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STUDIES OF THE CHROMOSOMES OF FORMOSAN RHOPALOCERA
1. Papilionidae and Hesperiidae

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An extensive entomological survey in Formosa was undertaken by some Japanese and U. S. entomologists during a period from March to April, 1965, as part of Japan-U. S. Co-operative Science Program. The present article reports the results of chromosomal studies of Formosan Rhopalocera which were collected during the period. This constitutes the first of a series of studies on the chromosomes of Formosan Rhopalocera in the above mentioned survey, with special regard to phylogenetic significance of cytological data. Formosa is noted for its remarkably prolific butterfly fauna. According to Shirôzu (1960), as many as 362 species have been reported from that country. The chromosomes of Formosan Rhopalocera have already been reported on by Maeki, Ogata, and Shirôzu ('65), concerning 25 species belonging to 6 families. The present study deals with the chromosomes of 55 additional species of Formosan butterflies.

All the specimens studied were collected and fixed with Allen's P.F.A.-3 solution by Ae. They were identified by Dr. T. Shirôzu, Kyushu University, who also took part in this survey. The sections, 10 micra thick, were made according to the routine paraffin method and stained with Heidenhain's iron-haematoxyline with counterstaining of light green. Camera lucida drawings were made at the magnification of 4200 times. The photomicrographs were taken by means of a MIKAS camera at a magnification of 5000 times.

In addition to the species described in this paper, we studied the chromosomes of the following species: *Chilasa epycides melanoleucus* (Ney), *Papilio paris hermosanus* Rebel, *Papilio hoppo* Matsumura, *Papilio dialis andronicus* Fruhstorfer, *Graphium doson postianus* (Fruhstorfer), and *Graphium cloanthus kuge* (Fruhstorfer), but failed to obtain satisfactory results, because the materials were inadequate for chromosome research.

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his invaluable advice and identification of all specimens used as materials in the present study. Mr. T. Takakura helped us in writing this paper.

Observations

A. PAPILIONIDAE

1. *Troides aeacus kaguya* (Nakahara & Esaki)

The haploid chromosome number is 31. Counts were made in 16 nuclei in the primary spermatocyte division and 7 nuclei in the secondary spermatocyte division, all from one male (F-160) emerged at Chu-ch'i, Chia-i Hsien on April 15 from a pupa, which was obtained at P'ing-tung Hsien. Many additional suitable nuclei were present and could have been counted. Thus the chromosome number of the Formosan *Troides aeacus kaguya* (*n*, 31) differs from that

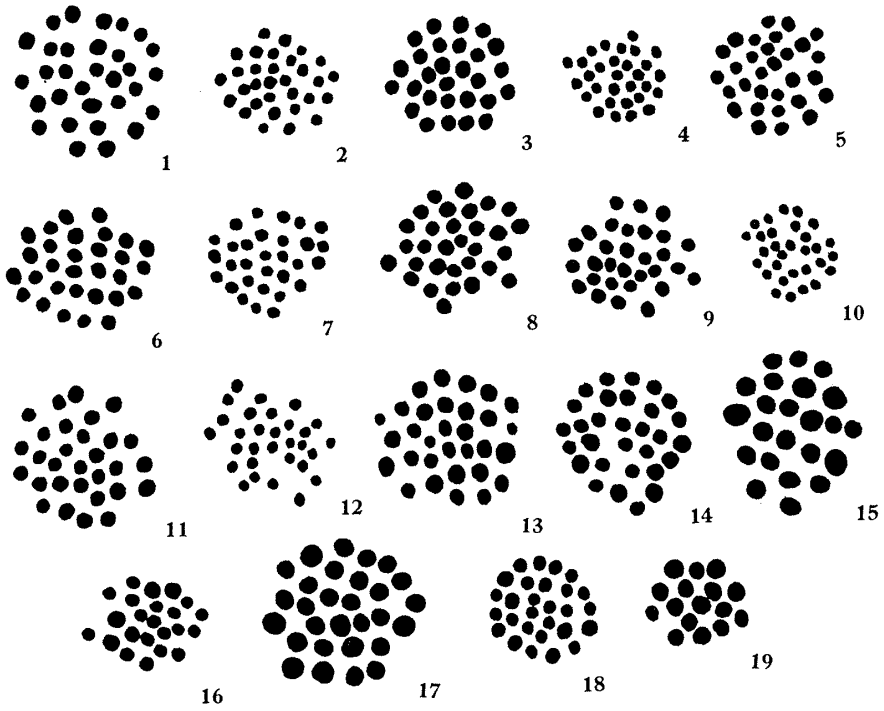
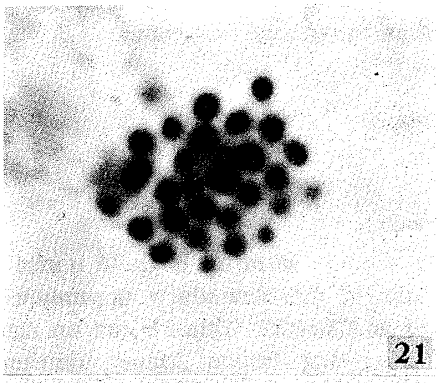


Fig. 1: *Troides aeacus kaguya* (I). Fig. 2: same (II). Fig. 3: *Byasa febanus* (I). Fig. 4: same (II). Fig. 5: *Princeps demoleus libanius* (I). Fig. 6: *Papilio xuthus koxinga* (I). Fig. 7: *Papilio protenor amaura* (II). Fig. 8: *Papilio castor formosanus* (I). Fig. 9: *Papilio thaiwanus* (I). Fig. 10: same (II). Fig. 11: *Papilio bianor takasago* (I). Fig. 12: same (II). Fig. 13: *Bibasis jaina formosana* (I). Fig. 14: *Daimio tethys moorei* (I). Fig. 15: *Suastus gremius gremius* (I). Fig. 16: same (II). Fig. 17: *Telicota ancilla horisha* (I). Fig. 18: same (II). Fig. 19: *Polytremis lubricans taiwana* (I).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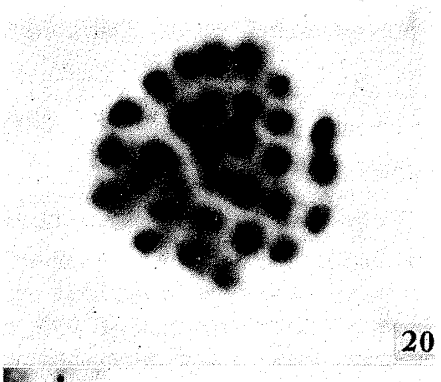
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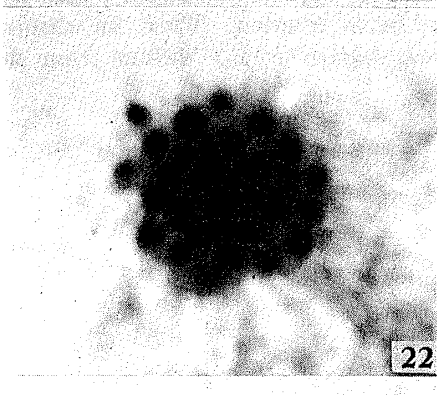
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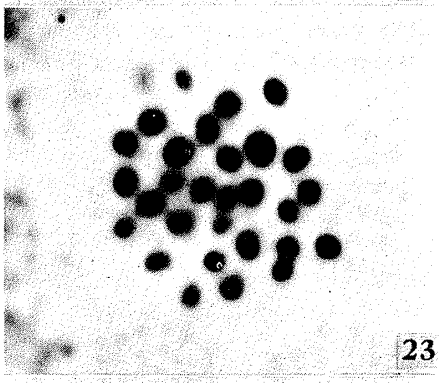
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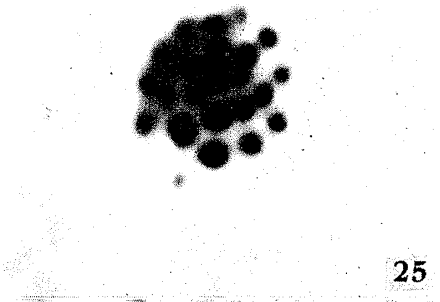
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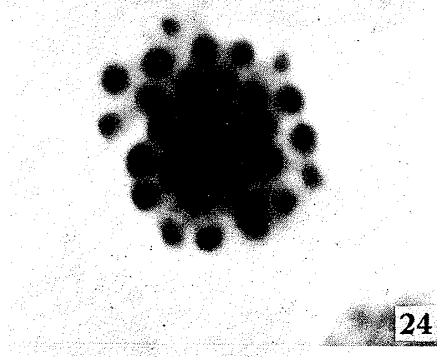
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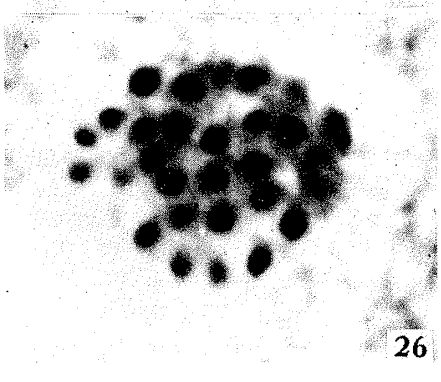
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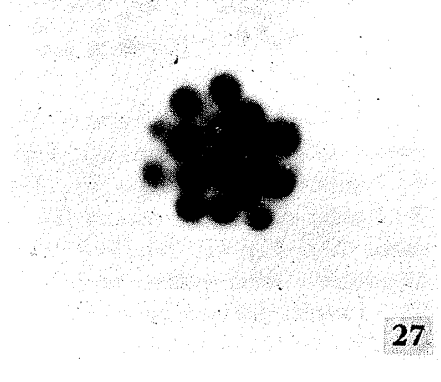
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of the Himalayan *Troides aeacus aeacus* (*n*, 30). Twelve chromosomes are somewhat larger than the other chromosomes.

2. *Byasa febanus* (Fruhstorfer)

The haploid chromosome number is 30. Counts were made in 15 nuclei (I) and 9 nuclei (II) in testes of 3 males (F-154, F-156, F-159) taken at Ch'i-hsin-liao, Chia-i Hsien on April 14. There appear to be 7 chromosomes somewhat larger than the other 23, the latter are similar in size.

3. *Princeps demoleus libanius* Fruhstorfer

The haploid chromosome number is 30. Counts were made in 10 nuclei (I) in testis of one male (F-14) taken at Taipei on March 30. A second male taken at the same time shows no meiotic divisions. Eleven chromosomes are some larger than the others.

4. *Papilio xuthus koxinga* Fruhstorfer

The haploid chromosome number is 30. Counts were made in 6 nuclei (I) in testis of one male (F-141) taken at Chu-ch'i on April 13. Ten chromosomes are somewhat larger than the others.

5. *Papilio protenor amaura* Jordan

The haploid chromosome number is 30. Counts were made in 10 nuclei (II) in testes of 2 males taken at Mt. Yang-ming Shan, T'ai-pei Hsien on March 28 (F-3) and at Fen-ch'i-hu, Chia-i Hsien on April 12 (F-138). Twelve chromosomes are somewhat larger than the other chromosomes.

6. *Papilio castor formosanus* Rothschild

The haploid chromosome number is 31. Counts were made in 20 nuclei (I) in testes of 3 males (F-161, F-164, F-170) taken at Ch'i-hsin-liao on April 15. Eight chromosomes are distinctly larger than the others.

7. *Papilio thaiwanus* Rothschild

The haploid chromosome number is 30. Counts were made in 10 nuclei (I) and 5 nuclei (II) in testis of one male (F-169) taken at Ch'i-hsin-liao on April 15. About 13 chromosomes are somewhat larger than the others. No meiotic divisions were found in another male collected on April 14.

8. *Papilio bianor takasago* Nakahara & Esaki

The haploid chromosome number is 30. Counts were made in 10 nuclei (I) and 10 nuclei (II) in testes of 3 males taken at Mt. Yang-ming Shan, on March 28 (F-1), at Kuan-tzu-ling, T'ai-nan Hsien on April 6 (F-71), and at Chu-ch'i on

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Explanation of the photographs of chromosomes in spermatocyte divisions on p. 118.

Fig. 20: *Troides aeacus* (I). Fig. 21: *Papilio castor* (I). Fig. 22: *Papilio thaiwanus* (I). Fig. 23: *Papilio bianor* (I). Fig. 24: *Bibasis jaina* (I). Fig. 25: *Suastus gremius* (II). Fig. 26: *Daimio tethys* (I). Fig. 27: *Polytremis lubricans* (I).

April 13 (F-142). About 10 chromosomes are some larger than the others.

B. HESPERIIDAE

1. *Bibasis jaina formosana* (Fruhstorfer)

The haploid chromosome number is 31. Counts were made in 6 nuclei (I) in testis of one male (F-18) collected at K'en-ting-kung-yuan, P'ing-tung Hsien on April 2. There are 16 large, 12 medium, and 3 small chromosomes. No secondary divisions were found.

2. *Daimio tethys moorei* (Mabille)

The haploid chromosome number is 30. Counts were made in 8 nuclei (I) in testes of 2 males collected at Fen-ch'i-hu on April 11 (F-128) and at Ch'i-hsin-liao on April 15 (F-175). There are nine large and 21 small chromosomes. No dividing cells were found in secondary spermatocytes.

3. *Suastus gremius gremius* (Fabricius)

The haploid chromosome number is 23. Counts were made in 5 nuclei (I) and 8 nuclei (II) in testes of 2 males (F-15, F-16) collected at Taipei on March 30. Five chromosomes are distinctly larger than the others.

4. *Telicota ancilla horisha* Evans

Table 1. *Chromosome numbers of Formosan Papilionidae, and Hesperiidae.*

Species	Number(n)	Phase	Reference
A. Papilionidae			
Troides aeacus kaguya	31	♂(I, II)	Present paper
Byasa polyeuctes termessus	30	♂(II)	Maeki, Ogata, Shirôzu, 1965
Byasa febanus	30	♂(I, II)	Present paper
Princeps demoleus libanius	30	♂(I)	〃
Papilio xuthus koxinga	30	♂(I)	〃
Papilio polytes pasikrates	30	♂(II)	Maeki, Ogata, Shirôzu, 1965
Papilio protenor amaura	30	♂(II)	Present paper
Papilio castor formosanus	31	♂(I)	〃
Papilio thaiwanus	30	♂(I, II)	〃
Papilio memnon heronus	30	♂(I, II)	Maeki, Ogata, Shirôzu, 1965
Papilio Bianos takasago	30	♂(I, II)	Present paper
B. Hesperiidae			
Bibasis jaina formosana	31	♂(I)	Present paper
Daimio tethys moorei	30	♂(I)	〃
Suastus gremius gremius	23	♂(I, II)	〃
Telicota ancilla horisha	29	♂(I, II)	〃
Polytremis lubricans taiwana	16	♂(I)	〃

(I)=meiosis I. (II)=meiosis II.

The haploid chromosome number is 29. Counts were made in 7 nuclei (I) and 5 nuclei (II) in testis of one male (F-143) taken at Chu-ch'i on April 13. Seven chromosomes are distinctly larger than the others.

5. *Polytremis lubricans taiwana* (Matsumura)

The haploid chromosome number is 16. Counts were made in 6 nuclei (I) in testis of one male (F-100) taken at Kuan-tzu-ling on April 7. There are apparently 1 small, 6 medium, and 9 large chromosomes. No normal divisions were found in secondary spermatocytes.

Discussion

Table 1 shows the chromosome numbers of 11 species of Papilionidae and 5 species of Hesperiidae for which counts have been recorded. The 13 species described in the present paper are shown in roman antiques in the Table. Almost all species so far studied of Papilionidae were found to possess the conventional *n*, 30 chromosomes. In the chromosomal study of Formosan Papilionidae, too, all species belonging to this family showed the 30 chromosome type in the haploid set, except the following two species with *n*, 31 elements, namely, *Troides aeacus kaguya*, and *Papilio castor formosanus*. *Papilio castor formosanus* joins the group of *P. hippocrates* (*n*, 31), *P. polyxenes* (*n*, 30-31), and *P. thoas* (*n*, 27) which do not possess the characteristic number of *n*, 30 in *Papilio*.

Maeki and Ae (1966) counted 30 chromosomes in second meiosis in the Himalayan *Troides aeacus aeacus*, while the *T. aeacus kaguya* of Formosa showed 31 elements. These two karyotypes of *T. aeacus* may be considered to be a chromosomal dimorphism which resulted from geographical isolation:

Troides aeacus aeacus (Nepal).....*n*, 30 (II)♂
Troides aeacus kaguya (Formosa).....*n*, 31 (I, II)♂

In the karyotypes of the above two subspecies, we could not recognize the diversity in the chromosomal size of each complement, apart from the numerical difference of only one element. There is, however, a possibility that *T. a. aeacus* and *T. a. kaguya* both have *n*, 30 and *n*, 31, namely it is a case of chromosomal dimorphism within a population, since these two subspecies are very close and we could examine one individual each.

Papilio castor (*n*, 31) and *Papilio nepheles* (*n*, 30) belong to the third Formosan *Papilio* group separated on the basis of male genitalia by T. Shirôzu (1960). However, the two species have been known to differ from each other in chromosomal aspects. Huge chromosomes suggestive of the fusion of ancestral chromosomes do not exist in the karyotypes of *P. nepheles*, *P. thaiwanus* and *P. memnon* which belonging to the same group which also includes *P. castor*. Since *Papilio castor* has the ancestral karyotype, it is considered to be a unique species among the Formosan *Papilio* for this reason. Interspecific hybrids between *P. castor* and *P. nepheles*, between *P. castor* and other related species would offer

an interesting and phylogenetically significant problem of how many unvialents arise as a result of non-pairing in the meiosis.

In the karyotypes of the following subspecies found in Formosa, Nepal, or Japan, the authors could not find any chromosomal difference: between *Byasa polyeuctus termessus* (Formosa) and *B. p. letincius* (Nepal), *Princeps demoleus libanius* (Formosa) and *P. d. demoleus* (Nepal), *Papilio polytes pasikrates* (Formosa) and *P. p. romulus* (Nepal), *Papilio protenor amaura* (Formosa) and *P. p. euprotenor* (Nepal), *Papilio protenor demetrius* (tailed form, Japan) and *P. p. amaura* (tailless form, Formosa), *Papilio bianor takasago* (Formosa) and *P. b. dehaanii* (Japan), *Papilio xuthus koxinga* (Formosa) and *P. xuthus* (Japan), *Papilio memnon heronus* (Formosa) and *P. m. thunbergii* (Japan).

The timing of male meiosis in relation to the course of phylogeny of Papilionidae is of particular interest to us. Generally, the meiosis cannot be observed in the imaginal stage of the heterocerous species so far studied. It is interesting to note that spermatocyte divisions were present in the imagines of *Byasa polyeuctes letincius* (Nepal), *Byasa latreillei latreillei* (Nepal), *Byasa febanus* (Formosa), and *Graphium phaon* (U.S.A.) and many other butterflies. On the other hand, all the species examined of *Parnassius, Luehdorfia, Graphium doson, Graphium sarpedon,* and *Byasa alcinous* finish the spermatogenesis before imaginal emergence as in the case of ordinary moths. In view of these facts, it seems probable that *Byasa* and *Graphium* occupy an intermediate position between the *Parnassius-Luehdorfia* and *Troides-Papilio* type in Papilionidae.

Two species belonging to *Suastus* and *Telicata* have been studied cytologically for the first time, and *Suastus gremius* is remarkable in having 23 chromosomes. This number was unknown in Hesperiidae. Two subspecies of *Daimio tethys*—— *D. t. tethys* (Japan) and *D. t. moorei* (Formosa), were both found to have 30 chromosomes. Also, the karyotype of *Polytremis lubricans* (*n*, 16) is remarkably similar to that of *Polytremis pellucida* from Japan in having a characteristic karyotype with 1 small element. In two species of *Bibasis, Bibasis jaina* has 31 chromosomes, in which 16 are larger and 3 are smaller than the remaining 12 chromosomes, while *Bibasis aquilina* from Japan has 29 chromosomes, which are the same in size.

Summary

1. The chromosome numbers and their sizes are reported of 8 species of Papilionidae and 5 species of Hesperiidae from Formosa. Among these species, 7 species were cytologically studied for the first time. The numerical and morphological features of their chromosomes are given in Table 1 and Figures 1–27. All the chromosome counts were carried out on the basis of the spermatocytes.

2. Spermatocyte divisions in the imaginal stage were present in *Byasa febanus.* In contrast, *Byasa alcinous* of Japan practically completes meiosis prior to eclosion like *Parnassius, Luehdorfia, Graphium doson, Graphium sarpedon* and Heterocera.

3. The karyotype of *Troides aeacus kaguya* (*n*, 31) from Formosa differs from that of the Himalayan *T. aeacus aeacus* (*n*, 30).

4. *Papilio castor formosanus* has an unusual number of chromosomes (*n*, 31), being exceptional as a member of *Papilio*.

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