138. Chromosomal Studies on Interspecific Hybrids of Butterflies (*Papilionidae*, *Lepidoptera*). VI

Crosses among *P. protenor*, *P. helenus*, and *P. memnon*

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The literature refers to important information on hybridization experiments of butterflies having been undertaken by Lorkovic, Remington, Clarke and Sheppard, and Ae on genetical and evolutionary bases. A research program along a similar scheme has been in progress by us in interspecific hybrids of *Papilio*, with particular concern to the behavior of chromosomes in hybrid meiosis in relation to the evolutionary process of chromosomes. Chromosome analyses have already been carried out in crosses among *P. polyctor*, *P. bianor*, *P. maackii*, *P. polytes*, *P. helenus*, *P. protenor*, *P. macilentus*, *P. paris*, *P. nepheles*, *P. aegeus*, *P. fuscus*, and *P. memnon* (Maeki and Ae 1966, 1970, 1975, 1976a, 1976b) with particular reference to pairing of the chromosomes at the male first meiosis. The accounts derived from the following hybrids: *P. protenor* $\times$ *P. helenus*, *P. helenus* $\times$ *P. memnon*, and *P. memnon* $\times$ *P. protenor* constitute the contents of the present article. They were produced artificially by Ae (1959, 1960, 1962, 1966, 1967), and supplied material for chromosome researches by Maeki. The routine paraffin-method was employed for this study (for detail, see Maeki & Ae 1966).

Results. Samples of the chromosomal findings of interspecific hybrids concerned in this study are shown in photomicrographs (Figs. 1–4). Three parental species of *Papilio*, *P. helenus*, *P. protenor* and *P. memnon*, were characterized uniformly by $n, 30$, with a similar pattern of the chromosome complement. In the following, are presented the chromosomal patterns for each hybrid group.

1. *Papilio protenor* ♀ (from Japan) $\times$ *Papilio helenus* ♂ (from Japan). Counts of chromosomes in hybrids were made in 87 M-I nuclei derived from five hybrid males (Nos. 1, 2 and 3 from Brood R-54-43; and Nos. 3 and 5 from Brood R-135). The M-I chromosomes of the hybrids showed a considerable variation in number, ranging from 32 to 47 (Figs. 1–2). The nuclei having $n, 32$ – 36 showed a

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frequency of 67%. The chromosome-number distributions so far examined at M-I from 87 nuclei were as follows:

<i>n</i> , 32	10 nuclei	<i>n</i> , 40	 2 nuclei
<i>n</i> , 33	10 "	<i>n</i> , 41	 2 "
<i>n</i> , 34	10 "	<i>n</i> , 42	 4 "
<i>n</i> , 35	18 "	<i>n</i> , 43	 2 "
<i>n</i> , 36	10 "	<i>n</i> , 44	 1 nucleus
<i>n</i> , 37	 6 "	<i>n</i> , 45	 1 "
<i>n</i> , 38	 5 "	<i>n</i> , 47	 1 "
<i>n</i> , 39	 5 "		

2. *Papilio helenus* ♀ (from Japan) × *Papilio memnon* ♂ (from Japan). Chromosome counts were available in 28 M-I cells coming from two hybrid males (Nos. 1 and 2 from Brood N-40-1). The M-I chromosome-numbers of the hybrids showed variations ranging from 39 to 52 (Fig. 4). The complement consisting of 12 bivalents and 36 univalents was very frequent, constituting 21% of the observed cells. The chromosome-number distributions observed at M-I in 28 cells were shown as follows:

<i>n</i> , 39	 2 nuclei	<i>n</i> , 45	 2 nuclei
<i>n</i> , 40	 3 "	<i>n</i> , 46	 3 "
<i>n</i> , 42	 2 "	<i>n</i> , 47	 2 "
<i>n</i> , 43	 3 "	<i>n</i> , 48	 6 "
<i>n</i> , 44	 3 "	<i>n</i> , 52	 2 "

3a. *Papilio memnon* ♀ (from Japan) × *Papilio protenor* ♂ (from Japan).

Figs. 1-4: Meiotic chromosomes of *Papilio* hybrids (M-I, ×3000). Figs. 5-6: Eupyrene and apyrene spermatozoa (×1500). 1-2: *P. protenor* × *P. helenus*, F₁. 3: *P. protenor* × *P. memnon*, F₁. 4: *P. helenus* × *P. memnon*, F₁. (1: *n*, 33, 2: *n*, 35, 3: *n*, 41, 4: *n*, 47).

3b. *Papilio protenor* ♀ (from Japan) × *Papilio memnon* ♂ (from Japan). The chromosomes were studied in 74 M-I nuclei derived from four hybrid males, such as No. 1 from Brood M-15-6; and Nos. 1, 2 and 3 from Brood R-120-8, which were produced from the following reciprocal crosses: *P. memnon* ♀ × *P. protenor* ♂, and *P. protenor* ♀ × *P. memnon* ♂. The chromosome-numbers of the above crosses showed at M-I a variation which ranged from $n, 41$ to $n, 54$ (Fig. 3). The formation of 19 bivalents was most frequent showing 14% of the observed cells. The occurrence of 11 and 13 bivalents ranked next which were 12% in each. The chromosome-number distributions so far observed in 74 M-I cells were obtained as below:

$n, 41$ 10 nuclei	$n, 48$ 8 nuclei
$n, 42$ 2 "	$n, 49$ 9 "
$n, 43$ 5 "	$n, 50$ 3 "
$n, 44$ 8 "	$n, 51$ 2 "
$n, 45$ 4 "	$n, 52$ 4 "
$n, 46$ 4 "	$n, 53$ 3 "
$n, 47$ 9 "	$n, 54$ 3 "

Remarks. The chromosomes of the F_1 hybrids from a cross, *P. polyctor* × *P. bianor*, showed a complete pairing at M-I, while those of the hybrids from *P. maackii* × *P. polytes* failed to complete their pairing (Maeki & Ae 1966, 1975). The situation of three *Papilio*-hybrids herein dealt with seems to be intermediate between the above two categories in the meiotic behavior of chromosomes, because pairing of the M-I chromosomes was incomplete. In the F_1

Fig. 7. Diagram showing the relationship of four *Papilio* species, based on analysis of meiotic chromosomal pairing in hybrid specimens.

hybrids from *P. macilentus* $\times$ *P. memnon*, the chromosome-number variation was shown as $n, 36-56$ in the M-I nuclei with a mode lying at $n, 44$ (Maeki & Ae 1976b). Based on the above data along with those presented by this study of three *Papilio*-hybrids, it may be stated that systematically *P. protenor* and *P. helenus* are most closely related, and that the systematic relations between *P. macilentus* and *P. memnon*, between *P. memnon* and *P. protenor*, and between *P. helenus* and *P. memnon*, rank in order, viewed from genic homology occurring among *P. macilentus*, *P. protenor*, *P. helenus* and *P. memnon*.

Hybrid specimens derived from *P. helenus* $\times$ *P. memnon* and from *P. memnon* $\times$ *P. protenor* were remarkable by producing many apyrene spermatozoa, together with a few bundles of eupyrene spermatozoa. The apyrene spermatozoa have been known to occur in association with aberrant spermatogenesis (Maeki 1953). Then, it seems possible to assess the systematic relationship of the parental species on the basis of the apyrene formation. The two features, genic homology and the apyrene-sperm formation, seem to correlate with each other in the *Papilio* hybrids, so far as our cytogenetic studies have gone. In three hybrid categories concerned here, especially the hybrids between *P. protenor* and *P. helenus* were remarkable by the formation of both apyrene and eupyrene spermatozoa, suggesting partial fertility in the hybrid males.

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