A Use of Chromosome Numbers in the Study of Taxonomy of the Lepidoptera and Notes on the Internal Reproductive Anatomy

By

Kōdō Maeki

(Biological Institute, Kwansei Gakuin University)

(With 6 Text-figures)

The following report deals with the chromosome numbers observed in the germ cells of 16 species of Papilionidae which are found in Japan. Further data are presented concerning the testicular form and color, and the stages of the life cycle at which meiotic divisions have been seen to have taken place. These data are correlated with the known morphological relationships of these species, and these relationships are more clearly defined.

Testicular form is classified into three major types: separate, gourd shaped, and conjugated. In the larvae of all species the testes are clearly separated, each testis being made up of four follicles. In certain groups this condition changes during development to a point where they become slightly adherent, having the appearance of a gourd or dumb-bell, or becoming completely conjugated and spherical. In these types the single testis has eight follicles. Table 1 shows the data concerning the distribution of these types during the various stages of development for the groups under discussion. These suggest a phylogenetic sequence wherein the separate condition appears most primitive, and the conjugated condition that of the derived forms. The latter form is found in most of the butterflies.

In addition to the different shapes of testes, coloration of their epithelial sheath was found to vary. Three distinct classes were found: yellow, pale blue, and red.

Table 1. Testicular form during development in the Papilionidae

Genus	Larva	Pre-pupa	Pupa	Imago
Parnassius	separate	separate	separate	separate
Luehdorfia japonica	separate	separate	separate	separate or slightly adherent
puziloi	separate	conjugated	conjugated	conjugated
Graphium	separate	separate	gourd	gourd
Byasa	separate	conjugated	conjugated	conjugated
Papilio	separate	conjugated	conjugated	conjugated

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 13, 1957 (Prof. T. Uchida Jubilee Volume).

The primitive species (*Parnassius, Luehdorfia, Graphium*) have yellow or pale blue testes, while the derived ones (*Byasa, Papilio*) have them red colored. These data, and the data on meiosis discussed in the following paragraphs, are presented in Table 2.

Table 2. Chromosome number and imaginal testicular form and color for sixteen species of Papilionidae

Species	Chromosome number	Testes Form	Testes Color
Parnassius eversmanni	62(I)	separate	yellow
P. glacialis	—	separate	yellow
P. stubbendorfii	—	separate	yellow
Luehdorfia japonica	31(I, II)	separate or adherent	pale blue
L. puziloi	30(I, II)	conjugated	pale blue
Graphium doson	30(I, II)	gourd	yellow
G. sarpedon	20(I, II)	gourd	yellow
Byasa alcinous	30(I, II)	conjugated	red
Papilio macilentus	—	conjugated	red
P. machaon	31(I)	conjugated	red
P. xuthus	30(I)	conjugated	red
P. protenor	30(I)	conjugated	red
P. memnon	30(I, II)	conjugated	red
P. helenus	30(I)	conjugated	red
P. bianor	30(I, II)	conjugated	red
P. maackii	30(I, II)	conjugated	red

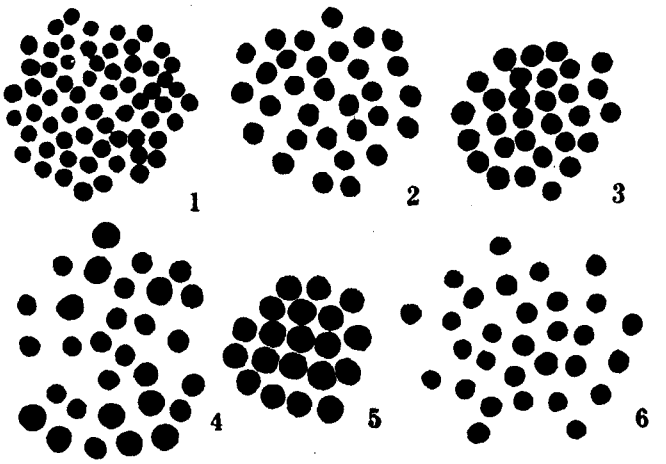
Meiosis is observed only in the pre-pupal and early pupal stages of many butterflies. This was found to hold true for all the species of *Parnassius, Luehdorfia, Graphium, Byasa* and *Pailipo macilentus*. In all the other members of the family considered here, spermatogenesis was not only found in these stages, but also in the imago. However, in the imaginal testes, meiosis was aberrant and lead to the formation of apyrene spermatozoa.

The chromosomes of all of the species are dot-like and the following haploid numbers were found: 20, 30, 31, and 62. The roman numerals given after the numbers for each species in Table 1 refer to the stage of spermatogenesis in which the determinations were made.

A correlation is found between morphology and chromosome numbers, with the most primitive species exhibiting the highest number of 62 (Fig. 1). The number 31, 30, and 20 occur in the progressively more specialized, or derived, forms.

Graphium sarpedon with 20 and *G. doson* with 30 chromosomes are closely related, and it can be observed that differences in chromosome numbers here appear to be associated with chromosomal fusion. *Graphium doson* was found to possess 30 chromosomes, 20 are small and 10 large dots (Fig. 4); whereas in *G. sarpedon* there are 20 dots, all of which are large (Fig. 5). The most probable explanation

is that the lower numbers have been derived by fusion of 20 smaller chromosomes into 10 larger ones. *Luehdorfia japonica*, peculiar to Japan, showed 31 chromosomes in both the primary and secondary spermatocytes (Fig. 2). On the other hand, there are 30 chromosomes in the haploid complex of *Luehdorfia puziloi* (Fig. 3).



All figures are primary spermatocytes. ×3500.
Figs. 1-6. *Parnassius eversmanni*, *Luehdorfia japonica*, *L. puziloi*, *Graphium doson*, *G. sarpedon*, and *Papilio bianor*.

In the course of the study on the chromosomes in the Papilionidae, the author chanced to collect *Parnassius eversmanni daisetuzaus* Matsumura on Mt. Daisetsu in Hokkaido. It was observed that this species was primitive in the testicular form and color, the stage of meiotic division and the chromosome number. In addition to these characteristics, this species spins a cocoon just like ordinary moths. From the facts mentioned above, it seems apparent that this species is of the most primitive type in the Papilionidae.

Based on the results of the present observations it is most probable that *Papilio macilentus* seems to be better assorted as a member of the genus *Byasa*.

Table 2 gives a phylogenetic arrangement of 16 species of the Papilionidae, on the basis of the observations of the chromosome number and testicular form and color.

The author is deeply grateful to Professor Sajiro Makino, Hokkaido University, for his helpful suggestion and kind guidance. The author's thanks are also due to Prof. Yoshio Ojima, Kwansei Gakuin University and Dr. R. H. T. Mattoni, California University at Los Angeles, for their valuable advice and encouragement.

Literature

Beliajeff, N. K. 1930. Z. I. A. V. 54 : 369-399.
Federley, H. 1938. Hereditas 24 : 397-464.
Lorkovic, Z. 1941. Chromosoma 2 : 155-191.
Maeki, K. 1953. Zool. Mag. (Tokyo) 62 : 445-449.
Makino, S. 1956. A review of the chromosome numbers in animals. Hokuryukan, Tokyo.