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CHROMOSOMAL STUDIES IN SOME INDIAN LEPIDOPTERA*

By

Y. GUPTA

With 63 Figures in the Text

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The chromosomal studies in *Lepidoptera* furnish several predominately striking points which deserve special attention. Up to now very little work has been done on the Indian species of *Lepidoptera*. It was, therefore, considered worth-while to make a survey of the chromosomes in these.

Material and Methods

An attempt has been made to study the chromosomes in the male germ cells of fifteen species, belonging to six families. All the forms studied came from the local populations of Allahabad, except for the two species of the genus *Vanessa*, which were collected from Simla hills. The larvae were usually found feeding upon the foliage of their respective food plants, except those of *Earias* species, which are internal feeders boring into fruits of *Hibiscus esculentus*. The following Table 1 gives the systematic position, food plants and time of collection of the caterpillars.

Freshly dissected testes from the fourth and fifth instar larvae mainly furnished the material for the present study. Of the various fixatives and stains tried Sanfelice and Heidenhain's Iron Haematoxylin yielded the best results. Feulgen's stain was used for the purpose of distinguishing between heterochromosomes and plasmosomes.

Observations

1. Vanessa indica

The diploid group of chromosomes has been studied in the polar view of mitotic metaphases of several spermatogonial divisions; thirty elements have always been found to constitute the diploid complement of the species (Fig. 1). This is a low-numbered species with distinctly larger chromosomes, which at metaphase assume the characteristic spherical shape of the lepidopteran chromosomes. These hardly show any perceptible difference in their morphology or size. Following the last spermatogonial division the premeiotic nucleus remains inactive for a while.

At the beginning of the prophase stage of the primary spermatocyte, the nucleus is found to contain fine, faintly-staining threads studded

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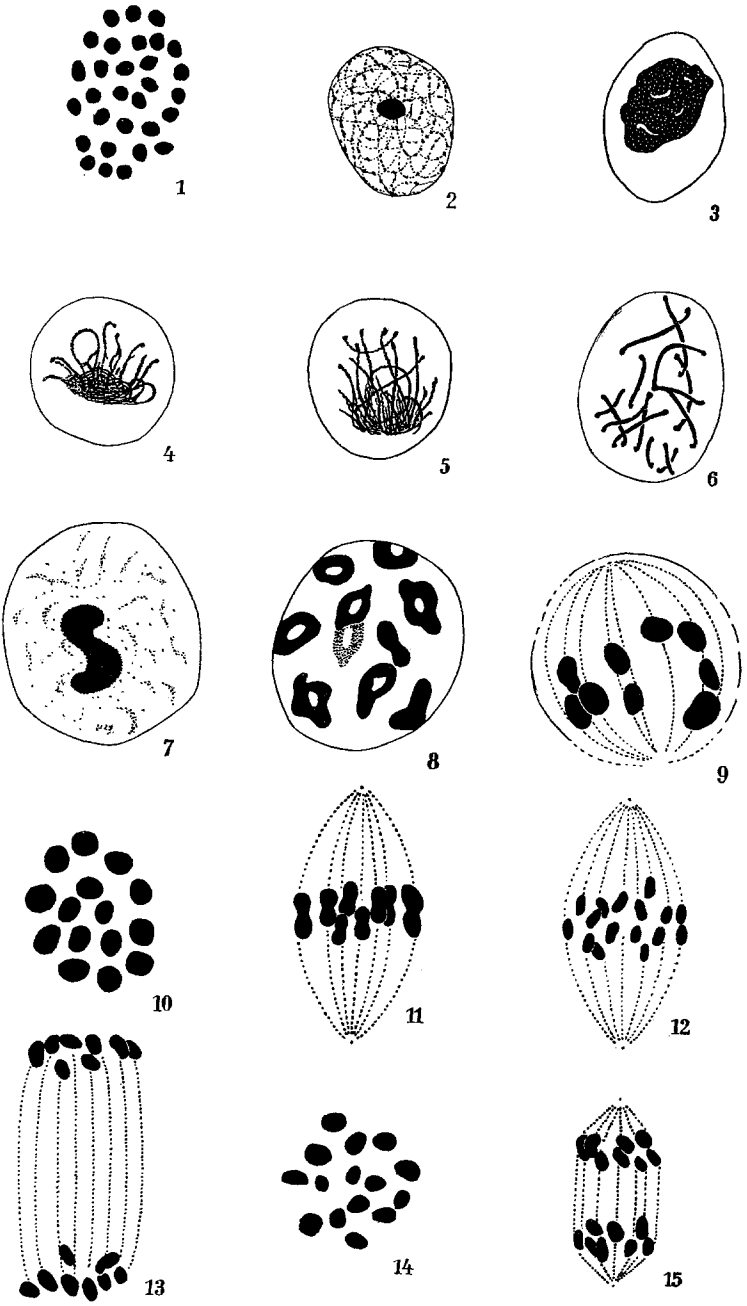
Table 1

Superfamily	Family	Species	Food plant	Time of collection
1. *Papilionoidea*	1. *Nymphalidae*	1. *Vanessa indica* HERBST	*Nasturtium*	June
		2. *Vanessa kashmirensis* MOORE	*Urtica divica*	September
		3. *Danais chrysippus* (LINN.)	*Calotropis* sp.	Oct. to Nov. and March
	2. *Pieridae*	4. *Belenois mesentina* CRAMER	*Capparis horidus*	March to April
		5. *Catopsilia pyranthe* HERBST	*Cassia occidentalis*	August to September
	3. *Papilionidae*	6. *Papilio demoleus* LINN.	*Citrus* sp.	March and July to August
2. *Sphingoidea*	4. *Sphingidae*	7. *Theretra oldenlandiae* (FABR.)	*Impatiens*	July to August
3. *Noctuoidea*	5. *Arctiidae*	8. *Pericallia ricini* (FABR.)	*Ricinus communis*	July to September
		9. *Utetheisa pulchella* (LINN.)	*Helotropium* sp.	July to September
		10. *Earias insulana* BOISD.	*Hibiscus esculentus*	September to November
		11. *Earias fabia* STOLL	*Hibiscus esculentus*	September to November
	6. *Noctuidae*	12. *Polytela gloriosae* FABR.	*Lilium* sp.	July to September
		13. *Prodenia littora* FABR.	*Ricinus communis*	August to September
		14. *Ophiusa melicerte* DRURY	*Ricinus communis*	August to September
		15. *Hoplotarache lunana* FABR.	*Cida cadifolia*	July to September

with coarse granules and a small plasmosome (Fig. 2). The whole chromosomal material is now massed together in the form of a large, tight knot situated in the centre of the nuclear cavity (Fig. 3), leaving the rest of the space entirely empty. This gradually expands into the unoccupied part of the nucleus by the emergence of double threads from the tightly coiled skein. These threads are much thicker than before and looped on themselves forming a bouquet (Fig. 4). Gradually the nucleus, by a further expansion of the synizetic knot and passing through a stage shown in Fig. 5, approaches the pachytene stage. In all the post-synaptic stages there is an indication of the occurrence of a pseudo-reduction caused by longitudinal pairing of the homologous threads. The nucleus shown in Fig. 6, represents entangled pachytene bivalents typically possessing the knobbed heteropycnotic ends. The counting of the threads is not quite easy, but approximately fifteen seem to be present. A longitudinal split in the chromosomes resulting in the formation



Figs. 1—15

of chromatids has not been observed. In the succeeding diffuse stage, major part of the nuclear material loses its staining reactions (Fig. 7), except for a prominent deeply-staining body. This body does not seem to be simply a plasmosome as it reacts positively with Feulgen-reagent. Most probably this body mainly consists of a plasmosome associated with highly condensed Feulgen-positive granules, which surround it entirely. Sometimes this appears bilobed or as two separate bodies. Probably the diffuse stage corresponds to normal diplotene. Before the commencement of diakinesis the Feulgen-positive granules of the deeply-staining body of the diffuse stage disappear and nucleolus once again becomes Feulgen-negative. Ultimately the plasmosome also loses its identity.

As the diakinesis ensues, the chromosomes become differentiated in the form of typical ring-shaped or dumb-bell-shaped bivalents (Fig. 8). The terminal connections between the homologues appear as typically chiasmal in character. The rod- and rings-haped bivalents seem to have one and two terminalized chiasmata, respectively. It has not been possible, however, to ascertain whether these chiasmata are formed at the ends of the bivalents or at some interstitial spot, becoming rapidly terminalized. It is noteworthy that, though the size of the nucleus in this species is very small in comparison to other species studied, the size of the individual chromosomes is comparatively bigger. This leads to the conclusion that the increase in the size of the chromosomes in *Lepidoptera* must be attributed to the corresponding decrease in number and not to the nuclear size. The diakinetic bivalents, by a further contraction, give rise to almost rounded chromosomes, which with the dissolution of the nuclear membrane and the formation of the spindle fibres (Fig. 9), become arranged on the metaphase plate. Primary spermatocytes at metaphase show, with great regularity, fifteen more or less equal sized chromosomes when the equatorial plate is seen in the polar view (Fig. 10). In the side view of the first metaphase, the bivalents are seen to be dumb-bell-shaped bodies arranged on the equatorial plane of the massive spindle (Fig. 11). During the early anaphase of the first spermatocyte (Fig. 12), the chiasmata resolve, so that the homologues

Figs. 1—15[1]. Chromosomal cycle during the spermatogenesis of *Vanessa indica*. – Fig. 1. Polar view of spermatogonial metaphase plate, containing thirty elements. Fig. 2. – Primary spermatocyte nucleus at early prophase stage. – Fig. 3. Synizetic nucleus. Fig. 4. – Typical bouquet stage. – Fig. 5. Further loosening of post-synaptic threads. – Fig. 6. Pachytene stage, showing fifteen bivalent threads. – Fig. 7. Diffuse stage. – Fig. 8. Diakinetic nucleus containing typical ring- and dumb-bell-shaped bivalents. – Fig. 9. Prometaphase stage showing disruption of nuclear membrane and formation of spindle. – Fig. 10. Polar view of metaphase I plate, exhibiting fifteen bivalents. – Fig. 11. Metaphase plate in side view. – Fig. 12. Early anaphase stage of the first spermatocyte. – Fig. 13. Primary telophase stage. – Fig. 14. Polar view of second spermatocyte metaphase plate, showing fifteen univalents. – Fig. 15. Second anaphase

[1] Approximate linear magnification of all the figures from 1 to 60 is 3000 ×.

of a bivalent separate along the median constriction seen in the side view of the first metaphase. No succession or precession of any chromosome has been observed, as the separated groups of chromosomes move towards the poles (Fig. 13).

The polar view of the equatorial plate of the secondary spermatocyte mataphase presents fifteen spherical univalents (Fig. 14). The second maturation spindle can be distinguished from the first by its smaller size. At the anaphase stage the chromosomes are seen on the spindle as separate, rounded bodies (Fig. 15). In telophase they form a densely staining mass around which a new nuclear membrane is formed. No unequal or heteromorphic pair is observed at any stage during the whole spermatogenesis.

The mitotic and meiotic cycles, in all the species studied, are more or less similar except for minor differences.

2. Vanessa kashmirensis

In contrast to the low chromosome number of *V. indica*, this species possesses a diploid chromosome complement made up of sixty-two elements (Fig. 16). The number of bivalents in the polar view of the first metaphase plate has been found to be thirty-one (Fig. 17). A comparative study of the primary spermatocyte metaphase plates in the two *Vanessa* species studied by me, reveals several points of special interest. The chromosomes in *V. kashmirensis* number more than twice of those in *V. indica* and at the same time each element in the former is almost half in size compared to any single element of the latter; thus the total amount of chromosomal substance in the metaphase plates of the two, roughly appears to be the same. Thirty-one univalents are always seen in the polar view of the secondary spermatocyte plates (Fig. 18).

3. Danais chrysippus

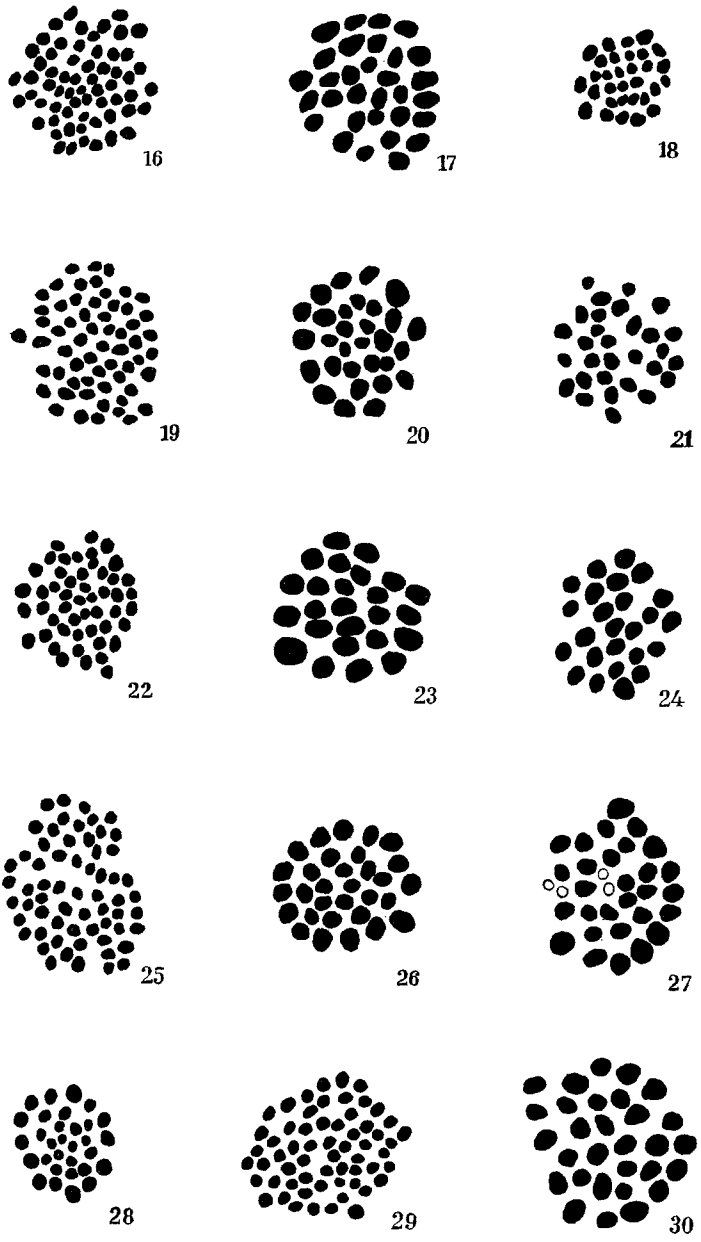
Spermatogonial metaphase count in this species is found to be sixty (Fig. 19). The primary and secondary spermatocyte metaphases invariably present thirty spherical elements (Figs. 20 and 21).

4. Belenois mesentina

In the best preserved spermatogonial plates, fifty chromosomes are seen in polar view (Fig. 22). Primary metaphases are very regular and clear exhibiting twenty-five bivalents (Fig. 23). The secondary spermatocyte metaphase plate again shows twenty-five rounded chromosomes (Fig. 24).

5. Catopsilia phyranthe

In the equatorial plates of the spermatogonial divisions sixty-two chromosomes have been made out (Fig. 25). Primary spermatocyte metaphase presents thirty-one bivalents (Fig. 26). There are, however,



Figs. 16—18. Spermatogonial first and second spermatocyte metaphase plates of *V. kashmirensis*, in polar view. — Figs. 19—21. Spermatogonial, first and second metaphase plates of *D. chrysippus*, in polar view. — Figs. 22—24. Polar views of metaphase plates in a spermatogonium, first spermatocyte and second spermatocyte of *B. mesentina*. — Figs. 25—28. *C. pyranthe*. — Fig. 25. Polar view of spermatogonial metaphase exhibiting sixty-two rounded chromosomes. — Fig. 26. Polar view of first spermatocyte metaphase, showing thirty-one bivalents. — Fig. 27. Same stage with abnormal chromosome number (33), bivalents uniformly shaded, univalents outlined. — Fig. 28. Secondary metaphase plate in polar view, revealing thirtyone univalents. — Figs. 29 and 30. Polar views of spermatogonial and first metaphase plates of *P. demoleus*

a few exceptions to this general rule. In this case a discrepancy in chromosome number has been observed within the cells of the same cyst. Certain cells of primary spermatocytes in metaphase contained thirty-three chromosomal bodies (Fig. 27), while in the neighbouring cells the normal number appeared. Although the origin of the extra elements has not been ascertained owing to lack of sufficiently good diakinetic nuclei, it seems more than probable that, a similar condition which has been observed in certain abnormal primary spermatocytes of *Philosamia ricini* (SRIVASTAVA and GUPTA, 1962), prevails in this form also. This means that the presence of a few univalents (originated by a lack of chiasma-formation or a precocious separation of the homologues of the bivalents) in the first metaphase plate, is responsible for this apparent increase in number. That no abnormality is introduced at the subsequent stages by the unusual resolution of the bivalents into univalents, has been concluded by the regularity in the appearance of thirty-one univalents in the second metaphase plates (Fig. 28) and by the normality in the anaphasic movement of chromosomes.

6. Papilio demoleus

Spermatogonial metaphase plates consist as a rule of sixty-small chromosomes (Fig. 29). The haploid number for this species has been established to be thirty after the countings on numerous first and second metaphase plates (Figs. 30 and 31).

7. Theretra oldenlandiae

The diploid chromosome count, as made in the polar view of the spermatogonial metaphase plate, is sixty-two (Fig. 32). Haploid count on the first and second meiotic metaphase plates is thirty-one (Figs. 33 and 34).

8. Pericallia ricini

In the polar view of the spermatogonial metaphase plate (Fig. 35) sixty-two spherical elements have been counted. Haploid complex of both primary and secondary spermatocytes consists of thirty-one chromosomes (Figs. 36 and 37).

9. Utetheisa pulchella

Mitotic metaphase plates present sixty-two elements (Fig. 38). Counting on the numerous first and second metaphase plates (Figs. 39 and 40) reveals thirty-one elements.

10. Earias insulana

Mitotic metaphase constantly shows sixty-two chromosomes (Fig. 41). Metaphase plates of the first and second maturation divisions present thirty-one elements (Figs. 42 and 43).

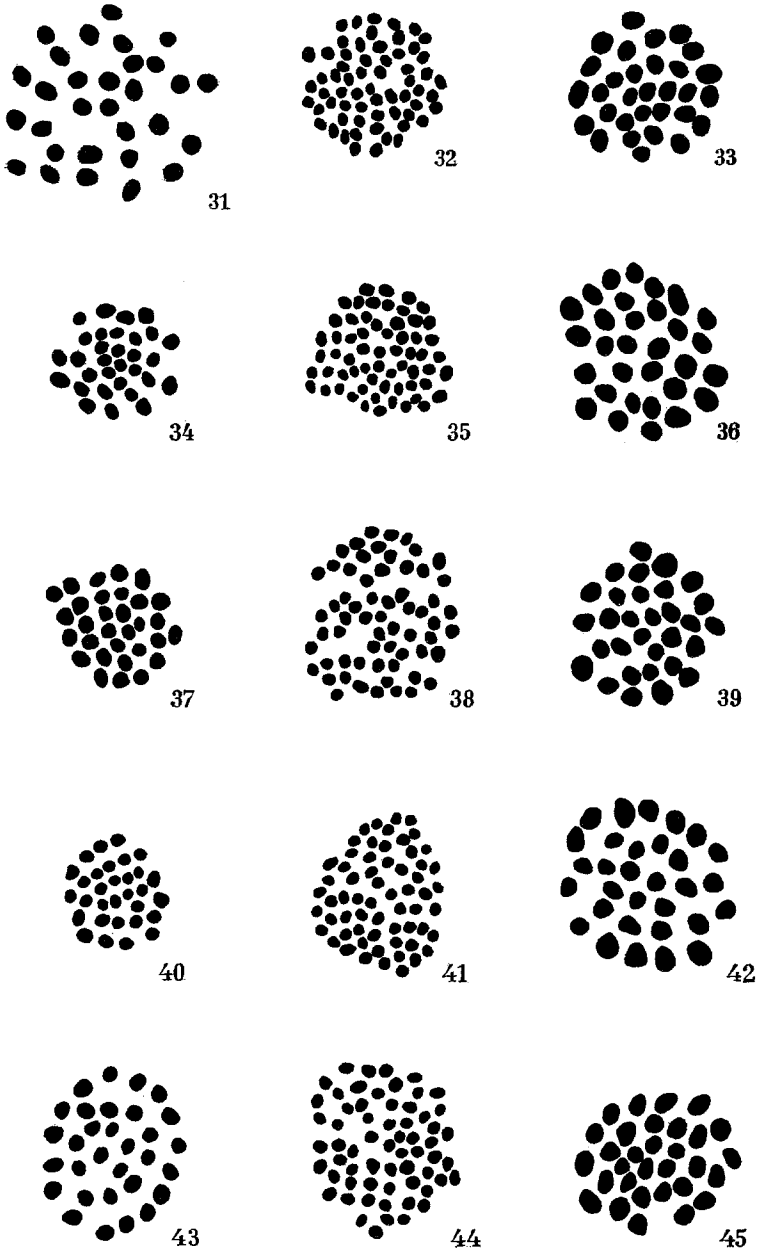


Fig. 31. Polar view of second metaphase plate of *P. demoleus*. — Figs. 32—34. Spermatogonial, first and secondary spermatocyte metaphase plates of *T. oldenlandiae*, in polar view. — Figs. 35—37. Polar views of spermatogonial, first and second metaphase plates of *P. ricini*. — Figs. 38—40. Polar views of metaphase plates of a spermatogonium, first spermatocyte and second spermatocyte of *U. pulchella*. — Figs. 41—43. Spermatogonial, first and second spermatocyte metaphase plates of *E. insu- lana*, in polar view. — Figs. 44 and 45. Spermatogonial and first spermatocyte and secondary spermatocyte metaphase plates of *E. fabia*, in polar view

11. Earias fabia

The spermatogenesis in this species is exactly like that of its congeneric form *E. insulana*. The number, size and morphology of the chromosomes in the two species appears to be more or less identical. Mitotic and meiotic metaphase plates of *E. fabia* are shown inFigs. 44—46.

12. Polytela gloriosae

The spermatogonial metaphase complement is composed of sixty-two chromosomes (Fig. 47). The first and second metaphase plates in polar view (Figs. 48 and 49) always present thirty-one elements.

13. Prodenia littora

The diploid chromosome count on spermatogonial metaphase plate is sixty-two (Fig. 50). Both primary and secondary metaphases invariably exhibit thirty-one chromosomes (Figs. 51 and 52).

14. Ophiusa melicerte

The male mitotic complement consists of sixty-two chromosomes (Fig. 53). Chromosomes of the first and second meiotic metaphases (Figs. 54 and 55), are found to number thirty-one.

15. Hoplotarache lunana

The spermatogonial metaphase plate is characterized by the possession of sixty-four chromosomes (Fig. 56). Primary spermatocyte metaphase plates usually display thirty-two bivalents. It is interesting to note that, this species furnishes yet another example of variation in chromosome number within the same testis. An abnormal feature is marked in quite a large number of first metaphase plates (nearly 40%), where more than thirty-two elements have been counted. Figs. 57—59 illustrate the polar views of three abnormal primary metaphase plates. It seems that in *H. lunana* a similar condition, as has been already described for *C. pyranthe*, prevails. In an abnormal metaphase plate (Fig. 57) thirty-one bivalents and two univalents, while in another (Fig. 59) thirty bivalents and four univalents have been counted clearly. A case intermediate between the two is shown in Fig. 58, where the components of a bivalent on the extreme right have not yet parted completely and remain connected to each other at one point, manifesting a dumb-bell shape while another bivalent has completely resolved itself into two univalents, which are seen lying in close vicinity but without any actual contact. In the secondary metaphase plates (Fig. 60) the normal haploid number (thirty-two) appears without any exception.

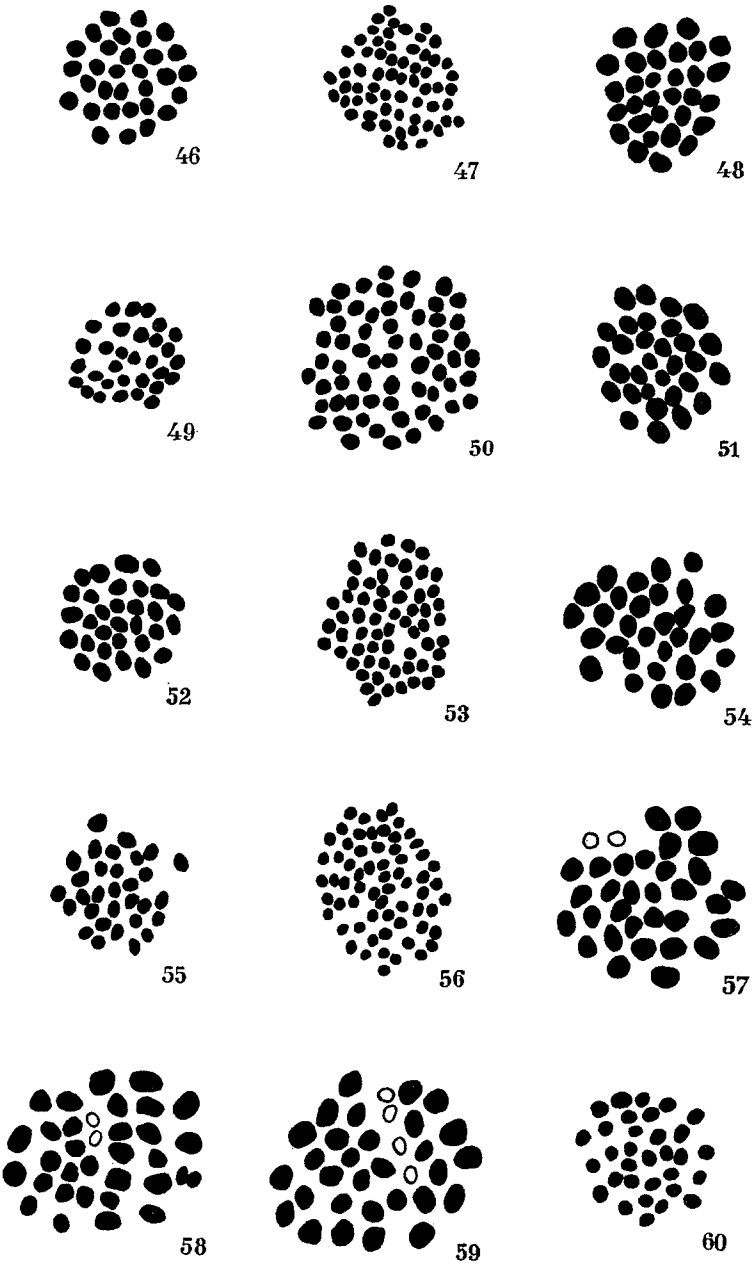


Fig. 46. Polar view of second spermatocyte metaphase plate of *E. fabia*. – Figs. 47—49. Polar views of metaphase plates of a spermatogonium, primary spermatocyte and secondary spermatocyte of *P. gloriosae*. – Figs. 50—52. Polar views of spermatogonial, first and second meiotic metaphase plates of *P. littoralis*. – Figs. 53—55. Spermatogonial, first and second metaphase plates of *O. melicerte*, in polar view. – Fig. 56—60. *H. lunana*. – Fig. 56. Spermatogonial metaphase plate in polar view, with sixty-four chromosomes. – Fig. 57. Polar view of primary spermatocyte abnormal metaphase plate, showing thirty-one bivalents and two univalents. – Fig. 58. Same stage with thirty spherical bivalents, one dumb-bell-shaped bivalent and two univalents. – Fig. 59. Same stage with thirty bivalents and four univalents. – Fig. 60. Polar view of second spermatocyte metaphase plate, showing thirty-two univalents

Discussions

Synizesis

A number of workers have reported that characteristic synizesis stage occurs regularly in the early meiotic prophase stages of the primary spermatocytes and oocytes of Lepidoptera. The nucleus in this phase exhibits an extreme condensation of the entire chromosomal material, which become collected into a single, densely-stained mass placed eccentrically, leaving the rest of the nuclear cavity practically empty. Some authors, on the other hand, consider this to be an artifact instead of a regular phase of the meiotic cycle. In 1910 COOK concluded that the contraction stage occurs undoubtedly as a perfectly normal phase. Earlier, MUNSON in 1906 had mentioned that the synizetic nuclei do not show any shrinkage; and as the synizetic contraction has been seen to occur always at a particular stage even in the best preserved material, it must not be regarded as an artifact produced by the action of the reagents. The results obtained by different fixing solutions used by WAGNER (1930), however, led him to a different inference. According to him the synizesis is no more than a fixation artifact. The present study does not support WAGNER'S opinion, as in all the fifteen forms investigated a constant occurrence of the typical synizesis stage has been observed by me, regardless of the fixative or the stain used.

Mode of Conjugation

It is surprising that, diverse interpretations have been put forth by different authors regarding the mode of pairing process between the homologous chromosomes forming bivalents. FEDERLEY in 1913, during his studies on *Pygaera* species, described an end-to-end conjugation of the homologues. COOK (1910) and KÜRIHARA (1929) also hold a similar view. The evidences and arguments furnished by the exponents of this view, however, do not seem to me convincing. SEILER (1914), on the basis of his investigations on the oogenesis and spermatogenesis of certain species of *Lepidoptera*, has put forth an effective theory. His postulates deviate very widely from those of the authors mentioned above. According to him a pseudo-reduction in the chromosome number takes place through a parallel conjugation of the homologous threads during synizesis, so that a haploid number of chromosomal bodies is seen in the subsequent pachytene stage. An identical situation has been noticed in all the species described in this presentation. The typical ring-shaped bivalents visible in the nucleus after the growth-phase justify the interpretation by SEILER. These figures arise when the conjugants of a bivalent repel each other all along their lengths excepting the terminal portions, which retain their original partnership, thus

creating a central space between the paired chromosomes. With a slight modification of this interpretation, I assume that the terminal connections of these ring bivalents probably represent the location of the chiasmata. MALAN (1918), CRETSCHMAR (1928) and KAWAGUCHI (1928) have supported the parallel conjugation hypothesis of SEILER. According to MAEDA (1939) the homologous chromosomes synapsed longitudinally during the synizetic contraction phase both in the males and the females of *Bombyx mori*. In the later stages, however, this longitudinal pairing is lost in the bivalents of the female where the two components now exhibit a terminal association only. Though WAGNER (1930) failed to demonstrate any circumstancial or observational evidence for a parallel conjugation in the early prophase stages (as described by SEILER and CRETSCHMAR), he was forced to accept the theory of longitudinal pairing after his studies on diakinetic bivalents. He has concluded that all the configurations assumed by the bivalents during diakinesis, can evidently be derived from a bivalent originally composed of two rods lying parallel to each other. It is worthmentioning that in 1945 FEDERLEY while studying the spermatogenesis of *Trichiura crataegi*, himself reported an unequivocal evidence of the occurrence of parallel conjugation.

After a critical study of the pre- and post-synaptic stages during the spermatogenesis of all the fifteen species investigated by me, I am led to believe that in *Lepidoptera* a pseudoreduction is essentially caused by a parallel conjugation of the homologous threads. This pairing takes place at the synizetic stage of the primary spermatocyte nucleus. It is true that, an exact number of the pachytene threads is not ascertainable in most of the species, but at the same time the presence of an approximately haploid number of chromosomal bodies is more than evident. In the low-numbered species, however, it has been possible to establish more or less the exact number, e.g., *Vanessa indica* (Fig. 6). A considerable increase in the thickness of the threads after synizesis again shows their double nature in the post-synaptic stages.

Chiasma-Formation

The question of the presence of chiasmata was barely touched upon in the earlier works of chromosomal cytology in *Lepidoptera*. In 1939 MAEDA carried out chiasma studies during the spermatogenesis and the oogenesis of *Bombyx mori*. He is of the opinion that distinct chiasmata are formed normally in the bivalents of the males, while in the oocytes they occur only exceptionally. WHITE (1954), however, has concluded the existence of a single terminalized chiasma in each bivalent of the oocytes sketched by MAEDA. Before making his studies on *Trichiura crataegi* in 1945, FEDERLY insistently denied the formation of chiasmata both in the male and female; but in 1945 he acknowledged that the

chiasmata are formed in male *T. crataegi* and are characterized by a rapid and complete terminalization. In 1953 SUOMALAINEN has reported the formation of chiasmata in both the males and females of *Cidaria*, but in his paper of 1963 he contradicts the occurrence of chiasmata during oogenesis. I have tried to ascertain the presence of chiasmata in the forms studied by me. In several of these, the diakinetic bivalents exhibit apparently typical chiasma-bearing shapes, such as rings, dumb-bells or V's. According to my interpretations the ring-shaped bivalents possess two terminalized chiasmata, while the dumb-bells and the V's bear only one. Unfortunately, it is not possible to observe any chiasmata at the metaphase stage owing to the extreme condensation and the small size of the chromosomes.

Morphology and Number of Chromosomes

In all the fifteen species investigated by me the chromosomes exhibit a remarkable uniformity in their size and shape. At the metaphase stage they are invariably seen in the form of small, spherical and compact bodies of nearly equal sizes, so that it is almost impossible to identify the corresponding elements in different equatorial plates. A morphological similarity in the lepidopteran chromosomes has also been noted by previous workers.

An interesting phenomenon of a correlation between the number and the size of chromosomes has been noticed during the studies on the spermatogenesis of *Vanessa indica* and *V. kashmirensis*. It becomes obvious at the first glance that the chromosomes in *V. indica* (Fig. 10) are less than half the number but nearly double the size than those in *V. kashmirensis* (Fig. 17). Thus a comparison of the caryotypes in these related species demonstrated that the size of the chromosomes is inversely proportional to their number. From parallel cytological investigations several other workers have already derived a similar theory (HARRISON and DONCASTER, 1914; BELIAJEFF, 1930; FEDERLEY, 1938, 1943; SUOMALAINEN, 1953, 1963; DE LESSE, 1955c). The observations of the other two congeneric forms studied herewith, *Earias insulana* and *E. fabia*, are also in favour of this view. The chromosome garnitures in both the species are identical morphologically as well as numerically (Figs. 42 and 45).

The data on the haploid number of chromosomes of *Lepidoptera* after eliminating the unreliable or uncertain ones have been plotted in the form of the accompanying histogram (Fig. 61); these comprise 393 species including the fifteen described herewith. The *Lepidoptera* (which are mostly characterized by high numbers) furnish an outstanding example of a group which shows a wide range in chromosome number. A glance at the histogram representing the distribution of chromosome numbers in the order at once discloses this striking feature, as the range extends

from n = 8 to n = 191. It is worth noticing here that two lepidopteran species now surpass the hermit crab, *Eupagurus ochotensis* (n = 127), which was formerly regarded to possess the highest haploid number in animals (NIIYAMA, 1951). The Lycaenid species *Lysandra nivescens* (n = 191) now attains a new record of possessing the most elevated num-

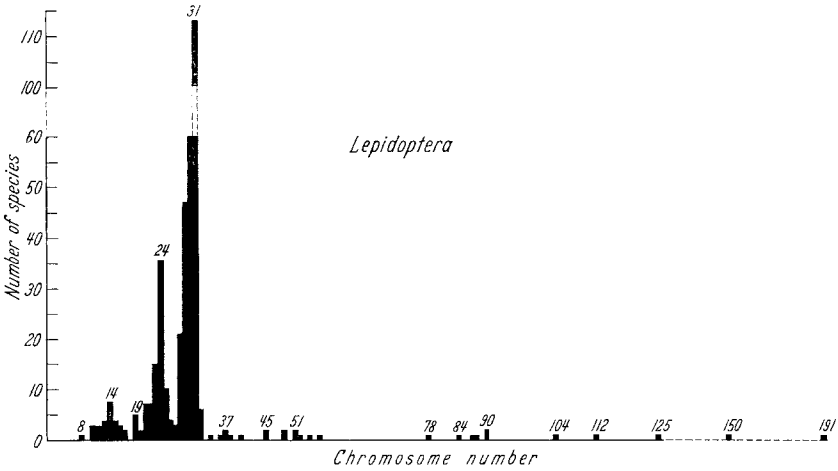


Fig. 61. Histogram, showing haploid chromosome numbers in order *Lepidoptera*

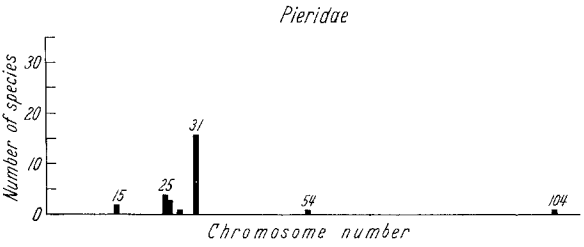


Fig. 62

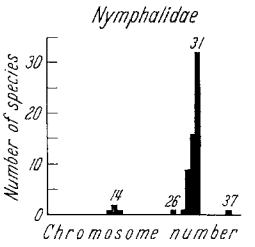


Fig. 63

Fig. 62. Histogram, showing haploid chromosome numbers in family *Pieridae*
Fig. 63. Histogram, showing haploid chromosome numbers in family *Nymphalidae*

ber known in Metazoa up to the present (DE LESSE, 1954b). Secondly, it is curiously noteworthy that the distribution of chromosome numbers is not even throughout. It is evident that the low numbers are more frequent whereas all the numbers higher than 32 are represented merely once or twice. Moreover, the numbers from 8 to 32 are found in a continuous series apart from 9 and 18 (not reported so far), while the distribution of higher numbers is more or less discontinuous and scattered. The third important point which may be derived from the survey of the chromosome numbers is that the majority of the species possess a haploid number of 31 chromosomes. Hence this must be considered as

the modal number for the *Lepidoptera*. Next to 31 other numbers in their order of frequency may be mentioned as 30, 29 and 24. The distribution of haploid chromosome numbers in the families *Pieridae* and *Nymphalidae* is illustrated in Figs. 62 and 63.

The *Lepidoptera* are also remarkable for striking variations in chromosome numbers even in related species or the different geographical races of the same species. The authors have put forward two different alternatives to explain the process through which such variations might have originated:

a) Deviations in related species are produced by polyploidy, and

b) Variations in number result from simple fusions or fragmentations of the chromosomes.

My findings on the spermatogenesis of two species of the genus *Vanessa*, however, appear to indicate support for the latter hypothesis. In my opinion *V. indica* presents a specific instance of the chromosomal fusions in *Lepidoptera*. I have discovered that the chromosome complement of Indian *V. indica* consists of fifteen pairs and not of thirty-one as reported by MAEKI and MAKINO (1953) and MAEKI (1961) for the Japanese individuals of the same species. These authors report this figure for the primary and secondary spermatocytes only, the spermatogonial chromosome number being undetermined. In view of these reports of the Japanese workers I have subjected my material to a close examination which has left me in no doubt that specimens of the Indian *V. indica* possess fifteen pairs of chromosomes only. For an absolute confirmation of the name of the species the specimens were sent to the British museum for identification. As the Japanese authors mentioned above have published no figures and given no details, it is not possible to institute a comparison between the chromosomes of the Japanese and the Indian representatives of this species. This raises the question of whether the Indian and Japanese species of *V. indica* are identical or stand for two geographical races possessing chromosome complements interrelated in such a remarkable way — one being double of the other in number. Since all the other species of *Vanessa* investigated cytologically so far (BELIAJEFF, 1930; FEDERLEY, 1938; LORKOVIĆ, 1941; and MAEKI, 1961), including *V. kashmirensis* studied by me, possess thirty-one pairs of chromosomes, the condition in the Indian *V. indica* must be considered secondary, having arisen probably through fusion of chromosomes. This is borne out by a comparison of the chromosome complents of *V. indica* (Fig. 10) and *V. kashmirensis* (Fig. 17). The chromosomes of the former are appreciably larger than those of the latter so that the total amount of the chromosomal material in the two species seems to be practically the same. Thus the chromosomal study of *Vanessa* species accords with the general observation that in lepido-

pteran related species an increase in number is accompanied by diminution in size of the chromosomes; hence polyploidy is obviously ruled out. It is most likely, therefore, that the chromosome complex of *V. indica* consists of compound chromosomes, each of which is represented by about two chromosomal units of the other species of *Vanessa*. The assumption of a diffuse kinetochore in *Lepidoptera* is in accord with this view, which in turn is in consonance with the fusion and fragmentation hypothesis put forth by several investigators (SEILER, 1914, 1922, 1925; HARRISON and DONCASTER, 1914; MALAN, 1918; SEILER and HANIEL, 1921; DEDERER, 1928; CRETSCHMAR, 1928; BELIAJEFF, 1930; FEDERLEY, 1938; WHITE, 1946, 1954, 1957; SUOMALAINEN, 1953, 1958, 1963; DE LESSE, 1954b, 1955a, 1959a, b; GOLYSHEVA, 1961). A number of instances of closely related species with chromosomes which are numerically so related as the multiples of the same basic number, have been previously reported by several workers. HARRISON and DONCASTER (1914) have reported the haploid numbers as 14 and 56 for two species of a Geometrid genus *Biston*, namely, *B. hirtaria* and *B. zonaria*, respectively. BELIAJEFF (1930) on the basis of a comparison of the chromosomal size in the two, concludes that this does not indicate polyploidy, the high number of *B. zonaria* having arisen through the fragmentation of the *hirtaria*-chromosomes. Moreover, in the genus *Biston* besides 14 and 56, the number 51 (*B. pomonaria* studied by MALAN in 1918) is also represented, which does not fall in the series of polyploidy at all. BELIAJEFF has given a similar explanation of the occurrence of a high number of chromosomes in *Dasychira pudibunda* (n = 87). According to him not only the reduced size of the chromosomes but also the exceptional largeness of two chromosomes in the chromosomal garniture of this species furnishes evidence for the occurrence of the fragmentation process. The large chromosomes have retained their originality and have not undergone fragmentation. DE LESSE in his recent series of papers has reported upon the chromosome numbers of several species of *Lysandra* and *Erebia* and shown that these do not, in either of the two genera, necessarily form a series representing the multiples of the same fundamental number. Besides, a noticeable reduction in size of the chromosomes of the species with high numbers has also been recorded. He has, therefore, come to the conclusion that all these do not fall into line with the hypothesis of a phenomenon of polyploidy and holds that the high chromosome numbers result from fragmentation.

LORCOVIĆ (1941, 1949) is the only worker who considers the species with high chromosome numbers as polyploid forms. He claims that the evidence favour the supposition of the occurrence of polyploidy in three lepidopteran genera — *Lycaena*, *Leptidea* and *Erebia*. LORKOVIĆ's hypo-

thesis was criticized by WHITE (1946, 1954, 1957). According to him the presence of single large bivalent both in *Lycaena bellargus* (n = 45) and *L. coridon* (n = 90) argues against their being polyploids (as one should expect two such bivalents in a tetraploid and four in an octaploid garniture). Moreover, these species are often placed under a separate

Table 2

Species	Haploid number	Investigators
Erebia calcarius	8	DE LESSE, 1954a, 1955b
E. cassioides murina, *E. tyndarus tyndarus*	10	DE LESSE, 1953b, c
E. medusa, *E. nivalis*	11	FEDERLY, 1938; LORKOVIĆ, 1941; DE LESSE, 1954a, 1955b, c
E. gorgone, *E. mnestra*	12	DE LESSE, 1953b, c
E. oëme, *E. meolens*	14	LORKOVIĆ, 1941; DE LESSE, 1953c
E. callias	15	DE LESSE, 1954a, 1955b, c
E. epiphron cassiope, *E. serotina*	17	LORKOVIĆ, 1941; DESCIMON and DE LESSE, 1954
E. pharte, *E. glacialis*, *E. pronoë*	19	LORKOVIĆ, 1941
E. melas, *E. gorge*, *E. aethiops*	21	LORKOVIĆ, 1941
E. nerine, *E. scipio*, *E. lefebvrei*	22	LORKOVIĆ, 1941; DE LESSE 1953b, c
E. hispana rondoni	24	DE LESSE, 1953b, c
E. lappona, *E. pandrose sthennyo*	28	FEDERLY, 1938; DE LESSE, 1953c
E. ligea, *E. disa*, *E. manto constans*, *E. euryale*	29	FEDERLY, 1938; DE LESSE, 1953c; DESCIMON and DE LESSE, 1954
E. ottomana	40	LORKOVIĆ, 1941
E. iranica	51	DE LESSE, 1955c

genus *Lysandra*, the members of which are characterized by the possession of a large number of small chromosomes presumably produced by repeated fragmentations of the basic chromosome set of the family *Lycaenidae* (modal haploid number = 24). This fact has been confirmed by the fairly recent studies of DE LESSE (1952, 1953a, 1954b) on some more species of this genus. During these investigations haploid numbers ranging from 45 to 191 have been encountered, which cannot be regarded as the multiples of the same basic number. The high numbers of *Leptidea* have been similarly attributed to fragmentations of the chromosomes, by WHITE; here again the occurrence of a single pair of large

chromosomes in the garniture of *L. duponcheli* renders the acceptance of polyploidy difficult. WHITE has raised an insuperable objection against the hypothesis of polyploidy through the fact that when all the forms of a lepidopteran genus are studied cytologically they usually present a graded series of chromosome numbers. From a glance at the serial arrangement of the haploid numbers in different species of the most extensively studied genus *Erebia*, given below, the correctness of WHITE'S statement becomes clear and consequently the idea of polyploidy is effectively negatived.

SUOMALAINEN (1953, 1958, 1963) did also not favour LORKOVIĆ'S conjecture of polyploidy. On the basis of relative numbers and sizes of chromosomes in closely related species, and the presence of a diffuse kinetochore, he was inclined to believe that a variation in chromosome numbers of related lepidopteran species is caused by fragmentation and fusion. There is, therefore, no creditable evidence for the occurrence of polyploidy in any species of *Lepidoptera* up to now. The accounts given by LORKOVIĆ are now considered doubtful and are interpreted differently.

Sex-chromosome mechanism

Information on the sex chromosomes of *Lepidoptera* is very meagre, as in most of the species these are cytologically indistinguishable. Unlike most other animal groups, a digamety in the males has not been established in any of the species described so far (including the present fifteen), as no heteromorphic pair of chromosomes or an odd chromosome has been discovered.

A remarkable phenomenon of heteropycnosis exhibited by one bivalent has been reported by some authors during the growthphase of the primary spermatocytes and oocytes of various species. According to these authors this heteropycnotic pair of chromosomes actually represents a homomorphic sex-chromosome pair, which can be distinguished from the autosomes only by its peculiar relation with the nucleolus. As regards the nature of this relationship there are two different views. The holders of the first view believe that the nucleolus of the growth phase nucleus is a chromosome nucleolus which itself represents the heteropycnotic sex-chromome pair (STEVENS, 1906; COOK, 1910; DONCASTER, 1911, 1912a, b; HARRISON and DONCASTER, 1914); while the exponents of the other view express the opinion that the nucleolus is composed of a plasmosome associated with a heteropycnotic pair of chromosomes (DEDERER, 1907, 1928 in the spermatogenesis of *Philosamia cynthia*; KAWAGUCHI, 1928 in the oogenesis of *Bombyx mori*; KÜRIHARA, 1929, and KAWAGUCHI, 1933, 1937). The authors mentioned above have stated that at the end of the growth-phase the nucleolus gives rise to a bivalent

which is not differentiated from the others in the subsequent stages. Keeping these reports in mind I have made a close examination of the deeply stained, Feulgen-positive irregular body observed during the diffuse stage in all the fifteen forms studied herewith, but I have failed to demonstrate the origin of any bivalent from it. It cannot be stated with absolute certainty that whether the Feulgen-positive material of this body represents the extremely condensed mass formed by all the chromosomes or it is a part of the nucleolus itself, but a close study reveals that the first probability seems to be more likely. I could not establish, therefore, a relationship between the nucleolus and the sex-chromosome pair during my investigations. This is in accord with the view of several other investigators who have denied the existence of any such relationship (FEDERLY, 1913; SEILER, 1914; KERNEWITZ, 1915; DEDERER, 1915 in the oogenesis of *Philosamia cynthia*; MALAN, 1918; MACHIDA, 1926; CRETSCHMAR, 1928; KAWAGUCHI, 1928, in the spermatogenesis of *Bombyx mori*).

Summary

1. Cytological studies have been carried out during spermatogenesis of fifteen species.

2. A peculiar synizesis phase occurs normally in the early prophase stage of primary spermatocytes.

3. The homologous chromosomal threads undergo a longitudinal pairing during synizesis.

4. Typical diplotene stage has not been discovered; instead, a diffuse phase intervenes between pachytene and diakinesis.

5. In the diakinetic bivalents the presence of one or two completely terminalized chiasmata can be justified. There are a few instances, however, where certain bivalents are precociously resolved intou nivalents disclosing most probably a failure of chiasma-formation in them. The question, therefore, whether definite chiasmata are formed in *Lepidoptera* cannot be answered with absolute certainty.

6. By a comparative study of the chromosomal garnitures in the congeneric forms investigated herewith, such as *Vanessa indica* and *V. kashmirensis*, and *Earias insulana* and *E. fabia*, it has been concluded that a number discrepancy in related species must be attributed to fusions and fragmentations of original chromosomes and not to polyploidy.

7. A male homogamety occurs in all the species studied.

8. Any relationship between a sex-chromosome pair and the nucleolus could not be established.

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Dr. Y. GUPTA,
Department of Zoology, University of Allahabad,
Allahabad, India