

DATA SUGGESTING ABSENCE OF LINKAGE BETWEEN TWO LOCI IN THE MIMETIC BUTTERFLY *HYPOLIMNAS BOLINA* (NYMPHALIDAE)C. A. CLARKE AND P. M. SHEPPARD<sup>1</sup>

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A large number of female forms of the polymorphic Batesian mimic *Hypolimnas bolina* (L.) have been described, but the situation is complicated because essentially similar phenotypes have been given different names in different geographical areas. In fact, the forms can be described in terms of four basic phenotypes and their combinations, together with minor modifications of pattern. We have used for the sake of clarity the varietal names for the four main forms given by Poulton (1924). In the present paper we discuss the genetics of three of these, the mimic f. *euploeoides* and two of the three nonmimetic forms, f. *nerina* and f. *naresi*.

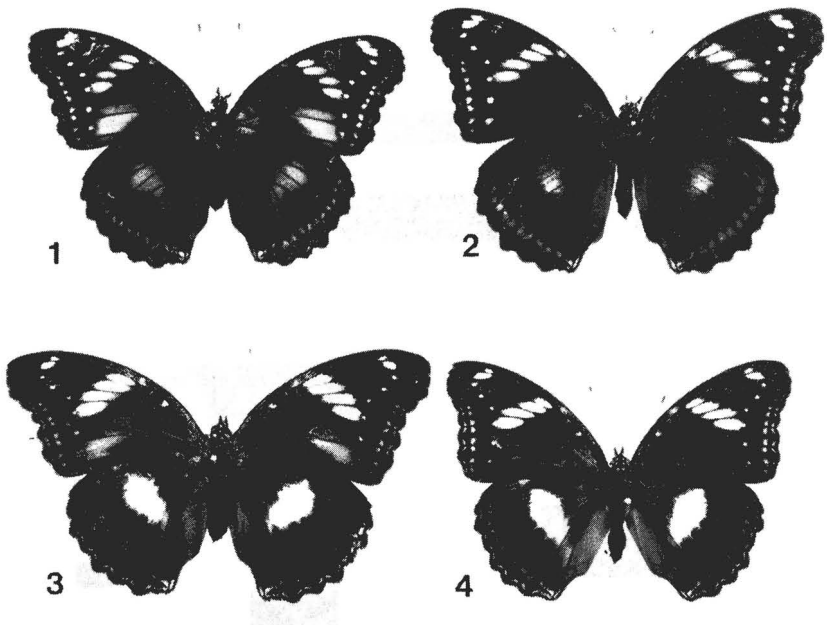
In a previous paper (Clarke & Sheppard, 1975) we showed that this polymorphism, sex-controlled to the female, is determined by two loci, one with two allelomorphs (*E* and *e*) and the other with three (*P*, *P<sup>n</sup>*, and *p*). It is possible that the locus with three alleles consist of two very closely linked loci with epistatic interactions between the allelomorphs.

The data used in the previous investigation gave a crossover value between the two loci *E* and *P* of 45.2%, which was not significantly different from independent assortment. Since all the evidence, including that from *Ephestia kuehniella* Z. (Traut & Rathjens, 1973), suggests the absence of chiasmata in female Lepidoptera, and in our broods the double heterozygote was the female, it seemed unlikely that the two loci are on the same chromosome. However, despite the absence of chiasmata in *E. kuehniella*, Robinson (1971) reports a brood in which crossing over had apparently occurred in the female of this species, and it therefore seemed important to investigate the possible linkage between *E* and *P* further. The present paper reports five broods in which the progeny were all female (a phenomenon surprisingly common in *H. bolina* (Clarke *et al.*, 1975)). One of the backcross broods (14228) in which the female was a double heterozygote produced 126 offspring and therefore could give information on linkage.

## MATERIAL AND METHODS

*Genotypes and corresponding phenotypes.* Clarke & Sheppard (1975) list all the genotypes and their corresponding phenotypes. One of these,

<sup>1</sup> ED. NOTE: Professor P. M. Sheppard died 17 October 1976. His obituary will appear in the next issue.



Figs. 1–4. Four female offspring of brood 14228. 1. f. *euploeoides-nerina* (genotype  $EeP^np$ ), wing span 85 mm. 2. f. *euploeoides* ( $EEpp$ ), wing span 89 mm. 3. f. *nerina* ( $eeP^np$ ), wing span 95 mm. 4. f. *naresi* ( $eepp$ ), wing span 78 mm.

*euploeoides-nerina* (sometimes called *aphrodite*, Fig. 1) was synthesised on many occasions by crossing both homozygous and heterozygous *euploeoides* ( $EEpp$  and  $Eepp$ , Fig. 2) with *nerina*, again when both homozygous and heterozygous ( $eeP^nP^n$  and  $eeP^np$ , Fig. 3). However, we never succeeded in showing that a wild *euploeoides-nerina* was in fact carrying both the allelomorph  $E$  and the allelomorph  $P^n$ , rather than being of some as yet unknown genotype.

In the present experiment the original female sent to us was a wild gravid *euploeoides-nerina* from Sarawak. Her progeny were mated to males from a stock of hybrid origin. These were of the genotype  $eepp$ , which in the female produces the form *naresi* (Fig. 4), the bottom recessive.

*Breeding methods.* The butterflies were bred in heated greenhouses in Liverpool using the methods described in Clarke & Sheppard (1975).

TABLE 1. Broods giving linkage data in *H. bolina*.

Brood No.	Provenance and Form of Mother	Provenance of Father	Offspring		
			♂♂		♀♀
14067	Wild Sarawak <i>euploeoides-nerina</i>	Wild Sarawak	0	17	11 <i>euploeoides-nerina</i> 6 <i>nerina</i>
14181	14067 <i>euploeoides-nerina</i>	hybrid <i>eepp</i>	0	19	11 <i>euploeoides-nerina</i> 8 <i>nerina</i>
14187	14067 <i>euploeoides-nerina</i>	hybrid <i>eepp</i>	0	4	3 <i>euploeoides-nerina</i> 1 <i>nerina</i>
14228	14181 <i>euploeoides-nerina</i>	hybrid <i>eepp</i>	0	126	34 <i>euploeoides-nerina</i> 28 <i>euploeoides</i> 32 <i>nerina</i> 32 <i>naresi</i>
14229	14181 <i>euploeoides-nerina</i>	hybrid <i>eepp</i>	0	10	2 <i>euploeoides-nerina</i> 2 <i>euploeoides</i> 2 <i>nerina</i> 4 <i>naresi</i>

## RESULTS

Table 1 gives the progeny of the original female and subsequent broods. The first matings at Liverpool were those of two of her *euploeoides-nerina* offspring, which were mated to males of hybrid stock homozygous *eepp*. Two of the resulting *euploeoides-nerina* progeny were again backcrossed to hybrid males *eepp*.

The original female *euploeoides-nerina*, or her wild mate, or both, were probably homozygous for *nerina* ( $P^nP^n$ ). Thus, brood 14067 appears to be a backcross with respect to *E*. If neither of the parents was homozygous  $P^nP^n$ , then one would expect at least one in three of the progeny to be neither *euploeoides-nerina* nor *nerina*. Since no other phenotype (*euploeoides* or *naresi*) appeared, it is likely that at least one of the parents was in fact homozygous  $P^nP^n$ . On the other hand, if the wild brood were an F2 for *E*, then the ratio should be at least three *nerina* to four non-*nerina* (*euploeoides* or *naresi*) among those insects that are not *euploeoides-nerina*.

That the wild male was certainly carrying *nerina* is shown by the next generation. His *euploeoides-nerina* daughter ( $EeP^nP^n$ ) mated to a *naresi* male (*eepp*) (brood 14181) produced no *euploeoides* or *naresi*, showing that she was homozygous *nerina*; her offspring must therefore have been heterozygous ( $EeP^np$  and  $eeP^np$ ). This was confirmed by the second backcross (brood 14228), which segregated *euploeoides-nerina* ( $EeP^np$ ), *euploeoides* ( $Eepp$ ), *nerina* ( $eeP^np$ ), and *naresi* (*eepp*)

in a good 1:1:1:1 ratio. The small brood 14229 is also consistent with this ratio. Thus, the series of matings not only shows that a female *euploeoides-nerina* from the wild was in fact produced by the combination of the allelomorphs *E* and  $P^n$ , but the broods also strongly support the view that the two loci segregate independently and there is no need to invoke crossing over in the female.

Since two of the forms (*nerina* and *naresi*) are nonmimetic, *euploeoides-nerina* is a poor mimic, and only *euploeoides* is a good one, it seems unlikely that the polymorphism is maintained by frequency-dependent selection due to the mimicry alone, particularly in the absence of close linkage. It therefore seemed important to look at other possible selective effects of the allelomorphs, such as viability and speed of development. The present second backcross broods can give us no information on the viability of the *euploeoides* and *nerina* homozygotes. However, they also give no evidence, under laboratory conditions, of differential viability between the four genotypes that could be tested.

There was a suggestion of differential speed of development, with an apparent excess of *naresi* emerging early and butterflies carrying *euploeoides* ( $EeP^np$  and  $Eepp$ ) coming out late. However, an analysis of variance revealed no significant differences with respect to the allelomorphs and their interactions, the comparison "*euploeoides*" ( $EeP^np$  and  $Eepp$ ) to non-*euploeoides* ( $eeP^np$  and  $eepp$ ) giving  $0.1 > p > 0.05$ , *nerina* ( $EeP^np$  and  $eeP^np$ ) to non-*nerina* ( $Eepp$  and  $eepp$ )  $p > 0.1$ , and the interaction  $0.1 > p > 0.05$ .

Because two of the probabilities were quite low we investigated similar large broods from Clarke & Sheppard (1975). As in the present brood, an insignificant excess of females carrying *euploeoides* came out late ( $p > 0.1$ ). Clearly, it would be worth investigating the matter further. We could not combine the analyses because under the different conditions of raising the broods the variances in the emergence times were heterogeneous.

#### SUMMARY

The complicated polymorphism in the mimetic butterfly *Hypolimnas bolina* can be described in terms of the four main phenotypes and their combinations. Genetic analysis from broods bred in the laboratory reveals that these are controlled by two unlinked loci, a matter that was previously in doubt.

No differences in the viability of the four genotypes tested could be detected. Although there was a suggestion that these allelomorphs affected speed of development, the differences were insignificant from the amount of data so far available.

## ACKNOWLEDGMENTS

We are grateful to the Science Research Council, the Nuffield Foundation, and the Royal Society for support. We would also like to thank Mr. Stephen Kueh for butterfly material from Sarawak.

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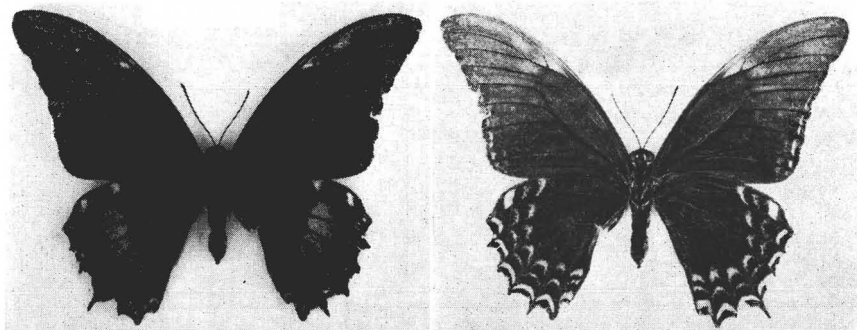
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CAPTURE OF *PAPILIO ANDROGEUS* (PAPILIONIDAE) IN SOUTHERN  
FLORIDA, A NEW RECORD FOR THE USA

A female *Papilio androgeus* (Cramer) was captured on 22 March 1976 in Broward Co., Florida (Figs. 1-2). The badly worn specimen was captured in an overgrown orange grove adjacent to Flamingo Road, near State Road 84, west of Ft. Lauderdale. I captured the specimen while she rested on weeds beneath the orange trees. According to Barcant (1970, *Butterflies of Trinidad and Tobago*, London) the main foodplant of the species is the orange tree, *Citrus sinensis* (Osbeck).

Irving Finkelstein and Ray Suydam, reported seeing two males of *P. androgeus* in the same orange grove on 23 March and 27 March, respectively. Charles V. Covell, Jr. saw one male of the same species about 1 mi. W of the above location on 23 May 1976.

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Figs. 1-2. 1, left, female *Papilio androgeus*, upper surface; 2, right, same specimen, under surface.