some Trinidad ancestry, suggests that the full yellow line is produced by the yb (yellow bar) allele: in brood CYHW all seven of the yellow barred ($N^B yb / N^B yb$) individuals have full yellow lines, and all ten of the yellow bar heterozygotes ($N^N Yb / N^B yb$) have the basal line characteristic of the N^N allele ($P = 5.1 \times 10^{-5}$). This relation is confirmed by one of the present F_2 broods. If the lines are divided into basal lines or basal dots (bl in the table) and those that are as strongly (pl) or more strongly (fl) developed than in East Brasil, the association with the yb allele is highly significant, the numbers being: bar and strong line 37; bar and basal line 5; no bar, strong line 7; no bar, basal line 120 ($P = 1.8 \times 10^{-24}$, two-tailed). The interaction of $ybyb$ and the N locus in producing the line is shown by another F_2 (NF2, table A2). The 93 insects in which the yellow line is scorable show the same association between absent (nl) or basal lines (bl) versus strongly developed lines (pl) and (fl) and the yellow bar: the numbers are (in the same order as before) 4, 22, 1, 16 ($P = 0.041$, two-tailed). On the other hand, if the lines are divided into those that are absent (nl), or expressed in any degree (bl, pl, fl), there is no association with the yellow bar (numbers 3, 13, 38, 29; $P = 0.72$, two-tailed). Thus $ybyb$ produces strong development of the line, but does not condition the development of the weak basal line or basal dot, which can be seen to be produced largely by the N^N allele, for among the insects without yellow bars the numbers with yellow or yellow and red forewing bands (N^N–) and no line or dot, yellow or yellow and red forewing bands and a yellow line or dot, no or wide red forewing bands ($N^B N^B$) and no line or dot, and no or wide red bands and a yellow line or dot are 24, 38, 5, 0, for which $P = 0.012$, one-tailed (heterozygous $Ybyb$ individuals included). The interaction of $N^B N^B$, which tends to remove the line, and $ybyb$, which tends to add it, can be seen within the no band and wide red band ($N^B N^B$) butterflies; those five without yellow bars all lack the line, those with yellow bars divide into no lines, basal lines (bl) and East Brasilian lines in the numbers 13, 9 and 4; for this the 2 × 2 contingency probability (5, 0, 13, 13) is 0.050, one-tailed.

Hence in this brood $ybyb$ can add a basal line or a full line to an $N^B N^B$ butterfly; that it does not always do so, when the pure $N^B N^B$ $ybyb$ East Brasilian insects always have a yellow line, shows either that other factors are segregating, or that the yellow line effect is linked to, rather

than being a pleiotropic effect of, *yb*. In brood CF2 the lines are generally more strongly developed (no insect completely lacks the line or yellow dot), and here there is a strong tendency for the combination $N^B N^B$ *ybyb* to produce hypertrophy of the line, all 23 individuals with very strongly developed lines being in this class, which totals only 37. Both the loss of the lines in some insects and the hypertrophy in others argue that the control over the development of the line which must be exercised in pure East Brasilian butterflies has been partly lost by segregation.

The yellow line is almost entirely missing in the backcross to Trinidad; only a few butterflies (not noted in table A2, appendix 4) show the basal part of it.

In summary, the yellow line of East Brasil is largely produced by the *yb* allele (or something linked to it) and the basal yellow dot (or basal yellow line) of Belém by the N^N allele (*Yb* tending to remove the line and N^B tending to remove the basal dot), although other factors clearly influence these marks as well.

These complexities in the inheritance of the yellow line make it difficult if not impossible in this cross to use the yellow basal spot to distinguish $N^N N^N$ from $N^N N^B$, as we shall do in the East Ecuador cross.

(h) *Inheritance of red versus orange*

As we mentioned in the description of the phenotypes, this character (plate 2) becomes hard to score in some of the hybrid broods although the pure races are distinct, and fades to orange on exposure to air; to judge from the ratios obtained in scoring trials, P. M. S. scores this character more accurately, J. R. G. T. tending to misclassify the more orange of the red individuals as orange. We have not tried to obtain ultimate accuracy, and there are slight variations between broods in the ratios obtained. Overall, the F_2 segregates red to orange just wide of the ratio 3:1 (207 red:92 orange) ($\chi_1^2 = 5.00$, $P = 0.025$), indicating that most of the segretation of this character is under the control of a single locus (*Or*) with red dominant but with some mis-scoring, fading, or interference from other loci. In accord with this, the F_1 is red. The backcross to Belém segregates red to orange in the ratio 59:68 ($P = 0.48$ for 1:1), with some suggestion that sex influences the colour (26 red, 44 orange females; 33 red, 24 orange males; $P = 0.031$, two-tailed). The backcross to Trinidad (which is red) should have been entirely red, but produced 19 orange butterflies:123 red, indicating that penetrance is altered in this cross. The occurrence of this anomalous orange colouring is asscciated with a change in the colour of the scales that clothe the underside of the band from white (as in East Brasil and Trinidad) to yellow (plate 3*l*). Among the wide banded butterflies in the Trinidad backcross, the numbers are (upper/under): red/white 64, red/yellow 4, orange/white 4, orange/yellow 4 ($P = 0.0064$, two-tailed). Given the difficulty in scoring both of these characters, the association may be even stronger than this.

Remembering the similar findings with respect to sex and yellow underside in the Trinidad cross (§ 3.2*i*), we therefore have two repeatable associations of orange colour: an increased frequency in females in some broods, and an association with yellow colour replacing white on the underside of wide red bands. There has also been one case of very strong association with the N^N allele. It is tempting to speculate that (i) the deployment of extra yellow pigment (which is the main function of the N^N allele) somehow converts red colour to orange, and (ii) there is a sex-linked or sex-limited gene involved. However, no simple model of a sex-linked gene modifying the effects of *or* will explain all the results obtained.

(i) White scales on underside of band

The phenotype with the yellow marks of the forewing band fused into a solid yellow patch (plate 2d), which appears in the Belém × Trinidad F_2 and backcross to Trinidad, does not occur in any brood of Belém × East Brasilian ancestry. We must conclude that both the Belém and East Brasilian races are homozygous FF (broken yellow band), this being not expressed in the East Brasilian race because it lacks yellow marks in this area of the wing.

However, both the East Brasilian and the Trinidad races have the underside of the forewing band smoothly clothed in white scales. In Trinidad the effect is produced by ff (fused band), which affects only white or yellow scales, and probably by cc (cell spot), which has the function of putting white scales on the underside of the band where it enters the main cell of the forewing. As the East Brasilian race is FF, which would produce broken patches of white scales on the underside, some other genes must be controlling the distribution of white. The backcross to Trinidad shows that it is the C (no cell spot) gene that is mainly responsible. Among the wide banded butterflies, there are 35 without cell spots in which the white (or yellow) scales are smoothly spread, 20 with cell spots in which the scales are confined to patches, only three without spots and patchy white, and seven with spots and smooth white ($P = 6.78 \times 10^{-8}$, two-tailed). There is also some association with sex ($P = 0.015$), males tending to have the scales more smoothly spread than females (patchy females, smooth females, patchy males, smooth males in the numbers 16, 16, 7, 26), and all seven of the smooth phenotypes with cell spots being male. In addition to controlling this distribution of white scales, the C gene presumably has the effect of preventing the white scales from appearing within the cell of the forewing, as they do in Trinidad, for the East Brasilian red band does not extend into this part of the wing.

(j) Inheritance of Ecuador triangle

The coloured triangle that appears, in white, just posterior to the cell spot in the East Ecuador race (plate 1d; figure 4c) is absent in both Belém and East Brasil, but appears in the F_2 hybrids between them, always in yellow, and sometimes looking like a wedge-shaped extension of the yellow forewing line where this is present. The triangle is sometimes very strongly developed (plate 3j, right), but is more usually represented by a small coloured dot.

The development of this mark is enhanced by the yb (yellow bar) gene, and in some genetic backgrounds is strongly suppressed by the c (cell spot) gene. Scoring triangles simply as present or absent, without regard to their size, the combined F_2 contains nine butterflies with a cell spot and a triangle, 84 with a cell spot only, 63 with a triangle only, and 140 with neither mark; the association is highly significant ($P = 5.4 \times 10^{-5}$, two-tailed). However, this association is not homogeneously distributed between the F_2 broods. It cannot be tested in YM59 and YM63 in which triangles are found in only two butterflies, is present but not significant in the very large multi-parental CF2 ($P = 0.10$), and is very strong in NF2 ($P = 2.6 \times 10^{-5}$, all two-tailed). Thus only in one brood is there clear evidence that cc suppresses the triangle; the effect is obviously real, but is perhaps present on some genetic backgrounds only. The association of the triangle with the yellow bar is more consistent, being found in CF2 (62 with both marks, 15 with triangle only, 27 with bar only, 24 with neither, $P = 0.0019$), and NF2 (among insects without cell spots only, phenotypes in same order 13, 2 16, 30, $P = 0.0010$) (both two-tailed).

It is possible that yb enhances the development of the triangle even when heterozygous, but

this is difficult to demonstrate as the *Ybyb* individuals can be distinguished (by the shadow of the bar) only in the absence of radiate. Among plain butterflies without cell spots in NF2, and all plain butterflies in CF2, the following association is found:

	yellow bar		
	none	shadow	full
full or part triangle	3	8	7
no triangle	15	18	8

Although the association is not significant with this sample size ($\chi_2^2 = 3.47$, $P = 0.18$), it is the case that the percentage of triangles is exactly intermediate in the heterozygotes (at 0.31) between those in the two homozygotes (0.17 and 0.46), arguing that *Ybyb* genotypes show about half the tendency to develop triangles that *ybyb* homozygotes do. At any rate, really strongly developed triangles, among radiate butterflies also, are usually accompanied by a fully developed yellow bar.

However, enhancement and suppression by *ybyb* and *cc* cannot be the whole of yellow triangle control, for the East Brasilian race, being *ybybCC*, should then have a very well developed triangle. At least one further gene must suppress the development of the triangle in East Brasil. As the triangle appears, in minute traces and often in one wing only, in only nine out of 127 butterflies in the Belém backcross (again strongly associated with *Cc*, only two cell spot butterflies manifesting it, against seven without the cell spot; 2×2 probability 0.015, one-tailed), and as around half of the *Cc* butterflies here must be *Ybyb*, it would appear that in Belém also there are additional factors repressing the triangle. In support of this argument, it can be observed that just over half (26 out of 48) of the *C–ybyb* individuals in the F_2 show some triangle development, which is more than would be expected if there were merely a single further repressor in East Brasil.

The simplest system giving a reasonable fit to the data is that the two races are homozygous at two unlinked but complementary loci, whose dominant alleles suppress the triangle. If East Brasil were homozygous *Tr-1 Tr-1 tr-2 tr-2*, and Belém were homozygous the other way (*tr-1 tr-1 Tr-2 Tr-2*), the F_1 would lack triangles, as is observed. The double recessive phenotype with a full triangle would then appear as one-sixteenth of the F_2, which is close to what is observed (among yellow barred butterflies in CF2 and yellow barred butterflies without cell spots in NF2, seven full triangles out of 57, compared with 3.6 expected). The partly developed triangles could be the genotypes that are homozygous recessive at one locus and heterozygous at the other (*tr-1 tr-1 Tr-2 tr-2* and *Tr-1 tr-1 tr-2 tr-2*); the overall segregation would then be 11 no triangles:4 part triangles:1 full triangles, compared with observed numbers (again within the same subset) of 29:21:7 ($\chi_2^2 = 7.56$, $P = 0.02$). According to this hypothesis, *Ybyb Cc Tr-1 tr-1 tr-2 tr-2* insects, which comprise one in eight of those Belém backcross insects without cell spots, would exhibit partly developed triangles. There are in fact seven such triangles out of 49 ($P = 0.83$). There is thus a tolerable but not exact fit to the hypothesis of two repressor loci in addition to *Yb* and *c*.

In the Trinidad outcross the triangle itself does not appear, but a yellow spot that could be interpreted as its distal extreme (or as the proximal extreme of the Belém spot; see §(*k*) below) appears in five butterflies, all of them radiate females with thin red bands and lacking cell spots (the remaining seven females and all eight males in this category lack this mark). From the

linkage of N and Yb (below), these butterflies are also probably $YbYb$, so that expression of this part of the triangle appears in this case to be conditioned by the genotype $YbYbCc$, female, and presumably by the segregation of one of the triangle suppressor loci at which Trinidad is homozygous recessive.

Neither of these two suppressors appears to be the allele T, which will de described in §3.7e (the East Ecuador cross), as the tt homozygote causes the triangle to appear in company with the cell spot, whereas here the cell spot represses the triangle. On this account, as the Belém race is known to be TT (§3.7e), it follows that East Brasil must be TT also. On the other hand, the different responses to cc may be conditioned by the genetic background, in which case parsimony would require only two triangle suppressor loci, Belém being tr-1 tr-1, TT and East Brasil Tr-1 Tr-1, tt.

In summary, the Ecuador triangle mark is suppressed independently in Belém and East Brasil by at least two complementary loci, with suppression being largely dominant, so that triangles appear only in the F_2. In addition yb enhances the expression of the triangle at least when homozygous; the triangle is largely suppressed in cc homozygotes. This pattern of inheritance strongly suggests that the Ecuador triangle was present in the common ancestor of the Belém and East Brasil races, which both suppressed it independently (see also §5.7).

(k) Inheritance of the Belém spot

The yellow mark between Cu1a and Cu1b (the Belém spot) (plate 2k, l), often absent in pure Belém butterflies, is often absent also in the hybrids, being largely missing for example in the large mixed F_2 (CF2). In the smaller, uniparental F_2 (NF2) the spot appears in sufficient numbers to be correlated with other features, and can be seen never to develop properly in the absence of the cell spot, the numbers being 19 with both marks, 60 with neither, 13 with the cell spot but no Belém spot, and one only with the Belém spot, but minute and on one wing only, and no cell spot ($P = 4.03 \times 10^{-10}$, two-tailed). As we should expect from the suppression of the Ecuador triangle in the presence of the cell spot, the Belém spot and triangle virtually never develop together: the one individual with the minute Belém spot on one wing has a well developed triangle, but otherwise there are no individuals with both marks (compared with 48 with neither, 16 with the Belém spot only, and 27 with the triangle only; $P = 0.0019$, one-tailed). In the backcross to Belém the cell spot is similarly a prerequisite for the development of the Belém spot (both marks, 39; Belém spot only, 1; cell spot only, 40; neither spot, 47).

The easiest way of interpreting this is to suppose that when a yellow mark appears in the space between Cu1a and Cu1b the cell spot gene controls its distribution, cc (cell spot) butterflies having it placed distally as the Belém spot, and C– (no cell spot) having it in the proximal position as the Ecuador triangle.

In the East Ecuador race the cell spot and triangle appear together, and we shall suggest that on that genetic background the switching effect of the C locus is reversed (§§3.7e, 3.8d).

The Belém spot is absent in all Trinidad backcross butterflies, except for those described above as having either its proximal part or the distal part of the triangle, and one further individual with a well defined Belém spot.

The Belém spot is also governed, but less clearly, by the yb and N^N alleles. The numbers in brood NF2 with yellow bars and Belém spots, yellow bars only, Belém spots only, and neither mark are 5, 15, 13, 56, $P = 0.75$, two-tailed, if five individuals that have large triangles extending into the area of the Belém spot are treated as unscorable, and 10, 15, 13, 56, $P = 0.072$, if

these individuals are treated as having Belém spots. The association is therefore not significant, and is probably being reduced by the linkage of yb to N^B (§3.4o), for $N^B N^B$ butterflies, lacking the yellow forewing band, may tend to lack the Belém spot.

(l) *Control of yellow pigment between veins Cu1a and Cu1b*

We can summarize the relations of the yellow bar, cell spot, triangle and Belém spot, which appear complicated, by thinking of three areas of pigmentation: the yellow bar, the cell spot, and the region between veins Cu1a and Cu1b (which contains the triangle and Belém spot; figure 4h). Then, on taking brood NF2 only, the Yb allele, which removes the yellow bar and most of the yellow line, is seen to have a very strong effect in removing the yellow pigment between Cu1a and Cu1b; the numbers are yellow bar and yellow Cu1a/b 20, yellow bar only 7, yellow Cu1a/b only 28, neither 41 (2 unscorable), for which $P = 0.0059$, two-tailed (a triangle or Belém spot or both here counting as yellow pigment between Cu1a and Cu1b).

If yellow pigment does appear in this area, then cc (cell spot) genotypes tend to have it placed distally, to form the Belém spot, and C– genotypes to have it placed proximally to form the triangle. The genotype $N^N N^N$ causes pigment to appear in the distal part, thus also producing a Belém spot. The genes Tr-1 and Tr-2 both suppress the triangle, but probably have no effect on the spot. Thus the phenotypes of the two races are produced by the following genotypes:

Belém: $YbYb$ (no bar, pigment partly removed from Cu1a/b), cc (cell spot, triangle partly suppressed, Belém spot enhanced), Tr-2 Tr-2 (triangle further suppressed), $N^N N^N$ (yellow forewing band, Belém spot enhanced).

East Brasil: $ybyb$ (yellow bar, yellow mark in Cu1a/b), CC (no cell spot, suppression of Belém spot, and possibly of other Cu1a/b yellow), Tr-1 Tr-1 (suppression of triangle), $N^B N^B$ (no yellow forewing band, Belém spot suppressed).

This way of looking at the control of these marks is also not merely convenient, but ties in closely with our hypotheses about the way in which the patterns evolved (§5.7).

(m) *Inheritance of tooth on forewing band*

The projections of the outer posterior part of the forewing band, known as 'tooth' can be seen segregating in these crosses (plate 3i, right). However, they are very difficult to score, there being also sorts of blunt and sharp projections in this part in the F_2. It would seem that several genes may be involved, or at the least a gene of such variable effect that it defies our discrimination.

(n) "*Ethilla*" *and* "*cydno*" *patterns*

In the F_2 the radiate marks in many individuals take on an appearance reminiscent of certain marks seen in the related species *Heliconius ethilla* and *H. cydno*. On the forewing the red marks may be invaded in the cell by a deep black wedge extending almost to the base of the wing, rounded off distally by an extension of red into the cell spot, producing a black 'keyhole' effect. This is characteristic of *H. ethilla* and of other 'tiger' patterned *Heliconius* (figures 12, 17). On the hindwing the red rays, instead of being narrow and sharp as they are in Belém, may become very wide, to the point of coalescing, with broad blunt tips and even concave margins proximally (plate 3j). They then require only some shortening to become the same shape as the brown lunular marks in the hindwing of *H. ethilla*.

When seen on the underside the broad fused rays of these butterflies, with the red bar that tops them off, frequently have the appearance of a large coloured oval crossed by a few black marks (plate 3k), a shape that is reminiscent of the great brown C-shaped mark that occupies this position on the underside of *Heliconius cydno* (not visible in figure 18).

Variation in both the forewing black wedge and the fusing of the hindwing rays appears to be continuous (around 69 % of brood CF2 exhibit each character) and to be uncorrelated either with each other or with any segregating major gene. We therefore believe them to be produced multigenically.

The close resemblance between these altered forms of the radiate marks and the patterns of the other two species strongly suggests that the patterns are homologous. A similar appearance of the pattern of a related species will be noted later in *H. erato* (§§4.9b, h).

In several plain individuals in the outcross to Trinidad, a shadow of a deeply divided red forewing mark can be clearly seen on the underside (it obviously cannot be due to heterozygosity for radiate, as this is dominant). When accompanied by the shadow of the cell spot, and a patchy distribution of the white marks on the underside of the band, the total pattern is very close indeed to that of *H. ethilla*.

(o) Linkage groups

None of the genes designated above shows any sign of being carried on the sex chromosome.

The test for autosomal linkage used for the Belém × Trinidad cross (*qv*), namely the non-appearance of recombinants in the F_2 and backcross, cannot be used so extensively: both of the backcrosses use male F_1 parents, which eliminates the test for loci in coupling; loci in repulsion can still be tested by the F_2, but the incompletely dominant alleles at the N locus, which for this purpose can be regarded as in repulsion with all other loci (by pretending that either N^N or N^B is dominant according to the case), cannot be safely treated both ways because of the difficulty in identifying with certainty the $N^N N^N$ homozygotes. For the remaining pairs, we have to use the weaker, more conventional test of failing to reject the null hypothesis of independent assortment.

From the pairs of loci introduced in repulsion, the following recombinant genotypes are found in the F_2: $bb N^B N^B$ (black forewing tip, 28), $bbybyb$ (no red band, yellow bar, 20), $ccdd$ (plain with cell spot, 27), $cc N^B N^B$ (wide red band, or black forewing tip, with cell spot, 24), $ccybyb$ (cell spot and yellow bar, 27), $ddoror$ (plain, orange marks, 34), $N^B N^B oror$ (wide band or black tip, orange marks, 30), $ororybyb$ (orange marks, yellow bar, 30). All these pairs are therefore in different linkage groups, barring massive contamination or mis-scoring or achiasmatic recombination. Those pairs involving cc or $oror$ are of course subject to quite extensive mis-scoring.

The recombinant class $bbdd$ (no red band, plain wing base) fails to appear in almost 300 F_2 butterflies; as in all other crosses, B and D are clearly in the same linkage group. This immediately establishes that B is not linked to C, nor B to Or, nor D to N, nor D to Yb.

Table 2 (upper right) shows exact one-tailed probabilities from contingency tables between all pairs of loci in the F_2, with N^N treated as completely dominant. There are two highly significant associations (B and D, and N and Yb) and no other association is significant; the several associations that are 'significant' the wrong way (recombinants exceed random expectation, probabilities printed in parentheses) are, with the exception of N and B, all concerned with the Or locus, and probably simply reflect scoring difficulties.

Thus for the loci in repulsion (upright numbers in the table), these tests confirm the above findings: the association of B and D is not due to chance, and the independence of the other pairs does not result from a combination of linkage and errors. There is also no association between the coupling pairs B–C, B–Or, D–N and D–Yb, which we have already concluded are unlinked to each other if there is no crossing over in the female (sloping numbers in the table). The remaining two pairs, which cannot be tested by any of the above arguments depending on the absence of female crossing over, are C–Or, and N–Yb (probabilities in bold in table 2). There is no evidence that C is linked to Or; but the extremely low probability shows that N is linked to Yb.

TABLE 2. EXACT 2×2 ONE-TAILED PROBABILITIES FOR ASSOCIATION BETWEEN LOCI IN THE BELÉM × EAST BRASIL F_2 (ABOVE THE DIAGONAL) AND (BELOW THE DIAGONAL) EXACT ONE-TAILED PROBABILITIES (1:1, PARENTAL:RECOMBINANT) FROM THE BACKCROSS TO TRINIDAD

(Probabilities are given in parentheses when the proportion of recombinant phenotypes *exceeds* expectation (0.5 in the backcross, 0.375 in the F_2 for a coupling pair, and 0.625 in the F_2 for a repulsion pair). The italic and bold figures are explained in the text.)

	D	B	N	Yb	C	Or
D	—	2.3×10^{-12}	*(0.46)*	*0.41*	0.25	*(0.0021)*
B	—	—	(0.031)	(0.44)	*(0.51)*	*(0.0082)*
N	*0.073*	—	—	7.0×10^{-48}	0.34	0.12
Yb	—	—	O(10^{-21})[1]	—	0.19	(0.027)
C	0.27	—	0.23	0.0024[1]	—	**(0.13)**

[1] Among plain individuals only.

Contingency tests on the backcross to Belém are not particularly helpful in confirming these results, as there is a scoring difficulty both for N and C. Or is clearly independent of B, as the recombinants exceed the parentals 70:57.

The cross of the F_1 with Trinidad, which is a substitute for the East Brasilian backcross, provides information about C, D, N and Yb; probability values for equality of coupling and repulsion are shown in table 2 (lower left). There are three significant or almost significant associations, a strong one between N and Yb confirming their linkage, and weak ones between N and D, and between C and Yb. These last both result from a large excess of only one parental class and, in view of the accumulated evidence of all the other crosses, the disturbance, formally significant only for C and Yb, is unlikely to be due to linkage. As C is not linked to N, it cannot be linked to Yb; as it is the 'shadow bar, no cell spot' class that is in excess, it seems that in this cross the cell spot is being repressed either by yb or by N^B, which is tightly linked to it. This is consistent with what we have found about the expression of the cell spot in the F_2, and would also explain the excess over expectation of insects lacking the cell spot in the Trinidad backcross (§(d), above).

There is no linkage between the locus Ub and the only loci against which it can be tested in the Trinidad backcross: the two-tailed probabilities (used because it is uncertain which are the parental combinations) for equality of coupling and repulsion are, for D, 0.085 and, for C, 0.42. It clearly cannot be tightly linked to Yb.

The complicated and erratic inheritance of the yellow forewing line and yellow basal spot makes it unprofitable to analyse their linkage relations. At least in some broods we have shown that the yellow line is strongly associated with the ybyb genotype (§(g) above). The linkage of this locus to N means that the yellow line could in fact be produced by the N^B allele, with its

tendency to do this being suppressed by other genes in those races (Trinidad and East Ecuador) which are $N^B N^B$ but which lack the yellow line. What is clear is that the $N^N Yb$ chromosome of Belém has a strong tendency to produce the basal spot, the $N^B Yb$ chromosome of Trinidad and East Ecuador (§3.7) a tendency to produce no yellow marks, and the $N^B yb$ chromosome of East Brasil a tendency to produce a full yellow line as well as the yellow hindwing bar. It is easiest to conceive of the yellow spot as an effect of N^N and the yellow line as an effect of yb.

It is therefore established that B and D are in one linkage group, N and Yb in another, and that C and Or are independent of these groups; there is no evidence at all that C and Or are linked to each other, and they are almost certainly independent. The position of F in relation to the other loci is not clear, as it does not segregate in these crosses. It is already known that it is not linked to B–D, nor to N (and hence to Yb), nor to Or. It remains possible that it is linked to C, or is even an allele of it, an attractive hypothesis in view of the similar effects of C and F on the white underside of the forewing band.

(p) Crossing-over rate between N and Yb

The rate of crossing over between B and D cannot be measured in these crosses. The crossing-over rate in males between N and Yb can be measured both from the (Belém × East Brasil) × Trinidad backcross and from the F_2.

Measurement from the backcross is comparatively straightforward. Among the 81 plain butterflies, which alone can be reliably scored for the heterozygous yellow bar, there is one apparent $N^N yb/N^B Yb$ male, which gives a crossover value of $1.2 \pm 1.2 \%$. Among the 61 radiate butterflies, there are five $N^B N^B$ that appear to lack the shadow of the yellow bar, which therefore seem to be recombinants; this would give a total recombination rate of $6/142 = 4.2 \pm 1.7 \%$. But all these five butterflies have the red hindwing bar of the radiate pattern very strongly developed, and probably this is simply obscuring the shadow of the yellow bar. Even the apparent plain recombinant is a very worn specimen, and it is not absolutely certain that he exhibits a shadow of the bar. If he does not, then 1.2% is a maximum recombination fraction.

The best estimate from the F_2, based on the hypothesis that there is no crossing over in females, has to be obtained by deriving the maximum likelihood equation; as the loci are in coupling, the F_2 is quite informative. If r is the recombination rate in males, then the gametic output of male butterflies is $N^N Yb = N^B yb = \frac{1}{2}(1-r)$, $N^N yb = N^B Yb = \frac{1}{2}r$, and of females $N^N Yb = \frac{1}{2}$, $N^B yb = \frac{1}{2}$. Multiplying these together, and summing within phenotypes, we obtain the top row of table 3. It can be seen that the expected numbers are exactly those given by Bailey (1961) for two loci with complete dominance, with $(1-r)$ substituted for θ, as is appropriate with zero recombination in one sex. From this it is easy to show, following Bailey, that the maximum likelihood estimate of recombination in males is given by the root of

$$nr^2 - (a + 4b + 4c + 3d)\,r + 3(b+c) = 0,$$

where n, a, b, c, d are observed numbers in row 3 of table 3, and that the information

$$I_r = n(3-2r)/2r(1-r)(3-r).$$

The standard error of r is then $1/\sqrt{I_r}$. In the present F_2, the observed numbers are $n = 299$, $a = 212$, $b = 5$ (carrying $N^N yb$), $c = 13$ (carrying $N^B Yb$), $d = 69$, so that the estimate of r is $11.85 \pm 2.18 \%$.

There is thus a considerable discrepancy between the estimates from the backcross and the

F_2, although their 95 % confidence limits do almost overlap if one includes the five radiate butterflies in the backcross among the crossovers. The estimate from the F_2 is in fact a maximum estimate, as there is some doubt about the scoring of two of the classes of recombinant, that is the radiate, no band individuals lacking the yellow bar (apparent bb; N^BYb/N^Byb) and the plain and radiate red + yellow band individuals with the yellow bar (apparent $B-$; N^Nyb/N^Byb),

TABLE 3. EXPECTED AND OBSERVED NUMBERS IN AN F_2 WITH THE LOCI IN COUPLING
AND NO RECOMBINATION IN FEMALES

offspring phenotypes	N^NYb	N^Nyb	N^BYb	N^Byb	total
expected number	$\frac{1}{4}n(3-r)$	$\frac{1}{4}nr$	$\frac{1}{4}nr$	$\frac{1}{4}n(1-r)$	n
expected number[1]	$\frac{1}{4}n(2+\theta)$	$\frac{1}{4}n(1-\theta)$	$\frac{1}{4}n(1-\theta)$	$\frac{1}{4}n\theta$	n
observed number	a	b	c	d	n

[1] In general, for two loci in coupling, with complete dominance, and recombination r_1 in males, r_2 in females, where $\theta = (1-r_1)(1-r_2)$ (Bailey 1961).

of which there are respectively 8 and 5. It is noteworthy that all radiate, no band (or black forewing tip) individuals with the yellow bar have the minute traces of the yellow marks from the band which normally remain in this phenotype placed proximally in the region of the cell spot, whereas the same phenotype without the yellow bar has these traces of yellow placed distally in the region where the outer parts of the yellow band of the Belém race occur. It is likely that this difference is due to a minor effect of the yb allele itself, but it may mean that some at least of the apparent N^BN^B individuals without the yellow bar are in fact N^NN^B with an extreme reduction of yellow in the band, in which event they are not of course crossovers. A rather stronger case can be made out for believing that some of the 'narrow red + yellow band' individuals may be wrongly scored. The genotype BbN^BN^B, which normally has the wide band of the Trinidad and East Brasilian races, can sometimes have a much narrower band in inter-racial hybrids. In this event, some of the red + yellow banded butterflies may be N^BN^Bybyb, and hence not recombinants.

We shall report later what appears to be a dominance reverser of N, which causes hetero-zygotes to look much like N^BN^B homozygotes (§3.7g); this or a similar gene could convert yellow banded individuals to black tips, but the F_2 is very unlikely to be segregating for a simple recessive converter of N^NN^B phenotypes, for this would give a ratio of 10:6 with and without yellow in the forewing bands, which differs markedly from the observed 217:82 ($P = 3.0 \times 10^{-4}$). But if this gene converted only N^NN^Bbb individuals (smudgy yellow band coverted to black tip, no effect on individuals with thin red bands) then the overall ratio would be 23 yellow bands:9 without yellow, which is a very good fit to observation ($P = 0.84$, two-tailed).

It is interesting, and possibly of significance, that such conversion or mis-scoring of N^NN^Bbb butterflies as N^BN^Bbb, allied to mis-scoring of N^BN^BB- as N^NN^BB- (because of intermediate width of the red band), would result in an excess of apparent recombinants over expectation between B and N (what is sometimes called 'negative linkage'); just such an excess has been observed in the F_2, and is statistically significant with a one-tailed test (table 2).

It must also be remembered that in the silkworm, which is the only lepidopteran in which there has been any extensive work on recombination, recombination fractions can vary from 7 to 37 % between the same markers (Hasimoto 1957; Turner 1979; Ebinuma & Yoshitake 1982); however, in the present broods, it is only the backcross that shows a deviant recombination

fraction, the two large F_2 broods (NF2 and CF2) being closely similar in their recombination fractions (five recombinant and 93 parental phenotypes in the former, 11 and 158 in the latter, 2×2 probability 0.86, two-tailed).

A final possibility is that there is an error in the pedigree record, and that the F_1 parents of the backcross were female (this assumes that the apparent crossover is mis-scored). Although this is unlikely, there are a few other apparent pedigree errors for broods bred in the same period and place: it is unlikely from the segregation that brood F1F3 (table A 1) had a Belém × East Brasil F_1 mother (yellow bars should have appeared and fused forewing bands should not), and the parent of CYHW seems to be attributed to the wrong brood (see footnotes to table A 2, appendix 4).

Three further small broods show a low recombination rate between N and Yb, by failing to produce any recombinants. Brood TBF2 (table A 1, appendix 4) is apparently a mating between two coupling heterozygotes; if the next butterfly to emerge had been a recombinant, the recombination fraction would have been $17 \pm 19 \%$. Broods CF3L (table A 1) and CYHW (table A 2) are both backcrosses with male heterozygous parents; the absence of recombinants in a total of 25 offspring gives a maximum recombination fraction of $3.8 \pm 3.8 \%$.

Whichever of the possible explanations of the discrepancy in the $N-Yb$ recombination fraction (misclassification in the F_2 as a result of the segregation of further loci, individual variation in recombination, or an error in recording the sex of the backcross parents) is true, we can conclude that N and Yb are tightly linked, possibly as far apart as 12 %, but quite probably much closer together than this. The two effects are not likely to be produced by a single gene, as all four possible combinations are known, $N^B Yb$ (Trinidad), $N^B yb$ (East Brasil), $N^N Yb$ (Belém), and $N^N yb$ (see §3.5 a below).

(q) Scoring of N genotypes

With no recombination in females, the doubly recombinant genotypes $N^N yb/N^N yb$ and $N^B Yb/N^B Yb$ cannot occur in the F_2, as no individual can receive a recombinant chromosome from his mother. If $N^N N^N$ homozygotes can be scored, it should be possible to confirm the absence of the $N^N yb/N^N yb$ class. According to the usual method of scoring (figure 5), this class should consist of yellow barred butterflies with a Y or TY band. In fact, no such class occurs in the F_2, which points to the conclusion that the mis-scoring that we know to be taking place consists in treating $N^N N^N$ as $N^N N^B$ heterozygotes and not the reverse.

The doubly recombinant $N^B Yb/N^B Yb$ genotype cannot in most instances be distinguished from $N^B Yb/N^B yb$ because of the dominance of Yb. Only in plain (i.e. not radiate) butterflies can these be distinguished by the shadow of the yellow bar in the heterozygote. In the F_2, of the three plain $N^B N^B$ individuals that lack the yellow bar, and that can be scored (the fourth is too damaged), all exhibit the shadow of it on the underside. That is to say they are all $N^B Yb/N^B yb$, as we should expect, and the double recombinant is missing.

(r) Summary

On being crossed with the Belém race, the East Brasilian race (plate 1 c) turns out to have a genotype similar to that from Trinidad (and Venezuela), the plain phenotype being due to the d allele, the wide red forewing band to N^B and B, and the red rather than orange colour to Or, as in that race. The expression at the N locus is somewhat altered, with many $N^N N^N$ homozygotes being not clearly distinguishable from heterozygotes. The East Brasilian race carries an

allele (*C*) that directly removes the cell spot (plate 2*j*) and spreads white scales smoothly on the underside of forewing band (the allele *f*, which performs this task in Trinidad, being absent). The allele N^B also tends to remove the cell spot.

The distinctive yellow forewing line and yellow hindwing bar (plate 2*h*) of the East Brasilian race are produced by the allele *yb*, which produces a shadow of a bar when heterozygous, and which is subject to considerable modification by other, undetermined, factors in its effects on the yellow line. A further locus (*Ub*) may produce partial expression of the yellow bar on the upperside in heterozygotes.

Three remarkable 'atavistic' effects appear in the F_2: a 'wedge and keyhole' effect in the radiate marks of the forewing, and a widening of the hindwing rays so that they appear more like coloured lunules, both being characteristic markings of the related species *H. ethilla* (plate 3*j*, *k*) (these rays on the underside tend also to resemble the markings of *H. cydno*); and a yellow triangular mark in the position of the white triangle characteristic of the East Ecuador race (which is also the position of a yellow wedge in the related *H. nattereri*) (plate 3*j*, right). The first two effects appear to be multigenic; the yellow triangle is produced by the recessive alleles of at least two complementary loci, is enhanced by *yb*, and sometimes completely suppressed by *c* (which simultaneously enhances the neighbouring Belém spot).

All these loci are independent, except for *N* and *Yb*, which are rather tightly linked, and (as before) *B* and *D*.

3.5 *Exceptional genes in the Belém stock*

(a) *Yellow hindwing bar*

We have already noted that some of our Belém stock carried a yellow hindwing bar, mingled with the radiate marks and in the same position as the East Brasilian yellow bar (plate 2*k*) (see §3.1, *Races and phenotypes*). It was variable in expression, and much more strongly expressed in males than in females, who tended to show only the tip of the bar, sometimes only on the underside, mingled with the orange (or red) bar of the radiate marks (see, for example, plate 3*h*, left).

This yellow bar is produced by a single recessive allele: a mating (YM10, table A 1, appendix 4) between a pure Belém female without the bar and a male from the same stock with a brilliant yellow bar produced equal numbers with and without the bar in both sexes (21 with the bar, 19 without); two matings of Belém × Trinidad F_1 butterflies with yellow barred Belém males (YM27 and YM39) produced 64 offspring without bars. As expected from these results, a mating between two butterflies without bars can produce a 3:1 segregation of no bars to bars (YM51; numbers 33:12), and the offspring of a pair of yellow barred butterflies appear to have been all yellow barred (the nine contaminants in YM48).

It is very likely that this recessive yellow bar gene is identical with the *yb* allele of East Brasil (§3. 4*f*), as both are linked to *N*. In brood YM51, a mating between two $N^N N^B$ butterflies without yellow bars, there is a strong association between the $N^N N^N$ genotype (Y band) and the yellow bar, all but two of the offspring in this class having the bar, which is absent in the rest of the brood ($P = 3.1 \times 10^{-9}$). It is likely that the two exceptional butterflies, which are both females, are also $N^N yb / N^N yb$ individuals which have failed to express the bar.

The bar also seems, like that of East Brasil, to produce a shadow in the underside of the hindwing when heterozygous. The effect cannot be seen on radiate butterflies, but one such shadow has been found on a plain butterfly in the Belém × Trinidad F_2 (YM21); the ratio in this case is not informative because the brood is multi-parental.

It seems likely that the yb allele has been introduced into the Belém population from the hybridization zone with the East Brasilian race, in the state of Maranhão, some distance to the east and south, as has the allele B, not found in our stock, but apparently carried by an individual captured at Belém, bearing a thin red band outside its yellow 'Y' band. It has clearly undergone recombination with the N locus, for it would have occurred originally in an $N^{B}yb$ chromosome, and is now travelling as $N^{N}yb$. The bar shows no difference of expression between males and females in East Brasil, nor on a mixed Belém × Trinidad background (§ (b) below), and the quite extreme repression in females appears to be an effect of the pure Belém genome. It is not seen in the East Brasil to Belém backcross, which contains 49 females and 44 males without the yellow bar, and 21 females and 13 males with some, usually slight, expression of the bar, which in this case is heterozygous. This shows, if anything, a slight increase in expression in females ($P = 0.24$, one-tailed). The marked enhancement of expression in males must be confined to $ybyb$ individuals on a full Belém genetic background.

(b) Dominance of N^{N}

A Trinidad female, released into the Belém stock greenhouse, mated with an unseen male to produce the F_1 brood F1BG; this brood and its descendants (all listed in table A 1, appendix 4) showed some exceptional phenotypes. Unfortunately the recorded pedigree seems to be in error in respect of the exact parentage of broods TBF2 and possibly of F1F3, and brood CYHW, recorded as the offspring of a female from TBF2, has so many features characteristic of an East Brasilian ancestry (notably a marked tooth in the forewing band (plate 3i, right)) that it is virtually certain not to belong to the present provenance (it is listed in table A 2, appendix 4, and discussed in § 3.4). However, we can extract some clear conclusions which do not depend on the precise pedigree.

The F_1 butterflies (brood F1BG) showed much stronger yellow than normal for an $N^{N}N^{B}$ butterfly, although not quite so strong as inevitably to cause misclassification as TY, and segregated into two phenotypes differing in the width of the thin red band (plate 3e); these have been listed as TY (narrowed) and TS (widened) in table A 1, appendix 4, but this is purely for convenience, as both of them differ from what is usually meant by those phenotypes.

A female of the 'TY' phenotype was backcrossed to a normal Belém male, to give brood YM16, which segregates roughly as a (Belém × Trinidad) × Belém backcross is expected to, but with some unusual phenotypes. The brood segregates equally into those with and those without red in the forewing band, so showing normal segregation for the B locus, but gives no clear segregation of $N^{N}N^{N}$ and $N^{N}N^{B}$, most of the yellow banded butterflies having a phenotype that could be regarded either as S or Y (plate 3i, left) and the yellow + red ones all having very narrow red marks, and a considerable range of intensity of yellow, frcm the full TY type to a dusty yellow band that could be classified as TS, except that the red is much too narrow. There appears therefore to be some interference with the expression of N, such that the two genotypes are not readily distinguished.

The appearance of two yellow barred butterflies in this backcross brood (YM16) shows that both the Belém parent and the F_1 were $Ybyb$. The yb allele segregates again in the descendants of two of the 'TS' butterflies from F1BG that were mated together. As it appears in the combination $N^{B}yb$, whereas the F_1 must have been $N^{N}yb/N^{B}Yb$ (or even $N^{N}Yb/N^{B}Yb$), it is likely that TBF2 is not, as recorded, the direct result of this mating, as this would require crossing over in both the male and female F_1 parents. It seems either that there is another, unrecorded,

generation in which the recombined chromosome first appeared, or even an unrecorded mating with a butterfly of East Brasilian provenance carrying $N^B yb$. However this may be, broods TBF2 and CF3L give a clear and surprising segregation into yellow barred butterflies with O or W phenotypes (therefore $N^B yb/N^B yb$) and butterflies with a shadow of the bar (exceptionally, this can be seen on the radiate individuals) in CF3L, or no apparent bar in TBF2, which have fully developed yellow marks indicative of the $N^N N^N$ genotype (with very thin red in those that are TY) (plate 3h, left). But this they cannot be: one of these males from TBF2 fathered CF3L, in which the segregation of O and W phenotypes shows that he must have carried N^B. The shadows of the bars in CF3L show that the apparent TY individuals must be carrying yb, and hence must be $N^N Yb/N^B yb$.

Hence the $N^N N^B$ individuals in this stock carry some factor that gives them the full Y or TY phenotype of $N^N N^N$ butterflies, or which, in the backcross, makes the $N^N N^N$ and $N^N N^B$ classes difficult to distinguish. We can regard this as a modifier of dominance at the locus. It may or may not be the same as the enhancer(s) of yellow described in §3.2c (plate 3h, right), and it is not clear whether it comes from the Trinidad or Belém stock. Both the East Brasilian (§3.4) and East Ecuadorian races (§3.7) carry factors that similarly make it hard to distinguish $N^N N^N$ from $N^N N^B$ butterflies.

(c) Summary

Some individuals in the Belém stock carried the yb (yellow bar) allele of the East Brasilian race, probably introduced by introgression of the wild populations; it was travelling in the otherwise unknown combination $N^N yb$, and was much more strongly expressed in males than in females (plate 2k).

In some broods we have detected a modifier of dominance that enhances the expression of yellow to give $N^N N^B$ butterflies, normally of an intermediate phenotype on Belém × Trinidad backgrounds, the appearance of $N^N N^N$ homozygotes (plate 3h).

3.6. *The cross Bolívia × Rio Madeira*

Populations of *melpomene* from the southern part of the upper Amazon basin are recognized as a distinct subspecies (*H. m. madeira*); they differ slightly from the Belém race already described in having the yellow band on the forewing somewhat reduced in size and with a greater tendency for the separate yellow spots that compose it to run together (figure 4d, right butterfly). Our stock of this race was founded by a female from Riozinho (west of Pimento Bueno, state of Rondônia). The Bolívia/Mato Grosso race (*H. m. penelope*), which mimics the sympatric race of *H. erato* discussed later, differs from the Belém race in having the radiate marks consistently red (rather than orange) and in having the yellow forewing band compacted into a comparatively solid yellow patch, like that produced by homozygosity for the Trinidadian allele f, rather than broken up into a group of yellow spots (figure 4e, plate 1i, cf. plate 2d). The red marks are also rather reduced in their extent. Our stock of this race was founded by a female from Areia Branca (km 575 of Cuiaba–Vilhena highway, state of Mato Grosso do Norte).

(a) The hybrids

We crossed an orange Madeira male with a red Bolivian female, obtaining a small F_1 of 31 butterflies (17 males, 14 females) and a smaller F_2 of only nine individuals (four males, five

females). Several of the reciprocal cross (three Madeira females with four Bolivian males), for which siblings of the first cross were used, were combined in one cage to give a large multi-parental F_1 of which 53 are now extant and scorable (23 males, 30 females). Of this F_1, at least five females, mated to sibs, produced a small F_2 of ten individuals (six males, four females), which for scoring we have combined with the other F_2 to give a brood of 19. Only two backcross individuals were obtained, from matings of females from the smaller F_1 with Rio Madeira males.

All the F_1, F_2 and backcross are radiate and have yellow forewing bands (plate 3a, right). Thus it is virtually certain that the major genes controlling these elements of the pattern are identical in both races. As the Rio Madeira race is so similar in appearance to Belém, it is likely that these two are genetically identical, and that all three races are homozygous $D^R N^N b$.

Most F_1 butterflies have broken bands like those of the Madeira parents; in a very few there is a tendency for the yellow marks to fuse together (plate 3a, right), most noticeably by proximal extensions of the Belém spot and of the spot anterior to it (i.e. of the yellow spots between M3, Cu1a and Cu1b (figure 4h)) up to the cubital vein, so that they almost fuse with the cell spot, but also (less obvious and not strongly correlated) by outward expansion of the cell spot toward the end of the cell and inward extension of the distal yellow spots in this area. The numbers are, approximately, broken $29 + 17$, intermediate $7 + 3$, partly fused $17 + 11$ (large mixed F_1 first, smaller single-parent brood second; phenotypes equally distributed between sexes). As no butterfly presents the extreme fusion seen in the Bolivian parents, the fused band can be re-garded as incompletely recessive to the broken Madeira band. Given the range of phenotypic variation in the F_1 and the small size and multiple parenthood of the F_2, we cannot be certain how many loci are involved: of the 19 F_2 butterflies, four have bands very close indeed to the Bolivian phenotype; the remainder are, like the F_1, partly fused or of the broken Madeira phenotype. This is so close to 3:1 as to give credence to a provisional hypothesis that complete fusion of the band is produced by a single recessive allele, which might or might not be identical with f from Trinidad. Both individuals in the Madeira backcross are, as expected, broken banded.

In Bolívia the yellow part of the band immediately outside the cell is narrow, and has a concave distal margin; the Rio Madeira race has this mark broader, with a rather convex margin. The F_1 are nearly all concave (plate 2a, right), and the F_2 segregates ten concave to nine convex. Again, there is a strong suggestion of a single gene, with concave being dominant, provisionally designated Cv.

Two other apparently segregating effects may be due to single recessive genes: reduction of the red radiate marks of the forewing effectively to three broad red lines (as in Bolívia), which appeared in six F_2 butterflies (the F_1 being closer to the Rio Madeira parent), and the reduced (Bolivian) red bar anterior to the hindwing rays, which appeared, uncorrelated with the re-duced forewing marks, in five F_2 individuals. However, both characters are difficult to score, and with an F_2 of only 19 individuals we cannot be certain that we are not observing the segregation of several factors.

Five orange butterflies appeared in the F_2; the remaining 14 of the F_2, the F_1 and the two backcross butterflies were red like the Bolivian parent (the F_1, one of the backcross individuals and half the red individuals in the F_2 being a rather duller red than the parent). It is reasonable to conclude that Bolívia carries $OrOr$, and Rio Madeira $oror$, and likely that the heterozygote in this cross is dull red.

(b) Summary

The Rio Madeira race is probably genetically very similar to the Belém race. The Bolivian race (plate 1*i*) carries *Or*, which produces red (not orange) colouring, and a single recessive factor (possibly the same as Trinidadian *f*) that fuses the yellow band. It differs also by a number of other relatively minor characters which serve to differentiate it, and which may each be under the control of a single gene. These are: concave margin to forewing band (*Cv*) (plate 3*a*, right), reduction of radiate marks on the forewing, and reduction of red bar on the hindwing.

3.7. *The cross Belém × East Ecuador*

The race of *melpomene* found at high altitudes on the eastern slopes of the Andes in central Ecuador (plate 1*d*; figure 4*c*) differs from that at Belém in having no radiate marks, and in having a forewing band that is red (not orange) and white. The band differs in shape from the Belém band in having a white triangle (the Ecuador triangle) to the posterior of the red dumbbell shaped cell spot, and an outer arc of white marks which is short (not extending posterior to vein M3) and is split from the cell spot by a black gutter, so that the overall appearance of the band is that of two marks, an outer white mark and a red and white inner mark produced by the cell spot and the Ecuador triangle. The Belém spot (the yellow spot towards the posterior angle of the wing) is absent in the East Ecuador race. A preliminary cross of the East Ecuador race with Trinidad butterflies was bred by Emsley (1965*b*), but as the phenotypes of the five F_1 progeny are not fully described (their colour for example is not mentioned) we cannot relate his results to ours. We obtained one male from a high altitude Ecuadorian population in the region of Palora (30 km south of Puyo in the Pastaza valley) (sent to us by Dr P. Brakefield) and mated it to four or more Belém females, to produce a large F_1 (the actual number of Belém females that produced viable offspring in this cross is not known) (appendix 4, table A 3, brood M1) (plate 3*b*). We produced three backcrosses to Belém (broods M3, M8 and M9), one of them a literal backcross in that the female parent was one of those that may itself have been a mother of the F_1, and an F_2 was produced by mating two F_1 butterflies (brood M6). A second, larger F_2 became contaminated by a fertile Belém female, from the phenotype ratios apparently mated to a Belém male; although the contaminants can be fairly clearly distinguished by their later dates of emergence, there is some overlap, and this brood has been used only for information on genic interactions (brood M4C, the largely uncontaminated portion, brood M4D, the later-emerging contaminated part).

A further Ecuadorian male was obtained from a lower altitude in the Pastaza valley where the population shows signs of being partly introgressed with the Upper Amazonian populations (race *H. melpomene aglaope*), which resemble the Belém population except in having a reduced yellow forewing band (figure 4*g*). This male differs from the non-introgressed individuals in having only the white outer part of the band clearly present; the areas normally occupied by the cell spot and triangle are filled with smudgy red marks heavily intermingled with black scales. This phenotype is known to collectors as "fraterna" Niepelt. This male fathered an F_1 on a Belém female (brood M7) from which we obtained three very small F_2 broods (M16, M18, M19), five backcrosses of F_1 males to Belém females, and one backcross of an F_1 female to a Belém male (M17, M21, M26, M27, M30, 1A). The effects of the Upper Amazonian alleles carried by this male are discussed in §3.8.

As we failed to establish a pure East Ecuadorian stock, we have no backcrosses to Ecuador.

Some further test crosses have been performed, but as the stock seemed to become progressively less fertile (down to a brood of one in one instance) these are not extensive (appendix 4, table A 3), and we do not have information on all the possible interactions of the loci discovered. Many of the racial differences can be explained by loci already encountered in the previous sections.

(a) Inheritance of radiate versus plain

As in all other crosses, the radiate marks of the Belém race are dominant, the plain colouring of the Ecuadorian race behaving, just as does that of Trinidad, as a single recessive allele (plate 2). Thus both of the F_1 broods are fully radiate, as are the backcrosses to Belém. The F_2 broods combined segregate 25 radiate:3 plain ($P = 0.11$), a satisfactory fit to 3:1. Broods 11A, 12A and 19B are matings between radiate and plain butterflies, which segregate in aggregate 9:14, radiate to plain, a satisfactory fit to a backcross ratio ($P = 0.40$). It is reasonable to suppose that we are observing the segregation of D^R and d, as before.

In the natural hybridization zone of the present race with the Amazonian race below it, individuals can be found with the forewing red marks but lacking the hindwing rays (that is to say, with the 'dennis' pattern, see figure 4f; variety "niepelti" Riffarth). The fact that they appear only in hybrid populations suggests that the D locus may be a complex of two loci, one controlling the forewing marks and the other the hindwing rays (Turner 1971a), the 'dennis' butterflies being recombinants. But from the absence of the reciprocal recombinant (rays alone) it is clear that the explanation cannot be as simple as this.

(b) Inheritance of red and yellow in the forewing band

The Belém forewing band is yellow, that of the non-introgressed Ecuador male parent is largely white, with red chiefly in the cell spot. In the F_1 with this parent all the forewing bands are yellow with a thin red edging to the most distal part (plate 3b), with a thin strip of white between the red and the yellow in a few individuals only. (This is also the phenotype of the F_1 from the introgressed male; as this brood does not segregate for these characters, the male was clearly homozygous for any dominant Ecuadorian genes. Accordingly all Belém backcrosses have been considered together in what follows, a procedure also justified by the fact that those from the introgressed and pure parent segregate in the same way. The introgressed male may have been heterozygous for recessive Ecuadorian genes, but the F_2s of this origin are so small that for convenience they too have been added to those of pure origin.) The ensuing segregation of the F_2 and backcross into bands with various mixtures of red, white and yellow is most simply explained by the segregation of three loci, of which two, N and B, have already been encountered in the Belém × Trinidad cross. Thus the F_2 broods can be divided into those with and without red in the forewing band, which segregate 16:12, a more or less satisfactory fit to a 3:1 ratio ($P = 0.059$), indicating the segregation of a recessive allele that removes red colouring from the forewing band. It seems probable both from its phenotypic effects and from its linkage (below) that the locus involved is the same as the one designated B in the Belém × Trinidad and Belém × East Brasil crosses (above), the Ecuador race being BB like the Trinidad race.

Similarly the F_2 broods can be divided into those in which the yellow forewing band is strongly developed and has a firm, entirely yellow spot within the main cell of the wing, those in which the cell spot is weakened by an invasion of black scales at its anterior edge, and those in which the forewing band lacks yellow scaling altogether, being red, or red with an admixture

of white. The numbers of these phenotypes are $7:12:6$ (one individual not scorable), a close approximation to $1:2:1$, and probably represent the three genotypes $N^N N^N$, $N^N N^B$ and $N^B N^B$ by analogy with the phenotypes segregating in the Belém × Trinidad cross (§ 3.2 above).

In the backcrosses to Belém the amount of red in apparent $B-$ genotypes is considerably lower than in the similar backcross from Trinidad or East Brasil; some have only one or two red scales, in one insect on one wing only, and even when these are included, one of the backcrosses from the pure F_1 (brood M8) shows a significant deficiency of red banded individuals (numbers $15:29$, $P = 0.049$, two-tailed). However, the overall segregation from the pure Belém back-crosses (broods M3, M8 and M9) is nearer to the expected backcross ratio ($48:68$, $P = 0.077$, two-tailed) and that of the introgressed F_1 backcrossed to Belém is a good fit ($64:67$). It is worth examining the hypothesis that *two* recessive loci remove the red marks from the band. In that case, if homozygosity at *either* will remove the red, the expected segregation in the backcross is $1:3$, which is an excellent fit for brood M8 ($P = 0.23$) but an extremely poor fit for the pure backcrosses combined ($P = 1.6 \times 10^{-4}$) and for the introgressed backcrosses ($P = 6.9 \times 10^{-9}$). There is thus little support for the idea that two major loci are involved in the racial difference, but it is clear that the genetic background in this cross reduces the amount of red in $B-$ genotypes, probably so far in some broods as to remove the red altogether from a few butterflies. Red colour is also much more restricted in the present F_1 than in the other racial crosses, both in amount and in distribution (see § (f)).

(c) Inheritance of the yellow or white forewing line

The yellow spot (plate $2j$, right half) at the base of the forewing in Belém butterflies, which is often manifested as a yellow line right along the cubital vein in hybrids with the plain phenotype, has already been shown to be produced by the $N^N N^N$ homozygote (in Suriname (Turner 1972); in Belém × Trinidad (§ 3.2f)). This is also the case in these broods. There is an extremely high correlation throughout between the presence of the basal spot or line and the full development of the cell spot which we take as the mark of an $N^N N^N$ butterfly; no presumed $N^B N^B$ butterfly has the basal yellow. But the invasion of the cell spot by black scales is sometimes very weak, and the cell spot sometimes appears to be reduced in size by other factors (or removed altogether in broods derived from the introgressed male (see § 3.8)). Our examination of the butterflies leads us to suspect that the presence of yellow basal spot is the more reliable way of distinguishing $N^N N^N$ from $N^N N^B$ butterflies in these broods, and the phenotypes have been scored accordingly. In all ten backcrosses combined this gives a segregation of homozygotes to heterozygotes of $112:132$ ($P = 0.22$); this supports the hypothesis that this is a single locus segregation, although there is a suggestion that expressivity may vary between broods, one having a large excess of apparent homozygotes (M8 segregating $33:11$, $P = 0.0013$) and another of apparent heterozygotes (M9 segregating $23:43$, $P = 0.019$).

This diagnosis of the phenotypes is confirmed by brood 3E, in which a male with a red, white and yellow band having a firm cell spot and a very short yellow basal line (visible because the wing was plain) was shown to be $N^N N^N$ by its producing seven offspring with firm cell spots and basal yellow marks when mated to a Belém ($N^N N^N$) female.

In a few $N^N N^B$ heterozygotes there is a white line in the position occupied by the yellow line in homozygotes. It appears in four of the 18 butterflies in the introgressed F_1, in four of the ten heterozygotes in the pure F_2, in four of the eleven heterozygotes in the uncontaminated part (brood M4C) of the other F_2, in one of the eleven heterozygotes in backcross M8, in both

heterozygotes in brood M5 and in one butterfly in test cross 11 A. In backcross M30 one $N^N N^N$ homozygote has a yellow basal spot that continues as a white line. The white line is not present in the pure F_1 or the other backcrosses. There is no simple explanation of these results: the white line appears to be an effect of variable penetrance of the N^N allele when heterozygous on genetic backgrounds that do not contain a large contribution from Belém and it may to some extent be conditioned by recessive genes from East Ecuador. In test cross M23, two butterflies, probably $N^N N^N$ and $N^N N^B$, have partial white bars (in the position of the yellow bar of East Brasil) on the undersides of the hindwings. There is no way of knowing how this phenotype arises.

(d) Inheritance of white colouring

The white colour which in the East Ecuador race occupies most of the band (except for parts of the cell spot which are red) is virtually absent in the F_1 and backcrosses to Belém, and appears in nine out of 28 butterflies in the uncontaminated F_2 (plate 2i); the good approximation to a 3:1 ratio ($P = 0.50$) indicates that white colour is mainly due to a single recessive allele which we shall designate wh. In the absence of the Ecuador backcross, this hypothesis is supported by broods 12A, 11A and 19B, which segregate 4:4, 5:3 and 6:2, with and without white in the upperside, from matings between red and white males and red or red and yellow females ($P = 0.31$ for 1:1 ratio overall). A thin strip of white colouring appears between the yellow marks and the thin outer red edging of the band in ten backcross butterflies (out of a total of 240) and also in a few F_1 individuals. This suggests either slight, variable penetrance of wh when heterozygous or the action of further genes of minor effect. Brood 2A was a mating between a yellow banded backcross butterfly and such a red, white and yellow individual. A strong white strip appeared between the red and yellow marks in one out of four offspring (the fifth is entirely yellow).

In the F_2, white colouring occurs only in the combinations red and white, and red, white and yellow. There are no yellow and white bands. Four hypotheses could explain this. (i) White might be a modification of red colouring, unable to appear in a yellow banded butterfly. In that case white should appear in one-quarter only of the red banded or red and yellow banded butterflies. The ratio is 9:16, which is significantly wide of a 1:3 segregation if white is recessive ($P = 0.015$). The segregation is a better fit to the ratios of white to non-white of 3:1 expected if (ii) white were dominant ($P = 0.16$), or 9:7 if (iii) white were produced by either or both of two recessives ($P = 0.45$). Dominance of white seems to be ruled out by its general absence in the F_1. The two-locus hypothesis could be tested by a backcross to East Ecuador, which we do not have. Certainly, if there are minor loci of partly dominant effect that produce traces of white in the F_1 and backcross, these would increase the number of white individuals in the F_2 above expectation. (iv) The fourth hypothesis is that wh is linked to B. On the basis of the present data this is just as tenable as the two-locus hypothesis.

In the F_2 butterflies, white markings appear not only in the outer part of the band (where they are sandwiched between the red and yellow if yellow pigment is present) and in the Ecuador triangle (when present), but also in the cell spot, which is normally red in East Ecuador. It would appear that the distribution of white colour, as well as its presence, is segregating, but with the present broods it is not possible to determine anything about its inheritance.

(e) Inheritance of the shape of the forewing band

The major differences in the shape of the forewing band of the East Ecuadorian and Belém races are shown in figure 4 (also plate 1*a, d*). The East Ecuadorian band is *short* and *split* (which Emsley (1965*b*) called 'divided'), in that the outermost arc of white markings does not extend posterior of vein M3, and stops around 2 mm outside the cross veins at the end of the cell, leaving the black gutter between this mark and the cell spot, which is a distinctive feature of the East Ecuador race. The band of the Belém race is *long*, in that the outermost arc of yellow markings, which occupy the same position as the outer white marks in East Ecuador, extend to the posterior by one more intervenular space, reaching almost to vein Cu1a, and *entire*, in that the yellow marks nearly touch the outer posterior corner of the cell, bringing them in close proximity with the cell spot. The Belém race has an extra yellow spot in the space between veins Cu1a and Cu1b, which we call the *Belém spot*; the Ecuador race has, in this same space but much nearer the body, in the angle between the cubital vein and vein Cu1b, a white triangle, which we call the *Ecuador triangle*. Both races have the *cell spot*, yellow in Belém, red or white in East Ecuador.

There is an extremely high correlation in all our broods of the short and split phenotypes, and of long and entire (plate 2, compare *(i)* and the left hand of *(j)*). There are a few exceptional butterflies, for example short bands that just touch the cell, but as there is always some minor variation in the shape of the marks in the forewing band, and as some of these exceptions occur in the F_1, it appears that we are dealing with variability of expression rather than with re-combination, and that 'short' and 'split' are effects of the same gene. This is not surprising: all that the gene is doing is reducing all round the size of the outer portion of the band. We shall therefore refer simply to the bands as 'short' and 'long'; the very small number of individuals that have partly developed marks in the space between M3 and Cu1a have been treated arbitrarily as long.

Short bands are inherited as a simple dominant. The pure F_1 (plate 3*b*) is entirely short, the backcrosses of this brood to Belém (M3, M8 and M9) segregate 58 short:58 long, and the pure, uncontaminated F_2 (M6) segregates 10:7 ($P = 0.21$ for 3:1). Two test crosses between long and short banded butterflies segregate 11 short:4 long ($P = 0.12$ for 1:1). We designate the short and long alleles as S and s respectively. As the introgressed F_1 segregated for this character, it and its descendants are discussed in §3.8*c*.

The *Ecuador triangle* (plate 3*b, f*) is inherited as a recessive. It is usually represented by fragments in the F_1, is largely absent in the backcross to Belém, and is fully developed in two of the 17 butterflies in the pure, uncontaminated F_2; a further three have the mark partly developed (expected number 4¼). In the less contaminated portion of the contaminated F_2 there are four fully developed triangles and four partly developed in a brood of 16 (expected number 4); there are four more in the more contaminated portion (M4D). Brood 11A, a mating between two F_2 butterflies, one with a fully developed triangle and the other with a small coloured mark in this position, appears to be a backcross, with two fully developed triangles, five fragments, and one completely absent. We call the allele responsible for the fully developed triangle *t*.

It is unlikely that *t* is the same as either of the triangle genes (*tr-1* and *tr-2*) noted in the East Brasil cross (§3.4*j*), as the triangle that these produce is almost entirely suppressed by the presence of the cell spot, whereas the present triangle inevitably occurs with the cell spot. However we

should note that (i) the difference might be an effect of the genetic background, and (ii) the hint of a multilocus segregation given by the numbers of fully and partly developed triangles in the present F_2 suggests that East Ecuador may differ from Belém at the *Tr-1* or *Tr-2* loci, either or both of which may be segregating in addition to *T*.

(f) Amount and distribution of red

In the crosses studied so far (involving Trinidad, Venezuelan, Belém, East Brasilian and Suriname populations), *H. melpomene* shows a radical difference from *H. erato* in the control of the forewing band. In *erato* (Turner & Crane 1962; Sheppard 1963; §4 below), a single gene alters the colour of the band from red to yellow, independently of the shape of the band, which is controlled by a quite different set of genes; thus one can have broken and fused bands of both colours. In *melpomene* the factor controlling the shape of the yellow marks (*F*) has been found to have no effect at all on the distribution of red, so that in the F_1 hybrids of Belém with Trinidad, with Venezuela and with East Brasil, and of Suriname with Trinidad, the red and yellow marks appear to have drawn up a battle line across the middle of the wing. Distal to this is the outer edge of the wide red band, not broken into spots in any way; inside the line is part of the yellow Belém band, broken up into spots (see figure 5, or plate 2*a–c*, *e*). Conversion of the genotype from *Ff* to *ff* fuses the yellow marks, but leaves the red marks unaltered (plate 2*d*). Conversion from $N^N N^B$ to $N^B N^B$ produces a victory for the red marks, which simply sweep across the wing to produce the full red band, a solid red patch whether or not the butterfly is *Ff* or *ff* (plate 2*h*).

With this type of control a broken red band like that seen in some *H. erato* is impossible. Yet in our Ecuadorian × Belém F_2 this is just what has appeared: a Belém-shaped band that is entirely red (plate 2*l*).

Although our broods are probably not sufficient to elucidate the genetics of this phenotype completely, especially as both mated representatives happened to be sterile, we can make some reasonable hypotheses.

The factors that affect band shape in the East Ecuadorian race control the distribution of all three colours: white, yellow and red. In the F_2 there are insects with bands that are entirely red (or red and white), but that show no tendency to have a wide, Trinidad-type band; the band is short and split, and the Ecuador triangle, converted to red, and cell spot, are well defined (plates 2*i*, 3*c*, left). In the F_1 with Belém there is no appearance of the edge of a wide red band in the posterior distal part of the wing as in the Trinidad × Belém F_1 broods; the red edging stops at vein M3 just as does the rest of the band (plate 3*b*). Therefore there are one or more genes in the East Ecuadorian race that cause an effect that we shall call *restriction of red*, the tendency of red pigment in the band to respond to genes controlling band shape. Its function is clearly to break up those parts of the band that are red into the characteristic Ecuador pattern; without it the Ecuadorian genotype $N^B N^B bb$ would produce a wide red band as in Trinidad. The three individuals with the red broken bands therefore appear to result from a combination of the genes that produce the Belém shaped band (*c*, *s*, *T* and *F*) with the factor(s) for restriction of red.

How many genes are involved in the restriction of red is not clear. Evidence will be presented elsewhere that the effect can segregate in a 1:1 ratio (Turner *et al.* 1985), suggesting a single pair of alleles. In that case the phenotype of the present F_1 butterflies suggests that restriction is dominant, and that East Ecuador is homozygous, say *RrRr*, and that Belém is homozygous *rrrr*.

As red is not restricted in any other of our F_1s, it would appear that the Trinidad, Venezuelan, East Brasilian and Suriname populations are also *rrr*.

In that event, our backcrosses to Belém should segregate 50 % of red banded butterflies in which the red is not restricted. That they have not done so may be the result of the considerable reduction in the amount of red in those butterflies. As the red is confined to a very narrow red edging to the most apical part of the band, much smaller in extent than the band itself, even in $N^N N^B B-$ genotypes it is not easy to say whether the genes controlling band shape are cutting off the red at vein M3 (in short bands) or vein Cu1a (in long bands); the red tends not to reach that far in any case.

We should, however, expect to see unrestricted red butterflies in the F_2. No butterfly in these broods has the thin red band characteristic of F_1 hybrids between Belém and the other races, but there is one butterfly (plate 3*c*, right) in the uncontaminated F_2 with a red and white band (apparent $N^B N^B B-$ *whwh*) in which the white apical part of the band is short and split, and the white Ecuador triangle well defined, but in which the red marks, although liberally sprinkled with black scales except in the cell spot and the vicinity of the triangle, are spread right across the outer part of the forewing (including most of the region between the cell spot and the band), to produce what is roughly a smudged version of the Trinidad/Venezuela band. One further very strange phenotype in the contaminated F_2 may represent an $N^N N^B$ heterozygote in which red is not restricted (plate 3*d*, right). It has a long outer red band roughly of the type found in the Belém × Trinidad and Belém × East Brasil F_1, edged inwardly with a short split white band and a red and white cell spot. The general appearance is what we should expect of an $N^N N^B$ genotype with unrestricted red, except for the red in the cell spot (seen in one male F_1 insect also) and the absence of yellow. It is possible, however, that yellow has been removed by the gene described in the next section. (If this is not the correct explanation of the phenotype, then the phenotype is long and split, which suggests recombination within the supposed S gene.) These two individuals, in 32 of the F_2 that have some red in the band and might therefore be expected to manifest the unrestricted phenotype, give a barely satisfactory fit to a 3:1 ratio ($P = 0.013$); they are of course a perfect fit to the expected 15:1 ratio for two recessive genes. The fact that the wide band is not completely manifested in the homozygous individual suggests that the control of this pattern is rather more complicated than we have suggested. However the Rr locus provides a reasonable working hypothesis until further crosses can be performed.

Even in the presence of the restricted red factors, a wide red band should have appeared if the *ff* genotype were segregating in the F_2; certainly this genotype should have produced fused yellow bands in which the cell spot is joined to the rest of the band (plates 2*d*, 3*h*). The complete absence of these indicates either that the East Ecuador race is, like Belém, carrying the genotype *FF*, or that *f* is an allele of or tightly linked to one of the loci known only from this cross, of which S is the most likely candidate. In that case East Ecuador would be $F^S F^S$, Belém and East Brasil *FF*, and Trinidad *ff*. Provisionally we shall assume that *F* is an independent locus.

(g) *Expression of* $N^N N^B$ *heterozygotes*

We have seen that $N^N N^B$ butterflies are often hard to distinguish from $N^N N^N$ in this cross, having a strongly developed yellow band; the allele N^N is almost dominant. This effect is seen also in the Belém × East Brasil cross (§3.4) and was produced in some broods only in the Belém ×

Trinidad crosses, which appeared to be segregating for a gene that enhanced the expression of yellow in heterozygotes to make them similar to homozygotes (plate $3h$). It seems to be that in some of the present broods this dominance is reversed, and that heterozygotes have so much red colouring that they can barely be distinguished from $N^B N^B$ homozygotes. Brood 19B is a mating between an obvious $N^N N^N$ male (complete with yellow basal spot; plate $3g$, left) and a female from the Belém backcross that from its phenotype is $N^N N^B$; from its parentage this female certainly cannot be $N^B N^B$. The eight offspring consist of three normal $N^N N^N$ homozygotes (one of them confirmed by producing a non-segregating $N^N N^N$ brood (3E) with a Belém female), three normal heterozygous phenotypes and one male and one female whose bands are a mixture of red, mostly distally, and white, mostly proximally and in the cell spot (plate $3g$, right). There are a few traces of yellow, stronger on the underside. The butterflies cannot be $N^B N^B$, and are apparently heterozygotes in which the strong development of white (due to $whwh$) has largely suppressed the usual yellow marks. The male $N^N N^N$ parent of this brood came from a mating (brood 11A) between two F_2 butterflies (brood M4D), one of which was either $N^N N^N$ or $N^N N^B$ (the basal yellow spot cannot be scored because the specimen was partly eaten by greenhouse scavengers), the other being a butterfly whose band is red with a few touches of white. Like its two grandchildren already described, this butterfly cannot have been $N^B N^B$, for it has produced an $N^N N^N$ offspring, and as it is improbable that it was $N^N N^N$ we must conclude that it too is a much reddened heterozygote, even more extreme than the other two. As this means that mating 11A is likely to be a cross between two heterozygotes, it is difficult to tell whether any of the red banded offspring are similarly modified butterflies, as $N^B N^B$ should in any case be segregating. The brood consists of one definite $N^N N^N$ homozygote (the male parent of 19B already mentioned), one butterfly that is either $N^N N^N$ or $N^N N^B$, one normal $N^N N^B$ heterozygote, and five strongly red banded butterflies, three of which contain yellow and white, and two of which white only. If all these five are regarded as $N^B N^B$ then the brood departs significantly from the expected ratio of 3 N^N–:1 $N^B N^B$ ($P = 0.027$, one-tailed), which certainly suggests that some of the heterozygotes carry excess red: one particularly interesting male has a red and white band with touches of yellow, almost identical to his nephew and niece in brood 19B which we described first (plate $3g$, left).

A further mating between two butterflies from brood 11A, the female with a red band, the male with a red and white band with traces of yellow, gave a similar segregation: two manifest heterozygotes, four red banded butterflies and two red and white bands showing traces of yellow (a further $N^N N^N$ homozygote is almost certainly a pure Belém butterfly included by mistake). Although the deficiency of heterozygotes is not significant in this case ($P = 0.14$, one-tailed), the segregation is consistent with extreme reddening of some of this class, and also of the male parent (brood 12A).

We conclude that a gene or genes, probably recessive, is capable of almost reversing the dominance at the N locus by greatly increasing the amount of red in heterozygotes. If it is a single gene, it cannot be at high frequency in the Belém stock as its effects are not seen in Belém backcrosses, and it therefore must originate in East Ecuador. (There is one insect apparently of this phenotype in brood M24, which has a Belém $N^N N^N$ mother and an $N^B N^B$ father, and so must be $N^N N^B$; possibly the Belém individual did carry the reversing gene.) The simplest explanation of the broods is that the red F_2 male was a homozygote and his mate heterozygous, causing half the $N^N N^B$ butterflies to be reddened in brood 11A. The $N^N N^N$ male from this brood that fathered 19B was apparently homozygous, and his backcross mate heterozygous,

again producing modification of half the $N^N N^N$ offspring to red banded. The father of brood 12A appears also to have been a homozygote, mated to a heterozygous sibling.

A similar effect has already been encountered in crosses with the Trinidad race, although there its presence was inferred from phenotypes, without its being observed segregating as in the present cross. Turner & Crane (1962) reported that $N^B N^B bb$ butterflies had a thin red forewing band when the genetic background was strongly Trinidad/Venezuelan; in later crosses, with a larger background contribution from Suriname or Belém (Turner 1972; present paper, §3.2*b*), this genotype has no red in the band (plate 2*g*).

(*h*) Inheritance of the Belém spot

The most posterior spot of the forewing band, called here the Belém spot (figure 4*h*, plate 2*j*, *h*), is absent in East Ecuador. At the date of writing (1979) it is very variable from a large yellow spot to completely absent, in the Belém stock; this possibly results from contamination or the accumulation of mutations, allied with the effects of random drift, since the stock was founded in 1971. It is strongly developed in most wild-caught individuals of this race, and probably in most of the stock individuals at the time of the experiments (1975).

The spot is variably present or absent in the F_1. Its inheritance in the backcross to Belém has been analysed by examining one large brood (M9) in detail. The Belém spot has been arbitrarily scored in four categories: large, small, minute (frequently asymmetrical on the two wings) and absent. Tabulation of the extant butterflies (not quite the whole brood) against segregation of the N and S loci produces the following pattern:

	long (*ss*)		short (*Ss*)	
Belém spot	$N^N N^N$	$N^B N^B$	$N^N N^N$	$N^N N^B$
large	7	10	3	2
small	0	4	1	3
minute	0	3	5	8
absent	0	1	4	6

Condensation into 2 × 2 tables (by combining the top and bottom pairs of rows) shows a strong association of the spot and the S locus ($P = 5.4 \times 10^{-5}$, two-tailed) but no clear association with N; there is however a suggestion that, if the band is long, then the genotype $N^N N^N$ tends to enlarge the spot ($P = 0.081$, two-tailed). It is clear from the large numbers of short banded individuals with large or small spots, and the smaller number of long banded ones with the spot minute or absent, that there are other factors, genetic or environmental, besides the S locus, influencing the spot, but their nature cannot be determined from the present cross.

(*i*) Inheritance of red versus orange

The red colour of the East Ecuador race versus the Belém orange colour (plates 1, 2) is inherited in the manner encountered in all other crosses, a single locus with orange recessive. Both F_1 broods are red. Two of the backcross broods (M26 and 1A) having a red Belém parent are entirely red; those that segregate (M8, M9, M21, M27 and M30) give 72 red:85 orange ($P = 0.34$ for 1:1). Those F_2 individuals that have been scored (M6 and M16) give a ratio of 13 red:11 orange; the rather poor fit to 3:1 ($P = 0.043$) probably only reflects scoring problems arising because the largest brood was not scored until it had begun to fade. It is reasonable to suppose that it is the *Or* locus that is segregating.

(j) Linkage groups

Linkage can be excluded if recombinants appear in brood 1A, which has a multiply hetero-
zygous female parent, or for loci in repulsion, if they appear in the F_2. The loci N and B are
shown to be unlinked by the appearance of the recombinant phenotypes 'no yellow line, no
red in band' ($N^N N^B bb$) and 'yellow line, red edge to band' ($N^N N^N B-$) in the numbers 10 and
14 respectively in brood 1A.

The following repulsion pairs are shown to be unlinked by the appearance of the double
recessive phenotype in the F_2 (including the contaminated one, as the phenotypes in question
could not have appeared by contamination, but excluding the introgressed F_2 for S and T
because of possible initial heterozygosity): B and T (one no red in band, triangle, *bbtt* plus
several less certain ones with partly developed triangles), D and S (three plain, long, *ddss*), N
and T (two yellow line, triangle, $N^N N^N tt$, plus a few more with partly developed triangles),
N and S (two red band, long, $N^B N^B ss$), Or and Wh (four orange, white, *ororwhwh*), Or and T
(five orange, triangle, *orortt*, plus one orange with a partly developed triangle), N and Wh (two
yellow line, white, $N^N N^N whwh$) and N and D (one yellow line, plain, $N^N N^N dd$). A further five
pairs that segregate in the large backcrosses with male F_1 parents (broods M3, M8, M9, M21,
M27 and M30; M17 and M26 excluded because of doubts about scoring or lack of segregation
for S and *or*) fail to show any significant association and are apparently unlinked. These are
(with the one-tailed binomial probability of obtaining the observed numbers of parental and
recombinant phenotypes if the ratio were in fact 1:1) B and S (85 parental, 82 recombinant;
$P = 0.88$) and N and S (87 parental, 79 recombinant; $P = 0.59$) and (with a 2×2 contin-
gency test, probabilities one-tailed, on account of scoring difficulties with the Or locus) Or and B
(no test, recombinants exceed parentals), Or and S ($P = 0.21$), Or and N (no test, recombi-
nants exceed parentals). Or and B show what would be a significant excess of recombinants
if the test were two-tailed ($P = 0.0014$). In broods M21, M27 and M30, which were scored
by P.M.S., who appears to be a more accurate assessor of orange and red, when fresher than
the others, recombinants exceed parentals for all three pairs, and again the excess is rather large
for Or and B ($P = 0.12$, two-tailed). Thus neither from the smaller subset scored by P.M.S.,
nor from the complete set of backcrosses, is there any indication of linkage; the excess of
recombinants between Or and B is apparently not due to mis-scoring, and is unexplained.

The remaining pairs have to be tested for association in the rather small F_2; in addition, as
apparent *oror* genotypes can be produced by fading and mis-scoring, all repulsion pairs in-
volving this locus have been re-tested in the F_2. Except for Or, which was not reliably scored
except in M18, and S and T, which were possibly heterozygous in the Ecuadorian male parent,
the small introgressed F_2 broods have been added to the pure brood (M6) in performing the
tests. The following pairs are unlinked (exact one-tailed probabilities for 2×2 tables given; no
test if the recombinant types exceed the parentals): Or and Wh (no test), Or and D ($P = 0.35$),
Or and T ($P = 0.56$), Wh and T ($P = 0.31$), Wh and S (no test), T and D ($P = 0.51$) and
(two probabilities given; by combining $N^N N^N$ with the heterozygotes, and combining $N^B N^B$
with the heterozygotes) N and Wh ($P = 0.23$; $P = 0.057$; χ^2_2 for 2×3 table without Yates's
correction is equal to 4.39, $P = 0.11$; re-tested because only one of the recombinant classes
appeared) and N and D ($P = 0.11$; $P = 0.39$; re-tested because only *one* double recessive
appeared). There is no strong reason for regarding N and Wh as linked; Or, D and N are
clearly independent as usual.

This leaves four pairs that are under suspicion of being linked from having produced no recombinant phenotypes in the F_2, and/or showing significant associations in the F_2. B and D produced no *bbdd* phenotypes (plain, no red in band) in any F_2 brood, but give a 2×2 probability of 0.25. However, this arises largely because there are only three *dd* individuals in the non-contaminated F_2. If we perform the alternative test of observing the plain (*dd*) butterflies in all the F_2 broods, including the contaminated one (plain butterflies could not have been produced by the contamination), we find that there are 12 of them, all B–. The expected numbers are 9 B–:3 *bb*, and the deficiency is significant ($P = 0.016$, one-tailed). There is thus a good case for thinking that D is linked to the locus that removes red marks from the forewing band, and that is our reason for regarding this as the same B locus found in the previous crosses.

There are no recombinants between *wh* and B (white, no red in band) in any F_2, and the association in the uncontaminated broods is significant ($P = 0.027$), as is the association between the coupling pair, *Wh* and D ($P = 0.026$). Without the backcross to East Ecuador, it is difficult to tell whether this results from *Wh* being linked to B and D, or from *wh* being expressed only in the presence of B, as suggested in §3.7*d* (above). An analysis of linkage between *Wh* and D (Bailey 1961) gives a marginally significant χ^2 (without Yates's correction, $\chi^2_1 = 3.11$, $P = 0.039$, one-tailed) and an estimated recombination fraction of 0.46 ± 0.15. These loci could therefore be linked rather far apart, with recombination of somewhere between 20 and 50 %. On the other hand if *wh* were expressed only with B–, there would also be an association between D and *wh*. For with no female recombination the F_2 brood must segregate radiate, red in band:radiate, no red in band:plain, red in band in the ratio 2:1:1, and *wh* acting as a modifier of B– would divide this further into the classes radiate, red, no white: radiate, red, white:radiate, no red, no white:plain, red, no white:plain red, white in the ratio 6:2:4:3:1, which on regrouping gives radiate, no white:radiate, white:plain, no white:plain, white in the ratio 10:2:3:1. The observed numbers are 19:6:0:3, for which χ^2_3 with Yates's correction is 5.82, $P = 0.12$. Therefore not only is the association of *Wh* and B compatible with either epistasis or linkage between the loci (particularly if white is produced also by some minor loci), but the association such as it is between D and *Wh* can be interpreted equally as linkage between D and *Wh*, or as the known linkage between D and B plus epistasis between B and *Wh*. We are therefore quite uncertain whether *Wh* is linked to B and D or not. The fact that the missing class in the F_2 (plain, no white) is still lacking in the larger number of plain butterflies in the contaminated F_2 tips the balance slightly in favour of linkage, and this is the scheme that we have, provisionally, adopted.

The association between T and S, which fail to produce the recombinant class triangle, long (*ttss*) in the F_2, is also significant (2×2 probability in brood M6 is 0.041, one-tailed). An alternative test is that if the loci are not linked, short banded butterflies, which cannot be produced by the contamination, should segregate 1:3 with and without the triangle in the pure and contaminated F_2. The actual numbers are 21:14 ($P = 1.2 \times 10^{-5}$, one-tailed), if partly developed triangles are regarded as *tt*. On the basis of this, the simplest interpretation, the triangle and short loci must clearly be linked. A more complicated interpretation can be obtained by regarding partly developed triangles as T–, with some additional recessive modifier producing the partial development. In that case the latter segregation is 11:24 ($P = 0.24$, one-tailed, for 1:3), and the T locus is independent of S, but S is linked to the modifier.

The simplest way of interpreting this scheme would be to suppose that T was identical with the triangle suppressor $Tr\text{-}2$ already found in Belém (§3.4j), and that the 'linked' modifier was simply an effect of S, which would have the effects of producing a part triangle and suppressing the Belém spot, in addition to its main effect of shortening and splitting the band. This would be consonant with the quite large fragments of the triangle that appear in about half the F_1 butterflies. Attractive as this scheme is, in view of the limited amount of data we shall adhere to the simpler hypothesis that S is linked to T, while noting that (a) we do not know how T is related to $Tr\text{-}2$ and (b) the alternative scheme is very plausible.

The simplest alternative schemes can therefore be summed up as

	Belém	$tr\text{-}1$	$Tr\text{-}2$	sT
	East Brasil	$Tr\text{-}1$	$tr\text{-}2$	sT
	East Ecuador	$tr\text{-}1$	$Tr\text{-}2(?)$	St
or				
	Belém	$tr\text{-}1$	$Tr\text{-}2(= T)$	s
	East Brasil	$Tr\text{-}1$	$tr\text{-}2(= t)$	s
	East Ecuador	$tr\text{-}1$	$tr\text{-}2(= t)$	S

The cross East Ecuador × East Brasil is required for an informed choice between them.

It is not possible to tell whether S and T are loosely or closely linked, nor indeed whether they are effects of the same gene. There appear to be no known individuals with long bands and triangles in the natural hybrid zone of the East Ecuador and Upper Amazon (long, no triangle) races, but this zone is nowhere nearly as intensively collected as the Guiana hybrid zone, so that they might be absent from collections, even with a rather high crossover rate. The race on the upper Río Santiago in Ecuador (not illustrated, but listed as race 6b in appendix 1) has the phenotype radiate, short yellow band, no cell spot, no triangle, and could therefore be homozygous $SSTT$. We shall, provisionally, treat the system as a pair of linked loci.

To summarize, in this cross the pairs B and D and S and T are linked; Wh may be linked to B and D, or may simply be a modifier of B. All other loci, checked in all possible pairwise combinations by one or more of the given criteria (appearance of recombinants in a backcross from a heterozygous female or, in repulsion, in an F_2, or contingency tests on a backcross from a heterozygous male parent or, in coupling, in an F_2), are independent of these two groups, and of each other. For the loci already encountered in other crosses (Or, N, B and D) this is fully consistent with the previous findings.

(l) Summary

The East Ecuadorian race resembles that from Trinidad in having a pattern consisting solely of a forewing band, produced by the alleles d (plain), N^B and B (red forewing band), Or (red) and Yb (no yellow bar or line). The characteristic shape and colour of the East Ecuadorian band (plate 1d) is produced by a gene that modifies most of the red colour to white (Wh) (this may be linked to B rather than being a modifier), one that splits off the outer part of the band from the inner part and shortens it (S) (plate 2i), another, either linked to S or a pleiotropic effect of it, that adds the triangular mark (t) (plate 3f), and another that adds the adjacent cell spot (c) (plate 2j). In most other races S appears able to split yellow and white markings only; the residue of red colour in the East Ecuadorian band is made to conform to the split

phenotype produced by S by the action of a further gene (Rr, restricted red), which also has the incidental effect of making the red marks respond to the F allele from Belém (the genotype of East Ecuador at this locus is a little uncertain) (plate 2l). The East Ecuador race also carries a recessive gene which almost reverses dominance at the N locus by making the N^N (Amazonian) allele recessive (plate 3g), and there is some suppression of expression at the B locus in some broods.

3.8. The genetics of the Upper Amazonian race

The Amazon basin is largely occupied by races of $H.$ *melpomene* that are yellow banded and radiate (figure 1, numbers 5–9). Those from the upper part of the basin differ from the Belém race in having a yellow band that at first sight looks rather like the outer, white portion of the East Ecuador band (figure 4g). However, a closer look shows that, apart from minor differences in the shape of its outer margin, the Upper Amazonian band is the same as the outer part of the Belém band, being *long* (extending to vein Cu1a), and *entire* (almost touching the cell), differing in both of these features from the East Ecuador band. What distinguishes the Upper Amazon band is that it lacks the cell spot and the Belém spot. This difference can be seen in figure 4, or by comparing the left (Belém) wing of the mosaic insect in plate 2j with the right (Upper Amazon) wing, and can be summarized as follows:

	Belém	Upper Amazon	East Ecuador
cell spot	yes	no	yes
Belém spot	yes	no	no
Ecuador triangle	no	no	yes
outer band	entire, long	entire, long	split, short
band colour	yellow	yellow	red and white
wing base	radiate	radiate	plain
'red' areas	orange (normally)	orange	red

We have not hybridized any butterflies from the Upper Amazon race, although we have bred it, briefly, in captivity, but as it hybridizes with the East Ecuador race where these meet on the slopes of the Andes, our partly introgressed Ecuadorian male (see §3.7) and another introgressed male to be reported elsewhere (Turner *et al.* 1985) provide enough information about the Upper Amazonian genes that they were carrying to give an extensive picture of the genetic make-up of the Upper Amazon race.

(a) Genetic affinities with the Belém race

An introgressed male bred by Gilbert was of Amazonian phenotype, that is, it was orange, radiate and yellow banded, but the band was of Ecuadorian shape (the form known as "mimetica" Neustetter to butterfly collectors). Elsewhere we will present evidence from crosses made with this butterfly to show that the radiate marks and yellow band, which must be of Upper Amazon origin, were produced by the alleles D^R, N^N and b (Turner *et al.* 1985). These are the same alleles as are found in the Belém race, a conclusion supported by the absence of any phenotypes that could be interpreted as due to interlocus complementation at the meeting places of the different Amazonian races within the Amazon basin. In those features in which they are similar, the Upper Amazonian and Belém races are genetically identical, as are apparently the Rio Madeira and Bolívian races (§3.6).

(b) Inheritance of the cell spot

The cell spot (plate 2*j*), absent in our introgressed male, although replaced by a red smudge, segregated 7 butterflies with the spot: 9 without in the F_1 mating to a Belém female (appendix 4, table A 3, brood M7). The male was clearly heterozygous for a dominant, or nearly dominant, gene suppressing the spot. As expected with a segregating F_1, some of the backcrosses to Belém segregate equal numbers with and without the spot (broods M17, M21, M26, M30 and 1A; total numbers 55 with:41 without; $P = 0.18$); one backcross (M27) consists entirely of recessives, with the cell spot. There is some ambiguity in brood M21, in which seven butterflies have partly developed spots, which have been scored in the recessive category for the total count. One of the small F_2 broods (M16) was a cross between two spotted individuals; as expected all five offspring have the spot. Another (M18) had both parents lacking the spot and produced one offspring with and one without the spot. This and brood 2A (3 without:2 with the spot from a mating between two non-spotted parents) confirm that the presence of the cell spot is recessive.

It seems reasonable to suppose, although there is no direct evidence on this point, that the absence of the cell spot in the Upper Amazon is produced by the same locus (*C*) and possibly by the same dominant allele, which removes it from the East Brasilian race.

(c) Inheritance of long forewing band and triangle

The introgressed Ecuador male was apparently an sT/St heterozygote, having itself a short band (short being dominant) and no triangle (triangle being recessive). Mated to a Belém female (sT/sT), this male produced, in brood M7, as expected equal numbers of short (8) and long (10) bands, and these butterflies backcrossed to Belém gave two kinds of brood, those segregating equally for long and short bands (broods M17, M21, M27 and M30; 24 long:32 short; $P = 0.35$), and those that are entirely long (ss) (broods M26 and 1A). The F_2 broods M16 and M18, which are both short × short matings, produced three short and four long individuals. The original male is shown to have carried the *t* gene by the segregation of three individuals with Ecuador triangles in one of the little F_2 broods (M16); as expected if *S* and *T* are linked (see §3.7*j*), all these individuals are short banded, although with the very small numbers (five individuals only), the association of the phenotypes is not significant ($P = 0.10$, one-tailed).

(d) Inheritance of the Belém spot

As remarked in the previous section, the Belém spot (figure 4*h*) is absent in the Ecuador race and variably present or absent in the Belém race. It is absent in the Upper Amazon race. This mark is influenced in this cross by the cell spot gene. In brood M26, a backcross to a Belém female that lacked the Belém spot, the presence of the Belém spot, at least in traces, is perfectly correlated with the presence of the cell spot, there being 12 individuals with both marks, 21 with neither; and none in the two other possible categories (2 × 2 exact probability 5.6×10^{-9}, two-tailed, four not scored). There is no correlation of the Belém spot with the genotypes $N^N N^N$ and $N^N N^B$. The presence of the cell spot in all individuals in brood M27 (also a Belém backcross) seems to be the cause of all butterflies having the Belém spot; as would be expected from our previous findings (§3.7*h*), the spot is larger in the long banded individuals than in the short.

From this and the East Ecuador crosses, we conclude that development of the Belém spot is largely independent of the N locus, but is positively influenced by the cc (cell spot) genotype, and the ss (long) genotype. It is possible that $N^N N^N$ enhances expression in $ccss$ genotypes.

Whether there are any further genes that condition these two genotypes to produce the spot is an open question: as some Belém ($ccss$) butterflies lack the Belém spot, it seems likely that there are.

This is also the case in the backcross (brood 1A) from the F_1 female to a Belém male possessing the Belém spot. With two exceptions, all individuals with the cell spot have a well developed Belém spot, and all other individuals lack both marks. The exceptions are a female with a cell spot and no Belém spot, which is believed to have been included in this brood as the result of a labelling error, and the right side of one of the mosaics (see the next section, §(e) below), which has no Belém spot, but a curious faintly developed cell spot. Although it has been tabulated as having the cell spot, it is possible that we are seeing a case of penetrance in a Cc heterozygote, or with some other developmental anomaly.

The cell spot and Belém spot could be controlled by separate genes; the backcross with a male F_1 parent (brood M26) shows that they would be very tightly linked. Provisionally the two effects will be treated as arising from one gene.

It is possible that the development of the cell spot and of the triangle are also correlated. First, the triangle lies between the areas occupied by the other two marks, and it is not unlikely that a gene affecting developmental processes on either side of the triangle should affect the middle ground as well. Secondly, the triangle and Belém spots occupy the two extreme ends of an area that is occupied by a single yellow mark in the related species *Heliconius nattereri*, which may have a pattern similar to the ancestral pattern of *H. melpomene* (see §5.7 below). Thirdly, some of the Ecuadorian hybrids in the present broods have a few fragments of the triangle, forming a mark like a C on its back, with the outer limb appearing as a small fragment of the Belém spot (plate 3*j*, left). Around 60 individuals, all with cell spots, have such marks; only in the introgressed F1 (M7) and its Belém backcross (M26) do the marks appear in sufficient numbers with a segregating cell spot for the association to be tested. There are 11 with both marks, 29 with neither, 13 with cell spots but no trace of the triangle, and none with fragments of triangles but lacking the cell spot. As the association is clearly significant (2×2 probability 6.5×10^{-5}, two-tailed), we can conclude that in some circumstances the cc (cell spot) homozygote permits the development of fragments of the Ecuadorian triangle, which seem to be invariably removed by the dominant allele C. It would appear (as we have shown in §3.4*k*) that there are genes in the Belém race (which has the cell spot and Belém spot, but no triangle) that modify the effects of cc into pigmenting the outer part of the area between Cu1a and Cu1b, so producing a Belém spot but no triangle; in East Ecuador (cell spot, triangle, no Belém spot) the effects of cc are reversed, so that it enhances the development of the inner area which forms the triangle, but produces no Belém spot in the outer area. One gene that is clearly instrumental in doing this is S, or something linked to it. The chromosome bearing S tends to suppress the Belém spot, and has also a major effect in producing a fully developed triangle, which we designate as the t gene. The C-shaped mark found in many of our hybrids must result from the breakdown of this proximal–distal developmental switch, so that bits of both the triangle and the Belém spot appear together.

(e) Mosaic phenotypes

The mating of an F_1 daughter of the introgressed male (brood M7) to a Belém individual, which gave our only backcross with a female parent, produced among 35 offspring two remarkable butterflies, the more spectacular of which is seen in plate $2j$, which were regular mosaics both for sex and for the phenotypes conditioned by the loci B, C and (with some scoring difficulty) N. It is hoped to discuss these insects fully elsewhere, but as they are mosaics for four unlinked factors, including sex, we can say that they clearly result from binucleate or multinucleate eggs rather than chromosome loss. Therefore, we have adopted the simplest explanation of their phenotypes as being from binucleate eggs and have treated each half as a separate individual when scoring the brood.

(f) Linkage groups

The linkage relations of the East Ecuadorian alleles encountered in this cross have been discussed in § 3.7j. Concerning the Upper Amazonian alleles, we have already shown that the data are consistent with the previously demonstrated linkage of S and T.

The C locus is apparently independent of all others. The backcross with a female heterozygous parent (brood 1A) segregates for this gene and for N and B. The parental combinations were CN^Bb and cN^NB, so that the appearance of the recombinant classes (numbers in brackets), no cell spot, yellow line (7), weak cell spot, no yellow line (9), no cell spot, red in band (6) and cell spot, no red in band (10), shows that C is not linked to N or B. It is further shown to be independent of Or and S in the Belém backcrosses with a heterozygous father by the exact one-tailed binomial probability for equality of coupling and repulsion of 0.43 for S (broods M17, M21 and M30; numbers 16:14) and by the one-tailed 2×2 probability (used because of scoring problems with both loci, for broods M21 and M30, both of which show variable manifestations of the cell spot) of 0.25 for Or (segregation 3:6:2:13).

This leaves the relations of C and D, T and Wh untested (the appearance of recombinants in the F_1 cannot be used as the Ecuadorian male was Cc). However, if C is unlinked to B and S it must also be unlinked to D and T. Wh might be linked to C if it were a modifier of B, rather than carried by the $B–D$ chromosome.

(g) Genetic composition of the Upper Amazon race

Our introgressed male therefore carried three 'foreign' alleles which must have been introduced into the population from the Upper Amazonian race: C, s and T. Thus the Belém and Upper Amazonian races are identical for the alleles s and T, as well as for the alleles b, D and N^N. Although there is no direct evidence from the breeding experiments, we think that few would quarrel with the suggestion that both races also carry Wh and or. These conclusions are supported by the fact that the extensive variable population in the middle of the Amazon Basin, formed by the meeting of the two races, contains none of the phenotypes that would be produced by complementation if the races carried different orange, or white, or triangle, or short, or band-colour loci.

The two races differ only in that the Upper Amazonian carries the gene C which has the effect of removing both the cell spot and the Belém spot from the band, and, we presume, in certain minor factors that we have not attempted to study that slightly alter the shape of the outer margin of the yellow band.

Descimon & Mast de Maeght (1971) suggested from an examination of the phenotypes segregating in the hybrid zone between the Upper Amazonian and East Ecuadorian races that these races might differ by four loci: (i) controlling the cell spot and triangle, (ii) controlling the radiate marks, (iii) controlling the colour (white or yellow) of the forewing band, (iv) controlling the distribution of red in the forewing band. This is quite a good approximation to our findings, except that (i) cell spot and triangle are separate although interacting loci (*C* and *T*), (iii) the conversion of red and white to yellow is achieved by three loci (*N*, *B* and *Wh*) rather than two, with white being a modifier of red rather than the reverse, and that in addition the races differ by the loci *S* and *Or*; the surmise about (ii) the *D* locus and (iv) the *Rr* locus is roughly correct. The suggestion they make that a similar set of genes controls this difference in *H. erato* is not quite so near the mark, as pattern control in this species is more complicated (§4.10).

(h) Summary

The only major gene differentiating the upper Amazonian from the Belém race is *C*, which removes the cell spot and the Belém spot (figure 4*h*).

On a Belém background the *c* allele, while enhancing the Belém spot, tends to suppress the Ecuador triangle, which lies in the same intervenular space; on an East Ecuador background the effect is reversed, and *c* enhances the triangle and suppresses the spot.

Two of our butterflies were mosaic for three or four unlinked factors, showing that they resulted from binucleate eggs, not chromosome loss (plate 2*j*).

3.9 Summary of genetics and of genotypes of the parental races

(a) The genes

The genetics of *H. melpomene* can be summarized in terms of alterations to the pattern of the Belém race (plate 1*a*; figure 4*d*), which has been used in all but one of the present crosses, and of which our stock has become a standard laboratory and ornamental animal in the U.K. (see for example *Reader's Digest* (British edn) Dec. 1982, pp. 100–101).

The broken yellow forewing band of the Belém race can be altered by the following loci:

C (Belém allele *c*, recessive), the dominant allele *C* removes the yellow dumb-bell shaped spot in the main forewing cell, and tends also to remove the spot nearest to the posterior angle of the wing (the Belém spot) (plate 2*j*, *k*).

S (Belém allele *s*, recessive): the dominant allele *S* shortens and narrows the outer arc of yellow marks that form the main part of the forewing band, so that instead of reaching vein Cu1a they do not extend beyond vein M3. This allele tends also to remove the Belém spot (plate 2*i*).

T (Belém allele *T*, dominant): the recessive allele *t* adds a triangular mark below the cell spot, in the angle of the cubital vein and vein Cu1b (plate 3*f*, *j*). The effect is probably enhanced by the *cc* genotype in an East Ecuadorian genome, but not in a Belém genome, where *cc* tends to suppress the triangle and enhance the Belém spot. It is probable that the full development of the triangle requires homozygosity for the recessive alleles at two (or more) further loci provisionally named *Tr-1* and *Tr-2*. *T* is linked to *S*, or, as the rate of crossing over has not been determined, may be a pleiotropic effect of the *S* locus.

F (Belém allele *F*, dominant): the recessive allele *f* causes the marks in the band to spread out to form a fused yellow patch, instead of being broken into spots as in Belém (plate 2*d*).

N (Belém allele N^N, codominant), B (Belém allele b, recessive) and Rr (Belém allele rr, recessive): alleles at these three loci can jointly convert the yellow forewing band of the Belém race to a red one.

The interaction of these loci appears complicated (see for example: plate $2a–g$; figure 5), but stems from very simple forms of gene action. The effects of N and B can be summed up as N^N yellow, N^B no yellow, B red, b no red. The interaction of the two loci is basically simple:

	bb (no red)	$B–$ (red)
$N^N–$ (yellow)	yellow band (plate $2k$)	mixed red and yellow band (plate $2a–e$)
$N^B N^B$ (no yellow)	no band (plate $2g$)	red band (plate $2\,h$)

The complications are merely imposed on this pattern. In the Belém × Trinidad cross, $N^N N^B$ heterozygotes are mostly reliably distinguished from $N^N N^N$ homozygotes by the intensity of the yellow markings, and often by the width of the red mark in the band if they are $B–$ (figure 5). In crosses between Belém and East Brasil or East Ecuador there is considerable variation in $N^N–$ individuals, but the two genotypes cannot be certainly distinguished by their forewing bands, although on an East Ecuadorian background $N^N N^N$ can be fairly reliably distinguished by a small yellow spot at the base of the forewing.

When the band is red ($B–$), loci such as F, C and S which normally control yellow or white marks have no effect on the distribution of red. For example, in Trinidad f and c serve to spread evenly the white scales on the underside of the band, but substitution of F only restricts distribution of these white scales, leaving the red as a solid band. In the F_1 Belém × Trinidad and Belém × East Brasil butterflies, the outer half of the solid red band appears, with part of the broken yellow band to the interior (plate $2a$, b). However, in the presence of Rr, which is found only in East Ecuador, the red marks will respond to whatever genes are controlling the distribution of yellow, so that cc will produce a red cell spot, S a short red band, and F a broken band of the Belém type (plates $2i$, $3c$, left).

Wh (Belém allele Wh, dominant): the allele wh, known only from East Ecuador, adds much white colouring to the forewing band; it probably only has this effect on areas that would otherwise be red, but may, alternatively, be linked to B (plates $2i$, $3c$).

Yb (Belém allele Yb, dominant): the recessive allele yb adds a broad yellow bar across the hindwing (plate $2h$); a shadow of the bar is visible in most heterozygotes. On some genetic backgrounds the gene is partly sex-limited, being more strongly expressed in males (plate $2k$). This locus is linked to N, with a recombination fraction of under 12 %. The yb allele also adds a strong yellow line along the cubital vein of the forewing, and tends to enhance the expression of the triangular mark at the tip of the line, if this is present for other reasons (plate $2j$, right).

Ac and Tc (Belém alleles ac and tc, recessive): the dominant alleles at these loci, which appear to be independent of C, produce partial suppression of the cell spot in Trinidad. They are possibly members of a multigenic system suppressing the cell spot in that race.

A single gene converts the distal edge of the yellow band from convex (Rio Madeira, Cv, dominant) to concave (Bolívia, cv, recessive). The genotypes of the other races are unknown.

Ub (Belém allele probably *Ub*, dominant): the recessive *ub* causes the hindwing bar to be represented by a light sprinkling of yellow scales on the upperside in *Ybyb* heterozygotes. The direction of the dominance is not quite certain.

D (Belém allele D^R, dominant): the radiate marks on the base of fore and hindwings are removed by the recessive allele *d* (plate 2*c*, *d*); the middle allele *D* removes the hindwing rays only (figure 4*f*). This is linked to *B* with a recombination fraction whose best estimate is around 30%.

TABLE 4. EFFECTS OF THE DOMINANT ALLELES AT VARIOUS LOCI ON SOME OF THE YELLOW MARKS IN *HELICONIUS MELPOMENE*

	yellow bar	yellow line (basal)	yellow line (distal)	cell spot	triangle	Belém spot
Yb	removed	?	removed	no effect	reduced	reduced (?)
C	no effect observed	no effect observed		removed	reduced[1] or enhanced[2]	enhanced[1] or removed[3]
T	?	?	?	probably no effect	reduced or removed	?
Tr-1	?	?	?	?	removed	?
Tr-2	?	?	?	?	removed	?
N^N	slightly enhanced	enhanced	no effect	enhanced[4]	probably no effect	enhanced[5]
S	?	?	?	?	?	removed
Ac	?	?	?	anterior half removed	?	enhanced
Tc	?	?	?	suppressed	?	?

[1] On East Ecuador background.
[2] On Belém or East Brasil background.
[3] On Upper Amazonian, Belém and East Brasil background.
[4] Especially when homozygous on Belém or Trinidad background.
[5] Possibly conditional on spot being present due to other loci.

Or (Belém allele *or*, recessive): any orange marks on the wing, radiate marks or forewing band, are converted to intense, but unstable, red by the dominant allele *Or* (plates 1, 2, 3*j*). There are also further factors, one of which may be sex-linked, affecting this colour difference.

Apart from the linked groups *S–T*, *N–Yb*, and *D–B–*(?*Wh*), all these loci are independent, although *F* is not certainly independent of *Wh* (if unlinked to *B–D*), *C* or *S–T*, and *Ub* is known to be independent only of *C* and *D*, and to be unlinked or at the most loosely linked to *Yb*. *Rr*, despite having given no sign of linkage, is so little studied that it may not be independent, and may perhaps be an allele of *F*.

The effects of some of the loci on the yellow marks, including the minor ones, are summarized in table 4. Table 5 tabulates the allelic composition of the races whose genetics is known, and assigns the linkage groups.

(b) *Linkage or pleiotropism?*

In two instances in *H. melpomene* we are unable to tell whether we are dealing with a single locus with multiple effects, or with a linked pair of loci. The three alleles D^R (radiate), *D* (dennis) and *d* (plain) may be three combinations of two loci, *D/d* producing presence or absence of the dennis (forewing) marks, and *R/r* producing presence or absence of the hindwing rays. Although the latter hypothesis is supported by the existence of phenotypes attributable to the combinations *DR*, *dr* and the missing *dR* in the related *Heliconius timareta* (Turner & Crane

1962), we have adhered, for the sake of consistency, to the convention established by Sheppard (1963) of referring to the series as multiple alleles. Certainly the linkage if such it be is very tight indeed, and no natural or experimental recombinants are known.

The pair S and T (short band and Ecuador triangle) may also be a pair of alleles, S^t (short, triangle, from East Ecuador) and s^T (long, no triangle in other races), but we have treated them here as two combinations of a linked pair (St and sT), because we have no evidence whether or not the linkage is tight. The race *ecuadorensis* (appendix 1, race 6b) has a phenotype consistent with its being homozygous ST (short, no triangle).

(c) Modifiers of expression

The interaction of N and B illustrated in §3.9a above is subject to modification by further, lesser known, genes. The heterozygous $N^N N^B B-$, mixed red and yellow class, can have its yellow considerably strengthened by the enhancer(s) of yellow found in the Belém × Trinidad cross, so that they become indistinguishable from $N^N N^N B-$ homozygotes (plate 3h). What appears to be a different yellow enhancer converts $N^B N^B bb$ (no band) insects to yellow band insects not clearly distinguishable from $N^N N^B bb$ heterozygotes.

Modifiers strengthening the red marks have been found in the East Ecuadorian cross, where they give $N^N N^B B-$ insects a red band like $N^B N^B B-$ (plate 3g), and probably in the Suriname × Trinidad cross (Turner & Crane 1962), where they add a thin red band to $N^B N^B bb$ (no band) butterflies. The first of these two genes has provisionally been designated rs.

With this potential for modification, it is clear that both the dominance at the N locus and its epistatic interaction with B can be quite readily altered. A change of the mixed red and yellow category to red would make N hypostatic to B (which may be the situation in *H. erato* where there is a recessive gene converting red bands to yellow). Changing it to yellow, thus making N hyperstatic to B, almost occurs on the East Ecuadorian × Belém F_1 background, where we noted that some Bb individuals showed little or no expression of red (plate 3b).

There is also clearly a system of modifying genes affecting the expression of rays on the hindwing: these are narrow on a pure Belém background, but become much wider, so that they resemble the marks of the related species *H. cydno* and *H. ethilla*, when outcrossed to the Trinidad or East Brasilian races (plate 3j, k).

Direct modification of gene expression by the sex of the butterfly has been found for yb in a pure Belém background (yellow bar much stronger in males) and for $N^N N^B$ in certain hybrid backgrounds (smudgy yellow band stronger in females).

(d) The genetic composition of the races

The very diverse patterns of the six races now thoroughly investigated can be explained almost entirely by the 11 major loci described (table 5). The three Amazonian races that are radiate differ in the shape of the yellow forewing band, which is fused in Bolívia and broken in Belém (probably a single gene difference), and which lacks the cell spot and Belém spot in the Upper Amazon, merely as a result of the alteration of one gene (C). The similar Suriname race (Turner 1971b) lacks the rays on the hindwing (a single change at the D locus) and is red rather than orange (again a single gene), as is Bolívia (which shows other, probably polygenic modifications as well).

The races outside the Amazon basin differ from those inside in being red, not orange, in lacking the radiate marks, and in having a red, not yellow, forewing band. Changes at four loci

(*Or, D, B* and *N*) effect most of this alteration. A few further alterations characterize the individual extra-Amazonian races. Trinidad/Venezuela spreads white on the underside of the band with the *f* allele; East Brasil adds a yellow bar and yellow line by means of *yb* and also carries the cell spot remover *C*, which spreads white on the underside of the band; and East Ecuador converts the wide red band to a split red and white band by means of *S* (which splits the band into two halves and shortens the outer half), *c* and *t* (which produce the inner part of the band in the cell spot and triangle areas), *Rr* (which restricts the red pigment to these areas) and *wh* (which converts large parts of the band to white).

TABLE 5. GENOTYPES OF THE THOROUGHLY STUDIED RACES OF *HELICONIUS MELPOMENE*

(Races are homozygous for the alleles shown, except for some polymorphism near the city of Belém itself, and some introgression at Brokopondo, the source locality of the Suriname stock. The West Ecuador race has so far been crossed only with a Trinidad × East Ecuador hybrid (Emsley 1965*b*), giving insufficient information about the identity of the alleles segregating. Linkage group I (the sex chromosome) contains the gene for the polymorphic enzyme 6-phosphogluconate dehydrogenase (Johnson & Turner 1979), but no certainly known wing colour loci. [ ], allele deduced from phenotype and composition of hybrid zones and of laboratory hybrids between phenotypically identical races, not from direct segregation in our crosses.)

	Amazonian races				extra-Amazonian races		
linkage group	Belém/Rio Madeira[1]	Guiana (Suriname)	Upper Amazon	Bolívia[1]	Trinidad/ Venezuela	East Brasil	East Ecuador
II	D^R	D	D^R	D^R	d	d	d
	b	b	b	b	B	B	B
III	N^N	N^N	N^N	N^N	N^B	N^B	N^B
	Yb	Yb	$[Yb]$	Yb	Yb	yb	$[Yb]$
IV	s	$[s]$	s	s	$[s]$	$[s]$	S
	T	$[T]$	T	T	$[T]$	$[T]^7$	t
V	c	c	C^2	c	c	C	c
VI	or	$[Or]$	$[or]$	Or	Or	Or	Or
VII[3]	F	F	$[f]^5$	f	f	F	F
unassigned[4]	Wh	$[Wh]$	$[Wh]$	Wh	$[Wh]$	$[Wh]$	wh
unassigned	rr	$[rr]$	$[rr]$	?	$[rr]$	$[rr]$	Rr
unassigned[6]	Ub	?	?	?	ub	ub	?

[1] The Rio Madeira race is assumed to have the same genotype as Belém; the genotype of the Bolivian race is deduced by crossing with Rio Madeira only.

[2] Assumed from phenotype and dominance to be this allele.

[3] May be in groups IV or V.

[4] Likely to be in group II; known to be independent of III, IV and VI.

[5] From the phenotype, could be either *F* or *f*. Appearance of some apparent *ff* butterflies in some central Amazonian hybrid populations suggests the presence of *f* or something similar.

[6] If dominance is the other way, Belém is *ub*, East Brasil *Ub*, and Trinidad/Venezuela unknown. Not in groups II, IV or, probably, III.

[7] At least one further unlinked locus controls the triangle. Belém and East Brasil are known to differ by two such loci; if one of these is the *T* locus, then East Brasil is *t*. Alternative schemes for Belém, East Brasil and East Ecuador are described in §§3.7*e*, *k*.

In some cases we have no direct evidence as to which allele is present in a particular race. For example, as we have crossed East Ecuador only with Belém, we have not proved experimentally that the absence of white is always due to the allele *Wh*: the other races might be homozygous *wh* and have some other locus suppressing the white. But this is not merely an unnecessarily complicated way of interpreting the results, it is contrary to the evidence provided by the natural hybrid zones and by crosses between phenotypically identical races. For if our two-locus model were true, then the blend zone between the Belém race and the Trinidad/

Venezuela race in the Guianas and our own F_1 or F_2 broods would contain butterflies with white marks produced by complementation of the two loci. That they do not shows that the Trinidad/Venezuela race is homozygous *Wh*. Similarly the absence of yellow bars in the blend zones within the Amazon basin shows that the Upper Amazon race carries *Yb*, like Belém, and their further absence in the Ecuador blend zone shows that the East Ecuador race must have this genotype too. Alleles assigned by this logical method, rather than direct crosses, are placed in square brackets in table 5.

(e) Suppressors of yellow marks

The rather complicated action of the genes that affect the yellow line, yellow bar, cell spot, Ecuador triangle and Belém spot (table 4) can be grasped more easily by imagining that the common ancestor of all the present races was a butterfly like that in figure 16, with yellow marks in all these positions, a construct introduced here merely as a mental aid, although we shall show (see Discussion (§ 5.7)) that it may not be far from the truth.

The allele *Yb*, carried by all races except East Brasil, removes all these marks: both the hindwing bar and the original long forewing stripe, which in modern *melpomene* never occurs as a single mark, but only as a line, triangle or Belém spot. In East Brasil, the genotype *ybyb* allows the line and hindwing bar to remain; the triangle is suppressed by *Tr-1*, and the Belém spot by N^B, which removes the outer yellow forewing band, and also by *C*, which in addition removes the cell spot. In Belém, the bar and line are largely lost (*Yb*), but homozygosity for N^N allows the basal part of the line to remain, and, in company with *cc* (cell spot), produces the Belém spot; on this background *cc* has the effect also of suppressing the triangle, although Belém probably carries an additional suppressor (*Tr-2*). The effect of *cc* (cell spot) can however be reversed by the genetic background (possibly including *Tr-1* and *Tr-2*). In the Upper Amazon race the cells spot remover (*C*) removes the Belém spot, as in East Brasil, but in East Ecuador *cc*, in complete contrast to Belém, appears in company with the triangle. In this race, the allele *t* largely produces the triangle and the linked allele *S* removes much of the outer posterior part of the band, including the Belém spot. The total effect is that when a yellow (or white) mark is permitted to appear in the space between Cu1a and Cu1b, it can be made to appear distally (a Belém spot) or proximally (a triangle): the *C* locus operates this switch, but the direction of switching can be controlled by other loci. In a few hybrids the switch, not surprisingly, fails, and parts of both marks appear (plate 3*j*, left).

The Trinidad race (plate 1*b*) removes all the yellow marks, largely with *Yb* and N^B, but retains *c*, whose action is suppressed by *Ac*, *Tc* and perhaps other loci, so that it produces no yellow cell spot, but probably regulates the distribution of white scales on the underside of the band in this region. The distribution of the white on the remainder of the band is controlled by *f*, which spreads the white scales out evenly. In East Brasil this even spread of white is produced largely by *C*, which overrides the effects of *F*, which would otherwise cause the white to form in patches. The white scaling probably functions as a social signal during roosting (J. L. B. Mallett & D. A. Jackson, personal communication).

4. GENETICS OF *HELICONIUS ERATO*

4.1. *Races and phenotypes*

The races of *H. erato* used in the present experiments are illustrated in colour in plate 1 *e–h, j–l*. The distributions are shown in figure 2. For quicker reference, see figure 6. Authors of the Latin names are given in appendix 1.

The race from Panamá (*H. erato petiverana*) (figure 2, no. 2; figure 6*a*; plate 1*l*), which is found throughout the wetter parts of neotropical Central America from around Bayano (80 km east of the Panama Canal) to Nayarit and Tamaulipas (México) (with strays even recorded in the U.S.A.), has a broadish red band on the forewing, and a strong yellow bar across the hind-wing; on the underside this bar turns forward at its tip, to form a loop of yellow with the yellow stripe along the anterior margin of the wing (the yellow stripe is found in all races of *erato* and of *melpomene*) (plate 4*d*, right). Our stocks came from the Canal Zone.

Populations in México differ from those in Costa Rica and Panamá in minor quantitative characters, notably a narrowing of the yellow hindwing bar (some authorities like to distinguish the Mexican and southern populations as *H. e. petiverana* and *H. e. demophoon*); our Mexican stock came from the village of Gomez Farias (25 km northwest of Ciudad Mante) in the state of Tamaulipas.

The race from East Brasil (*H. erato phyllis*) (figure 2, no. 15; figure 6*b*; plate 1*g*), found in Brasil, Argentina, Uruguay and Paraguay, from Buenos Aires and Córdoba in the south to Mato Grosso and Maranhão in the north, is a mutual mimic of the sympatric races of *melpomene* already described (plate 1, compare *c* and *g*). Its wide red forewing band is concave anteriorly and proximally, so that it does not enter the cell, and is extended into two tooth-like projections near the posterior angle of the wing. The yellow bar across the hindwing does not turn forward on the underside, but instead tends to extend to meet a group of little cream rectangular spots at the apex of the wing (plate 4*d*, left). Posterior to the bar there is often a row of little red triangles, usually better developed on the underside. There is a yellow line along the centre of the forewing. Our stocks came from São Paulo and Rio de Janeiro.

The Mato Grosso/Belém race (*H. erato amazona*) (figure 2, no. 8; figure 6*c*; plate 1*e*), which occurs across the southeast part of the Amazon Basin, immediately to the north of the last race, between Mato Grosso and Maranhão, and which is sympatric and mutually mimetic with the Rio Madeira and Belém races of *melpomene*, has the bases of both wings covered with orange marks superficially like the radiate pattern of *melpomene*, but differing in detail, particularly in the placing of the rays in the hindwing (compare plate 1*a* with *e*, or figure 1 with figure 2); the forewing band is a broken yellow one, superficially like that of the sympatric *melpomene*, but again differing in the detailed shape of the marks. Our stock came from Belém.

The race from Trinidad (*H. erato hydara*) (figure 2, no. 3; figure 6*d*; plate 1*f*), also found in wide areas of northern South America outside the Amazon basin (Colombia, Venezuela, the Guianas), and in a narrow strip along the lower Rio Amazonas in Brasil, is black with a broad red band on the forewing. It resembles very closely indeed the sympatric race of *melpomene*. Our stock came from the Piarco area of Trinidad, and in two broods we also used butterflies, carrying some genes brought in by hybridization with the following race, from Georgetown, Guyana, and from the lower Rio Trombetas, Brasil.

The Manaus (Guiana) race (*H. erato amalfreda*) (figure 2, no. 4; figure 6*e*; plate 1*k*), found in the interior of the Guianas and the area immediately to the south, as far as the Rio Amazonas
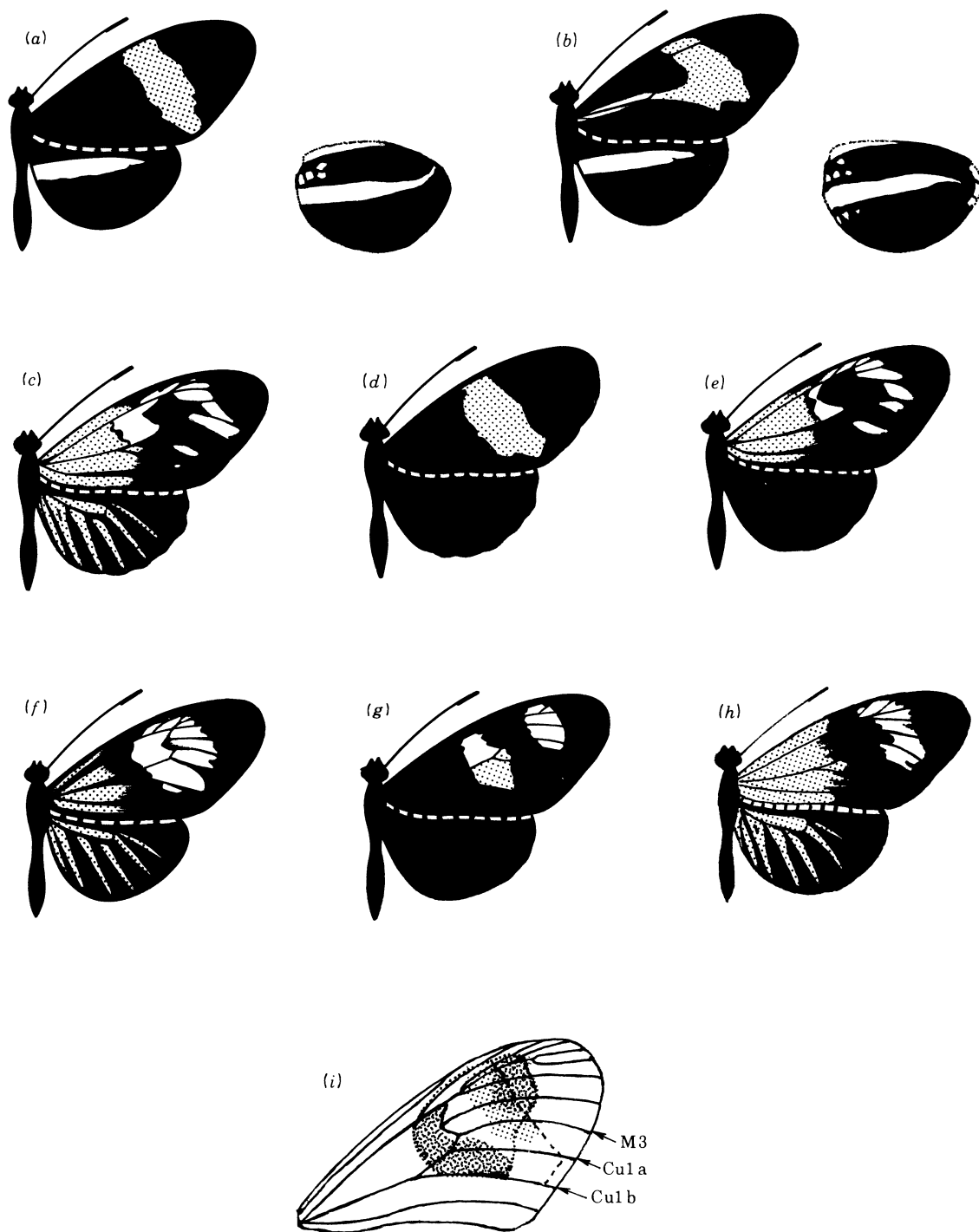
FIGURE 6. (a–h) Races of *Heliconius erato* hybridized in the present experiments (see also plate 1). (a) Panamá/ México, (b) East Brasil, (c) Mato Grosso/Belém, (d) Venezuela/Trinidad, (e) Manaus (Guiana), (f) Bolívia, (g) East Ecuador, (h) Upper Amazon. (i) Veination, with various types of forewing band: outer margin of long entire band (dashed line), outline of short entire band (dotted line), short split band (heavy stippling), and shortened band (light stippling). Detached hindwings are undersides.

between eastern Pará and Manaus, mimics the Suriname (Guiana) race of *melpomene* (figure 4*f*) with which it is broadly sympatric. The basal forewing marks, identical in shape with those of the Mato Grosso/Belém race, are intense red; the yellow broken band is like that of the Mato Grosso/Belém race, and the hindwing is black. Our stock came from the Ducke Forest Reserve of I.N.P.A., Manaus.

The Bolivian race (*H. erato venustus*) (figure 2, no. 9; figure 6*f*; plate 1*j*) is like the Mato Grosso/Belém race, except that the yellow marks on the forewing are fused into a solid band and the basal forewing marks and hindwing rays are reduced in extent and deep red in colour. The stock used in these experiments was from a population hybridized naturally with the Mato Grosso/Belém race at Riozinho (west of Pimenta Bueno) in southeastern Rondônia.

The East Ecuadorian race (*H. erato notabilis*) (figure 2, no. 12; figure 6*g*; plate 1*h*), from the eastern Andes in Ecuador above, roughly, 1100 m (Descimon & Mast de Maeght 1974), is a close mimic of the East Ecuadorian race of *melpomene*, from which it differs in the detailed shape of the marks and in the reversed position of the red and white colours in the inner forewing band (cf. plate 1*d*). As in *melpomene*, the features differentiating the East Ecuadorian race from Trinidad are that the band is *white* (with some red in most individuals), *split* by a black mark, *short*, in not extending toward the posterior angle, and *round*, in being extended in a generous convex curve toward the wing apex. This race must not be confused with the dissimilar West Ecuadorian race investigated by Emsley (1965*b*) (see figure 2, no. 11). Our hybrids derived from a single male introgressed with the Upper Amazonian race from the lower Andean slopes, captured south of Puyo in the Pastaza valley, Ecuador.

The Upper Amazonian race (*H. erato lativitta*) (figure 2, no. 6; figure 6*h*; plate 4*e*, right) resembles the Belém race in colour and radiate markings, but its yellow band is solid, does not enter the main cell of the forewing, and is *shortened*, in extending only as far as vein Cu1a. (This is not to be confused with the band being short: the Ecuadorian band is short in not extending to the posterior angle, but is *not* shortened, in that it extends to vein Cu1b.) Although we have bred this race, we have not successfully hybridized it, and what we know of its genetics stems from a gene controlling band shape carried by the above introgressed male from East Ecuador.

The unit characters used in scoring the broods, with what is known of their genetics from breeding experiments involving the Trinidad race and a Suriname population of the same race slightly introgressed with the Belém race (Turner & Crane 1962, using data of Beebe 1955; Sheppard 1963), are as follows.

Dennis versus plain (plate 1*k*): the red forewing marks of the Manaus race, versus their absence.

Radiate versus plain (plate 4*h*): a pattern like Dennis, but with red or orange rays on the hindwing; known to be a single allele dominant to plain.

Red versus orange (plate 1*j*, *k* versus plates 1*e*, 4*e*): the intense red of the extra-Amazonian races and the Manaus and Bolivian races, versus the orange of the Mato Grosso/Belém race. As in *melpomene* the red pigment is unstable and changes to orange in a matter of years, though in general the scoring is far easier in *erato*, even on old specimens.

Broken band versus solid band (plate 4*h* versus *a–d*): the forewing band may be broken into a series of spots, as in the Belém race, or fused into a solid patch, as in Trinidad or southeast Brasil. It is known that the difference is due to a single locus, with the broken band dominant.

Red band versus yellow (plate 4*i*): the forewing band may be red (or orange), or yellow; yellow was shown to be recessive to red, but the number of loci was not known.

Concave versus convex band (plate 4*e*): the red forewing band of the East Brasilian race is

markedly concave on its inner margin and hardly enters the main cell of the forewing; that of the Panamanian and Trinidad races has a considerable area of red within the cell, giving the margin a convex appearance. Hybrid butterflies show various intermediate phenotypes (plate 4*b*, right).

Tooth on forewing band (plate 4*c*): the posterior angle of the forewing band of the Panamanian and Trinidad races forms a broad red wedge; in the East Brasilian race this is divided by black pigment into two distinctly pointed marks; this is scored respectively as absence and presence of *tooth*.

Yellow forewing line (plate 4*a*, left): the yellow line along the centre of the forewing in the East Brasilian race, running from the base of the wing along the cubital vein to a point just beyond its first branch. It is absent in all other races, and appears in hybrids in various intermediate conditions (plate 4*a, b*).

Yellow hindwing bar (plate 4*a–e, j*): the yellow bar across the hindwing varies in its morphology, particularly on the underside of the wing. In the Panamanian race it turns anteriorly at its distal end (*bar forward*) so as to curve along the anterior wing margin (plate 4*d*, right). In the East Brasilian race it turns slightly backward (*bar backward*) and is accompanied by a group of *cream rectangles* at the apex of the wing (plate 4*d*, left). The Panamanian bar is also placed more posteriorly on the wing than the East Brasilian. In inter-racial hybrids the East Brasilian yellow bar is expressed as a number of variants, showing partial development of different kinds (plate 4*a–d, j*; figure 7). We shall describe these in detail under the appropriate crosses, as they are more easily understood in company with the genetic analysis.

Shadow of hindwing bar: as in *melpomene*, both wild and laboratory insects may have a shadow of the Panamá bar, visible because of a change in the reflecting properties of the scales, especially in the underside.

Red raylets: little red triangles, in the position of the bases of red rays, just distal to the yellow hindwing bar, are of frequent occurrence in the East Brasilian race, where they have generated the varietal name 'artifex'. They have segregated in some of our crosses.

In addition, in the cross involving the East Ecuador race, the following characters of the forewing band have been used, given here with their genetics, as far as it was detrmined by Emsley (1965*b*) in crosses with Trinidad.

White band versus red (plate 4*j*): the white forewing band from Ecuador, recessive to red, but controlled by an unknown number of factors in Emsley's broods.

Short band versus long (plate 4*f* versus *a–d*): the band extends toward the posterior angle of the wing much less in East Ecuador than in other races. Emsley showed that the short band was dominant, and probably unifactorial.

Round margin versus flat (plate 4*g*): the convex outer edge, which extends the band nearer to the apex in East Ecuador than in other races; shown by Emsley to be dominant and probably unifactorial.

Split band versus entire (plate 4*f* versus *a–d*): the division of the band into two by the black ground colour of the wing in East Ecuador, versus the single band of the Trinidad and East Brasilian races. The inheritance of this difference has not so far been accurately determined, but Emsley's data, although not his analysis, suggest a single factor.

Shortened band versus long (plate 4*e*): the shortening of the band, so that it does not extend to the posterior of vein Cu1a (as in the Upper Amazonian race), versus the long band which

extends as far as, or beyond, vein Cu1b (in the other races). This must not be confused with the *short* effect in East Ecuador, which withdraws the outer part of the band from the posterior angle of the wing, but does not affect which veins the band reaches (plate 4*f*). Emsley (1965*b*) did not investigate this character, which is introduced into some East Ecuadorian populations by hybridization with the Upper Amazonian race; in our crosses the shortened effect occurred only on the underside if the band was red, but on both surfaces if it was white (or yellow in pure Upper Amazonian butterflies).

Restriction to underside versus expression on both surfaces (plate 4*f*): in hybrids the splitting of the Ecuador band may be expressed fully only on the under surface, or may be expressed fully on the upperside of the wing as well.

The following two minor characters are associated with the East Ecuador cross.

Costal spot present versus absent: a small, deep red spot next to the thorax on the leading edge of the forewing, on the underside only (very pronounced in plate 4*f*, right).

Double versus single tip to yellow bar: an extra cream or yellow mark appears just anterior to the tip of the bar in the double phenotype (underside only).

4.2 The cross Trinidad × East Brasil

As we pointed out above, *H. erato* in Trinidad and East Brasil differ in the presence and absence of yellow lines, bars and rectangles, and in the shape of the red band on the forewing (figure 6*b*, *d*; plate 1). By crossing Trinidad butterflies with a stock from São Paulo, we obtained an F_1, an F_2 and the backcross to São Paulo. All were mixed broods with several parents (one male, two or three females for the F_1, two males, three females for the F_2, two males, one female for the backcross, presumably only one male being the actual parent as it is very rare for a female *Heliconius erato* to mate twice) (table A 4, appendix 5).

(a) Inheritance of cream rectangles

The cream rectangles at the outer angle of the hindwing (underside) in the East Brasilian race (plate 4*d*) are removed in Trinidad by a single dominant allele, designated *Cr*. The rectangles are generally absent in the F_1, although they appear weakly in ten of the 63 butterflies. In the F_2 and backcross they segregate fairly cleanly with some intermediates. Treating those showing partial or no rectangles as *Cr*–, and only full manifestations as *crcr*, the backcross segregates 14 dominants:22 recessives, and the F_2 27 dominants:12 recessives (the actual numbers, for no rectangles, intermediate, and full are 4:10:22 in the backcross and 20:7:12 in the F_2, two individuals having unscorable hindwings). These are a satisfactory fit to the expected ratios, if the rectangles have partial penetrance when heterozygous (respectively $P = 0.24$ for 1:1 and $P = 0.51$ for 3:1). The control of penetrance will be discussed below when we analyse the yellow hindwing bar.

(b) Inheritance of yellow forewing line

The yellow line in the forewing of the East Brasilian race (plate 4*a*, *b*) is produced mainly by a single recessive allele which we shall provisionally call *yl*. Thus the line is absent in the F_1, is fully developed in 18 out of 36 backcross butterflies, and in nine out of 41 F_2 individuals (expected number $10\frac{1}{4}$).

(c) *Effect of the cream rectangle gene on the forewing line*

Further variation in the forewing line is most easily explained by the action of the *Cr* locus. Individuals with a full yellow line (*ylyl*) but with weak or no cream rectangles (*CrCr* and *Crcr*) tend to have the line coloured red for at least its distal half, and sometimes to the base, and to have the yellow colour at the base of the line considerably weakened (plate 4*b*, left). This is true of all four of the apparent *Crcrylyl* butterflies in the backcross, and of all four similar individuals in the F₂. We conclude that the *cr* allele strengthens the yellow colour right along the forewing line, and suppresses red pigment in this region. Pure East Brasilian butterflies and hybrids with strong yellow lines do sometimes have red in the line, but normally only at the extreme tip where it runs into the band (plate 4*a*, left).

The effect of the *cr* allele in phenotypes that otherwise lack the yellow line (*YlYl* and *Ylyl*) is likewise to increase the amount of yellow. Considering only those butterflies that lack the fully developed forewing line, we find that in the backcross all eight of these butterflies that have also full cream rectangles have a small, weak yellow line of about one-quarter the normal length at the base of the forewing (plate 4*b*, right); in seven out of nine such butterflies that have weak or no cream rectangles, the yellow line is totally absent. Similarly, in the F₂ all eight cream rectangled butterflies have the short basal yellow line, and 21 out of 23 individuals with weak or no rectangles lack the line completely.

Thus according to the hypothesis that *Yl* and *Cr* jointly produce the forewing line, the phenotypes are:

	Cr– (no or weak rectangles)	*crcr* (full rectangles)
Yl–	no yellow line	short basal yellow line
ylyl	weak yellow line, strongly red distally	full yellow line, sometimes red at extreme tip.

Our broods have produced four anomalous butterflies which do not agree with this hypothesis. The anomaly is best illustrated by tabulating the phenotypes in the broods:

	no or weak rectangles	full rectangles
for the backcross		
full yellow line	0	14
weak yellow line, red tip	5	0
fraction of yellow line	*2*	8
no yellow line	7	0
for the F₂		
full yellow line	0	4
weak yellow line, red tip	4	0
fraction of yellow line	*2*	8
no yellow line	21	0

It can be seen that the perfect association between the rectangles and the strengthened and weakened yellow lines required by our hypothesis is marred by the numbers in italics. The yellow line of the anomalous insects is in fact different from the short basal line of the genotype *crcrYl–* in that it is not at the base of the wing, but lies medially along the vein, fading out both

distally and toward the base. Thus four simple hypotheses will explain the anomalies. First, their yellow lines may be produced by the action of a number of other loci, or of the environment, the butterflies being in fact *Cr– Yl–*. Secondly, they may be *crcrylyl* butterflies in which both the yellow line and the rectangles are weakened. Thirdly, they may be *crcrYl–* butterflies
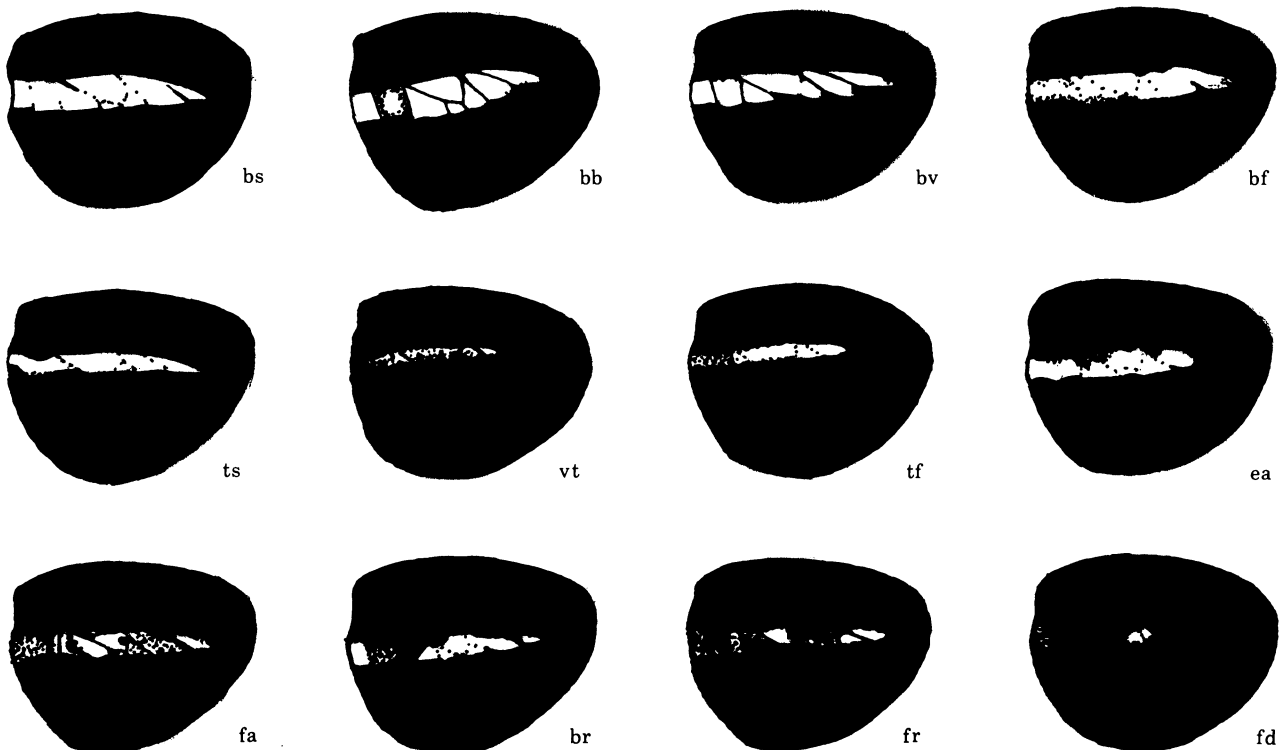
FIGURE 7. *Heliconius erato*: the main variations of the yellow hindwing bar in crosses involving the East Brasilian race. Abbreviations: bs, broad sharp; bb, broad sharp with black veins, slightly eaten; bv, broad with black veins (in East Ecuadorian crosses); bf, broad fuzzy; ts, thin sharp; vt, very thin fuzzy; tf, thin fuzzy; ea, eaten; fa, fuzzy eaten (in East Ecuadorian crosses); br, broken fuzzy; fr, broken fuzzy (in East Ecuadorian crosses); fd, fuzzy dots.

in which the rectangles are suppressed and the yellow line is shifted distally. Last, they may be *Cr– ylyl* butterflies, lacking the cream rectangles and having a weakened yellow line as expected, that for some reason have failed to develop the strong red in the outer part of the line. We believe that the four anomalous butterflies are in fact of this last genotype; for as we shall show when we discuss the yellow hindwing bar, there is good reason to think that there is a third locus capable of repressing parts of the line.

(d) Inheritance of yellow hindwing bar

The alleles *cr* and *yl* interact to produce not only the yellow forewing line but also the yellow hindwing bar, whose apparently complicated segregation in the crosses can be readily understood once the butterflies have been scored for yellow line and cream rectangles.

The yellow bars of F_1 butterflies are intermediate between the parental races, but vary considerably, from a barely visible fuzzy yellow dot at the outer end of the cell (completely absent in one individual only), to a thin yellow bar heavily invaded by black scales.

In the backcross, we can distinguish the following phenotypes, on the upperside (see also plate 4 *a–d*).

Broad sharp: the bar is about 3 mm broad, more or less straight along both its leading edge and its rear edge and divides clearly from the black background; this is the bar of the East Brasilian race (figure 7, bs).

Broad fuzzy: the band is the same width and shape, but particularly at its rear edge blends into the black background through a narrow zone of mixed black and yellow scales, thus appearing blurred at its edges (figure 7, bf).

Thin fuzzy: a bar with the same shape and edge as broad fuzzy, but only 2 mm or less in width (figure 7, tf).

Eaten: the band is up to 3 mm or more wide, but, instead of being smooth and straight at its leading edge, is eaten into by black scales and by mixed black and yellow scales, particularly toward the base of the wing; the rear edge is convex and comparatively sharply demarcated from the black background (figure 7, ea).

Broken fuzzy: an extreme form of eaten, in which the invasion of black breaks completely through the bar proximally, leaving it split into separate yellow patches (figure 7, br).

Fuzzy dot: the bar is represented only by a yellow dot with blurred edges lying just distally to the main cell of the wing or sometimes (in the F_2) by this and another distally placed dot (figure 7, fd).

The F_1 patterns vary from eaten through broken fuzzy to fuzzy dot (with one no bar). When examined for these patterns as well as the yellow line and cream rectangles, the backcross presents an almost regular segregation of the yellow bar (numbers of insects in brackets):

	Crcr (no rectangles)	*Crcr* (weak rectangles)	*crcr* (full rectangles)
Ylyl (no or weak basal line)	fuzzy dot (3)	broken fuzzy (4)	eaten (8)
ylyl (full or weak medial line)	thin fuzzy (2)	broad fuzzy (5)	broad sharp (14)

(note that for this purpose we regard the weak medial line as *ylyl*). The phenotypic classes are not absolutely distinct, and there is variation within them, so that we have had to re-classify two insects in order to produce a complete correspondence: the most extreme of the eight *crcYlyl* (eaten) butterflies has a bar that is not distinguishable from the most strongly developed of the *CrcrYlyl* (broken fuzzy) bars, and one of the three individuals with fuzzy dots does have *very* weakly developed cream rectangles (and has been scored as this elsewhere). Otherwise the bars fall well within their modal classes. Thus it can be seen that the Trinidad allele *Yl* has the effect of making the bar convex towards the rear and causing it to be invaded by black anteriorly and at the base, whereas the Trinidad allele *Cr* causes black scales to invade the bar from both sides, producing fuzzy edges. Homozygosity for both recessives (*crcrylyl*) is necessary for the development of a full East Brasilian bar, and both dominants together can reduce the bar to a yellow dot (and remove it completely in one F_1 insect).

It remains to be noted that there is a correlation within *Crcr* heterozygotes of the strength of development of the cream rectangles and of the yellow bar, so that complete removal of the rectangles (nearly complete in one exceptional insect) changes a broken bar to a dot, and a broad fuzzy bar to a thin fuzzy bar. It seems likely that there is an additional dominant allele

from Trinidad, which we will call *Ybs* (yellow bar suppressor), which can reduce the amount of yellow, both in bar and rectangles, in *Crcr* heterozygotes; in that case it is segregating in a 5:9 (or 4:10) ratio in this brood, a satisfactory fit to 1:1 ($P = 0.42$). This allele may also have the effect of weakening the yellow line, particularly by removing the red from the tip, in *Cr–ylyl* genotypes, thus explaining the two anomalous yellow line individuals that we have noted in this cross: both of the thin fuzzy butterflies with *no* rectangles have the weak medial yellow lines which we were unable fully to explain by the interaction of the *Cr* and *Yl* alleles.

In sum, our explanation of the backcross segregation is that the three loci produce phenotypes as follows (numbers of butterflies in brackets):

	Crcr; *Ybsybs*	*Crcr*; *ybsybs*	*crcr*;–
Ylyl	no yellow line, fuzzy dot, no rectangles (3)	no yellow line, broken fuzzy bar, weak rectangles (4)	short basal yellow line, eaten bar, full rectangles (8)
ylyl	weak medial yellow line, thin fuzzy bar, no rectangles (2)	weak medial yellow line red distally, broad fuzzy bar, weak rectangles (5)	full yellow line, broad sharp bar, full rectangles (14)

The existence of *Ybs* requires confirmation which will be provided by the F_2; the effects of the other two loci are very clear. It is salutory to think that with the slight overlap in phenotype classes, the segregation of the yellow bar, produced mainly by two loci, would have appeared 'polygenic' had the effects of these loci on the line and rectangles not allowed us to distinguish the genotypes.

(e) Interaction of the three yellow bar loci in the F_2

With certain assumptions we can now predict how the F_2 will segregate if the three loci *Cr*, *Ybs* and *Yl* all affect the yellow hindwing bar. Six phenotypes are already known from the backcross (tabulated above); these, and predictions about the remaining genotypes are laid out in table 6. The four new phenotypes have been induced by the following argument, based on the assumption that *Ybs* is fully dominant to *ybs*: first, no *CrCr* butterfly will have cream rectangles; secondly, no *Yl–* butterfly will have a yellow line; thirdly, if *Cr* is fully dominant in its effect on the yellow line, the phenotype of *CrCr*; *ybsybs*; *ylyl* will be similar to *Crcr*; *ybsybs*; *ylyl* (a weak yellow line, red distally); fourthly, if the three loci do account for the difference between Trinidad and East Brasil, then all three dominants together, in the genotype *CrCr*; *Ybs*–; *Yl*– should give a butterfly of the Trinidad phenotype with no yellow bar and no yellow line; fifthly, although the suppressing effect of *Ybs* on the cream rectangles will not be seen in *CrCr* homozygotes, which lack them in any case, it may still affect the yellow bar, so that this will be more strongly developed in *CrCr*; *ybsybs* butterflies than in *CrCr*; *Ybs*–, from which it follows that the former genotype will have a partly expressed bar, although it is not possible to say of what type. This leaves several phenotypes unpredicted; they are placed in parentheses in table 6.

This model makes two testable qualitative predictions about the F_2. First, the butterflies lacking both the yellow line and the cream rectangles (all *Cr–Yl–*) should segregate into three classes: no bar (*CrCr*; *Ybs*–), fuzzy dots (*Crcr*; *Ybs*–) and some other partly expressed bar. These phenotypes have appeared in the numbers 5, 9 and 4, the unpredictable type having a broken fuzzy bar, which is accordingly the phenotype of *CrCr*; *ybsybs*; *Yl*– and is so entered in the table.

Secondly, whereas in the backcross all phenotypes with the weak yellow line red distally had to have *weak* rectangles, this phenotype should now appear with *no* rectangles as well. There are three such butterflies with weak rectangles, and a single one with no rectangles, which is therefore believed to be the genotype *CrCr; ybsybs; ylyl* (the others being the same but *Crcr*). This single butterfly has a broad fuzzy bar, entered in brackets as the appropriate phenotype.

TABLE 6. PHENOTYPES IN THE *HELICONIUS ERATO* TRINIDAD × EAST BRASIL F_2,
AS EXPECTED FROM THE THREE LOCUS HYPOTHESIS

(Phenotypes in parentheses have been deduced from the F_2, not predicted from the backcross.)

	CrCrYbs–	*CrCrybsybs*	*CrcrYbs–*	*Crcrybsybs*	*crcr–*
Yl–	no yellow line no bar no rectangles (Trinidad) 9 (5) 5.48	no yellow line (broken fuzzy bar) no rectangles 3 (4) 1.83	no yellow line fuzzy dots no rectangles 18 (9[1]) 11.0	no yellow line broken fuzzy bar weak rectangles 6 (2[2]) 3.66	short basal yellow line eaten bar full rectangles 12 (8) 7.31
ylyl	(no yellow line, red dot) (very thin fuzzy bar) no rectangles 3 (1) 1.83	weak yellow line, red distally (broad fuzzy bar) no rectangles 1 (1) 0.61	weak yellow line (red dot) thin fuzzy bar no rectangles 6 (2) 3.66	weak yellow line, red distally broad fuzzy bar weak rectangles 2 (3) 1.22	full yellow line broad sharp bar full rectangles (East Brasil) 4 (4) 2.44

[1] Two of these butterflies have very weakly developed rectangles consisting of fewer than a dozen cream scales.
[2] These butterflies have only very weak rectangles consisting of fewer than half a dozen cream scales and are listed as 'no rectangles' in table A 4.
Numbers are, in order: expected ratio (**observed number**) expected number.

It remains to deal with the phenotype of *CrCr*; *Ybs–*; *ylyl* (bottom left in table 6); all that is obvious is that it should have no rectangles. In the F_2 those *Crcr* butterflies whose yellow line is medial and weak as a result of suppression by *Ybs–* have, unlike the similar backcross genotype, a small red dot at the place where the line normally ends just beyond the bifurcation of the main cubital vein (root of Cu1b, figure 6*i*). One of the three such butterflies has the dot but has the yellow line reduced to fewer than four yellow scales. This therefore is probably the missing phenotype, a view strengthened by the fact that in contrast to the other two butterflies which have some bright yellow with no black scales in their bar, this butterfly has a thin fuzzy bar with black scales mixed with the yellow throughout (figure 7, vt). Obviously the assignment of the two phenotypes each represented by only one individual is tentative.

Given this assignment of phenotypes, it is easy to predict that the F_2 should segregate in the ratio 9:3:18:6:12:3:1:6:2:4. As can be seen from table 6, the observed and expected numbers are a good fit (two butterflies with unscorable hindwings are omitted); χ^2 is 4.35 with 9 degrees of freedom, $P = 0.89$. It is further predicted that apparent *Ybs–* genotypes should segregate to *ybs ybs* in the ratio 3:1. The numbers are 17:10, which is satisfactory ($P = 0.23$).

There is one probably minor anomaly: among the *Yl–* butterflies the two attributed to *Crcrybsybs* have rectangles that are so weak as to be barely detectable (fewer than six scales on each side). Two of the butterflies attributed to *CrcrYbs–* (no rectangles) have similar weak rectangles. If they are regarded as having weak rectangles then they constitute the phenotype no yellow line, fuzzy dots, weak rectangles, which is not predicted by our hypothesis. However, one normally finds that F_2 broods in *Heliconius* have minor scoring problems owing to segregation of the genetic background. In addition, there is one identical individual in the backcross,

which suggests that what we are seeing is slight penetrance of the rectangles even when suppressed by *Ybs*. What is remarkable is that in this case, with the exception of two butterflies, the segregation is adequately described by three loci.

TABLE 7. EFFECTS OF THE TRINIDAD ALLELES OF THREE LOCI ON THE YELLOW
MARKS OF THE EAST BRASILIAN RACE OF *HELICONIUS ERATO*

	yellow line	yellow bar	cream rectangles
Cr	weakens the line; allows the distal part to become red	allows black scales to invade bar evenly, mixing with yellow scales mostly on the posterior edge	removes the rectangles when homozygous; allows some expression when heterozygous
Ybs	weakens the line; particularly any red in the distal part	reduces width of the bar along whole length	removes rectangles in *Crcr* individuals
		seems to have no effect on *crcr* individuals	
Yl^{t1}	considerably weakens the line particularly the distal part; removes line in conjunction with *Cr*	causes invasion of solid black areas, and of black scales mixed with yellow unevenly from the anterior edge, chiefly near the wing base	probably has no effect

[1] The superscript *t* for *Yl* is explained on page 513.

(*f*) Summary of effects of *Cr*, *Ybs* and *Yl* on the yellow marks

The absence of yellow marks in Trinidad *H. erato* (except for the marks on the body and the yellow costal stripe on the hindwing underside, which are present in all races), versus the presence of yellow line, yellow bar and cream rectangle in East Brasil, is therefore produced by the joint action of these three dominant or partly dominant genes, each of which effects some reduction in the yellow line and yellow bar. The concerted action of all three dominants is required for the complete removal of all the marks. The particular effects of each gene are summarized in table 7, and their effects on the yellow bar are illustrated in figure 7.

(*g*) Inheritance of shape of band

The red forewing band of the F_1 butterflies has the tooth (plate 4*c*) on the outer margin, as in the East Brasilian parent. In many butterflies the tooth is blunter than in East Brasil, and in a very few the two red points that compose it almost coalesce as in Trinidad, although it is never the very broad, blunt wedge of that race. In the backcross to São Paulo all the butterflies have the tooth, indicating that this character is dominant. The inner margin of the band in F_1 butterflies is never smoothly convex as in Trinidad. In 52 of the 63 butterflies there is a substantial amount of red in the main cell of the forewing, but even in the most extreme specimens the band is eaten into by black scales along the veins that bound the cell (plate 4*b*, right). We regard this as the intermediate phenotype; it varies from just distinguishable (by the black invasion) from a Trinidadian band, to barely distinguishable from a concave East Brasilian band. The remaining 11 butterflies have a concave band not distinguishable from that of East Brasil. The backcross to East Brasil segregates 21:15 for concavity as compared with the intermediate phenotype which is a satisfactory fit to 1:1.

The F_2 segregates as follows:

	concave	intermediate	convex
tooth	21	11	0
no tooth	0	0	9

If concave and intermediate bands are combined, the fit to a 3:1 ratio is good ($P = 0.81$). Both concavity and tooth are therefore produced by the same single dominant gene or at least are segregating as a single unit. From the backcross it appears that intermediates are likely to be heterozygotes, but eleven undoubted heterozygotes in the F_1 are concave, and there are almost ten too few intermediates in the F_2, with a corresponding excess of concave bands, showing that many heterozygotes are concave, and that the allele for convexity is often completely recessive.

Total association of two characters in an F_2 in *Heliconius* does not prove that they are produced by a single gene, as the absence of crossing over in females (Turner & Sheppard 1975) can stop the appearance of the recombinant phenotype even for loosely linked genes. However, this applies only to alleles in repulsion: the tooth and concavity are in coupling, so that if they were separate genes the recombinants should appear. Their absence shows that we are dealing with a single locus or with two that are very tightly linked. Parsimony requires us to assume a single locus until contrary evidence is produced.

(h) *Association of yellow line and band shape*

Full band concavity and yellow line are completely associated in the backcross. With use of the development of the yellow forewing line and the phenotype of the hindwing bar as diagnostic characters, all individuals that are *Yl–* have the red band developed extensively within the outer end of the main cell of the forewing, usually as a continuous red patch extending from the front to the rear of the cell; this is the condition that we tabulate as intermediate between full convexity and concavity, which we regard as heterozygous. On the other hand all individuals diagnosed as *ylyl*, whether the yellow line is fully expressed or not, have a fully concave band, which enters the cell either only at its extreme anterior and distal part or, in seven out of the 21 butterflies, as a little loop starting from the anterior and posterior extremities of the cell, but just failing to meet in the middle, like a ⊃ broken at its right end. The two most extreme members of this last group have very slightly more red in the cell than the least red of the convex individuals in the intermediate (heterozygous) class. We can if we like regard these three individuals as recombinants (the heterozygous parent of this brood being male and recombination therefore quite possible), but the case for doing so is very weak, as all three are at the extreme end of the continuous variation in their phenotype class; recombinant individuals with non-extreme band phenotypes have not appeared. Both of the male 'parents' of this brood (only one of which is likely to be the true father) are among the most extremely convex members of the F_1, almost resembling Trinidadian butterflies; the fact that a substantial number of their concave offspring have a slight amount of red in the cell probably shows polygenic inheritance for the amount of red in this region. The concavity of the red forewing band is therefore produced by the allele *yl*, or by a gene very closely linked to it (the maximum estimate of the recombination fraction, based on the assumption that the next offspring would have been a recombinant, is 0.03 for males). Similarly in the F_2 the expected recombinant phenotype 'convex band, yellow line' has not appeared; this confirms that the genes controlling these characters are linked, but gives no information about the recombination fraction as the genes would be in repulsion, producing absolute association in the F_2 because of the absence of recombination in females.

As is to be expected from the fact that band concavity and tooth are produced by one gene, there is also an absolute association in the F_2 between the tooth and the yellow line, the expected

recombinant 'no tooth, yellow line' also being absent. This cannot be investigated in the backcross because of the complete dominance of tooth.

(i) *Manifold effects of the yellow line gene*

The simplest explanation of the above facts is that the yellow line (with the demonstrated effects on the yellow bar) and the shape of the band are both controlled in this cross by a single gene locus, the effects of the East Brasilian allele on the shape of the band being largely dominant, those on the yellow line and yellow bar being recessive. To make the notation easier to understand, we shall designate the effects on band shape T (tooth) and t (no tooth), and call the East Brasilian allele yl^T (yellow line, tooth) and the Trinidad allele Yl^t (no yellow line, no tooth), the capital and lower case letters indicating dominance in the conventional way. The effects of the alleles are summarized in table 8.

TABLE 8. EFFECTS OF THE TRINIDAD AND EAST BRASILIAN ALLELES AT THE
Yl LOCUS OF *HELICONIUS ERATO*

allele	origin	band	yellow line	yellow bar
Yl^t	Trinidad	convex, no tooth	absent[1]	eaten into by black from anterior, chiefly in basal part[3]
yl^T	East Brasil	concave, tooth	present[2]	fully developed[3]
dominant effect		concavity (partial, variable penetrance of convexity in heterozygotes), tooth	absence	eaten

[1] Unless inserted by other loci.
[2] Unless repressed by other loci.
[3] Subject to modification by the Ybs and Cr loci.
For all these interactions see table 7.

There is of course no way of disproving that Yl and T are very closely linked loci rather than a single gene; in fact this can only be proved (by the appearance of recombinants) and never disproved. It may seem surprising that a single gene should have such widespread effects on the pattern. However, there is reason to believe that the pattern of *H. erato* is derived from an ancestral form rather like that of its close relative *H. charitonia* (see §5.7), which has a broad yellow bar across the hindwing and a broad yellow stripe extending right along the cubital vein and then right along one of its branches (Cu 1b) to the outer edge of the forewing (figures 14 and 15d). The yellow line of *H. erato* lies in the basal part of this yellow stripe, and the upper red tooth, particularly on the underside, can be seen to lie exactly in its distal part. The East Brasilian allele yl^T may be part of a developmental chain retained from a time when *erato* had yellow bars on both fore- and hindwings; the effect of the Trinidadian allele Yl^t is then to disrupt much of this developmental chain, affecting all the marks that were originally yellow bars, that is to say the forewing line, the tooth and the hindwing bar. If this hypothesis is correct, then it should be possible to show experimentally that this allele operates rather early in the development of the pattern.

(j) *Phenotypes of $yl^T Yl^t$ heterozygotes*

As the Yl alleles both have effects that are dominant and recessive, all genotypes should be recognizable, as follows:

$yl^T yl^T$ — the East Brasilian phenotype – tooth, yellow line, full yellow bar

$yl^T Yl^t$ tooth, no yellow line, eaten yellow bar

$Yl^t Yl^t$ the Trinidad phenotype – no tooth, no yellow line, no yellow bar.

Diagnosing the yellow line, yellow bar phenotypes according to the scheme in table 6, that is allowing for modifications of these characters by the other two loci, and then scoring for tooth, we obtain the following numbers in the F_2:

$$yl^T yl^T \quad 11, \qquad yl^T Yl^t \quad 20, \qquad Yl^t Yl^t \quad 8,$$

which is an excellent fit to $1:2:1$ (expected numbers $9\frac{3}{4}:19\frac{1}{2}:9\frac{3}{4}$). There is thus every reason to believe that this diagnosis of the genotypes is correct.

We are now able to investigate the penetrance of band concavity. The phenotypes are

$$
\begin{array}{lll}
yl^T yl^T & \text{concave} & 11 \\
yl^T Yl^t & \text{concave} & 9 \\
yl^T Yl^t & \text{intermediate} & 11 \\
Yl^t Yl^t & \text{convex} & 8
\end{array}
$$

from which it can be seen that all intermediates are heterozygotes, but that just under half of the heterozygotes have been misclassified with the concave homozygotes. Careful examination of these nine butterflies shows that all but one have red marks in the cell, but only to the extent that concave individuals do in the backcross, and in some instances to a smaller extent than the most extreme of the undoubted $yl^T yl^T$ concave butterflies. Thus the penetrance of Yl^t in heterozygotes is variable, sometimes producing an intermediate band, but equally often failing to cause an unambiguous alteration in the concavity produced by yl^T.

It is, again, possible to regard the mis-scored butterflies as crossovers, in which concavity has separated from the other effects of the gene, but in that case we should expect the reciprocal recombinant (intermediate band, full yellow line), which has not appeared in this brood.

Again, it is interesting to note that scoring the broods solely on the basis of band shape and the full development of the yellow line gives a result quite consistent with around 20 % recombination between yellow line and band shape, whereas the full analysis shows that the 'recombinants' are the results of mis-scoring the band and of the modification of the yellow line by the genes Cr and Ybs.

(k) Variable penetrance of Cr, Ybs and Yl characters in the F_1

We have noted that the F_1 is variable in the expression of band concavity, cream rectangles, yellow hindwing bar and, to a much lesser extent, tooth. The variation in concavity is clearly due to the uncertain penetrance of the recessive allele Yl^t, which also causes scoring problems in the backcross and F_2. The ten butterflies displaying weak rectangles are also presumably showing the weak penetrance of cr in heterozygotes even with Ybs– which probably accounts for two anomalous butterflies in the F_2 and one in the backcross.

Variation in the yellow hindwing bar is confined to those phenotypes appearing in the upper row of table 6; thus there is no sign of penetrance for this character by the recessive yl^T allele (East Brasilian). The segregation is:

	no bar	fuzzy dots	broken fuzzy bar	eaten bar
no cream rectangles	0	37	14	2
weak cream rectangles	1	2	5	2

Apart from the exceptional insect with no bar, there is an association, though not a perfect one, between expressed cream rectangles and increased strength of the bar, showing that they are being influenced by the same developmental process (with classes combined, exact 2×2 probability 0.044, two-tailed). The modal pattern, fuzzy dots, is that deduced in the F_2 and backcross to be $Crcr$; Ybs–; Yl^t–, which ought to be the genotype of the F_1, but there are appreciable numbers of butterflies in the F_1 that in the F_2 and backcross would have been classed as $ybsybs$.

The probable explanation is that we are witnessing general variation for the yellow marks, deriving from heterozygosity in one or other parent: the Ybs locus for instance has no effect on $crcr$ homozygotes, and could therefore be heterozygous in East Brasil without producing any visible polymorphism in natural populations. There may be other loci of similar effect that did not segregate in our F_2 or backcross, but, apart from the five butterflies with no bar or eaten bars, variation in the F_1 could be accounted for by the segregation of Ybs. As there is a slight overlap in the backcross between broken fuzzy and eaten, it is reasonable to suppose that in this very much larger F_1 brood the apparent eaten individuals are in fact merely the extreme members of the broken fuzzy class, especially as none has the fully developed rectangles characteristic of eaten in the F_2, and that the individual with no bar simply represents the other tail of the same distribution. It is pointless to analyse the segregation, as the brood is of mixed parentage.

It is not surprising that the F_2 segregates clearly for Cr and Yl: these are loci of large effect, and there is no indication that either parental race is heterozygous. However, the clean segregation of Ybs is a little surprising in view of the F_1, as the F_2 is also multiparental (the backcross raises no such problem, having only one female parent). It is likely that we have been lucky in this brood and that, as often happens, only one of the three female parents of the F_2 was fully fertile, and that she produced most of the offspring.

As the possibility of cryptic heterozygosity at the colour loci is a most interesting one, and is perhaps to be expected on account of the high heterozygosity in the allozymes of *Heliconius* and of other insects (Lewontin 1974; Turner *et al* 1979), it is important that in future experiments broods should be bred only from single females.

(*l*) *Shape of tip of yellow bar*

The East Brasilian yellow bar bends to the posterior at its tip, as it were to point at the cream rectangles (plate 4*d*, left; figure 6*b*). By contrast, on the underside of the Panamá race (and of those races found in Colombia and Ecuador west of the Andes) the tip of the yellow bar turns forward, to form when well developed a loop with the yellow costal stripe (plate 4*d*, right; figure 6*a*). In a few of our F_2 Trinidad × East Brasil butterflies the tip of the yellow bar curls forward in the Panamanian manner! The character cannot be scored on bars which are absent or reduced to dots, and is sometimes difficult to score on broken fuzzy and some of the shorter bars in other classes. Allowing only positive evidence that the bar turns forward, we find:

	broad sharp	broad fuzzy	thin fuzzy	eaten	broken fuzzy	total
bar turns back, or too short to score	3	4	2	6	5	19
bar turns forward	2	0	1	2	1	6

which is an excellent fit to a 3:1 ratio. If the broken fuzzy class, which is the hardest to score, is excluded, the numbers are 14:5, which is still an excellent fit. It is likely that Trinidad carries a recessive allele causing the forward turn of the bar, which we shall designate bf. Two butterflies in the backcross to East Brasil have very faint traces of the forward turn, possibly indicating slight penetrance when heterozygous. There may be a small amount of mis-scoring of this character: we have erred in the direction of placing slightly doubtful individuals in the 'back' category, as this produces a more reliable test for the independence of bf from the other loci.

(m) Linkage groups

We have already shown that the factors controlling the shape of the forewing band and certain features of the yellow line and yellow bar are either a closely linked group or, more probably, a single locus, Yl. The interaction of the three loci Cr, Ybs and Yl has been deduced with the assumption that they segregated to some extent independently: close linkage is already ruled out ex $hypothesi$, but loose linkage is still possible.

Using as complete a diagnosis of the genotypes as we can obtain, we find the following degrees of association in the backcross

	$Crcr$	$crcr$
$Yl^t yl^T$	7	8
$yl^T yl^T$	7	14

which, tested for equality of coupling and repulsion phenotypes, gives $P = 0.20$ (one-tailed), and

	$Ybsybs$	$ybsybs$
$Yl^t yl^T$	3	4
$yl^T yl^T$	2	5

which by inspection is clearly not significant. The backcross therefore gives no evidence of linkage between Yl and either Cr or Ybs.

There is similarly no strong indication of linkage between these three markers in the F_2. For Cr and Ybs, as can be seen from table 6, there are 11 parental types and 16 recombinants. For Ybs and Yl we find

	$Ybs-$	$ybsybs$
$Yl^t Yl^t$	5	3
$Yl^t yl^T$	9	3
$yl^T yl^T$	3	4

with the doubly recombinant genotype $Yl^t Yl^t ybsybs$ appearing three times. This genotype cannot appear in the F_2 if the loci are linked, because of the absence of recombination in females (Turner & Sheppard 1975). Further, $\chi^2_2 = 1.96$, $P = 0.38$ (without Yates's correction), showing no association. For Cr and Yl the segregation is:

	$CrCr$	$Crcr$	$crcr$
$Yl^t Yl^t$	3	5	0
$Yl^t yl^T$	6	6	8
$yl^T yl^T$	2	5	4

If the loci were linked, then the doubly recombinant genotypes at the top right and bottom left could not appear. One is absent: its expected number is only 2.46. The other is present: both

individuals are unique members of a phenotypic class in table 6, and the diagnosis of their genotype is therefore not absolutely certain. Neither the presence of one nor the absence of the other can be taken as conclusive evidence. However, combining these two individuals with the centre cell to make the table as unfavourable as possible to the null hypothesis, we still obtain a value of χ^2_4 of only 7.29, ($P = 0.06$, one-tailed without Yates's correction). Therefore there is every reason to believe that Cr, Ybs and Yl are carried in three different chromosomes.

The gene turning the tip of the bar forward (bf) is likewise independent of the other three; if it were linked then the doubly recombinant genotypes $bfbfcrcr$, $bfbfyl^Tyl^T$ and $bfbfybsybs$ could not appear in the F_2: there are respectively four, two and one of them.

Therefore with the possible exception of the locus $Yl-T$ perhaps consisting of several tightly linked genes, none of the factors in this cross is linked to any other.

(n) Summary

The Trinidad and East Brasil races differ by at least four unlinked colour pattern loci. Trinidad is homozygous bf; Cr; Ybs; Yl^t and East Brasil is homozygous Bf; cr; ybs; yl^T, with possible polymorphism for Ybs or some similar gene. The effects of the loci are, briefly: Bf, backward or forward turn of the tip of the bar (backward dominant) (plate 4d); Cr, presence or absence of cream rectangles (plate 4d), strengthening or weakening of yellow bar, strengthening or weakening of base of yellow line (first effect in all cases mainly recessive, but some penetrance in heterozygotes for the effect on rectangles and maybe on yellow bar) (plate 4a, b); Ybs, weakening or strengthening yellow line, weakening or strengthening yellow bar, removing or inserting weak rectangles in $Crcr$ heterozygotes (first effect dominant); Yl, manifold effects in weakening or strengthening the yellow line and bar, and altering the red forewing band from concave toothed to convex toothless (first effect in each case dominant or largely so, dominant effects on line and bar go with recessive effects on forewing band, and *vice versa*) (plate 4a, b). Table 7 shows the effects of three loci on the yellow marks; the full effects of the Yl locus are summarized in table 8.

4.3. *The Cross Panamá × East Brasil*

The Panamá (Central American) and East Brasilian races of *erato* are superficially alike, in having a red forewing band and a yellow hindwing bar. The Panamá race differs in lacking the yellow forewing line and the hindwing cream rectangles, and in having the yellow bar a different shape, being placed in a slightly more posterior position on the wing, and, on the underside, turning forward instead of backward at the tip; the red forewing band is convex, not concave, and ends in a blunt wedge, not in the pointed 'tooth' marks, at the posterior angle of the wing (figure 6a, b; plate 1g, l).

We have bred the F_1 and a backcross, using F_1 males mated to East Brasil females, consisting of three broods (table A 5, appendix 5), one of mixed parentage (East Brasil originating from São Paulo: brood B1), one from a single female (East Brasil from São Paulo: brood B1 (2)) and a third also from a single female (East Brasil originating from Rio de Janeiro: brood WK1). We have also a single F_2 brood (WK2), also as a result of using Rio de Janeiro stock, and having several F_1 female parents. The genetics of this cross turns out to be very like the cross Trinidad × East Brasil. In the backcrosses we have not counted two individuals in the mixed brood, one of which is so worn as to be scored for only a few characters, the other being a Panamanian phenotype and hence presumably a contaminant.

In addition, we have bred a number of further crosses from this stock, some of them involving butterflies with ancestry from the Trinidad and other races. Because of limitations of space, most of these were opportunistic matings designed to keep the stock going, from parents of only partly known origin, and for most of them the parents have succumbed to natural hazards in the greenhouse and been lost. Some useful information can be extracted from these broods, which are shown in table A 5, Appendix 5 (those known to be matings between sibs in the (Panamá × East Brasil) × East Brasil backcross) and in tables A 6 and A 10, appendix 5 (those that do or may contain genes from other races, including Trinidad). Conclusions relating to genes from other races will be discussed under the other race crosses.

(a) Inheritance of cream rectangles and Panamanian yellow bar

As before, the removal of East Brasilian cream rectangles (plate 4d) is achieved by a single gene, dominant in its effect. Rectangles are absent in the F_1; they segregate 38 without rectangles: 35 with fully developed rectangles in the backcross to East Brasil, and 23 without: 9 with rectangles in the F_2 (expected 24:8).

The Panamanian yellow bar does not appear in the F_1 or backcross, but is replaced by a 'shadow' bar on the underside, like that of a *Ybyb* (yellow bar) heterozygote in *H. melpomene*. In the F_2 full Panamanian bars, shadows and no Panamanian bars segregate 3:19:10, showing that the shadows are indeed the heterozygotes (expected numbers 8:16:8, $\chi^2_2 = 3.20$, $P = 0.20$). There is thus an allele from Panamá, largely recessive in effect, but producing a shadow in the heterozygote, which produces the yellow hindwing bar of that race.

The backcross shows that this gene behaves as an allele of the gene producing the Brasilian cream rectangles. All 34 backcross butterflies with rectangles lack the shadow of the bar (one more is not scorable) and all 38 without rectangles have the shadow; the association is too strong to require a statistical test. The association is also strong in the F_2:

	Panamanian bar		
rectangles	full	shadow	none
absent	3	19	1
present	0	0	9

The single exceptional individual may indicate that we are dealing with two extremely closely linked loci, this being a crossover; on the other hand, in the backcross the shadow is sometimes very faint and this F_2 insect may simply be an extreme example in which the shadow has become invisible. A reciprocal recombinant showing full rectangles and a shadow would be much more convincing evidence of crossing over.

There is ample confirmation of this allelism or linkage from the other broods; examining all those in which the rectangles and the Panamanian bar have segregated we find:
for apparent F_2 crosses for both genes (that is B and J2)

	Panamanian bar			
rectangles	full	shadow	none	not scorable
absent	6	28	—	2
present	—	—	12	

for apparent backcrosses for both alleles (that is C, H, K and N)

rectangles	Panamanian bar		not scorable
	shadow	none	
absent	36	0	7
present	2	37	

and for broods too small to classify as backcross or F_2 (that is I, O, P and Q)

rectangles	Panamanian bar		not scorable
	shadow	none	
absent	11	—	0
present	—	16	

The two anomalous butterflies in the backcrosses are not certain recombinants, as one has only a suggestion of the shadow and the other has extremely weak rectangles. Thus they probably represent aberrations of gene expression, rather than recombination. Of these broods, only the F_2 demonstrates absolute linkage, as there are no recombinants in the classes 'no rectangles, no bar' and 'rectangles, shadow bar' (the third class, 'rectangles, full bar' could not appear in any case if there was no female recombination). As the sex of the heterozygous parent is not recorded, or in C and H is known to have been female, the backcross and other broods show only that the loci are on the same chromosome, on account of the absence of female crossing over (Turner & Sheppard 1975). Thus the weight of evidence is that the remover of cream rectangles and the gene producing the Panamanian bar are either alleles or are tightly linked in repulsion, with no certain recombinants observed.

The question is now whether the locus segregating here is the same locus as segregates in the Trinidad × East Brasil cross. It may be that it takes the cooperation of several loci in a developmental pathway to produce the cream rectangles, and that mutations at different loci remove the rectangles in Trinidad and in Panamá. The inheritance of the yellow line provides evidence on this point.

(b) Inheritance of yellow line

As in the Trinidad × East Brasil cross, the yellow line (plate 4a, b) is controlled by the segregation of two loci, one affecting its full development, and the other, which is the one that also adds and removes the cream rectangles, having a modifying effect. In the backcrosses, we find a clear 1:1 segregation of full length yellow lines versus short basal lines or no lines, the numbers being 26 with a full line:37 of the other phenotypes. The modifying effects of the cream rectangle/Panamanian bar locus on these phenotypes varies from brood to brood. In the Rio (single parent) brood, all cream rectangle/no shadow individuals in the full yellow line class have the line strongly developed, whereas all no cream rectangle/shadow bar individuals have a weakened line that is red distally. Similarly, in the remaining butterflies the cream rectangle/no shadow phenotype always has a short basal yellow line, and the no cream rectangle/shadow bar phenotype has no line at all.

These effects are so similar to those reported for the Yl and Cr loci in the cross with Trinidad, that an excellent working hypothesis is that we are seeing the segregation of the same two loci

here. Calling the Panamanian allele that removes the rectangles and produces the Panamanian yellow bar Cr^p, the phenotypes (with numbers of butterflies in brackets) are

	$Cr^p cr$ (no rectangles, shadow bar)	$crcr$ (rectangles, no bar)
$Yl^t yl^T$	no line (9)	short basal line (4)
$yl^T yl^T$	weak line, red distally (7)	full line (8)

The single parent backcross using São Paulo stock is closely similar: the presumed $Yl^t yl^T Cr^p cr$ individuals all have minute traces of the yellow line; otherwise the phenotypes are the same. The numbers are, in the same order as above (top left to bottom right) 4, 1, 1, 5.

The mixed backcross shows some deviation from this pattern, segregating

	$Cr^p cr$ (no rectangles, shadow bar)	$crcr$ (rectangles, no bar)
$Yl^t yl^T$	no line (7)	no line (1)
	minute traces of line (4)	short basal line (7)
$yl^T yl^T$	full line (2)	full line (9)
	weak line, red distally (4)	

Thus there is some segregation within the presumed genotypic classes, which probably but not necessarily stems from the use of more than one female parent. At least in the top left cell, the segregation corresponds to the difference between the two single-parent broods, indicating that this difference is probably not an absolute one between the Rio de Janeiro and the São Paulo populations.

From the segregation of the yellow line there is thus every reason to believe that the same loci, Yl and Cr, are segregating as in the cross with Trinidad; the only apparent difference is that the Cr allele of Trinidad is replaced in Panamá with the allele Cr^p, which not only removes the rectangles (dominant) but also adds the Panamanian yellow bar (nearly recessive). Alternatively, the bar gene (p) may be another locus very tightly linked to Cr. The only way finally to show that the apparent Yl and Cr loci are the same in both Panamá and Trinidad crosses is to hybridize all three races at once, or at least to cross Trinidad with Panamá; until this has been done, identity of the loci remains an excellent working hypothesis.

(c) Effects of the Yl locus on the shape of the forewing band

If the segregating locus controlling the yellow line is indeed Yl, then it should affect the shape of the forewing band exactly as it does in the Trinidad cross (plate 4a, b). In the backcross, the single-parent São Paulo brood divides well into presumed $Yl^t yl^T$ butterflies with an intermediate band and presumed $yl^T yl^T$ which are concave; the most extreme intermediate insect has slightly less red in the cell than the most extreme 'concave', but as before these individuals are at the extremes of their respective distributions and do not provide good evidence of crossing over. In the single-parent Rio brood the concave individuals have more red in the cell than in the São Paulo brood, but there is no overlap of the phenotypes, all presumed $Yl^t yl^T$ butterflies being intermediate and all presumed $yl^T yl^T$ being the rather extreme concave phenotype. The brood of mixed parentage again divides into concave $yl^T yl^T$ individuals and intermediate $Yl^t yl^T$, but with two of these being indistinguishable from concave and in fact having less red in the cell than the most extreme $yl^T yl^T$ insects. In view of the evidence from the Trinidad F_2

that heterozygotes may often be concave in appearance, these two cannot be regarded as cross-overs. No 'good' recombinants, that is insects having a full yellow line with an undoubted intermediate band, have appeared. All backcross and F_1 butterflies are toothed, showing that this character is dominant in the cross, and the F_1 is intermediate for concavity with a great range of variation reaching between near convexity and near concavity.

In the F_2 we can now diagnose the full Yl genotypes, using yellow line phenotypes and tooth, the weak yellow line, red distally counting as $yl^T yl^T$, and the weak basal line as Yl^t-:

band...	convex	inter-mediate	concave
no yellow line, no tooth ($Yl^t Yl^t$)	10	—	—
no yellow line, tooth ($Yl^t yl^T$)	—	16	4
yellow line, tooth ($yl^T yl^T$)	—	—	2

The segregation is very similar to that in the Trinidad F_2, with most heterozygotes having an intermediate band, but some being concave. Absence of tooth is recessive, and produced by $Yl^t Yl^t$ only, but most of the apparent heterozygotes have a shorter tooth than is normal in East Brasil; this appears to grade more or less continuously into the full tooth of the homozygotes. The segregation appears to deviate from $1:2:1$ (expected numbers $8:16:8$, observed $2:20:10$), but does not reach the conventional significance level ($\chi_2^2 = 4.83$, $P = 0.09$). The deviation should not be regarded as other than due to chance.

Among the additional broods of known pure Panamá × East Brasil origin, three (broods J, M and N in table A 5, appendix 5) segregate as backcrosses of the type $Yl^t yl^T \times yl^T yl^T$, and produce together

band...	intermediate	concave
$Yl^t yl^T$ (no line, tooth)	13	—
$yl^T yl^T$ (line, tooth)	—	21

The criterion for division between the intermediate and concave class differs slightly between broods, but is clear within broods, there being no overlap. A further brood of this provenance (brood K in table A 5, appendix 5) behaves like an F_2 cross ($Yl^t yl^T \times Yl^t yl^T$) and segregates

band...	convex	inter-mediate	concave
$Yl^t Yl^t$ (no line, no tooth)	3	—	—
$Yl^t yl^T$ (no line, tooth)	—	16	1
$yl^T yl^T$ (line, tooth)	—	—	6

Here, four of the concave class have a fair amount of red in the cell, but only the single most extreme heterozygote has an equal amount of red. Finally, broods L and O, which are small, have not segregated but consist entirely of five toothed individuals without yellow lines ($Yl^t yl^T$), all of which have, as expected, bands intermediate for concavity. (Classification of the lines in all the above takes account of modification by the Cr locus.)

The total absence in all these broods of the recombinant type 'line, no tooth' and the strong associations of convexity and concavity with the other phenotypes confirm that the Yl locus is affecting all these characters, heterozygotes, as always, being intermediate with a very small amount of overlap between the intermediate heterozygous and concave homozygous classes. There is no material difference between the action of the Yl alleles in this cross and in the cross with Trinidad; this is excellent evidence that the same alleles are segregating in both crosses.

(d) *Yellow line phenotypes in the* F_2 *and later generations*

Using the complete genotype diagnosis for both the Cr and Yl loci, we find the following phenotypes in the F_2 (numbers of butterflies in brackets):

	Cr^pCr^p (no rectangles, full Panamá bar)	Cr^pcr (no rectangles, shadow bar)	$crcr$ (rectangles, no Panamá bar)
Yl^tYl^t (no line, no tooth)	no line (3)	no line (3)	no line (1) weak basal line (3)
Yl^tyl^T (no line, tooth)	—	no line (14) ? very weak basal line (1)	no line (2) weak basal line (2)
yl^Tyl^T (line, tooth)	—	weak line, red distally (1)	full line (1)

Compared with the Trinidad cross, the $crcr$ genotype is less able to put a weak yellow line at the base of the wing, giving several 'no lines' in the right column; a similar discrepancy appeared in the mixed backcrosses. Otherwise the interaction of the loci in both the Trinidad and the Panamá crosses is very similar.

The remaining broods of pure Panamá × East Brasil provenance show similar interactions of the two loci in governing the line (table A 5, appendix 5). The effect of segregation of the Yl alleles on a homozygous $crcr$ background is seen in brood J, where eight offspring with concave bands (yl^Tyl^T) have a full yellow line, and two with intermediate bands (Yl^tyl^T) have a weak basal line. Similarly, brood O shows the Cr locus segregating on an apparently uniform heterozygous Yl^tyl^T background, all four individuals having intermediate forewing bands: three offspring with cream rectangles and no bar ($crcr$) have the expected weak basal line, and one with no rectangles and a shadow bar (Cr^pcr) has no line. The rest of the broods segregate for both loci. Brood N is a backcross for both:

	Cr^pcr (no rectangles, shadow)	$crcr$ (rectangles, no bar)
Yl^tyl^T (no line, tooth)	no line (3) weak medial line (1)	weak basal line (3)
yl^Tyl^T (line, tooth)	weak line, red distally (2)	full line (8)

The anomalous individual with the weak medial line is definitely a Yl^tyl^T heterozygote, having a clearly intermediate band with a tooth. Brood K is an F_2 for the Yl locus and a backcross for Cr:

	Cr^pCr^p (no rectangles, Panamá bar)	Cr^pcr (no rectangles, shadow)
Yl^tyl^T (no line, tooth)	no line (1)	no line (1)
yl^Tyl^T (line, tooth)	—	very weak line, red distally (1)

(one further individual with a full line is not otherwise scorable). This is important in confirming that the genotype Cr^pCr^p Yl^tyl^T, missing in the F_2 (above), has no yellow line, as far as can be judged from a single example.

Thus in these crosses the action of the Yl^t allele from Panamá is identical with that from Trinidad: it removes part of the line both when homozygous and when heterozygous. The

action of the *Cr* locus is almost identical in the two crosses: *crcr* allows the basal part of the line to remain when the rest is removed by Yl^t, and Cr^p weakens the base of the line, allowing it to become red from the tip in $yl^T yl^T$ genotypes. Both Yl^t and Cr^p are required for the complete removal of the line.

(e) Control of the East Brasilian yellow hindwing bar

The yellow bar of East Brasilian butterflies is removed in Panamá, to be replaced by the different yellow bar generated by homozygosity for $Cr^p Cr^p$. Our broods are consistent with the hypothesis that the East Brasilian bar is mainly removed by the joint action of the *Cr* and *Yl* loci, as in Trinidad, but with some difference in gene action, particularly of the *Cr* locus on a $yl^T yl^T$ background.

On the basis of the full genotype diagnosis as above (figure 7), the backcross broods segregate: first the single-parent (São Paulo) brood

	$Cr^p cr$	$crcr$
$Yl^t yl^T$	thin sharp (trace of yellow line) (3)	eaten (1)
$yl^T yl^T$	broad fuzzy (2)	broad sharp (5)

second, the single-parent (Rio) brood

	$Cr^p cr$	$crcr$
$Yl^t yl^T$	broken (no yellow line) (9)	eaten (4)
$yl^T yl^T$	broad fuzzy varying to thin sharp (7)	broad sharp (8)

As with the yellow line, the mixed backcross brood (São Paulo) shows a mixture of the effects of the other two broods:

	$Cr^p cr$	$crcr$
$Yl^t yl^T$ (trace of line)	broad fuzzy (2)	eaten (8) broad fuzzy (1)
$Yl^t yl^T$ (no line)	eaten (6) broad fuzzy (1) thin sharp (1)	
$yl^T yl^T$ (line)	broad fuzzy (4) broad sharp (2)	broad sharp (9)

Notice that *between* the uniparental broods and *within* the multiparental one, the presence of a trace of the yellow line in the top left of the table is correlated with an increase in the amount of yellow in the bar. The phenotypes are not as clearly separated as in the Trinidad crosses, especially broad fuzzy and broad sharp which are not clearly separated: both of the classes here include intermediate butterflies with slight black scaling at the edges of the bar, and no broad fuzzy is as extreme in fuzziness as in the Trinidad cross. The thin sharp phenotype is like thin fuzzy in the Trinidad cross, but much less invaded by black on the edges (figure 7, ts), and the appearance of broken and dots is a little altered. With these differences, the effect of the loci in the two crosses is similar: Yl^t has the effect of causing black to invade the bar from in front, at the base, and loss of *cr* makes the edges of the bar fuzzy. However, $Cr^p cr$ genotypes may tend to have stronger bars than the corresponding *Crcr* genotypes.

If this diagnosis is correct, then the F_2 should segregate in the following manner: each parental type of Brasilian yellow bar, that is no bar and broad sharp, should constitute one-sixteenth of the brood: between them, we expect four such parental phenotypes among the 32 offspring: there are three of the first and one of the second type, an excellent fit. The full segregation is:

	$Cr^p Cr^p$	$Cr^p cr$	$crcr$
$Yl^t Yl^t$	no bar (3)	thin sharp (1) broken (1) dots (1)	broken (3) dots (1)
$Yl^t yl^T$	—	thin sharp (2) eaten (1) broken (10) dots (2)	eaten (1) broken (3)
$yl^T yl^T$	—	thin sharp (1)	broad sharp (1)

The additional broods from this provenance, analysed in the same order as for the yellow line, give the following effects of the two loci on the bar (the classes 'no line' and 'line' as always include modifications due to Cr):

brood J

	$crcr$ (rectangles, no bar)
$Yl^t yl^T$ (no line, tooth)	eaten (2) broken (5)
$yl^T yl^T$ (line, tooth)	broad sharp (6)

brood O

	$Cr^p cr$ (no rectangles, shadow)	$crcr$ (rectangles, no bar)
$Yl^t yl^T$ (no line, tooth)	eaten (1)	eaten (3)

brood N

	$Cr^p cr$ (no rectangles, shadow)	$crcr$ (rectangles, no bar)
$Yl^t yl^T$ (no line, tooth)	very thin fuzzy (1) eaten (3)	broad (slightly fuzzy) (1) broad (slightly eaten) (1)
$yl^T yl^T$ (line, tooth)	thin sharp (2)	broad sharp (8)

(one individual not scorable.) The broad sharp bands are very wide, much wider than in a normal East Brasilian butterfly.

brood K

	$Cr^p cr$ (no rectangles, shadow)	$crcr$ (rectangles, no bar)
$Yl^t Yl^t$ (no line, no tooth)	—	eaten (1) broken (2)
$Yl^t yl^T$ (no line, tooth)	broad, slightly eaten (2) eaten (7)	eaten (8)
$yl^T yl^T$ (line, tooth)	broad, slightly eaten (1) broad sharp (4)	broad sharp (1)

brood M

	Cr^pCr^p (no rectangles, Panamá bar)	Cr^pcr (no rectangles, shadow)
Yl^tyl^T (no line, tooth)	broken (or dots) (1)	eaten (1)
yl^Tyl^T (line, tooth)	—	thin sharp (1)

This last brood has again provided a missing genotype: Cr^pCr^p Yl^tyl^T, which has an extremely reduced broken bar, almost in the 'dots' category, combined with a full Panamanian bar.

TABLE 9. INTERACTION OF Cr AND Yl LOCI IN THE PANAMÁ × EAST BRASIL
CROSS IN *HELICONIUS ERATO*

	Cr^pCr^p no rectangles full Panamá bar	Cr^pcr no rectangles shadow Panamá bar	$crcr$ rectangles no Panamá bar
Yl^tYl^t band convex, toothless	no yellow line no E. Brasil bar	no or weak yellow line part E. Brasil bar (variable)	weak basal yellow line, or none part E. Brasil bar (variable)
Yl^tyl^T band intermediate (or concave), toothed	no yellow line[1] part E. Brasil bar	no or weak yellow line part E. Brasil bar (variable)	weak basal yellow line, or none part E. Brasil bar (variable)
yl^Tyl^T band concave, toothed	weak yellow line,[2] red distally full E. Brasil bar (superbar)	weak yellow line, red distally part E. Brasil bar, varying to full	full yellow line full E. Brasil bar

[1] Only one of this phenotype in the broods of certain provenance; two more in the 'additional broods'.
[2] This phenotype is from the 'additional broods'.

Thus there is considerable variation both between broods and within the genotypic classes in each brood, possibly indicating the segregation of 'polygenes' like the Ybs locus in the Trinidad cross, including almost total removal of the bar in two 'dots' individuals (Cr^pcr Yl^tyl^T and $crcr$ Yl^tYl^t) in the F_2. We can conclude that homozygosity for $crcr$ yl^Tyl^T produces the broad sharp bar of the East Brasilian race, and that, as far as can be judged from three individuals, homozygosity for Cr^pCr^p Yl^tYl^t completely removes the East Brasilian bar. All other combinations of the alleles (except for Cr^pCr^p yl^Tyl^T which has not been seen) give bars that are reduced in some way, the usual effect of Yl^t, apparently partly dominant, being to eat into the bar in the same way as in the Trinidad cross. The effect of Cr^p on the bar appears to be mainly seen in Yl^t– genotypes; the Cr^pcr Yl^t– heterozygotes often have bars that are reduced compared with $crcr$, but in several broods, particularly K, the Cr^pcr yl^Tyl^T heterozygote may show no significant reduction of the bar compared with an East Brasilian homozygote. The modal phenotypes of the East Brasilian bar, which are affected of course by the relative sizes of the different broods, and to which the Panamá bar must be added where appropriate, are:

	Cr^pCr^p (Panamá bar)	Cr^pcr (shadow Panamá bar)	$crcr$ (no Panamá bar)
Yl^tYl^t	no bar	thin sharp, broken, dots	broken
Yl^tyl^T	broken	eaten, broken	eaten
yl^Tyl^T	—	broad fuzzy, thin sharp	broad sharp

The simplest conclusion from the existing data, which clearly need to be supplemented by a backcross to Panamá (see §4.5b for similar backcross to México), is that the yellow bars of the two races are mutually replaced by the cooperative action of the Cr and Yl loci. In addition, Yl and Cr act on the yellow line, Cr on cream rectangles and Yl on the red band in a manner almost identical to the cross with Trinidad. The phenotypes for all these characters are summarized in table 9, and the action of the individual alleles in table 10. If p is regarded as a gene closely linked to Cr, then Cr is having the same effects as in the Trinidad cross, and p in addition to adding the Panamanian shadow bar, is almost exactly cancelling the reducing effects of Cr on the Brasilian bar in the heterozygous genotype $Cr–p$, $cr–P$; $yl^T yl^T$.

TABLE 10. EFFECTS OF THE PANAMANIAN ALLELES Cr^p AND Yl^t ON THE
EAST BRASILIAN PHENOTYPE

(Compare with table 7)

allele	Cr^p	Yl^t
forewing band concave margin	—	converts to convex margin (intermediate heterozygote)
forewing band tooth	—	removes tooth (intermediate heterozygote, tooth tends to be dominant)
yellow forewing line	weakens line, especially at base and produces red invasion distally (dominant)	removes line (dominant), except for parts produced by $crcr$
yellow East Brasilian bar	weakens or removes (but not on $yl^T yl^T$ background), heterozygotes intermediate (?) on other backgrounds	weakens by invasion of black from in front at base (dominant)
yellow Panamanian bar	adds bar (recessive, shadow in heterozygote)	?
cream rectangles	removes rectangles (dominant)	—

(f) Variability of hindwing bars in the F_1

The modal phenotype of the F_1 from São Paulo stock is thin fuzzy, but some are eaten or even at the extreme end of the broken class. In a few there is a broad smudge of yellow and black scales at the base of the wing, occupying the area that would be the Panamanian bar in a homozygote. The Rio F_1 is similar. The variability of the F_1 is thus probably not much less than of heterozygotes in the F_2, which suggests that it may be due to loss of canalization or to the segregation of alleles polymorphic in one or both parental stocks.

(g) Shape of the end of the hindwing bar

In the F_1 and all the backcross butterflies, the hindwing bar turns backward at the tip, as in East Brasil (plate 4d). In the F_2 the bar is too short to score in three specimens and a further three specimens have been destroyed. In the remaining 26, there are 18 with a backward turn and 8 with a forward turn (expected 3:1 ratio 19½:6½), confirming that as in Trinidad the forward turn of the bar is produced by a single recessive allele, presumably the same as the Trinidad allele bf.

(h) Inheritance of red raylets

These appeared in the F_2, and are discussed in §4.7h.

(i) Linkage groups

As in the cross with Trinidad, none of the loci are certainly linked. The total backcross segregation for *Cr* and *Yl* (male parents heterozygous) is

	$Cr^p cr$	$crcr$
$Yl^t yl^T$	24	13
$yl^T yl^T$	14	21

Tested for equality of coupling and repulsion phenotypes, this gives $P = 0.02$ (one-tailed); $\chi_c^2 = 4.01$ with Yates's correction, $P = 0.02$ (one-tailed). If this represents linkage then the recombination fraction is 37.5 %, with a standard deviation of ± 5.7 %. On the other hand in the F_2 there are four apparent $crcr Yl^t Yl^t$ butterflies, which, if the loci are linked and recombination is absent in females, should not appear; the other recombinant homozygote is missing, making this evidence less than conclusive. By combining two of the homozygous classes with the heterozygotes to eliminate small expected numbers, the F_2 segregation is

	Cr^p-	$crcr$
$Yl^t Yl^t$	6	4
$-yl^T$	16	5

for which $P = 0.30$ (one-tailed), which is not significant. The apparent association between the loci in the backcross is probably a spurious one, being the formally significant result that one expects from time to time when making a large number of tests. It is likely to have been enhanced because we kept a number of the singly and doubly heterozygous offspring for further breeding, and some of these died and have been destroyed by greenhouse scavengers; most of the parents of the broods in table A 5, appendix 5, which are crosses between various backcross butterflies, have been lost to the brood in this way.

Because of unrecorded parentage the remaining broods, with the exception of K, cannot be used to test the linkage. Brood K must have had one doubly heterozygous parent: it segregates

	$Cr^p cr$	$crcr$
$Yl^t Yl^t$	0	3
$Yl^t yl^T$	7	8
$yl^T yl^T$	5	1

Combining the upper rows to make a 2×2 table gives $P = 0.15$ by Fisher's exact test (two-tailed); combining the two lower rows gives $P = 0.22$ (for the whole table, $\chi_2^2 = 5.73$ (without Yates's correction), $P = 0.058$, which is unreliable because of low expected numbers). This is further confirmation that *Cr* and *Yl* are unlinked.

The *Bf* locus is again independent of the other two. In the F_2 the appearance of two representatives of the doubly recombinant genotype *bfbfcrcr* (forward turn and cream rectangles) shows that *bf* is independent of *cr*. The double recombinant $bfbf yl^T yl^T$ is absent in the F_2. The segregation is

	$Bf-$	$bfbf$
$Yl^t Yl^t$	5	4
$Yl^t yl^T$	3	12
$yl^T yl^T$	0	2

Combining the lower rows gives $P = 0.06$ (one-tailed), which in view of the independence of these characters in the Trinidad cross is unlikely to indicate linkage.

(j) Summary

The Panamá race has a genetic make-up very like that of the Trinidad race: it carries the alleles Yl^t and Cr, which remove the yellow lines and bars of the East Brasilian race and alter the shape of the forewing band, and the gene bf, which turns the tip of the hindwing bar forward (plate 4 a–d). It differs from Trinidad in carrying p, which places a large yellow bar across the hindwing, and which either is closely linked to Cr or is an allele at that locus (Cr^p). All other loci are independent, and their interactions are similar, although not identical, to those in the Trinidad × East Brasil cross.

4.4 Additional broods

Those broods of Panamá × East Brasil provenance that are known or suspected to have additional ancestry from the Trinidad or other races have been excluded from the above analysis of the Cr and Yl loci as we wished to establish how similar the action of the loci was in the Trinidad and Panamanian races; whereas the Panamanian Cr^p allele is clearly distinct from the Trinidadian Cr, this is not true of the respective Yl^t alleles. However, now that we have established by independent analysis that the Yl^t alleles from both these races are very similar if not identical, we shall analyse the effects of the two loci in the remaining broods, to confirm the other results. (Broods that segregate as well for alleles from the Amazonian races affecting the forewing band will be dealt with in §4.9.)

(a) Effect of Yl on shape of forewing band

As before, the phenotype 'line, no tooth' has not appeared, indicating that the teeth are a product of the yellow line allele, and permitting full classification of all genotypes, as before the short basal line being treated as 'no line.' It is convenient to score these broods in three groups, according to the segregation of the Yl alleles. First, broods G and J2 (tables A 6 and A 10, appendix 5) appear to be backcrosses of the type $Yl^t yl^T \times yl^T yl^T$ (that is, like a backcross to East Brasil):

	intermediate	concave
$Yl^t yl^T$ (no line, tooth)	19	3
$yl^T yl^T$ (line, tooth)	—	15

(three individuals not scorable). The male parent is the heterozygous phenotype, so crossovers could appear if the yellow line and band genes were separate. The three anomalous individuals (all from brood G) just overlap with the most extreme of the concave homozygotes, and appear to be extreme variants rather than recombinants. The separation of the classes in brood J2 is unambiguous. Second, one brood (R in table A 10, appendix 5) segregates as the other backcross (to Trinidad or Panamá), giving

	convex	intermediate
$Yl^t Yl^t$ (no line, no tooth)	4	—
$Yl^t yl^T$ (no line, tooth)	—	7

with the teeth of some individuals being rather blunt. Third, there are six crosses apparently

between two yellow line heterozygotes (broods C, H, Q, I, P and A in tables A 6 and A 10, appendix 5), segregating as an F_2:

	convex	intermediate	concave
Yl^tYl^t (no line, no tooth)	17	—	—
Yl^tyl^T (no line, tooth)	1	23	2
yl^Tyl^T (line, tooth)	—	—	21

(brood A is of recorded parentage: both are clearly heterozygotes). There are a few scoring difficulties. One heterozygote in brood H is not separable from the convex homozygotes, and one in each of broods C and H is not separable from the concave. There are three concave individuals in brood H that have the weak yellow line with the red tip, which we originally scored as Yl^tyl^T because the line is almost obliterated by the red marks of the radiate pattern which is segregating in this brood; careful re-examination revealed the line among the red scales. In some broods, particularly C, most heterozygotes can further be distinguished by having a blunt tooth. Last, there is one brood (T in table A 6, appendix 5) that appears to consist entirely of Yl^tyl^T heterozygotes (all no line, tooth); all of these have an intermediate band.

All the additional broods therefore confirm that the Yl locus affects the shape of the band in the way already described. There is a perfect association between the line and the tooth, and a strong one between these characters together and the degree of band concavity. There are no certain crossovers, and all the apparent ones are in one direction; there is therefore no reason to suppose that this is anything more than a single functional locus with diverse effects.

(b) Control of yellow line and yellow bar by Cr and Yl

The above-listed broods also segregate for the Cr locus (five backcrosses of the East Brasil type, one F_2, four homogyzous *crcr*). Rather than describe in detail all six combinations of segregations at the two loci that were obtained, we have tabulated the yellow line and yellow bar phenotypes according to complete genotype diagnosis, for all ten broods combined (table 11).

Brood J2 is particularly interesting in that it behaves as an F_2 for Cr and a backcross for Yl, and has produced one individual of the genotype that was missing in the pure Panamá × East Brasil broods: homozygous Cr^pCr^p yl^Tyl^T. This has a very weak yellow line, strongly red for the distal three-quarters of its length, and has the full yellow hindwing bars of both parental races producing, because of the difference in position, an extremely wide yellow bar which we have dubbed 'super bar'. We have one further obvious Cr^pCr^p yl^Tyl^T butterfly (unlabelled, but possibly from brood J2); this confirms the phenotype described (plate 4c, right). As the allele Cr^p must come from Panamá and yl^T from East Brasil, there is no possibility of Trinidadian or other ancestry (except in the genetic background), and this phenotype has been inserted in table 9. The two $Cr^pCr^p Yl^tyl^T$ butterflies are similar, except that their Brasilian bars are 'eaten', so producing a 'superbar' that is concave from the front. The same genotype in brood M had a more reduced East Brasilian bar, but was otherwise similar. Brood G shows a perfect segregation of Yl^tyl^T versus yl^Tyl^T on a uniform *crcr* background; all 14 heterozygotes have a weak basal line and an eaten bar, all 12 homozygotes have a full line and a full East Brasilian bar. Brood R segregates Yl^tYl^t and Yl^tyl^T on the same background: all 11 butterflies have a weak basal line and an eaten bar. Brood T is uniformly Yl^tyl^Tcrcr and all butterflies have weak basal yellow lines and rather broad eaten hindwing bars, as expected.

The effects of the two loci on the yellow line are so similar to what has already been found as to require no further comment.

TABLE 11. YELLOW LINE AND YELLOW BAR PHENOTYPES FOR ALL NINE Cr–Yl GENOTYPES OF *HELICONIUS ERATO* IN BROODS Q, R, I, P, G, H, C, J2, A AND T COMBINED

(Numbers of butterflies in brackets. Ratios are meaningless because of addition of several different kinds of brood.)

	Cr^pCr^p	Cr^pcr	$crcr$
	(full Panamá bar)	(shadow of Panamá bar)	(no Panamá bar)
Yl^tYl^t	—	no line (6)	very weak basal line (1)
		very weak basal line (2)	weak basal line (12)
	—	dots (3)	broken bar (6)
		broken bar (5)	eaten bar (7)
Yl^tyl^T	no line (2)	no line (17)	very weak basal line (1)
		very weak basal line (3)	weak basal line (44)
	eaten (eaten superbar) (2)	broken bar (1)	eaten bar (43)
		eaten bar (18)	thin fuzzy bar (1)
		thin fuzzy bar (1)	thin sharp bar (1)
yl^Tyl^T	very weak line, strong red distally (1)	weak line, red dot (6)	full line (sometimes red tip) (26)
		weak line, red distally (4)	
	broad sharp bar (superbar) (1)	thin, slightly eaten bar (3)	broad sharp bar (sometimes very broad) (26)
		thin fuzzy bar (1)	
		thin sharp bar (1)	
		broad fuzzy bar (2)	
		broad sharp bar (3)	

The overall segregation is very clear, slightly clearer in fact than in the pure Panamá × East Brasil broods, suggesting that the introduction of the genetic background of the Trinidad and of some of the Amazonian races may have improved canalization. The allele Yl^t shows as before a largely dominant effect of eating into the bar, slightly stronger in homozygotes, producing the phenotypes 'eaten' and 'broken', and Cr^p the effect of weakening the bar, seen least in yl^Tyl^T homozygotes, where Cr^pcr and Cr^pCr^p may not be distinguishable in this respect from the full East Brasilian phenotype. The genotype $Cr^pcryl^Tyl^T$ is particularly variable, although a little less so *within* broods: for example, all three 'thin, slightly eaten' bars are in brood C (table A 10), in which they form a quite distinct phenotypic class.

(c) Inheritance of tip of hindwing bar

The Panamá–Trinidad character of the yellow bar turning forward at the tip segregates in five of the additional broods (plate 4d).

Broods C, J2 and Q are apparently F_2 or backcrosses segregating 9:3, 9:5 and 7:3 backward turned bars to forward respectively. The expression of forward turn of the bar appears to be independent of the cream rectangles, as one butterfly in J2 has both characters. In brood R, seven out of the 11 butterflies seem to show the forward turn of the bar, but the mark gives the impression of being repressed, and is difficult to score. Brood H appears to be an F_2, with forward-turning bars in three out of 25 butterflies (there are two more doubtful ones, and the slight deficiency may be due to scoring difficulties in this brood). The gene *bf* therefore continues to behave as a single recessive factor, with some scoring problems.

In the remaining broods all butterflies have a backwardly turning bar.

(d) Linkage groups

Because of incompletely recorded parentage, only limited tests for linkage can be performed in the additional broods. Only one brood can be tested for the absolute linkage of *Cr* and *Yl* in a doubly heterozygous female. If the loci are linked, then a cross with such a mother, which is a backcross for *Cr* and an F_2 for *Yl*, either will not contain the recombinant classes *crcr* Yl^tYl^t or $Cr^pcr\ yl^Tyl^T$ if the loci are in coupling, *or* will not contain $Cr^pcr\ Yl^tYl^t$ or *crcr* yl^Tyl^T if the loci are in repulsion. Brood C is such a mixed backcross and F_2, and the father, which is preserved, was plainly of the genotype *crcr* Yl^tyl^T, which means that the mother, now lost, was the double heterozygote. All four of the prohibited genotypes have appeared in this brood (two of the coupling type, four of the repulsion), thus proving that the loci are on separate chromosomes, whether they were in coupling or repulsion.

TABLE 12. TESTS FOR LINKAGE IN VARIOUS OF THE ADDITIONAL BROODS OF *H. ERATO*

(Probabilities without χ^2 values were computed by Fisher's exact test
for a 2×2 contingency table, two-tailed.)

loci tested	brood no.	number of offspring scored	χ^2_2	P
Cr–Yl	C	12	0.4	0.8
Cr–Yl	H	25	0.6	0.7
Cr–Yl	J2	14	0.2	0.9
Cr–Yl	Q	10	2.0	0.4
Cr–Yl	I	6	—	1.00
Cr–Yl	P	7	—	1.00
Bf–Cr	J2	14	—	0.91
Bf–Yl	J2	14	—	0.91
Bf–Yl	Q	10	—	1.00
Bf–Cr	Q	10	—	0.37
Bf–Cr	C	12	—	0.51
Bf–Yl	C	12	—	0.47

The weaker test for statistical association of the loci can be performed on this brood and on three others which are mixed F_2–backcrosses (J2 being on F_2 for *Cr*, the other two being like C), and on two very small broods which are certainly F_2 for *Yl* and are probably backcrosses for *Cr*. The results are all non-significant, confirming the absence of linkage (table 12).

Four broods test for linkage of *Bf* with the other two loci. First, brood H is an F_2 for *Bf* and *Yl*; if the loci are linked, then, given the full dominance of *Bf*, the prohibited genotype is *bfbf* yl^Tyl^T (yellow line, tooth, bar turned forward) if the loci are in repulsion, and *bfbf* Yl^tYl^t (no yellow line, no tooth, bar turned forward) if the loci are in coupling. There is a single definite *bfbf* yl^Tyl^T in brood H. Linkage is therefore not ruled out in brood H, if the loci happened to be in coupling.

In a brood that is an F_2 for *Bf* and a backcross for *Cr*, the prohibited genotypes are *bfbf crcr* (rectangles with bar turned forward) for repulsion crosses and *bfbf* Cr^pcr (shadow of Panamá bar with bar turned forward) for coupling. These are also among the prohibited genotypes if the brood is a backcross for both loci with a doubly heterozygous mother. In brood C, which is the only brood of this type known to have had a doubly heterozygous mother (it may be a backcross or a mixed F_2–backcross), there are six of the second type, but none of the first; so from this brood the loci could be linked if they were in repulsion. This test cannot be used in the other broods because it is not clear which parent was the double heterozygote.

Testing three broods by Fisher's exact test for contingency gives the results shown in table 12;

brood H has not been tested because of difficulties in scoring $bfbf$. All the tests, both for Bf–Cr and Bf–Yl, are non-significant.

Thus the additional broods, like those of known provenance, show that the Bf, Cr and Yl loci are on separate chromosomes.

(e) Summary

These broods confirm all the findings of the broods of known provenance that they are able to test: (i) the pleiotropic action of the Yl alleles, affecting forewing line, hindwing bar, band tooth and band concavity (plate 4a, b), with all anomalous phenotypes being attributable to variation in expression rather than crossing over; (ii) the interaction of the Cr and Yl loci in causing mutual replacement of the East Brasilian and Panamanian hindwing bars; (iii) the recessive single allele inheritance of the forwardly turning bar of the Panamá race (plate 4d); (iv) the absence of linkage between Bf, Cr and Yl. The broods also show that the two kinds of hindwing bar are to a high degree independent developmentally, the genotype with both bars fully developed simply adding them together to produce a single wide 'superbar' (plate 4c, right), and that the allele Cr^p, while able to reduce the East Brasilian bar on Yl^t–backgrounds, has virtually no effect on the bar on a $yl^T yl^T$ background.

4.5. The Cross México × East Brasil

The México populations of *H. erato* are very like those from Panamá (above), but in the north, where our stock came from (Gomez Farias, figure 3), differ quantitatively, particularly in that the yellow hindwing bar is very narrow. It is a reasonable working hypothesis that, when crossed with East Brasilian stock, the Mexican race will turn out to be genetically closely similar to the Panamanian. Our crosses of Mexican butterflies were made long after we had prepared our interpretation of the Panamá crosses recounted above, and can therefore be used as independent verification of our hypotheses.

We have bred an F_1, an F_2, and two backcrosses to México (table A 5, appendix 5); the outcomes of all these crosses are easily predicted from our Panamanian results, which lead us to expect the segregation of three loci, Panamá being homozygous bf; Cr^p; Ylt and East Brasil homozygous Bf; cr; yl^T.

(a) The F_1

It is therefore predicted that the F_1 México × East Brasil hybrids will be $Bfbf$; $Cr^p cr$; $Yl^t yl^T$, which will be the phenotype concave or intermediate band, tooth, no yellow line, no cream rectangles, eaten Brasilian bar, shadow of Panamá (México) bar (plus backward turn of bar tip, probably not scorable). The nine extant specimens do indeed have this phenotype, except that the shadow of the Panamanian bar cannot be detected on one slightly worn male, and that some of the eaten bars are so extreme that they could be scored as broken.

(b) The backcross to México

The phenotype segregation should be (in equal numbers)

	$Cr^p Cr^p$	$Cr^p cr$
$Yl^t Yl^t$	(i) band convex, toothless, full Panamá bar, no E Brasil bar (Mexican phenotype)	(ii) band convex, toothless, shadow of Panamá bar, part of E Brasil bar

$yl^T Yl^t$ (iii) band concave or inter- (iv) band concave or inter-
 mediate, tooth, full   mediate, tooth, shadow
 Panamá bar, part   of Panamá bar, part
 E Brasil bar   E Brasil bar.

All classes should lack the yellow line and the cream rectangles, and should segregate $1:1$ into forward-turning ($bfbf$) and backward-turning ($Bfbf$) yellow bars. In the large backcross (brood MB2, table A 5, appendix 5), there are in fact eight such phenotypic classes, whose numbers are (in the numerical order given above, with backward turns given in each pair before forward turns) (i) 10, 6, (ii) 5, 6, (iii) 5, 12, (iv) 2, 1, giving a not quite significant departure from equality ($\chi_7^2 = 12.91$, $P = 0.07$). As predicted, there are no yellow lines or rectangles.

As there may be an overall departure from regular segregation, an analysis of the seven components of departure from equality (Bailey 1961) has been performed. In no case is there any indication of linkage between any pair of loci (in all three pairs the recombinant pheno-types outnumber the parentals) nor any significant association due to pleiotropic interaction (all P values exceed 0.24, two-tailed). The segregation at the Yl and Bf loci is regular ($P = 0.38$ and 0.77). Departure from regularity is shown by the triple interaction ($P = 0.08$, two-tailed), which cannot result from linkage, as no pair is linked, and the Cr locus, which segregates $33:14$ ($P = 0.008$). As simple scoring problems are unlikely to produce this result (the shadow bar being mistaken for a full Panamá bar) it is worth considering whether another locus may be involved.

If another recessive allele, unlinked to Cr, was also able to produce a full Panamá bar, then Panamá bars and shadows would segregate in a $3:1$ ratio, which is a good fit to that observed. However, this would produce in the F_2 the segregation

Panamá bar, no rectangles	6
shadow bar, no rectangles	6
no bar, no rectangles	3
Panamá bar, rectangles	1

which is almost certainly not the segregation observed (below). Thus linkage, scoring (mani-festation) and interference by another locus all seem to be excluded. It is possible that there is a viability effect, but a simpler explanation seems more likely. Inspection shows that the chief source of the disturbance is a deficiency of phenotypic class (iv), the F_1 phenotype. This is the natural choice as parent for continuing the experiments by producing a quasi-F_2, and it is probable that some of these butterflies were simply lost in the greenhouse by P.M.S. in un-successful and unrecorded attempts to breed from them.

The effects of the presumed loci on the Brasilian hindwing bar are much as would be expected from the Panamá cross. The bar is too weakly developed to be observed when the Panamá bar is present; when the Panamá bar is only a shadow then the Brasilian bar appears as a very thin version of the 'eaten' phenotype (see under the Panamá cross) or as an even more extreme 'broken' form in which only the distal part of the eaten bar is present. All three of the $Yl^t yl^T$ butterflies have the thin eaten bar, and the $Yl^t Yl^t$ butterflies are divided roughly equally between the two phenotypes; there are too few of the former class for us to say whether this indicates an influence of Yl^t on the form of the bar in this brood. In nine of the butterflies the Panamá bar is represented on the upperside by a faint dusting of yellow scales; the effect is absent in the remaining four specimens. This could indicate the segregation of a further locus, with a recessive allele that strengthens the bar on the upperside (for $1:1$, $P = 0.27$) (see also p. 551).

The smaller backcross (brood MB13, table A 5, appendix 5) segregates (in the order listed above) (i) 1, 0 (plus 1 not scorable for tip of bar), (ii) 1, 1 (iii) 2, 2, (iv) 2, 3. Here there is no sign of irregular segregation from the three locus hypothesis, the loci segregating

$$Yl \quad 4:9, \quad Cr \quad 6:7, \quad Bf \quad 6:6,$$

which are all satisfactory fits to 1:1 segregations. This lends further weight to the hypothesis that the deficiency of class (iv) in the larger brood results from chance or from the loss of breeding individuals.

(c) The F_2

In this cross the segregation should be in the ratio 1:2:1 for the phenotypes full Panamá (México) bar, no rectangles (Cr^pCr^p):shadow Panamá (México) bar, no rectangles (Cr^pcr):no Panamá (México) bar, cream rectangles $(crcr)$. The actual numbers are 9:15:5, a satisfactory fit (three individuals cannot be scored). In addition there are three individuals with the un-expected phenotype no Panamá (México) bar, no rectangles. These could be the result of recombination or variable gene expression; as in the previous broods, only the reciprocal phenotype with a shadow bar and cream rectangles would be convincing evidence of recombination (see below).

In four of the nine individuals with Panamá bars, the bar is rather weakly developed, with a heavy dusting of black scales; this may again indicate, as in the backcross, that further genes besides Cr^p are required for its full development.

It is also predicted that the Yl locus will segregate convex, toothless band, no yellow line (or only traces) (Yl^tYl^t):concave or intermediate toothed band, no yellow line (or only traces) (Yl^tyl^T):concave, toothed band, full yellow line (or slightly reduced) (yl^Tyl^T), in the ratio 1:2:1, with the variation in the yellow line resulting from the segregation of cr. The numbers are 6:21:7 (one further concave individual not otherwise scorable) ($\chi_2^2 = 1.31$, a satisfactory fit).

The forward turn of the hindwing bar, if produced by the recessive allele bf, should appear in one-quarter of the brood. Many individuals are difficult to score, but if we accept only positive evidence of a forward turn seven out of the 35 butterflies have a forward-turning bar (expected $8\frac{3}{4}$); if we exclude all butterflies with hindwing bars scored as broken or dots, then there are seven forward turns out of 25 butterflies (expected $6\frac{1}{4}$). With either method of scoring, the prediction is confirmed.

On the basis of the most probable assignment of genotypes, the Cr and Yl loci control the yellow line as follows:

	Cr^pCr^p (full Panamá bar, no rectangles)	Cr^pcr (shadow bar or no bar, no rectangles)	$crcr$ (no bar, cream rectangles)
Yl^tYl^t (convex, toothless)	no line (2)	no line (3) basal line (1)	—
Yl^tyl^T (concave or intermediate, tooth)	no line (5)	no line (8) basal line (5)	basal line (3)
yl^Tyl^T (concave, tooth)	red line (2)	red line (1) medial weak line (1)	full line (3)

The Panamanian alleles remove the line, both Cr^p and Yl^t being necessary for its complete removal, Yl^t being sometimes able to remove the line when heterozygous. Similarly the Brasilian yellow bar is reduced by joint action of these two loci (its traces in the presence of the full Panamá bar not being scorable):

	$Cr^p cr$	$crcr$
$Yl^t Yl^t$	dots (1)	—
	broken (3)	
$Yl^t yl^T$	broken (6)	
	eaten (6)	eaten (3)
	thin fuzzy (1)	
$yl^T yl^T$	thin fuzzy (2)	thin sharp (2)
		broad sharp (1)

The action of both loci on the yellow line and bar is so similar to that already seen in the cross with Panamá (table 9) as to make it clear that Mexican and Panamanian butterflies are genetically very much alike, and that the same loci are segregating in both crosses.

The absence of the apparent genotype $crcr\,Yl^t Yl^t$, although not statistically significant, suggests that Yl^t may have some ability to suppress the cream rectangles in this cross, converting this genotype to the phenotype no Panamá bar, no rectangles, which looks like a recombinant between Cr and p (above). One of the three butterflies in this class is $Yl^t Yl^t$ and the other two are $Yl^t yl^T$, indicating that the effect can probably occur on any Yl^t background; alternatively, it is possible that the shadow of the bar is sometimes suppressed in $Cr^p cr$ butterflies.

(d) Unlinked loci

The mothers of both backcrosses are F_1 butterflies and therefore heterozygous for all three of the loci *Bf*, *Cr* and *Yl*. The appearance of recombinant phenotypes in the backcrosses proves that, as in the Trinidad and Panamá crosses, these loci are not linked.

(e) Summary

Except for one irregularity of segregation in one of the backcrosses, which probably results from loss of part of the brood, it is clear that Mexican and Panamanian *erato*, regarded by some authorities as representatives of the same subspecies, and differing only in minor features, are genetically identical at the major loci controlling the colour pattern. Similar but much weaker evidence has been obtained (§ 3.3) for the identity of Venezuelan and Trinidadian populations of *melpomene*.

The backcrosses to México confirm our hypotheses about the genetics of the Panamá × East Brasil cross, which had to be drawn without the benefit of a backcross in that direction.

4.6. The Cross Rondônia × East Brasil

Populations of *H. erato* in the Brasilian state of Rondônia (formerly the territory of Guaporé) have a variable appearance probably resulting from hybridization between the Belém/Mato Grosso race and the Bolívia/Mato Grosso race; these races are identical except that the latter is red rather than orange (with the red marks rather reduced in extent) and has a yellow forewing band which is compact, somewhat intersected by black along the veins, whereas the Belém/Mato Grosso race has a broken yellow band (see Brown & Mielke 1972, fig. 63). Both races are

The invaded pattern segregates clearly as a single dominant allele. The F_1 are all invaded, although somewhat variable, and the backcrosses to East Brasil segregate 33 invaded:41 entire (for 1:1, $P = 0.42$). One of the F_3 broods (WK13) with both parents invaded, segregates 3 invaded:1 entire (actual numbers 29:6, $P = 0.38$). Some of the invaded individuals appear to have the band more broken by black than the original Rondônia parent. This may represent homozygosity for the 'invaded' gene, or the segregation of other factors. The F_4, a cross between an invaded and an entire butterfly, could be expected to segregate as a backcross: the numbers are 7 invaded:6 entire. The remaining F_3 brood (WK15) is a similar cross, and segregates 2 invaded:7 entire, which is a satisfactory fit to a backcross (the exact probability, two-tailed, is 0.18). Similarly, brood B (table A 10, appendix 5) is derived from this stock, and was a mating between an entire female (preserved) and what must have been an invaded male (now lost); it segregates 16 invaded:17 entire.

There is thus no doubt from the clear 3:1 and 1:1 segregations that invaded bands are produced by a single dominant gene, and that this was homozygous in the Rondônia parent.

Concavity and tooth (plate 4a, e) can be scored readily when the band is entire; on invaded bands we can treat absence of the large coloured spot in the cell as concavity. The F_1 is intermediate for concavity, having the mark in the cell more or less invaded by black, and has a jaggedness at the outer posterior part of the band, which we can interpret as a partial expression of the teeth. All backcross butterflies with entire bands have the teeth; they cannot always be scored on invaded bands, as they are often too restricted in size to enter the part of the wing where the teeth develop, but no butterfly in this class can be described as definitely lacking the teeth in the way that a Trinidad or Panamá butterfly does (that is, showing a toothless extension of the band into the rear angle of the wing). If we regard intermediate convexity on invaded bands as the presence of a more or less well developed coloured spot within the cell of the forewing, the segregation of all three characters is

	intermediate, slight or unscorable tooth	concave, full tooth
invaded	33	—
entire	—	41

As is usual, there is some overlap between the intermediate and concave categories, and some variation in the expression of invaded, so that three or four butterflies could be placed in the unoccupied cells of the table. However, as these seem to represent the extremes of phenotypic variation within each brood (especially as the Rondônia parent may have been highly heterozygous for genes affecting the expression of invaded in the direction of entire), and as they occur in broods with both male and female F_1, and hence heterozygous, parents, there is no strong case for regarding them as recombinants. The F_3 and F_4 broods are consistent with this pattern of segregation: all offspring are either invaded and intermediate (or convex), or entire and concave with manifest teeth, with the exception of ten individuals in brood WK13 and in brood WK15 which have invaded bands that are very weakly developed within the cell, and which could therefore be regarded as the crossover class 'invaded, concave'. The simplest hypothesis is that we are dealing with two alleles, one producing an invaded, convex, toothless

band, the other an entire, concave, toothed band, invaded being dominant to entire, toothed being dominant to toothless but showing some reduction in the heterozygote, and convexity showing an intermediate, variable heterozygote. The ten apparent crossovers probably represent the modifying effects of other loci or of the environment, rather than true crossing over, as the reciprocal crossover class 'intermediate, entire' is absent (11:0 being a significant departure from 1:1, with a two-tailed probability of only 0.00049). If the anomalous butterflies are moved into the 'intermediate, invaded' class, then brood WK13 is a good fit to an F_2 ratio, segregating convex broken:intermediate invaded plus concave invaded:concave entire in the numbers $7:(12+10):6$, compared with the expected numbers $8\frac{3}{4}:17\frac{1}{2}:8\frac{3}{4}$ ($\chi_2^2 = 1.67$, $P = 0.43$). There is, as always, variation in the intermediate class, two heterozygous butterflies in brood WK14, which is a backcross, being convex in appearance.

(d) Inheritance of yellow line

As in all other crosses involving East Brasil, the inheritance of the yellow forewing line (plate 4a, b) is intimately involved with the inheritance of the shape of the forewing band. Nearly all backcross, F_3 and F_4 butterflies with invaded bands lack the yellow forewing line (or have a weak line, usually confined to the base of the wing), and all butterflies with concave, entire bands have a fully developed line (one exceptional invaded backcross butterfly has a full line). In a total of 131 insects, this does not require a significance test. It is clear that we are dealing with a pair of alleles affecting all the major attributes of band shape and the yellow forewing line, or with two tightly linked clusters of loci (supergenes) with the same effect.

We may suppose that we are dealing either with another allele at the Yl locus, say Yl^I, with similar effect to Yl^t, except that it produces an invaded band as well as altering other parts of its shape, or with another completely separate locus, the Rondônia allele producing the invaded, convex band, no yellow line phenotype, and the East Brasil allele producing the entire, concave, toothed band and yellow line. No choice between these hypotheses is possible from the present broods, and for the moment we shall designate the factor that produces the invaded band and reduces the yellow line, I, writing it in bold type to emphasize that we reserve judgement on its identity.

(e) Inheritance of cream rectangles

The cream rectangles (plate 4d) of the East Brasilian race appear in this cross to be controlled by more than one factor, or at least to show some penetrance when heterozygous. There are weakly developed rectangles in seven of the 14 extant F_1 butterflies, and in the backcross broods WK8, WK9, WK11 and WK12 (all of which had F_1 parents without rectangles) there are 27 with more or less developed rectangles, four with very weak rectangles, and eight with no rectangles (only extant specimens with the hindwings in good condition being counted). This is wide of the expected 1:1 ratio. The third backcross (WK10) had an F_1 mother with cream rectangles, and all 14 of the scorable offspring have rectangles, although they are very weak in one specimen. The F_3 and F_4 do not segregate, being crosses between cream rectangled parents all of whose offspring have cream retangles. Because of the partial dominance of the absence of rectangles, it seems likely that Cr is segregating in this cross, but is being modified by other factors; it is also possible that some East Brasilian alleles affecting this character occur by gene flow in the Rondônia population.

The usual correlation between rectangles and the development of the yellow line is also weaker in this cross. In the backcrosses we have

	rectangles	weak rectangles	no rectangles
I–	weak line (3) basal line (14)	basal line (1)	basal line (1) no line (6)
$yl^T yl^T$ (not-*I*)	full line (27)	full line (4)	full line (4)

indicating a very strong influence of *I* on the line (above) with all 'not *I*' butterflies having full lines, whatever the state of their rectangles. However, within the *I* butterflies there is a moderate tendency for absence of rectangles to be accompanied by the complete disappearance of the line. This suggests that *Cr* is indeed segregating.

A curious feature of some of the *I* individuals with weak basal yellow lines is that a second yellow line, also faint, occurs along the anterior vein of the main forewing cell, in addition to the line in the usual position along the posterior vein. This character can be seen only on non-radiate individuals.

(*f*) Inheritance of yellow hindwing bar

Both the apparent *Cr* locus and the factor *I* affect the yellow bar on the hindwing. The bar is absent or appears only as traces in the F_1. The backcrosses segregate

	rectangles	faint rectangles	no rectangles
I–	no bar (6) faint yellow scaling (3) thin fuzzy (6)	no bar (1)	no bar (4) faint yellow scaling (1)
$yl^T yl^T$ (not-*I*)	eaten (2) broad sharp (25)	broad sharp with some black scaling (3) broad sharp (1)	broad fuzzy (2) broad sharp with some black scaling (2)

(see figure 7, bb for broad sharp with black scaling). The effects of *Cr*, or whatever else weakens and removes the rectangles, seem to be to weaken the bar, roughly to the extent observed in crosses with Panamá. If, instead of scoring the rectangles, we divide the brood into those with and those without a weakened bar (no bar versus thin bar in the *I* class, and bars with and without fuzziness or black scaling in the not-*I*), then the broods segregate 32 strong bars: 24 weak, which is a good fit to 1:1 ($P = 0.29$). This suggests that *Cr* is having its customary effects on the bar, but is being modified in its effects on the line and rectangles. In contrast to Yl^t, observed in Trinidad, and, as far as we can tell, in Panamá, the Rondônia factor *I* does not cause the bar to become eaten, but has the effect of reducing it to a faint dusting of yellow scales, or often removing it altogether. The F_3 and F_4 broods, in which all butterflies have rectangles, amply confirm this. All apparent yl^Tyl^T butterflies in the F_4 have a broad sharp bar, and six of the *I* butterflies have no bar; the seventh has a strong dusting of yellow scales (it also has a partly developed yellow line, lacking in the others, and has a forewing band intermediate

between invaded and entire). The F_3 brood WK15 shows a perfect segregation: seven $yl^T yl^T$ with broad sharp bars and two I with none. The second F_3 brood (WK13) is a cross between heterozygotes, and segregates

	crcr
homozygous *II*	no bar (5), faint scaling (2)
heterozygous *I*	no bar (7), faint scaling (14), thin fuzzy (1)
homozygous not-*I*, $yl^T yl^T$	broad sharp (6)

(homozygous and heterozygous I being distinguished by the convexity or intermediacy of the band), showing a strong reduction of the bar by I, which at least on this background is most pronounced in homozygotes. The effects of I seem to be largely dominant or epistatic to those of the Panamanian allele Yl^t, to judge from the brood of Rondônia × Panamá × East Brasil origin (brood B, table A10), which segregates:

	$Cr^p Cr^p$ (full Panamá bar)	$Cr^p cr$ (shadow of Panamá bar)	*crcr* (no Panamá (bar)
I, $Yl^t(yl^T)$	—	dots (1) broken fuzzy (1) thin fuzzy (4)	—
I, $yl^T(yl^T)$	no bar (1) (plus Panamá bar)	dots (1) broken fuzzy (3) thin fuzzy (2)	thin fuzzy (3)
not-I, $Yl^t yl^T$	—	eaten (4)	eaten (4)
not-I, $yl^T yl^T$	broad sharp (2) (+ Panamá bar = superbar)	broad sharp (4)	broad sharp (3)

(the allele in brackets is absent if I is an allele at the Yl locus, present if I is at another locus). Here the allele Cr^p can be seen, as in the East Brasil × Panamá broods, reducing the East Brasilian bar, but not on $yl^T yl^T$ backgrounds, where it combines both bars when homozygous to produce the 'superbar' (plate 4c, right), and Yl^t can be seen producing its usual eaten bar. The factor I seems, from the rather small numbers, to override the effect of Yl^t and to produce a thin fuzzy bar of a rather extreme type, becoming broken or reduced to dots in extreme individuals, whether it is heterozygous with Yl^t or yl^T.

(g) Inheritance of shape of tip of hindwing bar

In the backcross, F_3 and F_4 broods all scorable hindwing bars turn backward. Thus there is no positive evidence of the allele *bf*, which turns the bar forward, in the Rondônia race. However, it would not be expected to segregate in the backcrosses to East Brasil, and if present would have only a one in four chance of segregating in each of the F_3 broods. Thus we cannot tell for certain whether this allele was present in the Rondônia parent, and the genotype of this population remains in doubt.

The brood of Rondônia × Panamá × East Brasil ancestry (brood B, table A 10, appendix 5) segregates 8 forward-turning bars: 25 backward or unscorable. The almost perfect fit to an F_2

ratio (expected $24\frac{3}{4}:8\frac{1}{4}$), which has been seen in previous broods, suggests that *bfbf* not only turns the bar forward, but actually places the yellow pigment in that region of the wing, so that individuals that are unscorable are in fact *Bf–*. It is not possible to tell where the *bf* allele in this brood originated and as it is known to occur in Panamá we can still say nothing about the Rondônia population.

(h) Red versus orange

The fading of red to orange has proceeded too far in these butterflies for us to score them for these characters.

(i) Linkage groups

Because of the small size of the two crosses immediately following, linkage data from the present broods is combined with the other crosses in §4.9.

(j) Summary

In the Rondônia population the removal of the yellow lines and bars of the East Brasilian race is achieved mainly by two loci. The first of these, which may be *Yl*, also changes the shape of the band to the invaded shape characteristic of the Rondônia population; the allele has been designated *I* (invaded) and provisionally is not assigned to a locus. The second is possibly an allele of the *Cr* locus, although its ability to remove rectangles is much reduced, and its effects on the yellow lines and bars do differ from those of the *Cr* allele from Trinidad and Panamá. The radiate marks of the Rondônia population are produced by the dominant gene *R*, and the conversion of the forewing band from red to yellow by the recessive gene *y*.

4.7. The crosses Manaus (Guiana) × East Brasil and Manaus (Guiana) × Georgetown

The Guiana race of *H. erato*, which is a parallel mimic of the Guiana race of *melpomene*, bred from Suriname stock by Turner (1972), was obtained by us at Manaus. It is rather like the Rondônia *erato*, but has a yellow forewing band much more invaded by black (which we term a 'broken' rather than 'invaded' band) and lacks the rays on the hindwing; the red marks are a deep red colour (figure 6*e*; plate 1*k*). We obtained an F_1 by crossing a Manaus male to an East Brasilian female (from Rio de Janeiro), and backcrossed an F_1 female to an East Brasilian male (also from Rio). We also crossed a Manaus female to a male obtained from Georgetown, Guyana (figure 3) and obtained a small F_1 (five butterflies), one of which we outcrossed to an East Brasilian male, obtaining two offspring. The Georgetown male was of the Venezuela/Trinidad race (a black butterfly with a red forewing band), but showed signs of carrying genes from the Guiana race, which hybridizes somewhere in the centre of Guyana†, to the south of Georgetown, in that the red forewing band was rather more angular in outline than is normal in Venezuela. We also outcrossed another naturally hybrid butterfly from the lower Rio Trombetas in Amazonian Brasil with one of our (Rondônia × East Brasil) × East Brasil backcross males. This hybrid appears to be a member of an introgression zone between the Guiana race and the extension of the Venezuela/Trinidad race along the lower Amazonas (see figure 2). It is a plain black butterfly with a broken red forewing band. Breeding data are in table A 8, appendix 5.

† We use 'Guiana' to refer to the whole region of northeastern South America from eastern Venezuela to the Amazonas delta. Guyana is the political unit (formerly a British colony). Suriname is the former Netherlands colony. We refer to the French overseas *département* on this coast as 'Guyane', its official name.

(a) *Inheritance of the dennis pattern*

The extensive red marks at the base of the forewing in the Manaus race, known as the dennis pattern by analogy with the rather similar marks on fore- and hindwings in *melpomene*, are inherited as a single dominant factor. The F_1 Manaus × East Brasil are all dennis, the backcross to East Brasil segregates 11 dennis:10 plain; the Manaus × Georgetown F_1 is likewise entirely dennis (five butterflies) and the outcross to East Brasil segregates 1 dennis:1 plain. Thus dennis is dominant in crosses with both the East Brasilian and Venezuela/Trinidad races. It is possible that, as in *melpomene*, it is an allele of radiate, but this is not proved, and we shall provisionally designate the gene as a new locus, D.

(b) *Inheritance of colour of forewing band*

The yellow forewing band of the Manaus butterflies is recessive to the red colour both of Georgetown and of East Brasil (plate 4h). All F_1, backcross and outcross butterflies have a red band. The number of genes involved cannot be determined; it is quite probable that the same locus, Y, is involved as in the Rondônia crosses.

(c) *Inheritance of shape of band and yellow line*

An allele segregates in the backcross to East Brasil whose effects are indistinguishable from those of the Yl^t allele from Trinidad and which we shall therefore treat as being this same allele. The F_1 individuals all lack the yellow line, and are intermediate but variable for concavity (having partial or no development of the red spot in the cell); the teeth cannot certainly be scored as all the F_1 have broken bands. In the backcross all but one of the entire banded butterflies have some development of the teeth, as is expected from the usual dominance of this character; as usual, it cannot be scored on the broken banded butterflies. The concavity of the band segregates 11 intermediate:7 concave (three more are not scorable because they were lost in breeding attempts), and is strongly correlated, subject to modification by other loci (below) with absence of the yellow line and weakening of the yellow hindwing bar in intermediate butterflies, and strengthening of both these characters in concave butterflies. Thus there is every reason to believe that we are seeing the segregation of the genotypes $Yl^t yl^T$ and $yl^T yl^T$.

(d) *Inheritance of broken forewing band*

As has been previously observed in crosses of naturally hybrid Suriname populations (Belém × Venezuela/Trinidad) crossed with Trinidad (Sheppard 1963), the broken band (plate 4h, left) is inherited as a single allele, which we shall call Ly^B, dominant to an entire band (ly^b); the Manaus × East Brasil F_1 is entirely broken banded, and the backcross to East Brasil segregates 10 broken:11 entire. The mating of the broken banded female from the Rio Trombetas to an entire Rondônia × East Brasil backcross male was apparently a backcross for this factor, segregating 10 broken:20 entire (for 1:1, $P = 0.10$). The five Manaus × Georgetown F_1 butterflies had broken bands, rather variable in appearance, and the outcross of this F_1 to East Brasil segregated 1 broken:1 entire. Thus Ly^B is dominant in crosses both with the East Brasilian and with the Venezuela/Trinidad races, and is hyperstatic in its effect over the entire bands produced both by Yl^t (Venezuela/Trinidad) and by yl^T (East Brasil). For the possible allelism of this with the factor (I) producing the invaded band of Rondônia, see §4.8e.

(e) Inheritance of cream rectangles

The cream rectangles of the East Brasilian race (plate 4*d*) are removed in Manaus butterflies by a dominant allele, apparently at the usual *Cr* locus. They are absent in the F_1 with East Brasil, and segregate 9 without rectangles:9 with rectangles (three more not scorable) in the backcross to East Brasil. In the cross Manaus × Georgetown they are absent in both parents and offspring; in the outcross of this F_1 to East Brasil, and the mating of the Rio Trombetas butterfly to a Rondônia × East Brasil backcross insect, all offspring lack the rectangles although one of the parents has them, confirming that *cr* is recessive on these genetic backgrounds.

(f) Effects of the loci Cr, Ly and Yl on the yellow line and yellow bar

The alleles Yl^t and *Cr* have their usual effect in this cross, reducing both the line and the bar. In addition, the allele Ly^B has a strong effect in suppressing both these marks. The phenotypes in the backcross to East Brasil are (three not scorable):

	Crcr (no rectangles)	*crcr* (rectangles)
$Ly^B ly^b$; $Yl^t yl^T$ (broken, intermediate concavity)	no line (1) no bar (1)	no line (1) dots (inner margin) (1)
$Ly^B ly^b$; $yl^T yl^T$ (broken, concave)	no line (1) weak line (2) no bar (3)	weak line (2) dots (inner margin) (1) dots (1)
$ly^b ly^b$; $Yl^t yl^T$ (entire, intermediate concavity, tooth)	no line (2) weak line (3) dots (5)	weak line (4) eaten bar (4)
$ly^b ly^b$; $yl^T yl^T$ (entire, concave, tooth)	— (0)	full line (2) broad sharp (2)

In this brood the 'dots' phenotype consists of a fuzzy bar, distal to the cell, heavily invaded by black along the veins. The effect of Yl^t is as usual to produce an eaten bar, and of *Cr* to reduce this in strength; Ly^B further reduces the bar and line, apparently removing them altogether in conjunction with Yl^t and *Cr*.

The wild parent from the Rio Trombetas, as it is a broken banded hybrid from the Guiana and Venezuela/Trinidad races, was presumably *CrCr*; $Ly^B ly^b$; $Yl^t Yl^t$ in genotype. It was crossed with an obvious *crcr*; $ly^b ly^b$; $yl^T yl^T$ butterfly of Rondônia × East Brasil origin, and so apparently produced a brood segregating for the two genotypes *Crcr*; $Ly^B ly^b$; $Yl^t yl^T$ and *Crcr*; $ly^b ly^b$; $Yl^t yl^T$. All 30 of the offspring, of either genotype, lack the line and the bar, in contrast with the backcross, where the second genotype usually has a weak line and dots. This same genotype in the Manaus × East Brasil F_1 similarly lacks both the line and the dots.

(g) Red versus orange and shape of tip of bar

Neither of these differences segregates: the first because all parents in these crosses are red, and the second because, even if the Manaus population were to be *bfbf*, this could not be expressed in the backcross to East Brasil (*BfBf*).

(h) Inheritance of red raylets

Small red triangles, appearing to be the bases of otherwise absent red hindwing rays, are not uncommon in East Brasilian butterflies. We have not studied these in detail, but they did segregate in our (Manaus × East Brasil) × East Brasil backcross, being absent in both parents and present in six of the 21 offspring. The expected number for a recessive homozygote is $5\frac{3}{4}$; so it may be that this is a single, recessive allele (*rt*), polymorphic in the East Brasilian subspecies. Red raylets appear also in three out of 32 butterflies in the Panamá × East Brasil F_2 (although very weakly in two of them), which is almost significantly deficient from a 3:1 ratio ($P = 0.0503$); however, this brood had more than one female parent.

(i) Summary

The Manaus (Guiana) race carries two independent genes that tend to remove the yellow lines and bars of the East Brasilian race and that alter the shape of the forewing band. One of these is Yl^t, which has the same effects as in the crosses already described; the other, Ly^B, produces the broken band of the Manaus race (plate 4h, left). In addition this race carries the gene *Cr*, which has its familiar effect on the rectangles and yellow bar. The red 'dennis' marks on the forewing are produced by a single dominant gene (*D*) (plate 1k). The conversion of the forewing band from red to yellow is recessive, but the number of loci is not known.

The red raylets, found as a polymorphism in East Brasil, are possibly produced by a single recessive gene.

4.8. The cross Belém × East Brasil

The Belém/Mato Grosso race of *erato* has an orange radiate pattern and a broken yellow forewing band; it has no yellow line, no yellow bar, nor cream rectangles (figure 6c; plate 1e). It is like the Manaus (Guiana) race, except that the marks are orange and that there are rays on the hindwings. We crossed a male of the Rio de Janeiro stock of the East Brasilian race (figure 6b; plate 1g) with a female from Belém, obtaining an F_1 of 20 and an F_2 of 23 individuals (table A9, appendix 5). Several loci are segregating, giving 11 recognizably distinct phenotypes in the rather small F_2, which we shall interpret in the light of what is already known about the inheritance of similar patterns from Rondônia and Manaus.

(a) Inheritance of radiate

As in all other experiments the radiate pattern is inherited as a single dominant allele. The whole F_1 is radiate in pattern; radiate and plain butterflies segregate 17:6 in the F_2 (expected $17\frac{1}{4}:5\frac{3}{4}$).

(b) Inheritance of colour of forewing band

The yellow colour of the forewing band (Belém) is recessive to red (East Brasil) and segregates as a single factor. The F_1 has red forewing bands; the segregation in the F_2 is 18 red:5 yellow (expected $17\frac{1}{4}:5\frac{3}{4}$). This must result from the segregation of the Y locus or another very like it.

(c) Inheritance of cream rectangles

Rectangles are absent in the F_1; in the F_2 they appear in six individuals, although rather weakly expressed in all but two. This is a close fit to the expected ratio for a recessive, and it

seems that, subject to partial repression by other factors, the alleles *Cr* and *cr* are segregating in this as in other crosses.

(d) Inheritance of shape of forewing band, yellow line and yellow bar

The considerable differences in the shapes of the bands of the two races can be scored as three characters: the band is a solid area of colour (East Brasil) *or* is broken into a group of spots (Belém); the band is concave on its inner margin (East Brasil) *or* extends well into the cell (Belém); and the band has teeth on its outer edge (East Brasil) *or* it does not (Belém). The yellow line may be present, or weakly developed or absent, and the yellow bar may be broad sharp (East Brasilian type), 'dots' (like those in the Manaus cross, a fuzzy bar distal to the cell, heavily invaded with black along the veins), or absent. There are, as usual, scoring difficulties with concavity, especially in knowing how to cross-classify varying degrees of expression between broken and entire bands. The F_1 has the phenotype broken, convex to intermediate, tooth not scorable, no line, no bar. The F_2 segregates

broken, convex, no line, no bar	6
broken, intermediate or concave, no line, no bar	14
entire, intermediate or convex, tooth, weak line, dots	2
entire, intermediate or concave, full line, broad sharp bar	1

The only thing that is absolutely clear is that the last individual is an East Brasilian genotype, having the cream rectangles, and hence being *crcr* yl^Tyl^T, with all yellow marks fully developed: however, even it is rather intermediate in the concavity of its band.

It would be possible to set up a large number of more or less extravagant hypotheses to account for this brood. In fact, as the segregation appears to result from several loci, it would be very difficult to interpret even a much larger F_2, and a final description waits on the breeding of both backcrosses. Two reasonable hypotheses are that the Belém population resembles that at Manaus in being homozygous Ly^BYl^t, in which case the above is a two-locus segregation, or that the Belém population is homozygous ly^b like East Brasil, and has its own allele at the *Yl* locus, say Yl^B, which breaks the forewing band, in addition to having the usual effects on the yellow line and bar. Both hypotheses fit the data reasonably well. The first would give an F_2 consisting of

genotype	phenotype	ratio	expected number	observed number
Ly^B–Yl^tYl^t	broken, convex, no line, no bar	3	$4\frac{5}{16}$	6
Ly^B–Yl^tyl^T	broken, intermediate, no line, no bar	6	$8\frac{10}{16}$ ⎱	14
Ly^B–yl^Tyl^T	broken, concave, no line, no bar	3	$4\frac{5}{16}$ ⎰	
ly^bly^b Yl^tYl^t	entire, convex, no tooth, no line, eaten bar	1	$1\frac{3}{16}$	0
ly^bly^b Yl^tyl^T	entire, intermediate, tooth, weak line, eaten bar	2	$2\frac{14}{16}$	2
ly^bly^b yl^Tyl^T	entire, concave, tooth, line, full bar	1	$1\frac{7}{16}$	1

There is an anomaly in the penultimate class, which should have an eaten bar, but the reduction to 'dots' actually seen appears to be due to modification by *Cr*, both individuals being without rectangles. (The genotype *crcr* seems incapable of restoring the bar or the line to

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$Ly^B Ly^B$ genotypes in this brood: three of these have rectangles, but all lack the line and the bar.) Thus there is quite a good fit to this hypothesis.

The second, single locus hypothesis predicts an F_2 segregation of

genotype	phenotype	ratio	expected number	observed number
$Yl^B Yl^B$	broken, convex, no line, no bar	1	$5\frac{3}{4}$	6
$Yl^B yl^T$	broken, ?intermediate, (no line, no bar)	2	$11\frac{1}{2}$	14
$yl^T yl^T$	entire, concave, tooth, line, full bar	1	$5\frac{3}{4}$	3

to which the observed phenotypes can be fitted if we assume that the two individuals with dots and weak lines are $yl^T yl^T$, with the reduction in yellow marks caused by their $Cr-$ genotype, and that they are concave rather than intermediate (their concavity is in fact as great as that of the obvious $yl^T yl^T$ individual). For this ratio, $\chi_2^2 = 1.24$, $P = 0.54$.

Thus both hypotheses explain the data, and for the moment we cannot choose between them.

(e) Inheritance of shape of tip of hindwing bar

In all three individuals with hindwing bars the tip turns backwards; thus there is no indication of the segregation of bf in this cross.

(f) Inheritance of red versus orange

The F_1 is entirely red. In the F_2 it is recorded that only three individuals were obviously orange, suggesting that orange may be recessive but presents difficulties in scoring.

(g) Summary

The small F_2 brood in this cross is consistent with the Belém race (plate 1 e) differing from the East Brasilian race by the same two yellow line/yellow bar/band shape loci as the Manaus race (that is Ly and Yl), and also with the Belém race differing at only one such locus. It appears to carry Cr, with the usual effects. The radiate marks are the single dominant gene R, the yellow colour of the forewing band is produced by a single recessive (presumably y), and the orange colour, rather than red, is probably a single recessive gene.

4.9. Linkage, interaction and allelism in the Amazonian races

In view of the small size of some of the crosses, we shall consider problems of linkage and allelic identity for all Rondônia, Manaus and Belém crosses together.

(a) Linkage of yellow and radiate

The genes R (radiate) and y (yellow forewing band) are linked in repulsion and so can only be examined in F_2 or similar broods. Summing the Belém × East Brasil F_2, and both the F_3 broods of the Rondônia × East Brasil cross, all being matings between radiate, red banded butterflies that were heterozygous for both factors, we obtain

	radiate	plain
red band	35	20
yellow band	12	0

which has an exact probability of only 0.017 (two-tailed). (The individual probabilities are 0.2 for the Belém cross (one-tailed), and 0.1 for the combined Rondônia crosses.) Among the

broods of uncertain provenance (table A 10, appendix 5), two (D and S) are between radiate individuals (colour of bands not recorded) and segregate plain and yellow in the offspring, showing that at least one parent was doubly heterozygous. The segregation is

	radiate	plain
red band	9	6
yellow band	8	0

which is not formally significant ($P = 0.099$, two-tailed) but is fully consistent with the other broods. The combined probability for all the above broods is 0.00077 (two-tailed). The Rondônia × East Brasil F_4 is a cross between two radiate butterflies, one red banded and the other yellow banded. If the loci are linked in repulsion in these crosses, then the yellow banded parent has to be *RRyy*. In accord with this, the brood of 13 individuals is entirely radiate. Similarly, a yellow banded individual bred by Beebe (1955) from a natural hybrid population in Suriname (an Amazonian race × Venezuela/Trinidad) proved also to be homozygous radiate (analysis by Turner & Crane 1962).

The recombination fraction cannot be determined from the above, as the expected zero recombination in females would prevent the recombinant phenotype from appearing even with a high crossing over rate in males. Significantly, the radiate Amazonian races hybridize extensively round the borders of the Amazon basin with plain, red banded extra-Amazonian races; among the many thousands of such hybrids collected, especially in Guyane, we have seen not a single plain butterfly with a yellow band. *R* and *y* therefore appear to be very tightly linked.

(b) Additional red marks in the homozygous radiate phenotype

If *R* and *y* remain linked in repulsion, then all yellow banded butterflies must be *RR* homozygotes, and broods D and S (table A 10, appendix 5) are segregating *Ry/Ry*, *Ry/rY* and *rY/rY* butterflies. All the *Ry/Ry* (yellow banded radiate) insects, and none of the others, have red rays on the forewing, lying exactly between the veins, distal to the yellow band; they are always dusted with black, and are present to a variable degree (plate 4*l*). In some individuals the rays lying posterior to the yellow band are connected, on the underside, with the red marks in the basal half of the forewing (that is, the normal 'dennis' forewing part of the 'radiate' pattern), and can be seen as an extension of them. This phenotype seems to be peculiar to these broods, which are apparently related as both carry the pale eye mutant (§4.9*j*, below). A similar phenotype will be encountered in the cross with East Ecuador.

(c) Interaction of rY and ylᵀ

A further peculiarity of brood D is that all three of the rY/rY; $yl^T yl^T$ butterflies, but none of the butterflies that are *Ry/–* or *Yl*t–, have an extensive red tip in their otherwise fully developed yellow line (the brood is homozygous *crcr*: so this is not an effect of *Cr*) and an extensive mixture of red scales in the distal part of their fully developed hindwing bars (plate 4*k*, right). As with the red forewing rays, we can draw no conclusions, as the effect is unique to this brood and to brood S, where it is seen on the single $rr\ yl^T yl^T$ butterfly and on the single *rr Yl*t– (again, both are *crcr*).

(d) Criteria for allelism

When we cross two different races with a third one, we often find what appears to be the same gene segregating: for instance the radiate pattern segregates in the same way in crosses

both of Rondônia and of Belém butterflies with East Brasil. In such cases, we usually interpret this as the segregation of the same locus. The hypothesis that two such genes are in fact allelic can, in the absence of triple test crosses proving allelism, be justified in various ways. One can argue that such an elaborate pattern as, say, the radiate marks of *H. erato* is unlikely to be produced by two different mutations, and that therefore its segregation must always be controlled by the same alleles. Now while this may be true of some patterns, its application to all patterns depends on the assumption that the butterfly is a black surface on which patterns have been drawn in the course of evolution. The fallacy can be most easily seen by noting that the same argument 'proves' that all lethal alleles occur at one locus: it is unlikely in the extreme that anything so elaborate as a complete organism could be produced more than once by chance mutation, so that all 'organism versus non-organism' genes must occur at a single locus! It is likely to be true of patterns as well as organisms, that many loci contribute to the final product, and that mutations at many or all of these may be able to remove or alter the pattern. This is shown in *H. erato* by the *Ly* and *Yl* loci, both of which remove the yellow line and Brasilian yellow bar.

A better test of allelism is the occurrence of two similarly acting loci in the same linkage group in two races: we have used this to argue that *B* and *D* in *H. melpomene* are the same in various different crosses, as it is improbable, except in a polyploid, that two different chromosomes bear two pairs of similar loci.

Last, the allelism of two different genes, suppressing a pattern in two different races, can be tested by crossing the two races concerned. Non-appearance of the suppressed pattern in the F_1 for recessive suppressors and in the F_2 for dominant suppressors shows that two independent loci are not involved. For dominant suppressors two more complicated hypotheses are (i) that there are two linked suppressor loci and (ii) that there are so many suppressors that the multiple recessive does not appear in the F_2. The first hypothesis (linkage) cannot be eliminated except by parsimony, and we shall simply ignore it. The second (more than two loci) can be ruled out if in *both* races the character concerned segregates as a single factor in crosses with a third race possessing the suppressed character.

(e) Allelism at the Ly and Yl loci

The most difficult problem in allelism in our *H. erato* crosses is the allelic identity of genes that remove the yellow line and hindwing bar. They occur as follows:

race	effect on forewing band	designation in text
Manaus	broken, convex	Ly^B
Manaus	entire, convex, no tooth	Yl^t
Trinidad	entire, convex, no tooth	Yl^t
Panamá	entire, convex, no tooth	Yl^t
Belém	broken, convex, no tooth	Ly^B and Yl^t; or Yl^B
Rondônia	invaded, convex	I

East Brasilian genotype: homozygous $ly^b yl^T$, not I.

It is quite clear from the Manaus cross with East Brasil that two loci are involved in this cross. Although we have only limited crosses between Manaus and the Venezuela/Trinidad race, it is clear that these differ by only one yellow line locus, as yellow lines do not appear in the natural hybrids between these races in the Guianas. Our broods show Ly^B and ly^b segregating

in the Manaus × Georgetown cross, and so Manaus and Trinidad must both be homozygous Yl^tYl^t. Similarly, because of absence of the yellow line in naturally occurring hybrids between the Belém and Venezuela/Trinidad races, these can differ only at one yellow line locus. As Trinidad is homozygous ly^bYl^t, Belém must be Ly^BYl^t, or must have its own special allele at the Yl locus, removing the line and breaking the band, being say homozygous ly^bYl^B. Sheppard (1963) observed the B factor segregating in the Belém × Trinidad hybrids, but this of course gives no clue as to allelism. In the former case the Belém × East Brasil cross would segregate for both loci, in the latter only for the alleles Yl^B/yl^T. Our F_2 brood is consistent with both hypotheses and the question remains open.

The ly^b allele in Trinidad and in East Brasil is known to be recessive in both races (Sheppard 1963; present paper), and the dominant character (broken band) does not appear in the Trinidad × East Brasil F_1. Therefore this allele is identical in both races. Similarly, although the Panamanian entire band has not been tested against any of the broken races, so that neither the dominance nor the number of loci is known, the complete absence of broken bands in the F_1 and F_2 of the Panamá and México × East Brasil crosses makes it very likely that the Panamanian race is homozygous ly^b also.

No full tests of allelism have yet been performed for the Panamá and Rondônia yellow line alleles. Each race is shown by our crosses to differ by only one such locus from the East Brasilian race. Because of the extreme similarity of phenotypes, particularly in interaction with other loci, we assume that the Panamá allele is Yl^t, as in Trinidad. For the Rondônia allele, the first step in an allelism test has been taken. A butterfly of known Panamá × East Brasil ancestry, which was clearly a Yl^tyl^T heterozygote, was mated with a butterfly of known Rondônia × Panamá × East Brasil ancestry, which was heterozygous for the Rondônia gene (I) that removes the line and breaks the forewing band (brood B, table A 10). Either the parental genotypes are $Yl^tyl^T \times Yl^Iyl^T$ (all ly^bly^b), if the Rondônia I factor is at the Yl locus, or they are ly^bly^b; $Yl^tyl^T \times Ly^Ily^b$; yl^Tyl^T if I maps at the Ly locus.

As expected on either hypothesis, the brood segregates $1:1:1:1$ in four classes distinguishable by their band and yellow line phenotypes. If only the Yl locus is segregating, then the expected phenotypes are

	Yl^I	yl^T
Yl^t	invaded	entire
	convex	intermediate
	(*no tooth*)	tooth
	no line	no line
yl^T	invaded	entire
	intermediate	concave
	(tooth)	tooth
	no line	line

(based on the assumption that the invaded effect of Yl^I is dominant to Yl^t). On the other hand, if there are two loci we expect

	Ly^Iyl^T	ly^byl^T
ly^bYl^t	invaded	entire
	intermediate	intermediate
	(*tooth*)	tooth
	no line	no line

$ly^b yl^T$	invaded	entire
	intermediate	concave
	(tooth)	tooth
	no line	no line

assuming that the double heterozygote $Ly^I ly^b Yl^t yl^T$ will have an intermediate band. It can be seen that the test for the hypotheses, apart from the difference in the tooth phenotype, which is unfortunately not scorable on the invaded bands in this brood, is the segregation of the invaded, no line class into convex and intermediate bands on the one locus hypothesis, and its failure to segregate on the two-locus hypothesis. In fact the brood segregates

invaded	entire
convex (6)	intermediate (8)
no line	tooth
	no line
invaded	entire
concave (10)	concave (9)
to intermediate	tooth
no line	line

which is a satisfactory fit to equal partition ($\chi_3^2 = 0.58$, $P = 0.9$) and which corresponds better with the first than with the second hypothesis. However, the test is not strong, as it depends on an assumption about gene action in double heterozygotes: if being heterozygous for both postulated loci made the band convex, then the second hypothesis would stand equally.

As the Rondônia *I* factor produces invasion of the band akin to the broken band of Manaus, we shall assume that it maps at the *Ly* locus, pending further evidence. It may in fact be the Ly^B allele as in Manaus, modified in its expression by the rest of the Rondônia genome.

This interpretation of the *Ly* and *Yl* loci produces a self-consistent scheme for the control of the yellow line and yellow bar of the East Brasilian race: homozygosity for both loci (in addition to *cr*) is required for the development of the marks. Alteration of either locus removes or weakens them. The races are then homozygous on the following scheme (the *Cr* and *Ybs* loci being ignored):

	Ly^B (or Ly^I)	ly^b
Yl^t	no line	weak line
	no bar	eaten bar
	Manaus	Trinidad, ?Panamá
yl^T	no line	full line
	no bar	full bar
	?Rondônia	East Brasil.

(*f*) *Allelism of dennis and radiate*

The dennis and radiate patterns appear to be allelic as they are in *H. melpomene*. Although we have no direct evidence from breeding experiments, it is apparent from the total absence of yellow banded plain butterflies in the Guianas, where the Manaus race hybridizes with the Venezuelan race, that not only is *R* linked to *y* in the Amazonian races, but also *D* is linked to

whatever genes produce the yellow band of the Manaus race (which we have shown to be recessive). The simplest hypotheses explaining this fact are (a) that both D and R are linked to y and (b) that there are two chromosomes, R–y and D–y, there being *two* yellow loci. The second system might have been produced, when we remember that the radiate pattern contains the forewing marks that constitute the dennis pattern, by a chromosome duplication in the distant past. But for reasons of parsimony we shall adopt the first hypothesis that D and R are both linked to a single Y locus, and that they may well be alleles in a system like the D^R, D, d series in *melpomene*.

(g) Further allelism tests

The following pattern suppressors are known to be identical:

r in Trinidad, East Brasil and East Ecuador, as the dominant radiate pattern does not appear in the F_1 between these races;

Y in Trinidad and East Brasil, as the recessive yellow band phenotype did not appear in the F_2; this is known to be a single factor in the cross Belém × East Brasil, and is very probably a single factor in the cross Belém × Trinidad (Beebe (1955), analysed by Turner & Crane (1962));

Cr in Matto Grosso/Belém and Trinidad is probably identical, as it behaves as a single gene in both and does not appear in the hybrid zone between these races in Suriname and Guyane;

d in Trinidad and East Brasil, as the dominant dennis character does not appear in the F_1 between these races; the d allele is not known for certain to be recessive in Trinidad, but this is most probable as it is recessive in phenotypically similar populations in Guyana.

In other instances, we have assumed that when we see a gene of similar phenotypic action, we are dealing with the same gene. Although this is not rigorous, to do anything else would result in a ridiculous, and probably incorrect, complexity in the interpretation of our results. The hypothesis of identity is always in theory disprovable by the appropriate breeding experiment, and is therefore a good scientific hypothesis.

(h) Effects of Yl genes on the Panamanian bar

We have shown that brood B (table A 10) segregates for four genotypes at the Ly and Yl loci. It also segregates as an F_2 for the alleles Cr^p and cr. Heterozygotes for Cr^pcr show the usual shadow of the bar on the underside, but a few show yellow scaling in the position of the bar on the upperside as well. This effect may be associated with the Ly and Yl alleles, as it segregates

Panamá bar	$Ly^I ly^b$ $Yl^t Yl^t$	$Ly^I ly^b$ $yl^T yl^T$	$ly^b ly^b$ $Yl^t yl^T$	$ly^b ly^b$ $yl^T yl^T$
strong yellow on uppserside	1	1	0	0
weak yellow on upperside	3	4	3	0
no yellow on upperside	1	2	1	4

which can be condensed into a 2 × 2 table by adding the upper rows and the two left and two right columns, giving a value of $P = 0.23$, two-tailed, thus showing an insignificant association of yellow on the upperside with the allele Ly^I, or into a different 2 × 2 table by combining the three columns on the left and the upper two rows, giving a significant association of the absence of yellow on the upperside with the double recessive genotype $ly^b ly^b$ $yl^T yl^T$ (exact probability 0.029, two-tailed). For the whole table $\chi_6^2 = 8.65$, without Yates's correction, $P = 0.19$, two-tailed. It is therefore likely that the tendency of the Panamanian bar to be expressed on the

upperside in heterozygotes is repressed, at least in this brood, by the yl^T allele when homozygous, and possible that it is enhanced by the Ly^I allele. On the other hand, this effect might be an independent gene, like Ub in *melpomene*.

As there are only three Cr^pCr^p homozygotes in this brood, it is not possible to test for a similar effect in this genotype, but none is obvious. There is one further extraordinary effect, very obvious in the homozygotes, but visible in the heterozygotes that have upperside Panamá bars: the rear edge of the Panamá bar on the upperside, and to a lesser extent on the underside, is invaded by a series of short black lines running exactly up the middle of each intervenular space, making the Panamá bar look like the truncated base of the coloured rays or wedges that are found covering the hindwing in certain members of other subgenera, notably *Eueides* species and the green and blue forms of *H. (Laparus) doris*. The bar also closely resembles the yellow bar of the high Andean species *H. (H.) telesiphe* (in fact, our Cr^pCr^p homozygote with a concave invaded forewing band is quite a passable 'mimic' of that species!). No such pattern is known in any naturally occurring *erato*, and it seems possible that in this brood we have triggered a developmental system held in common with the other species but normally completely suppressed in *erato* (plate 4*k*, left). (See §3.4*n* for a similar effect in *melpomene*.)

(i) Unlinked loci

The most convenient tests for linkage between loci other than R and Y (above) are the appearance of recombinant classes in the Manaus × East Brasil backcross (heterozygous female parent) and in the three Rondônia × East Brasil backcross broods with female parents, for genes in coupling, and in the Belém × East Brasil F_2 for genes in repulsion. Like the N locus in *melpomene*, the Yl locus in *erato* is particularly useful in these tests as it has detectable heterozygotes, but cannot be tested in the Belém cross because it is uncertain whether it is segregating. In the Manaus × East Brasil backcross, there are three of the recombinant genotype $Crcryl^Tyl^T$ and five of the reciprocal $crcrYl^tyl^T$. In this cross, as in others, Cr and Yl are not linked. Similarly in this cross the recombinant genotypes $Ly^Bly^b\ yl^Tyl^T$, $ly^bly^b\ Yl^tyl^T$ and $Dd\ yl^Tyl^T$, $dd\ Yl^tyl^T$ appear in the numbers 5, 9 and 2, 5 respectively, showing that Ly and D are both unlinked to Yl (Ly^B causes some scoring difficulties by suppressing the effects of yl^T, but the recombinants all include ly^bly^b insects, which are not subject to this problem). Cr, D and Ly are likewise independent in this cross, the recombinants $Crcrdd$, $crcrDd$; $Crcrly^bly^b$, $crcrLy^Bly^b$; $Ddly^bly^b$, $ddLy^Bly^b$ appearing as 4, 3; 5, 3; 6, 5 individuals respectively. The Belém × East Brasil F_2 shows that Cr is probably independent of Y, as the loci are in repulsion, and as the recombinant phenotype 'cream rectangles, yellow bar' is represented by three yellow barred individuals with weak cream rectangles. From this it follows that Cr is independent of R as well.

The backcrosses of Rondônia × East Brasil females to East Brasil males (broods WK8, WK10, table A 7, appendix 5) confirm the independence of Cr and R, provided that Cr is indeed segregating. On the basis of the effects of the segregating locus on the yellow bar, which are less subject to scoring difficulties than those on the rectangles, the phenotypes radiate weak bar, radiate strong bar, plain weak bar and plain strong bar appear in the numbers 5:13:1:9 (only extant butterflies being used). There are more 'recombinants' than 'parentals', giving no indication of linkage, although the deficiency of plain weak indicates quite strong interaction or scoring problems. Similarly the I factor from Rondônia, whether it maps at Ly or Yl, is independent of Cr and R, the same backcrosses producing 'invaded, strong bar', 'entire, weak bar', and 'invaded, radiate', 'entire, plain' in the numbers 5, 0 and 11, 8 respectively. Loci

that are shown to be unliked by this test should of course show no association by contingency testing when the same backcross is performed with a male F_1 parent. For the pairs $Cr-R$, $Cr-I$ (strong and weak bars being used in both cases) and $I-R$, the χ^2_c values are respectively 2.0 ($P = 0.08$), 0.6 ($P = 0.22$) and 0.3 ($P = 0.29$), showing as expected no significant evidence of linkage (all one-tailed, without Yates's correction).

Of the remaining associations, that between I and Y is investigated by the F_3 broods, one of which is a double F_2 (brood WK13) and the other a mixed F_2–backcross with a doubly hetero-zygous male parent (brood WK15). The loci are in repulsion, and the recombinant phenotype 'entire, yellow' does not appear in the double F_2, suggesting linkage; however, the absence could be due to chance (the exact probability for contingency is 0.3, one-tailed), and there is no sign of linkage in the other brood, for which the two-tailed probability is unity. Whatever locus I maps at, there is no strong reason to suppose that it is linked to Y, especially as it has already been shown to be independent of R.

Finally, brood D (table A 10, appendix 5) is of uncertain provenance but is clearly an F_2 between butterflies of the genotype $Yl^t yl^T$; Ry/rY, probably originating in the Rondônia × East Brasil cross. It therefore tests for the linkage of Yl and R plus Y. These are the only wing markers segregating (cr is homozygous), so that the usual phenotypic interaction is absent, and the Yl genotypes are very easy to score on the standard criteria, apart from a rather wide range between concavity and convexity in heterozygotes. The segregation is

	Ry/Ry (radiate, yellow band)	Ry/rY (radiate, red band)	rY/rY (plain, red band)
$Yl^t Yl^t$ (no tooth, basal line, eaten bar)	4 (–)	3 (–)	3 (–)
$Yl^t yl^T$ (tooth, basal line, eaten bar)	1 (2)	3 (2)	0 (1)
$yl^T yl^T$ (tooth, full line, broad sharp bar)	1 (0)	0 (1)	1 (1)

(the numbers in brackets are those in brood S, apparently related to brood D because carrying the pale eye mutation).

Whether the loci were in repulsion or coupling in brood D, double recombinant genotypes (the corner cells of the table) have appeared, showing that the loci are unlinked. There is no convincing indication of linkage in brood S either.

The only loci whose linkage has not been tested by the combined evidence of all broods of Amazonian provenance (although many further pairs remain untested in particular races) are I and Ly, I and Yl, R and D (all of which are allelism problems), I and D (which is solved if I is an allele of Ly or Yl, which we assume it is), D and Y (which is tied to the $D-R$ allelism problem), and Ly and the Ry chromsome, and the linkage relations of Bf with the other loci, and also of orange pigmentation (bf segregates in brood B, but is subject to too many scoring difficulties to be useful). But the argument based on the absence of certain phenotypes in natural hybrid populations (in §(f) above), strongly suggests that R, D and y are tightly linked; as D is known to be independent of Ly, this further establishes the independence of Ly and the Ry chromosome.

(j) *Eye colour*

In broods D and S (table A 10, appendix 5), eight out of 16 and one out of seven butterflies show, when dead, eyes of a pale buff colour instead of the usual dark brown (plate 4l, left).

We have no record of their exact colour in living butterflies although the phenotype was readily seen to be a yellowish pink, and lacking data on the parentage of these broods we can say little about the inheritance of what is presumably an eye colour mutant. Brood S may be an F_2 or a backcross, and brood D appears to be a backcross. As brood D is an F_2 for Ry and Yl, the eye mutant can be tested for linkage to these, even in the absence of parental phenotypes, as one parent must be doubly heterozygous in each case. The segregations are

	Ry/Ry	Ry/rY	rY/rY
wild-type eye	1	3	4
pale eye	5	3	0

which is almost significant when condensed into a 2×2 table (exact probability 0.08, two-tailed), and which may therefore indicate linkage, and

	Yl^tYl^t	Yl^tyl^T	yl^Tyl^T
wild-type eye	1	2	5
pale eye	1	2	5

which is a perfect fit, showing independence of the loci.

Eye colour mutants are known also in *Papilio memnon* (Clarke & Sheppard 1973) and in *Bombyx mori* (see, for example, Chikushi 1972). Both the mutants known in the silk moth also change the colour of the egg chorion, but it is not possible from this to eliminate homology with the eye colour mutants in *Heliconius* and *Papilio*, as the change in *B. mori* is the dilution of a dark purple egg to either red or pale yellow; such an effect could probably not be expressed in *H. erato* and *P. memnon*, for the eggs are already yellow.

(k) Single loci or linked blocks?

The Amazonian races have three 'factors' that segregate as single units, but that have very diverse effects: the Ly locus, affecting band shape, yellow line and yellow bar, the Yl locus, affecting the same set of characters, and the chromosome producing radiate marks and a yellow forewing band.

It seems at first unlikely that a single gene could affect all these very different characters. However, this statement is based on our subjective impression of what constitutes a 'bit' of pattern in *Heliconius erato*: the red band, the yellow line and the yellow bar appear to be separate units to us, but there is no reason to suppose that each is necessarily produced by a totally independent developmental process. In the same way, the ordinary man, or even physician, may observe an apparently unrelated set of symptoms affecting what are to him diverse tissues and organs, which are in fact a syndrome produced by a single metabolic error, itself the outcome of a single mutation. It may be that the diverse effects of the Ly and Yl loci on marks of different colours on both wings are simply the result of the loci acting at an early or critical stage in the development of the pattern.

The same can be argued for the chromosome that we have called $R–y$ (radiate, yellow forewing band). One can imagine that the metabolic processes that cause red pigment to be spread all over the base of the wings withdraw it from the band, so that it becomes yellow. However, it is then difficult to imagine what is going on in Ry/rY individuals, which are radiate with red bands. We therefore prefer to regard these as two metabolically independent but tightly linked loci.

Our hypotheses about all these loci are capable of being tested, particularly by experiments on the genetic control of pattern development.

(*l*) *Summary*

Crosses involving the Amazonian races reveal the first linked block in *H. erato*: a chromosome containing *R*, *D* and *y*, apparently very tightly linked, with *D* and *R* possibly mapping at the same locus (like the D^R, *D*, *d* system in *H. melpomene*). The remaining major loci (*Yl*, *Ly*, *Cr*) are all independent, except for *Or* and *Bf*, about which we have insufficient information. Even in the absence of direct allelism tests, the assumption that genes producing similar phenotypes in two different races are in fact the same gene is not only justifiable as the simplest hypothesis but is in most cases supported by the absence of phenotypes attributable to complementary gene action in F_1 or F_2 hybrids. However, there are undoubtedly two yellow line removing loci (in addition to *Cr*); we believe the most likely composition of the races for these loci is (homozygous) Manaus $Ly^B Yl^t$, Trinidad and Panamá $ly^b Yl^t$, Rondônia $Ly^I yl^T$ and East Brasil $ly^b yl^T$.

We have noted an eye colour mutant and two curious 'atavistic' patterns, one consisting of red rays extending to the outer edge of the forewing, and the other of a yellow hindwing bar shaped like that of the related species *H. telesiphe* (plate 4*k*, *l*).

4.10. *The cross East Ecuador × East Brasil*

The high altitude race of *erato* on the eastern slopes of the Andes of central Ecuador (figure 6*g*) mimics the sympatric race of *melpomene* already discussed (plate 1*d*), from which it can be distinguished at a glance by the reversal of the red and white colours in the inner part of the forewing band (plate 1*h*). The pattern lacks the yellow bar, yellow line and cream rectangles of the East Brasilian race, and has a forewing band that is split into two halves by a black gutter, round at the tip, and predominantly white in colour.

Below this race, hybridizing with it on the slopes of the Andes, and extending for many hundreds of kilometres into the Amazon basin, is the Upper Amazonian race, which is like the other Amazonian races (Belém, Bolívia) in being radiate with a yellow forewing band. The radiate marks are orange, and the forewing band is *shortened*, not entering the forewing cell nor extending to the posterior of vein Cu1a (plate 4*e*, right; figure 6*h*).

Emsley (1965*b*) crossed individuals from an unspecified locality, which he described as members of the East Ecuadorian race, with butterflies from Trinidad; from the heterozygosity of the parents (revealed by segregation in the F_1) it seems that they were in fact from a partly hybridized population near the junction of the two races. Emsley obtained information on the inheritance of white colouring, split bands and the round band tip, which we shall discuss in the relevant subsections.

The single male parent used by us was sent by Dr P. Brakefield from the region of Palora (about 30 km south of Puyo) at around 1000 m in the Pastaza valley; like Emsley's butterflies, it was heterozygous and, from its phenotype, partly introgressed with the Upper Amazonian race, although predominantly East Ecuadorian in appearance. Its forewing band is not fully split, in that the halves just touch, and although white distally, the band is entirely red in the inner half. On the underside, only the outer half of the band is fully developed. (The aetiology of this phenotype is explained in §4.10*f* below.) On mating to an East Brasilian (São Paulo) female this male produced an F_1 which segregated for forewing band shape. We produced five F_2 broods from matings of F_1 butterflies (appendix 5, table A 11, broods E2, E6, E8, E10 and

E15) and six backcrosses to São Paulo, three of them with female F_1 parents to test directly for linked blocks (broods E3, E3A, E5, E7, E11 and E14).

There is also a large backcross to East Brasil (brood E12) which, having become contaminated with the East Brasilian stock, totally lacks the East Brasilian phenotype, which could not be distinguished from the stock butterflies. The brood also contains five butterflies that are clearly contaminants from brood E3 or E3A. Although it does not conform to the usual conventions of academic euphemism, we shall refer to this as the messy backcross; it cannot be used for testing ratios of the major genes, and is excluded from all data that involve totalling all the backcrosses. It has however been used to test certain other segregations and gene interactions where the absence of one class is immaterial; the contaminants have not been added to E3 or E3A, and are excluded from backcross totals on the grounds that any contaminants of the East Brasilian phenotype would not have been included in the brood, so that inclusion of the five butterflies would slightly bias the result. But two similar contaminants in brood E11, which is not subject to this problem, have been included in the grand totals.

To maintain as many alleles as possible we introduced butterflies of the East Ecuador × East Brasil cross into the greenhouse that contained our random mating stock of East Brasil × Trinidad × Panamá × Rondônia origin. Three butterflies from this stock were successfully mated, to produce broods E16, 2E and 5A. Broods 3D and 5D are sib matings within 5A. These give information on the alleles R, y and Yl^t not found in East Ecuador or East Brasil.

As the original East Ecuadorian male was not pure, and as we have no backcross in that direction, we may not have discovered all the genes that differentiate this race. But from the phenotypic effects of those that we have detected, it is likely that we have found most of them. We appear to have one allele at least from the Upper Amazonian race.

(a) Inheritance of cream rectangles

The removal of the East Brasilian cream rectangles (plate 4d) is effected, as in the other crosses, by a single dominant gene from the Ecuador parent, apparently without any penetrance of the rectangles in heterozygotes. The large F_1 entirely lacks the rectangles, which segregate 85 without rectangles:90 with in the backcrosses ($P = 0.76$ for $1:1$) and 31 without:4 with, in the F_2 broods ($P = 0.082$ for $3:1$).

(b) Inheritance of yellow forewing line

As in the cross with Trinidad and with Panamá, the East Brasilian yellow forewing line (plate 4a, b) is removed in the East Ecuadorian genome by the joint action of two loci, one of which also controls the cream rectangles. There are four main classes in the backcrosses, no line, weak basal line, weak line with red tip, and full line, which segregate in equality ($\chi_3^2 = 3.76$, $P = 0.29$), with some minor variants. The aggregate numbers from all backcross broods are

	no rectangles	rectangles
line 'removed'	no line (34)	basal line (34)
	trace of line (1)	weak line (3)
		no line (1)
line 'present'	yellow line, red tip (30)	full line (50)
	weak line (9)	weak line (1)
	basal line (9)	
	no line (1)	

(two individuals not scorable). Line 'removed' and line 'present' segregate roughly in equality ($P = 0.048$), the main source of the departure being an unexplained dearth of the 'removed' class in brood E7.

The total numbers of the line 'removed' and line 'present' classes in the combined F_2 (after allowance is made for the rectangle phenotype) are 24:10 (one not scorable) which is a good fit to a 3:1 ratio ($P = 0.67$). In these F_2 broods the yellow line, red tip phenotype has a singular appearance (plate 4c, left), seen otherwise only in one backcross individual, in which the line, both in its red and yellow parts, is expanded into a tapered wedge, ending distally in a red mark occupying the basal part of the cell spot, and connected with the forewing band by a red isthmus in the area normally occupied by the red part of the band in pure East Ecuador butterflies. Three of the line 'removed' cream rectangle butterflies have likewise a well developed line sprinkled with black scales, rather than the usual basal line. Both these effects presumably result from otherwise unidentified East Ecuadorian genes.

The similarities of phenotype with those encountered in the Trinidad and Panamá crosses strongly suggest that the loci concerned at Cr for the cream rectangles, and Yl for the yellow line, East Ecuador being homozygous Yl^eCr; we will call the Ecuadorian allele Yl^e to emphasise that it produces an effect on the hindwing bar different from the Trinidad and Panamanian allele Yl^t. The allelism of these two genes is demonstrated in § (f) below.

The interaction of the loci is similar in the messy backcross: yl^Tyl^T $crcr$ all have full lines, yl^Tyl^T $Crcr$ are divided into nine weak lines and 12 more or less weakened with a red tip, Yl^eyl^Tcrcr all have basal lines (19 individuals) and Yl^eyl^TCrcr all lack the line (nine individuals).

(c) Inheritance of yellow hindwing bar

If it is indeed the loci Cr and Yl that remove the yellow forewing line in East Ecuador, then the four classes tabulated above should have hindwing bars that are, respectively, absent or dots, eaten, broad fuzzy and full (East Brasilian). This is so, the phenotypes actually encountered being, as in all other crosses, characteristic of this particular race cross and slightly different from the others. The 'absent' or 'dots' class is usually a single, medial yellow dot, 'eaten' is a fuzzy bar mixed extensively with black scales but usually showing strong development of yellow distally and medially (the area that is fuzzy black and yellow in the present butterflies simply lacks yellow altogether in Trinidad and Panamá hybrids) (figure 7, fa) and 'broad fuzzy' is a well developed bar very like the East Brasilian bar but dissected by a network of black scaling along the veins like that in the Rondônia backcrosses WK11 and WK12 (figure 7, bv; plate 4j). The full bar is the standard East Brasilian phenotype. The numbers in the backcrosses, including the messy backcross and all contaminants are

	no rectangles (*Crcr*)	rectangles (*crcr*)
line 'removed' (Yl^eyl^T)	no bar (18)	broad fuzzy (1)
	single dot (24)	fuzzy eaten (33)
		thin fuzzy (8)
		broken fuzzy (16)
		dots (2)
		no bar (2)
line 'present' (yl^Tyl^T)	broad with black veins (71)	full bar (57)

As is often the case, the 'eaten' category is very variable; the division into the subclasses is somewhat arbitrary, and the two no bar phenotypes may have had the bar represented by

dots (they were scored as 'none' by P.M.S., but have been lost in an attempt to breed from them and cannot be checked).

In the F_2 the phenotypes are

	no rectangles (Cr–)	rectangles ($crcr$)
line 'removed' (Yl^e–)	no bar (8)	eaten (3)
	single dot (12)	broken fuzzy (1)
	two dots (1)	
line 'present' (yl^Tyl^T)	broad with black veins (10)	(full bar) (0)

Therefore the alleles that we believe to be Cr and Yl^e have the effects of causing black pigment to invade the bar along the veins and extensively along almost the whole length of the bar respectively. Together they can remove the bar entirely, but may leave a single, medial dot of variable size, whose total removal is probably effected by other loci. The removal of the yellow bar by Yl^e is much less 'clean' than the removal by Yl^t. The latter takes away yellow completely in certain areas; Yl^e simply causes these areas to become mixed with black scales (compare, in figure 7, ea with fa and br with fr). In addition in Yl^eyl^T butterflies the bar tends to be completely absent on the underside, whereas in Yl^tyl^T individuals it is only slightly less developed on the under surface than on the upper.

(d) Inheritance of tip of forewing band

The outer white part of the East Ecuadorian forewing band is markedly *round*, causing this part of the band to occupy an area much closer to the tip of the wing than in any other race; the outer margin of the East Brasilian band is *flat*, and much further from the wing tip (plate 4g). This difference is inherited as a single gene, with the round, distally placed margin dominant to the flat, proximally placed one, independently of all other elements of the band shape. The difference between the phenotypes is manifest on both surfaces of the wing, but is slightly easier to score on the under surface. The F_1 is entirely round, the backcrosses to East Brasil segregate 91 round to 84 straight ($P = 0.65$ for 1:1) and the F_2 is rather deficient in flat phenotypes at 33 round:2 flat ($P = 0.007$ for 3:1). We designate these alleles Ro and ro respectively.

In the cross East Ecuador × Trinidad, the round tip of the East Ecuadorian parent is dominant to the flat tip of the Trinidadian in the F_1, and segregates as a single gene in the backcross to Trinidad (43 round:41 flat) (Emsley 1965b); as round tips do not appear in crosses between East Brasil and Trinidad, it is clear that it is the Ro locus that is segregating in the Ecuador × Trinidad cross, the Trinidad race being *roro* like East Brasil. Some of Emsley's East Ecuador × Trinidad F_1 broods segregated for round and flat tips, showing that his East Ecuadorian parent butterflies, like ours, were from an introgressed population.

(e) Inheritance of shape of forewing band

The F_1 segregates equally for two shapes of the forewing band, showing that the male Ecuadorian parent was heterozygous (ratio 28:22, $P = 0.48$). One pattern is the East Ecuadorian *split band*, clearly expressed on the underside, but tending on the upperside to be an entire, very wide band, extending well into the main cell of the forewing (i.e. being convex), but tending to stop some distance short of the posterior angle of the wing. The split effect on the upperside tends to be manifested only as some black markings around the cross vein of the discal cell (plate 4f, left). The second phenotype is clearly the *shortened band* of the Upper

Amazonian race, not extending to the posterior of vein Cu1a, and not entering the forewing cell (i.e. being concave in the terminology of this paper). This also is clearly manifested on the underside of the wing, but is much less clear on the upperside, where red pigment develops to the posterior of vein Cu1a in most individuals (plate 4*i*, right).

On backcrossing to East Brasil these two shapes of band behave as alleles, and as alleles of the East Brasilian band, which is entire and long. The F_1 shows that both are dominant to the East Brasilian band. Broods E6, E8 and E10, matings between split, long and entire, shortened butterflies, show that the split and shortened bands are codominant. The split, shortened phenotype, which appears in these broods (the segregation is 8 entire shortened:5 split long:8 entire long:4 split shortened, χ_3^2 for 1:1:1:1 being 1.08, $P = 0.78$), consists, on the underside, of the outer portion of the band, well developed as far back as vein M3, and partly developed as far as Cu1a; the rest of the band appears as a faint red smudge, almost split from the outer portion; on the upper surface the band is much more strongly split than a split, long band, although the two halves do touch, and the inner half is quite strongly developed (plate 4*j*, left). Hence the split individuals in the F_1 do not carry shortened, and the shortened do not carry split, a hypothesis confirmed by the backcrosses to East Brasil, which segregate either split bands and East Brasilian bands if the F_1 parent is split (broods E5, E7, E11 and E14; total 40 split:64 entire; all long; for 1:1, $P = 0.024$; two contaminants excluded), or shortened bands and East Brasilian bands if the F_1 parent is shortened (broods E3 and E3A; total 31 shortened:38 long; all entire; $P = 0.47$), but never shortened and split bands in the same brood. The absence of the split shortened class, with these numbers, does not require a statistical test.

The deviation of the split banded backcrosses from equality is due in the main to the largest of them, brood E7, which segregates 14:35 ($P = 0.0038$). This appears to be purely a chance effect, although it does have a parallel in the Ecuadorian cross in *melpomene* which was performed at around the same time! The messy backcross segregates large numbers of long, entire and long, split butterflies (27:28, the ratio being uninformative because of the loss of the East Brasilian phenotype); the five apparent contaminants are all shortened, entire. The F_2 brood whose parents are both entire and shortened (brood E2) segregates as expected only shortened and long bands, all of them entire (no split bands) in a total of eight butterflies, and the F_2 that is a split × split cross (both long) contains two butterflies, both long, split (brood E15).

As must be the case with this scheme of inheritance, the East Ecuadorian male founder of the pedigree has the shortened, split phenotype, and must have been heterozygous for the two alleles, one from East Ecuador, the other from the Upper Amazon.

The apparent series of three alleles, long entire, shortened entire and long split, is most easily explained as a system of two loci, *Sd* producing the shortened effect, *St* the split effect, with the three races being East Brasil *sdst*, Upper Amazon *Sdst* and East Ecuador *sdSt*. If this is the correct explanation then the loci are tightly linked. No recombinants have appeared in the F_1, which numbers 50 individuals; if the next individual to emerge had been a recombinant, the crossover rate would have been 0.020, with an upper 95 % confidence limit of 0.058. The two loci are therefore very likely to be less than 6 % apart on the chromosome, and are probably much closer.

The locus or loci responsible for band shape in this cross are also tightly linked to the locus that partly removes the yellow line and bar. In all the backcrosses to East Brasil there is an overwhelming association between the two phenotypes attributed to the Yl^e allele (no line, no bar or dot, and basal line, eaten bar etc.) and either the split band or the shortened band,

according to the cross. The association is highly significant (71 Yl^e-, split or shortened: 101 $yl^T yl^T$, entire long). There are in addition three apparent recombinants in brood E11, which have reduced yellow lines and entire bands (the reduced lines normally accompanying split bands in this brood). However, in two of these individuals the forewing bands are *shortened*. This cannot represent recombination between St and Sd, as the father of the brood would have to be $Yl^e StSd/yl^T stsd$ to produce such a $Yl^e stSd$ offspring, and, although we cannot be certain of his phenotype as he is not preserved (P.M.S. might not have noted the shortened, split phenotype when setting up the mating as the full classification of phenotypes was not then recognized), this would have produced $StSd$ (shortened, split) offspring in the brood, and these are absent. In short, there is no way, even if St and Sd are not alleles, in which the father can have carried Sd, and the two apparent recombinants cannot be members of this brood. They probably came from brood E3A, which produced this phenotype in large numbers and which was housed in the adjacent greenhouse at the same time. The five shortened, entire butterflies in the messy backcross appear to be similar contaminants.

The third individual has a long, entire band, and is apparently carrying Yl^e, as the forewing line is weak and the yellow bar eaten into anteriorly by black scales. However, the butterfly does have some abnormalities in the shape of the posterior part of the band, which possibly indicate some developmental anomaly. We are inclined to believe that it is a recombinant, and, although the presence of proved contaminants puts the brood in doubt, it would have to be a recombinant even if it came from brood E3A. If this butterfly is taken on its face value as a member of brood E11, the recombination value between Yl and St is estimated as 0.029 ± 0.029 in males. As expected, the other backcrosses of this type, which have F_1 mothers (broods E5, E7 and E14), contain no recombinants. No recombinants between Sd and Yl have appeared in the two remaining backcrosses, both of which have entire, shortened F_1 fathers (broods E3 and E3A). If we assume that the next individual to emerge would have been a recombinant (and include the two contaminants in brood E11 as members of E3A), the maximum recombination fraction is 0.014 ± 0.014. The 95 % confidence limits for recombination between the three loci Yl, Sd and St are therefore all less than 9 %, and the loci are probably much closer than this.

As the supposed $Yl^e yl^T$ genotype nearly always contains either St or Sd, there is no way of knowing whether the Yl^e allele has an effect on the shape of the band like that of Yl^t in the crosses with Trinidad and Panamá. The one supposed authentic recombinant (above), with apparent genotype $Yl^e sdst/yl^T sdst$, has a band of roughly East Brasilian shape, of slightly abnormal outline posterior to vein Cu1a and lacking a well developed tooth, but quite clearly concave. However, with the expected variation in heterozygotes, it is not possible to conclude much from a single individual. The tooth phenotype is present in some form, as a jaggedness of the outer edge of the band at vein Cu1a, in nearly all backcross butterflies, and this effect of the yl^T allele is clearly largely dominant, as before.

There are obviously several equally plausible explanations of these results, besides the system of three loci proposed here. Either or both the band-shape 'loci' may be alleles of Yl (if our one recombinant should be a phenocopy). The effects on band shape attributed in the other crosses to the allele Yl^t may be the effects of a separate T locus, and the yellow line remover in East Ecuador may be not the Yl locus as we have supposed, but the Ly locus (which also has pleiotropic effects or linked genes influencing the band shape), or even a third locus that is neither Yl nor Ly. The Trinidad × East Ecuador cross (Emsley 1965*b*), which could settle the matter as Trinidad is known to be homozygous $Yl^t ly^b$, produced an F_2 of only one individual,

and is therefore inconclusive, but we shall show in §(f) below that it is almost certainly the *Yl* locus that is involved.

What is clear is that there are at least two chromosomes (*Yl* and *Ly*) in this species that both effect the partial removal of the yellow line and bar and produce considerable and various changes in the shape of the forewing band.

Emsley (1965*b*) reports the inheritance of split bands when East Ecuador is crossed with Trinidad in such a way that the phenotypes cannot be directly compared with ours: what appears to be our split class is scored as two classes (divided and semi-divided) in the F_1, and the phenotype is not mentioned at all in the backcross to Trinidad. However, in that cross he divides the brood into those with 'long' and those with 'short' (i.e. not extending toward the posterior angle) bands, and declares that length of the band is completely correlated with division, short bands always being divided. In our butterflies, as in his, split and 'short' bands appear to be joint effects of the *St* gene (the appearance of two characters, rather than a single character – the placing of a black gutter across the band from the outer posterior corner to the inner anterior corner – being purely subjective). We must emphasize that the *short* effect which is seen in split bands is quite different from the *shortened* effect of the allele *Sd*, which did not segregate in Emsley's broods. It would appear that Emsley scored the butterflies only on the upperside; as our insects would present similar difficulties if scored only on that surface (plate 4*f*, left), we believe that Emsley's results are consistent with ours: his F_1 was probably all split, and his backcross segregated 49 entire (which he scores as 'long'):35 split (scored as 'short') ($P = 0.16$ for 1:1). Hence, in the Trinidad cross, the *St* locus is segregating also.

St is shown to cause splitting of a yellow band as well as a red band by brood 5A, in which all four combinations of red and yellow, split and entire have appeared.

(f) *Allelism of Yl^t and Yl^e*

Our hypothesis that *Yl^e* and *Yl^t* are alleles rather than independent loci is supported, although not conclusively proved, by broods derived from the Rondônia × East Brasil × Panamá × Trinidad × East Ecuador hybrid stock. In these broods we observe the total association between a split forewing band and the eaten fuzzy hindwing bar (or absent bar if rectangles are also absent) which is expected from the linkage of *St* and *Yl^e* and which has been seen (with one possible crossover) in the backcross. However, unlike the backcross, the antithetical character is not the full East Brasilian hindwing bar (or its black-veined version) produced by *yl^T*, but the eaten or broken hindwing bar (respectively, with and without rectangles) which is seen in the Trinidad and Panamá crosses and is known to be produced by the gene *Yl^t* (figure 7, ea, br). This as expected shows total association with an entire forewing band, the numbers being (broods 2E and 5D) 13 entire with eaten or broken:12 split with fuzzy eaten or none. A mating between two split banded siblings, both having very weak fuzzy eaten bars of the Ecuador type (brood 3D), showed that *Yl^e* is dominant or hyperstatic to *Yl^t*, by segregating five individuals like the parents, and three entire banded butterflies with eaten or broken bands of the Trinidad type. Broods 2E and 5D therefore appear to be matings of the type *stYl^t/st–* × *StYl^e/st–*. There may be some synergism between *Yl^t* and *Yl^e* in that the supposed heterozygotes have very much reduced hindwing bars, usually completely absent if the rectangles are missing.

We confirmed that one such female from the hybrid stock, lacking the bar and the rectangles, carried both *Yl^t* and *Yl^e* by mating her to a pure East Brasilian male (brood E16). The offspring consisted of equal numbers of insects with split bands and basal yellow lines, with a reduced

hindwing bar of the thin fuzzy or eaten fuzzy phenotype encountered in the East Ecuador × East Brasil cross (or no line and no bar in the one individual that lacks rectangles) (figure 7, fa) and of insects with entire bands, yellow lines, and reduced hindwing bars of the Trinidad type (eaten if rectangles are present, broken if they are absent) (figure 7, ea, br). Clearly the female was carrying both Yl^e and Yl^t, the former linked in coupling to St, the $stYl^t/styl^T$ offspring having the Trinidad type of reduced bar, the $StYl^e/styl^T$ having the Ecuadorian type.

It is also clear that the mother did not carry the allele yl^T, as no yl^Tyl^T butterflies have appeared among 17 offspring ($P = 3.8 \times 10^{-6}$, one-tailed; or, on the more conservative hypothesis that they could have been detected only among the eight offspring that lack Yl^e, $P = 0.0020$). The simplest explanation for this is that Yl^e and Yl^t are alleles at the same locus, or are at least carried in the same chromosome. They might be different alleles with different effects on the yellow bar, or they might be the same allele, the difference being produced by closely linked loci.

An alternative explanation of these results is that the Ecuadorian allele which removes the yellow bar is at a completely different locus, say El, linked to St but not to Yl^t. In that case the broods are segregating El and el, and the mother of the last one, as she does not carry yl^T, must be homozygous Yl^tYl^t. According to this scheme, the entire banded offspring are $elst/elst$; Yl^tYl^t and the split banded $ElSt/elst$; Yl^tYl^t. However, as only one locus removing the yellow band (apart from Cr) segregates in the cross East Ecuador × East Brasil, our East Ecuadorian male parent, and hence one or both of the races that contributed to his make-up, must have been homozygous yl^Tyl^T, like East Brasil. But if the East Ecuadorian race had this genotype, then this locus should segregate in the cross with Trinidad, which is known to be Yl^tYl^t. Emsley (1965b) does not mention any yellow marks appearing on the hindwing in this cross, and although it is conceivable that the near-dominance of Yl^t and the effects of additional loci such as Ybs could have completely repressed the marks in a proportion of individuals, this cross lends no particular support to the two-locus hypothesis. In addition, the races in the lower Amazon are known not to carry yl^T, so that either in the Amazon basin, or at the junction between the East Ecuadorian and Upper Amazonian races, we might expect to see fragments of yellow bars appearing as a result of the segregation of the $elelyl^Tyl^T$ homozygote. No such hybrid butterflies are known, although an advocate of the two-locus hypothesis could point out that they would not occur if the change from El to el occurred in a different place from the change from yl^T to Yl^t. Thus, although the two-locus hypothesis is by no means disproved, belief in it does require belief in a few subsidiary hypotheses, which, while not individually improbable, do look rather like special pleading when taken together.

We therefore have provisionally adopted the simpler hypothesis, that Yl^e and Yl^t are alleles. It is clear that the difference between the Trinidad and East Ecuador crosses in the form of the 'eaten' bar, as both segregate so clearly within the same broods, is due to a difference in the yellow line remover itself, and not to the 'genetic background'. Possibly the difference in the effect of Cr on the bar in the two crosses is similarly the result of a local, Ecuadorian allele at this locus, but we have no evidence on this point.

(g) Effects of band shape genes and sex on the yellow bar

All the alleles influencing band shape in this cross (Sd, St and Ro) are correlated in some broods with minor effects on the yellow bar, all of which could be effects of the alleles themselves, or could be loci linked to the major alleles.

The *ro* allele, or a gene linked to it, can sometimes reduce the yellow hindwing bar. In one of the backcrosses (brood E3A) the presumed $Yl^e yl^T Crcr$ class is divided into four flat individuals with no hindwing bars and four round butterflies with a single yellow dot (probability for 2×2 contingency table 0.029, two-tailed). Further, in the presumed $Yl^e yl^T crcr$ class the eight round phenotypes have more strongly developed bars of the eaten phenotype than the four straight butterflies, in which the firm yellow spot within the bar is rather poorly developed, causing three of them to fall into the thin fuzzy category. It is interesting that the effect here is the reverse of what we might expect: a chromosome from East Ecuador is enhancing the bar, the reducing effect coming from East Brasil, where the bar is strongly developed. In the $yl^T yl^T crcr$ class, which has the East Brasilian phenotype, the bars of round individuals are not noticeably more developed than those of the flat, but all three of the round butterflies have a very strongly developed yellow forewing line, in two of them decidedly stronger than in any of the six flat individuals. There is no clear qualitative effect of *ro* on the yellow bars or lines of the presumed $yl^T yl^T Crcr$ class. It is possible that in this brood we have detected one gene in a polygenic system regulating the development of the yellow marks in East Brasil; if such a system is balanced, it should contain genes reducing yellow as well as enhancing it.

In the F_1, the two shapes of forewing band interact with the sex of the butterfly to determine the size of the very reduced hindwing bar, which is either absent or represented by a weakly developed yellow dot (rather strongly developed in one male). The segregation is:

	split		shortened	
	female	male	female	male
no bar	12	5	5	17
dot	2	10	0	0

The association between band shape and the development of the bar is highly significant ($P = 6.5 \times 10^{-4}$, two-tailed). This may be an effect of the *St* and *Sd* genes, or an effect of a further linked locus, or may indicate that each is linked to a different allele at the *Yl* locus. Within the split class, which is the only one segregating for the bar, there is a significant tendency for females to have the bar less developed than males ($P = 0.011$, two-tailed). This is the fourth case that we have noticed of sex influencing the development of a mark on some genetic backgrounds (the others being the yellow bar of Belém *melpomene* (§3.5a), the broken band of East Brasilian *melpomene* (§3.4i) and the yellow forewing band of Suriname and Belém *melpomene* (§3.2g)), in addition to a sexual influence on orange pigment (§§3.2h, 3.4h). This effect may have some evolutionary significance (see §5.7).

(h) Shadow of yellow bar

It is one of the differences between the East Brasilian bar and the Panamanian yellow bar that only the latter produces a 'shadow' when heterozygous. However, one of the three radiate individuals showing the Trinidad-style broken East Brasilian bar (genotype $Yl^t yl^T Crcr$) in brood E16 shows an effect analogous to the shadow. On the underside the yellow bar is represented by a distal and proximal yellow portion, between which is a pale brown shadow of the missing part of the bar, made much more obvious because the red hindwing rays have failed to develop there. One of the two extant radiate $Yl^t yl^T crcr$ butterflies shows a similar effect, but the rays have some tendency to develop in the shadow. It would appear that there are two stages

in the development of the yellow bar, the laying down of a 'prepattern', followed by the development of yellow pigment within the predetermined area, with the prepattern sometimes overriding the hindwing rays, possibly because developing earlier.

(i) Shape of tip of hindwing bar

The Trinidad and Panamá races are homozygous for the recessive allele bf, which causes the hindwing bar to turn forward at the tip on the underside (plate 4d, right). It appears that the East Ecuadorian race, or at least our introgressed male, does not carry this allele; although the absence of a true forward-turning bar in the F_2, among 13 butterflies whose bars are long enough to show this effect, could be due to chance, the probability of 13:0 being 3:1 is rather low ($P = 0.012$, one-tailed). However, things are not quite so simple as this. A male and female with eaten bars in the F_2 have a little extra cream crescent anterior to the tip of the bar, which gives the effect of the bar bifurcating into a tip that turns both ways. While these could be $bfbf$ homozygotes (11:2 being a good fit to 3:1; $P = 0.67$, two-tailed), a similar double tip appears rather commonly in the backcrosses to East Brasil, and almost exclusively in the supposed Yl^eyl^Tcrcr class (usually, like the two F_2 individuals, with eaten bars). It occurs in one or two out of three in this class in brood E3, in five out of eight in brood E3A, in one out of five in brood E11, in three out of seven (two not scorable) in brood E7, in three out of three in brood E5, and in 14 out of 19 in brood E12. It does not appear in the two members of this class in brood E14. In brood 10A, which is not a backcross, two out of three in this class are double. The apparent 1:1 segregation (27 double end:20 single) strongly suggests that there is a *dominant* gene in East Ecuador producing the double tip, but expressed only in the Yl^eyl^Tcrcr genotype, and hence not in the F_1. A single broad sharp individual (yl^Tyl^Tcrcr) in brood E3 has a bifurcated tip, possibly indicating a low level of penetrance in other phenotypes. This supposed gene clearly requires confirmation from further crosses, particularly a backcross to East Ecuador. Provisionally we shall designate the East Ecuadorian allele Fb, the East Brasilian fb.

(j) Inheritance of white in the forewing band

The distal part of the forewing band of the introgressed male that founded the present pedigree is white. White colouring does not appear in the F_1 or backcrosses to East Brasil, all of which have red bands, but segregates in an interesting way in the F_2. In the total of 35 butterflies, three have strongly developed white marks, and a further nine have a mixture of white scales among the red in the band; the white appears only in the distal part of the band, even in those bands that are split and which therefore have a proximal portion in the cell of the forewing. In one insect the 'white' scales appear to be decidedly yellow. This may indicate the segregation of genes introduced from the Upper Amazonian race, which has a yellow forewing band; Emsley (1965 b) reports yellow marks in this area in some of his hybrids, but as he does not give the location from which his parental butterflies were taken, we cannot tell how likely it was that they carried Upper Amazonian genes.

It would appear that white marks in the outer part of the band are produced either by a single recessive gene of variable expressivity (expected numbers $26\frac{1}{4}:8\frac{1}{4}$, $P = 0.28$) or possibly (and with a slightly better fit) by two recessive genes, homozygosity for *either* producing weakly developed white, homozygosity for *both* causing full development (expected ratio 9:6:1, expected numbers 20:13:2, $\chi^2_2 = 1.45$ with Yates's correction, $P = 0.48$). We shall designate

the locus *Wh* for the single-locus hypothesis, and Wh_1 and Wh_2 for the two-locus system. There is no way of telling how the white marks on the inner part of the band are produced in East Ecuadorian butterflies. These marks were absent in our male parent, and have not surprisingly failed to appear in the F_2.

The interesting thing about the action of the *white* gene is that it produces white marks only as far as vein Cu1a on the upperside. All three individuals with strongly developed white have shortened bands on the underside; on the upperside, as is common in this phenotype in these crosses, the band extends to the posterior of vein Cu1a, but is there entirely red. The result on the upperside is a particoloured band, white as far as Cu1a, and red thereafter (plate 4*j*, right). The white scaling likewise does not develop to the posterior of this vein in the weakly expressed phenotypes although the particoloured effect is not so obvious. It should be noted that the white is *not* restricted to the area of the outer portion of the East Ecuador band, which extends only as far as vein M3, but to the rather larger area of the Upper Amazonian band.

In the East Ecuador × Trinidad cross white is likewise recessive (Emsley 1965 *b*) although the number of loci cannot be determined from the data. However, the removal of white colouring in this species does appear to be effected by dominant genes at more than one locus, with the loci differing between races, as hybrids in the Guiana hybrid zone (between the Trinidad/ Venezuela, Guiana and Belém races) occur with white colouring, found in none of the parental races, in the band. Such specimens are quite common in collections, and one appeared among the offspring of a wild (not white) Suriname female bred by Beebe (see Turner & Crane 1962).

As in the East Ecuadorian crosses in *melpomene*, white colouring sometimes appears in our *erato* offspring in areas outside the forewing band. In one F_2 individual (in brood E2) the dot that constitutes the hindwing bar is white instead of yellow and is expressed only on the underside; in one backcross individual the fully developed forewing line contains a considerable amount of white (a $yl^T yl^T crcr$ individual in brood E7) (plate 4*j*, right). White pigment penetrates once in the forewing band of a backcross male (brood E11).

(k) Splitting of the forewing band on the upperside

In the East Ecuadorian race the band is split (and short) on both the upper and under surfaces of the wing; as in all other races, the distribution of marks on the two surfaces is very nearly coincident. This is not so in our F_1 and backcrosses to East Brasil, in which bands that are split and more or less of Ecuador shape on the underside are, on the upperside, more or less entire, although sometimes with some black dusting along the area that would be occupied by the black gutter, and usually, as on the underside, curtailed well away from the posterior angle of the wing (i.e. 'short') (plate 4*f*, left). It is clear that there must be genes in the East Ecuadorian race that cause the band to be split on the upperside as well. Two small broods from the general hybrid stock suggest strongly that there is a single recessive modifier of split that has this effect: both segregate entire bands, split bands (underside only) and split bands (both surfaces). The numbers are

brood	entire	split under only	split both	$P(1:1)$	$P(3:1)$	$P(1:3)$
3D	3	1	4	0.38	0.031	1.00
10A	1	6	2	0.29	1.00	0.0085

(the probabilities are for a backcross, an F_2 with expression of split on the upperside recessive, and an F_2 with split on the upperside dominant).

Although the parents are not fully recorded, it is fairly certain that the splitting of the band on the upperside (plate 4f, right) is produced by a single gene, having no effect on entire bands, and that the East Ecuadorian allele, which we shall call ur, is recessive, as it is not expressed in the F_1 or East Brasilian backcrosses. It is likely, on account of the lack of segregation of split (both surfaces) in the brood (5A) from which both parents came, that brood 3D is a mating between two split (underside only) butterflies, confirming that the modifier is recessive (only the mother is now extant). The gene is analogous, but not identical in action, to the Rr gene in $H.\ melpomene$.

From Emsley's (1965b) description of his East Ecuador × Trinidad F_1 (variable forewing bands divided arbitrarily into two classes, divided and semi-divided) it is likely that these also were only partly split on the upperside, and that the Trinidad race carries the allele Ur.

It is likely, again by analogy with $H.\ melpomene$, that yellow and white marks are split on the upperside independently of the presence of ur. In the F_2 the white marks are all restricted to the areas that they would occupy in a split band. We have only one brood in which yellow split bands appear (brood 5A): the four yellow bands in this brood are split on both surfaces (plate 4i, left), whereas all four of their red split siblings are split on the underside only. The significant association ($P = 0.014$, one-tailed) strongly suggests that Ur prevents red, but not yellow, from splitting on the upper surface. (To be strictly fair, we have to admit that one red banded butterfly is intermediate in phenotype, and that, if he is counted as split on both surfaces, the association has a probability of only 0.071, which is not formally significant. However, there is also a fairly large amount of yellow in the band of this individual.)

In the absence of ur, red shortened bands are not fully shortened on the upperside (§4.10j: we have no information on the effects of Ur), but the particoloured F_2 individuals (plate 4j, right) show that white marks are shortened on the upperside even when red are not, showing that white does not require the assistance of ur to be restricted by the $shortened$ gene.

(l) Radiate, and yellow forewing band

A radiate female of mixed East Ecuador × East Brasil × Panamá × Rondônia origin, mated to an East Brasilian male (brood E16), produced roughly equal numbers of radiate and plain, as did a further radiate × plain mating (brood 3D; total 17:8, $P = 0.11$); brood 5A, with a radiate mother and inadequately recorded father, appears to segregate 3 radiate:1 plain (18:2, $P = 0.18$), showing the usual inheritance of the radiate pattern as a single dominant allele. Yellow forewing band appears to be the usual recessive allele, linked to radiate, segregating six out of 20 in this last brood ($P = 0.77$), all of the yellows being radiate. This is only to be expected, as the genes must originate in the Rondônia population. While this does not necessarily indicate anything about the $plain$ allele from Ecuador (the allele in this cross may have been Panamanian or East Brasilian), it is necessary to establish this in order to test for linkage of other genes to radiate. In fact, the absence of radiate marks in Ecuador must be due to the allele r as in Trinidad, for if it were due to a separate locus radiate patterns would appear in the Trinidad × East Ecuador F_1, which they do not (Emsley 1965b).

(m) Extra forewing rays

Extra ray-like extensions of the radiate marks on the forewing have appeared in one brood (5A) derived from the general mixed stock (plate 4l, right). They are variably developed,

never very strong, and usually clearer on the underside, appearing in females even in the friction patch. In red banded butterflies they take the form of a red–brown stripe lying on the underside medially between vein Cu1a and Cu1b, and passing right across the forewing band, which as usual is whitish on this surface. This mark is present on all entire banded radiate butterflies, but not on butterflies that are plain or split banded. Among yellow banded butterflies this stripe appears in a brighter red, and occurs on either side of, but not across, the forewing band. It, and a well marked stripe just posterior to vein Cu1b in the friction patch, are sometimes visible on the upperside. In addition, black dusty rays lying medially between M3 and Cu1a, and between Cu1a and Cu1b, tend to invade the yellow band (plate 4*i*, left). These effects occur in all but one yellow banded butterfly, both split and entire; all are radiate.

We can say nothing about the cause of these patterns; they are reminiscent of the brown rayed patterns of the related genera *Dione*, *Agraulis* and *Dryadula*.

(*n*) Inheritance of the red costal spot

Most races of *H. erato* have an intense red spot, which like the red marks at the base of the hindwing, but unlike the red forewing band, does not fade to orange on prolonged contact with air, on the leading edge of the forewing next to the thorax, on the underside (plate 4*f*, right). The spot is normally absent in the East Ecuador race, and was absent in the male parent of our F_1. The presence of the spot seems to be mainly produced by a single dominant gene: all 50 F_1 butterflies have it, as do all but three of 234 butterflies in the East Brasil backcross (one individual is not scorable, and in one further butterfly the spot is reduced to two red scales on the left wing only; numbers include messy backcross and all contaminants). The combined F_2 broods segregate 23 with the spot: 8 without (one butterfly not scorable), an almost perfect fit to a 3:1 ratio. In crosses between Trinidad butterflies (in which the spot may be present or absent) and the East Ecuadorian and West Ecuadorian races of *erato*, which normally lack the spot, Emsley (1965*b*) found that the spot was usually dominant, although in some broods heterozygotes appeared to be distinguishable in having weaker spots. We designate the alleles at this locus *Cs* and *cs*.

(*o*) Red versus orange

Although this difference has not segregated in this cross, both the East Ecuadorian and East Brasilian races being red, one brood (2E) did have an orange mother: all her offspring (except one which is merely faded) are red, suggesting again that orange is recessive.

(*p*) Unlinked loci

Mutual independence of *Cr*, *Ro*, *Fb* and the *YlSdSt* linkage group can be shown by the appearance of recombinant phenotypes in the backcross broods with F_1 mothers (broods E5, E7 and E14). The phenotypes (and their total numbers) are: cream rectangles, line reduced, split (15) and no rectangles, yellow line, entire (23), showing that *Cr* is independent of the *St* chromosome; flat, line reduced, split (12) and round, yellow line, entire (22), showing that *Ro* is independent of this chromosome; cream rectangles, round (9) and no rectangles, flat (18), showing that *Cr* and *Ro* are independent; and round, bar tip backward (4) and flat, bar tip forward (3), showing that *Fb* is independent of *Ro*. As the *Fb* allele itself is expressed only in the $Yl^e yl^T crcr$ homozygote, its independence of *Yl* (and the linked *St*) and *Cr* cannot be tested in this way; however, its 1:1 segregation within this class makes it likely that it is unlinked, or at least only loosely linked.

In the Trinidad × East Ecuador cross (Emsley 1965 *b*) loci that we have argued here are identical with *Ro* and *St* are also unlinked: the recombinant phenotypes flat, split ('truncate, short' in Emsley's terminology) and round, entire (Emsley's 'round, long') are represented by four and two individuals respectively in the backcross with an F_1 mother; there is no association between the loci in the larger backcrosses with an F_1 father ($\chi_c^2 = 1.12$, $P = 0.15$, one-tailed).

The remaining pairs, involving the recessive Ecuadorian alleles *wh* and *cs*, can be tested only in the F_2, which on account of variable expression of both white and the costal spot does not provide particularly powerful tests. To take the costal spot first, the absence of the spot in some *Cscs* individuals in the backcross means that the presence in the F_2 of the recombinant double recessive phenotype, lacking the spot, for a repulsion pair cannot necessarily be taken as evidence of independence. We therefore resort to statistical tests, using the formula for χ_1^2 without Yates's correction, as in equation (5–42) in Bailey (1961), which allows for partial manifestation at one locus. The χ^2 values, one-tailed probabilities and (in parentheses) the number of the apparent double recessive phenotype, for the loci that are in repulsion with *cs* are: for *Cr*, $\chi_1^2 = 0.77$, $P = 0.19$ (8); for *Yl*, $\chi_1^2 = 0.95$, $P = 0.16$ (5); for *Ro*, no test, coupling phenotypes exceed expectation (0). There is therefore no evidence that *Cs* is linked to any of these loci. The recombinant phenotype, backward-turning bar, no costal spot (*fbfbcscs*), *within* the *Yl^t*–*crcr* class has not appeared, but, as there are only three individuals in this class with bars that are large enough to be scored, this cannot be taken as evidence of linkage. In the East Ecuador × Trinidad cross, *St*, *Ro* and *Cs* all segregated, but Emsley (1965 *b*) does not give enough information for us to judge whether *Cs* was independent of the other two loci.

(q) Inheritance and linkage of white colour

Testing the repulsion linkage of white colouring with the dominant East Ecuadorian alleles *Cr*, *Ro* and *Yl^e* presents some difficulties, as there are several plausible hypotheses about the inheritance of white. In these circumstances the best test is the naïve one which makes no assumptions about the mode of inheritance, that is the 2 × 2 contingency test. We can classify the phenotypes as either (i) any development of white, weak or strong *versus* red or (ii) fully developed white *versus* weak white plus red. The exact probabilities (one-tailed) for classification (i) are *Cr* 0.17, *Yl* 0.060, *Ro* 0.43; for classification (ii), recombinant (coupling) phenotypes exceed ten-sixteenths of the brood in all three cases, so that there is no test. Therefore the only indication of linkage is of white with *Yl*; the other two loci give no indication of linkage with this test, nor with any of the ensuing tests, which we have performed but refrain from printing. To test further for the linkage of white and *Yl*, we can adopt any one of four hypotheses

(i) White is produced by two unlinked loci, the double recessive being necessary for the development of the full white colour. Only one of the loci could be linked to *Yl*, and this is ruled out by the appearance of one multiply recombinant individual with full white colouring and a yellow line, genotype homozygous $wh_1wh_2yl^T$; as white band and yellow line are in repulsion, recombination of *both* white loci with *Yl* in the mother is required to produce this phenotype.

(ii) White is a single recessive gene, with variable manifestation, all individuals with any development of white being *whwh*. In that case there are 20 parental phenotypes (white, no line, and red, line) and 15 recombinants (red, no line, and white, line); tested for the expected segregation of 6:10 if the loci are not linked, this gives a one-tailed probability of 0.014.

(iii) White is a single recessive gene with variable manifestation, some individuals being misclassified, so that either some *whwh* butterflies are scored as red, or some *Whwh* butterflies have traces of white. In that case application of formula (5–42) of Bailey (1961) gives $\chi_1^2 = 3.99$, without Yates's correction, $P = 0.023$, one-tailed.

However, although the statistical tests under hypotheses (ii) and (iii) indicate that *wh* is linked to *Yl*, this is ruled out by the appearance of the white, yellow line individual described under (i), it being very unlikely that a butterfly with such strongly developed white is in fact a *Whwh* heterozygote.

(iv) White is a single recessive gene, but on the F_2 genetic background some heterozygotes show partial penetrance of white colouring. In that case the major *Wh* locus is independent of *Yl* (by the appearance of the double recombinant detailed under hypothesis (i)) but the tendency for white to appear in heterozygotes is strongly influenced by the *Yl* chromosome (partial white against full white plus red, 2×2 probability 0.029, one-tailed).

Thus under both the simple two-locus and one-locus hypotheses, the major white locus (loci) is (are) not linked to *Yl*. However, both these hypotheses produce an internal inconsistency in giving a statistically significant association of white and *Yl*, which is shown not to be the result of linkage. This makes hypothesis (iv) quite attractive, although the segregation of only three homozygotes falls significantly short of a $3:1$ ratio ($P = 0.027$, two-tailed).

The firmest conclusion that we are able to draw is that white colour is produced by recessive alleles at an undetermined number of loci, of which at least all the major ones are not linked to any of the other loci segregating, and that the *YlSdSt* chromosome appears to influence the development of white.

There is only a slight indication of linkage between the coupling pair white and *cs*. The exact 2×2 probabilities for full white *versus* partly developed white plus red and for any white *versus* red are 0.13 and 0.080 respectively. There is thus a hint that the *wh* locus is (or under a two-locus hypothesis, one of the two *wh* loci) is linked to *cs*, or, under the heterozygous penetrance hypothesis (iv, above), that homozygosity for the *cs* chromosome increases penetrance of white in heterozygotes. However, the effect is not formally significant, and no great weight can be attached to it.

(r) Independence of radiate

The mother of brood E16 was heterozygous for radiate, cream rectangles and the genotype Yl^tst/Yl^eSt, but as her parentage is unknown we do not know which are the parental combinations and cannot use the strongest test for independence, the occurrence of recombinants. However, two loci must be independent if at least three of the possible phenotypic classes appear. In fact in all three pairs, all four of the possible combinations have appeared, in the following numbers,

'A' locus	'B' locus	'AB'	'Ab'	'aB'	'ab'
R	Yl^eSt	5	6	4	2
R	*Cr*	4	7	1	5
St	*Cr*	1	8	4	4

showing that all three loci are independent. There is of course no certainty that the Ecuadorian alleles *Cr* and *r* are segregating in this brood as the mother is only partly Ecuadorian in origin. The independence of *R* and *St* (and *Yl*), which must be Ecuadorian, has not been previously established, and is consistent with the hypothesis that Yl^e is an allele of Yl^t.

Independence of the other two pairs merely confirms what is known from other crosses. The independence of *R* and *St* is further confirmed by brood **3D**, a cross between an *StStRr* female and an *Ststrr* male. In the absence of female recombination, only three of the four offspring classes can appear in such a cross if the loci are linked; all four have appeared.

Radiate has not been shown to be independent of the other uniquely East Ecuadorian genes *cs*, *wh*, *ur* and *Ro* (nor of *Fb*, if it exists).

(s) *Genetic constitution of East Ecuador and Upper Amazon races*

The East Ecuador race appears to have a genetic constitution rather like that of Trinidad, but carrying several genes which, to judge from the non-appearance of the East Ecuador phenotypes in crosses between other races, are unique to East Ecuador (at least among those races that we have bred). These are *Ro*, giving a rounded tip to the forewing band, *St*, splitting it in two, *wh*, turning the outer part white, and *ur*, causing the splitting of the band to affect the red marks, as well as the white, on the upperside. As with Trinidad, rays are absent by virtue of the *r* gene and yellow lines and bars by virtue of *Cr* and *Yl*, at least the latter being probably a local allele, *Yl^e*, different from the Trinidad one. As orange has not segregated in this cross, East Ecuador is apparently *OrOr* like Trinidad and East Brasil. The other yellow line remover locus must carry the allele *ly^b*, like East Brasil, as only one such locus has segregated. However, we have not accounted for the inner half of the East Ecuador band being partly white; perhaps *ur*, *St* and *wh* interact to produce this effect, but it is more likely that our original male, being a part hybrid with the Upper Amazon race, simply lacked the necessary gene.

From the Upper Amazon race our male carried, with certainty, only one gene: *Sd*, which produces the shortened forewing bar. We have bred this race, and confirm that it is orange (not red) when fresh, and so it is reasonable to suppose that its genetic constitution is homozygous *R* (radiate), *y* (yellow band), *Sd* (shortened), *Cr* (no rectangles), *ro* (flat tip), *Wh* (no white), *or* (orange). Its yellow line remover is unknown, but is unlikely to be *Ly^B* as this would break up the band somewhat. As white pigment does not require *ur* to respond to the shortening effect of *Sd*, and as yellow and white colour seem to be under very similar developmental controls, as witness the substitution of white for yellow in the lines and bars of some butterflies in the present cross, it is likely that *ur* is not needed by the Upper Amazon race for the yellow band to be shortened. But the absence of hybrids from the natural hybrid zone with bands that are split on the underside only suggests that this race is not introducing the alternative allele *Ur* into the hybrid zone.

(t) *Summary*

The East Ecuadorian cross establishes the existence of a second linked cluster in *H. erato*: a chromosome carrying the shortened forewing band (plate 4*e*, right) of the Upper Amazonian race (*Sd*), the split forewing band (plate 4*f*) of the East Ecuadorian race (*St*), and a yellow line remover that is likely to be, although the point is not fully proved, an allele at the *Yl* locus. The East Ecuadorian race has a cream rectangle remover, possibly the same allele of *Cr* as is found in Rondônia, but no special allele at the *Ly* locus. The rest of the characteristic forewing band is produced by further special genes: *Ro* (round tip) (plate 4*g*) and *ur* (splitting of band on upperside as well as underside) (plate 4*f*), *Ro* at least being independent of all others, and *wh* (white colour) (plate 4*j*), which may be more than one locus, and which may be linked to the *YlStSd* group.

In crosses with the Amazonian stock, red forewing rays have again appeared in some individuals, in this case reminiscent of related genera like *Dione* (plate 4*l*). There is a sex difference in the yellow marks in one brood.

4.11. *Summary of genetics and of genotypes of parental races*

The genetics of *H. erato* can be most easily understood in terms of alterations in the pattern of the East Brasilian race (plate 1*g*, figure 6*b*), which has been used in all our crosses (except that with Georgetown), and which we believe to be close to the ancestral pattern of all the races (see §5.7).

The yellow bar and yellow forewing line of this race can be removed by the following four loci.

Yl (East Brasilian allele *yl*^{T}). The allele *Yl*^{t} removes much of the yellow forewing line, particularly the distal part, and weakens the yellow hindwing bar, particularly anteriorly and at the base (figure 7, ea) (both effects dominant); this allele also alters the shape of the red forewing band, causing removal of the distal teeth (which appear to be part of the same developmental system as the forewing line) (largely recessive effect) and an alteration of the inner margin of the band from concave to convex (heterozygotes intermediate and very variable) (plate 4*a*, *b*). The allele *Yl*^{B}, which may occur in the Belém race although its existence is uncertain, would have the same effects, but in addition cause the band to break up into the broken band exhibited by that race (plates 1*e*, 4*h*; figure 6*c*). The allele *Yl*^{e} from East Ecuador has a similar effect to *Yl*^{t} but is distinguished by the type of intermediate line and bar that it produces in combination with the *crcr* genotype in hybrids (figure 7, fa) (these effects may of course be due to closely linked loci rather than to a separate allele). It may have some effects of its own on band shape, but we have provisionally attributed these to two linked loci *St* and *Sd*. The *Sd* locus (East Brasilian allele *sd*, recessive) is not known to recombine with *Yl* or *St*, and could be an allele of either. The *Sd* allele converts the forewing band to the shortened forewing band of the Upper Amazonian race (plate 4*e*, right; figure 6*h*). The *St* locus (East Brasilian allele *st*, recessive) appears to recombine with the *Yl* locus at a rate of around 3 %; the allele *St* converts the forewing band into the split form seen in East Ecuador (plates 1*h*, 4*f*; figure 6*g*).

Ly (East Brasilian allele *ly*^{b}, recessive). The dominant allele *Ly*^{B} strongly reduces both the yellow line and the yellow bar, and also alters the shape of the forewing band, causing it to become the broken, toothless band, extending well into the forewing cell, seen in the Manaus (Guiana) race (plates 1*k*, 4*h*, left; figure 6*e*). This is known to be a different locus from *Yl* as both segregate in the Manaus × East Brasil cross. In the Rondônia population the breaking up of the band is less extreme (plate 4*h*, right); this population may contain *Ly*^{B} modified by other loci, or its own allele, *Ly*^{I}, at this locus, or a specific allele, *Yl*^{I}, at the previous locus.

Cr (East Brasilian allele, *cr*, recessive). The dominant allele *Cr* from Trinidad weakens the yellow line and allows the distal end to become red, and weakens the yellow bar by permitting the invasion of black scales along its length, mostly on the posterior edge (figure 7, bf). It has no effect on the red forewing band, but does remove the cream rectangles from the distal edge of the hindwing on the underside (some variable penetrance in heterozygotes), and may be recognized by this effect (plate 4*d*). The Panamanian allele *Cr*^{p} has the same effects (all dominant) and in addition adds the yellow hindwing bar of the Panamá race (largely recessive, but producing a shadow on the underside in most heterozygotes). It is possible that this last effect is actually another closely linked locus (Panamanian allele *p*), but we have

no unimpeachable crossovers. The Cr alleles from East Ecuador and from Rondônia weaken the bar by causing the veins that traverse it to become black (figure 7, bb, bv); these are possibly different alleles from those found in Trinidad and Panamá.

Ybs (East Brasilian allele ybs, recessive). The dominant allele Ybs weakens the yellow forewing line, particularly any red in the distal part, reduces the width of the hindwing bar along its whole length (figure 7, tf, vt), and removes the rectangles in $Crcr$ heterozygotes.

In hybrids, various combinations of alleles at these four loci have been produced; their phenotypes, and the detailed action of the genes, are summarized in tables 7–11 and on pp. 556 to 558 for the East Ecuador cross. The important point to note in studying the racial differences is that the yellow or cream line, bar and rectangles of the East Brasilian race, being weakened by each of these loci, can be completely removed by a combination of several of the appropriate alleles. The combinations known to produce total removal are homozygous

$$
\begin{array}{ll}
Cr\ ly^b\ Ybs\ Yl^t & \text{in Trinidad} \\
Cr^p\ ly^b\ [ybs]\ Yl^t & \text{in Panamá} \\
\left.\begin{array}{l} Cr\ Ly^I\ [?]\ yl^T \\ \text{or } Cr\ ly^b\ [?]\ Yl^I \end{array}\right\} & \text{in Rondônia} \\
Cr\ Ly^B\ [?]\ Yl^t & \text{in Manaus} \\
Cr\ ly^b\ [?]\ Yl^e & \text{in East Ecuador}
\end{array}
$$

(the East Brasilian race being homozygous $cr\ ly^b\ ybs\ yl^T$).

Conversion of the shape of the forewing band between the East Brasilian, Belém/Manaus, Panamá/Trinidad, Rondônia and East Ecuador shapes is largely accomplished by the loci already described, all of which reside in two chromosomes, both of which carry genes affecting the yellow line and bar. To repeat, for the sake of completeness: the Yl chromsome contains factors provisionally identified as effects of the Yl^t allele, changing the East Brasilian band to a Trinidad/Panamá shape, and others, provisionally identified as the separate loci St and Sd, converting the band to East Ecuadorian or Upper Amazonian shape. The Ly chromosome contains factors, provisionally identified as effects of the Ly^B allele, converting the band to the broken shape found in Manaus. The genetics of the broken and invaded bands of Belém and Rondônia is less certain, but the bands appear to arise from the action of one or both of these chromosomes. The combination of the factors for the Upper Amazonian band and the East Ecuadorian band (Sd and St) produces a band consisting of the outer half only of the East Ecuadorian band (plate 4j, left).

In addition, there are two further independent loci affecting band shape:

Ro (East Brasilian allele ro, recessive). The dominant allele Ro, found only in East Ecuador (among those races bred by us), rounds the tip of the forewing band out toward the wing apex (plate 4g). Without it, the band has a flat edge in this region, making the outer part of the split East Ecuadorian type of band very narrow (plate 4f, left, plate 4i, left).

Ur (East Brasilian allele Ur, dominant). This locus determines whether the splitting of the band produced by St occurs on both surfaces of the wing, or only on the undersurface (plate 4f). In the presence of the East Ecuadorian allele ur, the splitting occurs on both surfaces of the wing; in the presence of Ur, the splitting, and also the shortening produced by Sd, occurs only on the undersurface. The effect is confined to red pigment; yellow and white forewing bands appear to be unaffected.

The red colour of the forewing band can be converted by two loci:

Y (East Brasilian allele *Y*, dominant). The yellow forewing band of the Amazonian races is produced by the recessive allele *y* (plate 4*i*).

Wh (East Brasilian allele *Wh*, dominant). The white colour of the outer part of the East Ecuadorian band (plate 4*j*) is produced by the recessive allele *wh*, and possibly by an additional locus with similar dominance and phenotypic relations. We do not know how the white colour of the *inner* part of the band is produced.

TABLE 13. GENOTYPES OF THE EIGHT THOROUGHLY STUDIED RACES OF *HELICONIUS ERATO*

The races are homozygous for the alleles shown. The West Ecuador race has so far been crossed only with Trinidad (Emsley 1965*b*) giving insufficient information about the identity of the alleles segregating. Linkage group I (the sex chromosome) contains the polymorphic enzyme 6-phosphogluconate dehydrogenase (Johnson & Turner 1979) but no known wing colour loci. [ ], Presumed from phenotype; no segregation in crosses that have been performed.

linkage group	Amazonian races				Extra-Amazonian races			
	Belém/Mato Grosso	Manaus	Rondônia (Bolívia)	Upper Amazon	East Ecuador	Panamá/México	Trinidad	East Brasil
II	Yl^t or Yl^B	Yl^t	yl^T (or Yl^I)	?	Yl^e	Yl^t	Yl^t	yl^T
	[*st*]	[*st*]	[*st*]	*st*	*St*	[*st*]	*st*	*st*
	[*sd*]	[*sd*]	[*sd*]	*Sd*	*sd*	[*sd*]	[*sd*]	*sd*
III[1]	*y*	[*y*]	*y*	[*y*]	*Y*	*Y*	*Y*	*Y*
	R	?	*R*	[*R*]	*r*	*r*	*r*	*r*
	?	*D*	?	?	[*d*]	[*d*]	*d*	*d*
IV	Ly^B or ly^b	Ly^B	$^4Ly^I$ (or ly^b)	?	ly^b	ly^b	ly^b	ly^b
V[2]	*Cr*	*Cr*	*Cr*	[*Cr*]	*Cr*	Cr^p	*Cr*	*cr*
VI[3]	?	?	?	?Bf^8	?Bf^8	*bf*	*bf*	*Bf*
VII[5]	[*ro*]	[*ro*]	[*ro*]	[*ro*]	*Ro*	[*ro*]	[*ro*]	*ro*
VIII[5,7]	[*Wh*]	[*Wh*]	[*Wh*]	[*Wh*]	*wh*	[*Wh*]	*Wh*	*Wh*
IX[5]	*Cs*	[*Cs*]	[*Cs*]	[*Cs*]	*cs*	[*Cs*]	*Cs*	*Cs*
unassigned	*or*	[*Or*]	[*Or*]	[*or*]	[*Or*]	[*Or*]	[*Or*]	*Or*
unassigned	?	?	?	[*Ur*]	*ur*	?	*Ur*	*Ur*
unassigned[6]	?	?	?	?	?	[*ybs?*]	*Ybs*	*ybs*

[1] *D* is almost certainly in group III, and is very probably an allele of *R*, as in *melpomene*.

[2] If *p* is a closely linked locus, rather than an allele of *Cr*, then Panamá is *p* and all other races *P*.

[3] Not shown to be independent of linkage groups III or IV.

[4] Ly^I might be Ly^B modified in expression by other loci.

[5] Independent of one another and of groups II and V, but not shown to be independent of groups III, IV or VI. There may be a further, independent locus with effects like *Wh* and one of the two loci may be in linkage group II. *Cs* does not control a major element of the mimetic pattern.

[6] Not in groups II or V.

[7] There is some doubt whether this is a single gene locus.

[8] At least one of these races is *Bf*.

The red (or orange) radiate marks of the Amazonian populations (Rondônia and Belém) (plates 1*e*, *j*, 4*h*) are added by a single dominant allele *R* (East Brasilian allele *r*, recessive), which is very tightly linked to the *y* allele for the yellow band. The variant of this pattern found in the Manaus race, in which the red marks appear only on the forewing (plate 1*k*), is produced by a single dominant allele (*D*); although there is no direct evidence on the point, it is likely on the evidence of natural hybrids that this is an allele of *R*, or is at least linked very closely to *y* (East Brasilian allele provisionally *d*, recessive).

Although there is less information on this point than in *H. melpomene*, as a result of fading, the

conversion of East Brasilian red to orange (plate 4*e*) appears in some crosses to be produced by a single locus *Or* (East Brasilian allele *Or*, dominant).

Besides these major loci converting the mimetic patterns, we have found several loci of comparatively minor effect, some of them being identified only tentatively.

Bf (East Brasilian allele *Bf*, dominant). The extreme tip of the hindwing bar, which points to the posterior in East Brasil, is turned forward toward the hindwing margin by the recessive allele, *bf*, which is found not only in Panamá, where it has this effect, but in Trinidad also, where it is without visible effect as the yellow bar is completely absent (plate 4*d*). (It is possible that a yellow barred race of *erato* still exists, adjacent to the Trinidad/Venezuelan race, somewhere in Venezuelan Guyana: the Holzinger collection in Wien contains a specimen of *melpomene* from near El Dorado in this region, which shows the heterozygous shadow of the yellow bar.)

Fb (East Brasilian allele *fb*, recessive). This has an effect similar to *bf*, but the mark that is added by the *Fb* allele, found only in East Ecuador, is of a different shape. The allele has this effect chiefly or only in *Yl^e–crcr* butterflies; we have not observed enough segregating broods to be certain that this is a single gene.

Cs (East Brasilian allele *Cs*, dominant). The red forewing spot, which appears close to the body of the underside (plate 4*f*, right) and which is generally regarded by workers on *Heliconius* as an orientation signal used during mating, is frequently absent in East Ecuadorian butterflies. It is removed by the recessive allele *cs*.

A polymorphism for red raylets (small red triangles on the hindwing) in East Brasil is possibly controlled by a single recessive gene (*rt*).

The eight races of *H. erato* that we have examined differ therefore at a total of a dozen major loci affecting the pattern (the exact number depends on what one calls 'major'). These loci are mostly unlinked, although in some cases we simply lack evidence on this point, with the exception of *R* (*D*?) and *y*, and *Yl*, *St* and *Sd*, the first set forming a very tightly linked group, the second a possibly somewhat looser one. Table 13 assigns the linkage groups and shows the genetic composition of the races.

DISCUSSION

5.1. *Features of muellerian mimicry*

At any one locality in the humid tropics, if one examines the local butterfly fauna, one finds that a number of distasteful and warningly coloured species share a common colour pattern; this is called a muellerian mimicry ring. Usually there are several such rings each with a very distinct pattern in any one area (figure 12). For example, Papageorgis (1974, 1975) noted that in mature and relatively undisturbed rain forest on the Río Llullapichis (a tributary of the Pachitea) in central Perú there were five such mimicry rings: (i) a 'transparent' ring, consisting of 26 species of ithomiid; (ii) a 'tiger' ring, consisting of 21 ithomiids, the danaid *Lycorea ceres*† and two 'tiger' *Heliconius*; (iii) an 'orange and yellow' ring which consists of *Heliconius erato*, *H. melpomene*, a form of *H. doris*, *H. elevatus*, *H. tales* and *H. aoede*; (iv) a 'blue and yellow' ring, consisting of *Heliconius sara*, *H. wallacei* and the other form of *H. doris*; and (v) an 'orange' or 'julian' ring, consisting of the heliconiines *Dryas iulia* and *Dione juno*. Members of all these rings from Trinidad, including *H. melpomene* and *H. erato*, have been shown to be distasteful to birds; the mimicry is effective at least to caged birds (Brower *et al.* 1963, 1971; Brower & Brower 1964). Within each of these rings, which also include some pericopid moths and various pierid and

† Authors' names for non-heliconians are given in appendix 3.

nymphalid butterflies (the last being probably batesian mimics), all the species bear a close resemblance to one another. In mature forests in Perú all five of these mimicry rings can be found flying together in the same places.

If one examines different geographical regions, not only may one find different mimicry rings present, but the details of the pattern of a particular mimicry ring may well be different. For example, in comparison with the situation in Perú, in Trinidad one finds also the 'transparent', 'tiger', 'blue and yellow' and 'julian' mimicry rings consisting to a large extent of the same species as in Perú but with somewhat different patterns and with some species added and some missing. The 'orange and yellow' ring is replaced by the 'postman' ring, consisting of the races of *H. melpomene* and *H. erato* with red forewing bands (figures 1 and 2).

Similarly, in the rain forest on the coast in southeastern Brasil, the same mimicry rings are present but again their pattern is modified: for example the 'tiger' pattern is noticeably altered by the addition of a white spot in the tip of the forewing, the 'postman' pattern has a yellow bar on the hindwing and the 'blue and yellow' pattern has a slight alteration in the shape of the yellow blotch.

Any theory of the evolution of muellerian mimicry has to explain both these phenomena: the presence of several mimicry rings in the same place, and the change in details of the colour pattern from area to area in the same ring.

This paper explains these aspects of mimicry in the light of our genetical observation and the Fisher–Nicholson theory of mimicry (Fisher 1927; Nicholson 1927; reviews by Turner 1977*b*, 1983*a*) in conjunction with current theories of palaeoclimatology; exploration of the problem in terms of ecological theories and field observations (such as the vertical stratification of the mimicry rings noted by Papageorgis (1974, 1975) and Benson (1981), the evolution of linkage, cladistics and punctuational evolution) is left for other studies (Brown 1981, 1982; Benson 1973, 1982; Turner 1977*b*, 1981, 1983*b*).

5.2. The evolution of muellerian mimicry

The foundation of the modern theory of muellerian mimicry was laid by Nicholson (1927) and by Fisher (1927, 1930), working on a suggestion by H. H. Turner (1924) (see Turner (1983*a*) for a historical analysis). They pointed out that predators make mistakes and that therefore an imperfect mimetic pattern can confer some protection. From this Fisher argued that warningly coloured species which already bore some resemblance to each other would undergo mutual convergence to become comimics.

Let us consider a pattern, the variation of which for the sake of illustration we shall represent on a linear scale. There is a species, say A, with a particular modal value on this scale, with some variation about this mode (figure 8). If the species is distasteful, then a number of patterns well outside the range actually found in the species would give some protection to an individual that happened to have any one of them, since an experienced predator would on occasion mistake them for the distasteful pattern that it had previously learned to avoid. The probability of such a mistake on the part of the predator will be less the further the pattern is from the mode. Thus not only will there be stabilizing selection for the modal pattern of the species, but there would be directional selection towards it in a hypothetical species with any such pattern outside the normal range.

Besides experienced predators, there will be those that have not learned to avoid the pattern or have temporarily 'forgotten' it; these last two categories of predator will be less numerous the
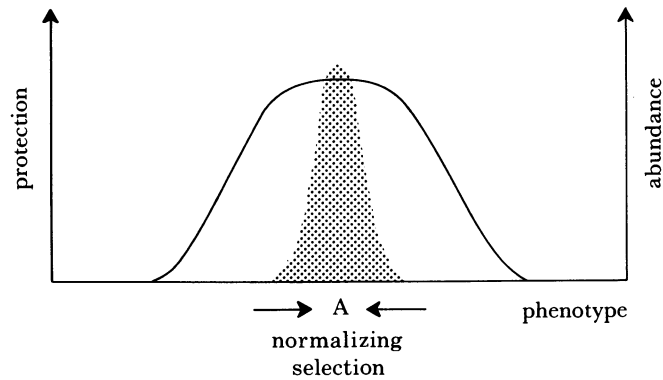
FIGURE 8. Frequency (abundance) distribution of a warningly coloured species (shaded) with the curve of protection (fitness) generated, both plotted against a linear measure of the phenotype (see text for details). The shape of the curve of protection is suggested by the experiments of Duncan & Sheppard (1965) and of Goodale & Sneddon (1977); its exact form is known to vary with the unpleasantness of the model.
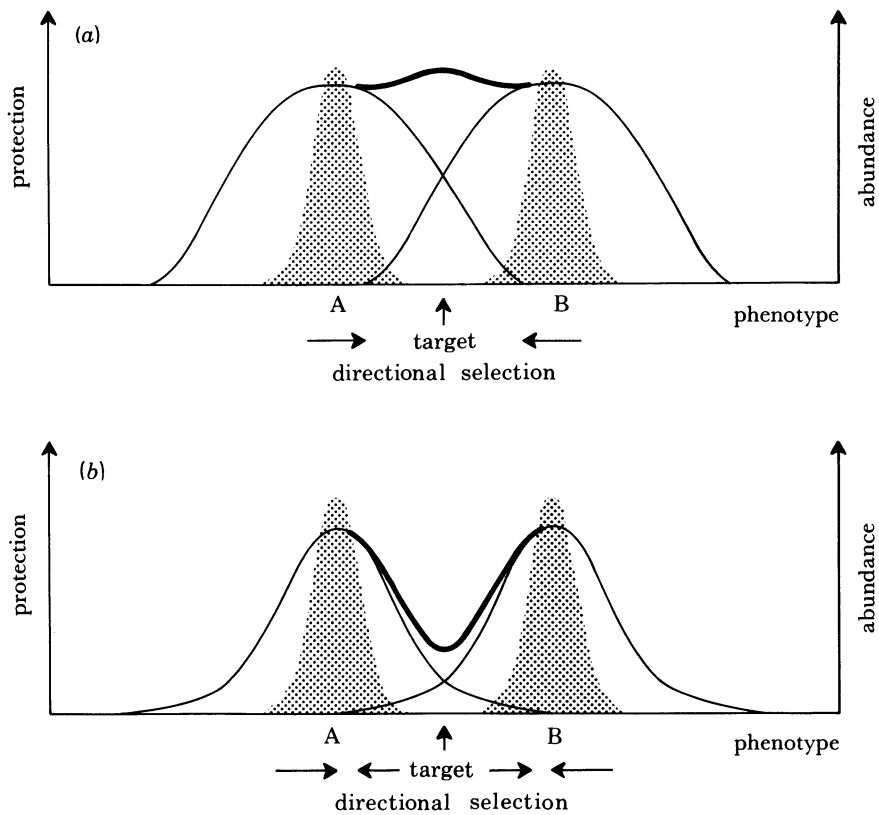
FIGURE 9. Gradual mutual convergence to a single 'target' phenotype of two warningly coloured species will occur when their curves of protection (thin lines) (assumed here to be equal) mutually overlap their phenotype distributions: any pattern occurring in the range of phenotypes between the two species is better protected (thick line) than it would be if only one of the species was present. The joint curve of potection (heavy line) might be convex (a) or concave (b) upward according to a variety of circumstances, but the target pattern will still be the same.

commoner or the more distasteful the protected prey is. By multiplying together the proportion of experienced predators and the probability of a pattern being mistaken for the protected one, we can obtain a distribution of the probability of escaping attack (figure 8).

If we now introduce a second species, say B, with a pattern not too dissimilar from the first, there will also be patterns on each side of it that have some probability of being mistaken for the main B pattern and avoided (figure 9). The probability of any pattern escaping attack is then some joint function of the two probability curves, which is greater than either single probability in the range where both are non-zero. If the curve for species A overlaps the pheno-type distribution of species B, this generates directional selection for B to converge towards A. That is to say that those individuals in the left tail of the phenotype distribution of B, which are more often mistaken for A, have greater viability than those in the right tail; i.e. the joint probability curve is now skewed to the left within the bounds of the phenotype distribution of species B. Obviously, if the situation is symmetrical as we have drawn it, then there will be equal selection pressure on both species to converge to a target pattern half way between the two. In a real situation, they may or may not respond to these equal and opposite selection pressures with an equal response, according to the genetic basis of variation in each species.

If the situation is asymmetrical, because one of the species is more numerous or more distasteful than the other, or for any other reason (e.g. if pattern B is more often mistaken for A than A is for B, or is less visible, or is easier to remember, or if in a seasonal climate one species has an earlier flying season than the other (see, for example, Rothschild 1971)), then the selection pressures will be unequal, and the less protected species is under greater directional pressure than the other (figure 10). In this case the final pattern is closer to the original pattern of the more protected species (or of the one with the earlier flying season), provided that both species have an equal ability to respond to selection. The better protected species could evolve the pattern of the other if this less protected species were totally unable to respond to the selection pressure and hence remained unchanged.

As the two patterns converge, so the survival value of the target pattern increases; finally, when the patterns of the species are identical, the survival of individuals in both is maximal, and the modal pattern, now common to both species, has the highest fitness of all. The two muellerian mimics may thus be said to generate their own peak of fitness, which has been manufactured by the fusion of the two lower peaks of fitness originally possessed by the two individual and differently patterned species.

If during the process of convergence a major mutant turns up in either species giving a pattern of greater survival value than its original (wild-type) allele, as assessed by the height of the joint probability curve, it will be at a selective advantage and will, other things being equal, replace its allele. Thus convergence can take place by selection both of the existing polygenic variation in the species and of mutations of large effect.

Consider the case in which the less protected species produces a mutant whose pattern is closer to that of the more protected species than it is to the pattern to which both species are converging (figure 10). The spread of this mutant in the population will increase the height of the peak of fitness around the better protected pattern, and hence change the final target pattern on which both converge to a point closer to that of the better protected species.

Hence the nature of the pattern that eventually becomes the common pattern of the mimicry ring is determined by four factors:

(i) the patterns possessed originally by the species;

(ii) the relative degree of protection afforded to each (other things being equal, the final pattern is closer to the better protected);

(iii) the store and architecture of variation in each species affecting the patterns (other things being equal, the final pattern is closer to the species with the lower ability to respond to selection);
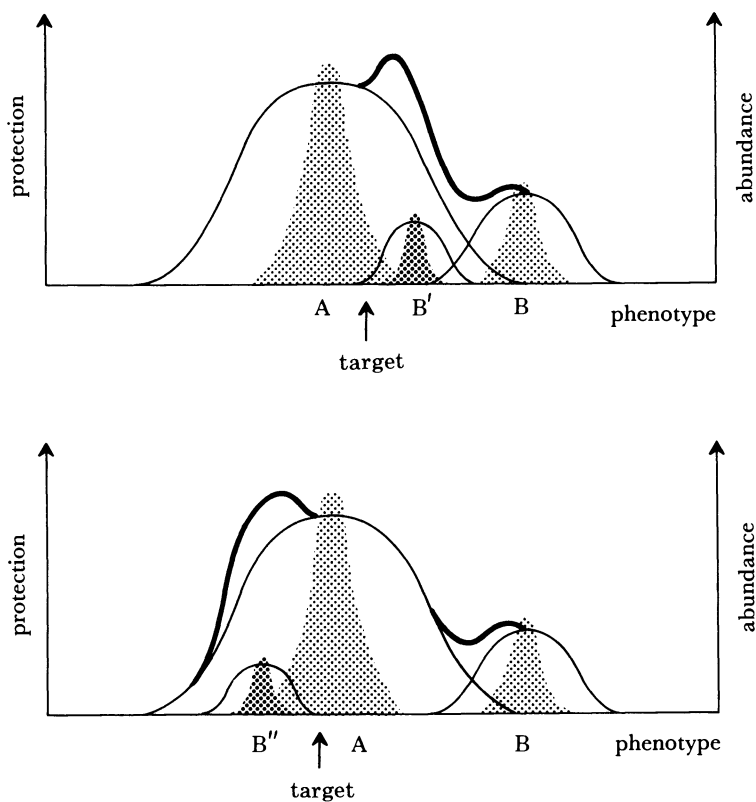
FIGURE 10. When one species is better protected than the other, the target pattern is closer to the better protected species (A); a major mutation (B') of the less protected (B) moves the target even closer to A, or at least further from B if the mutation 'overshoots' (B"). Symbols as in figure 9.

(iv) the unpredictable process of the establishment of major mutants.

This is the process of mutual convergence by small changes described by Dixey (1909 and earlier) and Fisher (1930). The model will work for real patterns (which have to be represented on a multi-dimensional, not a linear, scale) and for situations in which there are several species, which will tend to converge to some common pattern, whose nature is determined jointly by the four parameters just listed; this will produce a multi-species muellerian mimicry ring such as actually occurs in Nature. But it is now necessary to explain why one finds sympatric, distinct mimicry rings: from the argument so far developed, all warningly coloured species in one place should have the same pattern.

To explain this, we take a situation in which there are two species that the predator never confuses because their patterns are so different. The non-zero probability distribution generated by each species does not overlap the range of phenotypes of the other, and there is no selection for convergence to a common pattern. However, it is always possible that one of the species will produce a mutant which is a tolerable mimic of the other. Such a mutant will spread only if it is

fitter than its original (wild-type) allele, and this will be the case if its pattern lies in that part of the X-axis where the probability curve is higher than the modal probability for its own species (projection line in figure 11). This implies (i) that the two species cannot be equally protected (for then no such mutant could be at an advantage), (ii) that the mutation must be of the less protected species toward the better protected (no such mutation in the latter species can be at an advantage), and (iii) that, the better protected the more protected species is, the wider is the range of possible mutant patterns in the other species that will be at an advantage, and hence the greater the chance of some such mutant occurring.
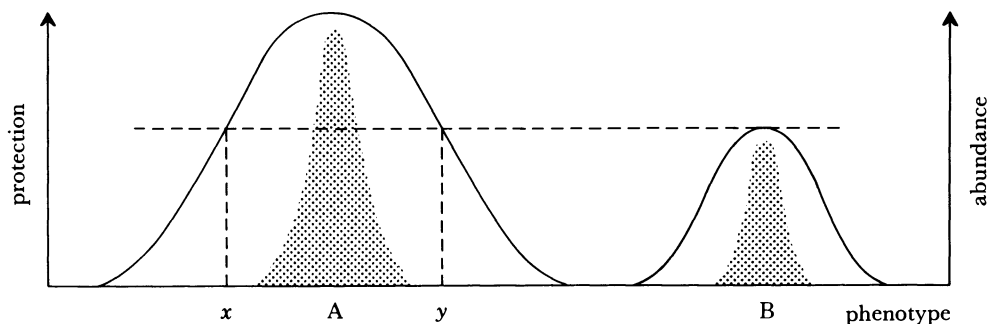
FIGURE 11. If the curves of protection of two warningly coloured species do not overlap each other's phenotype distribution in any way, then mutual convergence is impossible (even if the curves of protection overlap one another). But a major mutant of the less protected species (B) with a phenotype in the range xy will be at an advantage to the original pattern of B, and will increase in frequency. The phenotype of the mutation does not necessarily have to lie within the current range of variation of A, and need not be intermediate between A and B, provided only that it does not lie to the left of x or to the right of y.

Thus, even if two species have never been confused by predators, they may converge if the less protected can produce a suitable mutant. This is the process of convergence described by Marshall (1908) and Nicholson (1927), in which all the evolving is done by the less protected species.

However, the more dissimilar the two species, the more remote is this possibility. In the extreme case, it may require several mutations to produce an acceptable mimic, and the probability of these individually disadvantageous mutants establishing themselves simultaneously in the population is remote. The probability will of course be slightly increased if the several loci are tightly linked (as this is the situation in which new alleles at two loci do not establish themselves in a deterministic model unless their recombination fraction is below a critical value (see Bodmer & Felsenstein 1967)). Therefore several very distinct mimicry rings can coexist in the same habitat, provided that they are too distinct to be confused by predators and too distinct for all the species in the less protected rings to produce the appropriate convergent mutations in a reasonable period of evolutionary time.

Starting from a wide diversity of warningly coloured patterns not aggregated into mimicry rings, species with rather similar patterns will start to converge, producing several rather distinct mimicry rings, of which any one that is close to another ring (or any species of it) may tend to be captured, especially if the adjacent mimicry ring offers much better protection to its members; species outside these mimicry rings will tend to be drawn into the nearest, best protected ring, so that ultimately there are left a limited number of very distinct patterns. (A well established ring could of course lose one species to another ring without itself being absorbed, if that species happened to be the only one that produced a suitable mutation.)

2

It is in fact the case that the patterns exhibited by the different mimicry rings in the tropical American rain forests tend to be very distinct at any one place. Among the heliconids and ithomiids of South America the sympatric 'transparent', black, blue and yellow, 'postman' (black, red and yellow), 'tiger' and 'julian' (*Dryas iulia* and *H. aliphera*) patterns are very distinct (figure 12).
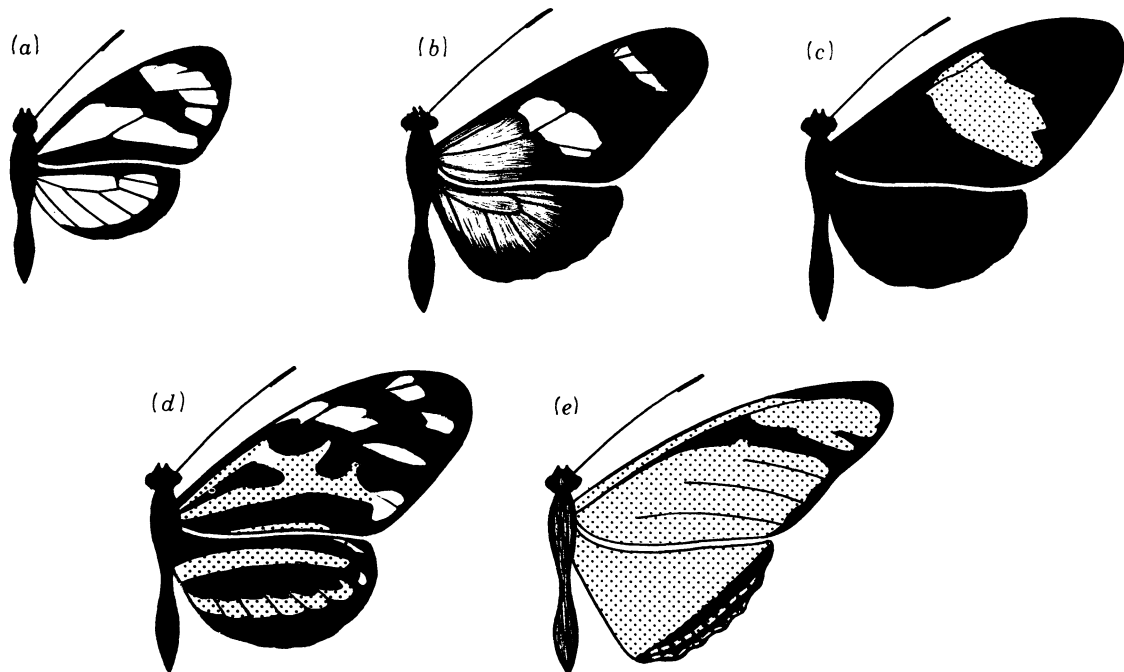
FIGURE 12. Distinctness of sympatric muellerian mimicry rings: representatives of the five South American rainforest rings as they appear in Trinidad are: (*a*) 'transparent' (*Ithomia pellucida*), (*b*) 'blue and yellow' (*Heliconius sara*), (*c*) 'postman' (*Heliconius melpomene*), (*d*) 'tiger' (*Heliconius ethilla*), (*e*) 'julian' (*Dryas iulia*). Colours are: 'transparent', black and transparent; 'blue and yellow', iridescent blue and yellow; 'postman', black and red; 'tiger', black, brown and yellow; 'julian', black and orange.

It is possible that two distinct rings, at least one of which is not numerous or well protected, which have been unable to converge, may be enabled to do so by the introduction into their habitat of a 'catalyst' species with a pattern intermediate between the two, provided that this is better protected than the less protected ring. All three patterns will then converge to some common target. A speculative example of this in *Heliconius* is described elsewhere (Turner 1976 *b*).

This general model will further explain how it is that, despite the strong stabilising selection on each mimicry ring, there can be variation in the modal pattern of each from area to area within tropical America. First, even within a mimicry ring there are differences in the modal patterns of each species, since complete identity seldom evolves. Consequently, any change in the relative abundance of the species as a result of ecological differences between areas will alter the optimum pattern and hence the target pattern. Secondly, changes in the relative abundance of two mimicry rings at the same places may allow previously disadvantageous major mutants in some species to become advantageous, so that a particular species can be captured from a temporarily less advantageous mimicry ring. During this process the modal phenotype of the capturing ring is under slight directional selection. (This, it is interesting to note, may be in a

direction somewhat *away* from the original pattern of the captured species, if the original major mutation 'overshoots' (figure 10). Thus the final pattern may *not* be intermediate between the two original patterns.) It might seem that the directional selection on the capturing ring is negligible, in view of the fact that the captured ring is not only less protected (*ex hypothesi*) but initially represented by a rare mutant. However, the initial rarity of the new mutant not only reduces directional selection on the capturing ring, but considerably reduces selection on any potential modifying genes in the captured species: while the new allele is still rare, the selection coefficients on unlinked modifiers will be negligible (O'Donald 1969). Once the new mutant becomes common, it will cause appreciable natural selection not only on modifying loci within its own population but on modifiers within the capturing ring, so that the patterns will undergo mutual convergence; this process will be subject to the constraints that we have already described, and other things being equal the captured pattern will evolve faster. However there will still in all probability be an appreciable change in the capturing ring.

The only way in which the captured species can improve the mimicry due to the new mutant while this is still rare is by the establishment of modifying genes that are closely linked to the mutant allele. This will produce what appears, on outcrossing, to be a single mutation giving a high degree of mimicry, but which is in reality a supergene. In this way a small supergene may evolve in a muellerian mimic, even in the absence of the balanced polymorphism under disruptive selection that is thought to cause the formation of supergenes in batesian mimics (Clarke & Sheppard 1960; Charlesworth & Charlesworth 1976). As suggested by Turner (1977*b*, 1979), some of the very tight linkages (*R*, *D* and *Y* in *erato*) and some of the apparent series of multiple alleles (the *D* locus in *melpomene*, the *Cr* locus in *erato*) may have arisen in this way.

Further, it is possible for the probability surfaces of two rings to overlap one another's phenotype distributions, but for only some of the elements of the pattern to have the genetic variance that will allow them to converge. There is some circumstantial evidence for this in the parallel geographical variation between members of the 'tiger' mimicry ring and the radiate *erato–melpomene* mimicry ring in parts of Amazonia. In flight, some of the 'tiger' patterns at Belém can easily be mistaken for the radiate pattern, especially at a distance. In the Guianas and south to the Amazonas all members of the radiate mimicry ring that are present (except for the curious polymorphic species *H. doris*) lose the rays on the hindwing, so presenting a pattern with red and yellow marks on the forewing and a black hindwing (no. 4 in figures 1 and 2). In this area almost all the 'tiger' species, regardless of family or genus, show a great increase in the amount of black on the hindwing, partly or completely losing the broad orange-brown bar that they have at Belém and through much of the Amazon basin (cf. figure 12*d*) (Moulton 1909; Fox 1956, 1960; Brown 1979, 1980). On the eastern slopes of the Andes in Ecuador, Emsley (1965*b*) noted that *melpomene* and *erato* had the radiate pattern at moderate altitudes, where 'tiger' butterflies were abundant, but in the higher forests, where 'tiger' species were absent and many of the butterflies had black and white patterns, *melpomene* and *erato* also had a mainly black and white pattern (East Ecuador races, no. 12 in figures 1 and 2; plate 1*d, h*; for further distribution details see Descimon & Mast de Maeght (1971)). Therefore it appears that the 'tiger' pattern and the radiate pattern are unable to converge except to a very limited extent because, although they can sometimes be confused, they lack the appropriate genetical variation. No close relative of *erato* has evolved an Amazonian 'tiger' pattern (Turner 1971*a*), although *H. hecalesia*, shown by its morphology to be a member of this group, has evolved a similar pattern that mimics some Central American and west Andean ithomiids

(Brown & Benson 1975 b); possibly *erato* lacks appropriate mutations. *H. melpomene* and *H. elevatus* (both radiate species) do have very close relatives which are 'tigers'; the reason that they themselves lack the ability to converge to the 'tiger' pattern is probably that this would now require several simultaneous major mutations. It appears that the genetical variation for convergence of black hindwings is available in both mimicry rings on a simple genetic basis (single mutation both in *melpomene* and *erato*), but not for convergence of the rest of the pattern.

A further example of partial convergence is found in Ecuador west of the Andes, where several species in distinct mimicry rings all have a general blue colour and a prominent white border to the hindwing. It is particularly noteworthy that, apart from the blue and the white hindwing border, the patterns are distinct (a red forewing mark in the 'postman' ring, a white one in the 'blue and white' ring and a yellow one in *H. sara*), and that three of the species (*erato, melpomene* and *sara*) have a white border nowhere else in their whole range in South America (no. 11 in figures 1 and 2).†

5.3. *The effect of a batesian mimic*

Batesian mimics can affect the evolution of muellerian mimicry in three ways. The presence of a palatable batesian mimic in a muellerian mimicry ring, as Fisher (1930) pointed out, is likely to reduce the advantage of the warning pattern and cause it to evolve away from the pattern of the mimic (see also Lea & Turner 1972). Various authors, for example Nur (1970) and Brower & Brower (1972), have asked how batesian mimicry is then possible, as one might think that the model species would evolve away from the potential mimic as quickly as the mimic converges towards it. In fact there is a simple explanation. Provided that the batesian mimic is not so common that the warning pattern confers *no* protection on the model, mutants of large effect in the model species, even those that change the pattern away from that of the mimic, will be at a disadvantage when rare because their bearers will not often be recognized as protected and will stand a greater chance of being sampled by predators. Consequently only very small changes in the pattern of the model will be advantageous. The same restriction does not apply to the palatable mimic, as its protection comes only from resemblance to the model, and not from being recognized as unpalatable in its own right. Consequently, a change of pattern of any magnitude that improves its resemblance to the model can be favoured. Thus the mimic can approach the model by large mutational steps, whereas the model can only move away from the mimic by small ones. (See also Turner 1984 b, c.)

The first effect of batesian mimics on a muellerian ring is therefore to cause slow evolution of the whole ring, while maintaining batesian resemblance. As the batesian mimics will differ in abundance and species composition from area to area, this will cause geographical divergence within mimicry rings.

If a warningly coloured species A, converging gradually toward a muellerian comimic B (as we have described above), has in addition a batesian mimic C, the resulting depression in the adaptive value of the pattern of A will cause it to converge more quickly towards the comimic B, and will reduce the rate of convergence of B on A. Thus the second effect of batesian mimics is to alter the target pattern during periods of convergence.

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† Throughout this paper statements about the geographical distribution and variation of heliconiines that are not supported by a citation from the literature are taken from field observations made severally and jointly by the authors, and from an extensive compilation from the major museums of Europe and of North and South America (by K. S. B. and J. R. G. T.). Maps of almost all species can be found in Brown (1977, 1979, 1982), Brown & Benson (1975 a, b, 1977), Brown & Holzinger (1973), Brown *et al.* (1974) and Turner (1971 a).

Finally, but only when there are several mimicry rings flying together, the depression in the selective value of a ring caused by batesian mimics will enhance the chances that new major mutants in the model species that produce a resemblance to another mimicry ring will be advantageous. Thus batesian mimics may promote the 'capture' of some or all of their model species by an alternative muellerian mimicry ring; only in this way can a model escape, at least temporarily, from its batesian mimic.
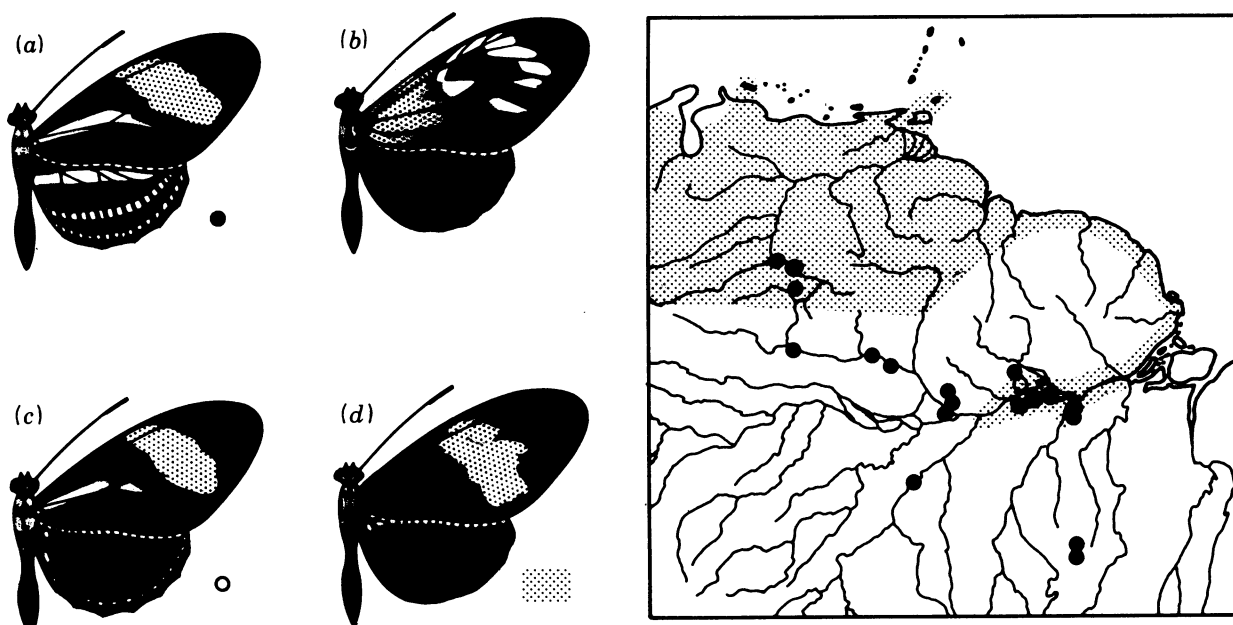
FIGURE 13. *Heliconius hermathena* (*a*) does not mimic *Heliconius erato* (*b*), *Heliconius melpomene* and other members of their mimicry ring through most of its distribution in Amazonia (filled circles on map) even though they fly together. At Faro (open circle on map) the pattern of the 'postman' ring (*d*) is already rather similar to *hermathena*, which in that area loses its yellow bars to become a 'postman' mimic (*c*). Map updated from Brown & Benson (1977), butterflies from Turner (1976*b*). Shading on butterflies: solid black, black; stippled, red; unshaded, yellow.

5.4. *Distributional evidence*

There is distributional evidence that a species can change allegiance from one mimicry ring to another, particularly if its own mimicry ring is reduced in size. *Heliconius erato* and *H. melpomene* belong to the same mimicry rings throughout their joint range in Latin America (figures 1, 2). In the Valle del Cauca in Colombia only *erato* is present. In the abundant fauna of warningly coloured butterflies in that area there is a mimicry ring consisting of a common blue *Altinote* (an acraeid) and *Heliconius cydno* (dark blue with a yellow hindwing bar); the local form of *H. sara*, blue with a single yellow forewing band (cf. figure 12), is an excellent mimic of these when in flight. *H. erato* in this area joins this mimicry ring by becoming blue, losing all its red marks, and retaining the hindwing yellow bar (figure 2, no. 1). The two control observations can be made in Middle America. First, in Panamá and Costa Rica, *erato* and *cydno* are sympatric but do not mimic one another, both belonging to apparently strong mimicry rings, *cydno* mimicking *H. sapho* (figure 18) and *erato* mimicking *melpomene* (figures 1, 2, no. 2). Second, in the northern part of its range in México, Guatemala and Honduras, and also in the extreme south of its range in the southern states of Brasil, and in Uruguay, Argentina and Paraguay, *erato* is again present without *melpomene*. In both these areas there are few other heliconids or other mimicry

rings and all are very unlike the black and red 'postman' pattern of *erato* (figure 2, nos. 2, 15). Despite the weakening of its own ring by the loss of *melpomene*, *erato* does not join any other mimicry ring because the other local warning patterns are too dissimilar for it to be able to do so.

A particularly beautiful example of changed allegiance, apparently resulting from a change in the relative size of two mimicry rings, is provided by the warningly coloured diurnal moth *Zygaena ephialtes* (Bullini *et al.* 1969; Sbordoni & Bullini 1971; Sbordoni *et al.* 1979). In northern Europe it closely resembles other members of its own genus; but in peninsular Italy, the warningly coloured ctenuchid moth *Amata phegea* (and some related species), which has apparently always been rather scarce in northern Europe and which has been extinct north of the Alps since the beginning of this century (Kirby 1898; Obraztsov 1958), is extremely common, being far more plentiful than the *Zygaena* species in that region. In peninsular Italy, *Z. ephialtes* (and also the related *Z. transalpina*) changes its colour, its black and white pattern and its behaviour to mimic the more abundant mimicry ring represented by *A. phegea*.†

An essential element of the model that we have developed is that patterns have to be rather similar before they can converge to become members of the same mimicry ring. Again, there is distributional evidence supporting this conclusion. The very local species *Heliconius hermathena* (figure 13) occurs in scattered localities in the Amazon basin, where its pattern of yellow bars with a broad red forewing band is quite unlike any of the local mimicry rings, including those of *erato* and *melpomene*, with which it is sympatric at least in the ecotone between its more open habitat and the forest habitat of the other two. Although it can readily be mistaken in the field for East Brasilian *melpomene* and *erato*, it is only occasionally sympatric with these at the extreme southern edge of its range (Brown & Benson 1977). Only an experienced taxonomist can detect differences between the various populations, even though they are clearly genetically isolated. It appears that this is an example of a pattern being so different from all the sympatric mimicry rings that it cannot converge to them by a single mutation. This view is confirmed by the population of *hermathena* at Faro on the Amazonas, one of the places where the species comes into contact with the black and red 'postman' mimicry ring of *melpomene* and *erato*, which is found in a restricted area along the river (figures 1, 2, no. 3). Here the yellow marks of *hermathena* are suppressed, so that it becomes an excellent comimic of *melpomene* and *erato*. The suppression of the yellow bars appears to result from a single mutation (Brown & Benson 1977), as could be predicted.

A similar case is presented by the widespread species *Heliconius charitonia* which has a pattern similar to *hermathena* but lacking the red bands (figure 14). Through almost its entire range in the U.S.A., the Antilles, Middle America and the Andes, this species keeps a non-mimetic pattern, varying only slightly in the width of the yellow bars (Brown & Comstock 1952; Comstock & Brown 1950) and showing no tendency to converge to any mimicry ring, all sympatric rings in North, South and Central America being considerably different. That it can converge to another similar enough pattern is shown on the Pacific slopes in Ecuador and northern Perú, where it mimics the local species *Heliconius atthis* and the ithomiid *Elzunia pavonii*, even to the extent of reducing the aspect ratio of its wings (figure 14). *H. charitonia* and *H. atthis* are known to fly together on the Río Tumbes in Perú (Poulton 1930; Lamas 1976).

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† It is only fair to point out that the original authors (Sbordoni *et al.* 1979) (and also Rothschild 1981) regard this case not as fitting into a general model of the evolution of muellerian mimicry but as representing a situation in between a batesian and a muellerian mimic (J. R. G. T.).

(The mimetic form appears to be partly sympatric with the non-mimetic one, and may be specifically distinct, under the name *Heliconius peruviana*; however, the relationship between *peruviana* and *charitonia* is extremely close.) *H. charitonia* might be expected to converge towards
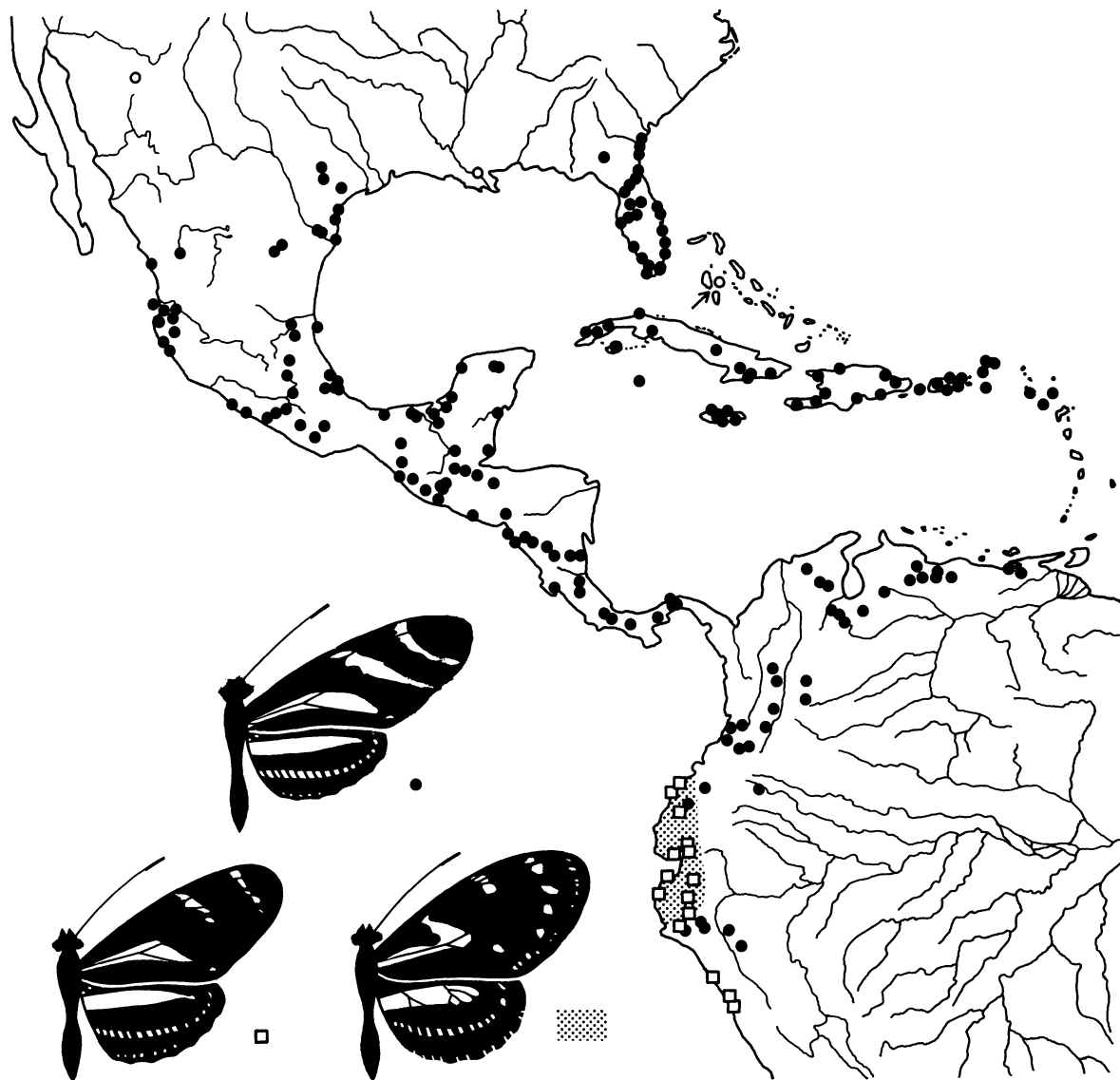
FIGURE 14. *Heliconius charitonia* is non-mimetic through most of its range (● collecting points) but in western Ecuador and northern Perú (□ collecting points) converges to mimicry of *Heliconius atthis* (illustrated) and *Elzunia pavonii* (not shown); the distribution of both these last two species is contained within the stippled area. *H. charitonia* collected at points marked ○ are probably strays or introductions of *charitonia* outside its breeding range. Butterflies are black and yellow with a little white.

the black and yellow ring comprising *H. pachinus*, *H. hewitsoni* and *H. sara* (figure 18*c*) in the Chiriquí region of Central America; we do not know why it does not: preliminary field observations suggest that *charitonia* and the other species may have separate habitats, *charitonia* flying more in the open and being commoner at higher altitudes.

H. melpomene and *erato* show an analogous failure to join another mimicry ring on the Río

Huallaga (Perú), where both have a yellow barred black and red 'postman' pattern (no. 2 in figures 1, 2) and yet occur in the valley with several radiate species, notably *H. elevatus* and *H. aoede* (Turner 1971a). The lack of convergence in this case is intriguing, as *melpomene* and *erato* have converged to the radiate pattern throughout the Amazon Basin. Similar failures to converge have been noted in other places on the periphery of the Amazon Basin (Brown & Mielke 1972).

5.5. *Polymorphism among muellerian mimics*

Pough *et al.* (1973) suggested that two species, both moderately distasteful to predators, which are muellerian mimics of one another might be batesian mimics of a third species if it is very much more distasteful than the other two. This raises the possibility that one or both of the less protected species may be polymorphic because of their batesian relationship to the third species.

Huheey (1976) has gone further in suggesting that whenever there is a difference in distastefulness the more palatable species is always a batesian mimic, in that its presence reduces the protection afforded to the more unpalatable one. However, if one examines his model one finds that the mimicry can never be muellerian, since even if the two species are equally distasteful the individual of one species gains no advantage from a resemblance to the other. Huheey's model also leads to the untenable conclusion that an unpalatable species is equally protected whether there be one or one million of them. The fallacy in this model is that Huheey compares the protection afforded to the more distasteful species in the presence of a less distasteful co-mimic with the protection that the more distasteful species would receive if all the individuals of the comimic were replaced by individuals of its own species. The proper comparison is between the protection afforded to the more unpalatable species (with constant population size) in the presence or absence of the comimic; the more unpalatable species is better protected if the comimic is present (Benson 1977; Sheppard & Turner 1977) (see also computer simulations in Turner (1984b, c)).

Therefore we reject the hypothesis that polymorphism in a distasteful species can be the result merely of its being rather less unpalatable than some of its comimics.

A polymorphism in a muellerian mimic could be maintained under the following circumstances. If two warningly coloured species (or mimicry rings) with different patterns have such ecological requirements that they tend not to fly together, then a third species with less restricted requirements that flies with both would be subject to selection for the possession of one pattern in one type of locality and the second pattern in the other locality. In the absence of migration the species would be monomorphic for the two forms in different places, but if it migrated to some extent between the two habitats it could be polymorphic (Brown & Benson 1974).

Extensive theoretical analysis of this kind of system (reviews by Hedrick *et al.* (1976) and by Christiansen & Feldman (1975)) shows that such spatial heterogeneity in natural selection tends under many circumstances to produce a balanced polymorphism or at least to retard the fixation of genes, especially when there is limited migration between localities. Temporal heterogeneity (fluctuations in selection) can also, under rather restricted conditions, produce a balanced polymorphism. Although no mathematical analysis has been made of a system appropriate to muellerian mimicry, that is with positive frequency dependence of natural selection, it is likely that a spatially heterogeneous environment, especially if it is temporally heterogeneous as well, would tend to maintain polymorphism (or at least impede fixation) in a muellerian mimic.

Brown & Benson (1974) have pointed out that this kind of spatial and temporal heterogeneity could account for the polymorphism in *Heliconius numata* and *Heliconius ethilla*, both members of the tiger mimicry ring. Both these species have forms that mimic different groups of mono-morphic ithomiid species, which form sub-rings within the tiger mimicry ring, differing consistently in the details of their pattern. For example, in Trinidad there are two forms of *H. ethilla*, the 'yellow' one mimicking the yellow tiger ithomiid *Tithorea harmonia*, and the dominant 'brown' one (Turner 1968*b*) mimicking the danaid *Lycorea ceres* and several similar ithomiids like *Mechanitis polymnia* (figures 12*d*, 17*b*). Brown & Benson (1974) and Bates (1862) have pointed out that the ithomiids involved in these rings tend to occur in concentrated 'pockets' in the mature forest, each consisting largely of one pattern. As these pockets appear and disappear over relatively short time spans, and as the *Heliconius* species are not restricted to the pockets, this subjects the *Heliconius* population to spatially and temporally fluctuating selection of a type that could be expected to maintain a polymorphism for the mimetic colour patterns.

Since all the species in the tiger mimicry complex have patterns that are in general rather similar, and therefore likely to be confused from time to time by predators, it is necessary to consider why the sub-rings have not converged to a single common pattern.

One does not expect that two mimicry rings, however similar in pattern, will converge if they occupy different areas, so that individual predators never encounter both. The situation with the ithomiid pockets is less extreme than this, since without movement of *Heliconius* between pockets the polymorphism would not be maintained. Furthermore, as the separation of the pockets is on a microgeographical scale, movement of both predators and ithomiids will ensure that there is some selection pressure for convergence of the patterns towards a common target. However, it is likely that, with temporal and spatial fluctuations ensuring changes of target pattern from time to time, the rate of convergence will be slow. Furthermore, the increased advantage of a warning pattern as it becomes more numerous is not simply pro-portional to the increase in numbers: once the pattern is so common that most predators are experienced then increasing the number of the warningly coloured species produces relatively little increase in protection. As the tiger mimicry ring is often particularly common, the selection pressure for convergence of the sub-rings may be further reduced. In addition, under reduced selection any counter-selection for other properties of the pattern will be relatively more effective in preventing convergence of the sub-rings.

5.6. *The evolution of mimicry in Heliconius melpomene and erato*

For most of the heliconiines that we have discussed in illustrating our model of the evolution of muellerian mimicry, we have no knowledge of the genetic basis for the evolutionary divergence of the patterns. However, we do have some information for many of the races of *melpomene* and *erato*. Thus although it is unlikely that we can ever discover the order in which various gene substitutions occurred or make a completely reliable reconstruction of the evolution of pheno-types within the species (cf. Turner 1981, 1983*b*), we can apply our evolutionary model to explain the general features of mimicry in these butterflies.

A general description of the evolution of *melpomene* and *erato* must explain how their races have diverged in the face of stabilizing selection on their patterns. Mere isolation by distance, or even severance of gene flow, does not in itself explain this divergence, as it cannot have the appropriate effect of altering stabilizing selection. Furthermore, the divergence cannot be

caused by pleiotropic effects of the genes for colour pattern, as the parallel phenotypic variation is shown by our genetical results not necessarily to be the result of substitution at homologous loci. Thus the broken yellow forewing band of the Belém races of the two species, versus the red band of the Trinidad races, results from quite different substitutions. In *melpomene* the difference is due to three loci, two reducing the amount of red and increasing the amount of yellow, and a third breaking the yellow only into separate patches. In *erato* this is achieved by one locus converting the red colour to yellow, and a second which breaks up both red and yellow bands into the spots that mimic the pattern in *melpomene*. Only by assuming that the pattern itself, regardless of its genetic control, affects the physiology of the butterfly, might we explain parallel divergence of pattern other than by selection for mimicry.

Application of the model suggests that the cause of divergence has been fluctuation in the relative abundance (including absence) of mimicry rings and of the relative species compositions of the individual rings in different areas. Very wide variations in species composition, adequate to account for the spectacular divergence observed in these butterflies, are particularly likely to occur if the species have been confined from time to time in isolated refuges. These refuges will behave like oceanic islands, losing and gaining species by a colonization–extinction cycle, and hence diverging from one another in the composition of their faunas (Mayr 1965; MacArthur & Wilson 1967; Turner 1977a, 1982). Such divergence is likely to have a much more marked effect on the evolution of mimetic patterns than are faunal fluctuations in a continuous forest, as the latter are 'temporary' (of the order of hundreds or even tens of years) whereas the extinction of a species or the evolution of a new endemic in a refuge is permanent until the refuge expands again, lasting, as we know from the absolute dating of the most recent cycle (van Geel & van der Hammen 1973), thousands of years.

There is ample independent evidence that such refuges have existed in the past, both in the Amazon Basin and in the peripheral areas, from the present distributions of several groups of rainforest organisms (Haffer 1969; Müller 1973; Prance 1973; Vanzolini & Williams 1970); Haffer (1974) gives extensive details of this evidence for birds, with a detailed study of jacamars and toucans. The races of *melpomene* and *erato* agree well in their distribution with the postulated refuges (Brown et al. 1974; Brown 1976b), particularly those for Amazonian birds (Haffer 1969). There is also ample palaeontological evidence for fluctuations of climate within the American tropics; in the mountains temperature and rainfall have fluctuated extensively during the Quaternary (Vuilleumier 1971; van Geel & van der Hammen 1973), and in the lowlands the grasslands have expanded and contracted at the expense of the forests; the dating available is fully consistent with the idea that the periods when the lowland forest was reduced in area were the same as the cold dry periods observed at higher altitudes (van der Hammen 1974; Flenley 1979). A computer simulation of world climate for the year 18000 B.P. (Gates 1976) shows a depression of around 4 °C in the Amazon Basin and a global decrease in rainfall (the simulation was designed to investigate sea surface temperature and global climate, the authors expressing no interest in proving the existence of refuges) (Climap project members 1976). (For extensive reviews see Prance (1982).) Thus the forest habitats of *Heliconius*, now more-or-less continuous in Amazonia, have in the past been split into more-or-less isolated forest refuges by Quaternary climatic fluctuations. That quite small areas of isolated rainforest can support permanent populations of *Heliconius* is shown by the presence of a thriving population of *erato* in the rainforest occupying the top of the Serra Negra (in northeastern Brasil, about 250 km west of Recife); this forest is only about 20 ha in extent, and is surrounded for

hundreds of kilometres by a dry deciduous thorn scrub unsuitable for permanent colonization by *H. erato*.

Further evidence comes from the presence of narrow belts of polymorphism at the junctions of different races, which have all the appearance of zones of secondary hybridization (cf. Benson 1973, 1982; Turner 1982). Turner (1971 *b*) has shown that the patterns appearing in the polymorphic belt of *melpomene* in the Guianas are those expected from the segregation of the genes known to differentiate the abutting races; the more extensive genetic investigation of the Belém race (one of the races involved) reported here does not alter this conclusion. The known differences between the equivalent races of *H. erato* reported above will also fully explain the varieties of this species collected in the same zone. Away from the zone of contact the amount of introgression seems to drop rapidly. Much less is known about the hybrid populations to the southeast of Belém, located somewhere near São Luis in the state of Maranhão. The known segregating forms can again be explained from what is known of the genetics of the hybridizing races (those from Belém and East Brasil), including what at first sight appears to be a curious systematic anomaly, a butterfly closely similar to the Trinidad/Venezuelan races with a wide red forewing band and no other markings. In *melpomene* we have evidence of introgression from this hybrid zone as far west as Belém, where we have shown the presence in a population of the Belém race of the East Brasilian gene *yb* (§3.5*a*) (and maybe of *Or*, though this may in fact be introduced from red populations on the Ilha do Marajó to the north). One of us (P.M.S.) captured a specimen with narrow red bands (TY phenotype, figure 5), possibly attributable to the East Brasilian allele *B*, at Belém in 1971.

If races have formed in refuges and spread out with the expanding forest, one would expect the zone of contact to be often at some ecological barrier, which impeded the spread of the race that first reached it. At Manaus the Guianian race of *erato* (figure 2, no. 4) is found on the north bank of the Rio Negro and radiate populations on the south bank. Brown & Mielke (1972) describe a similar separation in Mato Grosso of the Amazonian and extra-Amazonian races of *H. erato*, which are kept apart by a strip of open sandy field along the crest of the Serra dos Parecis. (Intermittent migration across the barrier will not produce introgression, for reasons explained elsewhere (Turner 1976*b*).)

In the Guianas, the position of the hybrid zone, at first sight arbitrary because it is not at a river or mountain range, again appears to result from an ecological barrier. The junction between the Amazonian and extra-Amazonian races of *melpomene* and *erato* runs east–west across Suriname about 40–60 km from the coast; the major rivers flow north and cut right across this line. The line itself appears to run along the white sand region, an area of variable width where the soil is almost pure glassmaker's sand. To the north of this are various clay and sand coastal soils, to the south the red forest earth of Amazonia. One of us (J.R.G.T.) in 1964 was unable to find *Heliconius* in the white sand areas, although they support a forest of impressive appearance, and also various types of second growth and scrub in which one would expect to see these butterflies. Janzen (1974) has reported that white sand forests are poor in nutrients and have small insect faunas (also Anderson 1981). Populations of *erato* and *melpomene* to the north of the white sand are predominantly of the extra-Amazonian type, sometimes with low frequencies of Amazonian genes, like the population at Moengo described by Sheppard (1963); to the south of the white sand the picture is reversed, and the populations of *melpomene* are predominantly Amazonian with low frequencies of the extra-Amazonian genes (for details of localities see Turner (1971 *b*)). The same is true of *erato*: the extra-Amazonian pattern occurs at

Republiek on the northern edge of the white sand, the Amazonian pattern immediately to the south of the sand near Kwakoegron (J.R.G.T. fieldwork; Leiden museum). It is likely that the butterflies have difficulty in colonizing the white sand zone and that the white sands of the Amazon Basin will turn out to be important zoogeographic barriers for *Heliconius* and for many other invertebrates. (There are some white sand areas in western Mato Grosso and coastal Guyane that are rich in *Passiflora* and *Heliconius* as a result probably of an unusually large amount of surface water; these do not act as barriers.)

The location of these hybrid zones at ecological barriers which impede gene flow strongly supports the theory that the races have expanded from previously restricted areas, almost halting their expansion on meeting an ecological barrier. To test this hypothesis, it will be important to see whether other hybrid zones occur near ecological barriers.

If faunal changes within forest 'islands' have been the driving force in causing the divergence of mimetic patterns, then those muellerian mimics that have not been confined to these refuges should not show such divergence. The members of the 'julian' mimicry ring (chiefly *Dryas iulia*, *Dione juno* and *Heliconius aliphera*) fly over the canopy of the rain forest and also extensively in open second growth and scrub habitats (Papageorgis 1974, 1975). Thus, unlike the forest *Heliconius*, which are our main subject, which fly beneath the canopy and tend not to come out into the open, and also unlike *H. hermathena*, which is confined to open vegetation within the rain forest, the members of the 'julian' ring probably have a more or less continuous distribution no matter what is the relative distribution of forest and other vegetation (Turner 1976 b). Thus they were probably not confined to refuges during Quaternary dry spells, and as a result show virtually no differentiation of their colour pattern on the tropical American mainland, *D. iulia* for instance being divisible into only two recognizable races, one occupying South America east of the Andes and the other Middle America and the Pacific Andean slopes (Clench 1975). The control observation is provided by *D. iulia* in the Antilles, the Bahamas and the Florida Keys: where its populations are split into real islands, *iulia* produces a large number of recognizable subspecies, one for each island or island group (Clench 1975).

Therefore we propose (cf. Turner 1965) that the spectacular race formation in *melpomene* and *erato* is the result of faunal changes within Quaternary forest refuges, acting on the mimetic patterns in accord with our model for the evolution of muellerian mimicry.

Our genetical results are fully consistent with this hypothesis: our model predicts that if the races have been formed because the populations of *melpomene* and *erato* changed from one well differentiated mimicry ring to another, rather than merely converging slowly to some other pattern, then most of the differences between races will be produced by genes of major effect, which is exactly what we find. A successful mutation of this sort is not of course expected to produce a perfect mimetic pattern at its first appearance: all that is needed is a mutation producing a good enough resemblance to the other ring to be at an advantage to its original (wild-type) allele. Accordingly, we find that the effects of the major genes are altered by polygenic modifiers which improve the mimetic resemblance: this is seen for example in *H. melpomene* in the modifiers that cause narrowing of the rays in the Belém race (plate 3k) and the reduction of red in Bolívia, and by the allele *Ybs* in *erato*, which removes the last traces of the yellow marks already largely suppressed by two other loci.

As the genetic differences between some races are due to approximately ten substitutions, and of more similar races to two or three, it may be that at least two cycles of isolation and expansion have gone into race formation in these species (Turner 1976 b).

If the present well differentiated races of *melpomene* and *erato* have indeed each spread out from a single centre, then as far as major colour pattern genes are concerned all populations of any one subspecies should be genetically identical. We have not been able to investigate this problem extensively, but our crosses have indeed failed to show any major genetic differences between *H. melpomene melpomene* in Venezuela and Trinidad nor between the Central American form of *H. erato* in Panamá and México. This does not of course in itself prove that the centre of evolution was a physically recognizable forest refuge.

It is important to note that the model that we are proposing differs from the conventional model of allopatric race formation as it appears in some text books. It is conventionally held that the severance of gene flow *permits* the divergence of allopatric populations under different local selection pressures. In our butterflies we are proposing that severance of gene flow is comparatively unimportant, and in any case ineffective by itself, and that the ecological differentiation resulting from the restriction of 'fauna flow' actually produces the different local selection and hence *drives* the process of divergence.

Differences in the ecological circumstances within 'island' forest refuges, generated by gain and loss of species, are likely to affect not only muellerian mimicry, but many features of the environment to which organisms are adapting, such as food plants, parasites and competitors. Hence there will be alterations in the genome involving many loci. Such changes might well be reflected in protein differences between the races. That this view is probably right is shown by the work of Spassky *et al.* (1971), who found considerable divergence at allozyme loci between races within the *Drosophila willistoni* group, which must have been isolated in the very same refuges as *Heliconius*. This divergence was not shown between localities within the same race, thus paralleling the racial differentiation in *Heliconius* (see also Turner *et al.* 1979).

It is possible to set up alternative historical schemes for the formation of *Heliconius* races, in which the existence of refuges is unimportant. Such a scheme, in which races appear para-patrically because of adaptation of their patterns to the background, and are prevented from speciating by the effects of environmental grain in breaking up coadapted gene complexes, has been explored elsewhere (Benson 1982). It may be possible to reconcile such parapatric models with the allopatric scheme presented here (Turner 1982, 1983*b*).

5.7. *Putative ancestral pattern*

In interpreting the present mimicry it would help if one knew the primitive pattern from which it arose. It is unfortunately impossible to know this with certainty in the absence of a fossil record. But there are three lines of evidence that can be used independently to guess at the primitive pattern in both species.

First, if the original pattern was like those now found in the Amazon Basin, then one must assume that the similarity between the disjunct extra-Amazonian patterns to the north *and* the south of the Basin is due either to independent origin and hence considerable convergence, or to migration over many thousands of miles round the western rim of the Amazon Basin. Independent origin is in fact rather unlikely as particularly in *melpomene* we have shown that apparently identical alleles produce the similar patterns to the north and the south. Therefore it is likely that the ancestral pattern was similar to the present extra-Amazonian races, with a red band on the forewing and a yellow bar on the hindwing, and that the Amazonian patterns are secondarily derived.

Secondly, within any group of species undergoing evolution of muellerian mimicry the

patterns least altered in the course of evolution will be likely to be those not associated with any mimicry ring. Both *melpomene* and *erato* have close relatives that are non-mimetic (figure 15). These relatives for *erato* are the non-mimetic races of *H. charitonia* and *H. hermathena*. Both have a pattern of longitudinal yellow bars on both wings; *hermathena* has in addition a red forewing
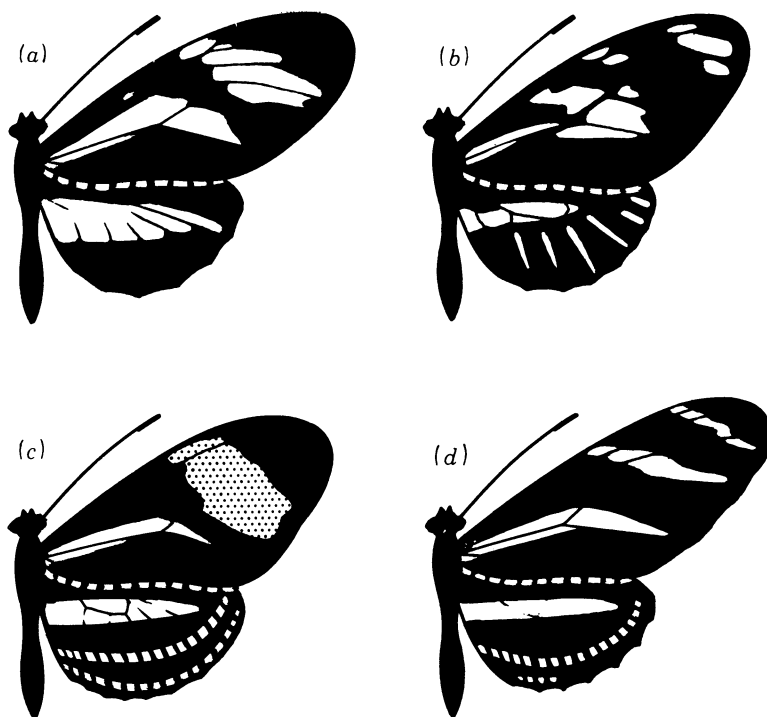
FIGURE 15. Non-mimetic relatives of *Heliconius melpomene* (top) and *Heliconius erato* (bottom): left to right (*a*) *Heliconius nattereri*, male (female shown in figure 17); (*b*) *Heliconius luciana* (yellow subspecies); (*c*) *Heliconius hermathena* (see also figure 13) and (*d*) *Heliconius charitonia* (see also figure 14). Shading: solid black, black; stippled, red; white, yellow.

band. For *melpomene* the relatives are the male of *H. nattereri* (Brown 1972), which has a pattern of longitudinal yellow bars on both wings, rather wider than in *charitonia*, and a form of *H. luciana* that also has a pattern of yellow longitudinal lines, plus some yellow cross bars, and in some individuals yellow hindwing rays (Lichy 1970). Thus, for both species, the primitive pattern appears to have been a black butterfly with longitudinal yellow bars or lines on the fore- and hindwings. Note that the argument is independent for the two species groups, as the four yellow barred species are allopatric and do not mimic one another.

Thirdly, a newly arisen advantageous mutant has a much greater chance of establishing itself in a finite population if it is dominant or at least partly expressed in the heterozygote (Crow & Kimura 1970). Once a mutant is established, it spreads at a much greater speed, initially, if it is dominant (James 1965). Thus, if different mutant alleles at one locus or mutations at various loci are roughly equivalent in their selective value in a changed environment, but vary in their expression when heterozygous, the one that spreads to become the new wild-type is very likely to be one that is dominant in its effect on fitness, or at least expressed to some degree in the heterozygotes (Merrill 1968). This is shown by the great rarity of newly established alleles for insecticide resistance and for industrial melanism which are recessive in effect (Kettlewell (1973), for industrial melanism). (This theory requires a large number of potential mutations,

a situation fully in accord with the findings of molecular genetics: a gene controlling a moderately sized protein such as a haemoglobin chain can in principle produce of the order of one thousand alleles, differing by a single residue in the protein, by mutations of single base pairs.) Therefore, during the evolution of muellerian mimicry as we have described it, most of the major mutants that have been established will have been those whose effect on the pattern was
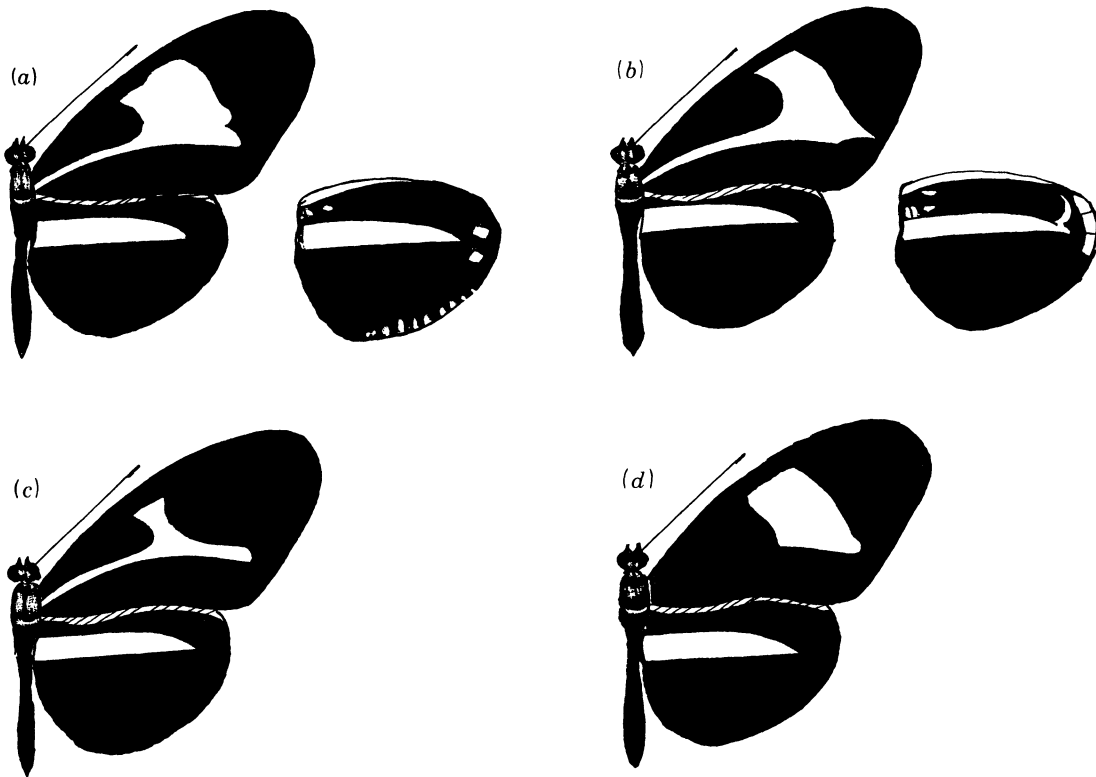
FIGURE 16. Putative ancestral patterns for *Heliconius melpomene* (*a*, *c*) and *Heliconius erato* (*b*, *d*), the alternatives resulting from intermediate dominance at some loci, and hence uncertainty about which allele is ancestral. Detached hindwings of the upper butterflies are undersides. Colours are black and yellow (or possibly white).

dominant or semidominant (although a small number of recessive alleles may have become established, see below). Therefore the bottom recessive phenotype for all loci affecting the pattern should be close to the ancestral one, although loci that now have dominant alleles fixed in all the races bred by us will not of course be detectable, and if the ancestral allele has been replaced independently in all races bred by us we shall not be able correctly to determine its nature.

In *melpomene* the fully recessive genotype is homozygous *db*; $N^B yb$; *st*; *c*; *or*; *f*; *wh*; *rr*, which gives a black pattern with longitudinal yellow bars on both forewings and hindwings (figure 16). A few variants in this are permissible: if N^N is taken as ancestral (the dominance at this locus being intermediate and to some extent reversible), the butterfly has a solid yellow band on the forewing, and if *S* and *T* are regarded as a single gene, then either *St* or *sT* could be ancestral, these respectively shortening and splitting the yellow band, or removing the Ecuador triangle; the *c* gene might tend to suppress the Ecuador triangle and enhance the Belém spot, or enhance the triangle and suppress the spot, as it is known to do both these things according to the genetic background. We have shown two of the various possible combinations, with and without the

yellow band, and with both the triangle and the Belém spot present; the reader's imagination can supply the remainder. There is some doubt about the effects of the allele wh: if it is a modifier of red colour then, like or, it can have no effect, and the markings are yellow; if it is merely linked to B then the butterfly might have white lines and bars instead.

In addition, in a few F_2 broods phenotypes have appeared that are found in neither parent race; they are most probably ancestral characters lost independently in the two races, suppressed in both by dominant genes. Some of these patterns confirm what is already constructed from the major recessive alleles: a yellow forewing line which appeared in the F_2 of West Ecuador × Trinidad (Emsley 1965b), fragments of a yellow hindwing bar which appeared in the F_2 of Suriname × Trinidad (Turner 1972), and the Ecuador triangle which appeared in the present Belém × East Brasil F_2 (§3.4j). Others, a series of small white marginal spots in the underside in the Suriname × Trinidad cross, are new, and have been added to the $N^N N^N$ butterfly in figure 16, as this genotype seemed to strengthen them. The independent suppression of the cell spot by at least two dominant alleles in Trinidad points in the same direction.

In $erato$ the fully recessive genotype is homozygous yl^T $stsd$; dry; ly^b; cr; bf; ro; wh; cs; ur; ybs; or, a black butterfly with longitudinal yellow stripes and a solid yellow forewing patch (figure 16). Again, permissible alternatives are Yl^t and Cr^p, which have much weakened yellow stripes, or cr–p (if these are two loci), which has a very broad one on the hindwing. The first genotype even produces (from the allele cr) a group of pale spots near the outer angle of the hindwing underside, closely similar to the pale spot in the upper drawing of $melpomene$.[†]

Since different loci seem to be responsible for the patterns in the two species, there is no reason to expect that the bottom recessives in both should have the same pattern; the similarity of the hypothetical ancestral patterns is therefore remarkable.

Furthermore, the three approaches all suggest that the ancestor was a black butterfly with a yellow bar on the hindwing and either a yellow or red band on the forewing. We incline to the belief that originally both species had something like the black and yellow pattern of the upper pair in figure 16 and were probably already comimics. At a later date, a red patch appeared on the forewing by substitution of dominant genes. Even later, further dominant mutants were established which produced the Amazonian patterns.

However, our hypothesis requires that $recessive$ alleles (as shown in our breeding experiments)

[†] As this reconstruction was made before all our crosses were set up, we have been able to use later genetical work to verify it. In both $melpomene$ and $erato$ all but four of the previously unknown alleles that are peculiar to the East Ecuadorian races and that give the forewing band its characteristic shape (that is S, Rr, Yl^e, St and Ro) are dominant, confirming our picture of the ancestral pattern and the notion that the next pattern to evolve had a simple, entire red band, the double Ecuadorian band being a later variant of this. The exceptions are wh (white) in both species, t (triangle) in $melpomene$, and ur (splitting on upperside) in $erato$. The t allele is closely linked to the dominant S, and may have been substituted in this way, or may even be a pleiotropic effect of S. On the other hand, t might be the ancestral allele, lost in all the other races; in this case it widens still further the yellow bar along the cubital vein in the reconstructed ancestral pattern, making it even more like $H. nattereri$. There seems to be no way of escaping the conclusion that the presence of ur in East Ecuadorian $erato$ represents substitution of a recessive allele, but if for the sake of principle we assert that ur is ancestral, this does not alter the ancestral pattern. The fact that white is recessive in both species suggests that the ancestral patterns were white rather than yellow. However, this conclusion does not follow inevitably: in $melpomene$ white either is a modifier of red colouring or is linked to the allele producing the red band; in $erato$ there is some association of white and Yl, which we have not been able to analyse fully. Further, in hybrids between Belém $melpomene$ and the blue and white $Heliconius cydno$ from Costa Rica (figure 18), we find that white is dominant to yellow (radiate is also dominant, as usual). (L. E. Gilbert and P. Brakefield have also obtained this hybrid, and confirm our observations.) Thus there is some genuine doubt about whether white or yellow is ancestral (indeed the ancestral species, like many extant ones, may have possessed both colours in different times and places), but as there is a majority of points in favour of yellow, we have retained this for the sake of argument (see also Brown 1972).

be later established, to convert the red extra-Amazonian forewing band to the yellow band of the Amazonian races. Now an advantageous recessive allele considerably increases its chances of spreading if its locus happens to be linked to one at which a dominant allele is already spreading. The effect is analogous to the hitch-hiking of a neutral allele linked to an allele that is spreading (Maynard Smith & Haigh 1974). Therefore, it is remarkable that in *both* species the recessives that according to our hypothesis are to be substituted are linked to dominants that should have been spreading at the same time (b with D^R in *melpomene*, y with R in *erato*).† ‡

It has been pointed out elsewhere (Turner 1978) that the difference in expression between males and females of the yb gene (in a Belém background) and of the N^N gene (in a Trinidad background) of *melpomene* (§§3.2g, 3.5a) is also compatible with the above evolutionary history: if sexual selection resists the spread of new patterns in males, then, where an allele is more strongly expressed in females, this is the substituted (non-ancestral) allele. This makes Yb (suppression of yellow bar) and N^N (increased yellow band) the substituted alleles, and yb and N^B ancestral, as is predicted by our reconstruction of the evolution of the patterns.§ This is also the case for the enhanced supression of the yellow bar in females in one of the *erato* crosses (§4.10g) and the enhancement of the broken band in females in *melpomene* (§3.4i), which argue, as does the rest of the reconstruction, that yellow bars and fused bands are the ancestral condition. The only exceptions are the intermittent increased frequency of orange in female *melpomene*, which may however be merely the result of a sex-linked gene (§§3.2i, 3.2h), and the enhanced expression of a fragment of the triangle mark in females of the East Brasilian crosses (§3.4j).

It is difficult to derive the 'tiger' pattern from the radiate or the yellow barred black and red 'postman' pattern, and yet several very close relatives of *melpomene*, including *H. numata*, which can be hybridized with it to produce a viable F_1 with a normal sex ratio, have this pattern. We suggest that this 'tiger' species diverged before *melpomene* gained its red forewing band. The ease with which the yellow pattern could be converted to a 'tiger' is shown by *H. nattereri*, whose female 'tiger' pattern is produced by minor alterations of the non-mimetic black and yellow male (figure 17). Further, it would follow that orange parts of the 'tiger' pattern should then be dominant to yellow; this is true for *H. ethilla* (Turner 1968b). The black and white patterns of *H. luciana* and *H. antiochus*, mutually mimetic relatives of *melpomene* and *erato*, respectively, can be derived from the putative primitive pattern by conversion of yellow to white (figure 18) (indeed yellow forewing marks and even the yellow hindwing bar occur in some races of *antiochus*).

This hypothesis about the ancestral patterns is supported by the fact that it makes sense of

† Some of the other linkages in *erato* and *melpomene* may have similar explanations (see Turner 1977b). That the linkage is the prior condition, affecting the probability of particular alleles entering the population, but not itself being produced by selection (the latter situation has been suggested for batesian mimics (Clarke & Sheppard 1960)) is indicated by crosses of the West Ecuadorian races of both *melpomene* and *erato* (Emsley 1965b): the alleles producing the yellow hindwing bar (underside) and the white hindwing margin are linked in *both* species, suggesting that the linkage is ancient, transcending the separation of the two species. (We know nothing about the relation of the West Ecuadorian hindwing bars with the hindwing bars in the present broods.) (P. M. S., J. R. G. T.).

‡ An obvious alternative hypothesis, which involves the substitution of dominant genes *only*, but leaves the linkage of the yellow (or non-red) band and radiate genes unexplained, is that the Amazonian races are independently derived from the striped yellow ancestor, having retained the yellow outer marks of the forewing and added the radiate marks to the base of the wing, without ever having possessed red forewing bands (Turner 1981, 1983b) (J. R. G. T.).

§ This point was not noted until early 1977, over 2 years after our original reconstruction on the basis of dominance (late 1974), and in this way can be regarded as strictly independent confirmation of our hypothesis (J. R. G. T.).

two otherwise rather odd facts about the genetic control of the yellow marks. That Yb in *melpomene* removes not just the yellow bar on the hindwing and the yellow line on the forewing (plate 2*h*), but the yellow triangle (plate 3*j*) and the Belém spot (plate 2*k*), is easily seen as the action of a single gene if the line, triangle and spot were all originally parts of a single longitudinal stripe right along the centre of the forewing from base to margin. Similarly the removal of the yellow hindwing bar, much of the yellow forewing line and the tooth in the red forewing band by the Yl locus in *erato* appears as the action of a gene on a single pattern, for the tooth, although it is now in red, can be seen, particularly on the underside, to be the tip of this same longitudinal yellow stripe (plate 4*d*) (also §4.2*i*).
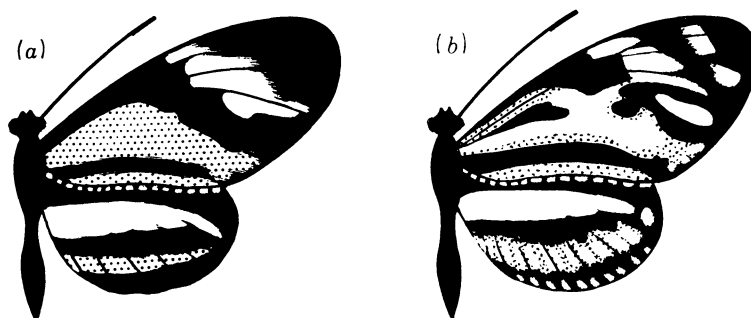
FIGURE 17. 'Tiger' patterns readily derived from the putative ancestral pattern of *Heliconius melpomene* (figure 16): (*a*) the female of *Heliconius nattereri* (left) (male is shown in figure 15*a*) and (*b*) *Heliconius ethilla* (yellow form). For the brown form of *ethilla* (dominant to yellow) see figure 11*d*. Shading: solid black, black; stippled, orange-brown; unshaded, yellow.

Both *melpomene* and *erato* have also produced what would once have been called atavistic patterns, reminiscent of related species but not found in any of the present races. These are the deep 'keyhole' pattern in *melpomene*, reminiscent of the related *H. ethilla* (figure 17) and other tiger *Heliconius*, the spread hindwing rays of *melpomene*, reminiscent of the hindwing lunules of *ethilla* and the brown hindwing C mark of *H. cydno* (plate 3*k*), the notched edge to the hindwing bar in *erato*, reminiscent of *H. telesiphe* (plate 4*k*), and the red or brown forewing rays of *erato* (plate 4*l*), reminiscent of related genera like *Dione*. These may indicate something of the patterns of even more remote common ancestors.

If our theory about the dynamics of the divergence of the patterns of the races of *melpomene* and *erato* in refuges is correct, then we should be able, at least for most races, to point to other distasteful butterflies that *melpomene* and *erato* are mimicking. If the mimicry evolved chiefly in refuges these would not have to be precisely sympatric with the present races of *melpomene* and *erato*, but they should at least occur in the same general area. In some instances there might be no apparent comimic, as the species that caused the divergent evolution of *melpomene* and *erato* several thousand years ago might now be extinct, or have been captured by another mimicry ring. However, for most races, there should be some plausible comimics.

The divergence of the East Brasilian races from the putative ancestral pattern by the addition of the red forewing band is an event now so remote that the agent causing it may not be discoverable, although the now allopatric populations of typical *H. hermathena* (figures 13, 15) in the Amazon Basin, readily mistaken for East Brasilian *erato* when on the wing, may be relics of this stage of evolution, and the East Brasilian comimic *H. besckei* (illustrated in Turner (1971*a*)) is a possible candidate.

Taking, as we have suggested above, the East Brasilian pattern (plate 1 *c, g*) as the ancestral one for the subspeciation, we suggest that the other races have diverged as follows (pattern numbers refer to figures 1 and 2):

(i) The 'radiate' patterns of Amazonia (nos. 4–9) have apparently evolved in mimicry of the many *Heliconius* species in that area that exhibit only this pattern, and in lower grade mimicry of the 'tiger' patterns shown by many heliconiines and ithomiines.

(ii) The 'postman' pattern of Colombia, Venezuela and Trinidad, and Perú (no. 3) and the double banded version in Perú (no. 13), which has a red forewing band like the putative primitive pattern but lacks all the yellow bars and lines, has possibly converged, as was suggested by Dixey (1894, 1913), to various members of the pierine genus *Pereute* which are black with a brilliant red bar across the forewing, and which are widespread in Colombia and Venezuela, and which may be unpalatable as are other pierines (Marsh & Rothschild 1974), or more probably to various members of the acraeine genus *Altinote*, often abundant and almost certainly distasteful.

(iii) The 'postman' pattern of western Ecuador (no. 11), which has blue iridescence and a white hindwing margin, and retains the yellow bar on the underside of the hindwing, is probably partly convergent to the sympatric forms of *sara, cydno* and *sapho*, as suggested above, and possibly partly convergent to the similar red, white and black *Pereute* species that flies in this area.

(iv) The 'postman' patterns of Central America and of various parts of Colombia and Perú (no. 2) are a little puzzling, in that they resemble the presumably primitive East Brasilian pattern (no. 15), but lack the yellow forewing line. In the only one that has been bred (the Central American race of *erato*), we have shown that the yellow line is removed by the combined action of the alleles Yl^t and Cr; however, these alleles also remove the hindwing bar, which is now replaced by the allele p, which either is closely linked to, or is a part of, the Cr locus. This suggests that there has been strong selection simultaneously for the removal of the forewing yellow line and the retention of the hindwing yellow bar, but it is not clear what local species has produced this pressure.

Thus, although we are not in a position to assert that *particular* butterflies have caused the divergence of the *melpomene* and *erato* races from their presumed ancestral pattern, there are sufficient possible candidates for this role to strengthen our belief that our model for the evolution of the two *Heliconius* species is correct in general outline and approximately correct in its details.

5.8. *Convergent and divergent evolution*

If the races of *melpomene* and *erato* should in the future become full species, then one would find two species groups, each containing a great diversity of patterns, but each species having a matching comimic in the other group. Should the evolutionary processes (the divergence of races in refuges) that we have been discussing in *melpomene* and *erato* have also occurred in the more distant past, then at the present we should expect to find such matching pairs of species. This indeed appears to be so. For example *cydno* and *sapho* are reciprocal comimics, paralleling one another geographically in much the same way as do *melpomene* and *erato*. Further reciprocal comimics are *luciana* and *antiochus*, and *pachinus* and *hewitsoni*. In each case, the first named in each mimetic pair is a close relative of *melpomene*, and the second of *erato* (figure 18).

This pattern of evolution of course requires that the forest should have contracted more than once in Amazonia, as indeed it must have done, for it is known that South America underwent multiple glaciations in the Quaternary, the cooler areas showing traces of three or four

glaciations, and the lower tropical parts (Agulhas Negras in Brasil and Mérida in Venezuela) showing geomorphological traces of one or two (Vuilleumier 1971). Each glaciation on higher ground was probably accompanied by changes in the distribution of the lowland forests.
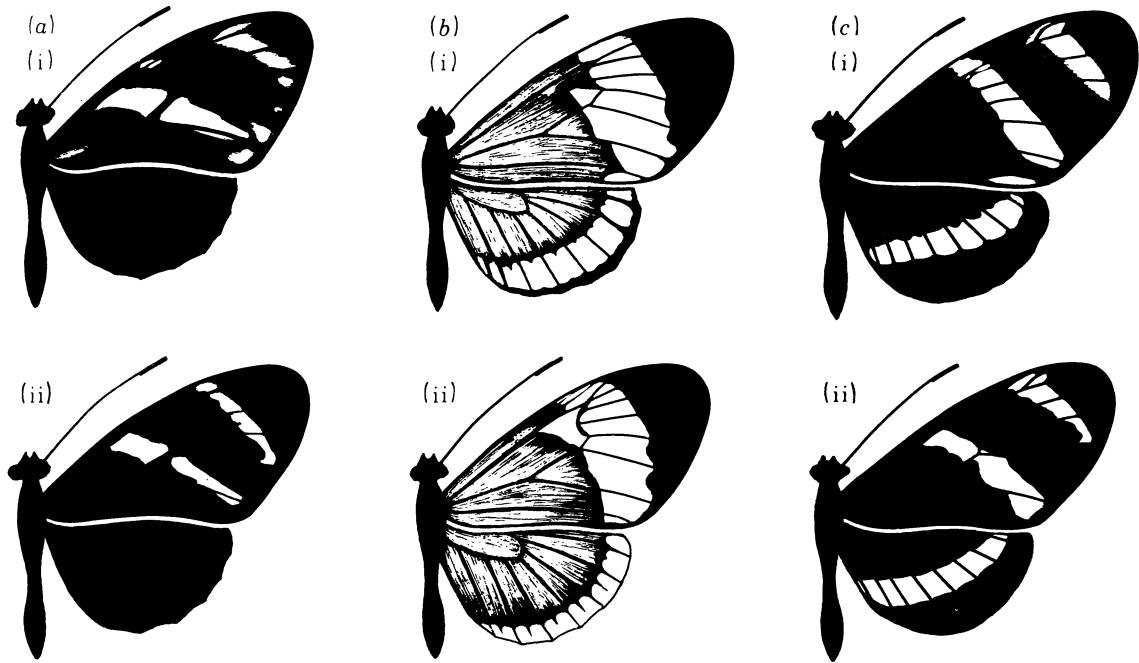
FIGURE 18. Three comimetic pairs of species, of which the upper member is a close relative of *Heliconius melpomene* and the lower member a close relative of *Heliconius erato*. The pairs are (a, i) *Heliconius luciana* and (a, ii) *Heliconius antiochus* (the white subspecies of each), (b, i) *Heliconius cydno* and (b, ii) *Heliconius sapho* (Panamanian subspecies of each), (c, i) *Heliconius pachinus* and (c, ii) *Heliconius hewitsoni* (these last are very close relatives of *cydno* and *sapho* respectively, both these species showing extensive variation). Colours: (a) black and white with slight blue iridescence; (b) white with iridescent blue; (c) black and yellow.

Matched mimetic pairs from different subgroups like those just described can be found throughout the genus *Heliconius* (Turner 1971a, 1976a; Brown 1981), and indeed between different butterfly families; clearly, the higher the taxonomic rank of the subgroups, the more likely it is that the resemblance results from convergence into the same mimicry group, rather than the parallel 'tracking' which we have postulated for *melpomene* and *erato*. Indeed, according to our model, such convergence *must* occur, and some of it may have taken place within the genus *Heliconius*; other instances of convergence, the most notable being of the 'tiger' heliconiids toward abundant ithomiids and danaids (Brown & Benson 1974; Brown 1976a) have clearly been between members of different families.

6. CONCLUSIONS

In our model of the evolution of muellerian mimicry, based on those of earlier workers, especially Nicholson (1927) and Fisher (1927, 1930), and detailed above, we have proposed that muellerian mimicry evolves in two steps:

(i) A major mutation in one of the potentially mimetic species produces an approximate resemblance to the other. Such a mutation must be of the less protected toward the more

protected, protection being a function of numbers, distastefulness, catchability and memorability. This may be termed one-way convergence.

(ii) As the mutation increases in frequency, the mimicry between its pattern and that of the other species will be improved by the selection of the existing polygenic variation in the population, or of new mutants with comparatively small effect. This may be termed mutual convergence, for both patterns will be altered even though, given an equally useable pool of genetic variation, the less protected species will evolve the faster.

It is clear that one or other phase may be omitted. Mutual convergence will not occur if the appropriate heritable variation is not available or, although this is very unlikely, if the original major mutation produces a mimic so good that it already perfectly resembles the other species within the predators' power to know the difference. Further, the phase of one-way mutational convergence may be omitted if the potential mimics are already so similar that predators sometimes mistake one for the other; this can happen for instance when two allopatric species whose patterns are already similar, either by chance or because they are related, extend their ranges to become sympatric.

If two species are too dissimilar to undergo mutual convergence, but are roughly equally protected, then mimetic mutants in both species have little chance of being established, and mimicry is unlikely to evolve. Finally, the probability of producing a mimetic resemblance by one or more mutations may be so low that muellerian mimicry does not evolve at all. Thus we can explain one of the features of muellerian mimicry: the existence in any one habitat of several distinct mimicry rings, showing generally great similarity *within* the rings, but a considerable difference in pattern between them.

At the beginning of the century two entomologists discussed with great skill and considerable insight, though naturally without modern genetic terms, the two phases of the evolution of muellerian mimicry. Marshall (1908) showed how what we regard as the first phase would be a one-way process, and would not occur if the species were equally protected (he treated protection purely as a matter of numbers, in which he was corrected by Dixey (1909) and Nicholson (1927)). Dixey (1894, 1909) argued that mutual convergence could occur if the 'mutants' were still mistaken for their own wild-type as well as for the other species. What is interesting is that there was considerable controversy between Marshall and Dixey, who each argued that the other's theory was incorrect! On the contrary, their models are not mutually exclusive and the processes are both, as we have shown, likely to occur during the evolution of a muellerian mimicry ring.

Figueredo Filho, Mrs A. Gill, Miss C. Green, Mr R. Kann, Mr M. Koello, Mr C. Madison, Mrs C. Sharrock, Mr A. Smith, and Dr J. Wolfson.

While all authors made a preliminary analysis of their own broods (as indicated in appendixes 4 and 5) the detailed analysis and interpretation of the results was done, as a consequence of Professor Sheppard's long illness and to ensure uniformity of scoring, by J.R.G.T.; it is a source of great regret to all of us that Professor Sheppard did not live to check the final analysis of the East Ecuador–Upper Amazon crosses (both species) nor the Mexican cross in *erato*, and did not see the final manuscript to these sections, nor the final revisions of the other crosses in *melpomene*. Errors therein are not Professor Sheppard's responsibility.†

Professor Sheppard, on 6 September 1976, requested that the following acknowledgement be added: 'Owing to delays for which I...was not responsible, I have been unable to take part in the analysis of the Ecuadorian and Mexican race crosses, or see the final manuscript. I am very grateful to Professor E.B. Ford, F.R.S., for agreeing to-day...to see the final stages through on my behalf, and also to thank him for all the encouragement and help that he has given me in the past.'

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† I have allowed the Discussion (§5) to stand much as it was authorized by Philip Sheppard in 1976 (J.R.G.T.).

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Appendix 1. Latin names and authors of the major races of *Heliconius* mapped in the text

(For full citations, see Neustetter (1929). Certain minor races are omitted; for example, the Trinidad forms can be distinguished as *flagrans* Stichel and *adana* Seitz.)

no. (figures 1 and 2)	distribution	subspecies of *melpomene* (figure 1)	subspecies of *erato* (figure 2)
1	Río Cauca (Colombia)	—	*chestertonii* Hewitson
2a	Middle America (Panamá etc.)	*rosina* Boisduval	{*petiverana* Doubleday[1] {*demophoon* Ménétriés
b	Northern Río Magdalena (Colombia)	?	*columbina* Staudinger
c	Upper Río Putumayo (Colombia)	*bellula* Stichel	*dignus* Stichel
d	Río Huallaga (Perú)	*amaryllis* Felder und Felder	*favorinus* Hopffer
3a	Venezuela, Colombia, Trinidad, Guiana coast	*melpomene* (Linnaeus)[1,2]	*hydara* Hewitson[1,2]
b	Vilcanota (Perú)	*euryades* Riffarth	*amphitrite* Riffarth
c	Lower Rio Amazonas	*melpomene* (Linnaeus)	*hydara* Hewitson[1]
d	Central Río Magdalena (Colombia)	*euryas* Boisduval	*guarica* Reakirt
e	Roraima region	*pyrforus* Kaye	*magnifica* Riffarth
4	Guiana (Suriname, Manaus)	*meriana* Turner[2]	*amalfreda* Riffarth[1]
5	Río Ucayali (Perú)	*cognata* Riffarth	*emma* Riffarth
6a	Upper Amazon basin	*aglaope* Felder und Felder[1]	*lativitta* Butler
b	East Ecuador (Upper Río Santiago)	*ecuadorensis* Neustetter[3]	*etylus* Salvin
7	Ilha do Marajó	to be named	*estrella* Bates
8a	Mato Grosso/Belém	*thelxiope* (Hübner)[1] *madeira* Riley[1]	*amazona* Staudinger[1]
b	Guyane/Amapá	*thelxiopeia* Staudinger	*erato* (Linnaeus)
9a	North Amazon basin, Rio Negro	*vicina* Ménétriés	*reductimacula* Bryk
b	Bolívia/Mato Grosso	*penelope* Staudinger[1]	*venustus* Salvin[1]
10	West Colombia	*vulcanus* Butler	*venus* Staudinger
11	West Ecuador	*cythera* Hewitson[2]	*cyrbia* (Godart)[2]
12	East Ecuador	*plesseni* Riffarth[1]	*notabilis* Salvin and Godman[1]
13	Río Perené (Perú)	*xenoclea* Hewitson	*microclea* Kaye
14	Upper Río Marañon (Perú)	to be named	*himera* Hewitson
15a	Bolívia	*amandus* Grose Smith and Kirby	*phyllis* (Fabricius)[1]
b	interior of Brasil	*burchelli* Poulton	
c	East Brasil	*nanna* Stichel[1]	

[1] Race used in breeding experiments in this paper.

[2] Race used in previous breeding experiments (Beebe 1955; Turner & Crane 1962; Sheppard 1963; Emsley 1965b; Turner 1972).

[3] The type of this name appears to be a hybrid between races 12 and 6a; race 6b appears to be strictly unnamed.

Breeders initials are given in the heading of each table.

Symbols and Abbreviations used in tables A 1–A 3

ns, not scorable; +, present; −, absent; Or, orange; BC, backcross.

Forewing lines: bl, basal line; fl, full line; hl, white line (with white in forewing band); nl, no line; pl, part line; v

Ecuador triangles: bt, trace of triangle (with broken forewing band); ft, full triangle; lt, large triangle (smaller than traces); pt, part triangle; tt, trace of triangle.

Forewing bands: br, broken; bt, broken (with trace of Ecuador triangle); fu, fused; hl, white in band (with white fo in forewing band; wu, white on underside (distinguished only in table A 3); Y, TY, S, TS, O, W: see figure yellow; O, no colouring; W, wide red).

Cell spots: nc, no cell spot; hc, part cell spot (distinguished from sc in some broods only); sc, cell spot.

Hindwing bars: hb, shadow of bar on underside; nb, no bar; tb, trace of bar; yb, full yellow bar.

Belém spot: scored only in some broods and not tabulated. Segregation is described in the text.

TABLE A 1 BELÉM × TRINIDAD AND BELÉM × VENEZUELA BROODS (P.M.S.

(No butterfly in this table has a full yellow forewing line or Ecuador triangle.)

brood no., type and total	provenance and phenotype of parents (♀/♂)	colour	forewing band, yellow bar	Y	TY	S	
						radiate	
YM2 F₁ (41)	Trinidad × Belém; Trinidad × Belém (orange) (same father as YM6)	red	broken nb				24
YM6 F₁ (50)	Trinidad × Belém; Trinidad × Belém (orange) (same father as YM2)	red	broken nb				28
YM10 (40)	Belém × Belém; normal (orange) phenotype × Belém phenotype with yellow hindwing bar (same father as YM12)	orange	broken yb broken nb	7/14 8/11			
YM12 F₁ (1)	Trinidad × Belém; Trinidad × Belém phenotype with yellow hindwing bar (same father as YM10)	red	broken nb				0
YM15 F₂ (3)	YM6 × YM6; both normal F₁ phenotype (same father as YM10; see also YM21)	red red ns	broken nb fused nb fused nb			0/1	0
YM16 BC (14)	F1BG × Belém; radiate, TS (yellow strong), red × Belém (orange)	red orange	broken nb broken yb broken nb	0/1 2/1	1/0 2/1[3]	3/0[2] 2/1[3]	0 1
YM19 BC (1)	Belém × YM6; Belém (orange) × normal F₁ phenotype (same father as YM15)	red	broken nb	[1]1/0			
YM21 F₂ (199)	YM6 × YM6; normal F₁ phenotypes (4 ♀♀ including mother of YM15; 3 ♂♂ including father of YM15, YM19)	red orange ns ns	broken nb fused nb ns nb broken nb fused nb broken ns fused ns ns ns	4/0 0/1 2/0 2/0 1/2	4/2 0/1 9/2 3/0 0/1	5/8 0/3 2/2 1/0 0/1	9 4 7 1 0
YM27 BC (59)	YM2 × YM10; normal F₁ phenotype × Belém phenotype with yellow hindwing bar	red orange ns	broken nb broken nb broken nb	4/5 4/4	1/6 8/4[3]	0/5 2/2 1/0	1 3
YM34 BC (31)	Belém × YM6; Belém × normal F₁ phenotype	red orange	broken nb broken nb	1/3 1/0	4/0 1/3	5/4 2/2	1 2
YM39 BC (5)	YM2 × YM10; normal F₁ phenotype × Belém phenotype with yellow hindwing bar	red orange	broken nb broken nb	0/1	0/3	1/0	
YM43 (36)	YM2 × YM21; normal F₁ phenotype × plain, W, fused	red orange ns	broken nb fused nb ns ns broken nb fused nb ns nb				1 2
YM44 (34)	YM21 × YM21; plain, W, fused, red × plain, W, fused, pale orange red	red red orange ns	broken nb fused nb fused nb ns nb				
YM46 BC (7)	YM6 × Belém; normal F₁ phenotype × unobserved stock male	ns	broken nb		2/1	1/2	1

part line; wl, white line.
naller than ft); nt, no triangle (including minute

h white forewing line); nw, no white; wh, white
ee figure 5 (Y, yellow; T, thin red; S, smudgy

(P.M.S., J.R.G.T.)

riangle.)

| | | fspring (♀/♂) | | | | |
| radiate | | | | plain | | |
S	TS	O	W	TY	TS	W
	24/17					
	28/22					
	0/1[1]					
/1	0/1					
						1/0
/0[2]	0/2					
/1[3]	1/0					
/8	9/14	3/5	12/8	2/4	6/10	7/3
/3	4/4	1/0	2/1	1/1	0/6	1/2
		2/2	1/0			
/2	7/6	0/2	1/0	2/0	2/0	0/1
/0			1/0		1/0	1/1
/1	1/3				1/0	
	0/2					
/5	1/3					
/2	3/6					
/0						
/4	1/2					
/2	2/0					
/0						
	1/0		0/1			
			1/3		1/3	1/3
			1/0			1[4]
	2/2		2/0		2/4[13]	
			2/1		1/0	0/1
			3[4]			
			0/1[1]			8/17[5]
						4/2
						1[4]
/2	1/0					
/0	1/0					

		ns	ns ns				
YM46 BC (7)	YM6 × Belém; normal F_1 phenotype × unobserved stock male	ns	broken nb		2/1	1/2	1,
YM47 BC (25)	offspring of YM46 contaminated with pure Belém stock	ns	broken nb	[6]4/10	2/2	2/2	1,
YM48 BC (9) (18)	offspring of YM46 contaminated with a mating YM10 × YM10, both yellow barred Belém phenotypes	ns	broken yb broken nb	2/7[7] 0/3	1/3	3/3	0,
YM49 (2)	YM21 × YM21; radiate, O, ns, red × radiate, O, fused, red	salmon pink orange?	broken nb broken nb			1/0	
YM51 (46)	YM27 × YM27; radiate, S, broken, orange × radiate, S, broken, red (same father as YM52)	red[8]	broken yb broken nb	5/7 2/0		9/14	[1]1,
YM52 (4)	YM21 × YM27; plain, TY, fused, red × radiate, S, broken, red (same father as YM51)	red?	broken nb broken tb fused nb	0/1	0/1 0/1		0,
YM57 (53)	YM49 × YM27 or YM34; radiate, S, broken, salmon pink × radiate, TY, broken, red	red	broken nb			12/7	9,
YM64 (7)	YM51 × YM51; both radiate, O, broken, red	red red	broken nb fused nb				
YM65 (42)	YM43 × YM51; plain, TS, fused, strong yellow, ?orange × radiate, O, broken, red	red orange	broken nb fused nb broken nb fused nb		0/1[1]	[1]1/0	0, 0, 7, 3,
BTFG F_1 (50)	Trinidad × Belém; Trinidad × Belém (colour ns) (or, less probably, the reciprocal)	ns	broken nb				18,
TBFT BC (18)	BTFG × Trinidad (or reciprocal)[10]	red orange	broken nb fused nb broken nb				0, 2, 12,
B1TN BC (5)	BTFG × Trinidad (or reciprocal) (may be same brood as TBFT)[10]	red	broken nb fused nb				0,
F1BG F_1 (4)	Trinidad × Belém; Trinidad × unknown[11]	red	broken nb		2/1		0,
TBF2 (8)	both F1BG; both radiate, apparent TY broken, red[12]	red orange ns	broken yb fused nb fused yb fused nb broken yb	0/1	1/1		
CF3L (8)	?TBF2 × TBF2; unrecorded × radiate, apparent TY, fused, orange	red orange	fused hb fused hb fused yb ns yb		0/1 1/1		
F1F3 (9)	ESF1 (table A2) × CF3L (or reciprocal); phenotypes not recorded[12]	red	broken hb fused hb broken ns fused ns				1,
CF3T (2)	Trinidad × CF3L; Trinidad × unrecorded	red	fused hb				
14C (13)	Quebrada Grande × Belém; Venezuela × Belém	red	broken nb				5,
19F (1)	Belém × 14C; Belém × normal F_1 phenotype	orange	broken nb			0/1	

[1] Contaminant, or possible contaminant (or misattribution in TBF2, see table A 2, footnote 7).
[2] S and Y not clearly distinguishable in this brood.
[3] One ♀ may be from YM34 or YM47.
[4] Not scored for sex.
[5] 3/2 not scored for fused; 1/0 not scored for colour.
[6] Includes pure Belém contaminants, many with yellow bars.
[7] Most or all contaminants in this category.
[8] A minority are rather orange (?fading).
[9] Also 4/2 radiate TS with strong yellow which may be from F1BG, and one obvious Belém × East Brasil F_1.
[10] Most TS fused bands have red in the areas that are usually yellow; both plain W butterflies have diagonal red stripe at
[11] See text (p. 476) for details of offspring phenotypes; there are further 1/1 radiate 'TY' and 0/2 radiate 'TS' preserve
[12] It is likely that the recorded pedigree of these broods is in error (see p. 476)
[13] One female has yellow bar shadow underside.

						1.
/2	1/0					
/2	1/2					
/3	0/5					
/0					[1]1/0	
/14	[1]1/0	4/4				
	0/1					
/7	9/15					
		4/8 1/4				
/0	0/1 0/1 7/2 3/3		0/3 0/5 9/0 6/0			
	18/26[9]					
	0/1 2/3 [12]2/0		4/2 0/2	2/0		
	0/1		1/0	1/0		0/2
	0/1					
						1/0
			1/0			1/0
		1/0				[1]1/0
		1/0 1/0	1/1			1/0
	1/0		0/3 1/0 1/0			0/1 0/2
			1/0			0/1
	5/8					
/1						

...ed stripe at apex of hindwing (unique to this brood).
...S' preserved that appear to be members of this brood.

brood no., type and total	provenance and phenotype of parents (♀/♂)	cell spot	colour	yellow	yellow+ thin red	no band	wide red	no band	wide red	ye th
				radiate, no yellow bar				radiate, yellow bar		r n
ESF1 F₁ (54)[1]	Belém × Espirito Santo; Belém × East Brasil (or reciprocal)	−	red		bl nt 24/26 bl pt 0/2					
CF2 F₂ (169)[2]	ESF1 × ESF1 (2 ♀♀, several ♂♂); all normal F₁ phenotype	+	red	bl nt 4/3 pl nt 1/0	bl nt 4/8 bl pt 0/1			fl nt 0/1	bl nt 1/1 fl nt 2/1 fl pt 0/1	
		+	orange		bl nt 2/1			fl nt 1/0 pl pt 0/1		bl
		−	red	bl nt 4/5 bl pt 2/1 pl nt 1/1 pl pt 1/0 pl lt 0/2	bl nt 17/14 bl pt 1/3	bl nt 2/3		pl nt 0/2 fl pt 2/0 fl lt 1/0	pl nt 1/0 fl nt 0/2 fl pt 3/1 fl ft 0/1	bl bl
		−	orange	bl nt 3/0 bl pt 1/1	bl nt 2/4 bl pt 1/1 pl pt 1/0	bl nt 1/0		bl nt 2/0 bl pt 0/1	pl nt 1/2 fl nt 0/1 fl pt 1/0	bl bl
NF2 F₂ (98)	ESF1 × ESF1 (1 ♀); both normal F₁ phenotype	+	red	bl nt 2/2 nl nt 1/1	ns ns 0/1 nl nt 1/2 bl nt 1/2			nl nt 0/1 pl nt 0/1 bl pt 0/1	bl nt 0/2 pl nt 0/1	
		+	orange	ns nt 0/1	bl nt 2/2 nl nt 2/0 ns ns 1/0			bl pt 0/1	nl nt 2/0	
		−	red	bl nt 5/1 bl ft 1/1 nl pt 1/1 ns ns 1/0	nl nt 3/1 nl pt 1/4 bl pt 2/1 bl nt 0/2		nl nt 1/0	pl nt 0/1 pl ft 0/1	nl nt 0/1 nl lt 1/0 bl pt 1/0	
		−	orange	bl nt 5/0 nl pt 1/0 nl nt 1/1				ns pt 0/1 nl pt 0/1	nl pt 0/1 nl ft 0/1 bl ft 1/1	bl
YM59 F₂ (12)[4]	ESF1 × ESF1; both normal F₁ phenotype	+	red		bl nt 0/1					
		+	orange							
		−	red		bl nt 1/2	bl nt 1/0				
		−	orange						pl nt 2/0	
YM63 F₂ (20)	ESF1 × ESF1; both normal F₁ phenotype	+	red	ns nt 0/2	bl nt 1/0					
		+	orange							
		−	red	ns nt 1/1 bl nt 1/0 ns pt 1/0	ns nt 1/1 ns pt 1/0	bl nt 1/0		pl nt 0/1		
		−	orange		bl nt 1/1					
F1TM backcross Trinidad (142)[6]	Trinidad × ESF1 (several ♀♀, probably only 1 or 2 fertile, 1 ♂); Trinidad × normal F₁ phenotype	+	red		nl nt 5/5		nl nt 5/5	nl nt 5/5	nl nt 1/2	nl
		+	orange		nl nt 1/0			nl nt 1/0		nl
		−	red		nl nt 5/8 nl pt 4/0			nl nt 1/2	nl nt 1/4	nl
		−	orange		nl nt 2/3 nl pt 1/0			nl nt 0/1	nl nt 1/1	nl
YM66[7] testcross (22)	YM64 (table A 1) × YM59 or YM63; radiate O fused, cell spot, no yellow bar × radiate, thin red+yellow band, ns cell spot, no yellow bar (mother's band may have been S)	+	red	nl nt 1/0	nl nt 0/1	nl nt 1/2 pl nt 0/1	nl nt 1/0			
		−	red	nl nt 2/1	nl nt 0/1	nl nt 3/2	nl nt 2/2			pl
F1BM backcross Belém (127)	Belém × ESF1 (4 ♀♀, 1 ♂); Belém × normal F₁ phenotype	+	red	bl nt 7/12	bl nt 6/6 bl pt 1/0					
		+	orange	bl nt 17/6 bl pt 1/0	bl nt 13/10					
		−	red	bl nt 8/6 bl pt 2/0	bl nt 2/9					
		−	orange	bl nt 5/2 bl pt 1/1	bl nt 5/4 bl pt 2/1					
CYHW testcross (7)	Both TBF2 (table A 1); plain, W, yellow bar, yellow line, cell spot, (red/orange ns) ×	+	red							
		+	orange							
		−	red							

	plain, no bar	plain, shadow of bar		plain, yellow bar	
e red	yellow+ thin red	yellow+ thin red	wide red	yellow+ thin red	wide red
t 1/1		bl nt 1/1		pl nt 2/0	
2/1					
0/1					
	bl nt 0/1	bl nt 3/3			fl nt 1/0
		bl pt 1/2			
		bl ft 1/0			
t 1/0	bl nt 4/5			pl nt 0/1	pl pt 0/1
0/2	bl pt 1/0				pl ft 0/1
3/1					fl nt 1/2
0/1					fl pt 1/0
t 1/2	bl nt 3/0	bl nt 1/1		pl nt 0/1	
0/1	bl pt 2/0	nl pt 0/1			
1/0					
t 0/2		nl nt 1/1			
t 0/1		bl nt 1/0			
t 2/0					nl nt 0/3
t 0/1		nl pt 1/0	nl nt[3] 2/1		nl pt 1/0
1/0		bl nt 1/3			bl pt 0/1
t 1/0		pl lt 1/0			
t 0/1	bl nt 2/0	bl pt 0/1	nl nt 0/1		bl pt 1/0
0/1					nl ft 0/1
1/1					
		bl nt 1/1			
		bl nt 1/0			
t 2/0		bl nt 1/0			
		bl nt 1/0			
					pl nt 0/1
					pl nt 1/0
		ns nt 2/1[5]			
		ns nt 0/1			
t 1/2	nl nt 8/5		nl nt 1/2		nl nt 5/3
	nl nt 2/0				nl nt 1/1
t 1/4	nl nt 5/7	nl nt 0/1	nl nt 8/8		nl nt 7/12
t 1/1	nl nt 1/1				nl nt 2/0
	pl nt 1/0	pl nt 1/0			
		bl br 2/1			fl fu 0/2
		bl br 1/2			fl br 2/0
		bl br 0/1			fl bt 1/0

				bl pt 1/1	bl pt 2/1				
CYHW testcross (7)	Both TBF2 (table A 1); plain, W, yellow bar, yellow line, cell spot, (red/orange ns) × unrecorded (see p. 476)[8]	+	red						
		+	orange						
		−	red						
		−	orange						

[1] Includes two males without yellow in the band and with a shadow of the cell spot, which are clearly contaminants. Of the 'pt' individ... a contaminant from the F_2; the other shows only a minute trace; a few individuals show traces of the yellow bar on the underside, and one o...

[2] Including radiate, yellow bar, red and thin yellow band, cell spot +, orange 0/1. The bl category in this brood includes some ns indi...

[3] One not scorable for shadow of bar.

[4] Contains some individuals from YM63.

[5] 'Male' not scorable for sex, shadow of bar, or red versus orange.

[6] The nl category contains a few basal lines (bl); in this brood *only*, the yellow bar is merely a light sprinkling of yellow scales in that re... the yellow bar unscorable, one plain wide red band, one radiate, wide red band, one radiate thin red+yellow band, all red (not orange) ... one an East Brasilian phenotype, and some radiate phenotypes which appear to be from F1BM.

[7] Among radiate individuals in this brood, no distinction is made between nl and bl; all yellow band individuals have a weak yellow band (S p...

[8] Attribution of parents to TBF2 almost certainly incorrect, as all progeny show phenotypes characteristic of strong East Brasil ancestry (...

		bl br 2/1			fl fu 0/2
		bl br 1/2			fl br 2/0
		bl br 0/1			fl bt 1/0
					fl fu 0/1
		bl br 2/1			fl br 1/0

ot' individuals, one has the triangle strongly developed and may be
and one or two of these have a sprinkling of scales on the upperside.
ne ns individuals.

in that region of the hindwing. Brood also includes three males with
t orange) and lacking cell spots. There are also some contaminants,

y band (S phenotype).
ancestry (e.g. tooth). All progeny nt unless otherwise described.

brood no., type and total	provenance and phenotype of parents (♀/♂)	colour	forewing band and basal spot	radiate yellow +spot	radiate yellow −spot	radiate red+yellow +spot	radiate red+yellow −spot	
M1 F1 (64)	Belém × Palora; Belém × East Ecuador (several ♀♀)	red	short				(see footnote 13) sc nt wh 0/1 sc tt wh 20/10 sc pt wh 22/8	
M3 BC (6)	Belém × M1; Belém × normal F1 phenotype (♀ same as one parent of M1)	red	short				sc nt nw 0/1	
		red	long			sc nt nw 1/0		
		orange	short			sc nt nw 0/1		
		orange	long			sc nt nw 0/2	sc nt nw 1/0	
M4C F2 (16)	M1 × M1; normal F1 phenotypes; possibly contaminated (see text)	red	short	sc nt nw 1/0 sc pt nw 0/1 sc ft wh 1/0	sc tt wh 0/1	sc ft wh 0/1 sc pt wh 0/1		sc p
		red	long	sc nt nw 0/1			sc nt lw 0/1 sc ft lw 1/0 sc ft nw 0/1	
		orange	short	sc nt nw 2/1				
M4D F2 (54)	same brood as M4C; heavily contaminated (see text)	red[8]	short	sc nt nw 0/1[2]	sc nt nw 1/1 sc pt nw 1/0		sc ft wh 1/1 sc nt wh 1/0	
		red	long				sc tt nw 1/0 sc nt wh 1/0	
		orange	short	sc pt nw 1/0			sc nt wh 1/0	
		orange	long		sc nt wh 0/1[4]		sc pt nw 1/0	
		red or orange	long	(cs nt nw 20/14)[1]				
M5 (F2?) (3)	not recorded	red	short					
		orange	short				sc nt wl 1/0 sc nt hl 0/1	
M6 F2 (17)	M1 × M1; both normal F1 phenotype	red	short	sc ft nw 0/1 sc nt nw 1/0	sc nt nw 0/2		sc nt wh 0/1	sc ft
		red	long				sc nt wh 1/0	
		orange	short		sc tt nw 1/0	sc ft nw 0/1	sc pt hl 0/1	
		orange	long	sc nt nw 0/1	sc nt nw 0/1	sc tt nw 0/1	sc nt wl 0/1 sc nt wh 1/0	
M7 F1 (18)	Belém × Pastaza; Belém × plain, red and white short band, no basal spot, no cell spot (i.e. East Ecuador introgressed Upper Amazon)	red	short				sc nt nw 2/0 nc nt nw 0/1 sc nt nw 0/2 sc pt wl 1/0 sc nt wl 1/0 sc pt wh 0/1	
		red	long				sc nt nw 0/1 nc nt nw 2/0 sc nt wh 1/0 nc nt wh 0/4 nc nt wl 1/1	
M8 BC (44)	Belém × M1; Belém × radiate, red+yellow band, short, cell spot, no triangle, ? no white	red[9]	short	sc nt nw 2/1		sc nt nw 1/1	sc nt nw 0/3	
		red	long	sc nt nw 3/6 sc tt nw 2/0	sc nt nw 2/1		sc nt nw 0/1	
		orange	short	sc nt nw 1/1	sc nt nw 0/1	sc nt nw 3/1 sc tt nw 1/0		
		orange	long	sc nt nw 7/1 sc tt nw 1/0		sc nt nw 0/1	sc nt nw 0/2 sc nt wl 1/0	
M9 BC (66)	Belém × M1; Belém × radiate, red+yellow band, no basal spot, short, (cell spot not recorded), no triangle, ? no white	red	short	sc nt nw 3/4 sc tt nw 2/1	sc nt nw 2/3 sc tt nw 2/1	sc nt nw 0/1	sc nt nw 0/2	
		red	long	sc nt nw 1/2 sc tt nw 0/1	sc nt nw 1/4		sc nt nw 0/3	
		orange	short	sc nt nw 1/0	sc nt nw 5/1	sc nt nw 1/0	sc nt nw 3/1	
		orange	long	sc nt nw 1/0	sc nt nw 2/1	sc nt nw 2/1	sc nt nw 4/4 sc tt nw 2/0	
		not scored	short	ns ns nw 1/0	ns ns nw 2/0	ns ns nw 1/0	ns ns nw 0/3	
M16 F2 (5)	M7 × M7; both radiate, red and yellow band, no basal spot, short, cell spot, part triangle, no white	red	short	sc ft nw 1/0	sc ft nw 1/0		sc ft wh[2,5] 1/0	
		red	long	sc nt nw 0/1	sc nt nw 0/1			

		plain		
ow	red[11] −spot	red + yellow + spot	red + yellow −spot	red[12] −spot
e				
/1 /10 2/8				
/1 /0				
1 /1	sc pt nw 0/1		sc pt wh 0/1	
1 0 1				sc ft wh 1/0
1 /0 0 0 0 0			sc pt wh 1/0 sc nt wh 0/1[2]	sc nt wh 2/0 sc ft wh 1/1 sc nt wh 1/0
0				sc pt wh 0/1[3]
/1	sc ft wh 0/1			
0			sc nt wh 1/0	cs ft wh 0/1
1 /0				
0 /1 2 0 1 1 /0 0 /4 1				
3 1 2 0				
2 3 1 4 0 3				
/0				

F2 (5)	red and yellow band, no basal spot, short, short, cell spot, part triangle, no white	red	long	sc nt nw 0/1	sc nt nw 0/1			
M17 BC (6)	Belém × M7; Belém × radiate, red, yellow and white band, no basal spot, short, no cell spot, no triangle	not scored	short		nc ns nw 0/2 / sc ns nw 1/0		sc nt wh 0/1 / sc tt wh 1/0 / nc nt nw 1/0	
M18 F2 (2)	M7 × M7; both radiate, red and yellow band, no basal spot, no cell spot, no triangle, short; ♂ only with white in band	not scored	short / long		sc nt nw 1/0			
M19 F2 (4)	M7 × M7; both radiate, red and yellow band, no basal spot, no triangle; ♀ long, no cell spot, ♂ not scored for these characters	see footnote 5	short / long				nc nt nw 0/1	nc n... / sc nt...
M21 BC (17)	Belém × M7; Belém (orange) × radiate, red and yellow band, no basal spot, short, no cell spot, no triangle, red, no white	red	short	sc nt nw 1/0	sc nt nw 2/0 / hc nt nw 0/1	hc nt nw 0/1	sc nt nw 1/0	
		red	long		sc nt nw 1/0	hc nt nw 0/1	sc nt nw 1/0 / sc nt wh 0/1	
		orange	short		sc nt nw 2/0 / hc nt nw 2/0	hc nt nw 1/0		
		orange	long		nc nt nw 1/0		hc nt nw 0/1	
M23 (6)	Both unknown; plain, red, yellow and white band, no basal spot, short, cell spot, no triangle, red × radiate, red, yellow and white band, short, cell spot not scored, no triangle	red	short		sc ft hl [6]1/0 / sc nt nw [7]2/0			sc ft
		red	long	sc nt nw 1/0				
M24 (4)	Belém × ?; Belém (orange) × plain, red band, no basal spot, short, cell spot not scored, full triangle, no white	red	short					sc nt
		orange	short				sc nt wh 2/0 / sc nt nw 1/0	
M26 BC (37)	Belém × M7; Belém (red) × unrecorded phenotype	red	long	sc nt nw 1/0 / sc tt nw 4/0 / nc nt nw 4/0	sc tt nw 1/1 / nc nt nw 4/7	sc nt nw 1/1 / nc nt nw 0/3	sc nt nw 0/1 / sc tt nw 2/0 / sc pt nw 0/1 / nc nt nw 4/1	
M27 BC (27)	Belém × M7; phenotypes not recorded	red	short	sc nt nw 3/1 / sc tt nw 0/1	sc nt nw 1/1 / sc tt nw 0/1	sc nt nw [2]1/0		
		red	long	sc nt nw 0/1 / sc tt nw 0/1	sc nt nw 2/0	sc nt nw 1/0	sc nt nw 1/2	
		orange	short			sc nt nw 1/0	sc nt nw 1/1	
		orange	long	sc tt nw 1/0	sc nt nw 1/1		sc nt nw 1/1 / sc nt nw 1/1 / sc nt wh 0/1 / sc tt wh 1/0	
M30 BC (7)	Belém × 'F₁' (= M7); Belém (red) × radiate, red (and yellow) band, short, no triangle, red (otherwise not recorded)	red	short		sc nt nw 1/0		nc nt wh 1/1	
		red	long	sc tt nw 0/1	sc tt nw 0/1	nc nt wl 0/1	nc nt nw 1/0	
		orange	short				nc nt wh 0/1	
1A BC (37)	M7 × Belém; radiate, red and yellow band, no basal spot, long, no cell spot, no white, red × Belém	red	long	sc nt nw 3/1 / sc nt nw 0/3[15]	sc nt nw 2/4 / nc nt nw 1/3	sc nt nw [14]1/4 / sc tt nw 1/4 / nc nt nw 2/1 / nc nt wh 1/0	sc nt nw [14]2/1 / (sc nt nw 1/0)[1] / nc nt nw 2/0	
2A (5)	1A × 1A; radiate, red and yellow band, traces of white, basal spot, long, no cell spot, no triangle, red × radiate, yellow band, no basal spot, long, no cell spot, no triangle, no white, red	not scored	long		nc nt nw 1/0	nc nt nw 1/0	sc nt nw [2]2/0 / nc nt wh 0/1	
3E	Belém × 19B; Belém ×	red	long				sc nt nw 0/1	

/1				
0				
/0				
				nc nt wh 0/1
/1	nc nt nw [5]1/0			
	sc nt nw [3]1/1[3]			
0				
0				
1				
/1				
	sc ft wh [6]1/0	sc ft wh 1/0		
0	sc nt wh 0/1[7]			
0				
/1				
0				
1				
/1				
2				
1				
1				
1				
0				
/1				
0				
/1				
2/1				
/0)[1]				
/0				
/0				
/1				

ID	Description	Color	Band					
	band, no basal spot, long, no cell spot, no triangle, no white, red							
3E (7)	Belém × 19B; Belém × radiate, red, yellow and white band, basal spot, short, cell spot, trace of triangle, red	red	long short			sc nt nw 0/1 sc tt nw 0/1 sc nt nw 0/3 sc tt nw 0/2		
11A (8)[10]	M4D × M4D; radiate, red and yellow band, ? no basal spot, long, cell spot, trace of triangle, no white × plain, red and white band, no basal spot, short, cell spot, full triangle, red	red (or ns)	short			sc pt wl 1/0		sc ft
12A (9)	11A × 11A; plain, red band, short (split), no basal spot, cell spot, full triangle, band white on underside × radiate, red and white band (traces of yellow), short (split), basal spot ns, ? cell spot, triangle not scored	red	short	(sc nt nw 0/1)		sc tt wh 0/1[2] sc pt nw 0/1[2]		sc pt sc ft
15B (1)	1A × 1A; radiate, red and yellow band, long, basal spot, no cell spot, no triangle, no white, red × radiate, yellow band, long, no basal spot, no cell spot, no triangle, no white, red	red	long	nc nt nw 0/1				
16D (4)	11A × 1A; plain, yellow band, short, basal spot ns, cell spot, no triangle, no white × radiate, yellow band, long, no basal spot, no cell spot, no triangle, no white, red	red orange orange	short short long	 sc nt nw 0/1		sc nt wh [2]1/0 sc tt wh 0/1 (sc tt wh 0/1[2])[1]		
19B (8)	1A × 11A; radiate, red and yellow band, long, no basal spot, cell spot, no triangle, no white, red × plain, red, yellow and white band, short, basal spot, cell spot, part triangle, red	red red (some app. faded)	long short			sc tt nw 1/0 sc tt nw 0/1	sc nt wh 1/0 sc tt wh 0/1	

[1] Possible contaminants (mixture of contaminants and brood members in M4D).

[2] Basal spot not scorable. Column assigned on rest of N locus phenotype.

[3] Band is red (or red and white) with traces of yellow. In brood 12A, white represented by traces only.

[4] Has weak basal spot, but appears to be $N^N N^B$.

[5] Probably orange; remainder of brood red.

[6] Part of hindwing bar, in white, on underside.

[7] One may be orange.

[8] Includes three of doubtful phenotype.

[9] Includes one not scorable for red colour.

[10] Includes female parent of 16D, which was only partly scored (see brood 16D for phenotype).

[11] Split if short; broken (as in Belém) if long.

[12] If short, the red band is split, as are any white areas within it. The long red bands are (a) in M6, a long wide (not split) red band mixed red band with some white mixture throughout, (c) in M4D, a long, *split* band, red distally and in the posterior of the cell spot, white p

[13] White limited in extent, absent in many. Total includes three individuals with long bands, all presumed contaminants (two Belém

[14] Includes half of a sexual and somatic mosaic.

[15] Includes halves of two sexual and somatic mosaics.

0	sc ft wh 1/0	sc pt wh 0/1	sc pt wh³ 1/2	sc ft wu 1/0
1² /1²	sc pt wu 0/1 sc ft nw 1/0		sc ft wh³ 1/0 sc tt wh³ 1/0	sc ft nw 1/0 sc ft wh 0/1
/0 1 /1²)¹				
/0 1		sc tt wh 0/1 sc pt wh 1/0		sc pt wh³ 1/1

...and mixed with a short, split, white band, (b) in M4C, a long broken
...ot, white proximally and in the anterior of the cell spot.
...two Belém and one apparently from a backcross).

Breeders initials are given in the heading of each table.

Symbols and Abbreviations used in tables A 4–A 12

ns, not scorable; Pan., Panamá; rect., rectangles; BC, backcross.

Yellow lines: bl, basal; nl, none; nr, none, red spot; rl, red line; vl, very weak basal line; wd, weak basal line, table A 8; see head of table); wn, weak or no line; wr, weak medial, red tip; yl, full.

Hindwing bars (see figure 7): b, tip turns back; bb, broad sharp with black veins, slightly eaten; bf, broad fuzzy; bv, broad with black veins; ea, eaten; f, tip turns forward (double in tables A 11 and A 12); fa, fuzzy eaten; fr, broken fuzzy (East Ecuador type); fs, faint dusting of yellow scales; nb, none; ts, thin sharp; tf, thin fuzzy;

Cream rectangles: cr, present; nr, absent.

TABLE A4. TRINIDAD × EAST BRASIL BROODS (P.M.S.)

(All broods multiparental (see p. 505)

brood no. and type	provenance and phenotype of parents (♀/♂)	bar tip	concave, toothed full rect.	weak rect.	no rect.	inte full rect.
TF1 F₁	São Paulo × Trinidad; East Brasil × Trinidad		38/25 (see text for description of			
TF2 F₂	TF1 × TF1; both intermediate, tooth, no rectangles, nl, fd	b	yl bs 1/1 bl ea 1/1	wr bf 2/1	wr bf 0/1 wr tf 1/0 nr vt 0/1 nl br 0/1	bl ea 3/1
		f ns	yl bs 1/1 nl nb 0/1[1]		wr tf 1/0 nl fd 3/1 nl nb 1/1	bl ea 2/0
B1T BC	São Paulo × TF1; East Brasil × intermediate, tooth, no rect., nl, fd, b	b	yl bs 7/7	wr bf 1/4	wl tf 1/1	bl ea 6/2[2]

[1] Rectangles ns.   [2] One ♀ is indistinguishable from b*r*.   [3] Very weak rectangles in one of these.   [4

TABLE A 5. PANAMÁ × EAST BRASIL, MÉXICO × EAST BRASIL, AND OTHER BROODS OF THAT PROVENAN

brood no. and type	provenance and phenotype of parents (♀/♂)	bar tip	concave, toothed full rectangles, no Panamá bar	no rectangles, shadow bar	concave to intermediat full rectangles, no Panamá bar	no re sha
SF1 (F₁)	Panamá × São Paulo; Panamá × East Brasil		11/3 (see text for description of offspring)			
RF1 (F₁)	2 ♀ Panamá × 1 ♂ Rio de Janeiro; Panamá × East Brasil		23/20 (see text for description of offspring)			
WK2 (F₂)	RF1 × RF1 composite brood	b b b	yl bs 1/0	wr tf 1/0	bl ea 1/0 bl br 1/1 nl bf 0/1 (no rectangles)	bl b nl tf nl e
		b f f				nl b nl b
		ns				nl tf
		ns ns				nl b nl fd
B1 (BC)	several ♀ São Paulo × ♂ SF1	b	yl bs 8/1	yl bs 0/1 yl bf 1/0 bl bf 0/1 wr bs 0/1	bl ea [8]4/4 bl bf 0/1	wr b bl b nl e nl b nl ts
B1(♀)	São Paulo × SF1	b	yl bs 2/2		bl ea 0/1	nl

basal line, red dot; wl, weak medial (excluding

broad fuzzy; br, broken fuzzy; bs, broad sharp;
fuzzy eaten (East Ecuador type); fd, fuzzy dot;
thin fuzzy; vt, very thin fuzzy.

M.S.)

		ring (♀/♂)			
	intermediate, toothed			convex toothless	
ll rect.	weak rect.	no rect.	no rect.	total
escription of phenotypes)					**63**
ea 3/1		nl br 0/1 nl fd 2/0 nl nb 1/0	nl br 0/3 nl fd 1/2 nl nb 0/2	**41**
ea 2/0		nl br 1/0	nl 1/0[4]	
ea 6/2[2]	nl br 3/1	nl fd 3/0[3]		**36**

these.   [4] Rectangles and bar ns.

PROVENANCE (P.M.S., K.S.B., W.W.B., M.C.S.)

		pring (♀/♂)			
	intermediate, toothed		convex, toothless		
es, bar	no rectangles, shadow bar	full rectangles, no Panamá bar	no rectangles, shadow bar	total
pring)					**14**
ffspring)					**43**
	bl br 1/0 nl tf 0/1 nl ea 0/1	bl br 0/2 nl br 0/1	nl tf 1/0 nl br 1/0	**32**
gles)	nl br 2/2			
	nl br 1/2	bl br 1/0 bl fd 1/0	nl fd 1/0 nl ns 0/2 (full bar) nl ns 0/1[1] (full bar)	
	nl tf 1/0			
	nl br 1/1[1] nl fd 1/1[1]			
	wr bf 2/1 bl bf 1/0 nl ea 2/4 nl bf 1/0 nl ts 0/1			**34[2]**

					bl bf 0/1		nl e
					wr bs 0/1		nl b
							nl t
B1(2) (BC)	São Paulo × SF1	b	yl bs 2/3			bl ea 0/1	nl tf
WK1 (BC)	Rio × RF1; East Brasil × intermediate, tooth, no rectangles, shadow bar, nl bf b	b	yl bs 4/2/2[1]	wr bf 4/2/1[1]		bl ea 3/1	nl b
J	B1(2) × B1(2)	b	yl bs [4]4/2			bl br 2/3	bl b
K	B1 × B1	b	yl bs 1/0	wr bs 2/2		bs ea 4/3	nl e
		b		wr bs [6]1/0		bl ea 0/1	nl e
L	B1 × B1	b					nl e
M	B1 × B1	b		wr tf 0/1			nl e
							nl b
							(fu
N	B1 × B1	b	yl bs 2/6	wr tf 1/0		bl bf 0/2	nl v
						bl ea 0/1	nl e
							wl e
							wr tf
O	B1 × B1	b				bl ea 1/2	nl e
MF1 (F$_1$)	México × São Paulo; México × East Brasil	ns					nl ea
MF2 (F$_2$)	MF1 × MF1	b	yl bs–ts 1/1[6]	wr–wl ts 0/1		bl ea [7]2/2	bl e
		b		rl ns 2/0 (full bar)		bl br 0/1 (no rectangles)	nl n (fu
		b					nl ea
		f	yl bs–ts 0/1	wr–wl tf 0/1			nl tf
		f					nl n (ful
		f					nl ea
		ns					
MB2 (BC)	MF1 × México; ♀ intermediate, tooth, no rectangles, shadow bar, nl ea	b					nl ea
		b					nl ns (ful
		f					nl ea
		f					nl ns (ful
MB13 (BC)	MF1 × México; ♀ concave, tooth, no rectangles, shadow bar, nl br	b					nl ea
		b					nl ns (ful
		f					nl ea
		f					nl ns (ful
		ns					

[1] Not sexed. [2] Including one Panamá phenotype (contaminant). [3] Have traces of yellow line. [4] One bar is ra

[6] Panamá shadow not scorable. [7] One ns for tooth, line, Panamá shadow. [8] Panamá shadow

nl ea 2/4 nl bf 1/0 nl ts 0/1			
nl tf 1/2[3]			11
nl br 6/3			28
bl br [5]2/3			13
nl ea 2/5 nl ea 0/2[6]	bl ea 0/1 bl br 2/0		26
nl ea 0/1			1
nl ea 1/0 nl br 0/1 (full bar)			3
nl vt 1/0 nl ea 0/2 wl ea 1/0 wr tf 1/0			17
nl ea 1/0			4
nl ea–br 4/5			9
bl ea–br 2/2 nl ns 2/2 (full bar) nl ea–br 3/1[6] nl tf 0/1 nl ns 0/1 (full bar) nl ea–br 1/2	bl–nl br–fd 0/1 (no rect.) bl–nl br–fd 1/0 (no rect.)	nl ea–br 2/0 nl ns 1/0 (full bar) nl ns 0/1 (full bar)	35
nl ea–br 1/0 nl ns 6/6 (full bar) nl ea–br 2/0 nl ns 2/3 (full bar)		nl ea–br 2/4 nl ns 1/5 (full bar) nl ea–br 3/2 nl ns 3/7 (full bar)	47
nl ea–br 0/2 nl ns 0/2 (full bar) nl ea–br 2/1 nl ns 2/0 (full bar)	nl ns 1/0 (rect. ns, full bar)	nl ea–br 1/0 nl ns 1/0 (full bar) nl ea–br 0/1	13

...e bar is rather thin.   [5] One not scored for rectangles.
...ná shadow not scored in one female.

(For more detailed description of lines and bars see table 11.)

brood	phenotype of parents (when known)	bar tip	offspring (♀/♂)				
			concave, tooth			intermediate, tooth	
			full rect., no Pan. bar	no rect., shadow bar	no rect., full bar	full rect., no Pan. bar	no rect., shadow bar
A	both intermediate, tooth, rectangles, no Panamá bar, bl, ea, b	b	yl bs 1/1			bl ts 0/1	
J2		b	yl bs 1/0	wr bf 1/0		bl ea [1]1/0	bl ea 1/0
							nl ea 1/3
		b					
		f		wr bf 1/0	wr bs 1/0	bl ea 1/0	vl ea 1/0
P		b	yl bs 1/2	wd tf 1/0		bl ea 0/1	nl ea 0/1
Q		b		wd bs 1/0		bl ea 1/0	nl ea 2/1
		f					nl ea 1/0
T		b				bl ea 5/5	

[1] Ambiguous, bar turns both ways at tip.

(The abbreviation wn stands for weak or no line; detailed scoring has not been performed for invaded butterflies in these broods: it is weak... butterflies is uncertain; some, particularly the radiate ones, probably have a weak basal line. Individual specimens, no longer extant, in ... line or bar have been omitted from the totals used in the text. In broods WK13 and WK15 it is possible tentatively to distinguish a con... invaded class (see p. 537).

brood and type	provenance and phenotype of parents (♀/♂)	forewing band	basal marks	offspring (♀/♂)			invaded convex tooth n...
				invaded, intermediate (very variable), slight or unscorable tooth			
				full rect.	faint rect.	no rect.	full rect
RF1 F1	East Brasil × Rondônia; East Brasil × Rondônia	red	radiate	very weak yellow line, no bar or slight trace of yellow scaling (see text)			
WK8 backcross	RF1 × East Brasil; radiate, invaded, convex red band, no rectangles, bl, fd, × East Brasil	red	radiate	bl fs 1/0 bl nb 0/1			bl nb 1/0 nl nb 2/1
			plain	bl tf 1/0			
WK9 backcross	East Brasil × RF1; East Brasil × radiate, invaded, intermediate red band, no rectangles, wl, nb (multiparental)	red	radiate	bl nb 1/0			nl nb 1/2
			plain	bl tf 0/2	bl nb 1/0 (rect. ns)		
				bl nb 1/0			
WK10 backcross	RF1 × East Brasil; radiate invaded intermediate red band, rectangles, nl fs × East Brasil	red	radiate	bl nb 1/1	bl nb 0/1 (rect.ns)		
				wl tf 0/2	bl nb 0/1 (line and rect. ns)		
			plain		bl nb 0/1		
WK11 backcross	East Brasil × RF1; East Brasil × radiate, invaded intermediate red band, no rectangles, nl nb	red	radiate				
			plain	bl fd 0/1			
WK12 backcross	East Brasil × RF1; East Brasil × radiate, invaded intermediate red band, no rectangles, nl, fs	red	radiate	bl nb [3]1/0 wn (fs–nb) 1/3[4]			yl nb 1/
			plain	bl tf 0/1			wn nb 1/ bl fs 1/0
WK13 F3	WK10 (or WK8 or WK12) × WK12; both radiate, invaded intermediate red band, rectangles, nl, fs	red	radiate	wn fs 6/3 wn nb [5]1/2			wn fs 1/ wn nb 1
			plain	wn fs 1/0 wn nb 3/1			wn nb 1
		yellow	radiate	wn fs 0/2 wn nb 1/1			wn fs 1/

	diate, tooth		convex, no tooth		
	...w rect., dow bar	no rect., full bar	full rect., no Pan bar.	no rect., shadow bar	total
			bl ea 0/1		4
	ea 1/0 ea 1/3 ea 1/0	nl ea 1/0 nl ea 1/0			14
	ea 0/1		bl ea 0/1		7
	ea 2/1 ea 1/0		bl ea 2/0	nl br 1/1	10
					10

(W.W.B.)

: it is weak or absent in all of them. The scoring of some nl (no line) extant, in which there is some uncertainty of scoring of the rectangles, guish a concave, invaded class, here lumped with the intermediate

(♀/♂)				
invaded, convex, tooth ns	entire, concave, tooth			
full rect.	full rect.	faint rect.	no. rect.	total
				14
	yl bs [1]3/0 yl bs 2/1	 yl bs 1/0		14
	yl bs 3/2[2] yl ea 1/0 yl bs 1/1 yl ea 1/0			17
	yl bs 4/1 yl bs 1/3			16
	yl bs 0/1	yl bb 0/1[1] yl bb 0/2	yl bb 0/1	6
yl nb 1/0 wn nb 1/1 bl fs 1/0	yl bs 2/2 yl bs 1/2	yl bb 1/0	yl bf 0/1 yl bb 1/0 yl bf 0/1	21
wn fs 1/0 wn nb 1/1 wn nb 1/1 wn fs 1/1	yl bs 1/2 yl bs 2/1			35

F_3	WK12; both radiate, invaded intermediate red band, rectangles, nl, fs		plain	wn nb ·1/2 [?] wn fs 1/0 wn nb 3/1	wn nb 1 wn nb 1 [?]
		yellow	radiate	wn fs 0/2 wn nb 1/1	wn fs 1/
			plain	bl tf 1/0	
WK14 F_4	WK13 × WK13; radiate, invaded concave yellow band, rectangles nl, nb × radiate, entire concave toothed red band, rectangles, yl, bs	red	radiate	wn fs 1/0 wn nb 2/1/1[5]	
		yellow	radiate	wn nb 2/0	
WK15 F_3	WK10 × WK10 (or WK12); radiate entire concave toothed red band, rectangles, yl bs × radiate, invaded intermediate, red band, rect. nl nb	red	radiate plain	wn nb 1/0 wn nb 1/0	
		yellow	radiate		
AP	WK8 × WK9; all entire, concave, tooth, full rectangles, yl, yb; plain × radiate; multiparental	red	radiate plain		

[1] Rectangles not certainly scorable.   [2] 1/1 Not now scorable for rectangles.   [3] Line also weakly   [4] Rectangles may have been faint or absent in the three males.   [5] Not sexed.

wn nb 1/1	yl bs 2/1	
wn nb 1/1		
wn fs 1/1		
	yl bs 0/1	13
	yl bs 3/2	
	yl bs 2/2	9
	yl bs 1/1	
	tl bs 0/1	
	yl bs 4/7	19
	yl bs 4/4	

lso weakly developed distally.
ot sexed.

APPENDIX 2. ALPHABETICAL LIST OF SPECIES OF *HELICONIUS* AND RELATED GENERA MENTIONED IN THE TEXT, WITH AUTHORS' NAMES

(For simplicity, authors' names are omitted in the text. Subspecific names have been completely ignored, except for *H. melpomene* and *H. erato* (see appendix 1). All are *Heliconius* (*sensu lato*) unless otherwise stated. For a summary of heliconiine systematics, see Brown (1981*b*).)

aliphera (Godart)	*iulia* (Fabricius) (genus *Dryas*)
antiochus (Linnaeus)	*juno* (Cramer) (genus *Dione*)
aoede (Hübner)	*luciana* Lichy
atthis Doubleday	*melpomene* (Linnaeus)
charitonia (Linnaeus)	*nattereri* Felder und Felder
cydno Doubleday	*numata* (Cramer)
demeter Staudinger	*pachinus* Salvin
doris[1] (Linnaeus)	*peruviana* Felder und Felder
elevatus Nöldner	*sapho* (Drury)
erato[1] (Linnaeus)	*sara* (Fabricius)
ethilla (Godart)	*tales* (Cramer)
hecalesia Hewitson	*telesiphe* Doubleday
hermathena Hewitson	*timareta* Hewitson
hewitsoni Staudinger	*wallacei* Reakrit

[1] There is a nomenclatural problem with these two Linnaean names (Turner 1967, 1984*a*); we are here following the commoner usage.

APPENDIX 3. AUTHORS' NAMES AND FAMILIES OF LEPIDOPTERA, OTHER THAN HELICONIINAE, DISCUSSED IN THE TEXT, ALPHABETICALLY BY GENUS

Amata phegea (Linnaeus)[1]	Ctenuchidae
Elzunia pavonii (Butler)	Ithomiidae[2]
Ithomia pellucida Weymer	Ithomiidae
Lycorea ceres (Cramer)[3]	Danaidae
Mechanitis polymnia (Linnaeus)	Ithomiidae
Tithorea harmonia (Cramer)	Ithomiidae
Zygaena ephialtes (Linnaeus)[1]	Zygaenidae
Zygaena transalpina (Esper)[1]	Zygaenidae

[1] Palaearctic; all others are neotropical.
[2] Many taxonomists now regard the Ithomiidae as a subfamily of the Danaidae.
[3] Recently renamed *Lycorea pieteri* Lamas, 1978, hence causing the species name to be *Lycorea cleobaea* (Godart).

APPENDIX 6. STATISTICAL SUMMARY OF BROODS

cross	table no.	number of sibships	total progeny
melpomene			
Belém × Trinidad/Venezuela	A1	33	980
Belém × East Brasil	A2	9	661
Bolívia × Rio Madeira	—	5	105
Belém × East Ecuador	A3	26	482
total	—	73	2228
erato			
Trinidad × East Brasil	A4	3	140
Panamá/México × East Brasil	A5	16	325
Additional broods	A6	5	45
Rondônia × East Brasil	A7	10	164
Manaus × East Brasil etc.	A8	5	78
Belém × East Brasil	A9	2	43
Uncertain provenance	A10	9	142
East Ecuador × East Brasil	A11	13	319
East Ecuador/Amazonian provenance	A12	6	65
total	—	69	1398
total, both species		142	3626

TABLE A 8. CROSSES WITH THE MANAUS RACE (K.S.B, W.W.B.)

('Manaus' and 'East Brasil' indicate the normal phenotypes for these races (figure 6). Except for the pure East Brasil and the father of WK16, the parents all lack the line and the rectangles. All offspring are red banded. Weak line (wl) is usually basal.)

brood and type	provenance and phenotype of parents (♀/♂)	red raylets	basal marks	broken[1] convex	broken[1] intermediate	broken[1] concave	entire, tooth convex	entire, tooth intermediate	entire, tooth concave	total
WK5 F1	Rio de Janeiro × Manaus; East Brasil × Manaus (Guiana)	no	dennis			nr nl nb ca. 20[2]				20
WK6 backcross	WK5 × Rio de Janeiro; dennis, red broken band, intermediate × East Brasil	no	dennis	(3 broken, otherwise not scored)		nr wl nb 0/1; nr nl nb 1/0		cr wl ea 0/1; nr wl fd 0/1		21
		yes	plain		cr nl fd 0/1; nr nl nb 0/1	cr wl fd 1/1; nr wl nb 0/1		cr wl ea 0/1; nr wl fd 0/1; nr wl ea 1/1; nr nl fd 2/0; cr yl bs 0/1; nr wl fd 1/0	cr yl bs 0/1[2]	
			dennis							
			plain							
WK3 F1	Manaus × Georgetown; Manaus × Trinidad/Venezuela (see p. 541)	no	dennis	nr nl nb 1/4						5
WK4 outcross	WK3 × Rio (or São Paulo); dennis, red broken band, convex × East Brasil	no	dennis plain				nr nl nb 1/0	nr nl nb 0/1		2
WK16	Rio Trombetas × [East Brasil × Rondônia] × East Brasil]; plain, red broken band, convex × radiate, entire red band, intermediate, tooth, cr yl bs	no	radiate plain	nr nl nb 4/2; nr nl nb 2/2			nr nl nb 4/5; nr nl nb 4/7			30

[1] On broken band concave is difficult to separate from intermediate and tooth cannot be scored reliably.   [2] Not sexed, band varies from concave to intermediate.

TABLE A 9. BELÉM × EAST BRASIL BROODS (K.S.B.)

brood and type	provenance and phenotype of parents (♀/♂)	forewing band	basal marks	broken convex, no rectangles	broken intermediate, no rectangles	entire, tooth intermediate, no rectangles	entire, tooth concave weak rectangles	total
F1(B) F1	Belém × Rio de Janeiro; Belém × East Brasil	red	radiate	nl nb 20[1]				20
WK7 F2	F1(B) × F1(B); both radiate, broken red band, convex to intermediate, nl nb	red	radiate	nl nb 2/0; nl nb 0/1[1]	nl nb 1/1; nl nb 2/2 (rectangles)	wl fd 0/1; wl fd 1/0	yl bs 0/1	23
		red	plain					
		yellow	radiate	nl nb 2/1 (faint rect.)	nl nb [2]5/2/1[1]			

[1] Not sexed.   [2] Two are intermediate to concave.

TABLE A 11. EAST ECUADOR × EAST BRASIL F

brood no. type and total	provenance and phenotype of parents (♀/♂)	band	costal spot	bar tip	split, 'short' rectangles round	flat	no rectangles round	flat
E1 (F₁) 50	São Paulo × East Ecuador; East Brasil × round, split, shortened, red and white band, nl, nb, no rectangles, no costal spot	red	present			nl fd 2/10 nl nb 12/4		
E2 (F₂) 8	E1 × E1; round, entire, shortened red band, nl fd, no rectangles, costal spot × entire band	red red red+white	present absent absent					
E3 (BC) 26	São Paulo (several ♀♀) × E1; East Brasil × entire, shortened, red band	red	present	b b f				
E3A (BC) 43	São Paulo × E1; East Brasil (unknown number of ♀♀) × round, entire, shortened, red band, no rectangles, nl, nb	red red	present ?absent	b b b f b b				
E5 (BC) 11	E1 × São Paulo; round, split, short, red band, nl, nb, no rectangles, costal spot × East Brasil	red	present	b b f	bl ea 1/2		nl fd 0/1	
E6 (F₂) 8³	E1 × E1; round, split, short, red band, nl, nb, no rectangles, costal spot × round, entire, shortened, red band, nl, nb, no rectangles, costal spot	red red+weak white	present present	b f b	wl ea 1/1			
E7 (BC) 48	E1 × São Paulo; round, split, short, red band, nl or ns, nb, fd or ns, no rectangles (or ns), costal spot (or ns) (4 ♀♀) × East Brasil	red	present	b b b b b f f	bl fa 1/1 bl fr 0/1 ns fa 0/1⁴ nl nb 1/0⁴ bl nb 1/0⁴	wl fa 0/2 bl fr 1/0	nl nb 0/1	nl fd 1/0 nl nb 0/
E8 (F₂) 4⁵	E1 × E1; entire, shortened, red band × split, short, red band	red red+weak white red+weak white	present present absent	b b b				
E10 (F₂) 13⁶	E1 × E1; round, split, short, red band, nl, nb, no rectangles, costal spot × round, entire, shortened, red band, nl, nb, no rectangles, costal spot	red red+weak white	present absent present absent	b b b b			nl nb 1/0	nl nb 1/
E11 (BC) 36	São Paulo × E1; East Brasil × round, split, short, red band, nl, nb, no rectangles	red	present	b b f	bl fa 0/1 bl fr 1/0	bl fa 0/1 bl fr 0/3 wl fr 1/0	nl fd 2/3 ⁷fd 1/0	nl fd 1/
E12 (BC) 60	São Paulo × E1 (or reverse); East Brasil × not recorded (contaminated)	red	present	b b b f f	bl tf 1/0 bl fa 1/6 bl fr 1/2	bl fa 0/1 bl fa 0/3 bl fr 1/3	nl nb 2/1	nl fd 0/ nl nb 1/
E14 (BC) 10	E1 × São Paulo; round, split, short, red band, nl, nb, no rectangles, costal spot × East Brasil	red	present	b	bl fd 1/0	bl tf 1/1		nl nb 2/
E15 (F₂) 2	E1 × E1; round, split, short, red band, nl, fd, no rectangles, costal spot × round, split, nl, nb, no rectangles	red	present	b	wl fr 0/1		nl nb 1/0	

All offspring and parents are plain.
Split and shortened refer to under surface only; short is lack of extension of band into posterior angle of w

BRASIL BROODS (P.M.S.)

	offspring (♀/♂)							
	entire, shortened				entire, long			
...ctangles	rectangles		no rectangles		rectangles		no rectangles	
flat	round	flat	round	flat	round	flat	round	flat
			nl fd 5/15 nl nb 0/2					
			nl nb 0/2 nl fd 1/0 nl fd 1/0 nl fd 1/0				wr bv 1/0 wl bv 0/1 wr bv 0/1	
	bl fa 0/1	bl tf 1/0 bl fa 1/0	nl nb 1/0 nl fd 2/4[1]	nl nb 1/0	yl bs 2/4	yl bs 2/1 yl bs 1/0	wr bv 2/0 wl bv 0/2	wr bv 0/1
	bl fa 0/1 bl tf 1/0 bl fa 2/3	bl fa 1/0 bl tf 2/0 bl br 0/1	nl fd 1/3	nl nb 2/1 nl nb 0/1	yl bs 2/1	yl bs 1/5	wl bv 2/2 wr bv 2/3	wr bv 1/1 wl bv 1/0 wr bv 1/0 nl bv 0/1[2]
						yl bs 3/0	wr bv 0/1 ns bv 1/0	wr bv 1/0 wl bv 0/1
			nl fd 0/1 nl fd 0/1				nl nb 1/0	
nl fd 1/0 nl nb 0/3					yl bs 4/2	yl bs 5/6	bl bv 3/1 wr bv 1/5	bl bv 3/2 wr bv 1/2
			nl fd 1/1				wr bv 1/0	
nl nb 1/0			nl fd 0/1 nl fd 0/1 nl fd 1/0				wr bv 2/1 wr bv 1/1	wr bv 1/0
nl fd 1/2	bl tf 1/0		nl fd 1/0		yl bs 2/0	bl bf 1/0 yl bs 1/6	wr bv 3/0	wr bv 1/4
nl fd 0/2 nl nb 1/3	bl fa 0/1[9] bl fa 0/1	bl fr 2/0		nl nb 1/0	yl bs 3/3	yl bs 0/0[8]	wr bv 3/3 wl bv 1/2	wl bv 2/4 wl bv 2/4
nl nb 2/1					yl bs 1/2		wl bv 0/1	

...r angle of wing: shortened is truncation at vein Cu1a.

2	nl, nb, no rectangles					

All offspring and parents are plain.

Split and shortened refer to under surface only; short is lack of extension of band into posterior angle of w

[1] One individual not extant; some minor characteristics (e.g. red spot) may differ for this class.

[2] Damaged, possible scoring error for line and costal spot.

[3] Includes 1/0 split, shortened, red, no rectangles, round, b, ns, fd, red spot, not scorable.

[4] Costal spot not scorable.

[5] Includes 0/1 split, shortened, red+weak white, red spot absent, no rectangles, round, b, nl, nb.

[6] Includes 0/1 split, shortened, red, red spot present, rectangles round, b, nl, nb and 0/1 split, shortene

[7] Trace of line.

[8] Not distinguishable from pure East Brasilian contaminants.

[9] Not scorable for split or rectangles.

angle of wing: shortened is truncation at vein Cu1a.

nb.
t, shortened, red+weak white, red spot present, no rectangles, round, b, nl, nb.

TABLE A 10. BROODS OF UNCERTAIN PROVENANCE, SEGREGATING FOR RONDÔNIAN AND EXTRA-AMAZONIAN ALLELES (P.M.S.)

offspring (♀/♂)

brood no. and total	provenance and phenotype (where recorded) of parents (♀/♂)	forewing band	basal marks	eyes	bar tip	invaded, convex, tooth ns — no rect., shadow bar	invaded, concave to intermediate tooth ns / entire, convex, no tooth — full rect., no Pan. bar	no rect., shadow bar	no rect., full bar	entire, intermediate tooth — full rect., no Pan. bar	no rect., shadow bar	no rect., intermediate	entire, concave, tooth — full rect., no Pan. bar	no rect., shadow bar	no rect., full bar
B 33	♀ of Panamá × East Brasil ancestry; ♂ ancestry includes East Brasil and Rondônia; ♀ entire, intermediate, toothed red band, plain, no line, no rectangles, shadow Panamá bar, bf, b	red	plain	wild	b; b; f; f; ns; ns; ns; ns	tf bl 0/2¹; tf nl ¹¹/1¹; br nl 0/1¹,²; fd nl 1/0	tf bl 1/0; tf bl 1/0; tf bl 1/0	br nl 0/1; tf bl 1/0; br nl ¹¹/0; tf bl 0/1¹; br nl 0/1¹; fd nl ¹¹/0	nb nl 0/1	ea bl 1/2; ea bl 0/1	ea bl 0/1¹; ea nl ¹¹/1¹; ea bl 0/1		bs yl 0/2³; bs yl ³1/0	bs wr 0/3; bs wr 0/1	bs wr 0/1; bs wr 0/1
U 5	♂ São Paulo; ♀ invaded red band, radiate; ♂ East Brasil	red	radiate	wild	ns		fd bl 1/0; fd bl ⁴1/1	fd bl 1/0 both concave	both intermediate						
C 12	♀ and ♂ derived from mixed Amazonian × Trinidad × Panamá stock; ♂ entire, intermediate red band, full rectangles, no Panamá bar, bl, ea, b	red; red	radiate; plain	wild; wild	b; b; f; f; b; f		br bl 0/1	fd nl 0/1		ea bl 1/0; tf bl 1/0	ea nl 2/1; ea nl 0/1		bs yl 0/1	tf wr 1/0; tf wr 2/0	
D 16	both radiate	red; yellow; red	radiate; radiate; plain	wild; pale; wild; pale; wild	b; b; b; b; b; b		ea bl 0/1 (tooth ns); ea bl 1/0			ea bl 1/1; bs yl 0/1; bs yl 1/0 (tooth ns); ea bl 0/1 (tooth ns)	bs yl 1/0 (tooth ns); ea bl 0/1 (tooth ns)		bs yl 1/0; bs yl 1/1; bs yl 1/1; bs yl 0/1; bs wr ⁶2/0		
H 25	—	red	radiate; plain	wild; wild	b; b; f; b; b; f		fd bl 1/0; br bl 1/1; bf bl 1/0; fd bl 0/1; fd bl 0/1			ea bl 1/1; ea bl 1/1; ea bl 1/1	tf nl 0/1; br nl 1/0; ea nl 0/1		bs bl 0/2; ea bl 0/1; bs yl 1/2	bs bl 2/1	
I 6	—	red; red	radiate; plain	wild; wild	f; f		br bl 0/1	br nl 1/0		ea bl 1/1			bs yl 1/0; bs yl 0/1		
R 11	♀ radiate, ♂ plain	red	radiate; plain	wild; wild	ns; ns		bf bl 0/3; bf bl 1/0			bf bl 0/2; bf bl 1/4					
S 7	both radiate	red; yellow; red	radiate; radiate; plain	wild; pale; wild; wild	ns; ns; ns; ns					bf bl 0/2; bf bl 0/2; tf yl 0/1			bs yl 1/0; bs yl ⁶1/0		
G 26	♀ São Paulo; ♂ possibly brood D; ♀ East Brasil; ♂ entire, convex red band, radiate, full rectangles, bl, bf	red; red	radiate; plain	wild; wild	b; b; b	(bs yl 1/0, no Panamá bar; band shape and rectangles ns)				ea bl 2/4; ea bl ⁵3/4			bs yl 2/5; ea yl 0/1⁶; bs yl 1/3		

¹ Traces of Panamanian bar on upperside.   ² Scoring of Brasilian ns for rectangles.   ³ Slight trace of red tip to line.
⁴ Could be nl.   ⁵ One individual ns for rectangles.   ⁶ Red tips to forewing line and hindwing bar.
² Scoring of Brasilian line and bar ambiguous.

TABLE A 12. MULTIRACIAL BROODS SEGREGATING FOR EAST ECUADORIAN AND AMAZONIAN GENES (P.M.S., J.R.G.T.)

(All offspring and parents have long bands, red (not orange) colouring (except for the mother of 2E which is orange) and red costal spots (except for five individuals in 3D and 5A which are not scorable for costal spot). All bars turn back at the tip unless scored as f.)

Header for offspring columns: offspring (♀/♂)

brood no. and total	provenance and phenotype of prents (♀/♂)	rectangles	band	radiate: split (undersurface) round	radiate: split (undersurface) flat	radiate: split (both surfaces) flat	radiate: entire round	radiate: entire flat	plain: split (undersurface) round	plain: split (undersurface) flat	plain: split (both surfaces) round	plain: split (both surfaces) flat	plain: entire round	plain: entire flat
2E — 7	radiate, yellow, entire, round band, no rectangles, nl, br, orange, red spot × not recorded	present absent absent	red red red+white (upper) and red+yellow (under)	ns nb 1/0 nl nb 1/0 ns fd 0/1			ns ea 0/1 ns br 1/1	ns br 0/1						
3D — 8	radiate, red, split (under) flat band, rectangles, bl tf (Ecuador style), red spot × plain, red split (under), flat band, rectangles, ns, tf (Ecuador style)	present	red			bl fa 1/1 ns fd 0/1 ns fa 1/0		bl br 0/1 nl ea 0/1		bl fa 0/1				bl ea 0/1
5A — 20[1]	radiate, red, split (under), flat band, rectangles, bl, fa, red spot × plain, red entire, flat band, rectangles, bl, bf, red spot[2]	present present present	red[3] yellow red/yellow		bl fa 1/1 ns fa 1/0 part split upper, bl, fa 0/1	bl fa 2/2		nl ea 2/0 ns ea 3/1[4] bl ea 1/1		bl fa 1/0				bl ea 1/0
5D — 4	5A × 5A; phenotypes not known	present	red+yellow red+weak white					bl ea 1/2[5] bl ea 0/1						
10A — 9	plain, red, split, round band, rectangles, 'broken sharp' bar × not recorded	present absent	red red						nl nb 3/1	bl fa f 1/0 bl fd 1/0	nl nb 1/0	bl fa f 1/0	bl ea 0/1	
E16 — 17	radiate, red, split, flat band, no rectangles, nl, nb, red spot × not recorded	present absent	red red		bl fa 2/2[6] nl nb 0/1			bl ea 3/0 nl br 2/1		bl fa 3/1				bl ea 1/0 nl br 1/0

[1] Includes two East Brasilian butterflies introduced as potential mates and accidentally preserved as part of the brood.
[2] Father must be incorrectly recorded.
[3] May include small traces of yellow.
[4] Male not scorable for rectangles.
[5] One of the males may be from 5A.
[6] Males not extant; not certainly fa rather than ea.

PLATE 1. For description see opposite.

PLATE 2. For description see opposite plate 1.

PLATE **3**. For description see opposite plate **4**.

PLATE **4**. For description see opposite.

APPENDIX 4. BROODS OF *HELICONIUS MELPOMENE* (TABLES A 1–A 3)

Breeders initials are given in the heading of each table.

Symbols and Abbreviations used in tables A 1–A 3

ns, not scorable; +, present; −, absent; Or, orange; BC, backcross.

Forewing lines: bl, basal line; fl, full line; hl, white line (with white in forewing band); nl, no line; pl, part line; wl, white line.

Ecuador triangles: bt, trace of triangle (with broken forewing band); ft, full triangle; lt, large triangle (smaller than ft); nt, no triangle (including minute traces); pt, part triangle; tt, trace of triangle.

Forewing bands: br, broken; bt, broken (with trace of Ecuador triangle); fu, fused; hl, white in band (with white forewing line); nw, no white; wh, white in forewing band; wu, white on underside (distinguished only in table A 3); Y, TY, S, TS, O, W: see figure 5 (Y, yellow; T, thin red: S, smudgy yellow; O, no colouring; W, wide red).

Cell spots: nc, no cell spot; hc, part cell spot (distinguished from sc in some broods only); sc, cell spot.

Hindwing bars: hb, shadow of bar on underside; nb, no bar; tb, trace of bar; yb, full yellow bar.

Belém spot: scored only in some broods and not tabulated. Segregation is described in the text.

TABLE A 1 BELÉM × TRINIDAD AND BELÉM × VENEZUELA BROODS (P.M.S., J.R.G.T.)

(No butterfly in this table has a full yellow forewing line or Ecuador triangle.)

brood no., type and total	provenance and phenotype of parents (♀/♂)	colour	forewing band, yellow bar	radiate Y	radiate TY	radiate S	radiate TS	radiate O	radiate W	plain TY	plain TS	plain W
YM2 F₁ (41)	Trinidad × Belém; Trinidad × Belém (orange) (same father as YM6)	red	broken nb				24/17					
YM6 F₁ (50)	Trinidad × Belém; Trinidad × Belém (orange) (same father as YM2)	red	broken nb				28/22					
YM10 (40)	Belém × Belém; normal (orange) phenotype × Belém phenotype with yellow hindwing bar (same father as YM12)	orange	broken yb / broken nb	7/14 / 8/11								
YM12 F₁ (1)	Trinidad × Belém; Trinidad × Belém phenotype with yellow hindwing bar (same father as YM10)	red	broken nb				0/1[1]					
YM15 F₂ (3)	YM6 × YM6; both normal F₁ phenotype (same father as YM10; see also YM21)	red / red / ns	broken nb / fused nb / fused nb			0/1	0/1					1/0
YM16 BC (14)	F1BG × Belém; radiate, TS (yellow strong), red × Belém (orange)	red / orange	broken nb / broken yb / broken nb	0/1	1/0 / 2/1	3/0[3] / 2/1[3]	0/2 / 1/0					
YM19 BC (1)	Belém × YM6; Belém (orange) × normal F₁ phenotype (same father as YM15)	red	broken nb	[1]1/0								
YM21 F₂ (199)	YM6 × YM6; normal F₁ phenotypes (4 ♀♀ including mother of YM15; 3 ♂♂ including father of YM15, YM19)	red / / / orange / / ns / / ns	broken nb / fused nb / ns nb / broken nb / fused nb / broken ns / fused ns / ns ns	4/0 / 0/1 / / 2/0 / 2/0 / 1/2 / /	4/2 / 0/1 / / 9/2 / 3/0 / / 0/1 /	5/8 / 0/3 / / 2/2 / 1/0 / / 0/1 /	9/14 / 4/4 / / 7/6 / / 1/3 / / 0/2	3/5 / 1/0 / 2/2 / 0/2 /	12/8 / 2/1 / 1/0 / 1/0 / 1/0 / 1/0	2/4 / 1/1 / / 2/0 /	6/10 / 0/6 / / 2/0 / 1/0 / 1/0	7/3 / 1/2 / / 0/1 / 1/1
YM27 BC (59)	YM2 × YM10; normal F₁ phenotype × Belém phenotype with yellow hindwing bar	red / orange / ns	broken nb / broken nb / broken nb	4/5 / 4/4	1/6 / 8/4[3]	0/5 / 2/2 / 1/0	1/3 / 3/6					
YM34 BC (31)	Belém × YM6; Belém × normal F₁ phenotype	red / orange	broken nb / broken nb	1/3 / 1/0	4/0 / 1/3	5/4 / 2/2	1/2 / 2/0					
YM39 BC (5)	YM2 × YM10; normal F₁ phenotype × Belém phenotype with yellow hindwing bar	red / orange	broken nb / broken nb	0/1		1/0 / 0/3						
YM43 (36)	YM2 × YM21; normal F₁ phenotype × plain, W, fused	red / / / orange / ns	broken nb / fused nb / ns ns / broken nb / fused nb / ns nb				1/0 / / / 2/2		0/1 / 1/3 / 1/0 / 2/0 / 2/1 / 3[4]		1/3 / / / 2/4[13] / 1/0	1/3 / 1[4] / / / 0/1
YM44 (34)	YM21 × YM21; plain, W, fused, red × plain, W, fused, pale orange red	red / red / orange / ns	broken nb / fused nb / fused nb / ns nb						0/1[1]			8/17[5] / 4/2 / 1[4]
YM46 BC (7)	YM6 × Belém; normal F₁ phenotype × unobserved stock male	ns	broken nb		2/1	1/2	1/0					
YM47 BC (25)	offspring of YM46 contaminated with pure Belém stock	ns	broken nb	[6]4/10	2/2	2/2	1/2					
YM48 BC (9) (18)	offspring of YM46 contaminated with a mating YM10 × YM10, both yellow barred Belém phenotypes	ns	broken yb / broken nb	2/7[7] / 0/3	/ 1/3	/ 3/3	/ 0/5					
YM49 (2)	YM21 × YM21; radiate, O, ns, red × radiate, O, fused, red	salmon pink / orange?	broken nb / broken nb				1/0				[1]1/0	
YM51 (46)	YM27 × YM27; radiate, S, broken, orange × radiate, S, broken, red (same father as YM52)	red[8]	broken yb / broken nb	5/7 / 2/0			9/14	[1]1/0 / 4/4				
YM52 (4)	YM21 × YM27; plain, TY, fused, red × radiate, S, broken, red (same father as YM51)	red?	broken nb / broken tb / fused nb	0/1	0/1 / 0/1		0/1					
YM57 (53)	YM49 × YM27 or YM34; radiate, S, broken, salmon pink × radiate, TY, broken, red	red	broken nb			12/7	9/15					
YM64 (7)	YM51 × YM51; both radiate, O, broken, red	red / red	broken nb / fused nb					4/8 / 1/4				
YM65 (42)	YM43 × YM51; plain, TS, fused, strong yellow, ?orange × radiate, O, broken, red	red / / orange	broken nb / fused nb / broken nb / fused nb			[1]1/0 / / 0/1[1]	0/1 / 0/1 / 7/2 / 3/3		0/3 / 0/5 / 9/0 / 6/0			
BTFG F₁ (50)	Trinidad × Belém; Trinidad × Belém (colour ns) (or, less probably, the reciprocal)	ns	broken nb				18/26[9]					
TBFT BC (18)	BTFG × Trinidad (or reciprocal)[10]	red / orange	broken nb / fused nb / broken nb				0/1 / 2/3 / [1]2/0		4/2 / 0/2		2/0	
B1TN BC (5)	BTFG × Trinidad (or reciprocal) (may be same brood as TBFT)[10]	red	broken nb / fused nb				0/1		1/0	1/0		0/2
F1BG F₁ (4)	Trinidad × Belém; Trinidad × unknown[11]	red	broken nb		2/1		0/1					
TBF2 (8)	both F1BG; both radiate, apparent TY broken, red[12]	red / / / orange /	broken yb / fused nb / fused nb / fused nb / broken yb	0/1 / / / 1/1			/ / / / 1/0		/ / 1/0			1/0 / 1/0 / / / [1]1/0
CF3L (8)	?TBF2 × TBF2; unrecorded × radiate, apparent TY, fused, orange	red / orange	fused hb / fused hb / fused yb / ns yb	0/1 / 1/1			1/0 / 1/0		1/1			1/0
F1F3 (9)	ESF1 (table A2) × CF3L (or reciprocal); phenotypes not recorded[13]	red	broken hb / fused hb / broken ns / fused ns				1/0		0/3 / 1/0 / / 1/0			0/1 / 0/2
CF3T (2)	Trinidad × CF3L; Trinidad × unrecorded	red	fused hb								1/0	0/1
14C (13)	Quebrada Grande × Belém; Venezuela × Belém	red	broken nb				5/8					
19F (1)	Belém × 14C; Belém × normal F₁ phenotype	orange	broken nb			0/1						

[1] Contaminant, or possible contaminant (or misattribution in TBF2, see table A 2, footnote 7).
[2] S and Y not clearly distinguishable in this brood.
[3] One ♀ may be from YM34 or YM47.
[4] Not scored for sex.
[5] 3/2 not scored for fused; 1/0 not scored for colour.
[6] Includes pure Belém contaminants, many with yellow bars.
[7] Most or all contaminants in this category.
[8] A minority are rather orange (?fading).
[9] Also 4/2 radiate TS with strong yellow which may be from F1BG, and one obvious Belém × East Brasil F₁.
[10] Most TS fused bands have red in the areas that are usually yellow; both plain W butterflies have diagonal red stripe at apex of hindwing (unique to this brood).
[11] See text (p. 476) for details of offspring phenotypes; there are further 1/1 radiate 'TY' and 0/2 radiate 'TS' preserved that appear to be members of this brood.
[12] It is likely that the recorded pedigree of these broods is in error (see p. 476)
[13] One female has yellow bar shadow underside.

TABLE A 2. BELÉM × EAST BRASIL BROODS (P.M.S., J.R.G.T.)

brood no., type and total	provenance and phenotype of parents (♀/♂)	cell spot	colour	radiate, no yellow bar				radiate, yellow bar		plain, no bar	plain, shadow of bar		plain, yellow bar	
				yellow	yellow+ thin red	no band	wide red	no band	wide red	yellow+ thin red	yellow+ thin red	wide red	yellow+ thin red	wide red
ESF1 F1 (54)[1]	Belém × Espirito Santo; Belém × East Brasil (or reciprocal)	−	red		bl nt 24/26 bl pt 0/2									
CF2 F2 (169)[2]	ESF1×ESF1 (2 ♀♀, several ♂♂); all normal F1 phenotype	+	red	bl nt 4/3 pl nt 1/0	bl nt 4/8 bl pt 0/1			fl nt 0/1	bl nt 1/1 fl nt 2/1 fl pt 0/1		bl nt 1/1		pl nt 2/0	
		+	orange		bl nt 2/1			fl nt 1/0 pl pt 0/1		bl nt 0/1	bl nt 3/3 bl pt 1/2 bl ft 1/0			fl nt 1/0
		−	red	bl nt 4/5 bl pt 2/1	bl nt 17/14 bl pt 1/3 pl nt 1/1 pl pt 1/0 pl lt 0/2	bl nt 2/3		pl nt 0/2 fl pt 2/0 fl lt 1/0	pl nt 1/0 fl nt 0/2 fl pt 3/1 fl ft 0/1	bl nt 4/5 bl pt 1/0			pl nt 0/1	pl pt 0/1 pl ft 0/1 fl nt 1/2 fl pt 1/0
		−	orange	bl nt 3/0 bl pt 1/1	bl nt 2/4 bl pt 1/1 pl pt 1/0	bl nt 1/0		bl nt 2/0 bl pt 0/1	pl nt 1/2 fl nt 0/1 fl pt 1/0	bl nt 3/0 bl pt 2/0	bl nt 1/1 nl pt 0/1		pl nt 0/1	
NF2 F2 (98)	ESF1×ESF1 (1 ♀); both normal F1 phenotype	+	red	bl nt 2/2 nl nt 1/1	ns ns 0/1 nl nt 1/2 bl nt 1/2			nl nt 0/1 pl nt 0/1 bl pt 0/1	bl nt 0/2 pl nt 0/1		nl nt 1/1 bl nt 1/0			
		+	orange	ns nt 0/1	bl nt 2/2 nl nt 2/0 ns ns 1/0			bl pt 0/1	nl nt 2/0					nl nt 0/3
		−	red	bl nt 5/1 bl ft 1/1 nl pt 1/1 ns ns 1/0	nl nt 3/1 nl pt 1/4 bl pt 2/1 bl nt 0/2		nl nt 1/0	pl nt 0/1 pl ft 0/1	nl nt 0/1 nl lt 1/0 bl pt 1/0		nl pt 1/0 bl nt 1/3 pl lt 1/0	nl nt[3] 2/1		nl pt 1/0 bl pt 0/1
		−	orange		bl nt 5/0 nl pt 1/0 nl nt 1/1			ns pt 0/1 nl pt 0/1	nl pt 0/1 nl ft 0/1 bl ft 1/1	bl nt 2/0	bl pt 0/1	nl nt 0/1		bl pt 1/0 nl ft 0/1
YM59 F2 (12)[4]	ESF1×ESF1; both normal F1 phenotype	+	red								bl nt 1/1			
		+	orange		bl nt 0/1						bl nt 1/0			
		−	red		bl nt 1/2	bl nt 1/0					bl nt 1/0			
		−	orange						pl nt 2/0		bl nt 1/0			
YM63 F2 (20)	ESF1×ESF1; both normal F1 phenotype	+	red	ns nt 0/2	bl nt 1/0									
		+	orange											pl nt 0/1
		−	red	ns nt 1/1 bl nt 1/0 ns pt 1/0	ns nt 1/1 ns pt 1/0	bl nt 1/0		pl nt 0/1			ns nt 2/1[5]			pl nt 1/0
		−	orange	bl nt 1/1							ns nt 0/1			
F1TM backcross Trinidad (142)[6]	Trinidad × ESF1 (several ♀♀, probably only 1 or 2 fertile, 1 ♂); Trinidad × normal F1 phenotype	+	red		nl nt 5/5			nl nt 5/5	nl nt 1/2	nl nt 8/5		nl nt 1/2		nl nt 5/3
		+	orange		nl nt 1/0			nl nt 1/0		nl nt 2/0				nl nt 1/1
		−	red		nl nt 5/8			nl nt 1/2	nl nt 1/4	nl nt 5/7	nl nt 0/1	nl nt 8/8		nl nt 7/12
		−	orange		nl nt 2/3 nl pt 1/0			nl nt 0/1	nl nt 1/1	nl nt 1/1				nl nt 2/0
YM66[7] testcross (22)	YM64 (table A 1) × YM59 or YM63; radiate O fused, cell spot, no yellow bar × radiate, thin red+yellow band, ns cell spot, no yellow bar (mother's band may have been S)	+	red	nl nt 1/0	nl nt 0/1	nl nt 1/2 pl nt 0/1	nl nt 1/0							
		−	red	nl nt 2/1	nl nt 0/1	nl nt 3/2	nl nt 2/2				pl nt 1/0	pl nt 1/0		
F1BM backcross Belém (127)	Belém × ESF1 (4 ♀♀, 1 ♂); Belém × normal F1 phenotype	+	red	bl nt 7/12	bl nt 6/6 bl pt 1/0									
		+	orange	bl nt 17/6 bl pt 1/0	bl nt 13/10									
		−	red	bl nt 8/6 bl pt 2/0	bl nt 2/9									
		−	orange	bl nt 5/2 bl pt 1/1	bl nt 5/4 bl pt 2/1									
CYHW testcross (7)	Both TBF2 (table A 1); plain, W, yellow bar, yellow line, cell spot, (red/orange ns) × unrecorded (see p. 476)[8]	+	red								bl br 2/1			fl fu 0/2
		+	orange								bl br 1/2			fl br 2/0
		−	red								bl br 0/1			fl bt 1/0
		−	orange								bl br 2/1			fl fu 0/1 fl br 1/0

[1] Includes two males without yellow in the band and with a shadow of the cell spot, which are clearly contaminants. Of the 'pt' individuals, one has the triangle strongly developed and may be a contaminant from the F2; the other shows only a minute trace; a few individuals show traces of the yellow bar on the underside, and one or two of these have a sprinkling of scales on the upperside.

[2] Including radiate, yellow bar, red and thin yellow band, cell spot +, orange 0/1. The bl category in this brood includes some ns individuals.

[3] One not scorable for shadow of bar.

[4] Contains some individuals from YM63.

[5] 'Male' not scorable for sex, shadow of bar, or red versus orange.

[6] The nl category contains a few basal lines (bl); in this brood only, the yellow bar is merely a light sprinkling of yellow scales in that region of the hindwing. Brood also includes three males with the yellow bar unscorable, one plain wide red band, one radiate, wide red band, one radiate thin red+yellow band, all red (not orange) and lacking cell spots. There are also some contaminants, one an East Brasilian phenotype, and some radiate phenotypes which appear to be from F1BM.

[7] Among radiate individuals in this brood, no distinction is made between nl and bl; all yellow band individuals have a weak yellow band (S phenotype).

[8] Attribution of parents to TBF2 almost certainly incorrect, as all progeny show phenotypes characteristic of strong East Brasil ancestry (e.g. tooth). All progeny nt unless otherwise described.

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TABLE A 3. BELÉM × EAST ECUADOR BROODS (P.M.S., J.R.G.T.)

brood no., type and total	provenance and phenotype of parents (♀/♂)	colour	forewing band and basal spot	radiate yellow +spot	radiate yellow −spot	radiate red+yellow +spot	radiate red+yellow −spot	radiate red[11] −spot	plain red+yellow +spot	plain red+yellow −spot	plain red[12] −spot
M1 F₁ (64)	Belém × Palora; Belém × East Ecuador (several ♀♀)	red	short				(see footnote 13) sc nt wh 0/1 sc tt nw 20/10 sc pt wh 22/8	sc nt nw 0/1			
M3 BC (6)	Belém × M1; Belém × normal F₁ phenotype (♀ same as one parent of M1)	red red orange orange	short long short long				sc nt nw 1/0 sc nt nw 0/1 sc nt nw 0/2				
M4C F₂ (16)	M1 × M1; normal F₁ phenotypes; possibly contaminated (see text)	red red orange	short long short	sc nt nw 1/0	sc nt nw 1/0 sc pt nw 0/1 sc ft wh 1/0 sc nt nw 2/1	sc tt wh 0/1	sc ft 0/1 sc pt wh 0/1 sc nt lw 0/1 sc ft lw 0/1 sc ft nw 0/1	sc pt nw 0/1		sc pt wh 0/1	sc ft wh 1/0
M4D F₂ (54)	same brood as M4C; heavily contaminated (see text)	red[2] red orange orange red or orange	short long short long long	sc nt nw 0/1[2] sc pt nw 1/0 (cs nt nw 20/14)[1]	sc nt nw 1/1 sc pt nw 1/0 sc nt wh 0/1[4]		sc ft wh 1/1 sc nt nw 1/0 sc tt nw 1/0 sc nt nw 1/0 sc nt nw 1/0 sc pt nw 1/0		sc pt wh 1/0 sc nt wh 0/1[2]	sc nt wh 2/0 sc ft wh 1/1 sc nt wh 1/0	
M5 (F₂?) (3)	not recorded	red orange	short short				sc nt wl 1/0 sc nt hl 0/1			sc pt wh 0/1[2]	
M6 F₂ (17)	M1 × M1; both normal F₁ phenotype	red red orange orange	short long long long	sc ft nw 0/1 sc nt nw 1/0 sc nt nw 0/1	sc nt nw 0/2 sc tt nw 1/0 sc nt nw 0/1	sc ft nw 0/1 sc tt nw 1/0	sc nt nw 1/0 sc nt wh 1/0 sc pt hl 0/1 sc nt wl 0/1 sc nt nw 1/0	sc ft wh 0/1		sc nt wh 1/0	cs ft wh 0/1
M7 F₁ (18)	Belém × Pastaza; Belém × plain, red and white short band, no basal spot, no cell spot (i.e. East Ecuador introgressed Upper Amazon)	red red	short long				sc nt nw 2/0 nc nt nw 1/0 sc nt wh 0/2 sc pt wl 1/0 sc nt wl 1/0 sc pt wh 1/0 sc nt nw 0/1 nc nt nw 2/0 nc nt nw 1/0 nc nt nw 0/4 nc nt wl 1/1				
M8 BC (44)	Belém × M1; Belém × radiate, red+yellow band, no basal spot, short, cell spot, no triangle, ? no white	red[2] red orange orange	short long short long	sc nt nw 2/1 sc nt nw 3/6 sc tt nw 2/3 sc nt nw 1/1 sc nt nw 7/1 sc tt nw 1/0	sc nt nw 2/1 sc nt nw 0/1	sc nt nw 1/1 sc nt nw 3/1 sc nt nw 0/1	sc nt nw 0/3 sc nt nw 0/1 sc nt nw 1/0 sc nt wl 1/0				
M9 BC (66)	Belém × M1; Belém × radiate, red+yellow band, no basal spot, short, (cell spot not recorded), no triangle, ? no white	red red orange orange not scored	short long short long short	sc nt nw 3/4 sc tt nw 2/1 sc nt nw 1/2 sc tt nw 1/0 sc nt nw 1/0 ns ns nw 1/0	sc nt nw 2/3 sc tt nw 2/1 sc nt nw 1/4 sc nt nw 5/1 ns ns nw 2/0	sc nt nw 0/1	sc nt nw 0/2 sc nt nw 0/3 sc nt nw 3/1 sc nt nw 4/4 sc tt nw 2/0 sc nt nw 0/3				
M16 F₂ (5)	M7 × M7; both radiate, red and yellow band, no basal spot, short, cell spot, part triangle, no white	red red	short long	sc ft nw 1/0 sc nt nw 0/1	sc ft nw 1/0 sc nt nw 0/1		sc ft wh[b,¹] 1/0				
M17 BC (6)	Belém × M7; radiate, red, yellow and white band, short, no basal spot, no cell spot, no triangle	not scored	short		nc nt nw 0/2 sc ns nw 0/1		sc nt wh 0/1 sc tt wh 1/0 nc nt nw 1/0				
M18 F₂ (2)	M7 × M7; both radiate, red and yellow band, no cell spot, no triangle, short; ♂ only with white in band	not scored	short long		sc nt nw 1/0						nc nt wh 0/1
M19 F₂ (4)	M7 × M7; both radiate, red and yellow band, no basal spot, no triangle; ♀ long, no cell spot, ♂ not scored for these characters	see footnote 5	short long				nc nt nw 0/1	nc nt nw[2]1/0 sc nt nw[2]1/1[2]			
M21 BC (17)	Belém (orange) × radiate, red and yellow band, no basal spot, short, no cell spot, no triangle, red, no white	red red orange orange	short long short long	sc nt nw 1/0 sc nt nw 2/0	sc nt nw 2/0 hc nt nw 0/1 sc nt nw 1/0 sc nt nw 2/0 hc nt nw 2/0	hc nt nw 0/1 hc nt nw 1/0	sc nt nw 1/0 sc nt nw 1/0 sc nt wh 0/1 nc nt nw 1/0 hc nt nw 0/1				
M23 (6)	Both unknown; plain, red, yellow and white band, no basal spot, short, cell spot, no triangle, red × radiate, red, yellow and white band, short, cell spot not scored, no triangle	red red	short long	sc nt nw 1/0			sc ft hl[2]1/0 sc nt nw[2]2/0		sc ft wh[2]1/0	sc ft wh 1/0	
M24 (4)	Belém × ?; Belém (orange) × plain, red band, no basal spot, short, cell spot not scored, full triangle, no white	red orange	short short				sc nt wh 2/0 sc nt nw 1/0	sc nt wh 0/1[7]			
M26 BC (37)	Belém × M7; Belém (red) × unrecorded phenotype	red	long	sc nt nw 1/0 sc nt nw 4/0 nc nt nw 4/0	sc tt nw 1/1 nc nt nw 4/7	sc nt nw 1/1 nc nt nw 0/3	sc nt nw 0/1 sc nt nw 2/0 sc pt nw 0/1 nc nt nw 4/1				
M27 BC (27)	Belém × M7; phenotypes not recorded	red red orange orange	short long short long	sc nt nw 3/1 sc tt nw 1/0 sc nt nw 0/1 sc tt nw 0/1 sc nt nw 1/0	sc nt nw 1/1 sc tt nw 0/1 sc nt nw 2/0 sc nt nw 1/1	sc nt nw[2]1/0 sc nt nw 1/0	sc nt nw 1/2 sc nt nw 1/1 sc nt nw 1/1 sc nt wh 0/1 sc tt wh 1/0				
M30 BC (7)	Belém × 'F₂' (= M7); Belém (red) × radiate, red (and yellow) band, short, no triangle, no cell spot, red (otherwise not recorded)	red red orange	short long short	sc nt nw 1/0 sc nt nw 0/1	sc nt nw 1/0 sc tt nw 0/1	nc nt wl 0/1	nc nt nw 1/1 nc nt nw 1/0 nc nt wh 1/0				
1A BC (37)	M7 × Belém; radiate, red and yellow band, no basal spot, long, no cell spot, no white, red × Belém	red red	long short	sc nt nw 3/1 sc nt nw 0/3[15]	sc nt nw 2/4 nc nt nw 1/3	sc nt nw[14]1/4 nc nt nw 2/1 nc nt wh 0/1	sc nt nw[14]2/1 (sc nt nw 1/0)[5] nc nt nw 2/0				
2A (5)	1A × 1A; radiate, red and yellow band, traces of white, basal spot, long, no cell spot, no triangle, red × radiate, yellow band, no basal spot, long, long, no cell spot, no triangle, no white, red	not scored	long		nc nt nw 1/0	nc nt nw 1/0	sc nt nw[2]0/0 nc nt nw 0/1				
3E (7)	Belém × 19B; Belém × radiate, red, yellow and white band, basal spot, short, cell spot, trace of triangle, red	red	long short				sc nt nw 0/1 sc tt nw 0/1 sc nt nw 0/3 sc nt nw 0/2				
11A (8)[16]	M4D × M4D; radiate, red and yellow band, ? no basal spot, long, cell spot, trace of triangle, no white × plain, red and white band, no basal spot, short, cell spot, full triangle, red	red (or ns)	short				sc pt wl 1/0	sc ft wh 1/0	sc pt wh 0/1	sc pt wh[5] 1/2	sc ft wu 1/0
12A (9)	11A × 11A; plain, red band, short (split), no basal spot, cell spot, full triangle, band white on underside × radiate, red and white band (traces of yellow), short (split), basal spot ns, ? cell spot, triangle not scored	red	short	(sc nt nw 0/1)			sc tt wh 0/1[2] sc pt nw 0/1[2]	sc pt wu 0/1 sc ft nw 1/0		sc ft wh[5] 1/0 sc tt wh[5] 1/0	sc ft nw 1/0 sc ft wh 0/1
15B (1)	1A × 1A; radiate, red and yellow band, long, basal spot, no cell spot, no triangle, no white, red × radiate, yellow band, long, no basal spot, no cell spot, no triangle, no white, red	red	long	nc nt nw 0/1							
16D (4)	11A × 1A; plain, yellow band, short, basal spot ns, cell spot, no triangle, no white × radiate, yellow band, long, no basal spot, no cell spot, no triangle, no white, red	red orange orange	short short long	sc nt nw 0/1			sc nt nw[2]1/0 sc tt wh 0/1 (sc tt nw 0/1[2])[2]				
19B (8)	1A × 11A; radiate, red and yellow band, long, no basal spot, cell spot, no triangle, no white, red × plain, red, yellow and white band, short, basal spot, part triangle, red	red red (some app. faded)	long short				sc tt nw 1/0 sc nt nw 0/1	sc nt wh 1/0 sc tt wh 0/1		sc tt wh 0/1 sc pt wh 1/0	sc pt wh[5] 1/1

¹ Possible contaminants (mixture of contaminants and brood members in M4D).
² Basal spot not scorable. Column assigned on rest of N locus phenotype.
³ Band is red (or red and white) with traces of yellow. In brood 12A, white represented by traces only.
⁴ Has weak basal spot, but appears to be N[B]N[B].
⁵ Probably orange; remainder of brood red.
⁶ One may be orange.
⁷ Includes three of doubtful phenotype.
⁸ Part of hindwing bar, in white, on underside.
⁹ Includes one not scorable for red colour.
¹⁰ Includes female parent of 16D, which was only partly scored (see brood 16D for phenotype).
¹¹ Split if short; broken (as in Belém) if long.
¹² If short, the red band is split, as are any white areas within it. The long red bands are (a) in M6, a long wide (not split) red band mixed with a short, split, white band, (b) in M4C, a long broken red band with some white mixture throughout, (c) in M4D, a long, split band distally and in the posterior of the cell spot, white proximally and in the anterior of the cell spot.
¹³ White limited in extent, absent in many. Total includes three individuals with long bands, all presumed contaminants (two Belém and one apparently from a backcross).
¹⁴ Includes half of a sexual and somatic mosaic.
¹⁵ Includes halves of two sexual and somatic mosaics.

Breeders initials are given in the heading of each table.

Symbols and Abbreviations used in tables A 4–A 12

ns, not scorable; Pan., Panamá; rect., rectangles; BC, backcross.

Yellow lines: bl, basal; nl, none; nr, none, red spot; rl, red line; vl, very weak basal line; wd, weak basal line, red dot; wl, weak medial (excluding table A 8; see head of table); wn, weak or no line; wr, weak medial, red tip; yl, full.

Hindwing bars (see figure 7): b, tip turns back; bb, broad sharp with black veins, slightly eaten; bf, broad fuzzy; br, broken fuzzy; bs, broad sharp; bv, broad with black veins; ea, eaten; f, tip turns forward (double in tables A 11 and A 12); fa, fuzzy eaten (East Ecuador type); fd, fuzzy dot; fr, broken fuzzy (East Ecuador type); fs, faint dusting of yellow scales; nb, none; ts, thin sharp; tf, thin fuzzy; vt, very thin fuzzy.

Cream rectangles: cr, present; nr, absent.

TABLE A4. TRINIDAD × EAST BRASIL BROODS (P.M.S.)
(All broods multiparental (see p. 505)

brood no. and type	provenance and phenotype of parents (♀/♂)	bar tip	concave, toothed			intermediate, toothed			convex toothless	total
			full rect.	weak rect.	no rect.	full rect.	weak rect.	no rect.	no rect.	
TF1 F1	São Paulo × Trinidad; East Brasil × Trinidad		38/25 (see text for description of phenotypes)							63
TF2 F2	TF1 × TF1; both intermediate, tooth, no rectangles, nl, fd	b	yl bs 1/1 bl ea 1/1	wr bf 2/1	wr bf 0/1 wr tf 1/0 nr vt 0/1 nl br 0/1	bl ea 3/1		nl br 0/1 nl fd 2/0 nl nb 1/0	nl br 0/3 nl fd 1/2 nl nb 0/2	41
		f ns	yl bs 1/1 nl nb 0/1[1]		wr tf 1/0 nl fd 3/1 nl nb 1/1	bl ea 2/0		nl br 1/0	nl 1/0[4]	
B1T BC	São Paulo × TF1; East Brasil × intermediate, tooth, no rect., nl, fd, b	b	yl bs 7/7	wr bf 1/4	wl tf 1/1	bl ea 6/2[2]	nl br 3/1	nl fd 3/0[3]		36

[1] Rectangles ns. [2] One ♀ is indistinguishable from br. [3] Very weak rectangles in one of these. [4] Rectangles and bar ns.

TABLE A 5. Panamá × East Brasil, México × East Brasil, and other broods of that provenance (P.M.S., K.S.B., W.W.B., M.C.S.)

brood no. and type	provenance and phenotype of parents (♀/♂)	bar tip	concave, toothed — full rectangles, no Panamá bar	concave, toothed — no rectangles, shadow bar	concave to intermediate, toothed — full rectangles, no Panamá bar	concave to intermediate, toothed — no rectangles, shadow bar	convex, toothless — full rectangles, no Panamá bar	convex, toothless — no rectangles, shadow bar	total
SF1 (F₁)	Panamá × São Paulo; Panamá × East Brasil		11/3 (see text for description of offspring)						14
RF1 (F₁)	2 ♀ Panamá × 1 ♂ Rio de Janeiro; Panamá × East Brasil		23/20 (see text for description of offspring)						43
WK2 (F₂)	RF1 × RF1 composite brood	b b b b f f ns ns ns	yl bs 1/0	wr tf 1/0	bl ea 1/0 bl br 1/1 nl bf 0/1 (no rectangles)	bl br 1/0 nl tf 0/1 nl ea 0/1 nl br 2/2 nl br 1/2 nl tf 1/0 nl br 1/1[1] nl fd 1/1[1]	bl br 0/2 nl br 0/1 bl br 1/0 bl fd 1/0	nl tf 1/0 nl br 1/0 nl fd 1/0 nl ns 0/2 (full bar) nl ns 0/1[1] (full bar)	32
B1 (BC)	several ♀ São Paulo × ♂ SF1	b	yl bs 8/1	yl bs 0/1 yl bf 1/0 bl bf 0/1 wr bs 0/1	bl ea [3]4/4 bl bf 0/1	wr bf 2/1 bl bf 1/0 nl ea 2/4 nl bf 1/0 nl ts 0/1			34[3]
B1(2) (BC)	São Paulo × SF1	b	yl bs 2/3		bl ea 0/1	nl tf 1/2[3]			11
WK1 (BC)	Rio × RF1; East Brasil × intermediate, tooth, no rectangles, shadow bar, nl bf b	b	yl bs 4/2/2[1]	wr bf 4/2/1[1]	bl ea 3/1	nl br 6/3			28
J	B1(2) × B1(2)	b	yl bs [4]4/2		bl br 2/3	bl br [5]2/3			13
K	B1 × B1	b b	yl bs 1/0	wr bs 2/2 wr bs [6]1/0	bs ea 4/3 bl ea 0/1	nl ea 2/5 nl ea 0/2[6]	bl ea 0/1 bl br 2/0		26
L	B1 × B1	b				nl ea 0/1			1
M	B1 × B1	b		wr tf 0/1		nl ea 1/0 nl br 0/1 (full bar)			3
N	B1 × B1	b	yl bs 2/6	wr tf 1/0	bl bf 0/2 bl ea 0/1	nl vt 1/0 nl ea 0/2 wl ea 1/0 wr tf 1/0			17
O	B1 × B1	b			bl ea 1/2	nl ea 1/0			4
MF1 (F₁)	México × São Paulo; México × East Brasil	ns				nl ea–br 4/5			9
MF2 (F₂)	MF1 × MF1	b b b f f f ns	yl bs–ts 1/1[6] yl bs–ts 0/1	wr–wl ts 0/1 rl ns 2/0 (full bar) wr–wl tf 0/1	bi ea [7]2/2 bl br 0/1 (no rectangles)	bl ea–br 2/2 nl ns 2/2 (full bar) nl ea–br 3/1[6] nl tf 0/1 nl ns 0/1 (full bar) nl ea–br 1/2	bl–nl br–fd 0/1 (no rect.) bl–nl br–fd 1/0 (no rect.)	nl ea–br 2/0 nl ns 1/0 (full bar) nl ns 0/1 (full bar)	35
MB2 (BC)	MF1 × México; ♀ intermediate, tooth, no rectangles, shadow bar, nl ea	b b f f				nl ea–br 1/0 nl ns 6/6 (full bar) nl ea–br 2/0 nl ns 2/3 (full bar)		nl ea–br 2/4 nl ns 1/5 (full bar) nl ea–br 3/2 nl ns 3/7 (full bar)	47
MB13 (BC)	MF1 × México; ♀ concave, tooth, no rectangles, shadow bar, nl br	b b f f ns				nl ea–br 0/2 nl ns 0/2 (full bar) nl ea–br 2/1 nl ns 2/0 (full bar)		nl ea–br 1/0 nl ns 1/0 (full bar) nl ea–br 0/1 nl ns 1/0 (rect. ns, full bar)	13

[1] Not sexed.　[2] Including one Panamá phenotype (contaminant).　[3] Have traces of yellow line.　[4] One bar is rather thin.　[5] One not scored for rectangles.
[6] Panamá shadow not scorable.　[7] One ns for tooth, line, Panamá shadow.　[8] Panamá shadow not scored in one female.

TABLE A 6. BROODS OF PROBABLE PANAMÁ × EAST BRASIL × TRINIDAD PROVENANCE (P.M.S.)

(For more detailed description of lines and bars see table 11.)

brood	phenotype of parents (when known)	bar tip	offspring (♀/♂)								total
			concave, tooth			intermediate, tooth			convex, no tooth		
			full rect., no Pan. bar	no rect., shadow bar	no rect., full bar	full rect., no Pan. bar	no rect., shadow bar	no rect., full bar	full rect., no Pan. bar.	no rect., shadow bar	
A	both intermediate, tooth, rectangles, no Panamá bar, bl, ea, b	b	yl bs 1/1			bl ts 0/1			bl ea 0/1		4
J2		b b f	yl bs 1/0	wr bf 1/0 wr bf 1/0	wr bs 1/0	bl ea ¹1/0 bl ea 1/0	bl ea 1/0 nl ea 1/3 vl ea 1/0	nl ea 1/0 nl ea 1/0			14
P		b	yl bs 1/2	wd tf 1/0		bl ea 0/1	nl ea 0/1		bl ea 0/1		7
Q		b f		wd bs 1/0		bl ea 1/0	nl ea 2/1 nl ea 1/0		bl ea 2/0	nl br 1/1	10
T		b				bl ea 5/5					10

¹ Ambiguous, bar turns both ways at tip.

TABLE A 7. RONDÔNIA × EAST BRASIL BROODS (K.S.B., W.W.B.)

(The abbreviation wn stands for weak or no line; detailed scoring has not been performed for invaded butterflies in these broods: it is weak or absent in all of them. The scoring of some nl (no line) butterflies is uncertain; some, particularly the radiate ones, probably have a weak basal line. Individual specimens, no longer extant, in which there is some uncertainty of scoring of the rectangles, line or bar have been omitted from the totals used in the text. In broods WK13 and WK15 it is possible tentatively to distinguish a concave, invaded class, here lumped with the intermediate invaded class (see p. 537).

brood and type	provenance and phenotype of parents (♀/♂)	forewing band	basal marks	invaded, intermediate (very variable), slight or unscorable tooth			invaded, convex, tooth ns	entire, concave, tooth			total
				full rect.	faint rect.	no rect.	full rect.	full rect.	faint rect.	no. rect.	
RF1 F₁	East Brasil × Rondônia; East Brasil × Rondônia	red	radiate	very weak yellow line, no bar or slight trace of yellow scaling (see text)							14
WK8 backcross	RF1 × East Brasil; radiate, invaded, convex red band, no rectangles, bl, fd, × East Brasil	red	radiate	bl fs 1/0 bl nb 0/1 bl tf 1/0		bl nb 1/0 nl nb 2/1		yl bs ¹3/0 yl bs 2/1	yl bs 1/0		14
			plain								
WK9 backcross	East Brasil × RF1; East Brasil × radiate, invaded, intermediate red band, no rectangles, wl, nb (multiparental)	red	radiate	bl nb 1/0		nl nb 1/2		yl bs 3/2⁵ yl ea 1/0			17
			plain	bl tf 0/2 bl nb 1/0	bl nb 1/0 (rect. ns)			yl bs 1/1 yl ea 1/0			
WK10 backcross	RF1 × East Brasil; radiate invaded intermediate red band, rectangles, nl fs × East Brasil	red	radiate	bl nb 1/1 wl tf 0/2	bl nb 0/1 (rect.ns) bl nb 0/1 (line and rect. ns)			yl bs 4/1			16
			plain		bl nb 0/1			yl bs 1/3			
WK11 backcross	East Brasil × RF1; East Brasil × radiate, invaded intermediate red band, no rectangles, nl nb	red	radiate					yl bs 0/1	yl bb 0/1¹ yl bb 0/2	yl bb 0/1	6
			plain	bl fd 0/1							
WK12 backcross	East Brasil × RF1; East Brasil × radiate, invaded intermediate red band, no rectangles, nl, fs	red	radiate	bl nb ²1/0 wn (fs–nb) 1/3⁴			yl nb 1/0	yl bs 2/2		yl bf 0/1 yl bb 1/0	21
			plain	bl tf 0/1			wn nb 1/1 bl fs 1/0	yl bs 1/2	yl bb 1/0	yl bf 0/1	
WK13 F₂	WK10 (or WK8 or WK12) × WK12; both radiate, invaded intermediate red band, rectangles, nl, fs	red	radiate	wn fs 6/3 wn nb ²1/2			wn fs 1/0 wn nb 1/1	yl bs 1/2			35
			plain	wn fs 1/0 wn nb 3/1			wn nb 1/1	yl bs 2/1			
		yellow	radiate	wn fs 0/2 wn nb 1/1			wn fs 1/1				
			plain	bl tf 1/0							
WK14 F₄	WK13 × WK13; radiate, invaded concave yellow band, rectangles nl, nb × radiate, entire concave toothed red band, rectangles, yl, bs	red	radiate	wn fs 1/0 wn nb 2/1/1⁵				yl bs 0/1			13
		yellow	radiate	wn nb 2/0				yl bs 3/2			
WK15 F₃	WK10 × WK10 (or WK12); radiate entire concave toothed red band, rectangles, yl bs × radiate, invaded intermediate, red band, rect. nl nb	red	radiate plain	wn nb 1/0 wn nb 1/0				yl bs 2/2 yl bs 1/1 tl bs 0/1			9
		yellow	radiate								
AP	WK8 × WK9; all entire, concave, tooth, full rectangles, yl, yb; plain × radiate; multiparental	red	radiate plain					yl bs 4/7 yl bs 4/4			19

¹ Rectangles not certainly scorable.　　² 1/1 Not now scorable for rectangles.　　³ Line also weakly developed distally.
⁴ Rectangles may have been faint or absent in the three males.　　⁵ Not sexed.

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TABLE A 11. EAST ECUADOR × EAST BRASIL BROODS (P.M.S.)

brood no. type and total	provenance and phenotype of parents (♀/♂)	band	costal spot	bar tip	split, 'short' rectangles round	split, 'short' rectangles flat	split, 'short' no rectangles round	split, 'short' no rectangles flat	entire, shortened rectangles round	entire, shortened rectangles flat	entire, shortened no rectangles round	entire, shortened no rectangles flat	entire, long rectangles round	entire, long rectangles flat	entire, long no rectangles round	entire, long no rectangles flat
E1 (F1) 50	São Paulo × East Ecuador; East Brasil × round, split, shortened, red and white band, nl, nb, no rectangles, no costal spot	red	present			nl fd 2/10 nl nb 12/4					nl fd 5/15 nl nb 0/2					
E2 (F2) 8	E1 × E1; round, entire, shortened red band, nl fd, no rectangles, costal spot × entire band	red red red+white	present absent absent								nl nb 0/2 nl fd 1/0 nl fd 1/0 nl fd 1/0				wr bv 1/0 wl bv 0/1 wr bv 0/1	
E3 (BC) 26	São Paulo (several ♀♀) × E1; East Brasil × entire, shortened, red band	red	present	b b f					bl fa 0/1	bl tf 1/0 bl fa 1/0	nl nb 1/0 nl fd 2/4[1]	nl nb 1/0	yl bs 2/4	yl bs 2/1 yl bs 1/0	wr bv 2/0 wl bv 0/2	wr bv 0/1
E3A (BC) 43	São Paulo × E1; East Brasil (unknown number of ♀♀) × round, entire, shortened, red band, no rectangles, nl, nb	red red	present ?absent	b b b f b b					bl fa 0/1 bl tf 1/0 bl fa 2/3	bl fa 1/0 bl tf 2/0 bl br 0/1	nl fd 1/3	nl nb 2/1 nl nb 0/1	yl bs 2/1	yl bs 1/5	wl bv 2/2 wr bv 2/3	wr bv 1/1 wl bv 1/0 wr bv 1/0 nl bv 0/1[2]
E5 (BC) 11	E1 × São Paulo; round, split, short, red band, nl, nb, no rectangles, costal spot × East Brasil	red	present	b b f	bl ea 1/2		nl fd 0/1						yl bs 3/0		wr bv 0/1 ns bv 1/0	wr bv 1/0 wl bv 0/1
E6 (F2) 8[3]	E1 × E1; round, split, short, red band, nl, nb, no rectangles, costal spot × round, entire, shortened, red band, nl, nb, no rectangles, costal spot	red red+weak white	present present present	b f b	wl ea 1/1						nl fd 0/1 nl fd 0/1				nl nb 1/0	
E7 (BC) 48	E1 × São Paulo; round, split, short, red band, nl or ns, nb, fd or ns, no rectangles (or ns), costal spot (or ns) (4 ♀♀) × East Brasil	red	present	b b b b b f f	bl fa 1/1 bl fr 0/1 ns fa 0/1[4] nl nb 1/0[5] bl nb 1/0[6]	wl fa 0/2 bl fr 1/0	nl nb 0/1	nl fd 1/0 nl nb 0/3					yl bs 4/2	yl bs 5/6	bl bv 3/1 wr bv 1/5	bl bv 3/2 wr bv 1/2
E8 (F2) 4[5]	E1 × E1; entire, shortened, red band × split, short, red band	red red+weak white red+weak white	present present absent	b b b							nl fd 1/1				wr bv 1/0	
E10 (F3) 13[6]	E1 × E1; round, split, short, red band, nl, nb, no rectangles, costal spot × round, entire, shortened, red band, nl, nb, no rectangles, costal spot	red red+weak white absent	present absent present absent	b b b b			nl nb 1/0	nl nb 1/0			nl fd 0/1 nl fd 0/1 nl fd 1/0				wr bv 2/1 wr bv 1/1	wr bv 1/0
E11 (BC) 36	São Paulo × E1; East Brasil × round, split, short, red band, nl, nb, no rectangles	red	present	b b f	bl fa 0/1 bl fr 1/0	bl fa 0/1 bl fr 0/3 wl fr 1/0	nl fd 2/3 ?fd 1/0	nl fd 1/2	bl tf 1/0		nl fd 1/0		yl bs 2/0	bl bf 1/0 yl bs 1/6	wr bv 3/0	wr bv 1/4
E12 (BC) 60	São Paulo × E1 (or reverse); East Brasil × not recorded (contaminated)	red	present	b b b f f	bl tf 1/0 bl fa 1/6 bl fr 1/2 bl fa 0/3 bl fr 1/3	bl fa 0/1	nl nb 2/1	nl fd 0/2 nl nb 1/3	bl fa 0/1[8] bl fa 0/1	bl fr 2/0		nl nb 1/0	yl bs 3/3	yl bs 0/0[9]	wr bv 3/3 wl bv 1/2	wl bv 2/4 wl bv 2/4
E14 (BC) 10	E1 × São Paulo; round, split, short, red band, nl, nb, no rectangles, costal spot × East Brasil	red	present	b	bl fd 1/0	bl tf 1/1		nl nb 2/1					yl bs 1/2		wl bv 0/1	
E15 (F2) 2	E1 × E1; round, split, short, red band, nl, fd, no rectangles, costal spot × round, split, nl, nb, no rectangles	red	present	b	wl fr 0/1		nl nb 1/0									

All offspring and parents are plain.

Split and shortened refer to under surface only; short is lack of extension of band into posterior angle of wing: shortened is truncation at vein Cu1a.

[1] One individual not extant; some minor characteristics (e.g. red spot) may differ for this class.

[2] Damaged, possible scoring error for line and costal spot.

[3] Includes 1/0 split, shortened, red, no rectangles, round, b, ns, fd, red spot, not scorable.

[4] Costal spot not scorable.

[5] Includes 0/1 split, shortened, red+weak white, red spot absent, no rectangles, round, b, nl, nb.

[6] Includes 0/1 split, shortened, red, red spot present, rectangles round, b, nl, nb and 0/1 split, shortened, red+weak white, red spot present, no rectangles, round, b, nl, nb.

[7] Trace of line.

[8] Not distinguishable from pure East Brasilian contaminants.

[9] Not scorable for split or rectangles.