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NOTES AND COMMENTS

ABSENCE OF CROSSING-OVER IN FEMALE BUTTERFLIES (*HELICONIUS*)

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SUMMARY

Absence of recombination between linked markers in female *Heliconius* is suggested by coupling backcross broods in *H. erato*, by a repulsion F_2 in *H. melpomene*, and by other crosses with this species. No recombinants have been found in the offspring of doubly heterozygous females in either species. This supports the contention that the absence of chiasmata at oogenesis in these butterflies prevents genetic crossing-over. Chiasmata are absent in all the female Lepidoptera examined by Suomalainen and others, but *Ephestia* seems to show the absence of chiasmata but the presence of genetic recombination in the female, and therefore would repay further study.

1. INTRODUCTION

IN all the Lepidoptera so far examined, chiasmata have not been found at meiosis in the females; this has been shown in members of five families of moths (Bombycidae, Geometridae, Phycitidae, Pyraustidae, Tortricidae) (Maeda, 1939; Suomalainen, 1965, 1969, 1971; Traut and Rathjens, 1973) and in one group of butterflies (Suomalainen, Cook and Turner, 1973).

The Lepidoptera should provide, therefore, useful material for the study of the relationship between chiasma formation and genetic crossing-over. The group should also be useful in studying the association between these two phenomena and the heterogametic sex, since in those species adequately studied the female appears to be heterogametic (with the possible exception of the Lycaenidae, see Ford, 1971).

Evidence for the absence of genetic crossing over in female butterflies and moths is sparse as has been pointed out in the extensive review of their genetics by Robinson (1971).

The butterflies in which female chiasmata have been shown not to exist are eight species of the genus *Heliconius* (in the wide sense) and two members of related genera. We here present evidence for the absence of genetic crossing over in the females of two of these species.

2. RESULTS

The best evidence for absence of genetic crossing-over in females comes from broods of *Heliconius erato* published by Emsley (1965). Table 1 sum-

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marises Emsley's data on the segregation of the factors controlling white hindwing margin and yellow hindwing bar (on the underside only) which occur in coupling in crosses between the race of this butterfly from the west of Ecuador and the race from Trinidad (see Turner, 1971*a*). It can be seen that the cross-over rate in male heterozygous parents, summing both backcross broods, is 20 ± 8 per cent, compared with no cross-overs among 17 offspring from the heterozygous female backcrossed to Ecuador stock. The difference in cross-over rates borders on formal significance with $P = 0{\cdot}06$ by Fisher's exact test for a 2×2 contingency table (one tailed). The cross-over rate from the F_2 is consistent with the conclusion that the cross-over rate in males is high and that in females is zero; assuming zero female recombination, the male recombination can be estimated from the F_2 as $2 \times 6/26 = 46 \pm 10$ per cent (the presentation of the original data does not permit maximum likelihood estimation). Thus the data although not extensive are fully consistent with a total absence of cross-over in females

TABLE 1

Linkage between genes for hindwing white margin and hindwing yellow bar in Heliconius erato *(West Ecuador crossed with Trinidad). From Emsley (1965)*

Cross	Heterozygous parent	Recombinants	Total brood
Backcross to Ecuador	Female	0	17
	Male	0	5
	Male	5	20
F_2	Both	5	18
	Both	1	8

between two markers which probably have a recombination fraction somewhere between 10 and 50 per cent in males.

An extensive series of crosses involving two markers in repulsion in the related species *H. melpomene* is also fully consistent with the hypothesis. We have now bred a large number of F_2 butterflies involving these two markers (Turner, 1972; Sheppard and Turner, in preparation). As the markers are fully dominant and introduced into the cross in repulsion (the *erato* markers just reported were in coupling and produced detectable heterozygotes), the only recombination class is the double recessive phenotype, and the cross is extremely inefficient at measuring recombination; however, we have reared 585 offspring from these crosses (table 2) and not a single recombinant phenotype has been obtained. Fortunately it was virtually impossible to obtain this phenotype by contamination, as no other cross in our experiments would have been expected to produce it. We would expect this phenotype to be completely absent from our F_2 broods if recombination was absent in one sex, as this would make it impossible for an individual to obtain the recessive markers in coupling from both parents. This will be the case no matter how high the recombination fraction is in the other sex. The same result could of course be obtained if the genes were very closely linked and recombination occurred in both sexes, but there is a small amount of independent evidence against this. First Turner (1972) bred a small repulsion backcross brood with a female parent in which no recombinants appeared among 11 offspring (brood 46 in Turner's

paper). Second, Turner and Crane (1962) report a series of broods with the same two markers in which three recombinants definitely appeared during the course of the experiments. Unfortunately the crosses were not set up in such a way that the recombination fraction can be simply calculated, but in an analysis to be published elsewhere we have shown that assuming zero recombination in females in these broods the recombination fraction in males is around 27 per cent, with a rather high standard error. Thus it is rather unlikely that the absence of cross-over phenotypes in our F_2 results

TABLE 2

Broods of Heliconius melpomene *showing absence of recombination between the loci* b *and* D

Races	Parents		Offspring		Source
	Female	Male	*bbdd*	others	
Suriname × Trinidad	*bD/Bd*	*bD/Bd*	0	91	Turner, 1972
Belém × Trinidad	bD^R/Bd	bD^R/Bd	0	195	Sheppard and Turner, in preparation
Espirito Santo × Belém	bD^R/Bd	bD^R/Bd	0	299	Sheppard and Turner, in preparation
Total of above F_2 broods			0	585	
Suriname × Trinidad	bD^R/Bd	*bD/bD*	0*	11	Turner, 1972
Suriname (north) × Trinidad	*bD/Bd*	*Bd/bd*	0	54	Turner and Crane, 1962
Suriname (north) × Trinidad	*bD/Bd*†	*Bd/bd*	0	94	Turner and Crane, 1962

* Recombinant class in this case should have been *bd/bD*.
† The phenotype was incorrectly described by Turner and Crane (1962).

The taxonomic status of the races used in setting up the original crosses is:

Trinidad—*H. m. melpomene*, Trinidad, West Indies.
Belém—*H. m. thelxiope*, Belém do Pará, Brasil.
Espirito Santo—*H. m. nanna*, Linhares, Espirito Santo, Brasil.

Suriname—the population at Brokopondo, south of the racial suture zone, mainly *H. m. meriana*, but slightly introgressed with *H. m. melpomene* and carrying the allele D^R at low frequency, possibly by introgression with *H. m. thelxiope*.

Suriname (north)—the population around Moengo, north of the racial suture zone, mainly *H. m. melpomene* but carrying the alleles *b*, *D* and D^R by introgression with *meriana* and possibly *thelxiope* (see Sheppard, 1963; Turner, 1971*a*, *b*, 1972).

simply from low recombination in both sexes, as a recombination fraction of 10 per cent in males and females, which is a reasonable estimate from the male value of 27 per cent, should still have given us about six recombinant phenotypes. Furthermore, two of the broods reported by Turner and Crane (tabulated at the bottom of table 2) were set up in such a way that half the recombinants occurring in the female parents could have been detected in the offspring, and none has appeared in a total of 148 butterflies.

As a word of caution it must be added that it is not absolutely certain that the marker designated *b* by Turner and Crane is exactly the same locus as that designated *b* in the later papers (Turner, 1972; Sheppard and Turner, in preparation), as its phenotypic expression in the two series of

experiments is slightly different; however, there is no doubt whatever from all the genetical work that it is linked to the marker *D* which is certainly the same in all experiments and the balance of probability is in favour of the alteration in phenotypic expression being the result of a change in genetic background (Sheppard, 1963; Turner, 1971*b*; Sheppard and Turner, in preparation).

3. Discussion

The evidence for absence of female crossing-over in *H. melpomene* and *H. erato* is not as extensive as we would like, but the results are fully consistent with the hypothesis that the absence of chiasmata in the females which has been cytologically demonstrated is accompanied by an absence of genetic crossing-over.

The evidence for the absence of crossing-over in female moths, where it would also be expected on cytological evidence, has recently been reviewed by Robinson (1971). The phenomenon has been known in silkworms since it was pointed out by Sturtevant in 1915 and it seems that in the extensive work on the genetics of this species there has been no evidence that would contradict this, providing one excludes very rare recombination where the mechanism may not be that normally found in meiosis (we have to admit that we have not attempted to read the extensive Japanese literature). Robinson quotes breeding results by T. L. Smith on the markers *light body* and *rolled scales* in the wax moth, *Galleria mellonella*, which gave a recombination fraction in the backcross for male parents of 2·3 per cent and in a similar female cross a recombination fraction of zero in the female. However, the recombination fraction is so small and the total number of offspring from the female parents (111) so comparatively small that the difference in recombination rates between the sexes does not reach statistical significance (χ^2 for contingency = 2·6, $P > 0{\cdot}05$, one tailed). The flour moth, *Ephestia kuehniella*, presents a particularly interesting exception. Robinson quotes experiments by A. Kühn in which the factors *ml* and *Sy* were tested by means of a mixed repulsion/coupling F_2; the recombinant wild-type phenotype which should have comprised one-quarter of the brood (higher than normal in an F_2 because *Sy* is lethal when homozygous) failed to appear in the 317 offspring. Unlike our repulsion F_2 with *Heliconius melpomene*, this does not demonstrate absence of recombination in one sex, as if females had zero recombination and males 50 per cent then the recombinant phenotype should still have appeared at a frequency of one-sixth of the total offspring, or in a small proportion if the loci were more tightly linked. This cross therefore demonstrates very tight linkage between these markers in both sexes. In fact, a further experiment conducted by A. Kühn and B. Berg, also reported by Robinson, seems to show conclusively that female recombination does occur in this species; a repulsion cross between the markers *b* and *bch* gave an F_2 consisting of 243 + +, 93 *b* +, 109 + *bch* and 12 *bbch*; segregation at both loci is in the normal 3 : 1 ratio, and the value of χ^2 for the contingency table is highly significant at 15·9. There can be little doubt that the loci are linked, and that recombination is occurring in the females to permit the appearance of the double recessive recombinant genotype *bbch*/*bbch*. Cytological observations of *E. kuehniella* showed normal Lepidopteran achiasmatic chromosomes in the female at meiosis (Traut and

Rathjens, 1973). There is clearly a contradiction between the genetical and cytological evidence that would repay further investigation.

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4. References

EMSLEY, M. G. 1965. The geographical distribution of the color-pattern components of *Heliconius erato* and *Heliconius melpomene* with genetical evidence for the systematic relationship between the two species. *Zoologica (New York), 49,* 245-286.

FORD, E. B. 1971. *Ecological Genetics.* Chapman & Hall, London.

MAEDA, T. 1939. Chiasma studies in the silkworm, *Bombyx mori* L. *Japan. J. Genet., 15,* 118-127.

ROBINSON, R. 1971. *Lepidoptera genetics.* Pergamon, Oxford.

SHEPPARD, P. M. 1963. Some genetic studies of Müllerian mimics in butterflies of the genus *Heliconius. Zoologica (New York), 48,* 145-154.

SUOMALAINEN, E. 1965. On the chromosomes of the Geometrid moth genus *Cidaria. Chromosoma (Berl.), 16,* 166-184.

SUOMALAINEN, E. 1969. On the sex chromosome trivalent in some *Lepidoptera* females. *Chromosoma (Berl.), 28,* 298-308.

SUOMALAINEN, E. 1971. Unequal sex chromosomes in a moth, *Lozotaenia forsterana* F. (Lepidoptera: Tortricidae). *Hereditas, 68,* 313-316.

SUOMALAINEN, E., COOK, L. M., AND TURNER, J. R. G. 1973. Achiasmatic oogenesis in the heliconiine butterflies. *Hereditas, 74,* 302-304.

TRAUT, W., AND RATHJENS, B. 1973. Das W-Chromosom von *Ephestia kuehniella* (*Lepidoptera*) und die Ableitung des Geschlechtschromatins. *Chromosoma (Berl.), 41,* 437-446.

TURNER, J. R. G. 1971*a*. Studies of Müllerian mimicry and its evolution in burnet moths and heliconid butterflies. In *Ecological genetics and evolution,* Ed. E. R. Creed, pp. 224-260. Blackwell Scientific Publications, Oxford.

TURNER, J. R. G. 1971*b*. Two thousand generations of hybridization in a *Heliconius* butterfly. *Evolution, 25,* 471-482.

TURNER, J. R. G. 1972. The genetics of some polymorphic forms of the butterflies *Heliconius melpomene* (Linnaeus) and *H. erato* (Linnaeus). II. The hybridization of subspecies of *H. melpomene* from Surinam and Trinidad. *Zoologica (New York), 56,* 125-157.

TURNER, J. R. G., AND CRANE, J. 1962. The genetics of some polymorphic forms of the butterflies *Heliconius melpomene* Linnaeus and *H. erato* Linnaeus. I. Major genes. *Zoologica (New York), 47,* 141-152.