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Chromosome Studies on Four Species of Moths

Ranjana Thakur, and D. C. Gautam*

Department of Biosciences, Himachal Pradesh University, Shimla 171005, India

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Summary Moths belonging to the order Lepidoptera have high numbers of small-sized chromosomes which are holocentric. The modal chromosome number in Lepidoptera ranges from n=29 to n=31 but variations have also been found. This order has two types of sex determination systems: (i) WZ ♀; ZZ ♂ and (ii) OZ ♀; ZZ ♂. Lepidopterans are the subject of great interest because of the pest status of many of its species. The present work was undertaken to study the chromosomes of the following moth species from Himachal Pradesh: *Cnaphalocrocis medinalis* G., *Euproctis chrysorrhoea* L., *Idaea aversata* L., and *Pygospila tyres* C. The haploid chromosome numbers observed in *C. medinalis*, *E. chrysorrhoea, I. aversata,* and *P. tyres* were 30, 31, 26, and 28, respectively. The karyotypes of all the species showed holocentric chromosomes. A long pair of chromosomes was found in the karyotype of all species. Other chromosomes showed a gradual decrease in size or were almost of the same size.

Key words Lepidoptera, Holocentric chromosomes, Karyotype, Indian moth.

The cytogenetics of Lepidoptera has attracted the attention of many scientists because of the pest status of the majority of the species in this order. About 130,000 Lepidoptera species are known all over the world but the cytogenetics of only a few of them have been investigated (Traut *et al.* 2007). The species of this group have high numbers of small-sized chromosomes. Chromosomes lack a distinct centromere and are thus holocentric in nature (Bedo 1984).

The modal chromosome number in Lepidoptera ranges from n=29 to n=31 (Robinson 1971). Variations in the haploid chromosome number have also been found to range from n=7 in *Erebia aethiopellus* to n=220 in *Lysandra atlantica* (White 1973).

One system of sex determination in Lepidoptera is WZ/ZZ in which females are WZ and males are ZZ; the other system is OZ/ZZ in which females lack the W chromosome (Traut and Mosbacher 1968). The cytogenetic work on Indian Lepidoptera was started in the early sixties by Gupta (1964). Later, many workers from India investigated the chromosome numbers of Indian lepidopteran species (Rishi 1973, 1975, Nayak 1975, Rishi and Rishi 1977, 1978, 1979, 1985, 1990, Narang and Gupta 1982, Rishi *et al.* 1992, Mohanty and Nayak 1982, Sharma and Bajwa 1992, 1995a, b). So far, no work has been reported on the cytology of Lepidoptera from Himachal Pradesh, India. The present investigation was thus undertaken to study the chromosomes of four species of moths, which are pests of rice, apple, dandelion and other malvacaeous plants in this region.

Materials and methods

The moth species were collected by the light trap method from the Chhota Shimla locality (Latitude 31°5′N; Longitude 77°10′E and Altitude 2050 m above sea level) of the Shimla district of

* Corresponding author, e-mail: dcgautam_hpu@hotmail.com
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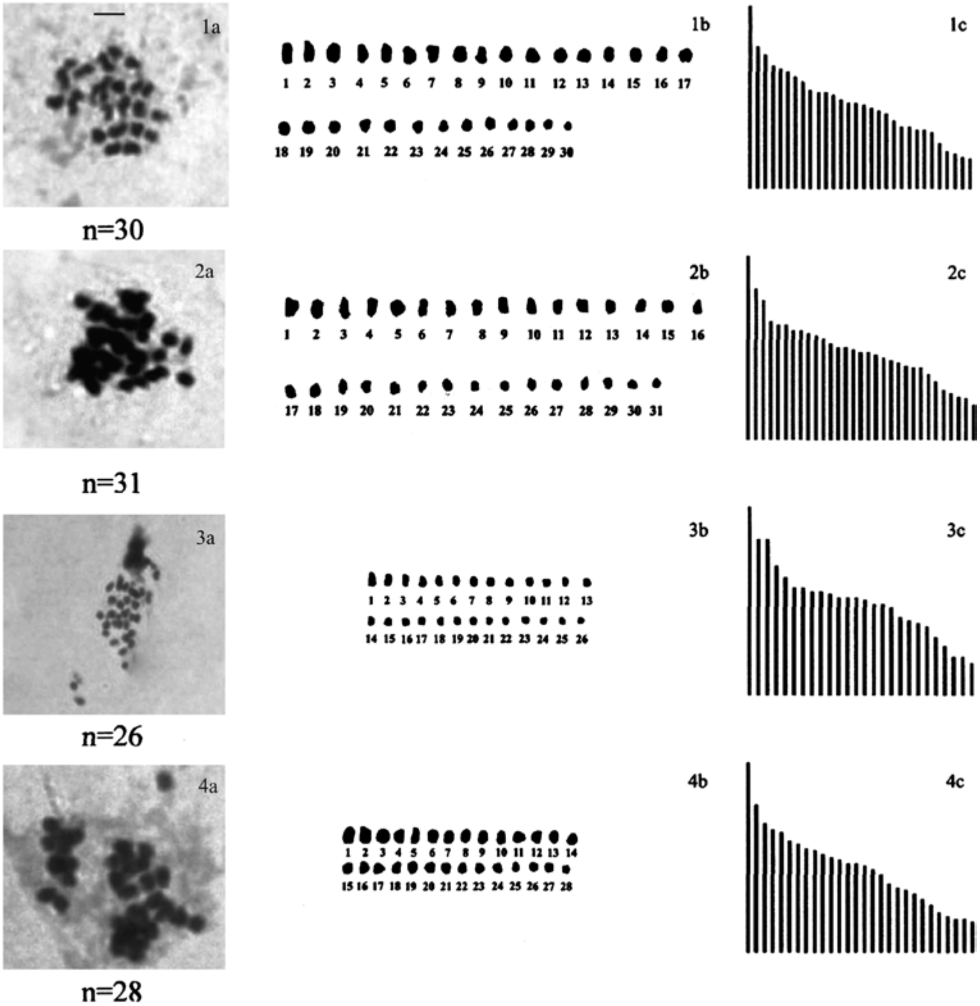
Himachal Pradesh. These moth species are *Cnaphalocrocis medinalis* G., *Euproctis chrysorrhoea* L., *Idaea aversata* L., and *Pygospila tyres* C.

The germinal tissue was dissected out from the adult moths and was cleared of fat bodies in a drop of 0.9% normal saline solution. It was then pretreated in 0.7% sodium citrate for half an hour and the material was fixed in a solution of 1 : 3 acetic acid : ethanol for 20 to 30 min at room temperature.

The material was squashed and uniformly spread after keeping it in a drop of 45% acetic acid

Table 1. Chromosome number (*n*) in four moth species.

Name of species	Haploid chromosome number (*n*)
Cnaphalocrocis medinalis	30
Euproctis chrysorrhoea	31
Idaea aversata	26
Pygospila tyres	28



Figs. 1a–4c. 1a, 1b, 1c, Metaphase I plate, karyotype and idiogram (n=30) of *Cnaphalocrocis medinalis.* 2a, 2b, 2c, Metaphase I plate, karyotype and idiogram (n=31) of *Euproctis chrysorrhoea.* 3a, 3b, 3c, Metaphase I plate, karyotype and idiogram (n=26) of *Idaea aversata*. 4a, 4b, 4c, Metaphase I plate, karyotype and ideogram (n=28) of *Pygospila tyres*. Bar represents 5 μm.

Table 2. Mean* actual lengths and mean relative lengths of chromosomes.

Chromosome No. and TCL	*Cnaphalocrocis medinalis* (*n*=30)		*Euproctis chrysorrhoea* (*n*=31)		*Idaea aversata* (*n*=26)		*Pygospila tyres* (*n*=28)	
	AL±S.E.** (μm)	RL±S.E. (%)	AL±S.E. (μm)	RL±S.E. (%)	AL±S.E. (μm)	RL±S.E. (%)	AL±S.E. (μm)	RL±S.E. (%)
1	1.79±0.11	7.10±0.29	1.76±0.06	6.65±0.35	1.52±0.13	7.37±0.35	1.62±0.18	8.01±0.82
2	1.39±0.11	5.50±0.27	1.45±0.07	5.43±0.24	1.26±0.09	6.36±0.22	1.26±0.11	6.23±0.45
3	1.31±0.09	5.20±0.17	1.34±0.04	5.00±0.17	1.26±0.09	6.36±0.22	1.10±0.03	5.48±0.09
4	1.21±0.05	4.81±0.06	1.13±0.04	4.24±0.09	1.05±0.05	5.35±0.17	1.05±0.04	5.23±0.17
5	1.18±0.05	4.72±0.10	1.10±0.03	4.15±0.12	0.95±0.05	4.81±0.91	1.02±0.04	4.96±0.28
6	1.15±0.04	4.63±0.10	1.10±0.03	4.15±0.12	0.87±0.04	4.42±0.08	0.95±0.05	4.55±0.27
7	1.10±0.03	4.53±0.11	1.05±0.04	3.94±0.09	0.87±0.04	4.42±0.08	0.92±0.04	4.41±0.26
8	1.05±0.04	4.22±0.14	1.05±0.04	3.94±0.09	0.84±0.03	4.30±0.10	0.89±0.05	4.27±0.20
9	0.97±0.04	3.90±0.12	1.02±0.03	3.83±0.07	0.84±0.03	4.30±0.10	0.87±0.04	4.16±0.16
10	0.95±0.04	3.80±0.15	1.00±0.05	3.72±0.08	0.82±0.02	4.19±0.13	0.84±0.03	4.05±0.18
11	0.95±0.04	3.80±0.15	0.97±0.08	3.62±0.11	0.79±0.00	4.08±0.16	0.81±0.02	3.94±0.18
12	0.92±0.04	3.69±0.13	0.92±0.55	3.43±0.12	0.79±0.00	4.08±0.16	0.79±0.04	3.79±0.19
13	0.87±0.04	3.48±0.09	0.89±0.05	3.33±0.12	0.79±0.00	4.08±0.16	0.76±0.02	3.67±0.16
14	0.84±0.03	3.38±0.09	0.89±0.05	3.33±0.12	0.77±0.02	3.93±0.16	0.76±0.02	3.67±0.16
15	0.84±0.03	3.38±0.09	0.87±0.06	3.24±0.13	0.74±0.03	3.77±0.15	0.74±0.03	3.54±0.17
16	0.82±0.02	3.29±0.09	0.84±0.03	3.16±0.09	0.74±0.03	3.77±0.15	0.71±0.04	3.40±0.17
17	0.78±0.04	3.16±0.10	0.84±0.03	3.16±0.09	0.71±0.04	3.63±0.17	0.66±0.04	3.13±0.13
18	0.76±0.02	3.08±0.11	0.82±0.02	3.08±0.11	0.63±0.04	3.21±0.11	0.58±0.05	2.72±0.15
19	0.74±0.03	2.96±0.12	0.79±0.04	2.96±0.12	0.60±0.04	3.07±0.08	0.55±0.04	2.72±0.15
20	0.67±0.04	2.75±0.14	0.76±0.02	2.87±0.09	0.58±0.03	2.95±0.08	0.53±0.04	2.61±0.15
21	0.61±0.04	2.43±0.09	0.74±0.04	2.76±0.09	0.55±0.04	2.81±0.17	0.50±0.03	2.50±0.12
22	0.61±0.04	2.43±0.09	0.71±0.03	2.66±0.11	0.47±0.05	2.38±0.20	0.45±0.04	2.21±0.19
23	0.58±0.03	2.33±0.09	0.69±0.04	2.55±0.10	0.40±0.04	2.00±0.20	0.40±0.04	1.95±0.20
24	0.58±0.03	2.33±0.09	0.69±0.04	2.55±0.10	0.31±0.09	1.57±0.10	0.34±0.04	1.69±0.19
25	0.55±0.02	2.25±0.11	0.63±0.04	2.37±0.13	0.31±0.09	1.57±0.10	0.31±0.03	1.55±0.15
26	0.45±0.04	1.81±0.16	0.56±0.02	2.10±0.11	0.26±0.00	1.34±0.05	0.29±0.03	1.44±0.14
27	0.37±0.04	1.46±0.15	0.48±0.03	1.77±0.12			0.29±0.03	1.44±0.14
28	0.34±0.04	1.34±0.12	0.45±0.04	1.66±0.12			0.26±0.00	1.30±0.04
29	0.31±0.03	1.24±0.10	0.42±0.04	1.56±0.13				
30	0.29±0.02	1.14±0.07	0.40±0.04	1.46±0.14				
31			0.34±0.04	1.27±0.13				
TCL ±S.E. (μm)	24.98±0.91		26.70±0.87		19.66±0.82		20.15±0.63	

* Mean of ten chromosomal plates. ** ±Standard error about the mean.

for 15 to 20 min. The slides and cover slips containing the material were air dried in a dust free chamber for 2 to 3 d before staining with 2% Giemsa for 20 min. The slides were mounted in DPX and observed under a research monocular microscope. Well-spread metaphase I plates were selected for photomicrography under a LEICA DMLS2 microscope fitted with a LEICA DFC 320 camera, and measurements were taken from ten well-spread plates.

Results

The haploid chromosome numbers observed in *Cnaphalocrocis medinalis*, *Euproctis chrysorrhoea, Idaea aversata*, and *Pygospila tyres* were found to be 30, 31, 26, and 28, respectively (Table 1, Figs. 1a, 2a, 3a, 4a). The mean actual lengths and percent relative lengths of the metaphase I chromosomes of the moth species are given in Table 2.

The karyotypes of all the species (Figs. 1b, 2b, 3b, and 4b) showed that the chromosomes do not have a localized centromere. Idiograms were constructed from the relative length data. The idiograms (Figs. 1c, 2c, 3c, and 4c) revealed a gradual decrease in chromosome size, where some chromosomes were of equal length. There was one long pair of chromosomes in all the species.

Discussion

The modal chromosome number in Lepidoptera ranges from *n*=29 to *n*=31 (Robinson 1971). Two of the species studied in the present investigation, *Cnaphalocrocis medinalis* and *Euproctis chrysorrhoea*, showed their chromosome numbers in this range. The findings of the present study differ from those reported by Mohanty and Nayak (1982). There were 30 bivalents observed in the present study in *Cnaphalocrocis medinalis* while Mohanty and Nayak (1982) reported 2*n*=58. Even in other members of the Pyralidae, *e.g.*, *Ectomyelois ceratoniae*, the diploid chromosome number has been reported as 2*n*=62 (Mediouni *et al.* 2004).

Euproctis chrysorrhoea belongs to the family Lymantriidae and has *n*=31. These findings are in accordance with the earlier reports of Mohanty and Nayak (1983) on the chromosomes of genus *Euproctis* sp. There is wide variation in chromosome number in this family. In *Orgyia thyellina* of this family, the haploid chromosome number was found to be eleven (*n*=11) (Traut and Clarke 1996).

Idaea aversata (*n*=26) and *Pygospila tyres* (*n*=28) showed a deviation from the general modal chromosome number. The observed chromosome numbers of *Idaea aversata* and *Pygospila tyres* also differ from earlier reports on the chromosome numbers in other members of their families *e.g.*, Geometridae and Crambidae, respectively. Suomalainen (1965) and Ennis (1976) have reported the haploid chromosome number as thirty (*n*=30) and sixty-one (*n*=61) in *Cidaria* sp. and *Nepytia canosaria* of the family Geometridae, respectively. *Pygospila tyres* is the member of the family Crambidae. The chromosome number of this species observed in the present study differs from the other moth species, *Ostrinia scapularis* (*n*=31), of the same family (Kageyama and Traut 2003).

Out of the four species reported here, two species showed the chromosome numbers as *n*=30 (*C. medinalis*) and *n*=31 (*E. chrysorrhoea*); the other two species had the chromosome numbers as *n*=26 (*I. aversata*) and *n*=28 (*P. tyres*). Lorkovic (1941) has hypothesized polyploidy as a means of increase in chromosome number in some lepidopterans. This idea appears to have been excluded here. White (1973) and Suomalainen (1969a) stated that fusion and dissociation are responsible for the evolution of chromosome number in Lepidoptera rather than polyploidy, and that fusion is more frequent than fission because there are more species with numbers below 31 than there are ones with numbers above 31. The karyotypes observed here are typical of Lepidoptera in general.

Thus, the observed chromosome numbers showed the trend towards the evolution of lower chromosome numbers, which must have been achieved through chromosomal fusion. Many authors have given evidence for holocentric chromosomes in Lepidoptera (Bauer 1967, Suomalainen 1969b, Murakami and Imai 1974). However, Bigger (1975 and 1976), Rishi and Rishi (1979), and Gus *et al.* (1983) have reported the presence of a localized centromere in such chromosomes through the use of improved cytological techniques. The current study, however, lends support to the holocentric nature of lepidopteran chromosomes based on their orientation. Although the karyotype in Lepidoptera is generally uniform with a slight gradation in size among the chromosomes, all the species studied had one long pair of chromosomes.

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