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A CHROMOSOMAL STUDY OF EIGHTEEN SPECIES OF BUTTERFLIES FROM AUSTRALIA (LEPIDOPTERA, RHOPALOCERA)

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This paper is an initial attempt to contribute to the chromosomal study of Australian butterflies. Australia composes a characteristic area in the geographical distribution of animals. The literature shows that as many as 370 species of Rhopalocera have been described from Australia (Waterhouse '32, Barrett & Burns '51, Common '64). The present authors report the chromosome features in 18 species of butterflies from this country.

Practically all material reported in this paper was collected at Cairns in Queensland and fixed with Allen's P.F.A.-3 solution by M. Ogata during the period from 27 September to 1 October, 1967, the only exceptions being *Hypocysta adiante*, *Ocybadistes walkeri* and *Trapezites eliena* collected at Sydney by M. Ogata on 21 and 22 September, 1967. 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 a magnification of 4200 diameters. The photomicrographs were taken with a MIKAS camera at a magnification of 5000.

We are especially grateful to Dr. Atuhiro Sibatani of CSIRO, Sydney, Australia, for arranging transportation for one of us (M.O.) during the field collecting in Australia.

Observations

A. Papilionidae

1. *Cressida cressida* Fabricius. The haploid chromosome number is 30, on the basis of counts made in 8 nuclei of the primary spermatocyte division in the testis of a single male (Au-36). No secondary spermatocyte divisions were found. There are 13 very large, 12 large, 4 small and 1 very small chromosomes (Fig. 1).

2. *Papilio ulysses joesa* Butler. The haploid chromosome number is 32. Counts were made in 17 nuclei (I) and 4 nuclei (II) from a single male (Au-26). There are 7 very large, 23 large and 2 small chromosomes (Figs. 2, 3, 29, 30).

B. Pieridae

3. *Delias mysis* Fabricius. The haploid chromosome number is 25. Counts were made in 14 nuclei (I) and 31 nuclei (II) from a single male (Au-21). There are 15 distinctly large and 10 somewhat smaller chromosomes (Figs. 4, 5, 32, 33).

4. *Appias melania* Fabricius. The haploid chromosome number is 32. Counts were made in 15 nuclei (I) and 10 nuclei (II) from a single male (Au-29). Apparently 4 chromosomes are small, 16 large and the others medium (Figs. 6, 7, 34, 35).

5. *Catopsilia pomona* Fabricius. The haploid chromosome number is 31. Counts were

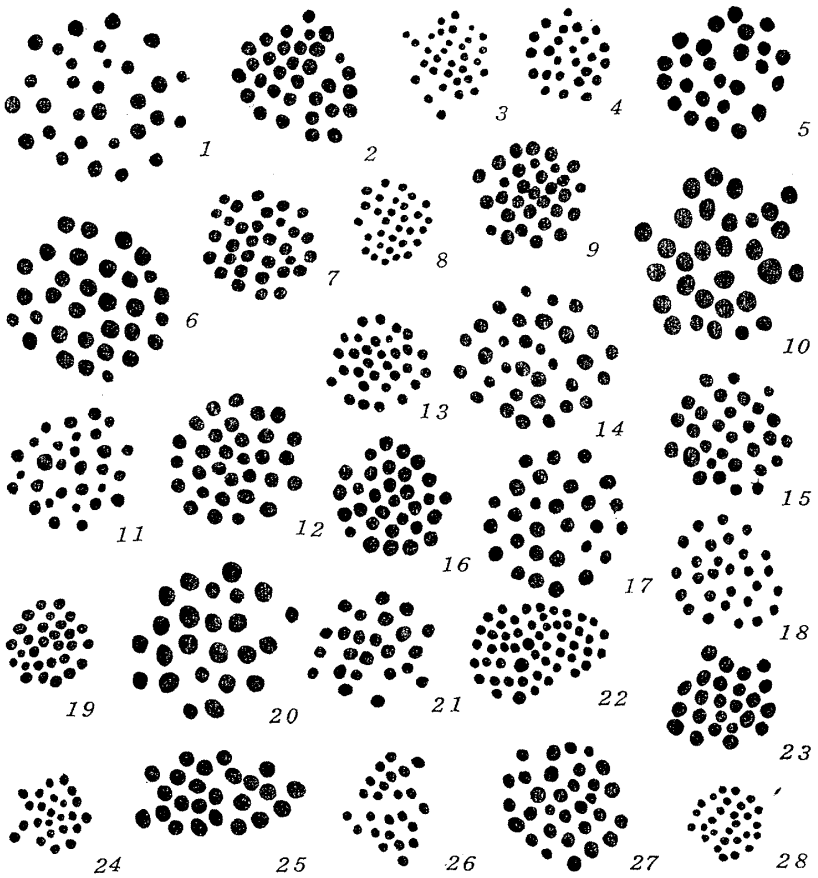

Fig. 1. *Cressida cressida* (I). Fig. 2. *Papilio ulysses* (I). Fig. 3. Same (II). Fig. 4. *Delias mysis* (II). Fig. 5. Same (I). Fig. 6. *Appias melania* (I). Fig. 7. Same (II). Fig. 8. *Catopsilia pomona* (II). Fig. 9. *Cupha prosope* (I). Fig. 10. *Neptis praslini* (I). Fig. 11. Same (II). Fig. 12. *Catopsilia pomona* (I). Fig. 13. *Cethosia chrysippe* (II). Fig. 14. *Vindula arsinoe* (I). Fig. 15. *Neptis shepherdi* (I). Fig. 16. *Cethosia chrysippe* (I). Fig. 17. *Melanitis leda* (I). Fig. 18. Same (II). Fig. 19. *Hypocysta adiante* (II). Fig. 20. *Narathura micale* (I). Fig. 21. Same (II). Fig. 22. *Holochila helenita* (I). Fig. 23. *Thysonotis hymetus* (I). Fig. 24. Same (II). Fig. 25. *Thysonotis cyanea* (I). Fig. 26. Same (II). Fig. 27. *Trapezites eliena* (I). Fig. 28. *Ocybadistes walkeri* (II). [×4200×*ca.* 0.6.]

made in 15 nuclei (I) and 10 nuclei (II) from a single male (Au–22), and meiotic divisions were numerous. There are 18 large and 4 small chromosomes, with the remainder medium in size (Figs. 8, 12, 31).

C. **Nymphalidae**

6. *Cupha prosope* Fabricius. The haploid chromosome number is 31. Counts were made in 10 nuclei of primary spermatocytes from a single male (Au–24). There are 15 large, 11 medium-sized and 5 small chromosomes (Figs. 9, 36).

7. *Neptis praslini staudingercana* Nicéville. The haploid chromosome number is 30. Counts were made in 13 nuclei (I) and 12 nuclei (II) from a single male (Au–27). Four chromosomes are small, 14 medium, 11 large and 1 distinctly the largest (Figs. 10, 11, 40, 41). Many dividing cells were observed in both first and second divisions of meiosis.

8. *Neptis shepherdi* Moore. The haploid chromosome number is 30. Counts were made in 9 nuclei (I) from a single male (Au–33). There are 2 small, 13 medium, 1 large and 14 medium large chromosomes (Figs. 15, 42).

9. *Vindula arsinoe ada* Butler. The haploid chromosome number is 32. Counts were made in 27 nuclei (I) from a single male (Au–28). There are apparently 9 large and 8 small chromosomes, all the others being medium in size (Figs. 14, 39).

10. *Cethosia chrysippe* Fabricius. The haploid chromosome number is 31. Counts

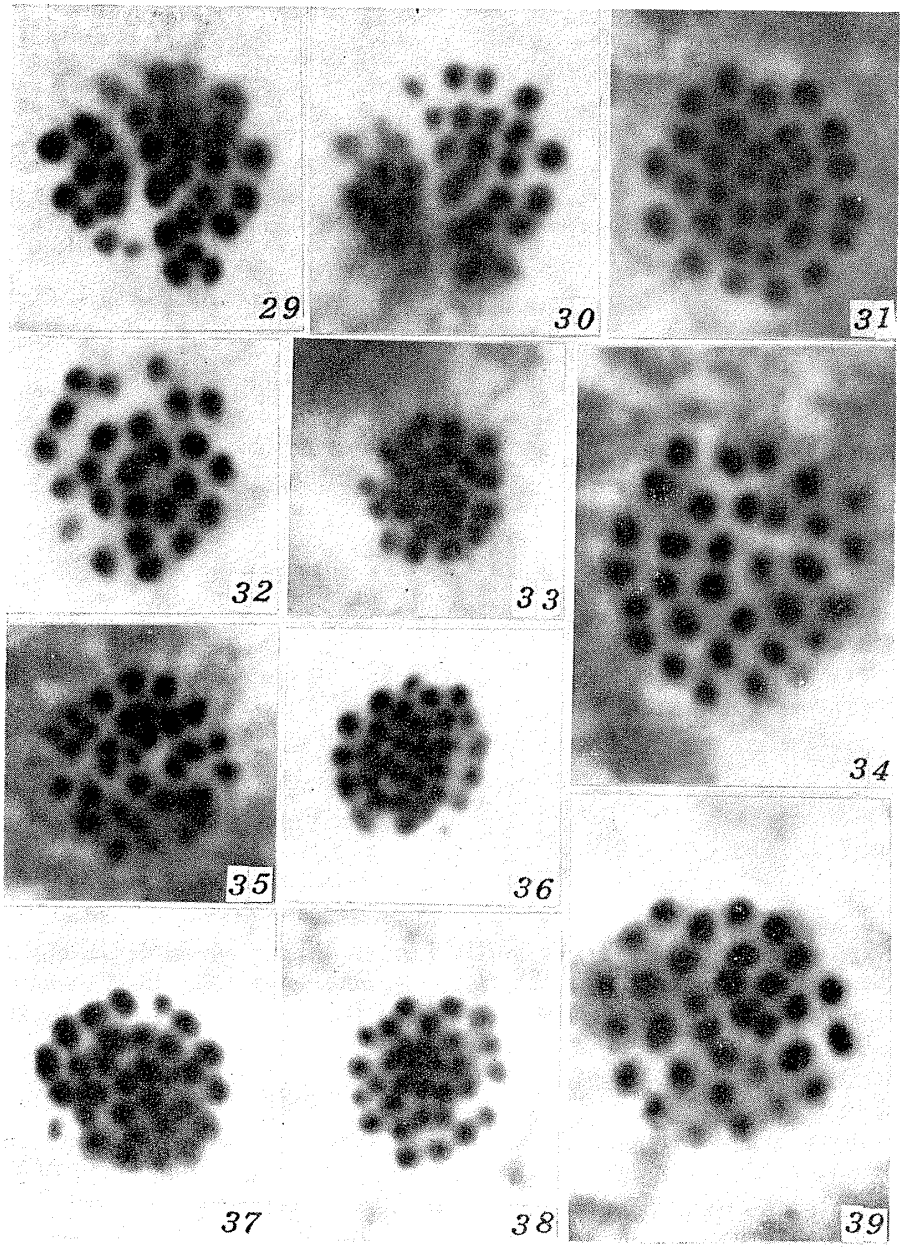

Fig. 29. *Papilio ulysses* (I). Fig. 30. Same nucleus (with different focus). Fig. 31. *Catopsilia pomona* (I). Fig. 32. *Delias mysis* (I). Fig. 33. Same (II). Fig. 34. *Appias melania* (I). Fig. 35. Same (II). Fig. 36. *Cupha prosope* (I). Fig. 37. *Cethosia chrysippe* (I). Fig. 38. Same (II). Fig. 39. *Vindula arsinoe* (I). [×5000×*ca.* 0.9.]

were made in 15 nuclei (I) and 11 nuclei (II) from a single male (Au–34). There are 22 large and 1 small chromosomes, with the rest medium in size (Figs. 13, 16, 37, 38).

D. **Satyridae**

11. *Melanitis leda bankia* Fabricius. The haploid chromosome number is 28. Counts were made in 8 nuclei (I) and 5 nuclei (II) from a single male (Au–25). There are 11 large, 11 medium and 6 small chromosomes (Figs. 17, 18, 43).

12. *Hypocysta adiante* Hübner. The haploid chromosome number is 29. Counts were made in only 4 nuclei (II) from a single male (Au–2). Apparently 4 chromosomes are small, 6 large, and the others medium (Figs. 19, 45). The gonad had a few dividing cells, of which none was in the primary division.

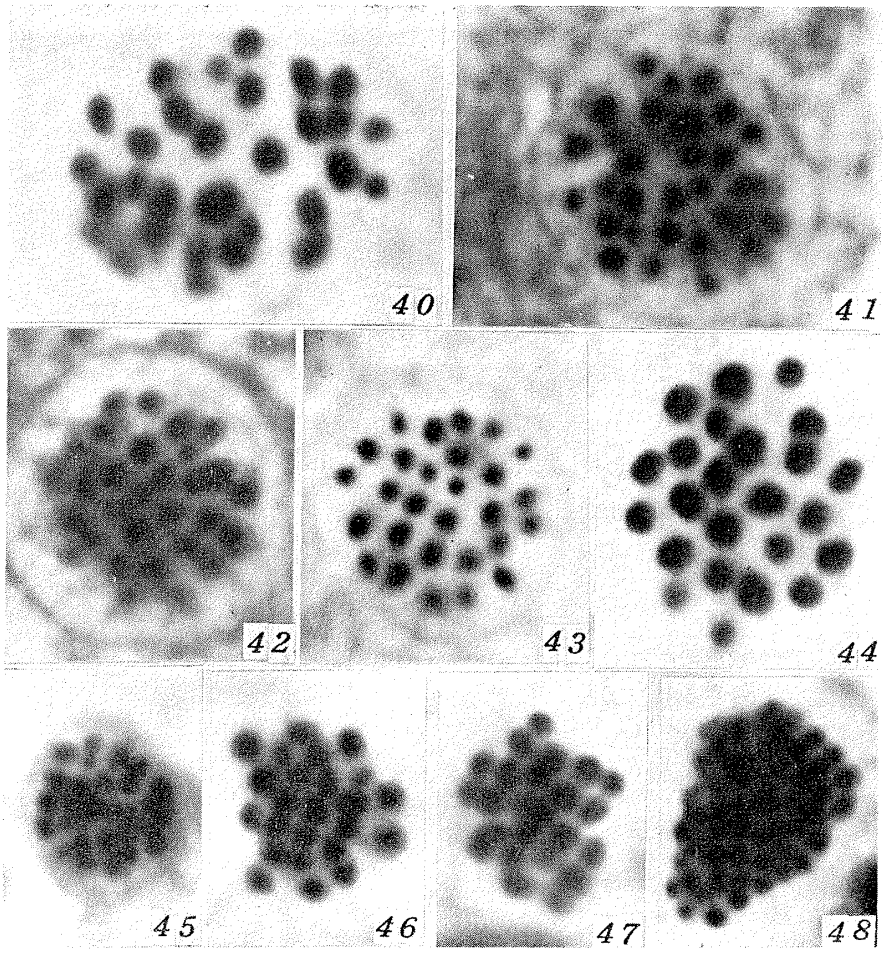

Fig. 40. *Neptis praslini* (I). Fig. 41. Same (II). Fig. 42. *Neptis shepherdi* (I). Fig. 43. *Melanitis leda* (I). Fig. 44. *Narathura micale* (I). Fig. 45. *Hypocysta adiante* (II). Fig. 46. *Narathura micale* (II). Fig. 47. *Thysonotis hymetus* (I). Fig. 48. *Holochila helenita* (I). [×5000×*ca.* 0.9.]

E. Lycaenidae

13. *Narathura micale amphis* Waterhouse. The haploid chromosome number is 24. Counts were made in 15 nuclei (I) and 5 nuclei (II) from a single male (Au–20). Seven chromosomes are larger than the other 17 (Figs. 20, 21, 44, 46).

14. *Holochila helenita* Semper. The haploid chromosome number is 53. Counts were made in 5 nuclei (I) from a single male (Au–31). A few dividing cells were observed in first division of meiosis. There are 1 very large and 2 moderately large chromosomes, the remaining 50 elements being small in size (Figs. 22, 48).

15. *Thysonotis hymetus taygetus* Felder. The haploid chromosome number is 24. Counts were made in 12 nuclei (I) and 10 nuclei (II) from a single male (Au–30). Several of the chromosomes are more or less smaller than the others (Figs. 23, 24, 47).

16. *Thysonotis cyanea arinia* Oberthür. The haploid chromosome number is 24. Counts were made in 5 nuclei (I) and 3 nuclei (II) from a single male (Au–32). All the chromosomes are large in size (Figs. 25, 26).

F. Hesperiidae

17. *Ocybadistes walkeri sothis* Waterhouse. The haploid chromosome number is 28. Counts were made in 3 nuclei (II) from a single male (Au–8). All the chromosomes are small in size (Fig. 28).

18. *Trapezites eliena* Hewitson. The haploid chromosome number is 31. Counts

were made in 5 nuclei (I) from a single male (Au–9). Apparently 4 chromosomes are small, 9 large, and the others medium in size (Fig. 27).

Discussion

Table 1 shows the chromosome numbers observed in the 18 species of Australian butterflies dealt with in the present paper. So far, almost all species studied of *Papilio* have been found to possess the conventional haploid chromosome number 30. Until recently, some exceptions with different numbers of elements have appeared in the literature, namely, *Papilio thoas* (n=27) of North America (Maeki & Remington '60), *Papilio hippocrates* (n=31) of Japan (Maeki '53), *Papilio polyxenes* (n=30–31) of North America (Maeki & Remington '60) and *Papilio castor* (n=31) of Formosa (Maeki & Ae '68).

Table 1. The species studied and their chromosome numbers.

Species	n
A. Papilionidae	
1. *Cressida cressida* Fabricius	30 (I)
2. *Papilio ulysses joesa* Butler	32 (I, II)
B. Pieridae	
3. *Delias mysis* Fabricius	25 (I, II)
4. *Appias melania* Fabricius	32 (I, II)
5. *Catopsilia pomona* Fabricius	31 (I, II)
C. Nymphalidae	
6. *Cupha prosope* Fabricius	31 (I)
7. *Neptis praslini staudingereana* Nicéville	30 (I, II)
8. *Neptis shepherdi* Moore	30 (I)
9. *Vindula arsinoe ada* Butler	32 (I)
10. *Cethosia chrysippe* Fabricius	31 (I, II)
D. Satyridae	
11. *Melanitis leda bankia* Fabricius	28 (I, II)
12. *Hypocysta adiante* Hübner	29 (II)
E. Lycaenidae	
13. *Narathura micale amphis* Waterhouse	24 (I, II)
14. *Holochila helenita* Semper	53 (I)
15. *Thysonotis hymetus taygetus* Felder	24 (I, II)
16. *Thysonotis cyanea arinia* Oberthür	24 (I, II)
F. Hesperiidae	
17. *Ocybadistes walkeri sothis* Waterhouse	28 (II)
18. *Trapezites eliena* Hewitson	31 (I)

Papilio ulysses joesa (n=32) from Australia joins the exceptional group which does not possess the characteristic number of *Papilio* (n=30). In the karyotype of this species, we recognized 2 small chromosomes. It is noteworthy that *P. ulysses* is an unusual species in view of its karyotype in contrast to a number of other species of *Papilio*. On the other hand the male genitalia show no remarkable difference from those of other closely related species like *Papilio peranthus* and *P. lorquinianus*, as M. Ogata pointed out (Ogata '68). However further discussion on the significance of the karyotype of *Papilio ulysses* will be impossible unless we can obtain sufficient material for further investigation.

Cressida cressida is well-known as an endemic species in Australia, and its taxonomic position is between *Parnassius* and *Battus-Byasa* groups. The chromosomes of *Cressida cressida* (n=30) are the same in number as those of the related *Battus philenor* of North America (Maeki & Remington '60), *Byasa alcinous* of Japan (Maeki '57), *Parnassius apollo* of Finland (Federley '38) and Yugoslavia (Lorkovic '41) and *Parnassius smintheus* of North America (Maeki & Remington '60).

The 3 *Delias* species studied previously, namely, *D. aglaia* of Formosa (Maeki, Ogata & Shirôzu '65) and Nepal (Maeki & Ae '66), *D. eucharis* of Nepal (Maeki & Ae '66) and *D. descombesi* of Nepal (Maeki & Ae '66) have a common haploid number, 25, except for *D. descombesi* with n=27–32. Also, the species of Australia, *Delias mysis* reported in the present paper has the same number, n=25. Four species of the genus *Appias*, namely *A. drusilla*, *A. lyncida*, *A. indura*, and *A. melania* are characterized by having a haploid number of 32, but show some difference in karyotype. In *A. drusilla* (Maeki & Remington, '60) from North America all the chromosomes are of the same size. In *A. lyncida* of Formosa two chromosomes are smaller than the others (Maeki, Ogata & Shirôzu, '65), while *A. indra* of Formosa has 9 large and 4 small elements (Maeki & Ae, '68). *A. melania* of Australia, dealt with in this paper, is remarkable in having 16 large and 4 small chromosomes. These differences in the size of chromosomes may be the result of chromosomal mutations during speciation in the genus *Appias*. The chromosomes of *Catopsilia pomona* are the same in number as those of *C. crocale*, *C. pomona* of Formosa and Nepal (Maeki, Ogata & Shirôzu '65, Maeki & Ae '66), and *C. pyranthe* of Nepal (Maeki & Ae '66), having a haploid number of 31, which is the only known number of chromosomes found in *Catopsilia*.

A previous report (Maeki & Ae '69) shows that the haploid chromosome number in the *Neptis* species (*N. aceris*, *N. columella*, *N. pryeri*, *N. philyra*, *N. hylas*) is consistently 30, except for 2 species. *Neptis soma*, one of the exceptional species, has a karyotype of 29 chromosomes which consist of 17 large, 8 medium and 4 small elements, while *Neptis ananta* has a karyotype which varies from n=31 to n=33 at first division of meiosis.

The newly examined *Neptis praslini* and *Neptis shepherdi* also have 30 chromosomes in haploid set. However, these species show slight differences in karyotype. Of the chromosomes of *N. praslini* 4 are small, 14 medium, 11 large and 1 distinctly the largest, while *N. shepherdi* possesses 2 small, 13 medium, 14 large and 1 very large chromosomes. (In order to avoid confusion, the old specific names of the genus *Neptis* used in our previous report are adopted here instead of the new ones based on the nomenclature revised recently by Eliot. It must be noted that *N. soma* in Shirôzu, 1960, "Butt. Formosa in colour" is now renamed *N. nata*.)

The karyotype of 32 chromosomes in *Vindula arsinoe* which include 9 large and 8 small elements is unique among Nymphalidae. Also, the karyotype of *Holochila helenita* with a haploid number of 53 is unique in Lycaenidae.

Summary

1. The chromosome numbers and sizes are reported for 2 species of Papilionidae, 3 species of Pieridae, 5 species of Nymphalidae, 2 species of Satyridae, 4 species of Lycaenidae and 2 species of Hesperiidae all from Australia. Among these species, 16 species are cytologically studied for the first time. The numerical and morphological features of their chromosomes are given in Table 1 and Figures 1–48. All the chromosome counts were carried out on the basis of the spermatocytes.

2. *Papilio ulysses joesa* has an unusual number of chromosomes (n=32), which is exceptional for a member of the *Papilio*-group.

3. It is noteworthy that *Cressida cressida* has n=30, *Vindula arsinoe ada* n=32, and *Holochila helenita* n=53.

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SOME GENERA AND SPECIES OF THE HEMEROBIIDAE (NEUROPTERA)

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Of the extensive materials of the Hemerobiidae which I examined since the publication of my "Systematic Studies", 1960, the parts appertaining to the Nearctic and Neotropical faunas were reported upon in two papers appearing in the Proceedings of the United States National Museum (1965 a and b). Specimens from Formosa and Ryukyu were treated of in my last paper in this Journal (1966). The remainder of the material coming from different parts of the world includes several noteworthy genera and species, and descriptions of and remarks upon these forms are now brought together in this paper.