Karyotype and meiotic mechanism in Muga silkmoths, Antheraea compta Roth. and A. assamensis (Helf.) (Lepidoptera: Saturniidae)

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Abstract

The chromosome number in males of *Antheraea compta* (Wild) and *A. assamensis* (semi-domesticated) is 30, while in females it is 30 (XY) in *A. compta* and 29 (XO) in *A. assamensis*, the latter concomitantly does not reveal sex chromatin in interphase nuclei of both somatic and germ cells. This confirms that the Y chromosome, which forms the sex chromatin in females of *A. compta*, has been lost in the other species. Meiosis in males shows discrete chiasmata while in females of both species it is achiasmatic. Meiotic details of the species are given and the evolutionary inter-relationship is discussed.

Introduction

Despite its great commercial importance the family Saturniidae has received little attention in cytogenetical studies because of the difficulty in procuring the different stages of these insects, the generally high number and small size of the chromosomes characteristic of Lepidoptera, and the drawbacks of the sectioning and squashing techniques available to earlier workers. So far preliminary information regarding chromosome numbers in this family is available for 25 species out of about 1000 recorded (see Traut & Mosbacher, 1968; Robinson, 1971; Ennis, 1976; Gupta & Narang, 1980). The nature of the centromere has been reported in some lepidopterans either as localized or diffuse in light as well as electron microscopic studies (Suomalainen, 1953; Bauer, 1967; Friedländer & Wahrman, 1970; Danilova, 1973; Murakami & Imai, 1974; Bigger, 1975, 1976; Rishi & Rishi, 1979). The sex chromosome mechanism in Lepidoptera is generally XX ♂/XY ♀ or XX ♂/XO ♀ and rarely XX ♂/XY_1Y_2 ♀ (Smith, 1945; Traut & Mosbacher, 1968; Suomalainen, 1969a, 1971; White, 1973; Ennis, 1976; Narang & Gupta, 1979b; Gupta & Narang, 1980).

In Saturniidae details of the meiotic cycle have been described in one species only, *Philosamia cynthia* (Dederer, 1907, 1915; Narang & Gupta, 1979b). The achiasmatic mechanism, characteristic of lepidopteran females, has also been reported in *P. c. ricini* (Narang & Gupta, 1979b).

Two species of the genus *Antheraea, A. compta* and *A. assamensis*, have been worked out for detailed chromosome studies for the first time; the former is a wild species and the latter is a semi-domesticated one of great commercial importance because of the precious golden-hued muga silk that it produces.

Material and methods

The larvae and the live cocoons of *A. assamensis* were collected at the muga seed farm Khanapara (Assam) during October-November, 1978 and September-October, 1979 on the host plant 'Som',

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Machilus bombycina. Material of the other species, *A. compta,* was collected from the forest near the village Hahim, District Kamrup (Assam) from 'Sualu', *Litsaea polyantha* during September-October, 1979. The slides were prepared from testes, ovaries and brain tissues of both larvae and pupae by the heat-dry smear method (Narang & Gupta, 1979a) with certain modifications. The detailed procedure is as follows. The tissue is pretreated for 10–15 minutes in hypotonic solution (0.9% sodium citrate) and then fixed in methanol: acetic acid (3:1) for 15–30 minutes. The material is then placed on prewarmed (45 ° C to 50 ° C) slides (already cleaned in chromic acid) in 3–4 drops of 45% to 50% acetic acid. The tissue begins to swell and becomes translucent, and is teased into small fragments with the help of fine needles. A drop of fixative is added to the suspension for better spreading. The suspension with little intact tissue or cell clumps is allowed to stay for 15 to 30 seconds and is then drawn into a Drummond pipette or dropper leaving a thin circular film on the slide containing a monolayer of the cells. The drawn suspension is again placed on the warm slide, now at new sites. After about 30 seconds the excess is again drawn up. The process may be repeated 2–3 times over the available space of the slide. The residual liquid is at last drawn up and discarded. During the above process the slide is kept warm to accelerate evaporation of the liquid as otherwise the cells overlap and the tissue gets distorted if left too long in acetic acid. The slides are air-dried for at least 24 hours and then stained with 2–5% Giemsa diluted in Sørensen's buffer (pH 6.8) for 15–30 minutes. However for the diffuse stages, late prophase I of females and diplotene of males, the slides were treated with glacial acetic acid for 30–45 seconds (to reduce cytoplasmic staining), air-dried and stained in 10–15% Giemsa. For revealing the position of the centromere 0.05 ml of a 0.05% colchicine solution was injected into the larvae and pupae, which were then kept for 3–4 hours in the dark before being sacrificed.

Observations

Both species revealed similar chromosomal complements and meiotic cycles as far as analysed, except for the loss of the Y-chromosome in the females of *A. assamensis.*

Karyotypes (Figs. 1–8)

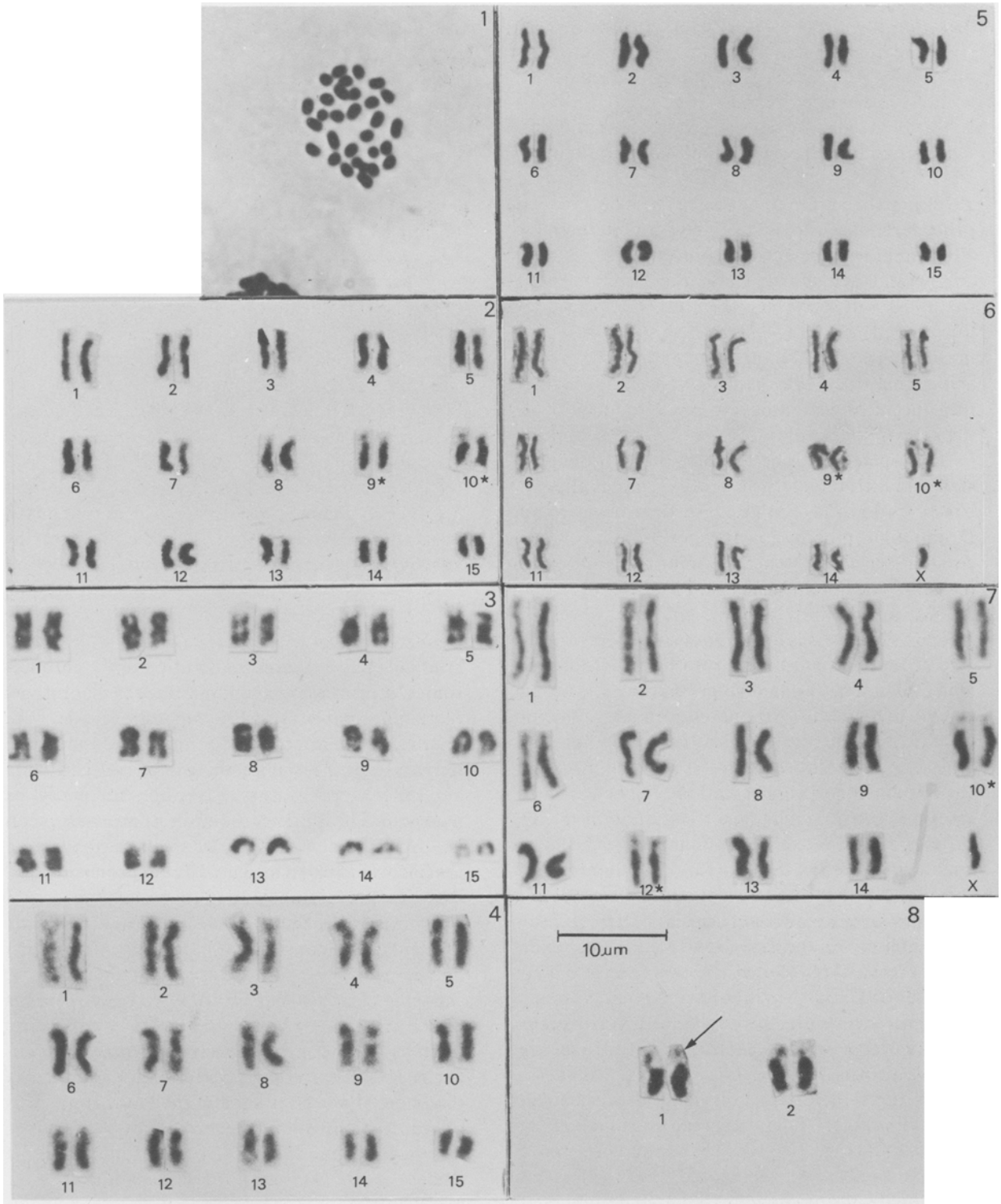
The diploid chromosome number is 30 except in the females of *A. assamensis,* in which it is 29. The chromosomes intergrade in length. At mitotic metaphase (Fig. 1) they appear spherical and rod-shaped; their actual length ranges from 0.94 μm to 2.01 μm. The karyotypes have been prepared from mitotic premetaphase stages of testis, ovary and neuroblast cells with the chromosomes in decreasing order of length (Figs. 2–8). The length of the different chromosomes was expressed as percentage of the total length of the haploid complement. The average relative lengths of the chromosomes of *A. compta* calculated from 7 cells were 9.07, 8.27, 7.97, 7.56, 7.38, 7.13, 6.90, 6.71, 6.45, 6.29, 6.12, 5.67, 5.24, 4.86 and 4.3%, while in *A. assamensis,* (15 cells), they were 9.42, 8.63, 8.06, 7.72, 7.24, 7.05, 6.88, 6.63, 6.4, 6.12, 5.86, 5.61, 5.25, 4.82, and 4.25%. From the above data it is evident that on the basis of relative length of the chromosomes the karyotypes of the two species are similar. Primary constrictions (localized centromeres) were observed in the chromosomes of *A. compta* at mitotic premetaphase of neuroblast cells (Fig. 3) prepared after colchicine treatment. In this species chromosomes Nrs 1 to 9 are submetacentric and the remaining, Nrs 10 to 15, are acrocentric. The karyotypes of the two species are further characterized by the presence of two pairs of satellite chromosomes (Fig. 8) whose serial numbers fall in between 9 and 12. The sex chromosome mechanism was found to be XX ♂: XO ♀ in *A. assamensis* on the basis of identification of the X chromosome as the smallest element of the complement, unaccompanied by the Y in the female sex.

Sex chromatin

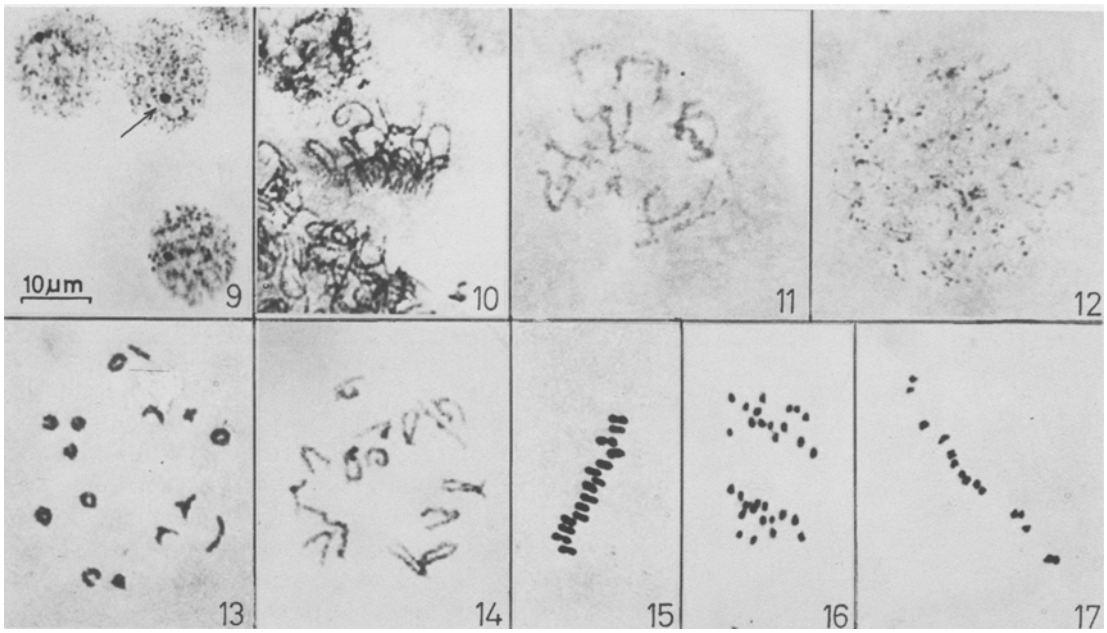
The interphase nuclei including those approaching prophase display a distinct positively heteropycnotic and peripherally placed sex chromatin body (Fig. 9) in germ as well as somatic cells of only the females of *A. compta.* Such a body is lacking in *A. assamensis.*

Meiotic cycle

The meiotic cycle of the two species is similar and the important stages can be characterized as follows:



Figs. 1–8. Mitotic chromosomes of *Antheraea compta* (1–4, 8) and *A. assamensis* (5–7); (1) Mitotic metaphase, 2n = 30; – (2–7) Karyotypes prepared from spermatogonia (2, 5), ♀ neuroblasts (3, 6) and oogonia (4, 7); – (8) Satellite chromosomes from oogonial prometaphase, arrow; secondary constriction.
*Satellite chromosomes

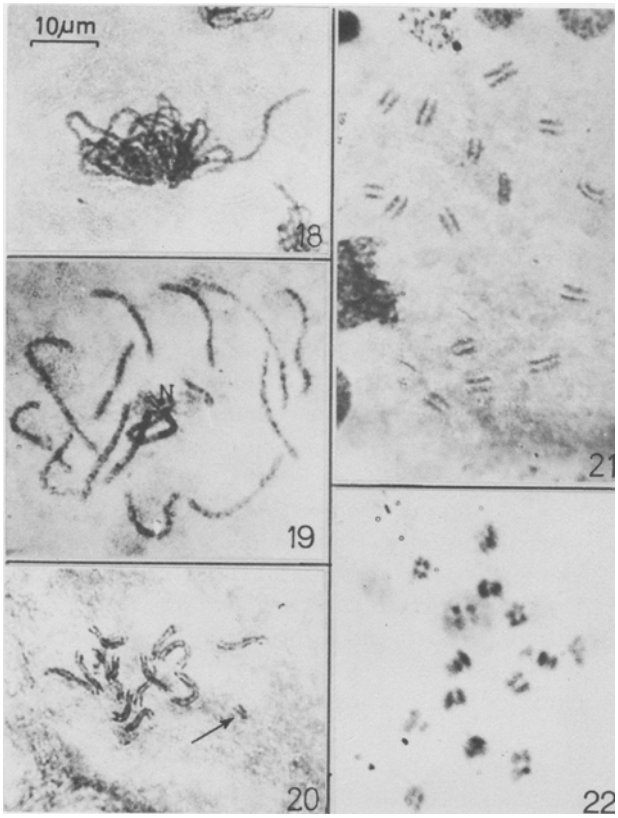


Figs. 9–17. Interphase nuclei of female (9, arrow: sex chromatin), and male (10–17) meiotic chromosomes of *Antheraea compta* (9, 14) and *A. assamensis* (others): (10) Bouquet stage; (11) Late pachytene with 15 bivalents; (12) Diffuse stage; – (13) Diakinesis; – (14) Late diplotene; – (15) Metaphase I with 15 bivalents; (16) Anaphase I; – (17) Metaphase II with 15 chromosomes.

During male meiosis the leptotene chromosomal threads undergo tight coiling to form a synizetic knot which loosens and clear looped post-synaptic threads, forming a characteristic monopolar bouquet (Fig. 10), become visible. During mid-prophase the bouquet disorientates and the thickened chromosomes spread out in the nuclear cavity (Fig. 11). Fifteen bivalents can be distinguished. The bivalents now enter the diffuse (early diplotene) stage (Fig. 12), during which their stainability is reduced and they appear diffuse. Diakinesis in *A. assamensis* (Fig. 13) and late diplotene in *A. compta* (Fig. 14) reveal rod-shaped, cross-shaped, and ring-shaped bivalents. The rings having two chiasmata range in number from 4 to 6 with an average number of 5 per cell. The mean chiasma frequency per cell thus is 20. Metaphase I (Fig. 15) shows fifteen dumb-bell shaped bivalents with terminalized chiasmata. They undergo disjunction at anaphase I (Fig. 16) and 15 homologues move towards each pole. Meiosis II (Fig. 17) is an equational division showing the haploid number of the chromosomes.

In female meiosis also, the early pachytene is characterized by the formation of a monopolar bouquet (Fig. 18). The polarization of the chromosomes disappears in mid- and late pachytene. During pachytene (Fig. 19) two of the bivalents are regularly seen attached to a nucleolus and in the karyotype their serial numbers lie in between 9 and 13. Fifteen chromosomal threads can easily be made out. The X chromosome in *A. assamensis* can be distinctly identified as the smallest isopycnotic univalent (Fig. 20) which usually appears bent upon itself. From late pachytene to premetaphase I a clear reductional split is visible in each of the bivalents, the constituent homologues of which remain parallelly aligned all along their length, showing their achiasmatic nature. In *A. compta* fifteen achiasmatic bivalents were observed during premetaphase (Fig. 21) indicating complete pairing of the sex chromosomes. Metaphase I and further stages could not be studied in the yolky eggs.

The development of the nurse cells after pachytene is, however, different from that of oocytes as studied in *A. assamensis*. The chromosomes on reaching metaphase (Fig. 22), when, interestingly, the nucleolus persists, undergo multiplication to form endopolyploid cells whose ultimate fate is disintegration while nursing the oocytes.



Figs. 18–22. Female meiotic chromosomes of *Antheraea compta* (21) and *A. assamensis* (others): (18) Bouquet stage; – (19) Pachytene, two bivalents attached to nucleolus, N; – (20) Late pachytene, arrow: bent univalent X chromosome; – (21) Prometaphase I with 15 achiasmatic bivalents; – (22) Metaphase I, nurse cell.

Discussion

The chromosome numbers of about 25 species of saturniids have been reported so far. The haploid number ranges from 13 in *Philosamia cynthia* (Dederer, 1907, 1915; Traut & Mosbacher, 1968) to 49 in *Antheraea pernyi* (Kawaguchi, 1933; Jolly *et al.*, 1970) with a probable modal number of 31 suggested for the family Saturniidae (Narang & Gupta, 1979a) as well as for Lepidoptera (Suomalainen, 1969b). In the genus *Antheraea* the haploid chromosome numbers of 9 species, including the present report, out of the 32 on record (Crotch, 1956) are now known (see Gupta & Narang, 1980). The lowest haploid chromosome number (15) is found in *A. compta* and *A. assamensis*, the highest (49) in *A. pernyi*, 30 in *A. polyphemus* and 31 in the other five (*A. mylitta, A. roylei, A. frithi, A. yamamai* and *A. sivalica*).

From this information it is evident that the haploid number 31, the suggested modal number for the Saturniidae, is the commonest in *Antheraea* and seems to represent the modal number of the latter also. While comparing the size of the chromosomes among different species of *Antheraea*, it has been observed that in *A. assamensis* and *A. compta*, with less than half the number of the chromosomes of the other species, the size of the chromosomes is nearly double that of *A. mylitta* or *A. roylei*, both with n = 31. This indicates the occurrence of chromosomal fusions or fragmentations (at least not polyploidy) during the evolution of *Antheraea* species. Jolly *et al.* (1974), considering the presence of diffuse centromeres in Lepidoptera, believed *A. assamensis* to be an ancestral form on the ground of its very low chromosome number and its morphological features. They suggested the evolution of other species with higher chromosome numbers from it by chromosome fragmentations. However, it is being reported for the first time in *A. compta* that the chromosomes have localized centromeres, which is in disagreement with the above view of frequent occurrence of fragmentations in the genus. Besides, it seems more convincing that such a low chromosome number (n = 15), present only in *A. compta* and *A. assamensis*, both closely related (see discussion below), might have evolved from a more commonly occurring chromosome number (n = 31) by a mechanism of chromosomal fusion rather than vice-versa.

The sex chromosome mechanism in saturniids has been reported only in *Anisota* (= *Dryocampa*) *rubicunda* and two subspecies of *Philosamia cynthia*. It is XX ♂ : XY ♀ in these two, (Traut & Mosbacher, 1968; Ennis, 1976) and XX ♂ : XY ♀ and XX ♂ : XO ♀ in the Titabar and Borduar-Dhenubhanga populations respectively of *P. c. ricini* (Narang & Gupta, 1979b; Gupta & Narang, 1980). In the present paper the sex chromosome system was found to be XX ♂ : XY ♀ in *A. compta* and XX ♂ : XO ♀ in *A. assamensis*, on the basis of chromosome number, chromosome size and sex chromatin. *A. compta*, which possesses an even number of chromosomes in both sexes and is also sex-chromatin positive in the females is likely to have an XX ♂ : XY ♀ system. The smallest pair of chromosomes in this species, assuming the ho-

mology of the karyotypes of the two species, is expected to represent the sex chromosomes likewise.

The presence of a sex chromatin body in the interphase cells of females of *A. compta,* and its absence in that of *A. assamensis,* accompanied by loss of a chromosome from the set, shows that the body is formed by the Y chromosome as suggested earlier for some of the other saturniids (Gupta & Narang, 1980). The karyotypes of the two species differ only by presence or absence of the Y-chromosome, whereas the meiotic cycles show similar characteristics. *A. assamensis,* the semi-domesticated form, in every probability seems to have evolved from *A. compta,* the wild form, by the loss of the Y-chromosome.

The nature of the kinetochore in lepidopterans has been a controversial issue. The earlier workers described it as diffuse by light-microscopic studies (Federley, 1945; Suomalainen, 1953, 65, 69a; Murakami & Imai, 1974). Bauer (1967) studied the effect of ionizing radiation on *Pieris brassicae* and *Philosamia cynthia* and reported the survival of chromosomal fragments in the next generation. On this evidence he reported the chromosomes to be holocentric. However, recent workers have studied lepidopteran chromosomes by light microscopy after employing hypotonic and colchicine pretreatment or by electron microscopy. Their work has revealed primary constrictions/localized centromeres in mitosis and/or meiosis II (Danilova, 1973; Gassner & Klemetson, 1974; Bigger, 1975, 1976; Rishi & Rishi, 1979 and the present paper). Friedländer & Wahrman (1970) studied the meiosis I chromosomes of *Bombyx mori* by electron microscopy and interestingly did not find the centromere, localized or diffuse. They, however, cautioned that the possibility of the presence of 'some kind of a centromere' concealed within the chromosomes should not be disregarded. No clear report has so far appeared on the presence in Lepidoptera of a diffuse centromere having a definite structure observable by electron microscopy comparable to that described in mitotic chromosomes of the hemipterans (Buck, 1967; Comings & Okada, 1972). Therefore it is urgently recommended that earlier reports of diffuse centromeres in mitosis and meiosis II of lepidopterans should be subjected to reinvestigation adopting the modern methodology. In the reports on hemipterans (*loc cit.*) the diffuse kinetochore plate or material has not been seen to be present during meiosis I. This situation is perhaps similar to that existing in meiosis I of *Bombyx mori* (Friedlander & Wahrman *op. cit.*): It seems likely that the kinetochore in the meiosis-I chromosomes of hemipterans and lepidopterans has become too diffuse to be seen. This possibility has also been suggested by Gassner & Klemetson (1974). However, their alternative suggestion,that the centromere may not be a requirement in meiotic disjunction, seems untenable since the role of this organelle is known to be essential in congression and disjunction of chromosomes in animals.

It has been reported in some of the lepidopterans that there occurs regularly a characteristic synizesis stage during early prophase of primary spermatocytes and oocytes (Doncaster, 1912; Maeda, 1939; Gupta, 1964) which for a long time had, however, been considered to be an artifact (Wagner, 1931).

In both the species reported here, there is a clear synaptic knot, formed by extreme coiling of the chromosomal threads. The existence of the diffuse stage in meiosis I of both species is also known from some other lepidopterans (Maeda, 1939; Gupta, 1964). This stage has been described as a regular feature of meiosis in several plants and animals studied (Klasterska, 1976, 1978).

Meiosis in the two species studied here is chiasmatic in males and achiasmatic in females, the bivalents of the latter exhibit a clear reductional split from late pachytene to premetaphase I. This has also been reported in some other lepidopterans (see White, 1973 pp. 493–494).

It is desirable that karyotypic and meiotic studies in more species of *Antheraea* should be carried out for further exploration of the chromosomal evolution in the genus.

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