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STUDIES OF THE CHROMOSOMES OF FORMOSAN RHOPALOCERA

4. Danaidae and Satyridae

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The three previous papers of this series described the results of chromosomal investigation of 8 species of Papilionidae, 5 species of Hesperiidae, 8 species of Pieridae, 6 species of Lycaenidae, 1 species of Riodinidae, 10 species of Nymphalidae and 1 species of Libytheidae in Formosan butterflies which were collected by Japanese entomologists in a trip during the period from March to April, 1965, as part of Japan-U. S. Co-operative Science Programs. The present report deals with the chromosomes observed in male germ cells of both 8 species of Danaidae and Satyridae as the fourth serial paper on chromosomal survey of Formosan Rhopalocera.

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 5 species: *Ypthima baldus zodina* Fruhstorfer, *Palaeonympha opalina macrophthalmia* Fruhstorfer, *Lethe dura neoclides* Fruhstorfer, *Neope pulaha didia* Fruhstorfer, and *Neope armandii lacticolora* (Fruhstorfer), but failed to obtain satisfactory results, because the materials were inadequate for chromosome research.

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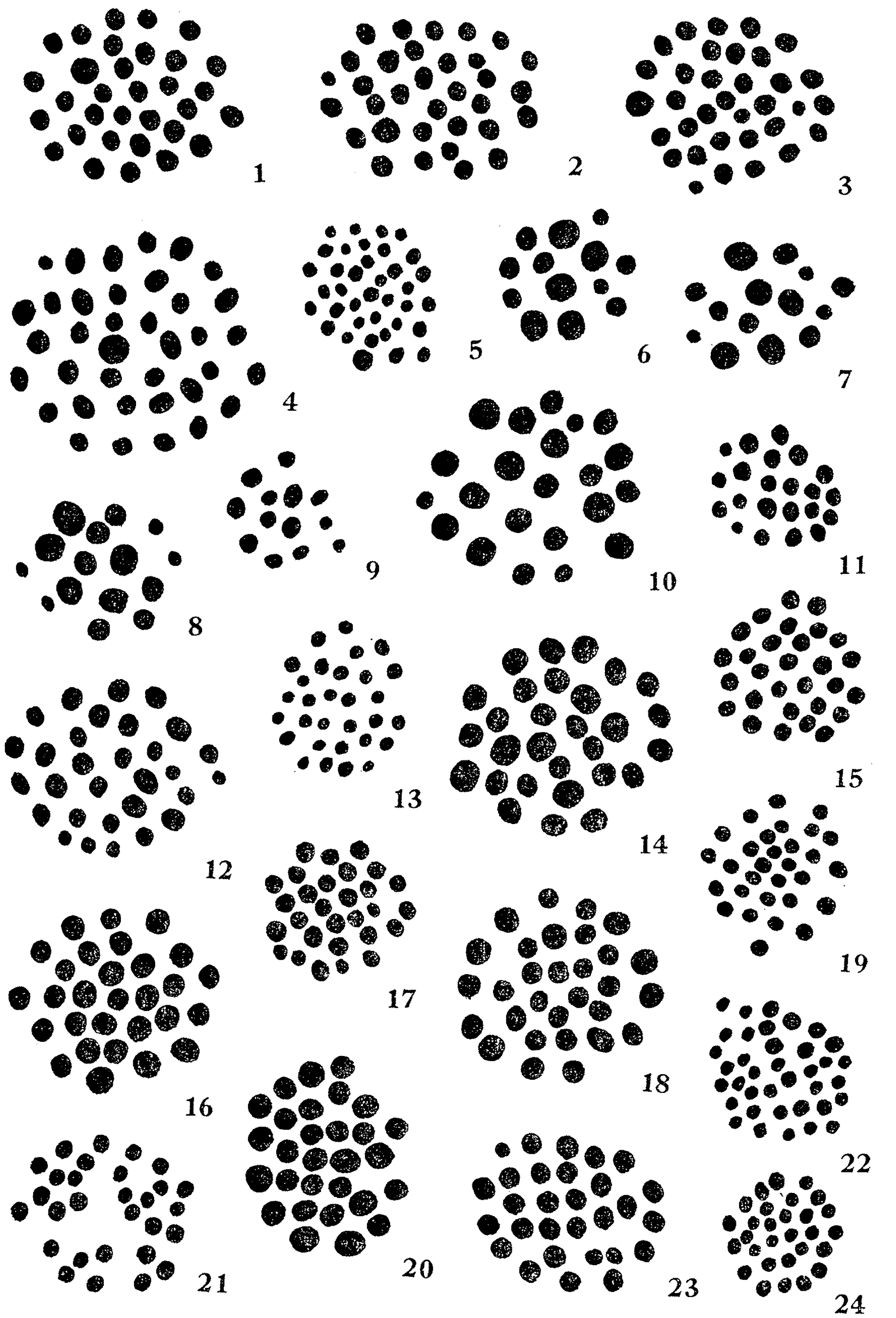
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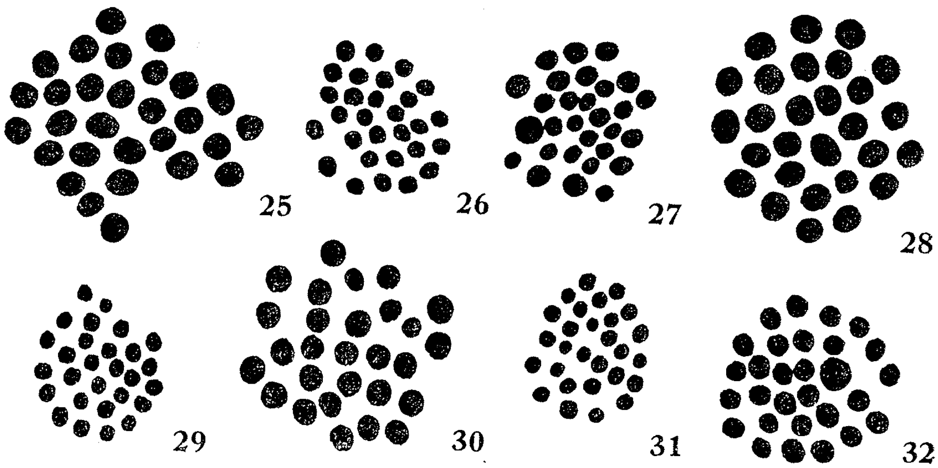
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Fig. 1: *Tirumata hamata septentrionis* (I, n=32). Fig. 2: same (I, n=33). Fig. 3: same (I, n=34). Fig. 4: *Tirumata limniace limniace* (I). Fig. 5: same (II). Fig. 6: *Radena similis similis* (I,n=13). Fig. 7: same (I, n=14). Fig. 8: same (I, n=15). Fig. 9: same (II, n=13). Fig. 10: *Parantica melaneus swinhoei* (I). Fig. 11: same (II). Fig. 12: *Idea leuconoe clara* (I). Fig. 13: same (II). Fig. 14: *Euploea sylvestor swinhoei* (I). Fig. 15: same (II). Fig. 16: *Euploea tulliolus koxinga* (I). Fig. 17: same (II). Fig. 18: *Euploea leucostictos hobsoni* (I). Fig. 19: same (II). Fig. 20: *Ypthima multistriata* (I). Fig. 21: same (II). Fig. 22: *Ypthima perfecta akragas* (II). Fig. 23: *Lethe europa pavida* (I). Fig. 24: same (II).

Fig. 25: *Lethe mataja* (I). Fig. 26: same (II). Fig. 27: *Lethe insana formosana* (I). Fig. 28: *Lethe europa pavida* (I). Fig. 29: same (II). Fig. 30: *Mycalesis francisca formosana* (I). Fig. 31: same (II). Fig. 32: *Mycalesis horsfieldi panthaka* (I).

Observations

A. DANAIDAE

1. *Tirumata hamata septentrionis* (Butler)

The haploid chromosome numbers are 32, 33 and 34. The following nuclei were counted in the first meiosis in the testes of two males taken at Fen-ch'i-hu, Chia-i Province (F-140) on April 12 and at Chu-ch'i (F-144) on April 13.

First metaphase	
n, 32	10 nuclei
n, 33	7 nuclei
n, 34	2 nuclei
Total	19 nuclei

The basic number of chromosome in this species seems to be 32. There are 1 large, 1 small chromosomes and the others are medium in size. The cause of the numerical variation lies in the presence of the supernumerary chromosome. The supernumeraries vary from 1 to 2 in number and are characterized by a minute element. The nuclei having 32 chromosomes are most frequent, occupying 53 % in 19 nuclei observed.

2. *Tirumata limniace limniace* (Cramer)

The haploid chromosome number is 37. Counts were made in 10 nuclei (I) and 6 nuclei (II) of the testes of one male (F-29) taken at K'en-ting Park, P'ing-tung Province on April 2. There are 1 huge, 13 large, 1 small chromosomes and others are medium in size.

3. *Radena similis similis* (Linnaeus)

The haploid chromosome numbers are 13, 14 and 15 in the first meiosis and conventional 13 in the second meiosis. As a result of counting, 4 nuclei had *n*, 13 (I), 10 nuclei *n*, 14 (I), 7 nuclei *n*, 15 (I) and 4 nuclei had *n*, 13 (II) in the testes from one male (F-7) taken at Mt. Yang-ming, T'ai-pei Province on March 28. The chromosomes of *n*, 13 nuclei consist of 5 huge, 6 large and 2 small elements. The *n*, 14 and *n*, 15 nuclei are composed of additional 1 or 2 supernumerary chromosomes and the *n*, 13 nucleus. The supernumeraries vary from 1 to 2 in number.

4. *Parantica melaneus swinhoei* (Moore)

The haploid chromosome number is 22. Counts were made in 10 nuclei (I) and 6 nuclei (II) in the testes of 2 males taken at K'en-ting Park, P'ing-tung Province (F-57) on April 4 and at Ch'i-hsin-liao, Chia-i Province (F-168) on April 15. There are 3 first largest, 6 second largest, 3 third largest chromosomes, and 3 are smaller than the rest.

5. *Idea leuconoë clara* (Butler)

The haploid chromosome number is 30. Counts were made in 15 nuclei (I) and 5 nuclei (II) in the testes of 3 males taken at K'en-ting Park, P'ing-tung Province (F-19, F-55, F-56) on April 2 and 4. There are 13 large, 5 small chromosomes and the others are medium in size. Many dividing cells were observed in both first and second meiosis.

6. *Euploea sylvestor swinhoei* Wallace

The haploid chromosome number is 29. Counts were made in 10 nuclei (I) and 5 nuclei (II) of the testes of 4 males taken at K'en-ting Park, P'ing-tung Province (F-37) on April 3 and at Ch'i-hsin-liao, Chia-i Province (F-163, F-165, F-167) on April 15. Six chromosomes are apparently larger than the others.

7. *Euploea tulliolus koxinga* Fruhstorfer

The haploid chromosome number is 29. Counts were made in 10 nuclei (I) and 6 nuclei (II) of the testes of 3 males taken at K'en-ting Park, P'ing-tung

Province (F-43, F-54) on April 3 and 4 and at Ch'i-hsin-liao, Chia-i Province (F-166) on April 15. There are 4 first largest, 6 second largest, 15 medium and 4 small chromosomes. Many dividing cells were observed in both primary and secondary spermatocytes.

8. *Euploea leucostictos hobsoni* (Butler)

The haploid chromosome number is 30. Counts were made in 20 nuclei (I, II) of the testes of one male (F-96) taken at Kuan-tzu-ling, T'ai-nan Province on April 7. Eight chromosomes are somewhat larger than the others.

B. SATYRIDAE

1. *Ypthima multistriata* Butler

The haploid chromosome number is 29. Counts were made in 10 nuclei (I) and 8 nuclei (II) of the testes from seven males taken at Mt. Yang-ming, T'ai-pei Province on March 28 (F-8) and at Kuan-tzu-ling, T'ai-nan Province on April 6 (F-66, F-79) and at Fen-ch'i-hu, Chia-i Province on April 10-12 (F-118, F-134) and at Ch'i-hsin-liao, Chia-i Province on April 14 (F-150, F-152). The specimens showed many dividing cells in both first and second meiosis. Eight chromosomes are larger than the others.

2. *Ypthima perfecta akragas* Fruhstorfer

The haploid chromosome number is 37. Counts were made in 3 nuclei of the second meiosis of the testes from a single male (F-135) taken at Fen-ch'i-hu, Chia-i Province on April 12. There are 10 large chromosomes and the others are small. This is a remarkably large number of chromosomes among the Satyrid species. No first meiotic divisions were observed in this specimen.

3. *Lethe europa pavida* Fruhstorfer

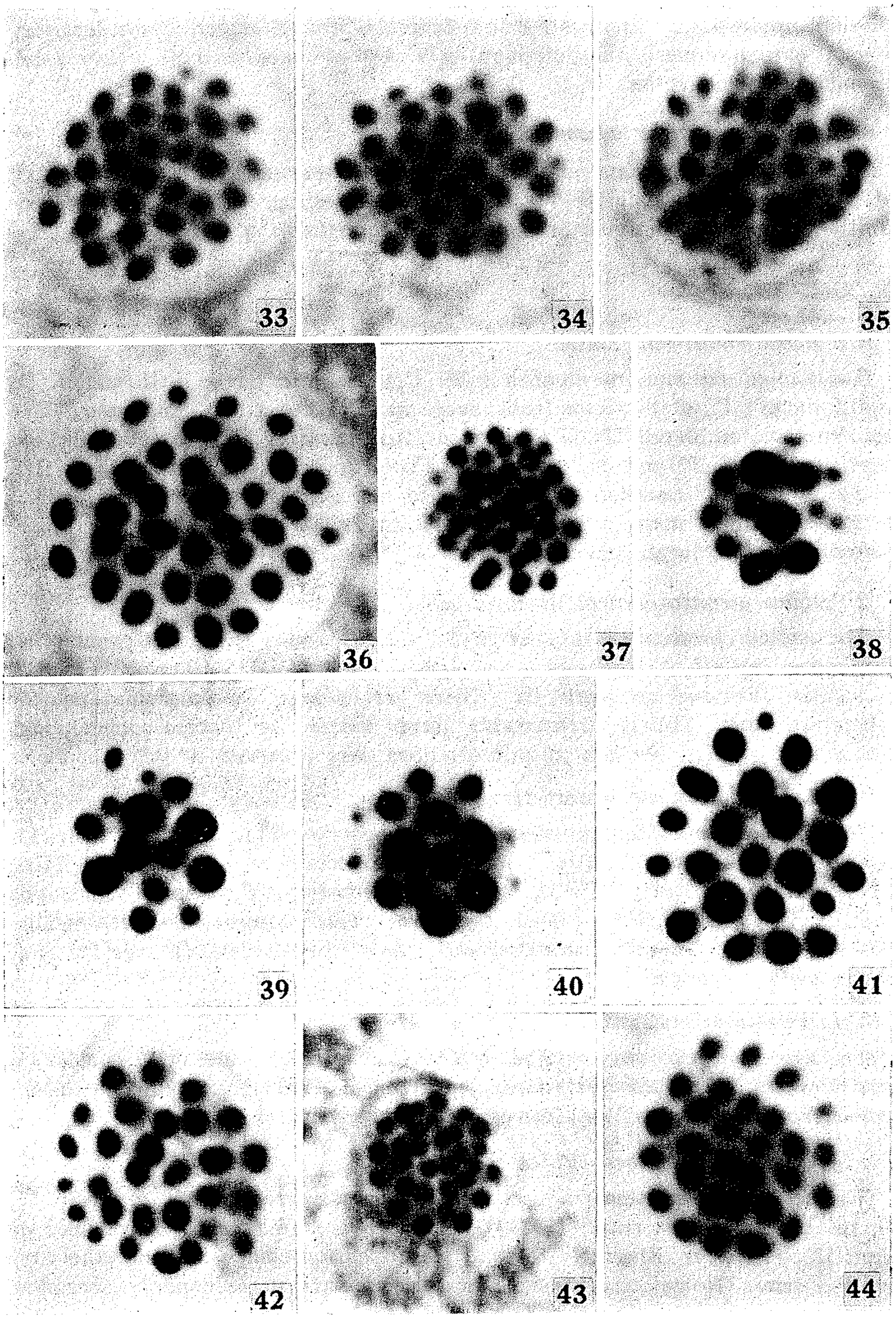
The haploid chromosome number is 29. Counts were made in 16 nuclei (I) and 10 nuclei (II) of the testes from 4 males collected at K'en-ting Park, T'ai-pei Province on April 2 (F-20) and at Kuan-tzu-ling, T'ai-nan Province on April 7 (F-92, F-98a, F-99). Distinctly three chromosomes are much smaller than the others. These specimens showed many dividing cells at the first and second meiotic periods.

4. *Lethe mataja* Fruhstorfer

The haploid chromosome number is 29. Counts were made in 10 nuclei (I) and 10 nuclei (II) of the testes from a single male (F-116) taken at Fen-ch'i-hu, Chia-i Province on April 10. All are large chromosomes.

5. *Lethe insana formosana* Fruhstorfer

The haploid chromosome number is 29. Counts were made in 5 nuclei (I) of the testes from one male (F-139) taken at Fen-ch'i-hu, Chia-i Province on April 12. There are distinctly 1 big, 11 large chromosomes and the others are small in size. No meiotic divisions were observed in the secondary spermatocytes.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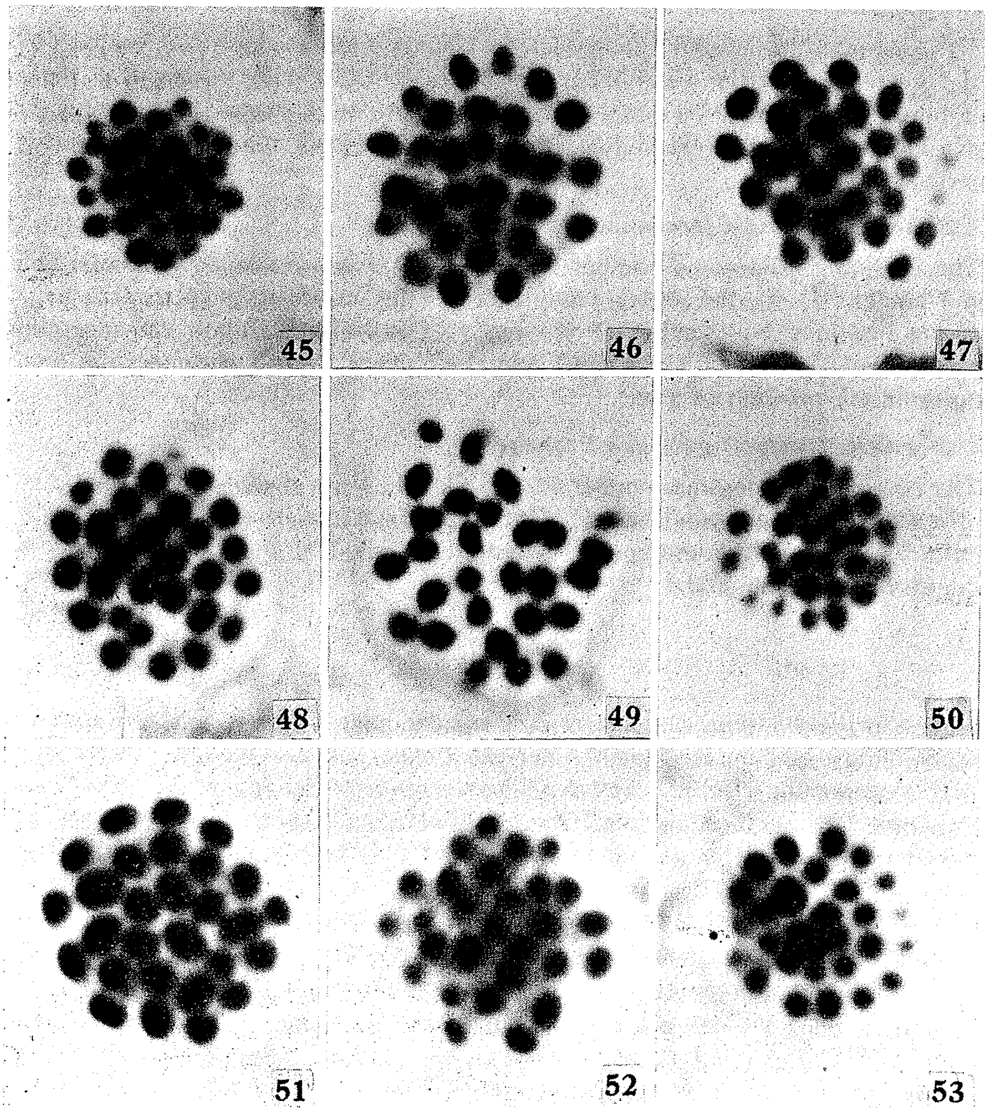

Fig. 33: *Tirumata hamata septentrionis* (I, n=32). Fig. 34: same (I, n=33). Fig. 35: same (I, n=34). Fig. 36: *Tirumata limniace limniace* (I). Fig. 37: same (II). Fig. 38: *Radena similis similis* (I, n=13). Fig. 39: same (I, n=14). Fig. 40: same (I, n=15). Fig. 41: *Parantica melaneus swinhoei* (I). Fig. 42: *Idea leuconoë clara* (I). Fig. 43: same (II). Fig. 44: *Euploea sylvestor swinhoei* (I). Fig. 45: *Euploea tulliolus koxinga* (II). Fig. 46: *Euploea leucostictos hobsoni* (I). Fig. 47: *Ypthima multistriata* (I). Fig. 48: *Lethe europa pavida* (I). Fig. 49: *Lethe mataja* (I). Fig. 50: same (II). Fig. 51: *Lethe verma cintamani* (I). Fig. 52: *Mycalesis francisca formosana* (I). Fig. 53: *Mycalesis horsfieldi panthaka* (I).

6. *Lethe verma cintamani* Fruhstorfer

The haploid chromosome number is 28. Counts were made in 10 nuclei (I) and 5 nuclei (II) of the testes from two males (F-130, F-133) collected at Fen-ch'i-hu, Chia-i Province on April 12. Apparently one chromosome is small in size. These specimens showed many dividing cells at the first and second meiotic periods.

7. *Mycalesis francisca formosana* Fruhstorfer

The haploid chromosome number is 28. Counts were made in 10 nuclei (I) and 7 nuclei (II) of the testes from three males collected at Kuan-tzu-ling, T'ai-nan Province on April 6 (F-68) and at Fen-ch'i-hu, Chia-i Province on April 10 (F-117, F-120). There are distinctly 14 large, 2 small chromosomes and the others are medium in size.

8. *Mycalesis horsfieldi panthaka* Fruhstorfer

The haploid chromosome number is 29. Counts were made in 8 nuclei (I) of the testes from a single male (F-173) taken at Ch'i-hsin-liao, Chia-i Province on April 15. One chromosome is apparently big, and the others are about the same in chromosomal size.

Discussion

Uutil the present time, six species of Danaidae have appeared in the literature so far published: they are North American *Danaus gilippus* having *n*, 29 (Maeki & Remington '60), North American *Danaus eresimus* having *n*, 30 (Maeki & Remington '60), South American *Ituna lamirus* having *n*, 30 (de Lesse '67), South American *Lycorea cleobaea* having *n*, 30 (de Lesse '67), Japanese *Caduga sita* having *n*, 47 (Maeki '60), and Formosan *Euploea mulciber* having *n*, 29 (Maeki, Ogata, and Shirôzu '65). In this paper 8 species from Formosa are add to the cytologically studied Danaidae by the present authors.

From the cytological point of view, two species are of interest to us, and they are included in above species reported in this paper. They are *Radena similis* (*n*, 13, 14, 15) and *Tirumata hamata* (*n*, 32, 33, 34). These variations of chromosomes are due to the presence of one or two supernumerary chromosomes which are found to occur in the nuclei of *Radena similis* and *Tirumata hamata*. Among the species of Danaidae so far studied cytologically, the supernumerary chromosomes have only been observed in the above species examined in this study. *Euploea tulliolus* (*n*, 29) and *E. mulciber* (*n*, 29) have 10 large, 15 medium and 4 small chromosomes. These two species strongly resemble each other in their karyotypes among the other in studied 4 species of the Formosan *Euploea* (*leucostictos, tulliolus, sylvestor, mulciber*). Also, the karyotype of *Tirumata hamata* (*n*, 32, 33, 34) has a similarity to that of *T. limniace* (*n*, 37) in the characteristic karyotype having 1 huge and 1 small chromosomes, which are much different from the others in size. *Radena similis* has a smaller number of chromosomes (*n*, 13, 14, 15). The karyotypes of first meiosis constantly include 5 huge chromosomes and 2 small chromosomes in *n*, 13 nuclei, or 3 small ones in *n*, 14 nuclei, or 4 small ones in *n*, 15 nuclei exist in addition to the huge elements. The nuclei of the secondary spermatocytes have only *n*, 13 chromosomes and their cytoplasms contain one or two supernumerary chromosomes. Therefore, *Radena similis* of Formosa possesses a basic number of *n*, 13 in the first and second meiotic phases and the numerical variation from *n*, 13 to *n*, 15 at the first meiosis is due to the occurrence of the chromosomal fragmentation. From all these considerations, the above mentioned facts and the fact that all nuclei of secondary spermatocytes possess of *n*, 13 chromosomes, it would seem that the supernumerary chromosome is subject to disappearance through meiosis. In view of the numerical variation of the chromosomes in the two species of *Radena similis* and *Tirumata hamata*, it may be concluded that the species show an

Table 4.* *Chromosome numbers of Formosan Danaidae, and Satyridae.*

Species	Number (*n*)	Phase	Reference
A. Danaidae			
Tirumata hamata septentrionis	32, 33, 34	♂ (I)	Present paper
Tirumata limniace limniace	37	♂ (I, II)	〃
Radena similis similis	13, 14, 15 13	♂ (I) ♂ (II)	〃
Parantica melaneus swinhoei	22	♂ (I, II)	〃
Idea leuconoë clara	30	♂ (I, II)	〃
Euploea sylvestor swinhoei	29	♂ (I, II)	〃
Euploea mulciber barsine	29	♂ (I, II)	Maeki, Ogata, Shirôzu, 1965
Euploea tulliolus koxinga	29	♂ (I, II)	Present paper
Euploea leucostictos hobsoni	30	♂ (I, II)	〃
B. Satyridae			
Ypthima multistriata	29	♂ (I, II)	Present paper
Ypthima perfecta akragas	37	♂ (II)	〃
Lethe europa pavida	29	♂ (I, II)	〃
Lethe rohria daemoniaca	29	♂ (I, II)	Maeki, Ogata, Shirôzu, 1965
Lethe mataja	29	♂ (I, II)	Present paper
Lethe insana formosana	29	♂ (I)	〃
Lethe verma cintamani	28	♂ (I, II)	〃
Neope muirheadi nagasawae	28	♂ (I, II)	Maeki, Ogata, Shirôzu, 1965
Mycalesis francisca formosana	28	♂ (I, II)	Present paper
Mycalesis horsfieldi panthaka	29	♂ (I, II)	〃
Melanitis leda leda	28	♂ (I, II)	Maeki, Ogata, Shirôzu, 1965
Penthema formosanum	28	♂ (I, II)	〃
Elymnias hypermnestra hainana	26	♂ (I, II)	〃

* Tables 1 (Papilionidae, Hesperiidae), 2 (Pieridae, Lycaenidae, Riodinidae) and 3 (Nymphalidae, Libytheidae) are in Parts 1, 2 (Kontyû, 36: 120, 130) and 3 (Kontyû, 37: 96) of this series of papers.

unstable status of the karyotype.

It is an interesting fact that the karyotype of the each species belonging to Danaidae has a wide variety of number and size of chromosome as shown in Table 4 and Figs. 1-19.

Two species of *Ypthima*, *Y. multistriata* (*n*, 29) and *Y. perfecta* (*n*, 37), show some discrepancy in the number of chromosomes. *Y. perfecta* has a karyotype of 37 chromosomes which consist of 10 large and other quite small elements, while, *Y. multistriata* has 29 chromosomes, which include 8 large elements. In the above two species the small elements are much different from each other. The small elements of *Y. perfecta* (*n*, 37) are distinctly smaller than those of *Y. multistriata* (*n*, 29). Four out of five species of genus *Lethe*, namely *L. europa*, *L. rohria*, *L. mataja* and *L. insana* are characterized by having *n*, 29 chromosomes. The remaining *L. verma* exceptionally has *n*, 28 chromosomes, but *L. verma* does not possess huge element, which generally results from the fusion of ancestral chromosomes as observed by several researchers in the Rhopalocera. Dr. T. Shirôzu also pointed out some differences in phallus of the male genitalia in *L. verma* in comparison of those of other species in *Lethe* (Shirôzu, 1960). The above species also show some difference in karyotype. *L. europa* has 3 chromosomes smaller than the others, *L. rohria* has 10 small chromosomes, *L. mataja* does not have small elements, *L. insana* has 7 small elements and *L. verma* has 1 small chromosome in their karyotypes.

In two subspecies of *Mycalesis francisca*, the karyotype of 29 chromosomes in *M. f. perdiccas* (in Japan) include 13 first largest, 14 second largest and 2 small elements, while *M. f. formosana* (in Formosa) reported in this paper has 28 chromosomes, consisting of 14 first largest, 12 second largest and 2 small elements. Their karyotypes are remarkably similar to each other, especially in having the characteristic two small elements. The one first largest chromosome of *formosana* could be the result of a fusion of two second largest chromosomes of *perdiccas*. In view of the numerical variation in the chromosomes in the two subspecies of *M. francisca*, it may be concluded that the species shows a dimorphism of the karyotype due to geographic isolation. The result of the present study has shown a difference in number of chromosomes between the Hong Kong (*n*, 16) and the Formosan subspecies (*n*, 29) of *Mycalesis horsfieldi panthaka*. The Hong Kong butterfly has 2 small and 14 large chromosomes (Maeki & Ae, 1968) and the Formosan subspecies reported in this paper has 1 huge and 28 large chromosomes. These differences are too great to consider as the result of chromosomal mutations within one species. Therefore, more studies are necessary to clarify these differences.

Summary

1. In this report, the number and relative sizes of chromosomes are described in 8 species each of Danaidae and Satyridae from Formosa. Of these species, 13 species were cytologically studied for the first time.

2. It is noteworthy that *Tirumata hamata septentrionis* (*n*, 32, 33, 34) and *Radena similis similis* (*n*, 13, 14, 15) from Formosa have supernumerary chro-

mosomes in the karyotype.

3. *Mycalesis francisca perdiccas* (*n*, 29) from Japan and *M. f. formosana* (*n*, 28) from Formosa shows a dimorphism of the karyotype due probably to geographic isolation.

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