# THE CHROMOSOMES OF A WILD SILKMOTH, ARCHAEOATTACUS EDWARDSII, WITH A RECORD HIGH CHROMOSOME NUMBER FOR SATURNIIDAE

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**ABSTRACT.** The diploid chromosome number of *Archaeoattacus edwardsii* (White), new to chromosome cytology, is 128, the highest so far reported in the family. The karyotype is characterised by the presence of two exceptionally larger (probably sex) chromosomes. The sex mechanism is XX  $\delta$  : XY  $\Im$ . The females show a prominent sex chromatin body in germ as well as somatic cells. The evolutionary status of the species has been discussed.

Besides the usually high number and smaller size of the chromosomes, the cytogenetical studies in moths also involve difficulties regarding the procurement of stages from caterpillar to adult, as the mitotic and the male meiotic divisions are passed before the adult stage. So far, the chromosome numbers of only 30 saturniid species (including the present report), belonging to 16 genera (Table 1) out of about 1000 species recorded, are known. Interestingly, the chromosome number in *Archaeoattacus edwardsii* (White) is the highest. The karyotype and the sex chromatin in this species are described here for the first time.

## MATERIAL AND METHODS

For the present study the cocoons were collected from Khasi Hills (Meghalaya) during Sept.–Oct. 1979. The slides were prepared from the gonads and brain tissues of both sexes. Some cocoons were also raised to the adult stage for the systematic determination of the species. Giemsa-stained cytological preparations were made by heat-dry smear technique (Narang & Gupta, 1979a), using, however, a 5% concentration of the stain. Some of the preparations were made after injection of 0.1 ml of 0.05% colchicine for 2.30 to 3 h.

## **OBSERVATIONS**

The diploid number of chromosomes has invariably been found to be 128 at mitotic metaphase in germinal (Figs. 1 & 2) as well as neuroblast cells (Fig. 3) in as many as 15 cells of 6 males and 20 cells of 3 females. The same chromosome number has been confirmed by the count of 64 bivalents in 5 male diakinetic cells (Fig. 4). The karyo-

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FIGS. 1–5. Chromosomes of Archaeoattacus edwardsii: 1, spermatogonial metaphase; 2, oogonial metaphase from colchicinized individual showing localized centromeres; 3, mitotic metaphase from neuroblast cell ( $\mathfrak{P}$ ); 4, diakinesis ( $\mathfrak{F}$ ); 5, oogonial interphase showing sex chromatin (SC). Arrows indicate larger (sex ?) chromosomes. Bar represents 10  $\mu$ m.

type is further characterized by the presence of two exceptionally larger chromosomes. The average length of these chromosomes is nearly the same (i.e., 2.5  $\mu$ m in male and 2.5 & 2.7  $\mu$ m in female). The smaller chromosomes range in length from 0.5  $\mu$ m to 1.25  $\mu$ m.

The mitotic metaphase chromosomes of *edwardsii* have been found to be of monocentric type as studied in the colchicinized preparations. Fig. 2 reveals an interstitial centromere in some of the chromosomes; whereas, the position of the centromere in others is not discernible, due to their smaller sizes.

A prominent, large-sized and positively heteropycnotic sex chromatin body has been clearly observed in interphase and prophase cells of brain and germ tissues of the females. The average size of sex chromatin at the interphase stage (Fig. 5) is  $1.15 \ \mu$ m.

#### DISCUSSION

The presence of the same and even number of the chromosomes in both the sexes and the formation of a sex chromatin body in the females indicates the sex chromosome mechanism in *Archaeoattacus edwardsii* to be XX  $\mathcal{S}$  : XY  $\mathcal{P}$ , as concluded for eight other saturniid species (Gupta & Narang, 1980a; Gupta & Narang, 1981); the sex chromatin, formed both in germ and somatic cells, represents the Y-chromosome.

Bauer (1943) and White (1957) suggested that the big chromosome frequently found in Lepidoptera is a sex chromosome, at least in those species which have a very high chromosome number. In the family Saturniidae to which the present species belongs, the modal chromosome number is n = 31, which has been reported in 18 species out of the 30 investigated so far (Table 1). The two exceptionally larger chromosomes of this species, with n = 64, seem to, likewise, represent the sex chromosomes. The difference in the size of these chromosomes compared to the autosomes is probably because the sex chromosome mechanism in this group is nicely balanced and fission or other such rearrangements in sex chromosomes may disturb the mechanism. This is also clear from the fact that there are very few cases in Lepidoptera in which multiple sex chromosomes are known.

In the tribe Attacini of the subfamily Saturniinae, seven species, four from genus *Hyalophora* and one each from *Philosamia*, *Callosamia* and *Archaeoattacus*, have so far been cytogenetically worked out. The haploid chromosome number in all the species of *Hyalophora* is 31, while it is 13–14 in *Philosamia cynthia* (Drury) (in its different races), 19 in *Callosamia promethea* (Drury) and 64 in *A. edwardsii*. Further work in other genera and species of Attacini is urgently needed for exploring the evolutionary trends in this tribe.

S. No.	Name of the species	Haploid chromosome number	References (First report)
	SUBFAMILY: SATURNIINAE		
	TRIBE: SATURNIINI		
1.	Actias selene	31	Deodikar et al. (1969)
2.	A. luna	31	Unpublished
3.	Antheraea assamensis	15	Deodikar et al. (1962)
4.	A. compta	15	Gupta & Narang (1981)
5.	A. frithi	31	Jolly et al. (1977)
6.	A. mylitta	31	Sinha & Jolly (1967)
7.	A. pernyi	49	Kawaguchi (1933, 34)
8.	A. (=Telea) polyphemus	30	Cook (1910)
9.	A. roylei	31	Jolly et al. (1970)
10.	A. sivalica	31	Jolly et al. (1978)
11.	A. yamamai	31	Kawaguchi (1933)
12.	Cricula trifenestrata	31	Narang & Gupta (1979a)
13.	Dictyoploca cachara	30	Narang & Gupta (1979b)
14.	D. japonica	31	Oba (1942) (cited by Makino, 1951)
15.	D. simla	31	Unpublished
16.	Graellsia isabelae	31	Templado et al. (1975)
17.	Loepa katinka	28	Narang & Gupta (1979c)
18.	Saturnia pyri	30	Pariser (1927)
19.	Sonthonnaxia maenas	31	Narang & Gupta (1979d)
20.	Eriogyna pyretorum	30	Gupta & Narang (1980b)
21.	Eudia (=Saturnia) pavonia	29	Kernewitz (1915)
	TRIBE: ATTACINI		
22.	Archaeoattacus edwardsii	64	(Present work)
23.	Philosamia cynthia	13–14	Dederer (1907, 15); Deodikar & Thakar (1958)
24.	Hyalophora (=Platysamia) cecropia	31	Bytinski-Salz (1938)
25.	H. euryalis	31	Bytinski-Salz (1938)
26.	H. gloveri	31	Bytinski-Salz (1938)
27.	H. columbia	31	Bytinski-Salz (1938)
28.	Callosamia promethea	19	Cook (1910)
	SUBFAMILY: HEMILEUCINAE		
	TRIBE: HEMILEUCINI		
29.	Automeris io	31	Cook (1910)
	SUBFAMILY: CITHERONIINAE		
30.	Anisota (=Dryocampa) rubicunda	31	Ennis (1976)

TABLE 1. Known haploid chromosome numbers for species of Saturniidae.

In the sister tribe Saturniini the probable modal number as indicated by the available data (Table 1) is n = 31, which is also the modal number for Lepidoptera as a whole (Suomalainen, 1969; White, 1973). From this it can be assumed that the same number, n = 31 (at present known only from genus *Hyalophora*), might be the ancestral number of the tribe Attacini, and the lower chromosome number of *Philosa-mia cynthia* (n = 13–14) and *Callosamia promethea* (n = 19), and the higher number for *A. edwardsii* (n = 64) might have evolved from this number by the mechanism of chromosomal fusions and fissions respectively (see discussion below). The presence of a single pair of exceptionally larger (probably sex) chromosomes and simple XX  $\delta$  : XY  $\varphi$  sex mechanism in *A. edwardsii* speaks against an incidence of polyploidy.

Bigger (1975, 76) clearly showed localized centromeres in the mitotic chromosomes of five butterflies and pointed out that there are two types of centromeric organizations. According to him at early mitotic metaphase the chromosomes exhibit a monocentric organization, either exclusively or, more probably, with one portion of the diffuse centromere exerting a dominant influence over the remainder. He further stated that with the advancement of metaphase the influence of this centromere is either lost or superceded by the combined influence of the rest of the centromere. Several other authors (e.g., Danilova, 1973; Gassner & Klemetson, 1974; Rishi & Rishi, 1979; Gupta & Narang, 1981) have also reported clear primary constrictions (localized centromere) at mitotic and/or meiotic II metaphases in several Lepidoptera. But there has appeared absolutely no report, either by light microscopy or by electron microscopy, about the localized nature of the centromere at metaphase I in this group. This dual centromeric organization and the presence of morphologically distinguishable sex chromosomes in Lepidoptera probably explains why the chromosome numbers in this group are less variable than the diffuse kinetochore (e.g., in *Luzula*) would at least theoretically allow.

It is a point of common observation in saturniids, like several other lepidopterans (see Gupta, 1964; Suomalainen, 1979), that the chromosomes of high-numbered species are always smaller than the chromosomes of low-numbered species. This evidence and the presence of the non-localized nature of centromere, at least at metaphase I, probably indicates the occurrence of fission. The possibility of the other structural rearrangements like inversions, translocations, etc. cannot, however, be disregarded.

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