

39. Chromosomal Studies on Interspecific Hybrids of Butterflies (*Papilionidae*, *Lepidoptera*). XIV

Crosses among *Papilio alphenor*, *P. polytes*, *P. protenor*,
P. helenus, *P. memnon*, and *P. nepheles*

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A large amount of cytogenetic work has been published in interspecific hybrids of moths by Federley 1915, 1916, 1927, 1929; Bytinski-Salz and Guenther 1930, Bytinski-Salz 1934, Kawaguchi 1928, and others. In contrast, knowledge on butterfly hybrids has been in a very restricted condition (Lorkovic 1950, 1968). The significance of hybridization lies in crossing between members of populations having different adaptive gene complexes. During the past several years we have been studying on this subject with interest in the significance of chromosomal pairing in male meiosis of F_1 hybrids of *Papilio*, on the standpoint of the analysis of the genic homology. In the previous papers, relevant data have been presented on the meiotic behavior of chromosomes observed in F_1 hybrids produced artificially in the following crosses among *Papilio polyctor*, *P. bianor*, *P. maackii*, *P. polytes*, *P. hipponous*, *P. paris*, *P. helenus*, *P. protenor*, *P. macilentus*, *P. nepheles*, *P. aegeus*, *P. fuscus*, *P. memnon*, *P. lowi*, *P. ascalaphus*, *P. polymnestor*, *P. rumanzovia*, *P. machaon hippocrates*, *P. machaon gorganus*, *P. machaon britannicus*, *P. xuthus*, *P. benguetana*, *P. polyxenes* and *P. zelicaon* (Maeki and Ae 1964, 1966, 1970, 1975, 1976a-d, 1977a-c, 1978a-b, 1979a).

In the present article are presented some aspects on the chromosomal features of F_1 -meiosis with regard to the species relationship in the crosses between the following six species, *Papilio alphenor*, *P. polytes*, *P. protenor*, *P. helenus*, *P. memnon*, and *P. nepheles*. Each species was found to have uniformly $n, 30$ which was common through other species of *Papilio*. The hybridization experiments were responsible to Ae (1964, 1971, 1972, 1973, 1974), while Maeki engaged in the chromosome study. The studies were exclusively subjected to the routine paraffin section-method as done in the previous cases.

Results. The feature of meiotic pairing of chromosomes in male

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F₁-hybrids was the subject mainly concerned in the following observations.

1a) *Papilio alphenor* ♀ (from Philippines) × *Papilio polytes* ♂ (from Hong-Kong). Chromosome counts were made in 28 cells at M-I derived from 4 hybrid males (Nos. 4, 5, 13 and 15 from Brood Le-33). The chromosome-number distributions in the M-I metaphase coming from the 28 cells are as follows:

n, 30 26 cells
n, 31 2 "

1b) *Papilio alphenor* ♀ (from Philippines) × *Papilio polytes* ♂ (from Japan). 1c) *Papilio polytes* ♀ (from Japan) × *Papilio alphenor* ♂ (from Philippines). In this cross were observed 52 M-I cells which came from 5 hybrid males in the above reciprocal crosses (Nos. 4, 6 and 7 from Brood Le-57-14, and Nos. 4 and 5 from Brood 0-40-7-24-12). The chromosome-number distributions studied in the 52 M-I cells are as below:

n, 30 29 cells
n, 31 14 "
n, 32 7 "
n, 33 2 "

1d) *Papilio polytes* ♀ (from India) × *Papilio alphenor* ♂ (from Philippines). Counts of chromosomes in the above cross were carried out in 26 M-I cells which were derived from 3 hybrid males (Nos. 4, 6 and 12 from Brood 0-34-14). The chromosome-number distributions observed in them are as follows:

n, 30 17 cells
n, 31 7 "
n, 32 2 "

1e) *Papilio alphenor* ♀ (from Philippines) × *Papilio polytes* ♂ (from Formosa). The chromosomes were studied in 39 M-I cells obtained in 4 hybrid males (Nos. 4, 17, 22 and 24 from Brood Le-30-14). The chromosome-number distributions so far observed in those cells were obtained as below:

n, 30 17 cells
n, 31 16 "
n, 32 6 "

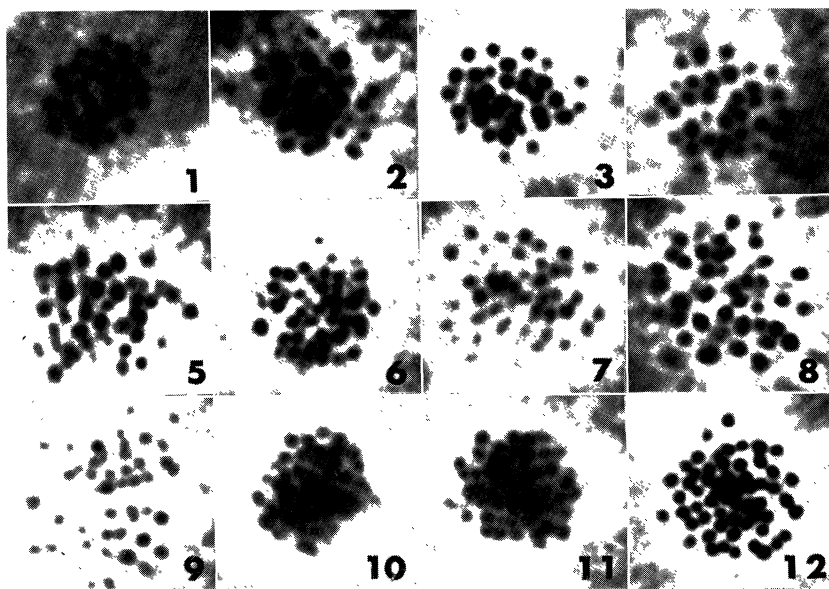
The chromosomes of the 145 M-I cells studied in the above 16 hybrid males coming from the crosses, 1a)–1e), between Philippine *P. alphenor* and Hong-Kong, Japanese, Indian, and Formosan *P. polytes*, showed a variation in number ranging from 30 to 33 (Fig. 1). The metaphase complement showing 30 bivalents was most frequent in occurrence, constituting 62% of the observed cells. This is indicative of that the parental species under study are nearly related with each

other. The distribution of the chromosome-numbers obtained in them are summarized as follows:

$n, 30$89 cells
$n, 31$39 "
$n, 32$15 "
$n, 33$2 "

2a) *Papilio protenor* ♀ (from Japan) × *Papilio alphenor* ♂ (from Philippines). 2b) *Papilio alphenor* ♀ (from Philippines) × *Papilio protenor* ♂ (from Japan). Chromosomes of F_1 -hybrids were studied in 67 cells at M-I derived from 7 hybrid males in the above reciprocal crosses (Nos. 4, 7, 13 and 14 from Brood R-i-184-15-14; Nos. 1, 2 and 7 from Brood Le-44). The chromosome-number of the M-I cells in this cross ranged in variation from $n, 32$ to $n, 39$ (Figs. 2-4). The cells showing 36 chromosomes were most frequent, being 34% of cells observed. The chromosome-number distributions obtained in 67 M-I metaphases are shown below:

$n, 32$1 cell	$n, 36$23 cells
$n, 33$12 cells	$n, 37$4 "
$n, 34$13 "	$n, 39$1 cell
$n, 35$13 "		



Figs. 1-12. Meiotic chromosomes of *Papilio* hybrids (M-I, ×3000). 1: *P. alphenor* × *P. polytes*, F_1 . 2-4: *P. alphenor* × *P. protenor*, F_1 . 5-6: *P. alphenor* × *P. memnon*, F_1 . 7-9: *P. alphenor* × *P. helenus*, F_1 . 10-12: *P. nepheles* × *P. protenor*, F_1 . 1: $n, 30$. 2: $n, 33$. 3: $n, 35$. 4: $n, 36$. 5: $n, 45$. 6: $n, 47$. 7: $n, 49$. 8: $n, 50$. 9: $n, 53$. 10: $n, 55$. 11: $n, 59$. 12: $n, 60$.

3) *Papilio alphenor* ♀ (from Philippines) × *Papilio helenus* ♂ (from Japan). In this cross, 3 hybrid males (Nos. 1, 2 and 5 from Brood Le-57-2-28-10-2) provided at M-I 28 cells which were available for chromosome analysis. The chromosomes of these cells showed a fluctuation in number ranging from n , 37 to n , 53 (Figs. 7–9). The cells having the numbers from 47 to 50 were most frequent, giving 50% of cells so far studied. The chromosome-number distributions in the M-I metaphases observed in them were obtained as follows:

n , 37	1 cell	n , 46	1 cell
n , 40	1 "	n , 47	3 cells
n , 41	1 "	n , 48	3 "
n , 42	2 cells	n , 49	5 "
n , 43	1 cell	n , 50	3 "
n , 44	1 "	n , 52	1 cell
n , 45	2 cells	n , 53	3 cells

4) *Papilio alphenor* ♀ (from Philippines) × *Papilio memnon* ♂ (from Formosa). In this cross, 19 nuclei at M-I cells derived from a single hybrid-male (Brood Le-85-1) provided materials for chromosome study. The n number of the M-I cells in the above cross showed a variation ranging from 40 to 51 (Figs. 5–6). The chromosome-number distributions in the M-I metaphase based on these cells are as follows:

n , 40	1 cell	n , 47	2 cells
n , 43	3 cells	n , 48	2 "
n , 44	1 cell	n , 49	2 "
n , 45	3 cells	n , 50	3 "
n , 46	1 cell	n , 51	1 cell

5) *Papilio nepheles* ♀ (from Formosa) × *Papilio protenor* ♂ (from Japan). Chromosome counts were possible in 16 nuclei at M-I cells which were obtained in a single male of hybrid (No. 1 from Brood Ch-1-3). The n -chromosomes of the 16 M-I cells fluctuated in number from 51 to 60 (Figs. 10–12). The metaphase complements having 59 or 60 chromosomes were most frequent in incidence, being 44% in the observed cells. This indicates the fact that *P. nepheles* and *P. protenor* are very remotely related with each other. The chromosome-number distributions in the 16 M-I nuclei are as follows:

n , 51	1 cell	n , 57	2 cells
n , 54	1 "	n , 58	1 cell
n , 55	2 cells	n , 59	4 cells
n , 56	2 "	n , 60	3 "

Remarks. The variations in chromosome-number and the modal numbers here obtained in hybrid males from the above 5 series of crosses which were made between the members of the black-*Papilio*

group were obtained as shown in the following:

Cross	Number variation	Mode
<i>alphenor</i> × <i>polytes</i>	30–33	30
<i>alphenor</i> × <i>protenor</i>	32–39	36
<i>alphenor</i> × <i>helenus</i>	37–53	47–50
<i>alphenor</i> × <i>memnon</i>	40–51	—
<i>nepheles</i> × <i>protenor</i>	51–60	—

The above data may allow the following statements: Among the crosses examined here *Papilio alphenor* and *P. polytes* are related most closely, and the relation between *P. alphenor* and *P. protenor*, that between *P. alphenor* and *P. helenus*, that between *P. alphenor* and *P. memnon*, and that between *P. nepheles* and *P. protenor* rank in order, on the basis of genic homology so far observed in the 6 species of *Papilio* concerned here. A diagram (Fig. 13) is given to illustrate the above feature.

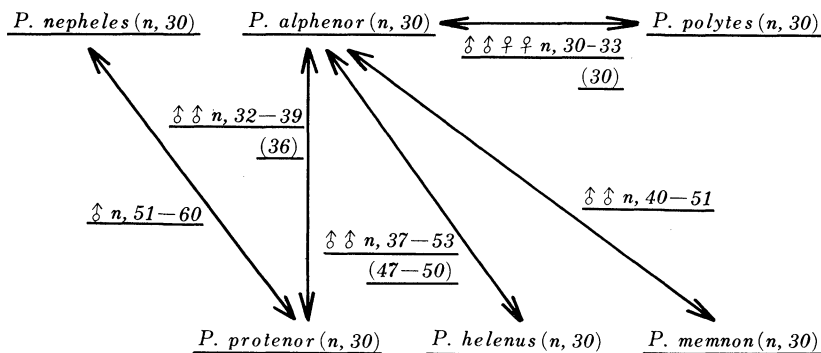


Fig. 13. Diagram illustrating the relationship of 6 species of *Papilio* here studied, based on the analysis of meiotic pairing of chromosomes in hybrids.

According to Shirozu (1960), *Papilio helenus* was noted as a species of the II-group, *P. nepheles* and *P. memnon* were a member of the III-group, *P. protenor*, *P. alphenor*, and *P. polytes* belonged to the IV-group, on the basis of the morphological resemblance of male genitalia. The relationships between each 2 species belonging to different groups on the basis of the genitalian morphology seemed to be remotely related in those of chromosomal pairing of hybrids rather than in those seen among the morphologically identical species.

In the F_1 -hybrids, the metaphase features of chromosomes having $n, 30$ consisting all of bivalents (Fig. 1) and those having $n, 60$ all being of univalents (Fig. 12) are shown to have a distinct appearance from one another. The chromosomes of the above cases fundamentally differ in nature and morphology, while their behaviors are rather

identical. It seems apparent that the important factor for the formation of the equatorial plate is due probably to the balance of chromosomes in relation to the spindle organization.

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