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A CHROMOSOMAL STUDY OF SEVENTEEN SPECIES OF BUTTERFLIES FROM HONG KONG (Lepidoptera, Rhopalocera)

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In recent years entomological expeditions have been undertaken by Japanese researchers to various countries in Asia: for example, to Formosa, Nepal, Borneo, Malay, Hong Kong and Korea. So far no information has been available on the chromosomes of lepidopterous insects of Hong Kong. This paper is an initial attempt to contribute to the chromosomal study of butterflies of the Chinese Continent.

All the species studied here were collected and fixed locally by Ae during his trip in April, 1965, under the Japan-U. S. Cooperative Science Program. As a fixative, only Allen's P. F. A.-3 solution was used. The sections, 10 micra in thickness, were made by 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 diameters. The photomicrographs were taken by means of a MIKAS camera. In addition to the 17 species dealt with in this paper, examinations were conducted of *Graphium antiphates antiphates* Cramer, *Pieris rapae crucivora* Boisduval and *Astictopterus jama chinensis* Leech but, with no fruitful findings.

Acknowledgement is made here of partial financial support for this investigation through a grant from the Japan Society for the Promotion of Science as part of the Japan-U. S. Cooperative Science Program. The authors are also grateful to Dr. T. Shirôzu, Kyushu University, who also took part in the trip, for identifying the collected specimens and to Mr. T. Takakura for helping in writing this paper.

Observations

A. PAPILIONIDAE

1. *Menelaides aristolochiae goniopeltis* Rothschild

The haploid chromosome number is 29, on the basis of counts made in 15 nuclei of the primary spermatocyte division in the testes of 3 males collected at Sai Tam Reservoirs in Hong Kong Island on April 23 (F-203, F-204, F-205).

No secondary spermatocyte divisions were found. These 15 nuclei show exactly 29 elements without exception. Distinctly, one chromosome is larger than the others.

B. NYMPHALIDAE

2. *Cupha erymanthis erymanthis* Drury

The haploid chromosome number is 31, on the basis of counts made in 10 nuclei (I) and 8 nuclei (II) in the testis of single male collected at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 21 (F-195). There are 4 small and 27 large chromosomes in the haploid set.

3. *Precis atlites atlites* Linnaeus

The haploid chromosome number is 31, based on counts made in 10 nuclei (I) in the testes of single male collected on April 22 at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories (F-197a, F-197b, usually all species belonging to genus *Precis* have 2 testes in the imaginal stage). Apparently 5 of the chromosomes are smaller than the others.

4. *Precis lemonias lemonias* Linnaeus

The haploid chromosome number is 31, from counts made in 10 nuclei (I) and 5 nuclei (II) from a male collected at Tai Tam Reservoirs in Hong Kong Island on April 23 (F-208). There are 2 huge, 15 large and 14 small chromosomes in the haploid complement.

5. *Neptis columella columella* Cramer

The haploid chromosome number is 30. Counts were made in 8 nuclei (I) in testis of 1 male collected at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 22 (F-198). There are 16 small, 13 large and 1 huge chromosomes in the haploid complement.

6. *Tacoraea nefte seitzi* Fruhstorfer

The haploid chromosome number is 30, from counts made in 10 nuclei (I) and 8 nuclei (II) from a single male obtained at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 21 (F-183). There are distinctly 25 large and 5 somewhat small in chromosomal size.

7. *Charaxes polyxena polyxena* Cramer

The haploid chromosome number is 25, from counts made in 18 nuclei (I) and 12 nuclei (II) from testes of 2 males collected at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 22 (F-194, F-199). Apparently 5 of the chromosomes are larger than the other chromosomes.

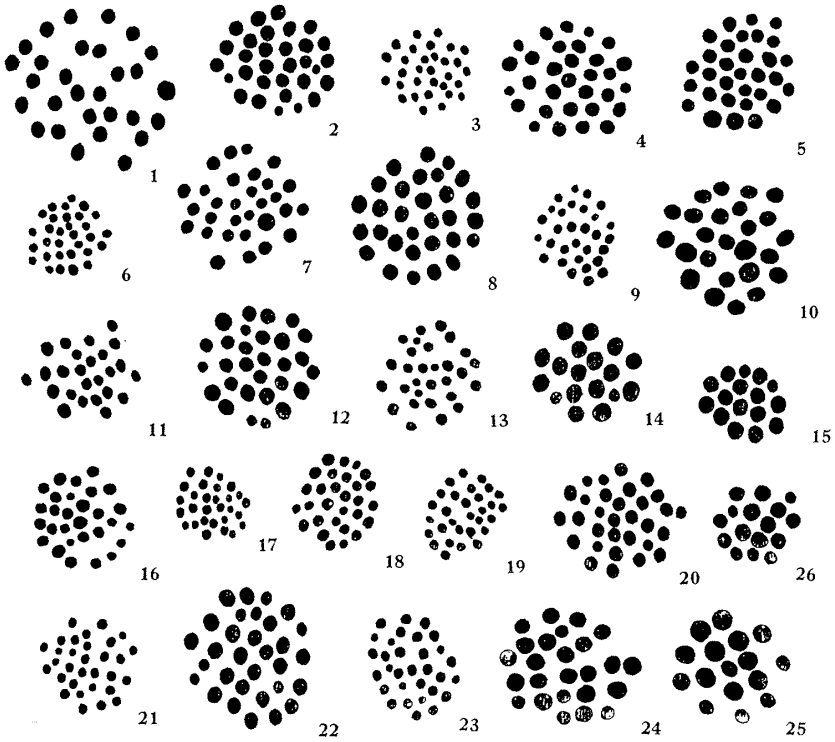

Fig. 1: *Menelaides aristolochiae goniopeltis* (I). Fig. 2: *Cupha erymanthis erymanthis* (I). Fig. 3: same (II). Fig. 4: *Precis atlites atlites* (I). Fig. 5: *Precis lemonias lemonias* (I). Fig. 6: same (II). Fig. 7: *Neptis columella columella* (I). Fig. 8: *Tacoraea nefte seitzi* (I). Fig. 9: same (II). Fig. 10: *Charaxes polyxena polyxena* (I). Fig. 11: same (II). Fig. 12: *Faunis eumeus eumeus* (I). Fig. 13: same (II). Fig. 14: *Mycalesis mineus mineus* (I). Fig. 15: *Mycalesis horsfieldii panthaka* (I). Fig. 16: *Lethe europa beroe* (I). Fig. 17: same (II). Fig. 18: *Lethe confusa apara* (I). Fig. 19: *Ypthima baldus baldus* (II). Fig. 20: *Abisara echerius echerius* (I). Fig. 21: same (II). Fig. 22: *Zemeros flegyas flegyas* (I). Fig. 23: same (II). Fig. 24: *Celastrina puspa barneyi* (I). Fig. 25: *Pelopidas conjuncta conjuncta* (I). Fig. 26: same (II).

C. AMATHUSIIDAE

8. *Faunis eumeus eumeus* Drury

The haploid chromosome number is 29, based on counts made in 15 nuclei (I) and 6 nuclei (II) from testes of 2 males obtained at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 21-22 (F-182, F-192). There are 8 large, 4 small and others medium in chromosomal size.

D. SATYRIDAE

9. *Mycalesis mineus mineus* Linnaeus

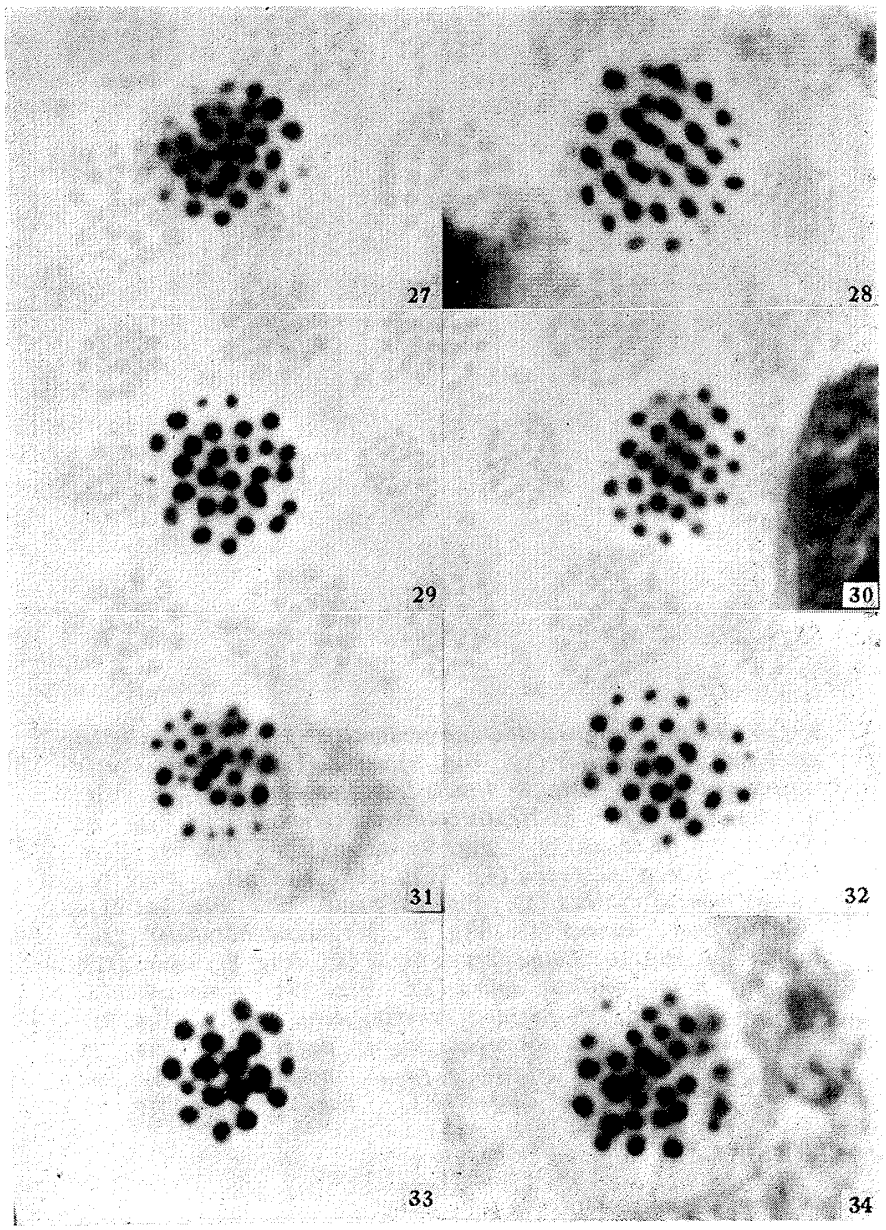

Fig. 27. *Precis lemonias* (I).
Fig. 28. *Tacoraea nefte* (I).
Fig. 29. *Faunis eumeus* (I).
Fig. 30. *Cupha erymanthis* (I).
Fig. 31. *Lethe europa* (I).
Fig. 32. *Precis atlites* (I).
Fig. 33. *Mycalesis mineus* (I).
Fig. 34. *Neptis columella* (I).

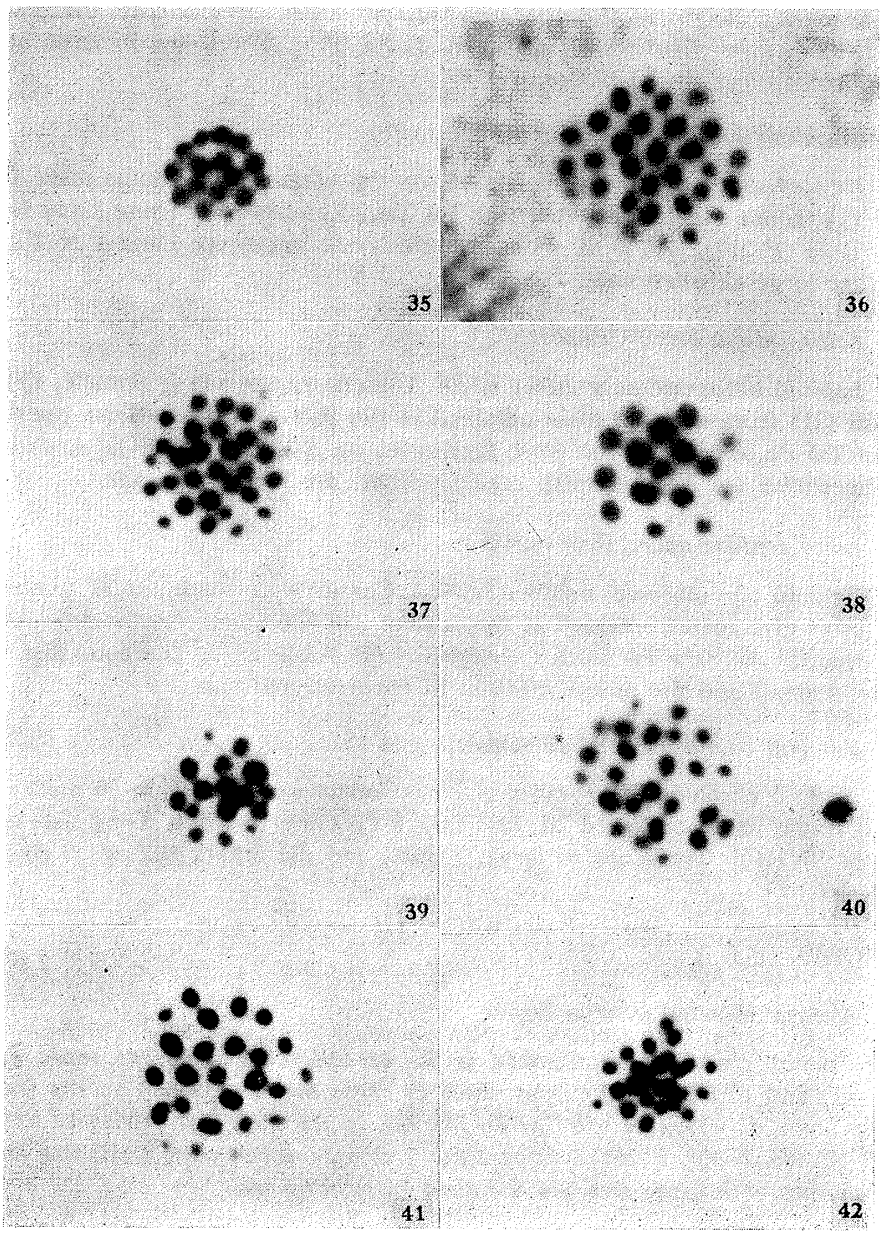

Fig. 35. *Mycalesis horsfieldii* (I).
Fig. 36. *Zemeros flegyas* (I).
Fig. 37. *Abisara echerius* (I).
Fig. 38. *Pelopidas conjuncta* (I).
Fig. 39. same (II).
Fig. 40. *Celastrina puspa* (I).
Fig. 41. *Charaxes polyxena* (I).
Fig. 42. same (II).

The haploid chromosome number is 18, from counts made in 6 nuclei (I) from a single male collected at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 21 (F-191). There are 16 large and 2 small chromosomes in the haploid set.

10. *Mycalesis horsfieldii panthaka* Fruhstorfer

The haploid chromosome number is 16, on the basis of the counts made in 10 nuclei (I) from a single male taken at Tai Tam Reservoirs in Hong Kong Island on April 23 (F-210). As with above *Mycalesis*, the karyotype consist of 2 small and other large chromosomes.

11. *Lethe europa beroe* Cramer

The haploid chromosome number is 29. Counts were made in 6 nuclei (I) and 3 nuclei (II) from a single male obtained at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 21 (F-184). Apparently 8 chromosomes are large, 6 small and the others are medium in size.

12. *Lethe confusa apara* Fruhstorfer

The haploid chromosome number is 29. Counts were made in 10 nuclei (I) from testes of 2 males obtained at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 21 (F-186, F-187). There are distinctly 9 large, 6 small and the others medium in chromosomal size.

13. *Ypthima baldus baldus* Fabricius

The haploid chromosome number is 29. Counts were made in 10 nuclei (II) from a single male collected at Tai Tam Reservoirs in Hong Kong Island on April 23 (F-211). There are 10 large, 2 small and the others medium in chromosomal size.

E. RIODINIDAE

14. *Abisara echerius echerius* Stoll.

The haploid chromosome number is 31, on the basis of counts made in 18 nuclei (I) and 10 nuclei (II) from testes of three males obtained at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 21 and 22 (F-188, F-201, F-202). Apparently 7 chromosomes with small size and 24 chromosomes with large size are observed in haploid set.

15. *Zemeros flegyas flegyas* Cramer

The haploid chromosome number is 31, on the basis of counts made in 15 nuclei (I) and 7 nuclei (II) from a single male collected at Sai-Kung in the New Territories on April 21 (F-185). The karyotype of *Z. flegyas* is quite similar to that of above *A. echerius*, there are distinctly 24 large and 7 small chromosomes in haploid complement.

F. LYCAENIDAE

16. *Celastrina puspa barneyi* Corbet

The haploid chromosome number is 25, based on counts made in 5 nuclei (I) from a single obtained at the foot of Tai Mo Shan, overlooking Tai Po Market, in the New Territories on April 22] (F-193). There are 9 large, 5 small and the others medium in chromosomal size.

G. HESPERIIDAE

17. *Pelopidas conjuncta conjuncta* Herrich-Schaffer

The haploid chromosome number is 16, based on counts made in 6 nuclei (I) and 7 nuclei (II) from a single male obtained at the foot of Tai Mo Shan, overlooking the Tai Po Market, in the New Territories on April 21 (F-189). There are distinctly 11 large and 5 somewhat small in chromosomal size.

Discussion

Table 1 shows the chromosome numbers of 17 species of butterflies from Hong Kong which the present paper deals with. All these species have furnished new material for cytology.

A previous report (Maeki & Remington '60) shows that the chromosome number in Papilionidae is consistently *n*, 30, except for several species. *Menelaides aristolochiae* has a karyotype of 29 chromosomes, which include a huge element. It is noteworthy that *M. aristolochiae* is an unusual species: firstly, in view of its karyotype in contrast to a number of other species in Papilionidae, and secondly, from a viewpoint of the male genitalia, which are extremely different from those of other genera related to *Menelaides*, namely *Byasa*, *Atrophaneura* or *Troides*, as Dr. T. Shirôzu pointed out (Shirôzu, 1960). Among a number of species of Papilionidae so far studied cytologically, *n*, 29 chromosomes are only known in *Parnassius mnemosyne*, examined by Federley ('38) in Finland. But it is evident that these two species are not in any way closely related. The unusual karyotype of *M. aristolochiae* has probably derived from one fusion of two ancestral chromosomes of the same size, in the course of evolution.

Five species of genus *Precis*, namely *P. almana*, *P. orithya*, *P. iphita*, *P. atlites* and *P. lemonias* are characterized by having *n*, 31 chromosomes. However, the above species show some difference in their karyotype. *P. atlites* has 5 chromosomes smaller than the others and *P. lemonias* has 2 large, 15 medium and 14 small chromosomes. These newly known karyotypes of *P. atlites* and *P. lemonias* differ from those of the previously known karyotypes of the other three species. *P. almana* of Formosa has 2 smaller chromosomes (Maeki, Ogata & Shirôzu, 1965), and that of Japan has 31 chromosomes of the same size (Maeki, 1960) but 2 of them may be a little smaller than the others. *P. orithya* has 4 smaller chromosomes and *P. iphita* has 5 small, 14 medium and 12 large chromosomes (Maeki, Ogata & Shirôzu, 1965). These differences in the size of chromosomes may be the result of chromosomal mutations during the speciations in the genus *Precis*.

Table 1. *The species studied and their chromosome numbers.*

Species	Chrom.-No. (*n*)
A. Papilionidae	
1. *Menelaides aristolochiae goniopeltis* Rothschild	29 (I)
B. Nymphalidae	
2. *Cupha erymanthis erymanthis* Drury	31 (I, II)
3. *Precis atlites atlites* Linnaeus	31 (I)
4. *Precis lemonias lemonias* Linnaeus	31 (I, II)
5. *Neptis columella columella* Cramer	30 (I)
6. *Tacoraea nefte seitzi* Fruhstorfer	30 (I, II)
7. *Charaxes polyxena polyxena* Cramer	25 (I, II)
C. Amathusiidae	
8. *Faunis eumeus eumeus* Drury	29 (I, II)
D. Satyridae	
9. *Mycalesis mineus mineus* Linnaeus	18 (I)
10. *Mycalesis horsfieldii panthaka* Fruhstorfer	16 (I)
11. *Lethe europa beroe* Cramer	29 (I, II)
12. *Lethe confusa apara* Fruhstorfer	29 (I)
13. *Ypthima baldus baldus* Fabricius	29 (II)
E. Riodinidae	
14. *Abisara echerius echerius* Stoll.	31 (I, II)
15. *Zemeros flegyas flegyas* Cramer	31 (I, II)
F. Lycaenidae	
16. *Celastrina puspa barneyi* Corbet	25 (I)
G. Hesperiidae	
17. *Pelopidas conjuncta conjuncta* Herrich-Schaffer	16 (I, II)

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

Butterflies belonging to the genus *Neptis* probably have a common characteristic number of *n*, 30, as already confirmed in 4 species (*N. philyra, N. pryeri, N. aceris* and *N. hylas*), and the newly examined *Neptis columella* also has 30 chromosomes in the haploid set. The karyotype of 25 chromosomes in *Charaxes polyxena* which include 5 large elements is unique in Nymphalidae. The existence of these unusually large chromosomes in *C. polyxena* strongly suggests the reduction in the chromosome number due to the fusion of 10 of the ancestral elements into the 5 large chromosomes.

The chromosomes of Satyridae are remarkable in that they show a wide numerical range (*n*, 8, 10, 11, 12, 14, 15, 17, 19, 21, 22, 23, 24, 25, 27, 28, 29, 30, 32, 40, 51), which is the characteristic of the family. Our discovery of $n=18$ in *Mycalesis mineus* and $n=16$ in *Mycalesis horsfieldii* is important, in view of the fact that they were lacking in the above series of various chromosome numbers in Satyridae. It was found that *Lethe europa* and *L. confusa* have a uniform karyotype with a constant number of chromosomes, which include 8-9 large and 6 small elements as in the cases of the previously reported *L. diana* of Japan (Maeki, 1953) and *L. rohria* of Formosa (Maeki, Ogata & Shirôzu, 1965). The chro-

mosome number of *Ypthima baldus* (*n*, 29) of Hong Kong is the same as that of *Y. argus* (*n*, 29) of Japan, but differs from that of the Japanese *Y. motschulskyi* (*n*, 27).

Calephelis virginiensis of North America has *n*, 45 and 9 chromosomes are distinctly larger than the others (Maeki & Remington, 1961a). That was the only example of known chromosome number in Riodinidae. The two species belonging to Riodinidae of Hong Kong have both *n*, 31 and 24 chromosomes are larger than the remaining 7 chromosomes. It is difficult to compare these two different karyotypes, but if 15 large chromosomes of the Hong Kong Riodinidae are presumed to be the result of the fusion of 30 small chromosomes of the North American *C. virginiensis*, the difference between these two karyotypes only concerns a shortage of one small chromosome in *C. virginiensis*.

In *Celastrina argiolus* of Europe and Japan, the $n=25$ chromosomes are reported by Federley ('38), Lorković ('41), Maeki and Makino ('53). *C. puspa* reported in this paper has $n=25$ chromosomes including 9 large and 5 small elements. The chromosome number of *Pelopidas conjuncta* (*n*, 16) was the same as that of *P. mathias* of Japan, which until then was the only known number of chromosomes in *Pelopidas*.

On the basis of relative numbers and sizes of chromosomes in related species of butterflies studied by many researchers (i. e. Beliajeff '30, Federley '38, Gupta '64, de Lesse '60, Lorković '41, Maeki '53, Maeki and Remington '60, Maeki, Ogata and Shirôzu '65, Maeki and Ae '66), it seems to be reasonable that a variation in the chromosome number of related lepidopterous insects is caused by fusion and fragmentary disappearance of ancestral chromosomes.

Summary

1. In this report, the numbers and relative sizes of chromosomes are described of 1 species of Papilionidae, 6 species of Nymphalidae, 1 species of Amathusiidae, 5 species of Satyridae, 2 species of Riodinidae, 1 species of Lycaenidae and 1 species of Hesperiidae, all from Hong Kong.

2. All species here considered have furnished new material for cytology. It is noteworthy that *Menelaides aristolochiae* has *n*, 29, *Charaxes polyxena n*, 25, and two species of Riodinidae, *n*, 31.

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Federley, H., 1938. Chromosomenzahlen finnlandischer Lepidopteren. I. Rhopalocera. Hereditas 24: 397-464, 47 figs.

de Lesse, H. 1960. Speciation et variation chromosomique chez les Lepidopteres, Rhopaloceres. Ann. Sci. Nat. Zool. Biol. Anim. (ser. 12) 2: 1-223, 222 figs.

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—— 1959. A chromosomal study in fifteen species of the Japanese Pieridae. Kwansei Gakuin University Annual Studies 7: 361-168, 32 figs.
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Maeki, K., & C. L. Remington, 1960a. Studies of the chromosomes of North American Rhopalocera. 1. Papilionidae, Journ. Lepid. Soc. 13: 193-203, 2 pls., 7 figs.
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Makino, S., 1956. A review of the chromosome numbers in animals. rev. ed. 300 pp. Hokuryukan, Tokyo.
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先島諸島の原尾目

今立源太良・鎌田昭次・砂川毓雄

先に今立・西平によつて，宮古，西表両島から，*Eosentomon sakura*，石垣島から *E. kumei* が報告されているが（昆虫，29：234，31：26），最近あらたに次のような記録がえられたので紹介しておく．

1. *Eosentomon sakura* Imadaté et Yosii カマアシムシ
石垣市開南，1 mature junior, 4-VIII-1967, 砂川採集．分布：台湾～北海道．

2. *E. udagawai* Imadaté
平良市福山，2♂1♀, 14-VIII-1967, 砂川採集．四国・南九州から未記録のこの種が宮古島から発見されたのは，分布圏が似ている *E. asahi* が沖縄本島から記録されている（昆虫，29：234）のと共に，きわめて興味深い．この系列の種の最南端の記録である．毛序は馬場ほか（昆虫，35：64）の西日本型に一致する．分布：宮古島，九州，本州，北海道．

3. *Berberentulus tosanus tosanus* (Imadaté et Yosii) トサカマアシムシ
西表島いなば，1♀, 14-III-1967, 鎌田採集．沖縄列島からクシカマアシ亜目の種が見出されたのは，これが最初の例である．鹿児島県開聞岳以北，新潟県村上以南の各地に普通．亜種を異にする *B. tosanus babai* Imadaté が台湾に，近縁の種が東南アジア各地に分布している．分布：西表島，九州，四国，本州．