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The Genetics of the Mimetic Butterfly Hypolimnas bolina (L.)

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Phil. Trans. R. Soc. Lond. B 1975 **272**, 229-265
doi: 10.1098/rstb.1975.0084

THE GENETICS OF THE MIMETIC BUTTERFLY *HYPOLIMNAS BOLINA* (L.)

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(*Received* 17 *February* 1975)

[Plates 1 and 2]

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Vol. 272. B. 917. 17 [Published 13 November 1975

Hypolimnas bolina is a Nymphalid butterfly having a west to east distribution from Madagascar to Easter Island, and a north to south one from Japan to Australasia. It is highly migratory in some areas. In much of the western part of its range the female is both monomorphic and a mimic of *Euploea*. Further east it is frequently polymorphic with the majority of the forms being non-mimetic. The polymorphism is sex-limited to the female and controlled by two unlinked loci, one with two allelomorphs, *E* and *e*, determining the extent of the dark pigmentation, the other with three allelomorphs, *P*, P^n and *p*, determining the presence and distribution of orange-brown. Only butterflies of the genotypes *EEpp* and to a lesser extent *Eepp* are satisfactory Batesian mimics of their *Euploea* models. The details of the mimetic pattern are under multifactorial control, following those of their local model, as is much of the variation within the non-mimetic forms, particularly with regard to the distribution of white and blue scaling.

The mimetic form is dominant or semi-dominant, depending on the background genotype, and there is little epistatic interaction between the gene responsible and the allelomorphs at the second locus. Here, the form with the greatest amount of orange-brown pigment is to all intents and purposes dominant to the other, and that with restricted brown pigment is semi-dominant to the form without any, although in certain genotypes it is entirely recessive.

Unlike the situation found in previous studies of *Papilio dardanus*, *Papilio memnon* and *Papilio polytes*, the sex-controlled polymorphism is not determined by a supergene. It is suggested that the absence of such a supergene results from the fact that there is only one mimetic form and that over much of the species' range the butterfly is monomorphic for this. Thus the polymorphism at two or more loci necessary for the selection of increased linkage is absent throughout much of the range of the species.

The genetic control of the mimicry has more in common with that in *Papilio polyxenes*, where there is also only one model but in which there is no polymorphism throughout the whole of the species' range, rather than in only a large part of it as in *H. bolina*.

1. Introduction

In previous papers we have investigated the genetic control of mimetic polymorphism in *Papilio dardanus* Brown, *Papilio memnon* L. and *Papilio polytes* L. We have concluded that the major forms are controlled by various combinations of allelomorphs in a supergene. Furthermore, the detailed resemblance between model and mimic is enhanced by the selection of specific modifiers of the pattern controlled by the supergene. We also found that complete dominance was commoner between sympatric than it was between allopatric forms. We concluded that the evolution of the supergene was the result of selection for linkage (see Clarke & Sheppard 1971, 1972). Conn (1972), working on the genetics of the mimetic Dipteran *Merodon equestris*

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(Fab.), has also found evidence for a linkage disequilibrium between loosely linked loci in favour of increasing the proportions of mimetic forms, thus suggesting that a supergene is being evolved.

In order to test the general validity of our conclusions it was clearly necessary to investigate genetic situations in a genus other than *Papilio*, as pointed out in Clarke & Sheppard (1972). The present study concerns the genetics of *Hypolimnas bolina* (L.), a Nymphalid butterfly in which the polymorphism is sex-controlled to the female as in the Papilios, which has a wide geographical distribution but which differs from the Papilio situation both by virtue of there being only one main mimetic form, and the fact that this is monomorphic over a large part of the species' range.

2. Materials and methods

Living butterflies and occasionally eggs of *H. bolina* have been sent to us by air mail from various localities. The method of packing them was the same as for *P. memnon* (Clarke, Sheppard & Thornton 1968). The inclusion of maximum and minimum thermometers in some of the packages showed that they were not subjected to excessively low temperatures. The butterflies we have used in our investigations have come from Sri Lanka (Ceylon), Hong Kong, Sarawak, Australia, Papua New Guinea and Fiji. In addition, we have examined preserved specimens in the British Museum (Natural History) the Hope Museum, Oxford and the Natural History Museums at Perth, Adelaide and Sydney.

Unlike the Papilios, we failed to hand-mate *H. bolina*, but the species will mate readily if allowed to fly freely in a greenhouse or less so in artificially lighted cages. The latter were 1.14 m wide by 3.91 m long with a height of 1.65 m, and each was lit by four 80 W white fluorescent lamps, with a limited amount of daylight from the glass roof of the cage. The greenhouses and cages were kept at a temperature of about 26 °C but varying between 38 and 21 °C.

The butterflies destined for mating were fed on a mixture of honey and water, marked with cellulose paint and released into the breeding area where flowers, together with honey and water on plastic sponges and in bird feeders, were provided. The females when they mated were transferred to silk organza bags enclosing the foodplant and fed daily on honey and water (as described for breeding Papilios). Under these conditions they would live for several weeks and often produced large numbers of very small blue-green eggs. A few females produced yellowish eggs which also proved fertile.

The females laid on *Asystasia gangetica*, Sweet Potato (*Ipomaea batatas*) and *Sida rhombifolia*. They will also lay, but less readily, on nettle (*Urtica dioica*). The eggs destined to hatch turned dark in about 4 days. The hatchability of eggs and the survival of larvae in relationship to the sex ratio of the adult offspring have been described elsewhere (Clarke, Sheppard & Scali 1975).

The larvae were reared in organza sleeves and fed on the plants mentioned above, and also in winter on *Urtica urens* (annual nettle) which proved acceptable but less satisfactory. They were allowed to pupate in the silk organza bags or were transferred to plastic boxes when nearly full grown. The larvae were similar in pattern from all localities except from Sri Lanka and Hong Kong. Here in our experience they differed in having a black angular pattern on the dorsal side in the last instar. The pupae were moved to cages to give the newly emerged insects room to expand their wings.

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Because of the labour involved in rearing individual broods, stocks homozygous for certain genotypes, or those of known geographical origin, were allowed to breed freely from time to time in one or other of the breeding areas. For this reason the provenance of some of the parents is designated 'Fiji', 'Sri Lanka', etc., or, if from a homozygous stock, 'all-*naresi*'.

Under the conditions in which we reared the species, the generation time from mating to the emergence of the first adult was in general between 29 and 48 days, with a tendency to be slightly longer in the winter. Part of the variability may be due to the time between mating and laying which can be over 8 days. Another factor is probably the slower development during the winter, when temperatures are lower and the foodplant less satisfactory, as judged by the higher mortality of the larvae.

3. Description of the forms

(*a*) *Male*

Although the appearance of *H. bolina* males varies geographically and seasonally (see p. 257), this variation is not very extensive. They are very similar to some other species of *Hypolimnas*, having a black ground colour shot with purplish blue, a round bluish-white patch on the hindwing and an oval one on the forewing. There are often small white spots close to the apex of the forewing. In addition, narrow white lunules on the distal margin of the fore- and hindwings are present (plate 1*a*).

(*b*) *The basic female forms*

A large number of female forms of *H. bolina* have been described, a situation complicated because essentially similar phenotypes have been given different names in different geographical areas. In fact the forms of this highly polymorphic butterfly can be described in terms of four basic phenotypes and their combinations, together with minor modifications of pattern. We propose for the sake of clarity to use the varietal names for the four main forms given by Poulton (1924).

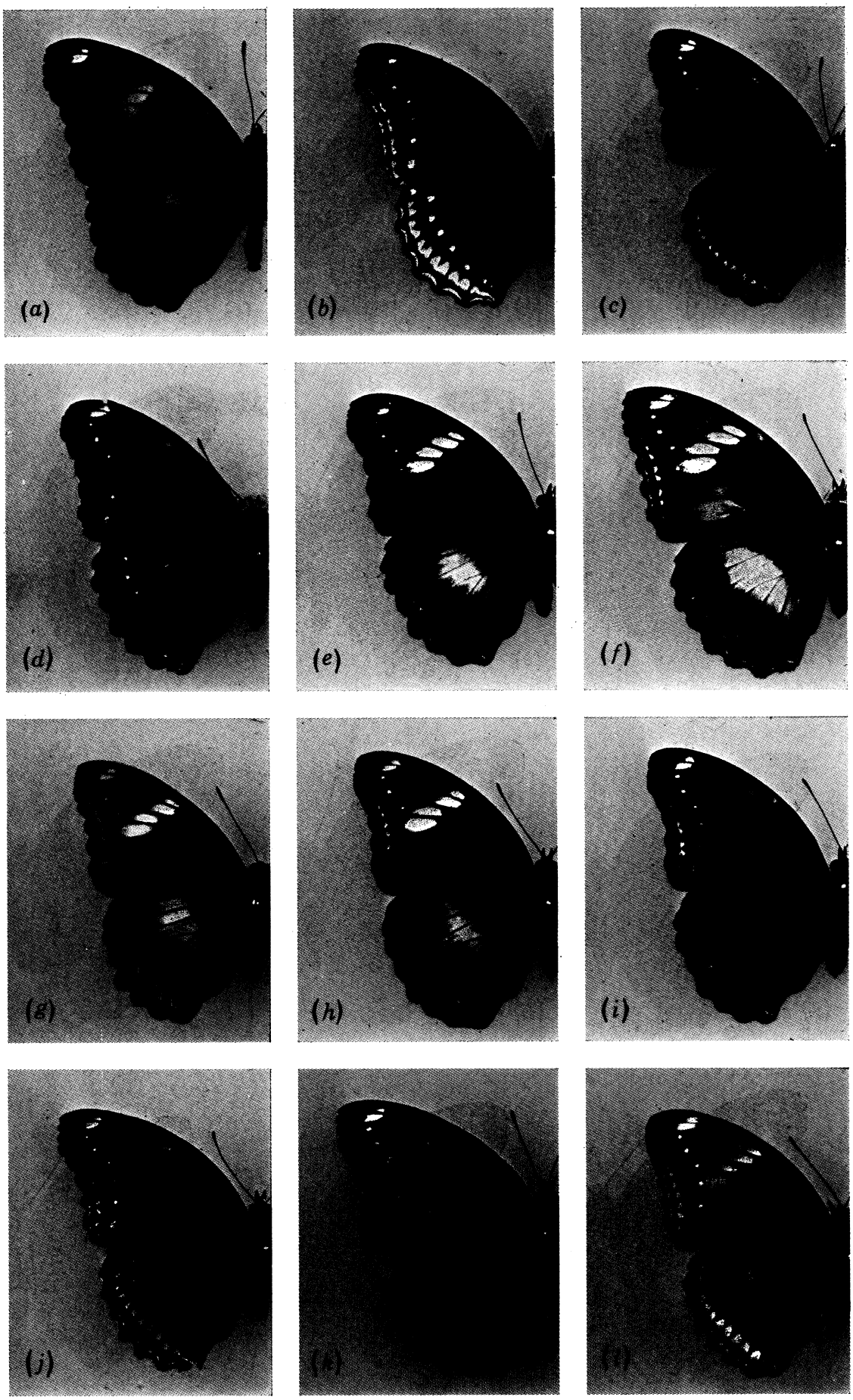
(i) *The mimetic form*, euploeoides

The ground colour is dark brown, with a pattern of marginal and submarginal white spots on the distal border of both fore- and hindwings. There is a variable amount of blue spotting extending from close to the costal border of the forewing out towards the distal margin, in much the same position as the pale mark in the males. The details of the pattern tend to follow the local *Euploea* models.

Description of plate 1

In plates 1 and 2 the wing span from the apex of one forewing to the other of each set butterfly is given to the nearest millimetre.

(*a*) ♂ 72 mm, Fiji
(*b*) ♀ f. *euploeoides* 75 mm, Sri Lanka
(*c*) ♀ f. *euploeoides* 62 mm, Fiji, brood 12668
(*d*) ♀ f. *kezia* 76 mm, Hong Kong, brood 12805
(*e*) ♀ f. *naresi* 81 mm, Fiji
(*f*) ♀ f. *nerina* 84 mm, Sarawak, compare with 2*f*
(*g*) ♀ f. *pallescens* 63 mm, Fiji
(*h*) ♀ f. *pallescens* 86 mm, Fiji, brood 12671
(*i*) ♀ *euploeoides*-like (heterozygous for *naresi*) 79 mm, brood 12862, compare with 2*a*, *e* and *l*.
(*j*) ♀ *euploeoides-nerina* 93 mm, brood 12596, compare with 2*c*
(*k*) ♀ *euploeoides-nerina* 86 mm, Australia, brood 12968, compare with 2*c*
(*l*) ♀ *euploeoides-pallescens* 81 mm, compare with 2*h* and *i*



For description see opposite.

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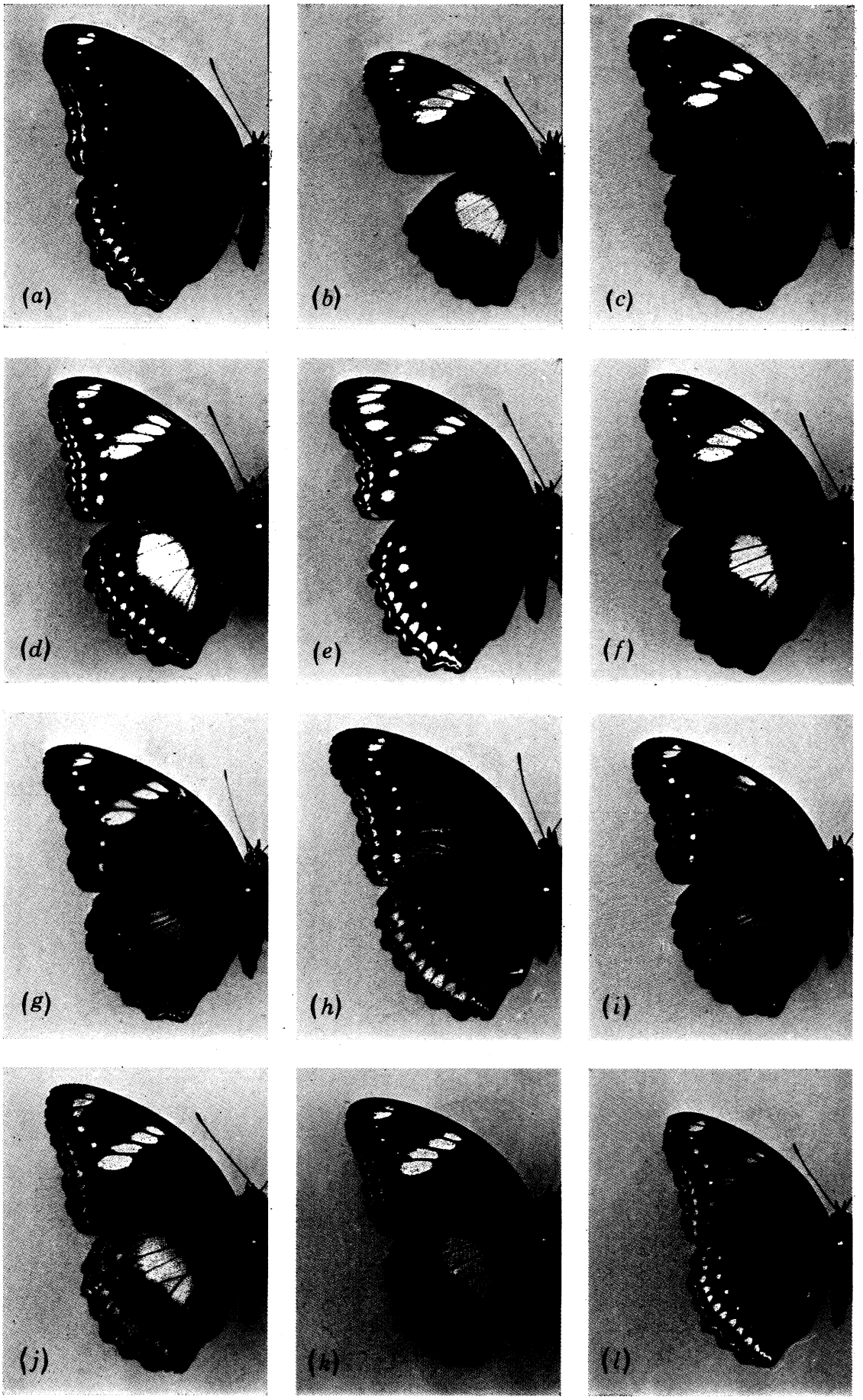

For description see opposite.

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The mimics from India and Sri Lanka have a well-marked pattern of creamy white spots, particularly on the hindwing, and very reduced blue spotting near the costal margin of the forewing (plate 1*b*). Those from Fiji are essentially similar to the ones from Sri Lanka except that the white spots bordering the wings are very much reduced (plate 1*c*).

The Hong Kong females are similar to those from Fiji with respect to the white spotting round the border of the wings. However, the reduced blue forewing costal spots are replaced by a white subapical forewing bar with a variable amount of blue in the position of the bluish white forewing spot in the male (plate 1*d*). This subapical bar is even more conspicuous and has more blue in some specimens from the Philippines. In contrast, further south, in the region of the Moluccas and New Guinea, immaculate black or brown *euploeoides* are sometimes to be found.

(ii) *The male-like form*, naresi

The basic pattern of this form is black, with rows of white spots bordering the distal edge of the fore- and hindwing. In addition, on the forewing there is a subapical white bar which is sometimes tinged with blue but can be plain white. It is in a similar position to the oval blue-white patch on the forewing of the male. There is a white patch in the centre of the hindwing, again in a similar position to that of the male. This can be white or shot with blue, it is less round than that of the male, is variable in size and can extend from the costal margin almost to the inner margin of the hindwing, unlike that of the male (plate 1*e*). In those specimens in which the hindwing patch is small and both it and the forewing patches heavily suffused with blue, the insect can look extremely like the male in pattern, particularly if the rows of spots bordering the margin of the wings are much reduced.

In some specimens we have bred from Fiji the blue on the hind-wing is replaced by brownish scales which may extend into the white spots at the border ('brown *naresi*'). In addition, there is sometimes some brown scaling near the inner margin of the forewing in a position similar to the more extensive patch found in *nerina* (see below). There may or may not also be a tinge of similar brown scaling near the apex of the forewing. Such an insect, though not of pure Fiji stock, is shown in plate 2*b*.

(iii) *Form* nerina

F. *nerina* is extremely similar to f. *naresi* in the distribution of white and shows the same degree of variation. However, it differs in having a bright orange-brown patch of scales two-thirds of the way along the inner border of the forewing, which gives the insect its characteristic appearance (plate 1*f*). The patch can vary considerably in size and when it is in its most reduced form the insect is very difficult to distinguish from the brown *naresi* mentioned above.

Description of plate 2

(*a*) ♀ *euploeoides*-like (heterozygous for f. *naresi*) 86 mm, brood 12862, compare 1*i*, 2*e* and *l*
(*b*) ♀ brown *naresi* 61 mm
(*c*) ♀ *euploeoides-nerina* 98 mm, Australia, compare 1*j* and *k*
(*d*) ♀ f. *nerina* 73 mm, compare 1*e*
(*e*) ♀ *euploeoides*-like (heterozygous for *naresi*) 87 mm, compare 1*i*, 2*a*, *l*
(*f*) ♀ f. *nerina* 69 mm, brood 13074, compare 1*f*
(*g*) ♀ *euploeoides-nerina* 93 mm, compare 1*k* and 2*c*
(*h*) ♀ *euploeoides-pallescens* 91 mm, brood 12582, compare 1*l* and 2*i*
(*i*) ♀ *euploeoides-pallescens* 92 mm, compare 1*l* and 2*h*
(*j*) ♀ f. *pallescens* 75 mm, brood 12858, compare 2*k*
(*k*) ♀ f. *pallescens* 70 mm, brood 12763, compare 2*j*
(*l*) ♀ *euploeoides*-like (heterozygous for *naresi*) 70 mm, brood 12862, compare 1*i*, 2*a*, *e*

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(iv) *Form* pallescens

In this form the pattern of white is similar in position and variability to that in *naresi* and *nerina*. The base of the fore- and hindwing tends to be dark brown, but the rest of the areas which are dark in *naresi* are replaced by orange-brown. The extent of the dark areas and the tone of the brown can vary from light yellowish (plate 1*g*) to dark chestnut (plate 1*h*). The rows of dots bordering the wings are not white but suffused with brown.

(*c*) *Intermediates between the mimetic and non-mimetic female forms*

(i) Euploeoides-*like* (euploeoides-naresi)

Besides the two main forms *euploeoides* and *naresi*, intermediates are found. These vary between typical *euploeoides* with an indistinct subapical white bar suffused with black and blue scales, to insects with quite a marked forewing bar. Such insects have a reduced white area on the hindwing which can vary between the presence of a few scales to a round pale area shot with blue (plate 1*i*).

(ii) Euploeoides-nerina

Intermediates also occur between *euploeoides* and *nerina*. The white areas of this form are again reduced and shot with blue, as in *euploeoides-naresi*. However, the pale hindwing area tends to be elongated, with brown scales also present in some specimens. In a number from Australia the area is entirely brown. The orange forewing patch typical of *nerina* is often much reduced (plate 1*j*), but in some butterflies from Australia, where *nerina* is particularly common, the patch is very conspicuous (plate 1*k*).

(iii) Euploeoides-pallescens

Intermediates also occur between *euploeoides* and *pallescens*. These have the general colouring and the same variability as *pallescens*, but differ from that form in that the brown scaling masks the white patches on the fore- and hindwings. Thus one obtains a brown insect with a ghost-like pattern of lighter brown replacing the areas that are normally white (plate 1*l*).

4. Distribution of *Hypolimnas bolina*

The species is very widely distributed, being found from Madagascar in the west to Easter Island in the Pacific. Furthermore, it extends as far north as Okinawa and as far south as New South Wales in Australia. It also seems to wander extensively since it is found from time to time in Southern Japan (Dr S. A. Ae, private communication). It is also intermittently found in North Island, New Zealand, more than 1900 km from its likely origin in Australia (Ramsay & Ordish 1966; Ramsay 1971). The view that these butterflies come from Australia is supported by the fact that there is only one female form (f. *nerina*) found in New Zealand, whereas if the specimens came from the islands to the north one would expect other female forms, particularly f. *pallescens*, to be caught occasionally (see distribution maps – figures 1–6). Furthermore, *H. bolina* inhabiting the Pacific islands is not likely to have been selected for a migratory habit, because of the equable climate and the loss that would be incurred with so little land area available. However, the Australian 'race' is known to be migratory since it invades New South Wales from the north most years and disappears in the winter (Common & Waterhouse 1972).

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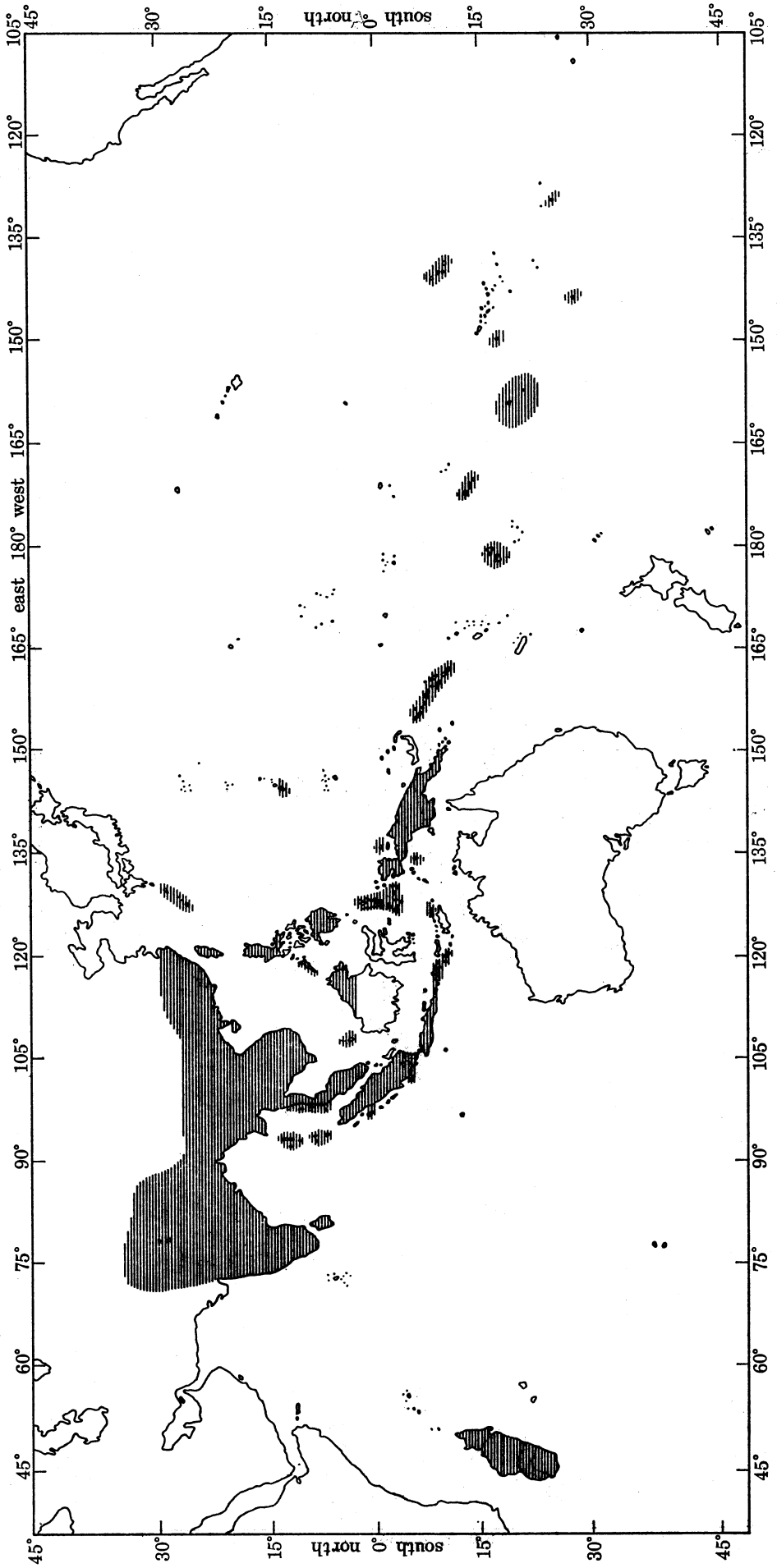

FIGURE 1. The distribution of the mimetic form *explosoides*.

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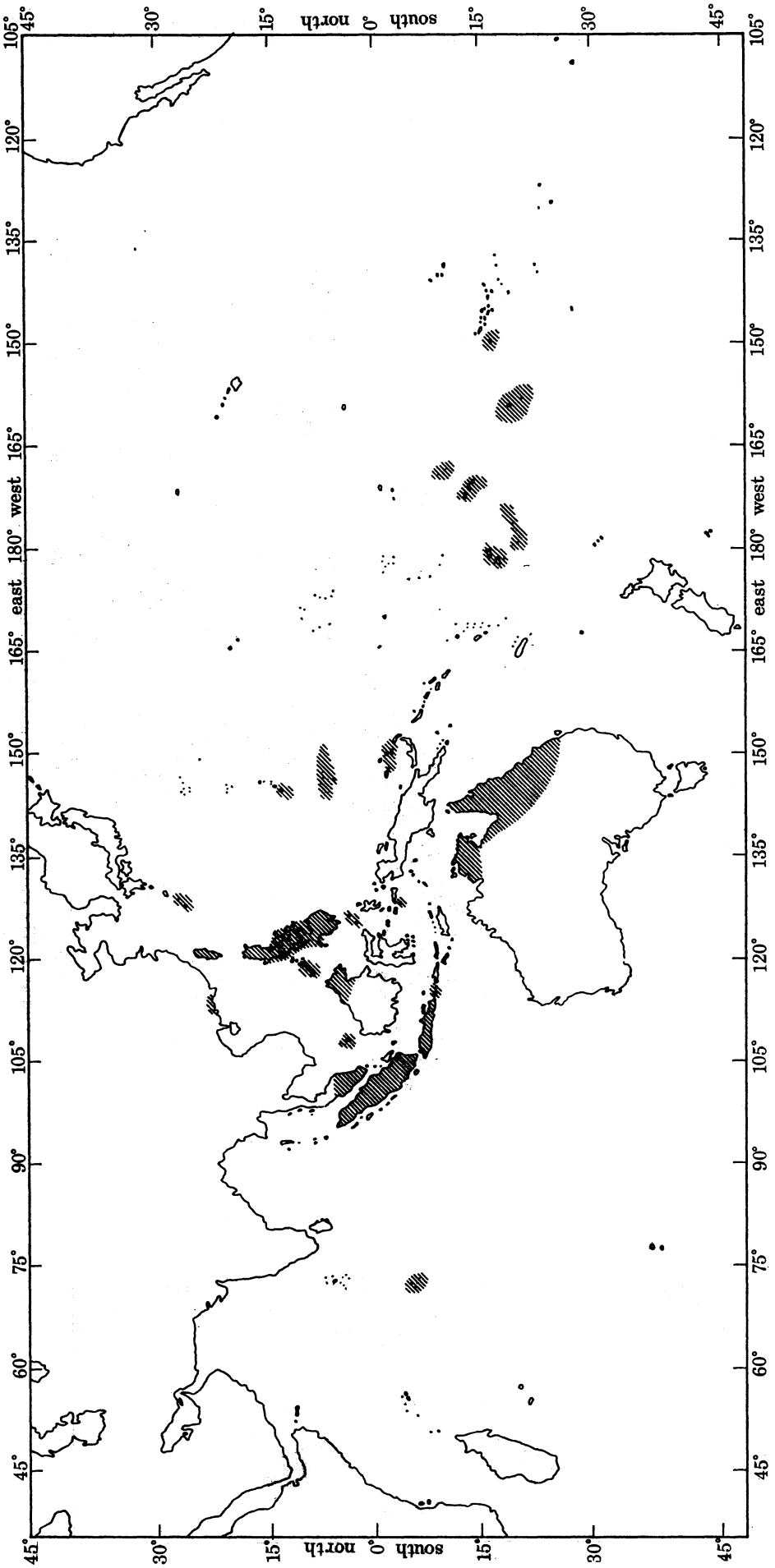

FIGURE 2. Distribution of f. *narei*.

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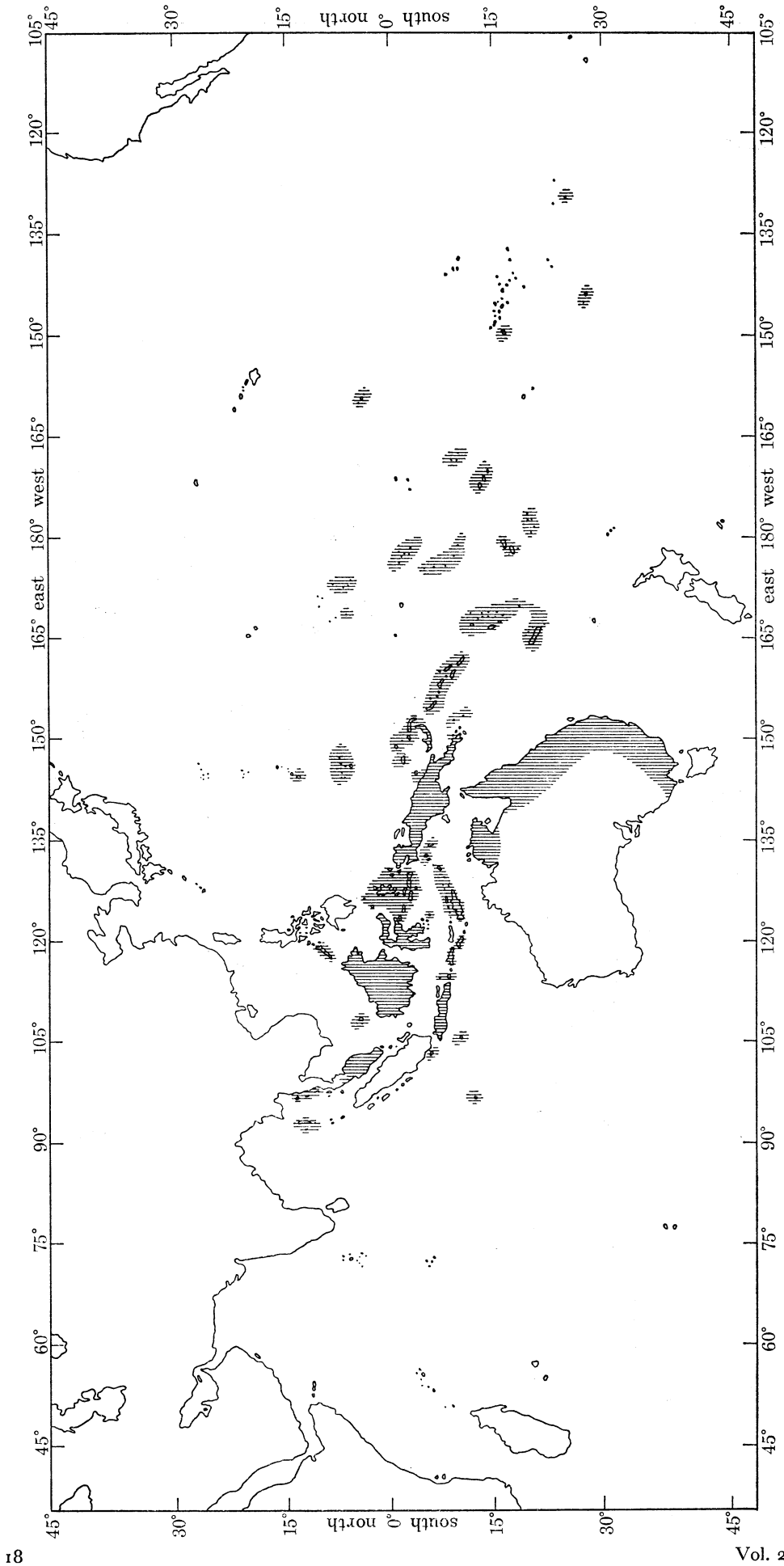

FIGURE 3. Distribution of f. *nerina*.

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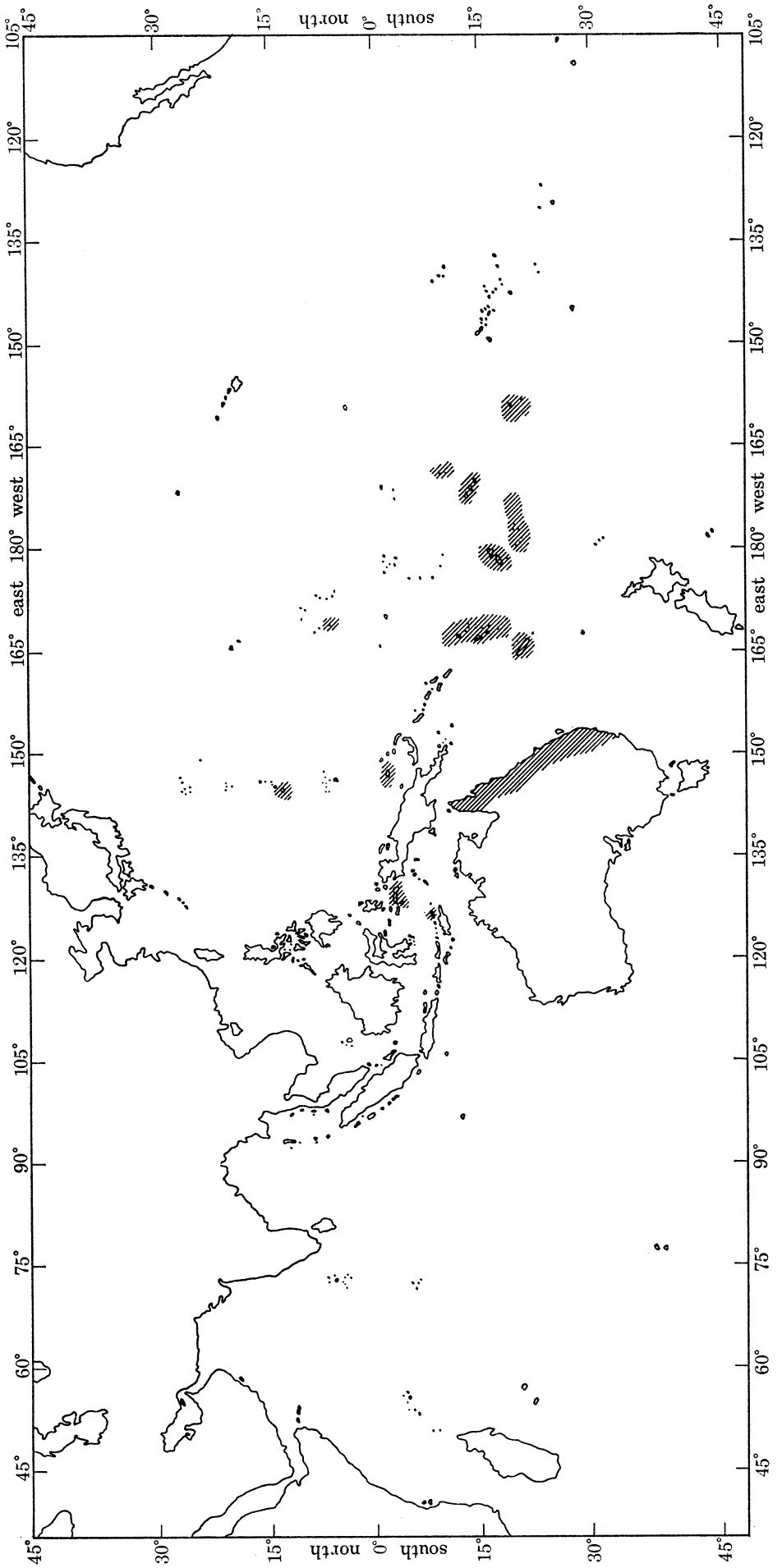

FIGURE 4. Distribution of f. *pallescens*.

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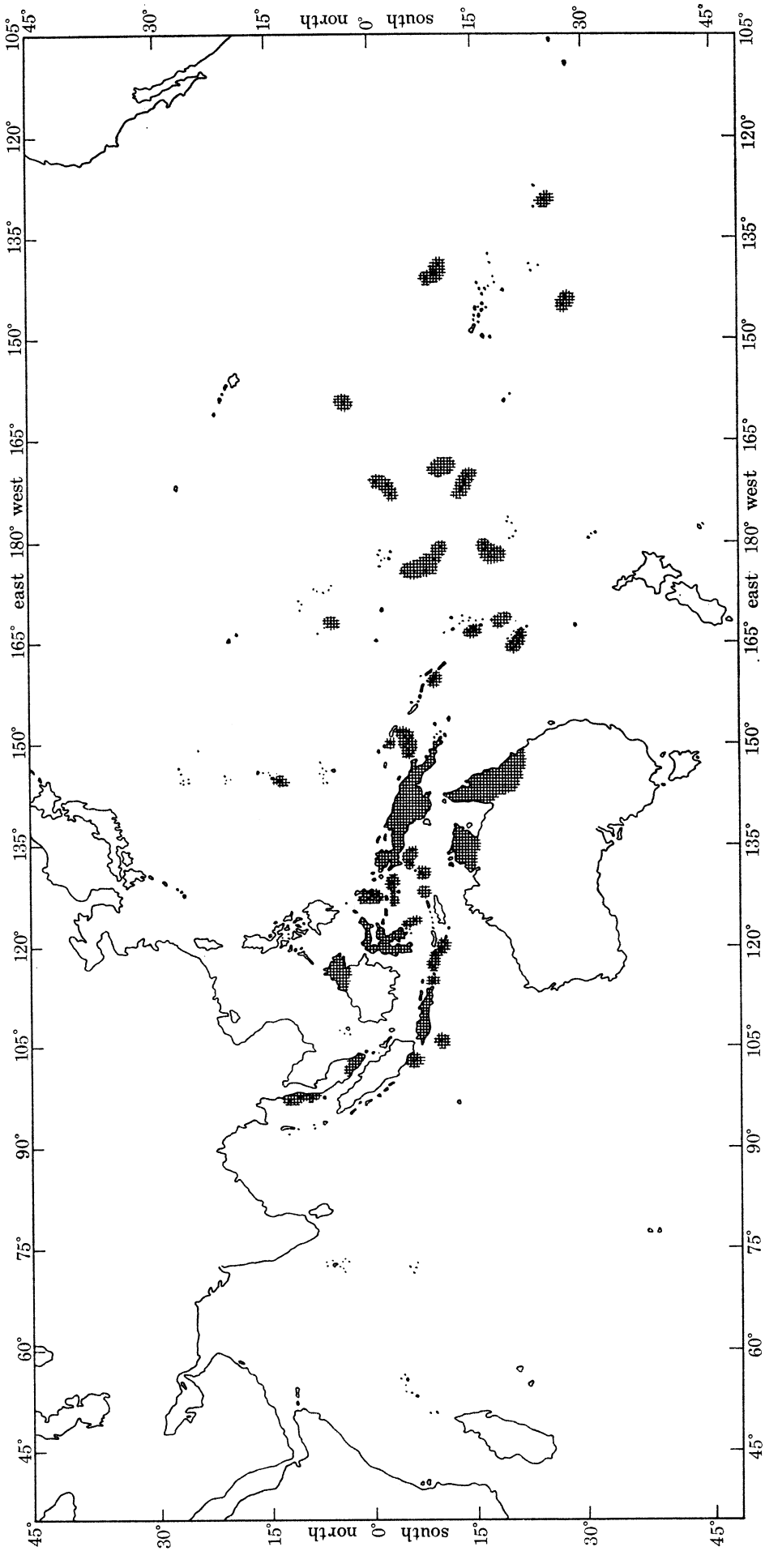

FIGURE 5. Distribution of *euploeoides-nerina*.

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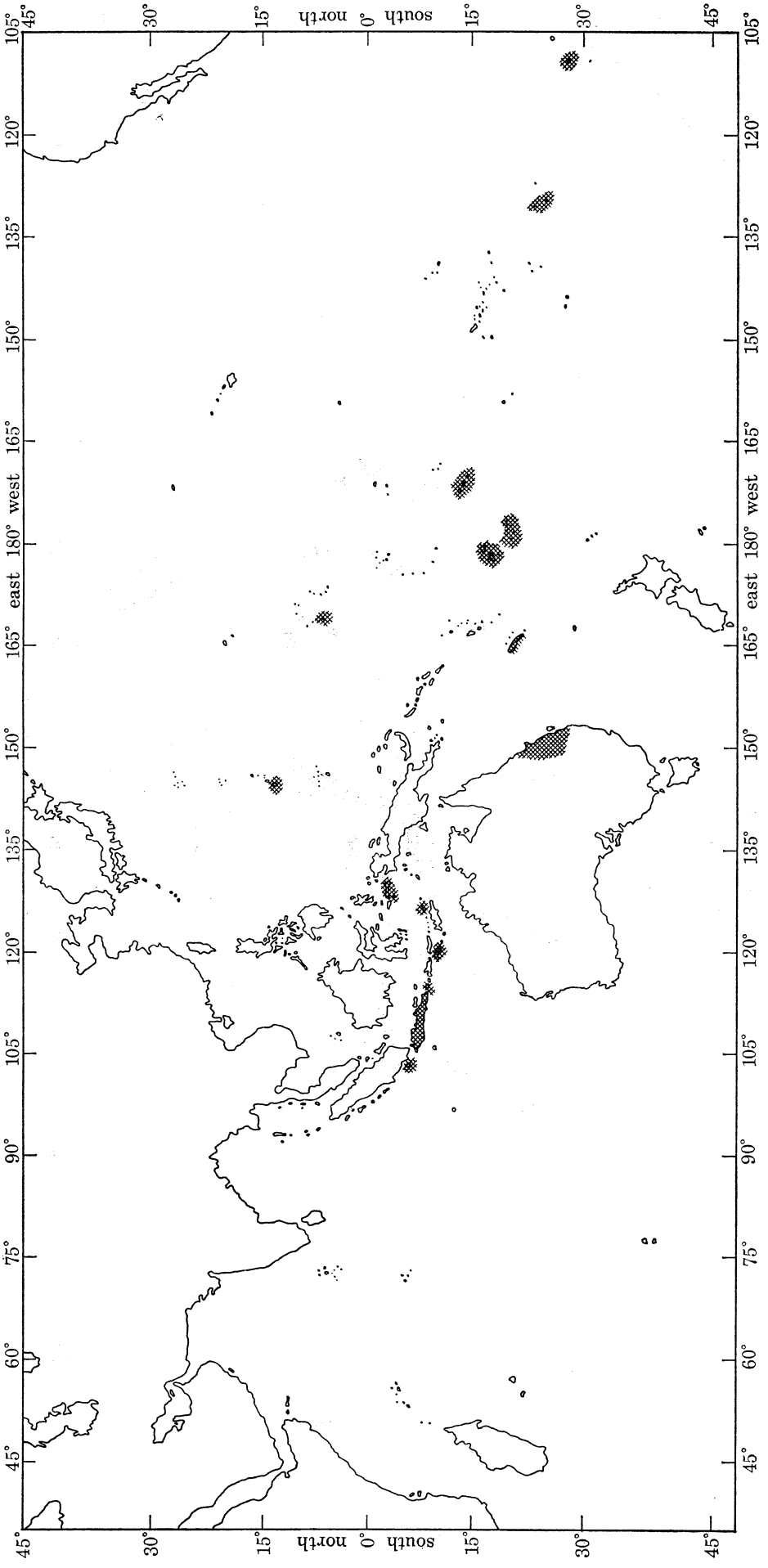

FIGURE 6. Distribution of *euploeoides-fallescens*.

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It would be extremely interesting to know whether there is a northern migration in the autumn since such a reverse movement is well documented among European Lepidoptera (Lack & Lack 1951) and *Danaus plexippus* in America (Urquhart 1960).

In order to determine the geographical distribution of the various female forms of *H. bolina* (but not of course their frequencies, which would require random samples) we have examined the specimens in the British Museum (Natural History) including the Rothschild collection, in the Hope Museum at Oxford and in the Adelaide and Sydney Museums. The distributions, as far as we can determine them from the museum specimens, material sent to us and private communications, are given in figures 1–6 and the localities in table 1. The most striking feature of the distributions is the monomorphic mimetic population stretching from the east coast of China to the west coast of India together with the outlying population in Madagascar. *Euploeoides* in this monomorphic area is a particularly good mimic of its *Euploea* models even in Madagascar where the models are absent.

F. *nerina* is almost ubiquitous from the Andaman Islands in the west to Easter Island in the east. A rare form, *pseudomisippus*, possibly a mimic of *Danaus chrysippus* (L.), is known from New Guinea (Seitz 1927). One labelled Fanning Island is present in the British Museum collection, together with forms intermediate between it and *nerina* labelled New Ireland and New Guinea respectively. *Pseudomisippus* is probably a modification of *nerina*.

F. *pallescens* has a much more restricted distribution, being chiefly found in the islands of the Pacific from New Caledonia to Easter Island. It is also known from the Bismarck Islands, the South Moluccas and, as a rarity, from Australia (Burns 1969, and private communication).

F. *naresi* is as widely distributed as f. *nerina* but is slightly more sporadic in its occurrence, being absent from New Guinea and rare in Australia, as judged by the collections. However, we know from our breeding work that it must be present in New Guinea.

The intermediates between *pallescens* and *nerina* on the one hand and *euploeoides* on the other are shown on the maps where they occur. The phenotypes intermediate between *euploeoides* and *naresi* are heterozygotes, but some heterozygotes are indistinguishable from *euploeoides*. We have therefore combined the intermediates with typical *euploeoides* (p. 232) on the distribution map. With respect to the other two intermediates the genotype can be determined with much greater accuracy from the phenotype. The presence of some combinations in the absence of the basic forms is explained by our breeding results (see below).

5. Palatability to birds

It has been known for some years that warningly coloured species of butterfly reared on certain foodplants contain toxic substances (see, for example, Rothschild 1972; Brower, Brower & Corvino 1967; and Brower, McEvoy, Williamson & Flannery 1972). Attempts have therefore been made to try and find out whether this applies to *H. bolina*, and a number of experiments were undertaken to test for the presence of toxins and the palatability of the butterfly to birds.

(*a*) *Tests on pigeons* (Columba livia)

The work on pigeons was carried out by Dr D. N. Kellett of the Huntingdon Research Centre. One pigeon which had fasted for 24 h was fed by oral gavage on a fine paste made from 2 ml of water and two crushed butterflies; a second was given a similar paste made from four butterflies; a third was fed on a suspension made from two crushed larvae and a fourth on

TABLE 1. LOCALITIES OF THE VARIOUS FORMS OF *H. BOLINA*

(The localities are given as on the original data labels except that where several labels have different spellings we have chosen one. Because of the variability in the spelling of the place names we have given what we believe to be the latitude and longitude to the nearest degree for all those which do not cover large, well-known areas. This should allow the reader to identify the localities either on our figures 1–6 or on a larger scale atlas. The four places we have been unable to locate are given at the end of the table.)

locality	latitude	longitude	*euploeoides*, map no. 1	*naresi*, map no. 2	*nerina*, map no. 3	*pallescens*, map no. 4	*euploeoides-nerina*, map no. 5	*euploeoides-pallescens*, map no. 6
Madagascar	.	.	+	.	.	.	.	.
Mahanoro	20 S	49 E	+	.	.	.	.	.
Punjab	28–34 N	69–79 E	+	.	.	.	.	.
N.W. Himalayas	.	.	+	.	.	.	.	.
South India	.	.	+	.	.	.	.	.
Ceylon	.	.	+	.	.	.	.	.
Chagos Is.	6 S	72 E	.	+	.	.	.	.
Simla	31 N	77 E	+	.	.	.	.	.
Berhampore	19 N	85 E	+	.	.	.	.	.
Durbunga	26 N	86 E	+	.	.	.	.	.
Darjeeling	27 N	88 E	+	.	.	.	.	.
Sikkim	27 N	88 E	+	.	.	.	.	.
Bengal	24 N	89 E	+	.	.	.	.	.
Assam	22–27 N	90–95 E	+	.	.	.	.	.
Andaman Is.	12 N	92 E	+	.	+	.	.	.
Khasi Hills	25 N	93 E	+	.	.	.	.	.
Nicobar Is.	8 N	94 E	+	.	.	.	.	.
Sumatra	6 S–6 N	95–106 E	+	+	.	.	.	.
Burma	21 N	96 E	+	.	.	.	.	.
Pegu	17 N	96 E	+	.	.	.	.	.
Mergui Is.	12 N	96 E	+	.	+	.	+	.
Siam	6–20 N	97–106 E	+	.	.	.	.	.
Kingo I.	12 N	97 E	.	.	+	.	.	.
Cocos Keeling I.	12 S	97 E	.	.	+	.	.	.
Nias I.	2 N	98 E	+	.	.	.	.	.
Penang	5 N	100 E	+	.	.	.	.	.
Enggano I.	5 S	103 E	+	.	+	.	+	+
Singapore	1 N	104 E	+	+	+	.	+	.
Christmas I.	10 S	106 E	.	.	+	.	+	.
West China	.	.	+	.	.	.	.	.
South China	.	.	+	.	.	.	.	.
East China	.	.	+	.	.	.	.	.
Natuna Is.	4 N	108 E	+	+	+	.	.	.
Hainan	19 N	110 E	+	.	.	.	.	.
Java	.	.	+	+	+	.	+	+
South Borneo	0	113 E	.	.	+	.	.	.
Hong Kong	22 N	114 E	+	+	.	.	.	.
Macao	22 N	114 E	+	+	.	.	.	.
Kangean	7 S	115 E	.	.	+	.	.	.
Bali	8 S	115 E	+	.	+	.	+	+
Lombok	9 S	116 E	+	+	.	.	.	.
North Borneo	5 N	117 E	+	+	+	.	+	.
Sumbawa	8 S	117 E	+	.	+	.	+	.
Palawan	9 N	119 E	+	+	+	.	.	.
Tanggari	3 S	119 E	.	.	+	.	+	.
Celebes	2 S	120 E	.	.	+	.	+	.
Sumba	10 S	120 E	+	.	+	.	+	+
Formosa	23 N	121 E	+	+	.	.	.	.
Luzon	16 N	121 E	.	+	.	.	.	.
Tikao	12 N	124 E	.	+	.	.	.	.

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TABLE 1 (*cont.*)

locality	latitude	longitude	*euploeoides*, map no. 1	*naresi*, map no. 2	*nerina*, map no. 3	*pallescens*, map no. 4	*euploeoides-nerina*, map no. 5	*euploeoides-pallescens*, map no. 6
Philippines	.	.	+	+	.	.	.	.
Kalidupa	5 S	124 E	.	.	+	.	+	.
Mindanao	7 N	125 E	.	+	.	.	.	.
Talisse	2 N	125 E	.	.	+	.	.	.
Sula Is.	2 S	125 E	.	.	+	.	.	.
Timor	9 S	125 E	.	.	+	.	.	.
Sanguir	3 N	126 E	.	+	.	.	.	.
Buru	3 S	126 E	+	.	+	.	+	.
Wetter	8 S	126 E	.	.	+	.	.	.
Talaut	4 N	127 E	.	+	+	.	.	.
Ternate	1 N	127 E	.	.	+	.	+	.
Batchian	1 S	127 E	+	.	+	.	+	.
Kisser	8 S	127 E	+	.	+	+	.	+
Loo Choo Is.	26 N	128 E	+	+	.	.	.	.
Morty I.	2 N	128 E	+	.	.	.	.	.
Halmaheira	1 N	128 E	+	.	+	.	.	.
Gebi	0	128 E	.	.	+	.	.	.
Obi	2 S	128 E	+	.	+	.	.	.
Amboina	3 S	128 E	.	+	+	+	+	+
Moa	8 S	128 E	.	.	+	.	+	.
Ceram	3 S	129 E	+	.	+	+	+	+
Saparua	3 S	129 E	.	.	.	+	.	.
Dammer	7 S	129 E	.	.	+	.	.	.
Teon	7 S	129 E	.	.	+	.	.	.
Babba	8 S	130 E	.	.	+	.	+	.
Weigeou	0	131 E	.	.	+	.	.	.
Tenimber	7 S	131 E	.	.	+	.	.	.
Darwin	12 S	131 E	.	+	+	.	+	.
Key Is.	5 S	132 E	.	.	+	.	+	.
Aru Is.	6 S	134 E	+	.	+	.	+	.
Schouten Is.	1 S	136 E	+	.	.	.	.	.
New Guinea	.	.	+	.	+	.	+	.
Guam	13 N	145 E	+	+	+	+	+	+
Vulcan I.	4 S	145 E	.	.	+	.	.	.
Admiralty Is.	2 S	147 E	.	.	+	+	.	.
Rook I.	6 S	148 E	.	.	+	.	.	.
Matthias I.	1 S	149 E	.	.	+	.	.	.
Queensland	.	.	.	+	+	+	+	+
New South Wales	.	.	.	.	+	+	.	.
Caroline Is.	8 N	150 E	.	+	+	.	.	.
Squally I.	2 S	150 E	.	.	+	.	.	.
New Hanover	3 S	150 E	.	.	+	.	+	.
Bismarck Is.	4 S	150 E	.	+	.	.	.	.
New Britain	6 S	150 E	.	.	+	.	+	.
Goodenough I.	9 S	150 E	.	.	+	.	+	.
New Ireland	3 S	152 E	.	.	+	.	.	.
Duke of York I.	4 S	152 E	.	.	+	.	.	.
Woodlark I.	9 S	153 E	.	.	+	.	.	.
Rossel I.	11 S	154 E	.	.	+	.	.	.
Solomon Is.	5–11 S	155–162 E	+	.	+	.	+	.
New Caledonia	20–22 S	164–167 E	.	.	+	+	+	+
New Hebrides	13–20 S	166–170 E	.	.	+	+	+	.
Loyalty Is.	21 S	167 E	.	.	+	+	.	.
I. of Pines	23 S	167 E	.	.	+	.	.	.
Jaluit	6 N	169 E	.	.	+	+	+	+
Ellice Is.	5–11 S	176–180 E	.	.	+	.	+	.
Gilbert Is.	1 S	176 E	.	.	+	.	.	.

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TABLE 1 (*cont.*)

locality	latitude	longitude	*euploeoides*, map no. 1	*naresi*, map no. 2	*nerina*, map no. 3	*pallescens*, map no. 4	*euploeoides-nerina*, map no. 5	*euploeoides-pallescens*, map no. 6
Fiji	17 S	179 E	+	+	+	+	+	+
Friendly Is.	15 S	177–173 W	.	+	+	+	.	+
Phoenix Is.	2 S	174–171 W	.	.	.	.	+	.
Samoa	13 S	171 W	+	+	+	+	+	+
Tutuila	14 S	171 W	.	+	.	.	.	.
Tokelau	10 S	169 W	.	+	+	+	+	.
Cook Is.	18–23 S	163–156 W	+	+	.	+	.	.
Aitutaki	19 S	160 W	+	.	.	.	.	.
Fanning I.	4 N	159 W	.	.	+	.	+	.
Hervey I.	19 S	159 W	+	.	.	.	.	.
Tahiti	17 S	149 W	+	+	+	.	.	.
Rapa I.	28 S	144 W	+	.	+	.	+	.
Marquesa Is.	9 S	140 W	+	.	.	.	+	.
Pitcairn I.	25 S	130 W	+	.	+	.	+	+
Easter I.	27 S	109 W	.	.	.	.	.	+
Killong	.	.	+	.	.	.	.	.
Sudest Is.	.	.	+	.	+	.	.	.
Tiandoe	.	.	.	.	+	.	.	.
Suer	.	.	.	.	+	.	.	.

one similarly made from three pupae. All the butterflies had been reared on *A. gangetica* and/or nettle (*U. dioica*). No subsequent ill effects occurred in any of the birds.

(*b*) *Tests on the Japanese quail* (Coturnix coturnix japonica)

These experiments were also carried out by Dr Kellett. This species was used because it was available at Huntingdon and because the birds are partially insectivorous. They have, however, the disadvantage of being rather resistant to cardiac glycosides (Rothschild & Kellett 1972).

Three quail were placed in a cage with three male butterflies and a further three birds in another cage with three females. In the first cage one of the quail ate the three males within 30 min. In the other, one quail killed and ate one of the females, but the others remained untouched. These two birds were separated from their groups and put in individual cages. The one that had eaten the males was offered three male and three female butterflies each day over the next four days and ate all of them readily without detectable toxic manifestations. The other bird was offered three female butterflies the next day and killed one but did not eat it. The other two females were untouched 24 h later. Furthermore this bird did not touch either male or female butterflies which were offered to it over the next three days.

A colony of 80 quail were then given six butterflies. These were ignored except by two quail, each of which ate one. One of these birds was separated and put in a cage with two *H. bolina* and a Buff Ermine moth (*Spilosoma lutae* (Hufn.)). The quail ate both the butterflies and the moth within 15 min of their being presented.

The moth, and the related White Ermine (*Spilosoma lubricipeda* (L.)), are known to be distasteful to birds (Rothschild 1963). We have confirmed this repeatedly, very few being touched either by sparrows (*Passer domesticus* (L.)) or robins (*Erithacus rubecula* Hart.), though they were readily eaten by the local blackbirds (*Turdus merula* L.). Subsequently, in the experiments with quail both Buff and White Ermine moths were offered to all the quail.

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Only the exceptional segregated bird accepted them, and it ate all that were given to it. The other birds did not even try the moths.

(*c*) *Tests on larval foodplants*

Miriam Rothschild and Neville Marsh (private communication) tested for the presence of toxins in *Asystasia gangetica* and Sweet Potato (*I. batatas*) and also in pupae of *H. bolina* reared on them. After injecting extracts they found no substances toxic to locusts and mice in *A. gangetica* and the insects reared on it. In experiments using Sweet Potato there was evidence on one occasion of a toxic substance in both the plant and the insect. However, Rothschild & Marsh believe that these may be false positives due to the fact that this particular stock of locusts may have been unhealthy. Subsequent tests were negative. Furthermore, a tame magpie (*Pica pica* (L.)) particularly sensitive to a wide range of insect toxins readily ate the butterflies reared on Sweet Potato.

(*d*) *Observations using wild birds*

We have found using moths discarded from our mercury vapour trap that some birds avoid many warningly coloured species. *H. bolina* butterflies were therefore placed on a lawn among the moths and observed from a window.

In the first experiment four females and two males (all reared on *A. gangetica* and/or nettle) were used. Three of the females and one of the males were eaten by a male blackbird whose territory included the area of the moth trap, and one female was taken by a sparrow. The other male flew away.

In the second test we put out two males reared on Sweet Potato and three females reared on *A. gangetica*. The same blackbird attacked the male but then left it alone, and did not touch the three females. On two subsequent visits he also ignored these insects.

A starling (*Sturnus vulgaris* L.) attacked a female, damaged it but did not eat it, ignoring as well the other females and the males, including that crippled by the blackbird. Another male butterfly, from a brood fed on Sweet Potato, was then added to the group. A magpie arrived and ate this male, together with the one damaged by the blackbird, and the three female butterflies. The remaining male flew off.

Three more females from the *A. gangetica* brood and a male from the Sweet Potato brood were then put out. These were all eaten by the male blackbird when it returned to the moth trap.

The many sparrows on the lawn during this period (except one, see above) ignored all the butterflies and concentrated on eating the non-warningly coloured moths.

We feel that these experiments give no convincing evidence of toxic or distasteful qualities in *H. bolina*, although more systematic experiments are required with the wild birds when material is available. It would be particularly informative to repeat the experiments using the *Euploea* models to make sure that these are distasteful to the predators being tested.

6. Autosomal inheritance of the major genes (tables 2 and 3)

(*a*) *f.* pallescens

In *H. bolina* the female is the heterogametic sex (see Clarke *et al.* 1975). Brood 12364 shows that f. *pallescens* is not Y-linked, since the female offspring of a *nerina* segregated for *pallescens*, which they could not have done if the locus were on the non-pairing part of the Y chromosome.

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Brood 12703 shows that *pallescens* is not X-linked since a female manifesting this form (in combination with *euploeoides*), mated to a male which apparently was not carrying the factor controlling *pallescens*, produced a female of this form.

(*b*) *f.* nerina

Broods 12596 and 12597 exclude Y-linkage since a *euploeoides* female which could not be carrying *nerina* when mated to a male with *nerina* sisters produced female offspring carrying *nerina* combined with *euploeoides*.

Broods 12338, 12364, 12380 and 12391 exclude X-linkage, since *nerina* females when mated to males not carrying this form produced *nerina* among their offspring.

(*c*) *f.* euploeoides

Brood 12537 shows that *euploeoides* from Fiji is not Y-linked, since a female *pallescens* produced female progeny which included *euploeoides-pallescens*.

Brood 12741 shows that *euploeoides* derived from Sri Lanka is also not Y-linked since the *euploeoides* female progeny must have derived *euploeoides* from their father.

Brood 12728 shows that *euploeoides* from Fiji is not X-linked since a female carrying this form mated to a male that could not have done so produced female offspring carrying the form. For similar reasons brood 12674 shows that *euploeoides* derived from Sri Lanka also cannot be X-linked.

The results show that *pallescens*, *nerina* and *euploeoides* are all autosomally inherited.

7. Sex-controlled inheritance of pattern

Since the genes controlling *pallescens*, *nerina* and *euploeoides* are not Y-linked, and none of the characters appears in the male, the patterns must be sex-controlled to the female. The female form *naresi* is extremely variable, some specimens being almost indistinguishable from the male pattern. However, some show characters never found in the male and these presumably are sex-controlled.

8. Dominance and epistatic relationships between the female forms (table 2)

(*a*) *Male like form* (naresi) *to the other female forms*

(i) *To* pallescens

Brood 12411, which was a mating between a *pallescens* and a pure Fiji male from the same stock, produced *pallescens* and *naresi* in a ratio consistent with 3:1. Furthermore, that *pallescens* is dominant is confirmed by 12537 and subsequent generations. Brood 12537 produced 33 females all of which carried *pallescens* and yet sib matings of it (12671, 12696, 12697 and 12700) segregated for *naresi*. Furthermore, in broods 12696 and 12697, where segregation was uncomplicated by the presence of other forms, *pallescens* and *naresi* gave good approximations to a 3:1 ratio. Thus *naresi* is recessive to *pallescens*.

(ii) *To* nerina

Broods 12707 and 12708 were matings between *nerina* females from Sarawak and Fiji males which could not be homozygous for *nerina*, and were almost certainly homozygous *naresi* (see below), and yet they produced 32 *nerina*, showing that *nerina* is dominant.

TABLE 2. SEGREGATION OF FEMALE FORMS IN *H. BOLINA*

(The table gives the data from the broods discussed in the sections on autosomal inheritance, dominance and epistatic relationships between the female forms, except for a few broods which also give information on linkage. These are to be found in tables 3–6. In addition, the ancestral broods of those in the subsequent tables are given.)

brood no.	form and provenance of mother	provenance of father	offspring: males	offspring: females	
12183	*naresi*, Fiji	Fiji	57	78	35 *pallescens* 43 *naresi*
12233	*nerina*, Sarawak	Sarawak	0	24	*nerina*
12274	*pallescens*, 12183	12183	13	6	4 *pallescens* 2 *naresi*
12338	*nerina*, 12233	12183	0	30	23 *nerina* 7 *naresi*
12350	*nerina*, 12233	12183	0	96	71 *nerina* 25 *naresi*
12358	*nerina*, 12350	12183	0	1	*nerina*
12364	*nerina*, 12350	12183	0	12	7 *pallescens* 1 *nerina*-like 4 *naresi*
12365	*nerina*, 12350	12183	0	12	5 *pallescens* 3 *nerina* 4 *naresi*
12380	*nerina*, 12233	Fiji	0	12	*nerina*
12391	*nerina*, 12338	Fiji	3	51	22 *nerina* 29 *naresi*
12400	*nerina*, New Guinea	New Guinea	31	31	24 *nerina* 7 *naresi*
12411	*pallescens*, Fiji	Fiji	15	25	21 *pallescens* 4 *naresi*
12457	*nerina*, 12400	12400	26	44	42 *nerina* 2 *naresi*
12458	*nerina*, 12400	12400	28	40	39 *nerina* 1 *naresi*
12529	*naresi*, 12391	12411	0	5	2 *pallescens* 3 *naresi*
12537	*pallescens*, Fiji	Fiji (wild)	38	33	16 *euploeoides-pallescens* 17 *pallescens*
12540	*naresi*, Fiji	Fiji	5	10	*naresi*
12550	brown *naresi*, Fiji	Fiji (wild)	26	23	2 brown *naresi* (see p. 250) 21 *naresi*
12582	*euploeoides*, Sri Lanka	12411	0		4 *euploeoides-pallescens*
12590	*euploeoides*, Sri Lanka	Fiji	0	33	23 *euploeoides-pallescens* 10 *euploeoides*-like
12596	*euploeoides*, Sri Lanka	New Guinea	0	81	40 *euploeoides-nerina* 38 *euploeoides*-like 3 unscorable
12597	*euploeoides*, Sri Lanka	New Guinea	1	13	9 *euploeoides-nerina* 4 *euploeoides*-like
12606	*nerina*, Sarawak	Sarawak	2	40	*nerina*
12642	*naresi*, 12529	12550	0	18	*naresi*
12649	*euploeoides*, Sri Lanka	12550	0	33	*euploeoides*-like
12659	*naresi*, 12550	12550	3	3	*naresi*
12668	*euploeoides-pallescens*, 12537	12537	13	14	6 *euploeoides-pallescens* 2 *euploeoides* 3 *euploeoides*-like 2 *pallescens* 1 *naresi*
12671	*pallescens*, 12537	12537	12	11	2 *euploeoides-pallescens* 5 *pallescens* 4 *naresi*

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TABLE 2 (*cont.*)

brood no.	form and provenance of mother	provenance of father	offspring: males	offspring: females	
12674	*euploeoides-nerina*, 12596	12550	0	40	7 *euploeoides-nerina* 16 *euploeoides-naresi* 8 *nerina* 9 *naresi*
12694	*pallescens*, 12537	12537	8	6	3 *euploeoides-pallescens* 3 *pallescens*
12696	*pallescens*, 12537	12537	17	13	10 *pallescens* 3 *naresi*
12697	*pallescens*, 12537	12537	29	26	21 *pallescens* 5 *naresi*
12698	*nerina*, Sarawak	Sarawak	4	58	*nerina*
12700	*euploeoides-pallescens*, 12537	12537	47	33	15 *euploeoides-pallescens* 5 *euploeoides* 4 *euploeoides*-like 6 *pallescens* 3 *naresi*
12703	*euploeoides-pallescens*, 12590	12540	0	1	*pallescens*
12707	*nerina*, 12606	12540 or 12550	0	15	*nerina*
12708	*nerina*, 12606	12550	1	17	*nerina*
12728	*euploeoides-pallescens*, 12537	12540 or 12550	14	11	4 *euploeoides-pallescens* 4 *pallescens* 1 brown *naresi* (see p. 250) 1 *naresi* 1 unscorable
12730	*euploeoides*, Sri Lanka	12668	0	17	*euploeoides*
12740	*naresi* (hybrid)	Sri Lanka	0	68	*euploeoides*-like
12741	*naresi* (hybrid)	Sri Lanka	0	60	*euploeoides*-like
12744	*naresi* (hybrid)	Sri Lanka	0	5	*euploeoides*-like
12748	*naresi*, 12642	Sri Lanka	0	47	*euploeoides*-like
12762	*nerina*, Sarawak	12668	0	13	*euploeoides-nerina*
12763	*nerina*, Sarawak	12668	0	58	12 *euploeoides-pallescens* 11 *euploeoides-nerina* 1 *euploeoides** 14 *pallescens* 18 *nerina* 1 *naresi*† 1 unscorable
12769	*naresi*, 12696	12659	13	19	*naresi*
12775	*nerina*, Sarawak	12668	0	48	13 *euploeoides-pallescens* 10 *euploeoides-nerina* 13 *pallescens* 12 *nerina*
12776	*nerina*, Sarawak	12694	0	24	10 *euploeoides-pallescens* 13 *pallescens* 1 *nerina*
12777	*nerina*, Sarawak	12668	0	52	31 *pallescens* 21 *nerina*
12806	*nerina*, Sarawak	Fiji	0	18	4 *euploeoides-pallescens* 5 *euploeoides-nerina* 6 *pallescens* 3 *nerina*
12811	*pallescens*, 12668	Fiji	11	6	4 *euploeoides-pallescens* 1 *euploeoides* 1 *naresi*
12816	*nerina*, Sarawak	12537	0	7	3 *euploeoides-pallescens* 3 *pallescens* 1 *nerina*

* Probably a non-manifesting *euploeoides-nerina*. † Probably a non-manifesting *nerina*.

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TABLE 2 (*cont.*)

brood no.	form and provenance of mother	provenance of father	offspring: males	offspring: females	
12862	*euploeoides*, 12730	12769	0	22	*euploeoides*-like
12904	*euploeoides*-like, 12811	Fiji	29	15	8 *euploeoides-pallescens*
					7 *pallescens*
12968	*euploeoides-nerina*, Australia	Australia	50	45	20 *euploeoides-nerina*
					25 *nerina*
12980	*pallescens*, 12904	Fiji	6	4	3 *pallescens*
					1 *naresi*
13021	*euploeoides-pallescens*, Fiji	Fiji	1	3	*euploeoides-pallescens*
13045	*pallescens*, 12980	Fiji	21	19	2 *euploeoides-pallescens*
					4 *euploeoides*-like
					8 *pallescens*
					5 *naresi*
13052	*nerina*, Australia	Fiji	0	1	*pallescens*
13053	*nerina*, Australia	Australia	9	5	*nerina*
13062	*nerina*, Australia	Australia	10	10	5 *euploeoides-nerina*
					5 *nerina*
13072	*naresi*, Fiji	Australia	17	20	*nerina*
13074	*nerina*, 13072	13072	6	9	7 *nerina*
					2 *naresi*
13076	*nerina*, 13072	13072	25	16	9 *nerina*
					7 *naresi*
13084	*pallescens*, 13045	Australia	26	25	3 *euploeoides-pallescens*
					3 *euploeoides-nerina*
					8 *pallescens*
					11 *nerina*
13087	*naresi*, Fiji	Fiji (all *naresi*)	10	15	*naresi*
13091	*nerina*, 13062	13045	23	15	8 *euploeoides-nerina*
					7 *nerina*
13100	*naresi*, 13074	Fiji (all *naresi*)	11	15	*naresi*
13101	*naresi*, 13076	all *naresi*	22	23	*naresi*
13105	*nerina*, 13053	13021	7	6	4 *euploeoides-pallescens*
					2 *nerina*
13115	*naresi*, 13076	13084	1	1	*naresi*
13190	*naresi*, 13115	all *naresi*	11	8	*naresi*

Brood 12350 suggests also that *nerina* is dominant to *naresi* in most individuals, but that in some *naresi* can be dominant. The female parent of this brood was derived from Sarawak and had 23 sibs, all of which were *nerina*. When mated to a Fiji male which must either have been homozygous *naresi* or heterozygous for *pallescens*, it produced 71 *nerina*-like and 25 *naresi*-like individuals. This is significantly different from a 1:1 ratio, and cannot be a 3:1 since the male cannot have been carrying *nerina*. Thus it appears that all the progeny are heterozygous for *nerina* but that this form is recessive in about 25% of the individuals. The absence of *pallescens* suggests that the male is homozygous *naresi*. Brood 12338 gives an exactly comparable result. Brood 12380 is again a similar mating but only *nerina* were produced.

The hypothesis that *nerina* is frequently dominant to *naresi* is supported by broods 12358, 12364 and 12365, in which a *nerina-like* individual mated to a male that could not have been carrying that form produced *nerina*. In these broods the situation is slightly complicated by the fact that two of the males at least were heterozygous for *pallescens*, and this form appeared among the progeny. Nevertheless, in the non-*pallescens nerina* and *naresi* segregated in what was not significantly different from a 1:1 ratio.

The matings discussed are crosses between individuals from widely different geographical areas. However, there appears to be misclassification or reversal of dominance within pure New Guinea stock. Thus in brood 12400 a wild *nerina* female gave rise to progeny consistent with a ratio of 3 *nerina* to 1 *naresi*. Subsequent matings showed that at least some of the *nerina* were heterozygous for *naresi*. However, two sib matings, 12457 and 12458, between them produced 81 *nerina* and 3 individuals which were scored as being *naresi*, again suggesting misclassification or reversal of dominance, but not on the scale shown in the Sarawak × Fiji crosses.

Brood 12391, derived from a *nerina* known to be heterozygous for *naresi*, mated to a Fiji male that could not be heterozygous for *nerina*, gave a good 1:1 ratio, showing that the two forms differ by a major gene. Even here there was some excess of *naresi*, suggesting again reversal of dominance or misclassification.

A further difficulty which results in misclassification is that *naresi* can sometimes be mistaken for *nerina*. Some true *naresi* have a flush of brown which may be present only on the hindwings, or on the forewings close to the inner margin (where *nerina* has an orange patch), or both. Such insects in their extreme form can be very difficult to distinguish from *nerina* when the orange is weakly expressed (see also Poulton 1924). We have not been able to detect any clear-cut segregation with respect to these characters, suggesting that they may be under multifactorial control. Such difficulties in scoring are illustrated in brood 12550. The mother of this brood was a wild female which was originally classified as *nerina* but gave rise to 23 *naresi* offspring, suggesting that in fact it was a *naresi* female. Nevertheless, at least two of these offspring had a trace of brown near the inner margin of the forewing where *nerina* has an orange patch. On occasion in subsequent broods similar insects appeared when the offspring of 12550 were used, but they never produced unequivocal *nerina*. Thus not only can *nerina* be misclassified as *naresi*, at least in heterozygotes, but *naresi* can on occasion be classified as *nerina* even if great care is taken (see also pp. 233, 254 and plate 2*b*).

(iii) *To* euploeoides

Broods 12740, 12741 and 12744 suggest that *euploeoides* is dominant or nearly dominant to *naresi*. In each of these matings a male from Sri Lanka was mated to a female *naresi* of hybrid origin from large broods producing only *naresi*. The resulting female insects look essentially like *euploeoides*; however, they have a variable amount of white in the subapical bar of the forewings, in which some at least are almost as white as in *naresi* itself, whereas in others it is only represented by a trace of blue scaling and is thus identical with many pure Sri Lanka *euploeoides*. Broods 12649 and 12748 are F_1 hybrids between pure Sri Lanka and pure Fiji stock, and differ only in that 12748 is a reciprocal cross of 12649. Nevertheless, the offspring differ considerably in the whiteness of the subapical forewing bar, suggesting that the parents differed in modifiers of dominance. In all these five broods the central area of the hindwing is usually dark brown as in *euploeoides* but a trace of white or blue scaling is present in a proportion of individuals, thus indicating the presence of *naresi* in the heterozygote. In the hybrids the rows of white spots round the margins of the fore and hindwings are much reduced. This is unconnected with heterozygosity since the *euploeoides* mother of brood 12862 (which was a homozygote, see below) also had reduced white spots. We can therefore say that *euploeoides* is nearly dominant to *naresi* but that the heterozygotes (referred to in the tables as '*euploeoides*-like') are recognizable in the majority of individuals.

Broods 12668 and 12700 suggest that a high proportion of *naresi*/*euploeoides* heterozygotes are

also recognizable in pure Fiji stock, indicating that the absence of complete dominance is not due to the effects of hybridization between individuals from different geographical areas. The sib matings involved should give a ratio of one homozygous *euploeoides* to two heterozygotes among the non-*pallescens* individuals. There were in fact seven insects that appeared to be homozygous and seven heterozygous (p. 250), suggesting that some of the heterozygotes were indistinguishable from homozygotes.

Brood 12730 was a mating between a *euploeoides* from Sri Lanka and a Fiji male from brood 12668 that could not be carrying *pallescens*, as judged by its offspring, but could have been genetically *naresi* or heterozygous or homozygous for *euploeoides*. The offspring were all typical *euploeoides*, with the fore- and hindwing border of white spots intermediate between the Fiji and Sri Lanka phenotype. Brood 12862 was a mating between one of these females and a male from an all *naresi* brood and produced 22 *euploeoides*-like individuals, which could not be separated into two distinct types (see plates 1*i*, 2*a*, 2*l*). This shows that the female was a homozygote, having received *euploeoides* both from Sri Lanka and Fiji stocks. The result not only demonstrates that *euploeoides* is dominant or semi-dominant to *naresi* but also that *euploeoides* derived from Sri Lanka and Fiji respectively are probably controlled by the same allelomorph (see also p. 256). The brood also shows that the reduction in the size of the white submarginal spots is due to modifiers introduced by the Fiji race and not to differences in allelomorphism between the Sri Lanka and Fiji stocks.

(*b*) *f.* pallescens

(i) *To* nerina

Brood 12777 was a mating between a *nerina* from Sarawak and a Fiji male known not to be carrying *nerina*. The brood segregated *nerina* and *pallescens* in a 1:1 ratio. Since these *pallescens* must have been carrying *nerina* and are not certainly distinguishable from *pallescens* not carrying *nerina* from other broods (see p. 260), *pallescens* must be dominant or epistatic to *nerina*.

(ii) *To* euploeoides

Brood 12590 was a mating between a *euploeoides* female from Sri Lanka and a Fiji male. It produced 33 females in which the forewing subapical white bar was heavily suffused with orange and the hindwing white area of *pallescens* was also replaced by orange. Ten offspring were *euploeoides*-like and therefore heterozygous for *naresi*. Thus butterflies carrying both *euploeoides* and *pallescens* are distinguishable and intermediate between the two forms.

Brood 12537 was a mating between a Fiji *pallescens* which could not have been carrying *euploeoides* and a wild Fiji male. It segregated 17 typical *pallescens* and 16 females that resembled the *euploeoides-pallescens* of brood 12590. In a sib mating of this brood (12668) both typical *euploeoides* and *naresi* segregated, showing that the intermediate females were in fact heterozygous for both *pallescens* and *euploeoides*. This demonstrates that the intermediate phenotypes in brood 12590 are not due to hybridization since similar insects have been produced in a cross not involving two geographical races.

(*c*) *f.* nerina

(i) *To* euploeoides

Broods 12596 and 12597 were matings between Sri Lanka *euploeoides* and males from New Guinea which had *nerina* sisters. The progeny were *euploeoides*-like, but different from pure *euploeoides* in having a more conspicuous subapical forewing bar. They also segregated in a 1:1 ratio for individuals with and without traces of the *nerina* orange forewing patch. Thus the

broods were probably segregating for *euploeoides-nerina* and *euploeoides-naresi*, and *euploeoides* is semi-dominant (or epistatic) to *nerina*.

Crosses between a *nerina* female from Sarawak and a Fiji male whose sisters segregated for *euploeoides* (broods 12762 and 12763) also produced recognizable *euploeoides-nerina* heterozygotes very similar to those derived from Sri Lanka females. These broods and a number of other similar ones suggested that the heterozygotes were more variable in the degree to which *nerina* was expressed compared with the Sri Lanka crosses.

Euploeoides-nerina is common in Australia and the progeny of two wild females (broods 12968 and 13062) segregated in a 1:1 ratio for *euploeoides-nerina* and *nerina*. Subsequent broods showed that several of the *nerina* were homozygous, suggesting that the original ratio resulted from the segregation of *euploeoides* in a homozygous *nerina* stock. We were unable to obtain *epuloeoides*-like insects from the descendants of the broods because many of the females proved infertile when crossed to pure *naresi* stock. However, our interpretation is supported by the fact that on crossing a *nerina* female (brood 13091) from Australia with a Fiji male carrying the gene for *euploeoides* we synthesized eight insects phenotypically very similar to the Australian *euploeoides-nerina*.

TABLE 3. INFORMATION ON POSSIBLE LINKAGE BETWEEN f. *EUPLOEOIDES* AND f. *PALLESCENS*

			offspring		
brood no.	mother	father	males	females	
12687	*euploeoides-pallescens*, 12590	12540	0	1	*naresi*
12703	*euploeoides-pallescens*, 12590	12540	0	1	*pallescens*
12705	*euploeoides-pallescens*, 12582	12550	0	12	3 *euploeoides-pallescens* 4 *euploeoides*-like 2 *pallescens* 3 *naresi*
12728	*euploeoides-pallescens*, 12537	12540 or 12550	14	10	4 *euploeoides-pallescens* 4 *pallescens* 1 *nerina** 1 *naresi*
12795	*euploeoides-pallescens*, 12705	12659	0	1	*euploeoides*-like
13244	*euploeoides-pallescens*, 13166	13190	20	32	6 *euploeoides-pallescens* 5 *euploeoides*-like 15 *pallescens* 6 *naresi*

* Could be brown *naresi*, see p. 250, or a crossover, see p. 254.

9. INDEPENDENT ASSORTMENT, LINKAGE AND ALLELOMORPHISM (TABLES 3–5)

(*a*) *f.* pallescens *and f.* euploeoides (*table* 3)

In order to test for linkage between *pallescens* and *euploeoides* we crossed females of pure Sri Lanka *euploeoides* stock with Fiji males carrying *pallescens* and mated the *euploeoides-pallescens* offspring of this and a subsequent generation to males with all *naresi* sisters (broods 12687, 12703, 12705 and 12795). In addition, we crossed a Fiji male carrying *euploeoides* with a Fiji female carrying *pallescens*, the resulting *euploeoides-pallescens* females being similarly mated to males from a pure *naresi* brood (brood 12728). We also mated a *nerina* from Australia with a Fiji male carrying *euploeoides* and *pallescens*, and subsequently back-crossed to *naresi* for two generations. The second back-cross (brood 13244) gave information on linkage equivalent to that in brood 12795, since in both the allelomorphs were in coupling whereas in the other broods they were

in repulsion. The resulting segregations showed that *euploeoides* and *pallescens* are not controlled by the same locus but that the loci concerned both segregate. The overall ratios of the four phenotypes expected in such matings suggested that both *euploeoides* to non-*euploeoides* and *pallescens* to non-*pallescens* segregated in a satisfactory 1:1 ratio and that there was no evidence of linkage, but the amount of information on these points is small.

TABLE 4. INFORMATION ON POSSIBLE LINKAGE BETWEEN f. *EUPLOEOIDES* AND f. *NERINA*

			offspring		
brood no.	mother	father	males		females
12674	*euploeoides-nerina*, 12596	12550	0	40	7 *euploeoides-nerina* 16 *euploeoides*-like 8 *nerina* 9 *naresi*
12675	*euploeoides-nerina*, 12596	12550	0	72	20 *euploeoides-nerina* 16 *euploeoides*-like 26 *nerina* 10 *naresi*
12677	*euploeoides-nerina*, 12596	12550	0	38	4 *euploeoides-nerina* 8 *euploeoides*-like 11 *nerina* 15 *naresi*
12678	*euploeoides-nerina*, 12596	12540	0	5	1 *euploeoides-nerina* 3 *euploeoides*-like 1 *naresi*
12679	*euploeoides-nerina*, 12596	12540 or 12550	0	3	2 *nerina* 1 *naresi*
12680	*euploeoides-nerina*, 12596	12550	0	83	19 *euploeoides-nerina* 23 *euploeoides*-like 21 *nerina* 20 *naresi*
12684	*euploeoides-nerina*, 12596	12540	0	21	9 *euploeoides*-like 6 *nerina* 6 *naresi*
12685	*euploeoides-nerina*, 12596	12540 or 12550	0	2	1 *nerina* 1 *naresi*
12692	*euploeoides-nerina*, 12596	12540 or 12550	0	18	3 *euploeoides-nerina* 6 *euploeoides*-like 6 *nerina* 3 *naresi*
12773	*euploeoides-nerina*, 12680	12659	0	10	2 *euploeoides-nerina* 4 *euploeoides*-like 3 *nerina* 1 *naresi*
12792	*euploeoides-nerina*, 12680	12659	0	61	16 *euploeoides-nerina* 15 *euploeoides*-like 25 *nerina* 5 *naresi*
12794	*euploeoides-nerina*, 12680	12659	0	4	1 *euploeoides-nerina* 2 *euploeoides*-like 1 *nerina*
13118	*euploeoides-nerina*, 13091	13087	1	5	1 *euploeoides*-like 2 *nerina* 2 *naresi*
13243	*euploeoides-nerina*, 13166	all-*naresi*	8	10	6 *euploeoides-nerina* 1 *euploeoides*-like 1 *nerina* 2 *naresi*

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(*b*) *f.* nerina *and f.* euploeoides (*table* 4)

In order to test for linkage between *nerina* and *euploeoides* we took a *euploeoides* female from Sri Lanka and mated her to a male with *nerina* sisters from New Guinea. The resulting *euploeoides-nerina* females were crossed with males from pure *naresi* stock. A number of the resulting *euploeoides-nerina* female offspring were again backcrossed to all-*naresi* stock. We also synthesized *euploeoides-nerina* using stock obtained from Australia and Fiji and back-crossed to *naresi*.

Tests of the segregation ratios between the broods (pooling broods with expected classes less than 5) showed considerable heterogeneity (χ^2_{15} 28.32 $P < 0.02$). Partitioning the χ^2 showed no heterogeneity with respect to the segregation of *euploeoides* to non-*euploeoides* but considerable heterogeneity in the segregation of *nerina* to non-*nerina* (χ^2_7 20.61 $P < 0.01$). Since the overall segregation is in agreement with the expected 1:1 ratio the heterogeneity is almost certainly due to misclassification of *nerina* for non-*nerina* in some broods and non-*nerina* for *nerina* in others, as has already been suggested can happen (p. 250). In order to estimate the crossover value between *euploeoides* and *nerina* it was therefore necessary to treat each brood independently using the method suggested by Bailey (1961) for such cases. Each crossover value was weighted by the reciprocal of its variance and a mean crossover value estimated which was 46.65 ± 3.1 %, giving no support for linkage. When investigating *pallescens* and *nerina*, triple back-cross broods were produced (see below) which give further data. The crossover value between *euploeoides* and *nerina* estimated from these using the maximum likelihood method is 45.2. Thus the genetic analysis shows little evidence for linkage between *euploeoides* and *nerina*.

(*c*) *f.* nerina *and f.* pallescens (*table* 5)

In order to test for linkage between *nerina* and *pallescens*, *nerina* females from the all-*nerina* brood (12698) from Sarawak were mated to males from Fiji, none of whose sisters were *nerina* but some were *pallescens* and some *euploeoides-pallescens*. In addition, heterozygotes in which the *nerina* came from Australia were also produced. The resulting *pallescens* and *euploeoides pallescens* females were crossed to males all of whose sisters were *naresi*. In these broods *pallescens* to non-*pallescens* segregated in a satisfactory 1:1 ratio, as did *euploeoides* to non-*euploeoides*. All the non-*pallescens* females were quite clearly *nerina* (or *euploeoides-nerina*) except for three specimens in which the orange *nerina* patch in the forewings was very much reduced together with four in which it was absent. Thus there is a striking deficiency of *euploeoides*-like and *naresi* on the assumption of independent assortment. In fact, if the seven doubtful females were carrying the allelomorph determining *nerina* there were no crossovers, and 7 % of the females carrying *nerina* were misclassified as not possessing it. Thus the genes determining *pallescens* and *nerina* are either allelomorphs of one another or are extremely closely linked.

Assuming there is no misclassification we can estimate the crossover value both in broods segregating for *euploeoides* and those where it is not. In the six broods in which the mother was not heterozygous for *euploeoides* there is one *naresi* among 30 females. Since *nerina* cannot be recognized in the presence of *pallescens* the *pallescens-nerina* crossover class cannot be distinguished from the non-crossover *pallescens* individuals. Consequently the crossover value is $\frac{1}{15} \times 100$, not $\frac{1}{30} \times 100$. This gives a crossover value of 6.7 %.

Among the broods segregating for *euploeoides* there are no *naresi* but there are six *euploeoides*-like. If correctly classified these will be crossover classes and the estimated crossover by maximum likelihood is 6.9 % (see below). Thus the two kinds of broods agree in giving a crossover

value of about 7 %. Alternatively, if there are no crossovers there has been a 14 % misclassification of *euploeoides-nerina* as *euploeoides* but no misclassification of *nerina* as *naresi* (that is to say 7 % misclassification overall).

TABLE 5. INFORMATION ON POSSIBLE LINKAGE BETWEEN f. *EUPLOEOIDES*, f. *PALLESCENS* AND f. *NERINA*

			offspring		
brood no.	mother	father	males		females
12858	*euploeoides-pallescens*, 12763	12769	0	58	11 *euploeoides-pallescens* 16 *euploeoides-nerina* 15 *pallescens* 16 *nerina*
12859	*euploeoides-pallescens*, 12776	12769	0	44	11 *euploeoides-pallescens* 6 *euploeoides-nerina* 3 *euploeoides*-like 11 *pallescens* 13 *nerina*
12860	*euploeoides-pallescens*, 12775	12769	0	11	2 *euploeoides-pallescens* 4 *euploeoides-nerina* 1 *euploeoides*-like 1 *pallescens* 3 *nerina*
12861	*pallescens*, 12763	12769	0	2	1 *pallescens* 1 *nerina*
12867	*pallescens*, 12763	all-*naresi*	1	2	*pallescens*
12868	*pallescens*, 12763	all-*naresi*	0	14	7 *pallescens* 6 *nerina* 1 *naresi*
12870	*pallescens*, 12806	all-*naresi*	0	6	4 *pallescens* 2 *nerina*
12871	*euploeoides-pallescens*, 12763	all-*naresi*	0	18	5 *euploeoides-pallescens* 4 *euploeoides-nerina* 1 *euploeoides*-like 3 *pallescens* 5 *nerina*
12878	*pallescens*, 12816	12769	0	2	*pallescens*
13095	*pallescens*, 13052	all-*naresi*	0	4	2 *pallescens* 2 *nerina*
13166	*naresi*, 13101	13105	16	23	4 *euploeoides-pallescens* 5 *euploeoides-nerina* 9 *pallescens* 5 *nerina*
13181	*naresi*, 13101	13105	1	4	2 *euploeoides-pallescens* 1 *euploeoides-nerina* 1 *nerina*
13183	*naresi*, 13100	13105	6	4	2 *euploeoides-pallescens* 1 *euploeoides-nerina* 1 *nerina*
13188	*naresi*, 13100	13105	2	4	2 *euploeoides-pallescens* 1 *euploeoides*-like 1 *nerina*
13201	*euploeoides-nerina*, 13091	all-*naresi*	2	1	*euploeoides-nerina*

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10. The relationship of the loci controlling *euploeoides*, *nerina* and *pallescens*

Using a maximum-likelihood approach we can estimate the crossover values in the triple backcrosses (table 5). That between *euploeoides* and *nerina* is 45.2%, agreeing well with the estimate above, where *pallescens* was not involved. The estimated crossover value between *nerina* and *pallescens* is 6.9%.

Because of the possibility of misclassification of *nerina* with respect to non-*nerina* it will be necessary to test some of these putative crossover individuals to make sure that they are not carrying *nerina* and thus show that the loci are distinct and we are not dealing with allelomorphs. Up to date we have managed to mate three such females but they have failed to produce progeny.

If the six *euploeoides*-like individuals really are due to crossing over then the two loci are involved. If we assume that the estimated crossover value of 45% suggests that the locus controlling *euploeoides* is also on the same chromosome (see above), then the absence of *naresi* in the triple backcross indicates that this must be the double crossover class and the order of the loci must be *euploeoides*, *nerina*, *pallescens*. However, against this view is the fact that the estimated crossover value between *euploeoides* and *nerina* is so near 50% that even if all three loci were linked one would expect at least some double crossovers (*naresi*) among the six putative ones. Because *nerina* is more difficult to detect in the presence of *euploeoides* (p. 234) the most likely hypothesis is that the locus controlling *euploeoides* is unlinked to that controlling *nerina* and *pallescens*, and that the latter has three allelomorphs with *pallescens* dominant or almost dominant to *nerina*, and *nerina* dominant to the third phenotype which lacks brown pigment.

11. The relationship of f. *euploeoides* from Sri Lanka, Fiji, Hong Kong, Australia and Sarawak (table 6)

In order to test whether the somewhat different *euploeoides* mimics from Sri Lanka (plate 1*b*) and Fiji (plate 1*c*) are controlled by the same locus we mated (brood 12730) a male from a pure Fiji brood (12668) in which *euploeoides* appeared, with a *euploeoides* female derived from Sri Lanka. Three of the resulting *euploeoides* females were mated to males all of whose female sibs were non-*euploeoides* (broods 12847, 12862 and 12869). The resulting 58 females were all *euploeoides*-like but showed considerable phenotypic variation. Some of them were very like Fiji *euploeoides* (compare plate 1*c* and plate 2*a*) while others showed evidence of being heterozygous for non-*euploeoides* (compare plate 1*c* with plates 1*i* and 2*l*). In the broods there seemed to be no clear-cut differentiation into two *euploeoides* patterns (Fiji and Sri Lanka). Since all the progeny carried *euploeoides* the genes from the two countries are apparently allelomorphic, and because there were not two distinct types of *euploeoides* the results are consistent with this phenotype being controlled by the same allelomorph in both localities, the differences arising from modifiers of the mimetic pattern.

A female *euploeoides* from Hong Kong (12805) produced 22 females, all of which were *euploeoides*-like. In order to test whether the form was controlled by the same locus as *euploeoides* from Sri Lanka and Fiji we crossed the insects to pure Fiji males from brood 12781 and attempted to cross the progeny (broods 12876 and 12877) back to broods not carrying *euploeoides*. Only one, brood 12907, was partially successful, producing two *euploeoides*-like females. Thus the brood is consistent with allelomorphism between Hong Kong and Fiji.

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TABLE 6. THE GENETIC RELATIONSHIP BETWEEN f. *EUPLOEOIDES* FROM SRI LANKA, FIJI AND HONG KONG

brood no.	mother	father	offspring: males	offspring: females	
12768	*naresi*, 12696	12659	4	6	3 *nerina* 3 *naresi*
12781	*euploeoides*, 12668	Fiji	8	9	*euploeoides*-like
12805	*euploeoides*, Hong Kong	Hong Kong	0	22	*euploeoides*
12847	*euploeoides*, 12730	12768	0	25	*euploeoides*-like
12862	*euploeoides*, 12730	12769	0	22	*euploeoides*-like
12869	*euploeoides*, 12730	all-*naresi*	0	11	*euploeoides*-like
12873	*naresi*, 12768	12769	4	9	1 *nerina* 8 *naresi*
12876	*euploeoides*, 12805	12781	0	3	*euploeoides*
12877	*euploeoides*, 12805	12781	0	14	*euploeoides*-like
12907	*euploeoides*, 12877	12873	0	2	*euploeoides*-like

We have attempted to test whether the *euploeoides* genes from Australia and from Sarawak are also allelomorphic with those from Sri Lanka and Fiji. Although we have managed to obtain suitable F_1 hybrids we have not yet been able to get the appropriate backcrosses.

12. QUANTITATIVE VARIATION

The details of the patterns of the various female forms show a great deal of continuous variation which we have found impossible to quantify. It has therefore been impossible to make a rigorous genetic analysis and, in particular, to measure the component of variance due to the environment. Nevertheless it has been possible to get some idea of the mode of inheritance of the variability.

(*a*) *Blue sheen*

It has been reported that in India (Poulton 1924) and in Sri Lanka (Woodhouse 1950) in both sexes the insects in the dry season tend to be bluer and larger than those in the wet season. Particularly striking in this respect were the progeny of a large female *euploeoides* caught by us in Sri Lanka in August 1974 and brought back alive to lay in one of our greenhouses. The offspring were typical of the dry season form being large and having a considerable amount of blue, particularly the vast majority of the males. The second generation, raised on the same foodplants in the same greenhouse, produced 11 dry-season forms and 4 wet among the males. In another greenhouse 6 dry and 29 wet forms were produced. Equivalent broods raised in our cages produced 7 dry and 1 wet. Our results are consistent with the hypothesis that an unidentified environmental factor controls this variation. The only one we could identify as being associated with the production of the two forms, though not necessarily determining them, was day-length, the greenhouse producing a majority of the wet season form having a 16 h day whereas the others had a day length of 12 h or less.

Since equivalent dry season males have not been produced during our extensive breeding of other races (all producing males analogous with the wet season form) the results suggest that the ability to react to the environmental factors is genetically determined. Because previous F_1 individuals and backcrosses to the other races have not produced males of the dry form, the

character is probably recessive. We have been unable as yet to produce F_2s or backcrosses to Sri Lanka stock because of the absence of Sri Lanka males (see Clarke *et al.* 1975).

In our broods other than the pure Sri Lanka stock mentioned above there is considerable variability in the amount of blue both in males and females between families. Furthermore, within broods there is a tendency for the larger females to be the bluer and in at least one these were the insects which emerged latest.

When a brood segregates for the various polymorphic forms, *euploeoides-nerina* is on average the most blue, closely followed by *euploeoides*, *nerina* and *naresi*, which are about equal, with *euploeoides-pallescens* tending to be less blue than these, but bluer than *pallescens*. Thus the segregation of the major genes affects the amount of blue, which itself is probably controlled by modifiers as well as the environment.

(*b*) *The light areas*

The modifiers affecting the amount of white in the marginal and three rows of submarginal spots of the fore and hindwing borders appear to be different from those affecting the white in the subapical forewing bar and the hindwing patch, since all three characters appear to vary independently. That the variation has a genetic basis is shown by comparing pure Sri Lanka material with individuals derived from backcrosses into Fiji stock. Such back crossing greatly reduces the amount of white in the border (compare plate 1*b* with plate 2*a*).

The light forewing bar can vary from a continuous band divided by the thin black lines of the wing veins into rectangular spots (plate 2*b*) to a series of more interrupted spots as a result of an extension of the black pigment (plate 1*e*). In the most extreme cases, a black triangular area divides the bar at the middle into two groups of spots.

The variation is seen both within broods and between them and affects all the major phenotypes about equally, except that insects heterozygous for *euploeoides* always have the bar even more reduced as the result of the manifestation of the major gene (plate 2*c*).

The light area in the hindwing can vary from a large distinct patch (plate 2*d*) to a small roundish area invaded by dark scales (plate 1*e*). The variation in the expression of the spot is found both within and between broods and affects about equally all the major forms excluding those which are carrying *euploeoides*, in which the area is much reduced (plate 1*i*, plate 2*e* and 2*l*) or absent (plate 2*a*) as the result of the presence of the major gene.

The three characters white submarginal border, forewing bar and hindwing patch appear to vary independently of one another within broods as well as between them, suggesting that the genetic and environmental factors affecting the characters are independent. Since all three are controlled by the extension of dark pigment this absence of correlation suggests that much of the variation is genetic in origin.

(*c*) *Brown pigment in f.* pallescens

The shade of the brown pigment in *pallescens* can vary from light fawn (plate 1*g*) to dark chestnut (plate 1*h*). This variation can occur both within and between broods. Furthermore, brood 12183 was particularly variable and a pale female from this brood mated to a sib produced *pallescens* which on average were lighter in colour than those in brood 12274, in which the female parent was dark, suggesting that anyhow part of the variation is genetically determined.

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(*d*) *Hue of the brown areas in relation to their size*

The orange brown pigment found on the forewing of f. *nerina* varies in colour in a manner parallel with that in *pallescens*. However, in f. *nerina* the hue is correlated with the size of the pigmented area – the smaller this is the darker the pigment (compare plate 1*f* and plate 2*f*). Thus the hue of the orange appears to be controlled by the genes determining the size of the area.

In the original brood carrying *pallescens* (12183) there appeared to be a positive correlation between the reduction in the pale area in the hindwing and the darkness of the brown pigment. This association was also found in some of the subsequent broods derived by sib matings. However, a few pale insects were produced which also had a very reduced pale hindwing area. Thus the data suggest that some of the factors controlling the size of the pale area in the hindwing are also affecting the brown hue of *pallescens*.

13. Discussion

(*a*) *Formal genetics*

The genetic investigation has shown that the mimetic female form of *H. bolina* mimicking different species of *Euploea* in different parts of its range, is controlled by a single major gene, which is dominant, or nearly dominant, in effect.

The second most widespread of the female forms is *nerina*. This is controlled by a single locus and the form is dominant or nearly dominant. However, the heterozygote can be rather variable and occasionally the allelomorph fails to manifest in this condition and then the form behaves as a recessive.

The major form with the most restricted range is *pallescens*, which is confined to the South Moluccas, Australia and some Pacific islands. This form, which is very variable in the shade of the brown pigment, is dominant, the heterozygote being indistinguishable with certainty from the homozygote.

Naresi, the male-like form, is probably the ancestral pattern, since it is shared with some other members of the genus. The interpretation of the genetic control of *naresi* depends on whether *nerina* and *pallescens* are controlled by separate loci or by two allelomorphs at a single locus. If two loci are involved then *naresi* is the triple recessive, being homozygous for the absence of the allelomorphs controlling *euploeoides* (*E*), *nerina* (*N*) and *pallescens* (*P*). If, on the other hand, the putative crossovers in our data are due to misclassification, then *naresi* is a double recessive, being homozygous for the absence of the allelomorph controlling *euploeoides* (*E*) and those controlling *nerina* (P^n) and *pallescens* (*P*). The possible genotypes of the major forms are given in table 7.

Considering the interactions between the major genes, one finds that *euploeoides* is partially epistatic to *nerina*, particularly when the latter form is in the heterozygous condition. The allelomorph *E* in the presence of the *nerina* homozygote produces a *euploeoides*-like insect with a brown patch on the inner margin of the forewing typical of *nerina* (but tending to be smaller) and frequently with brown scaling in the centre of the hindwing, a character not present in *nerina* or *euploeoides*. In the *nerina* heterozygote the brown may be considerably reduced and in a few specimens absent, in which case *euploeoides* is fully epistatic to *nerina*. Thus in the majority of cases these double dominants are poor mimics of the *Euploea* models (plates 1*k*, 2*c*, 2*g*).

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Euploeoides and *pallescens* in combination, whether in the homozygous or heterozygous condition, produce a phenotype intermediate in appearance. Thus the white areas characteristic of *pallescens* are very much reduced or absent and the black scaling of *euploeoides* is converted to brown, except in those restricted areas where *pallescens* is black. The actual shade of brown is very variable, as it is in *pallescens* (plates 1*l*, 2*h*, 2*i*).

It is not certain whether *pallescens* and *nerina* are controlled by separate loci, but if they are, then *pallescens* is fully epistatic to *nerina* in some insects, but not all. Thus if one compares *pallescens* females known to be heterozygous for *nerina* with their progeny not carrying *nerina* (plate 2*j* and table 5), then on average the *nerina* patch is both larger and of a lighter orange hue in those heterozygous for *nerina* (plate 2*k*). However, only about 50% can be certainly scored as heterozygous for *nerina* by their phenotype. On the other hand, if they are allelic, as the data suggest, then *pallescens* is not completely dominant in about 50% of insects (see table 7).

TABLE 7. POSSIBLE GENOTYPES OF THE MAIN PHENOTYPES (SEE TEXT)

form	two locus hypothesis	three locus hypothesis
euploeoides and *euploeoides*-like	*EEpp*, *Eepp*†	*EEnnpp*, *Eennpp*†
pallescens	*eePP*, *eePP*^{n}‡, *eePp*	*eeNNPP*‡, *eeNnPP*‡, *eennPP*, *eeNNPp*‡, *eeNnPp*‡, *eennPp*
nerina	*eeP*^{n}*P*^{n}, *eeP*^{n}*p*§	*eeNNpp*, *eeNnpp*§
naresi	*eepp*‖	*eennpp*‖
euploeoides-pallescens	*EEPP*, *EEPP*^{n}, *EEPp*, *EePP*, *EePP*^{n}, *EePp*	*EENNPP*, *EENnPP*, *EEnnPP*, *EENNPp*, *EENnPp*, *EEnnPp*, *EeNNPP*, *EeNnPP*, *EennPP*, *EeNNPp*, *EeNnPp*, *EennPp*
euploeoides-nerina	*EEP*^{n}*P*^{n}, *EEP*^{n}*p*¶ *EeP*^{n}*P*^{n}, *EeP*^{n}*p*¶	*EENNpp*, *EENnpp*¶ *EeNNpp*, *EeNnpp*¶

† This heterozygote is often recognizable.
‡ These genotypes can sometimes be scored for the presence of *nerina*.
§ Sometimes misclassified as *naresi*.
‖ The brown *naresi* form is sometimes difficult to distinguish from *nerina*.
¶ Sometimes misclassified as *euploeoides*-like.

It has frequently been suggested that crossing-over does not normally occur in female Lepidoptera, a hypothesis borne out by the cytological work of Suomalainen (see Suomalainen, Cook & Turner 1973) and genetic investigations in *Bombyx mori* (Tazima 1964) and *Heliconius melpomene* and *H. erato* (Turner & Sheppard 1975).

On the other hand, although Traut & Rathjens (1973) have cytological evidence for the absence of chiasmata in *Ephestia kuehniella* Z., there is genetic evidence for crossing-over in this sex in which 12 double recessive individuals were produced in an F_2 brood in repulsion (see Robinson 1971). E. Suomalainen (private communication) found no evidence of chiasmata during meiosis in five *H. bolina* females, two of which were from all-female producing stock (Clarke *et al.* 1975). If this evidence can be taken as indicating the absence of crossing-over in the female, then the locus controlling the *euploeoides* phenotype must be on a different chromosome from that controlling *pallescens* and *nerina*, since recombination occurred when the female parent was the double heterozygote. On the hypothesis of no crossing-over in the female the apparent recombination between the loci controlling *nerina* and *pallescens* (table 5) must be accounted for by misclassification of *nerina* as non-*nerina* in six individuals heterozygous for *euploeoides* and one not heterozygous for this allelomorph.

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In view of the difficulty of classifying *nerina* in the presence of the black pigment controlled by *E*, the absence of significant linkage between either *euploeoides* and *nerina* or *euploeoides* and *pallescens*, combined with the cytological evidence, it seems likely that only two loci are involved with two allelomorphs (*E* and *e*) at one locus and three (*P* controlling *pallescens*, P^n controlling *nerina* and *p*) at the other (see table 7).

The locus designated *E* controlling the distribution of dark scales could be complex, but there is no evidence for this since there are no known varieties whose patterns suggest that they could have arisen by crossing over between genes controlling its different elements. Furthermore, the modification of the pattern produced by *E* merely consists of an extension of the dark pigment and therefore is similar to the kind of modification found in industrial melanism, and in the major difference between *Papilio machaon* and *Papilio polyxenes*, where supergenes have never been invoked (see p. 263).

It is possible that the second locus (*P*) is complex since more than two allelomorphs are known, but again no insects likely to have been derived by crossing over have been observed in collections. Thus here again there is no evidence for the presence of a supergene.

(*b*) *Modifiers*

We have shown that the differences in the detail of the mimetic form *euploeoides* are not determined by different allelomorphs but by modifiers affecting the amount of white on the submarginal border and in the subapical bar on the forewing, and the amount of iridescent blue (compare plate 1*b*, *c* and *d*).

The pattern of *nerina* is also affected by modifiers determining the size of the *nerina* patch, the size of the white subapical bar on the forewing, of the white central patch on the hindwing and of the marginal white spots, and the amount of iridescent blue. There also appears to be genetic variation in the hue of the brown areas (compare plate 1*f* and plate 2*d*, *f*).

Pallescens is the most variable form of all, particularly as regards the colour which may be anything between a deep chestnut and a pale yellowish fawn. There is also great variation in the amount of black pigment and in the size of the white areas (the subapical bar and the hindwing patch). All this variation (compare plate 1*g*, *h*, and plate 2*j*) appears to be partly under genetic control.

Naresi (plate 1*e*) varies in the amount of white in the subapical forewing bar, in the size and shape of the hindwing patch, the presence or absence of a small quantity of brown in this area and on the forewing, and in the amount of blue scaling. Thus *naresi* can vary from a black and white insect (with the exception of those with a little brown on them, plate 2*b*) to one with so much blue that it is almost indistinguishable from the male. This quantitative variation is again influenced by modifiers.

These differences between individuals described for the main forms are also found in insects carrying combinations of the allelomorphs controlling them. This will be seen from a comparison of the appropriate insects illustrated in plates 1 and 2.

(*c*) *Comparisons*

H. bolina has always been assumed to be a Batesian mimic, since it is polymorphic and has a non-mimetic male, but the palatability of the insect needed testing since it is very long-lived, which is unusual in Batesian though quite common among Müllerian mimics. The preliminary tests described in this paper give no evidence of the presence of the toxins so often found in

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distasteful butterflies and showed that those from larvae reared on plants known not to contain toxins were eaten readily by some birds.

H. bolina resembles the mimetic *P. dardanus*, *P. memnon* and *P. polytes* in that it is polymorphic, with the forms sex-controlled to the female, and in that one of the forms resembles the male in pattern. It differs from the other species in that there is only one mimetic form but resembles them in that the details of this mimetic pattern vary from locality to locality and tend to parallel those of the local models. *H. bolina* also differs from the Papilios in that the populations are monomorphic for the mimetic form over almost half the range of the species, and in that some races are highly migratory. In the races of mimetic Papilios inhabiting areas where there are few or no suitable model species present, the populations tend to be monomorphic non-mimetic, as *P. dardanus* in Madagascar and *P. memnon* in Japan. Alternatively, the population remains polymorphic but the mimicry breaks down, as does *P. dardanus* in Kenya. *H. bolina* in Madagascar, on the other hand, is of the monomorphic mimetic form even though the models are absent. Furthermore, remarkably, the pattern remains an excellent mimic of *Euploea* from India, the subcontinent whence the Madagascan form almost certainly came. The reason for the maintenance of this resemblance may be that the species is a recent immigrant, or alternatively there is some sort of stabilizing selection unassociated with mimicry. The possibility that it is maintained by the presence of migrating birds which have had experience of the model in India can be ruled out because of the absence of such migration on a large scale. It is not due to regular migration of the butterfly from India, because of (1) the distance, (2) a form found on the Chagos islands is different and (3) the species is not regularly found on the other islands between Madagascar and India.

The genetic control of the polymorphism in *H. bolina* shows many of the features of that found in *P. memnon* (Clarke *et al.* 1968) and *P. polytes* (Clarke & Sheppard 1972) in that the mimetic form is dominant or nearly so to the male-like form. It is also similar to the Papilios (including *P. dardanus*) in that the details of the mimetic pattern, adjusted so that of the local model, are due to the operation of modifiers. In these respects the control of the mimetic pattern agrees with the deduction from our previous work that it should be dominant or semi-dominant and the details controlled by independent modifiers. The previous results with Papilios suggested to us that the polymorphism in *H. bolina* would be likely to be controlled by a supergene with tight linkage between the controlling loci. In fact, this turned out not to be so, for there is apparently no linkage between the locus controlling the mimicry and that determining the other polymorphic forms. With independent assortment selection could not maintain the very marked 'linkage disequilibrium' (non-random distribution of allelomorphs at different loci with respect to one another) found in the three Papilio species we have investigated. The evolution of a supergene depends on the presence of a polymorphism at two or more loci, since the selection for increased linkage between the loci depends on the presence of a linkage disequilibrium resulting from selective processes. Consequently the absence of a supergene in *H. bolina* may be in part due to the fact that it is only in certain localities that such selection can occur. Thus there can be no selection for a supergene in the large continental area where *H. bolina* is monomorphic mimetic, nor in Australia, where the species is virtually polymorphic at only one locus (*pallescens* and *naresi* are so rare that their selective effect for increased linkage would be negligible). Selection for a supergene could only occur in those semi-isolated island populations of south east Asia and the Pacific where the species is highly polymorphic. Here there could be selection for increased linkage between the loci but it may be much less powerful than in the other species

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studied. This is because there is only one mimetic form, whereas in the other species (including *P. polytes*) there are several in which different combinations of the same characters make up the different mimetic patterns. Thus any recombination will produce a poor resemblance to the model and this will be strongly selected against. In *H. bolina* on the other hand, since the whole pattern is determined by a single gene, any selection for increased linkage will be for reducing the frequency in which the allelomorph is in an unfavourable combination with a locus not producing an alternative mimetic pattern (e.g. *E* with *P* or P^n). The deleterious effects if they occur could be just as easily avoided by the selection of modifiers giving appropriate epistatic relationships. If the apparent allelomorphism is due to linkage between two loci *N* and *P* (see table 6) not controlling the mimicry, then it could be due to selection for linkage, but is more likely to be due to the two loci having arisen by duplication, since they both control the same character, i.e. the distribution of orange-brown pigment.

It is clear that the polymorphism where it occurs is partly affected by selective factors unassociated with mimicry. Frequency-dependent selection due to mimicry could maintain one non-mimetic form but it is difficult to see how it could maintain two in the presence of only a single mimetic one. Furthermore, in Australia the allelomorph *E* controlling the mimetic pattern is maintained at high frequency despite the fact that it does not produce a good mimic because the population is almost homozygous for P^n. Thus there seems to be good reason for believing that the form *euploeoides-nerina* is not unreservedly disadvantageous, and therefore would not necessarily generate strong selection for increased linkage between the loci *E* and P^n. The apparent advantage of *euploeoides-nerina*, anyhow under some circumstances, as judged by its high frequency in Australia and elsewhere, may explain why there has not been selection for *euploeoides* becoming fully epistatic to *nerina*. That the selection maintaining the allelomorph *E* is not always associated with selection for mimicry is further strengthened by Poulton's (1924) observation that the mimicry is poor in parts of Fiji, the mimics being too dark. Thus here apparently there has been no selection for appropriate modifiers, as there would have been if mimicry had been important. The absence of selection for mimicry in some areas but not others could be due to the models being protected only in some areas because of variations in the toxicity of the foodplants utilised by the larvae (Rothschild 1972; Brower *et al.* 1967), a matter that needs investigation.

The genetic results are in agreement with the theory of Fisher & Ford (see Ford 1953) on the evolution of mimicry and with ours on the evolution of dominance by the accumulation of modifiers, deduced originally from the study of *P. dardanus*, and supported by work on *P. memnon* and *P. polytes*. In contrast *H. bolina* has not evolved a supergene by selection in the way we suggested had occurred in the three Papilios. However, the present investigation does not disprove the hypothesis that supergenes will evolve in complex mimetic situations. If they do not evolve, we would have to postulate that they are a special characteristic of Papilios, not necessarily the outcome of mimicry. In fact, the mimetic situation in *H. bolina* is probably more comparable to that found in *P. polyxenes* and *Papilio glaucus*, where in both species there is a single mimetic pattern resembling in these cases the blue-black model *Battus philenor*. *P. polyxenes* is monomorphic and mimetic in the female and non-mimetic, or a poor mimic, in the male. The black pattern is controlled by a single locus and the sexual dimorphism by an independent one, which gives the mimetic pattern in the female only. The details of the mimetic pattern, with respect to the amount of blue and yellow, are controlled by modifiers (Clarke & Sheppard 1955). The genetic control of the mimetic pattern is much like that of *H. bolina* from Sri Lanka, where

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it is monomorphic. *P. glaucus* is polymorphic and the mimicry is controlled by a single locus which is apparently Y-linked (Clarke & Sheppard 1962). The species is monomorphic non-mimetic outside the range of its model and is polymorphic elsewhere, verging on a monomorphic mimetic condition where the model is very common. The details of the mimetic pattern, such as the amount of blue, are controlled by independent modifiers. Thus even in Papilios a supergene is not characteristic of mimicry when only one model is involved.

In order to find out whether or not the evolution of supergenes is a general phenomenon of complex mimicry, it will be necessary to investigate a genus, other than *Papilio*, which is polymorphic for several mimetic forms, thus exactly paralleling the situation in *P. dardanus*, *P. memnon* and *P. polytes*. A suitable species, as we have already pointed out (Clarke & Sheppard 1971) would be *Pseudacraea eurytus*, but we have so far found this impossible to breed in large numbers.

We are greatly indebted to the following for sending us living material of *H. bolina* from various areas: Dr Thelma Gunawardane, Dr R. W. Hornabrook, Col. and Mrs B. R. Johnston, Mr P. B. Karunaratne, Mr Stephen Kueh, Dr and Mrs J. Price and Mr Garry Sankowsky.

We are extremely grateful to Mr J. K. Hulme, Director of Ness Gardens, University of Liverpool, for keeping us constantly supplied with larval food plants.

We wish to thank the Keeper of Entomology at the British Museum, Mr T. G. Howarth and Mr Holloway, together with Professor G. C. Varley of the Hope Museum, Oxford, for access to collections and the Zoological Society of London for facilities.

Our thanks are due to Lady Clarke, Mrs B. Bradley, Mrs W. Cross, Mrs V. Dodd, Mr D. Fanoni, Mrs A. C. L. Gill, Mrs J. Mills, Mrs C. Sharrock and Mrs A. Williams for general help, to Mr D. J. Kidd for drawing the maps, and to Dr D. N. Kellett and The Hon. Miriam Rothschild for help with palatability tests.

The transport of living butterflies by post was greatly helped by the generous gift of polystyrene boxes from Mr H. J. G. Andrews of T. Wall and Sons (Ice Cream) Ltd.

This work could not have been carried out without the most generous grants from the Nuffield Foundation, The Science Research Council, The Royal Society and the Briggs–Bury bequest to the Department of Medicine, University of Liverpool.

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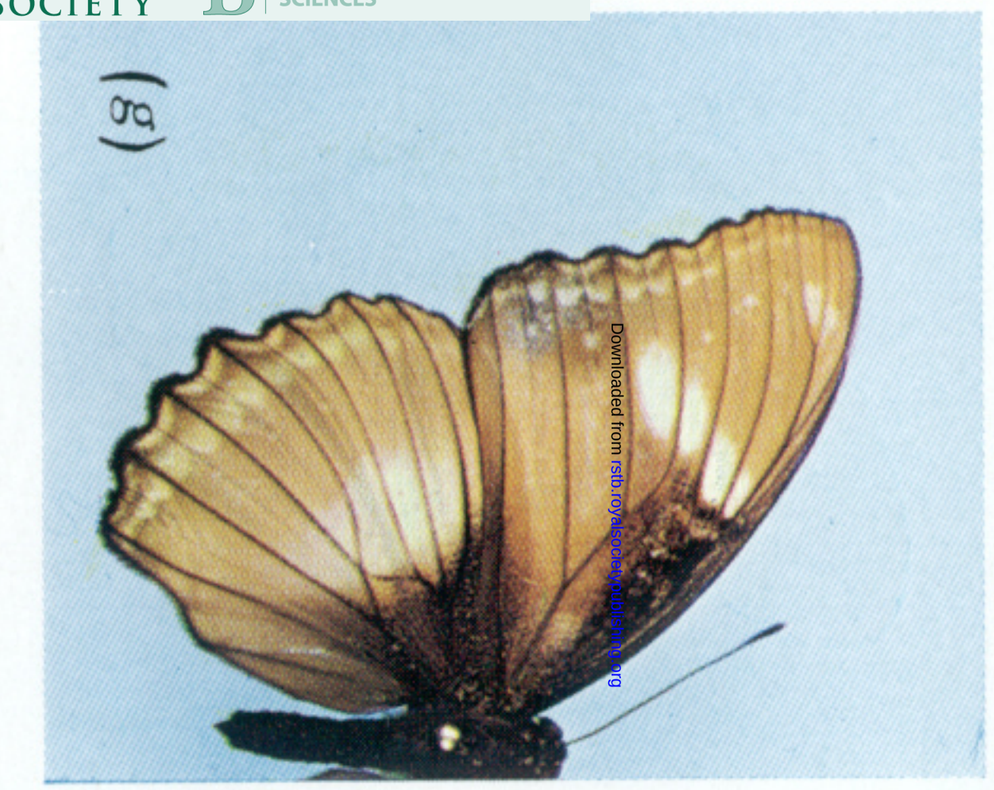

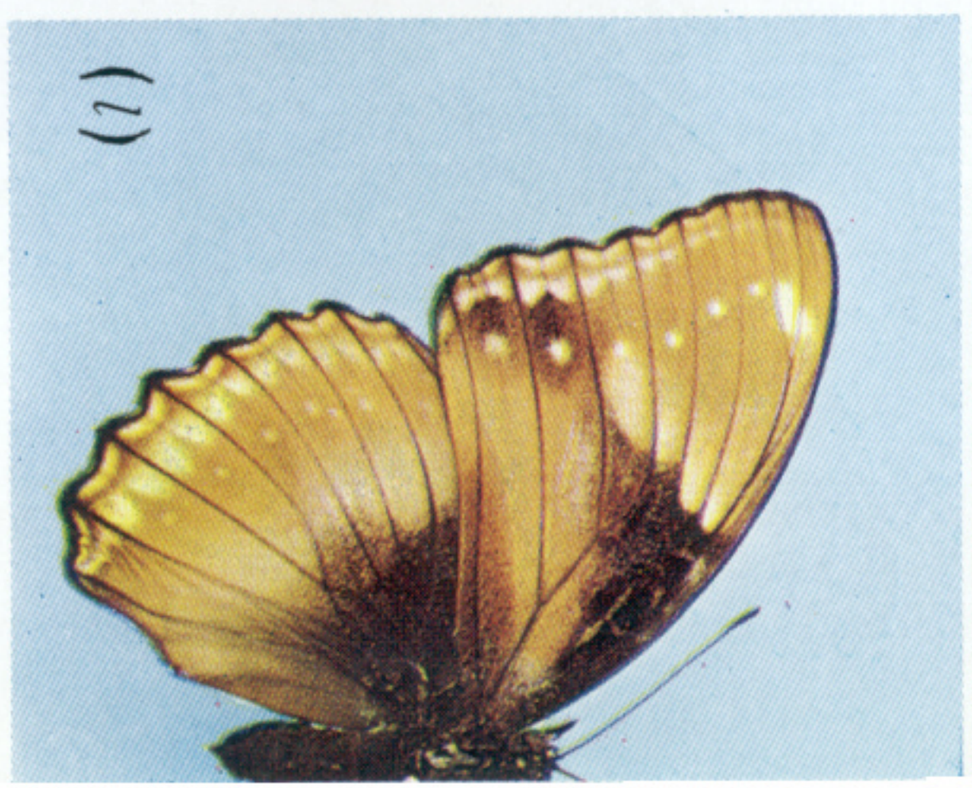

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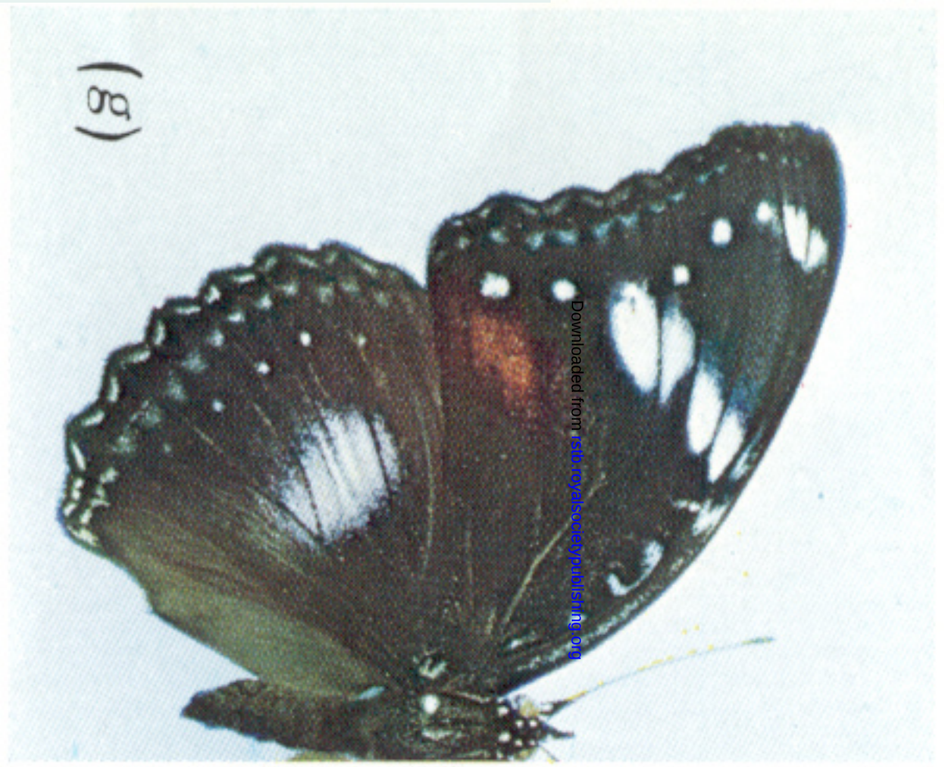

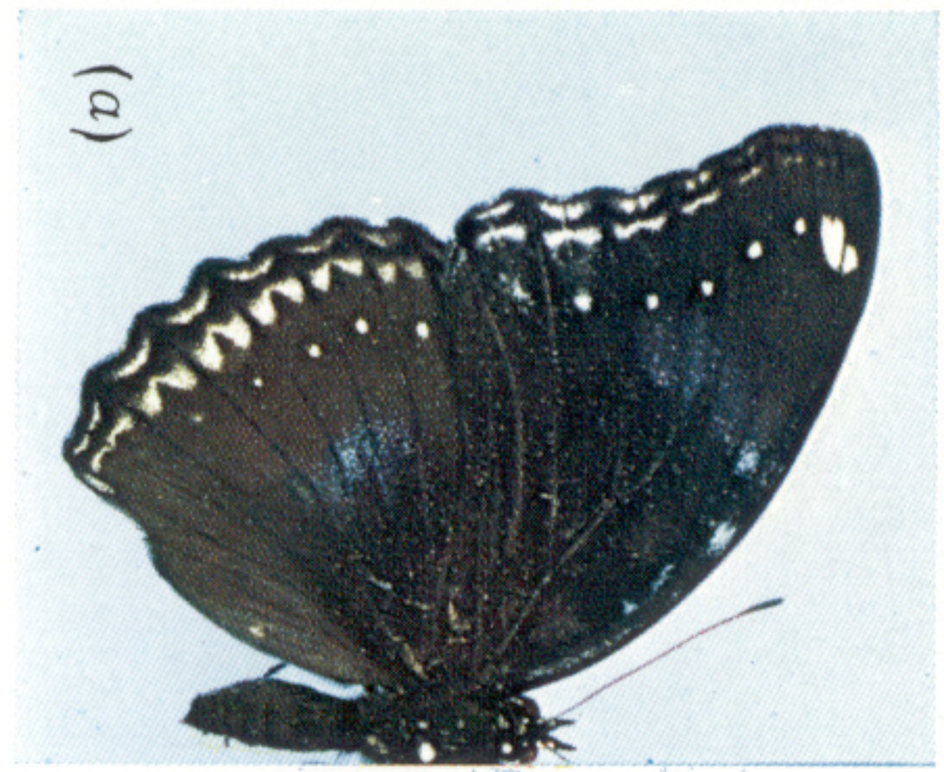

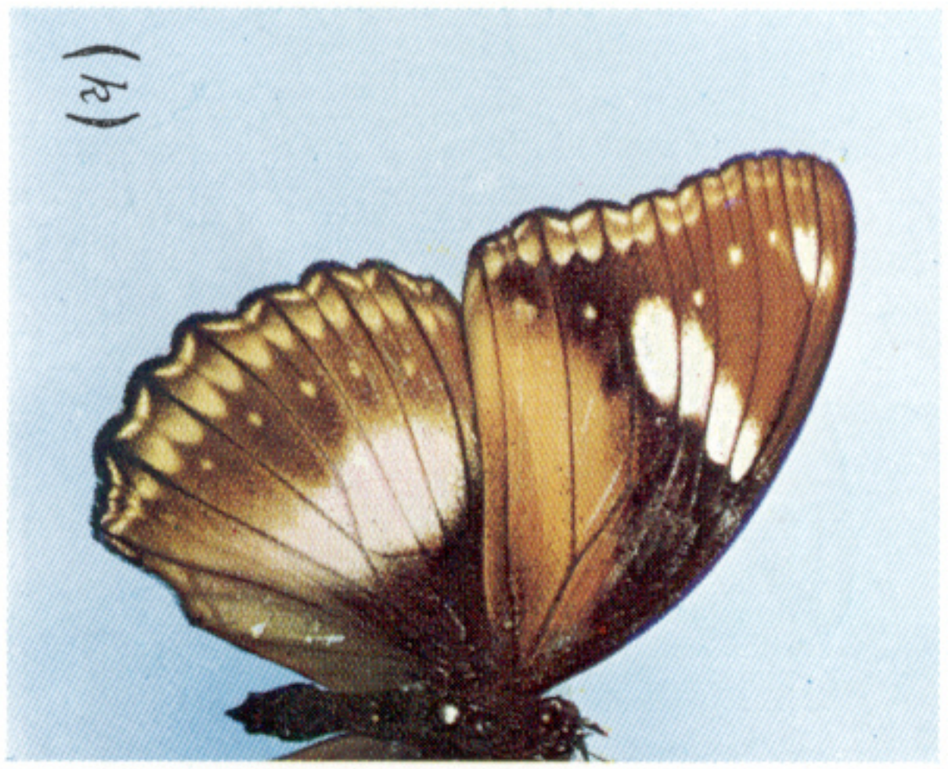

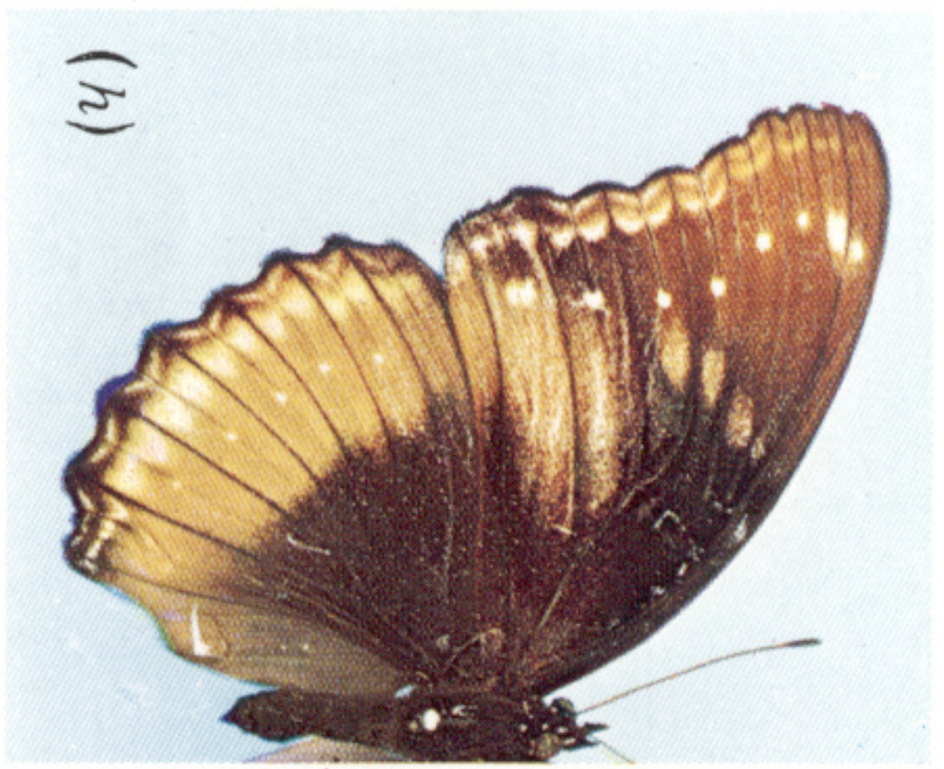

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