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Linkage and cytogenetic studies in the swallowtail butterflies *Papilio polyxenes* Fab. and *Papilio machaon* L. and their hybrids

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[Plates 1–4]

By the hand-pairing technique, F_1 hybrids, back crosses and, for the first time, F_2 hybrids have been obtained between *Papilio polyxenes* Fab. (black North American swallowtail) and *Papilio machaon* L. (yellow old world swallowtail). Three types of investigation were carried out in parent species and hybrids: (1) linkage, (2) the presence of heteropyknotic bodies, and (3) pachytene chromosomes.

(1) It was conclusively demonstrated that the loci controlling adult wing pattern and larval spot colour are not linked.

(2) The two species differ with respect to the heteropyknotic body in somatic cells. In *P. polyxenes*, a body is present in cells from females but absent in those of males, whereas in *P. machaon* the body is present also in a high proportion of males. This difference was studied in the various hybrids.

(3) The morphology of the W (= Y) chromosome was found to differ in the two species and this chromosome can, therefore, be traced in crosses. The data also suggest that in *P. machaon* the W consists of two parts which have been tentatively designated as W_1 and W_2, both pairing with the Z (= X) chromosome. An additional nucleolar bivalent is present in some *P. machaon* individuals and this may be responsible for the polymorphism of the heteropyknotic body in the males.

The fact that there are about 700 species of Papilios, many of which can be hybridized, means that comparative studies on heterochromatin and chromosomes are possible, and the findings may be relevant to heteropyknotic bodies in other orders.

Introduction

In extensive hybridization studies between the black swallowtail *Papilio polyxene* Fab. from the east coast of the United States and the yellow one, *Papilio machaon* L., from England and the continent of Europe, there have in the past been obtained many F_1s and back crosses but never an F_2 (Clarke & Sheppard 1953, 1955*a*, 1956).

This paper reports that the F_2 has now been bred, and consequently more data are available to look for possible linkage between the adult phenotype (black or yellow) and the larval spot colour (yellow in *polyxenes* and red in *machaon*). Moreover, the hybrids are now of additional interest since we have found that the two species differ in their sex chromatin constitution.

P. polyxenes has the heteropyknotic body first discovered by Smith (1945) in the somatic cells of the female whereas it is lacking in the male. This is the usual situation in the Lepidoptera. *P. machaon*, on the other hand, is one of the few exceptions to this generalization, Traut & Mosbacher (1968) having first shown that a heteropyknotic body was present in both males and females of this species in the few specimens which they examined.

Traut & Rathjens (1973) produced evidence strongly suggesting that the heteropyknotic body in females is derived from the W (= Y) chromosome, females being the heterogametic sex in the Lepidoptera, but there has been no explanation of the presence of the heteropyknotic body when it occurs in males.

In the present paper, therefore, in addition to the linkage data, information will be given about the segregation of the heteropyknotic body in *polyxenes* and *machaon* and their hybrids and a suggestion offered as to its nature in the male. We also report new findings on the W chromosomes in the two parent species at the pachytene stage of meiosis and on the way they pair with the Z (= X).

We thought these preliminary findings were worth reporting because *polyxenes* and *machaon* will hybridize readily with other *Papilio* species and the whole genus has great experimental potential as so many inter-specific crosses can be obtained (see for example Clarke & Sheppard 1953, 1955*a*, *b*, *c*, 1956, 1957, 1962, 1964, 1972, 1975; Clarke, Sheppard & Thornton 1968; Ae 1964, 1966, 1967).

Materials and methods

1. *Breeding*

The butterflies were hand-mated (Clarke 1952; Clarke & Sheppard 1956) and bred in heated greenhouses by using the normal foodplants for this group of swallowtails. The *machaon* (plate 1*c*) originated from Finland and the *polyxenes* (plate 1*a* and *b*) from the east of the United States. Plates 1*d* and *e* show the F_1 hybrids.

† The ZZ/ZW terminology is commonly used to denote the sex chromosomes of males and females in species with female heterogamety in place of XX (female) and XY (male) in species with male heterogamety.

The full-grown larva of *P. polyxenes* has a number of yellow spots on the mainly reen dorsal surface, while in *P. machaon* these are red and in the F_1 hybrids they re usually orange (plate 1*f*). We recorded the colour of the spots in the back cross nd F_2 broods as far as they could be determined by eye. The sexes of the butterlies were recorded with respect to the phenotype of both the larva and the imago. The adult of *P. machaon* has a mainly yellow background and that of *P. polyxenes* nainly black, and there is a clear-cut segregation in the F_2 and the back cross to *nachaon* which was recorded as yellow or black. A note was also made of occasional ex mosaicism as judged by abnormalities of the distribution of yellow or black in he pattern (plate 1*g*).

To assess fertility, the outcome of each hand mating was noted with respect to whether the female laid any eggs, whether they showed signs of development as udged by changes in their colour but failed to hatch, or whether the larvae natched successfully. When they did hatch, the mating was classified as 'fertile', egardless of whether or not butterflies subsequently emerged since larval mortality lue to environmental causes is often very high. Matings from which no larvae natched were classed as 'infertile'.

2. *The heteropyknotic bodies*

Cells from the middle part of the alimentary tract of freshly killed larvae, pupae and adult butterflies were studied for the presence or absence of heteropyknotic bodies (Smith 1945). Two methods were used, (*a*) for permanent, (*b*) for semi-permanent preparations.

(*a*) The material was fixed in 95% ethanol for 1 hour. It was then either stained immediately or preserved for a further few days in 70% ethanol. Staining was carried out by the Feulgen squash technique. The alimentary tracts were taken via 50% ethanol into water and hydrolysed in 5 N HCl at room temperature for 25 min. They were next stained in Feulgen stain (Gurr) for between 1 and 2 hours. The tissues were then briefly rinsed in tap water (2 changes) and distilled water (2 changes) before being placed in ice-cold 45% acetic acid. Small pieces were gently squashed and the preparations were made permanent by the dry ice technique (Conger & Fairchild 1953).

(*b*) Aceto-orcein squashes were made at all stages of development. When the embryo was used, the egg was placed in a drop of tap water on a slide and the embryo extracted by means of a needle and forceps. The head and skin were then rejected and the other tissues stained. When the animal was a larva, pupa or imago, the whole alimentary tract was removed and fixed in a 3:1 solution of absolute alcohol to glacial acetic acid. Tissues fixed by this method produced good results for at least 3 months. To stain the cells a very small piece of material was placed on a slide, teased out with needles and immersed in a drop of 2% orcein in 45% acetic acid. After 10 to 15 min a coverslip was placed on the slide and pressed gently to make a squash preparation.

3. *Chromosomes*

Chromosomes in the pachytene stage of meiosis were obtained from female and male larval gonads, the former often giving the more satisfactory preparations. Cells in the pachytene stage were most often found in larvae of a size ranging from 30 to 44 mm and with a width of head capsule from 2.7 to 3.7 mm, though neither measurement was a very reliable indicator of gonadal development.

The gonads were fixed in Carnoy's fluid (ethanol:chloroform:acetic acid 6:3:1) for 30 min, transferred to a drop of acetic acid and teased into fine pieces, this resulting in the nuclei becoming dissociated. The material was allowed to dry on the slide and then stained with acetic lactic orcein (see Traut 1976). Photographs were taken by using a phase-contrast microscope.

From every individual dissected, Malpighian tubule preparations were also made as a control for the presence or absence of heterochromatin bodies in somatic nuclei.

Results

1. *Breeding*

Table 1 gives the overall findings for fertility and for the sex ratio and wing colour in the adult butterflies, and table 2 information on linkage between larval spot colour and adult wing colour.

(*a*) *Fertility*

It is apparent that as defined under Materials and Methods there appear to be small differences between the fertility of the pure stocks, the F_1s and the back crosses; the striking comparison is with that of the F_2s, which are highly infertile (heterogeneity $\chi^2_5 = 27.8$, $p < 0.001$) (Woolf 1957). The six classes are pure *machaon*, pure *polyxenes*, F_1s, back crosses to *machaon*, back crosses to *polyxenes* and F_2s.

(*b*) *Sex ratio*

It is also apparent from table 1 that there is no significant disturbance in the sex ratio in any of the types of mating including the F_2s.

Description of plate 1

Average wing span of set butterflies given in millimetres.

(*a*) *P. polyxenes* ♀, 89 mm. (*b*) *P. polyxenes* ♂, 81 mm. (*c*) *P. machaon* ♂, 80 mm; (the wing pattern in the two sexes is similar). (*d*) F_1 hybrid ♀ (*P. polyxenes* ♀ × *P. machaon* ♂), 86 mm; (the reciprocal cross is similar). (*e*) F_1 hybrid ♂ (*P. polyxenes* ♀ × *P. machaon* ♂), 83 mm; (the reciprocal cross is similar). (*f*) Full-grown larvae of *P. machaon* (red spots), of *P. polyxenes* (yellow spots) and of the F_1 hybrid (orange spots); length of full-grown larvae about 40 mm. (*g*) F_2 hybrid ♀ showing sex mosaicism, 75 mm.

(*b*)

(*d*)

(*f*)

(*g*)

FIGURE 1. For description see opposite.

	mother	father	results of matings: no. of matings	female did not lay	female laid infertile eggs only	some eggs darkened but none hatched	no. of broods producing larvae	(*b*) phenotype and sex of adult offspring: black females	yellow females	black males	yellow males
pure stock	*polyxenes*	*polyxenes*	11	2	2	0	7	67	0	69	0
	machaon	*machaon*	23	5	2	0	16	0	54	0	35
F_1s	*polyxenes*	*machaon*	12	4	0	0	8	72	0	64	0
	machaon	*polyxenes*	13	4	2	2	5	36	0	27	0
back crosses to *machaon*	*machaon*	F_1 (*polyxenes* female × *machaon* male)	8	2	1	0	5	7	12	10	4
	machaon	F_1 (*machaon* female × *polyxenes* male)	5	1	1	2	1	0	0	0	0
	F_1 (*machaon* female × *polyxenes* male)	*machaon*	3	3	0	0	0	0	0	0	0
back crosses to *polyxenes*	*polyxenes*	F_1 (polyxenes female × *machaon* male)	1	0	0	0	1	0	0	0	0
	polyxenes	F_1 (*machaon* female × *polyxenes* male)	1	1	0	0	0	0	0	0	0
	F_1 (*machaon* female × *polyxenes* male)	*polyxenes*	1	0	0	0	1	0	0	0	0
F_2s	F_1 (*polyxenes* female × *machaon* male)	F_1 (*polyxenes* female × *machaon* male)	32	6	17	5	4	29	8	33	9
	F_1 (*machaon* female × *polyxenes* male)	F_1 (*machaon* female × *polyxenes* male)	4	4	0	0	0	0	0	0	0

The fertility is assessed as far as larval hatching only, as many larvae die from disease. The data on the actual butterflies are relevant to the sex ratio and to the dominance of wing colour.

(*c*) *Adult wing colour*

Table 1 also shows that the results are in agreement with the previous findings of Clarke & Sheppard (1955*a*), that the yellow adult colour is an autosomal recessive and that the ratios do not depart from those expected. Similar results were found with the related species *Papilio brevicauda* (black) and *Papilio zelicaon* (yellow) (Clarke & Sheppard 1955*a*).

(*d*) *Sex mosaics*

P. polyxenes, the F_1s and the black phenotypes of subsequent hybrids are sexually dimorphic, the females having greatly reduced yellow in the submarginal row of spots on the fore and hindwings. The yellow phenotypes, however, are not sexually dimorphic. Consequently in the black phenotypes, sex mosaicism can sometimes be detected by irregularities in the pattern. These were present in one F_2 brood, there being three insects where the abdomen and most of the wing pattern were female but where there were yellow patches on the wings indicating the presence of male tissue (plate 1*g*).

Because the yellow pattern is recessive, the yellow tissue in the sex mosaics might not be male but could result from the cells not possessing the allele determining black. If this were the case, the yellow patches might occur anywhere on the wings or body, whereas they are in fact confined to those areas in which the sexes differ in colour.

Mosaics of this type may be due to mitotic non-disjunction occurring in the wing buds. One cell line would have no Z chromosome and therefore die and the other, being ZZW, would produce a patch of male colour (see Ford 1946). Species hybrids might be expected to be predisposed to non-disjunction.

(*e*) *Larval spot colour* (table 2)

It has already been shown (Clarke & Sheppard 1955*a*, 1956) that the yellow larval spot colour is recessive to the red, and the information given in table 2 is in agreement with this. None of the larvae in the mating female *machaon* × male *polyxenes* or female *polyxenes* × male *machaon*, had yellow spots, showing that the locus is autosomal, the female being the heterogametic sex. The spots in the F_1 were variable orange to red, and in the F_2 and back crosses they could be scored as red, orange or yellow, but with some degree of difficulty. The segregation of yellow to non-yellow is in good agreement with Mendelian expectation, but there is some evidence that a proportion of heterozygotes are misclassified in some broods, there being a deficiency of larvae scored as orange in these.

(*f*) *Linkage*

The data in table 2 provide evidence for independent assortment between the loci controlling larval spot colour and adult colour pattern. Combining the orange and red spot classes there is no evidence of linkage in the F_2 broods, nor, classifying

larvae for red or orange, in the back cross to *machaon*. Furthermore, that the loci are on different chromosomes is made probable by the fact that some yellow spotted larvae can give rise to yellow adults (the double recessive combination) in the F_2 broods. Since the two characters are in repulsion, yellow adults from yellow spotted larvae could only result from crossing-over occurring in both sexes, and there is evidence for the absence of crossing-over in female Lepidoptera (see Turner & Sheppard 1975; Traut 1977; Suomalainen, Cook & Turner 1973).

TABLE 2. INFORMATION ON LINKAGE BETWEEN LARVAL SPOT COLOUR AND ADULT WING COLOUR

adult wing colour		black			yellow		
larval spot colour		red	orange	yellow	red	orange	yellow
brood no. and type of mating							
13223, F_2	males	0	0	2	0	0	1
	females	0	1	2	0	0	0
13302, F_2	males	7	7	6	4	3	1
	females	7	5	6	2	0	3
13312, F_2	males	0	2	0	0	0	0
	females	3	0	1	0	0	0
13324, F_2	males	1	6	2	0	0	0
	females	0	4	3	0	2	1
13292, back cross to female *machaon*	males	3	7	0	4	0	0
	females	2	5	0	4	8	0

2. *The heteropyknotic bodies*

(*a*) *In the parent species*

Cytological examination of 11 pure *polyxenes* adult females showed that 10 of them had a single chromatin body (plate 2*a*) and 1 had an additional smaller body in a few cells. In none of the 16 males was the body present (plate 2*b*). In pure *machaon*, on the other hand, of the 15 adult females examined, 4 had a single heteropyknotic body (plate 2*c*) (the usual condition in the Lepidoptera), 10 had 2 and 1 had up to 3 in some cells. When more than one body was present, one was usually larger than the other(s) (plate 2*d*).

Of the 17 *machaon* males examined, only 3 were negative in all cells looked at (the condition usually found in the Lepidoptera), while in the remaining 14 the findings were as follows: 1 body per cell (3 individuals); 0, 1 or 2 bodies per cell (5 individuals); 1 or 2 bodies per cell (6 individuals). Thus the stock showed the presence of heteropyknotic bodies, in the majority of the males. As already stated, Traut & Mosbacher (1968) reported that *P. machaon*, unlike most Lepidoptera, had a heteropyknotic body in the male. Our observations, however, suggest that the species is polymorphic for this character and that when the bodies are present they can vary in number and size between cells (plate 2*e* and *f*).

Thus the stocks of the two species used for the hybridization studies differed in respect of the heteropyknotic bodies, particularly in the males.

(*b*) *In the F_1 broods*

The cytological results of the F_1 broods are given in table 3. All the females were positive in both types of cross, and a proportion of them had more than one body. When the female parent was *polyxenes* 2 bodies per cell were found in 5 out of 14 female offspring. On the other hand, when the mother was *machaon*, 9 of the

TABLE 3. SEX CHROMATIN DATA IN F_1 BROODS

			offspring			
			males		females	
brood no.	mother	father	positive	negative	positive	negative
13138	*polyxenes*, 13065, not tested but female sibs all positive	*machaon*, 13015, not tested	4	2	9	0
13160	*polyxenes*, not tested	*machaon*, not tested	1	1	4	0
13134	*polyxenes*, not tested	*machaon*, not tested	0	2	1	0
14349	*polyxenes*, Smith positive	*machaon*, Smith negative	3	0	0	0
13835	*machaon*, not tested	*polyxenes*, not tested	6	1	10	0

10 female progeny had up to 2 bodies in some cells, and in one butterfly the heteropyknotic body was absent in many cells but present in some. Thus the proportion of cells with more than one body in the F_1 females appears to be significantly greater when the mother is *machaon* (Fisher's exact test, $p = 0.01$).

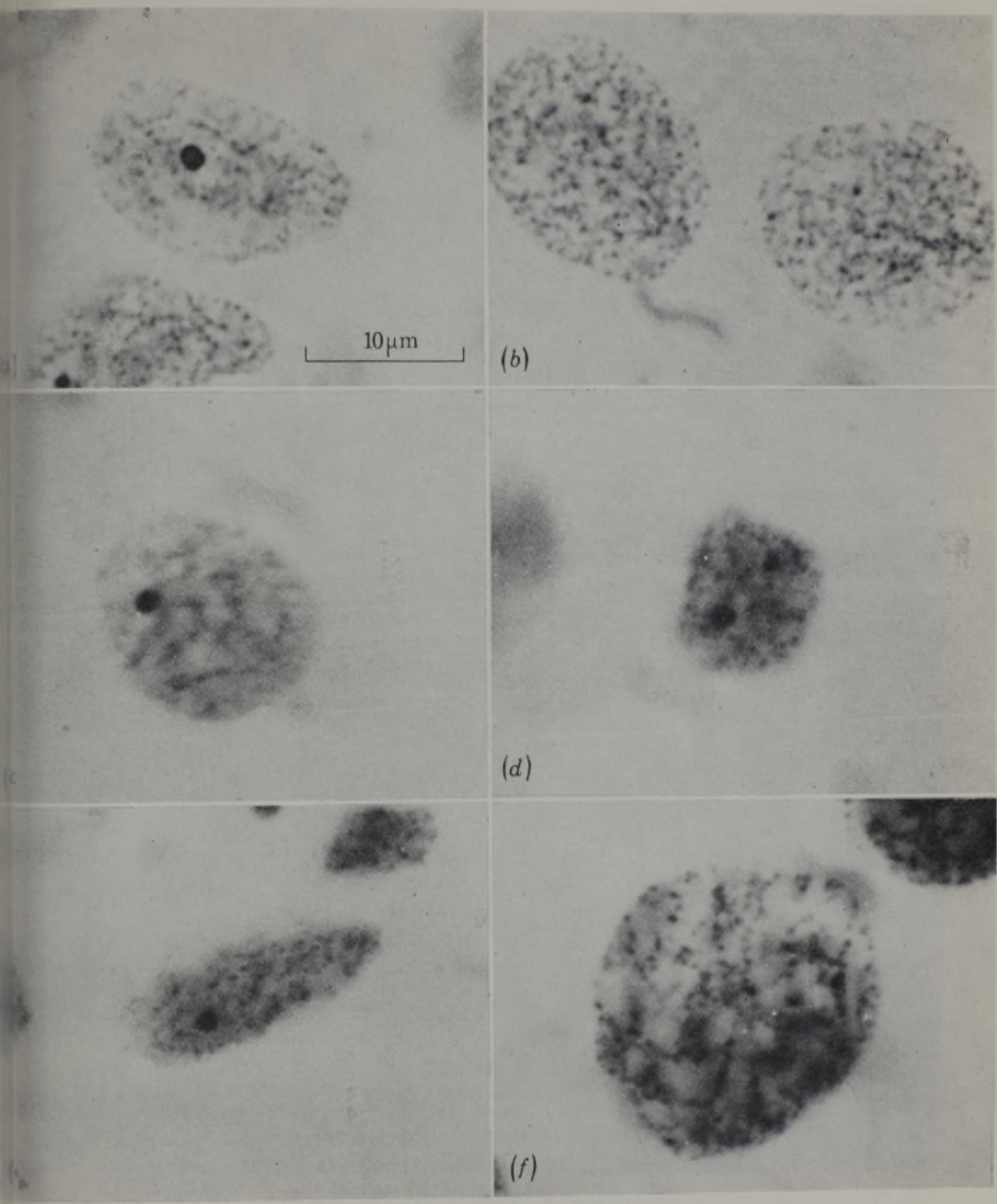
In the males where the mother was *polyxenes* and the father *machaon*, two of the four broods show segregation for the heteropyknotic body, and the other two broods are too small to exclude it.

In only one of the F_1 families (14349) is the heteropyknotic body phenotype of the parents known, and here there is an unexplained result because the father was scored as heterochromatin negative and yet three of his male offspring were positive. The significance of this finding remains uncertain until we understand more fully the nature of the heteropyknotic body in male *machaon* and hybrid butterflies.

The data are too limited for firm conclusions but are consistent with the segregation in the males being 1:1 in both types of F_1.

(*c*) *In the back cross to* machaon

Only one back cross, that to a female *machaon*, was examined cytologically, and all the female offspring were positive (table 4), 5 of the 7 having more than one

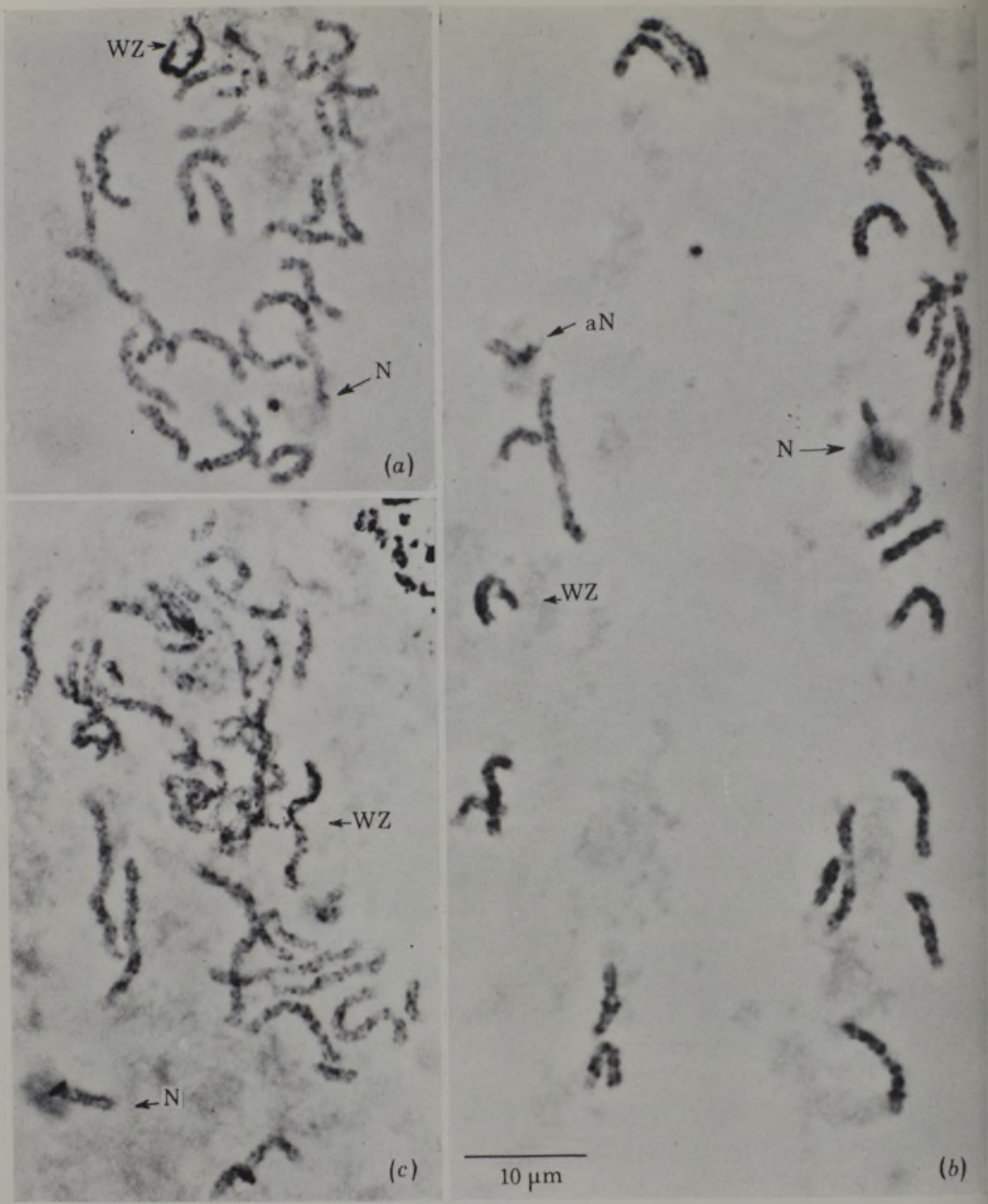


Nuclei from gut cells

(*a*) *P. polyxenes* ♀ showing single heteropyknotic body. (*b*) *P. polyxenes* ♂. No heteropyknotic body. (*c*) *P. machaon* ♀ showing single heteropyknotic body. (*d*) *P. machaon* ♀ showing two heteropyknotic bodies. (*e*) *P. machaon* ♂ showing single heteropyknotic body. (*f*) *P. machaon* ♂. No heteropyknotic body.

All the photographs on this plate are at the same magnification.

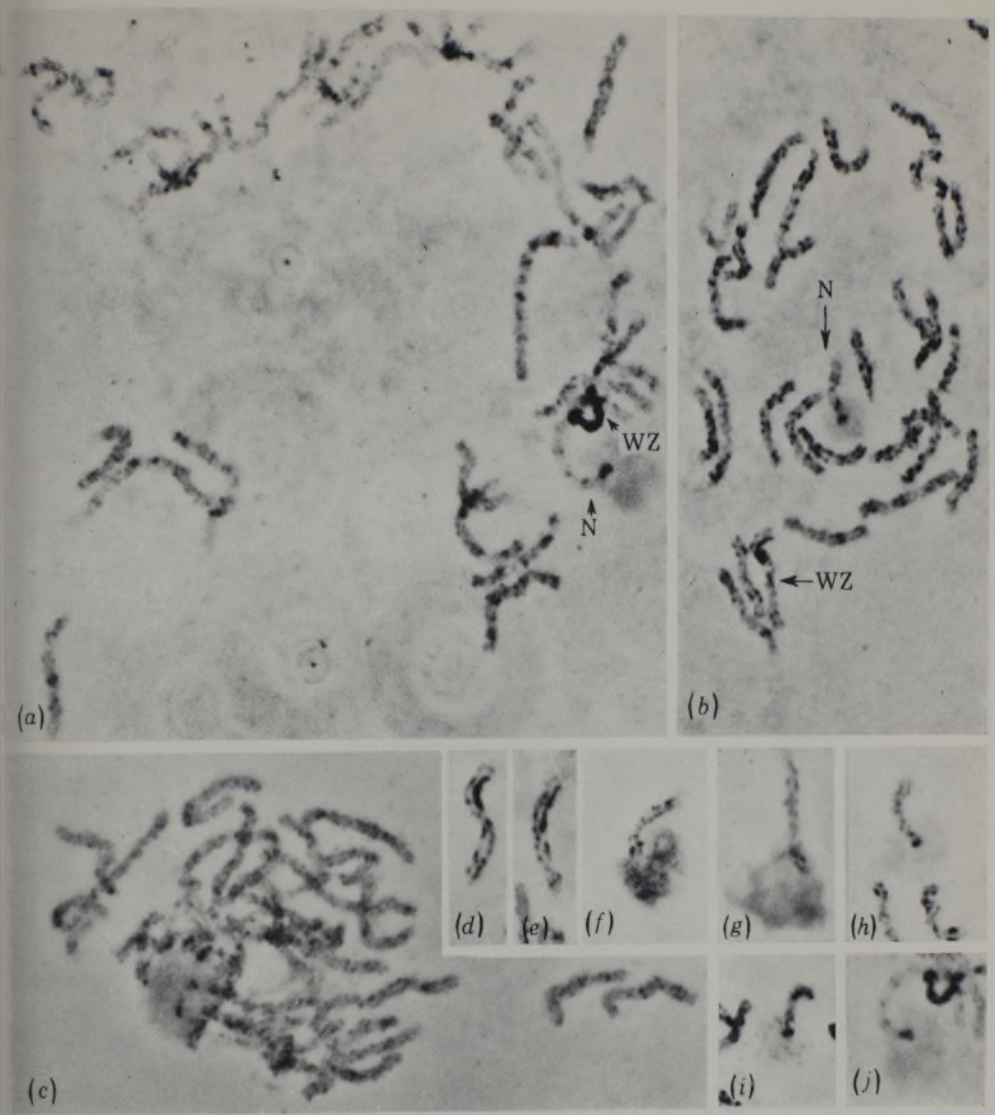
(*Facing p.* 392)



Chromosomes of larvae in pachytene

(*a*) *P. polyxenes* ♀. All homologous chromosomes are paired and chromomere patterns match in most bivalents. Note looped heteropyknotic segment of W chromosome (see also plate 4*a*). WZ = sex chromosome bivalent. *N* = nucleolar bivalent, which is attached to the circular nucleolus. (*b*) *P. machaon* ♀. WZ = sex chromosome bivalent. Note W chromosome is not looped. N = nucleolar bivalent. aN = additional nucleolar bivalent. The W is tentatively considered to be of two parts, one heterochromatic and one euchromatic, paired to different parts of the Z (see plate 4*d*, *e*). (*c*) F_1 hybrid ♀ (*P. machaon* ♀ *P. polyxenes* ♂). Note *machaon* type W. N = nucleolar bivalent. No aN present.

All the photographs on plates 3 and 4 are at the same magnification.



Chromosomes of larvae in pachytene

(*a*) Female F_1 hybrid (*P. polyxenes* ♀ × *P. machaon* ♂). The autosomes are correctly paired but in the WZ bivalent the W forms a striking loop, and pairing is prevented. The differences in the W between *polyxenes* and *machaon* enable the source of this chromosome to be identified in the various hybrids. N = nucleolar bivalent.

(*b*) Female back cross hybrid [(*P. machaon*♀ × *P. polyxenes* ♂) ♀ × *P. polyxenes* ♂]. The heteropyknotic segment of the W chromosome is short and *machaon*-like. N = nucleolar bivalent. aN is not shown in this preparation.

(*c*) Male F_1 hybrid (*P. machaon* ♀ × *P. polyxenes* ♂). Heteropyknotic body absent. The nucleolus is visible though the nucleolar bivalent cannot be distinguished.

(*d*) and (*e*) Tentative W_1W_2/Z bivalents in back cross females [(*P. machaon* ♀ × *P. polyxenes* ♂)] × (*P. polyxenes* ♂).

(*f*)–(*j*) Nucleolar bivalents showing similarity in females of parent forms in various crosses. (*f*) *P. machaon*. (*g*) F_1 (*P. machaon* ♀ × *P. polyxenes* ♂). (*h*) Back cross to *P. polyxenes* ♂ of F_1 ♀ (*P. machaon* ♀ × *P. polyxenes* ♂). (*i*) *P. polyxenes*. (*j*) F_1 hybrid (*P. polyxenes* ♀ × *P. machaon* ♂). The characteristic W of *P. polyxenes* can also be seen in this photograph.

heteropyknotic body. In the males the ratio was again consistent with a 1:1 segregation. Neither parent of this back cross was examined cytologically but the male sibs of the father of this brood (brood 13292) segregated for the heteropyknotic body.

(*d*) *In the F_2 broods*

There were four F_2 broods (table 4) in all of which the heterochromatin phenotype of the female parent is unknown, though in two there is information on that of the male parent, one having 1 or 2 very small heteropyknotic bodies in about

TABLE 4. SEX CHROMATIN DATA IN THE BACK CROSS TO FEMALE *machaon* AND IN F_2 BROODS

brood no. and type of mating	mother	father	offspring: males positive	offspring: males negative	offspring: females positive	offspring: females negative
13292, back cross to female *machaon*	*machaon*, not tested	13160, F_1 hybrid (ex female *polyxenes* × male *machaon*) not tested but his male sibs segregated for the chromatin body	1	5	7	0
13223, F_2	13138, not tested	13138, negative in most cells but $\frac{1}{3}$ of cells contained 1 or 2 very small bodies	0	2	0	0
13302, F_2	13160, not tested	13160, negative	0	4	8	0
13312, F_2	13160, not tested	13160, not tested	0	2	3	0
13324, F_2	13134, not tested	13134, not tested	0	12	10	0

a third of the cells and the other having none. In these four F_2 broods, all the females, unlike the situation in the F_1, had one body like the *polyxenes* grandparent. None of the F_2 males was positive and thus here the results appear to be significantly different from the F_1. The reason for the males being negative is unknown. It might be due to chance but there are other possibilities. For example, failure of the endoreduplicated chromosomes to stick together (Rathjens 1974) would result in the body not being visible; furthermore, the chromosome responsible for the heteropyknotic body might not undergo reduplication in step with the rest of the chromosomes. In support of this, it has been shown in *Drosophila* species (Berendes & Keyl 1967) that heterochromatin replicates less quickly than euchromatin during the process of endoreduplication.

In neither (*c*) nor (*d*) was there any association between the presence or absence of the heteropyknotic body and wing colour (black or yellow). This contrasts with

what we found in another swallowtail, *Papilio glaucus*, which is polymorphic black or yellow in the female. In this species, the black females possess and the yellow females (and the males) lack, the heteropyknotic body. Here, however, the inheritance of the wing colour is quite different, being Y-linked (Clarke, Sheppard & Mittwoch 1976) whereas in *P. polyxenes* and *P. machaon* it is autosomal.

3. *Chromosomes*

The numbers and types of larvae which yielded analysable pachytene preparations are listed in table 5.

The number of bivalents in *P. polyxenes* varied between 27 and 30 and in *P. machaon* between 27 and 31. Maeki & Remington (1959) report a haploid chromosome number of 30 or 31 in *P. polyxenes* while that for *P. machaon* has been given as varying between 30 and 32 (for review see Robinson 1971; Maeki 1976).

In the F_1 hybrid *polyxenes* female × *machaon* male, our counts ranged between 29 and 31. In the back cross to *polyxenes* ((*machaon* female × *polyxenes* male) × *polyxenes* male), the count was 30. As pachytene preparations are not as adequate for chromosome counting as those in metaphase part of the variation may be due to technical reasons.

TABLE 5. LIST OF LARVAE YIELDING ANALYSABLE PACHYTENE PREPARATIONS

species or cross	no. of females	no. of males
P. polyxenes	2	0
P. machaon	2	3
polyxenes × *machaon*	3	0
machaon × *polyxenes*	2	1
(*machaon* × *polyxenes*) × *polyxenes*	2	0

In all crosses, female parent is listed first.

(*a*) *Pairing of the homologues*

Homology of the maternal and paternal chromosome sets was investigated (i) by studying the extent of chromosome pairing in pachytene, and (ii) by comparing the chromomere pattern of the synapsed chromosomes.

Except for the WZ bivalents (see below) all chromosomes were found to be paired in their full length in pure *P. polyxenes* (plate 3*a*), in *P. machaon* (plate 3*b*) and in the back cross mentioned above (plate 4*b*). In both types of F_1 hybrid (plates 3*c*, 4*a*), cells with all the chromosomes fully paired could also be found, but incomplete pairing, i.e. zygotene-like stages, were more common than in the pure species and in the back cross.

Except for the WZ, the chromomere pattern of the paired homologues corresponded well, there being only minor differences in the hybrids; similar differences were occasionally also present in the parent species. They could be due to technical shortcomings or there may be real structural differences.

The overall findings for the autosomes, therefore, indicate extensive homology between the *polyxenes* and *machaon* chromosome complement.

(*b*) *The female sex chromosome*

There is only one bivalent which displays a markedly asymmetric pattern (WZ and W_1W_2/Z in plate 4*a*, *d*, *e*) in all preparations of female larvae of pure and hybrid origin. It does not occur in the male pachytene and therefore can be identified as the WZ bivalent. One of the paired strands of this bivalent contains a large heteropyknotic segment which distinguishes it from all other chromosomes of the male and female complement and thus must be interpreted as the W chromosome. It is different in the two species. When derived from *P. polyxenes*, the heteropyknotic segment appears to be folded back on itself and to form a ring. It sometimes gives the impression of an opened ring (see plate 4*a*) and is imperfectly paired with the Z. On the other hand, when the heteropyknotic segment is derived from *P. machaon*, either in pure broods or in hybrids (plate 3*b* and *c*), it appears to be well paired to the Z chromosome.

There is, however, an additional difference between the W chromosomes in the two species. It will be seen (plate 3*b*) that in a pure *machaon* female, and (plate 4*d*, *e*) in hybrids where the W is derived from *P. machaon*, the W appears to consist of two different chromosomes, both paired to different regions of the single Z chromosome. If this can be confirmed by investigation of metaphase I, the sex chromosomes of *P. machaon* females are likely to be W_1W_2/Z (see Suomalainen 1969), and in our material W_1 is heterochromatic and W_2 euchromatic in pachytene.

The heterochromatic W chromosome in *P. polyxenes* and *P. machaon* can confidently be assumed to give rise to one heterochromatin body in the female somatic cells. It is not known, however, whether the part of the *machaon* W which appears euchromatic in pachytene (tentatively called W_2 here) takes part in the formation of the heterochromatin body. It might do so, since in another species, *Bombyx mori*, which has a female specific heterochromatin body, no heterochromatic counterpart can be seen in pachytene (Traut 1976).

(*c*) *The autosomal bivalents*

In both species and in all types of hybrid studied one of the autosomal bivalents regularly carries a nucleolus which is terminal (e.g. plate 3*b*, plate 4*f–j*). At the nucleolar end there are two small heterochromatic knobs, one on each of the paired chromosomes. They probably represent the nucleolus-associated heterochromatin. These knobs do not form conspicuous individual heteropyknotic bodies in somatic cells.

There is, however, yet another special bivalent which has only been found in pure *machaon* females and in an F_1 female which had a *machaon* mother. Each of the bivalents carries a nucleolus-like structure in the middle region and there is a small knob associated with it. This bivalent is therefore tentatively called the

'additional nucleolar bivalent' – 'aN' (plate 3*b*). It cannot be recognized in all nuclei and it is not found in *P. polyxenes* or in the back cross to *P. polyxenes*. It has not yet been studied in the F_1 *P. polyxenes* female × *P. machaon* male. This bivalent is predominantly euchromatic in pachytene but because it is variable it is a possible candidate for the heteropyknotic body in somatic tissues of male *P. machaon* as well as for the additional heterochromatin body in somatic tissues of the female. Its presence or absence may thus account for the heterochromatin polymorphism we have noted. Until more material can be studied, however, this is only a preliminary suggestion.

Discussion

The independent assortment of the genes controlling larval spot colour and the adult phenotype in *P. polyxenes* and *P. machaon*, is a not unexpected finding. In each phase of their development, the butterflies have evolved different methods of avoiding predation: warning coloration in the case of the larvae, brown/green camouflage in the pupal stage and mimicry in the case of the adult female *polyxenes*. Furthermore, with such a large number of chromosomes, the chance of the genes being on the same chromosome is small.

On the subject of fertility it should be noted that in the pure species, F_1s and back crosses, only about 50% of matings produce larvae and yet chromosome pairing appears to be essentially normal. It therefore seems unlikely that the 50% infertility here is due to chromosomal causes, and more probable that it is the result of other factors such as abnormality in the maturation of the egg or sperm, disease, or that the artificial nature of hand-pairing interferes with some aspects of sexual selection.

The chromosomes of the larvae destined to produce the F_2 butterflies were not examined, since as many adults as possible were needed. However, we know that pairing is normal in both types of F_1 and that the sex ratio in the F_2 broods is 1:1. Haldane (1922) pointed out that there was a deficiency of the heterogametic sex in many hybrids, and since this does not apply in our material (though it does in many Papilios) this is further evidence in favour of an essentially normal functioning of the chromosomes in these F_2 hybrids. Some of the other factors mentioned above, therefore, are probably operative to a greater degree to explain the infertility.

It seems certain that the sex chromatin body seen in the somatic cells of female *P. polyxenes*, as well as the principal heteropyknotic body in female *P. machaon* and in all the female hybrids, is derived from the W chromosome, this containing a large proportion of heterochromatin.

Since during the development of insects, endoreduplication brings about successive doublings of the chromosome material in the absence of cell division (White 1973), each sex chromatin body represents many copies of the W chromosome and is thus visible in somatic cells, even though all individual chromosomes are small.

As has been stated, however, it is more difficult to explain the additional

heteropyknotic bodies which are present in both sexes of *P. machaon* (see above) but not in *P. polyxenes*. If the observations concerning the additional nucleolar bivalent in *P. machaon* are confirmed, it might fall into the category of a B chromosome* because these have been shown in a variety of plant species to be denser than the normal complement of A chromosomes (Rees & Hutchinson 1973). The matter might be further investigated by looking at mitotic preparations by use of recently improved techniques (Bigger 1975).

The significance of heteropyknotic bodies in the various phyla and orders is of considerable interest. In placental mammals, the Barr body found in the nucleus of females represents the inactivated X (either maternal or paternal) and the fact that this occurs may be the reason why the female does not synthesize double the amount of X-linked enzymes compared with males. However, the Barr body is confined to mammals, whereas heteropyknotic bodies, sometimes associated with sex but unconnected with dosage compensation, extend to other orders. For example, snakes resemble butterflies in that the W chromosome forms a heteropyknotic body in the somatic cells of females (Ray-Chaudhuri, Singh & Sharma 1971). In *Drosophila*, on the other hand, where the males are XY and the females XX, it is generally stated that there is no heteropyknotic body in the female somatic cells, and it has been shown here that the single X chromosome in the males synthesizes the same amount of RNA as is produced by two female Xs (Mukherjee & Beermann 1965). However, Berendes & Keyl (1967) reported a heteropyknotic body in the nuclei of nerve cells in both sexes; this was thought to contain the bulk of the heterochromatin, including that of the sex chromosomes.

The family Papilionidae consists of about 700 species and it is known that many of them, particularly in the genus *Papilio*, can be hand-paired and hybrids raised. Very few have so far been investigated for the heteropyknotic body but this is easy to do, and we now think it is possible to get reliable results without killing the insects (Daker 1977); this would allow much more efficient prospective studies. Moreover, techniques have been much improved for the analysis of butterfly chromosomes, so that the whole field seems to be a promising one for comparative studies.

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* A B chromosome is one which is additional to the normal complement and is present in some members of a species but not in others.

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