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RESEARCH ARTICLE

CHROMOSOMAL ANALYSIS OF MULBERRY SILKWORM *(BOMBYX MORI.* L) FROM JAMMU REGION.

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Abstract

In the present study, the chromosomal analysis of mulberry silkworm, *Bombyx mori* L. was carried out in order to study its chromosome behaviour and karyotype from larval gonadal tissue. The observed diploid number was 2n=56. The mean total length, RL% and TCL% were measured at metaphase stage of gametogenesis. The chromosomal behaviour during meiotic stages viz., leptotene, zygotene, pachytene, diplotene, diakinesis and anaphase were also analyzed. Results indicate that the samples studied were males with a diploid chromosome number of 2n=56.

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Introduction:-

The order Lepidoptera includes moths and butterflies. It is a species rich order and among this order are some commercially important insects like silkmoths and many serious agricultural pests too.The mulberry silkworm is a commercially important insect which is completely domesticated and one of the most genetically studied insect and well known as lepidopteran model insect (Nagaraju and Goldsmith, 2002) apart from its importance as a producer of silk-"the queen of textiles".

As in most Lepidoptera, the chromosomes of *Bombyx mori* L. are holocentric (Murakami and Imai, 1974) i.e. they possess centromeres throughout the chromosome body. These chromosomes are highly condensed and appear dot-shaped at mitotic and meiotic metaphase stages. Their diffused centromeres and lack of special features make them difficult to identify individually. The chromosome number is known with n=28 (Kawaguchi,1928) and 2n=56 (Kawamura 1979, Yoshido *et al* 2005). In the present investigation, an attempt has been made to prepare a karyotype of *Bombyx mori* L. on the basis of decreasing chromosome length from meiotic metaphase complement. The chromosome behavior during the meiotic stages was also studied from the larval gonadal cells.

Material and Methods:-

Rearing of silkworm larvae:-

The IIIrd instar larvae of mulberry silkworm, *B.mori* L. were collected from RSRS Miransahib, Jammu and transported to Cytogenetics Lab., Dept. of Zoology., University of Jammu. About 20 larvae were reared in rearing cage for further development (fig. 1). The temperature and relative humidity was maintained as 26˚C and 80% RH for the IIIrd instar, 24-25˚C and 75% RH for IV instar and 23-24˚C temperature and around 70% RH for the V instar, the larvae were fed with mulberry leaves during larval period.

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Cytological preparation of slides:-

The gonads of IV and V instar larvae was used for cytological preparations (fig 2). The slides were prepared by using the technique of Murakami and Imai 1974 with slight modifications. Briefly, the larval gonads were dissected in colchicine-hypotonic solution (0.01 % coach if I me in 0.45% sodium citrate solution) and then put in fresh hypotonic solution for 30-45 minutes at room temperature, the gonads were transferred to glass slide and torn into pieces in 60% acetic acid and fixed in carnoys fixative for 2-3 minutes. The material was squashed and stained with Aceto-orcein for 10 minutes. The prepared slides were scanned under Olympus camera aided microscope and photographed by CH20i BIMF microscope attached with Sony -SSC-DC378P camera under 1000x magnification. Histogram was prepared by taking chromosome pair number on X-axis and corresponding relative length percentage on Y-axis. The chromosomes were paired on the basis of size only as they lack any primary or secondary constriction.

Results:-

Spermatogonial metaphase (fig.3) showed the diploid chromosome number of 2n=56, the chromosomes were numerous, darkly stained, highly condensed and dot-like without conspicuous centromere. The karyotype prepared on the basis of decreasing chromosome lengths showed 28 pairs of chromosomes with their homologous chromosomes. Sex chromosomes were not distinguished from others. The karyotype (fig.4) was prepared from well spread spermatogonial metaphase that revealed the presence of 56 elements and showed 28 pairs of chromosomes gradually decreasing in length. Histogram (fig. 5) was prepared on the basis of decreasing value of RL% from chromosome pair 1 to 28. The maximum TCL% i.e., 5.66% was found in first chromosome and the minimum 1.548% was found in the last chromosome pair. The RL% varied from 27.33% in the last chromosome pair to 100% in the first chromosome pair. The total complement length of the haploid set was 132.35µm (Table 1). The other meiotic stages observed were leptotene, zygotene, pachytene, diplotene, diakinesis, anaphase and metaphase (side view)(Fig 6 to 13). The leptotene stage possessed chromosomes in the form of long and thin threads which crossed and inter-crossed each other forming a network. In zygotene stage, (fig. 7) all chromosomes are attached to the inner nuclear membrane. End to end synapsis of homologous chromosomes is also seen. The early pachytene stage showed cross-over between homologous chromosomes as in fig 8. The chromosomes are extended and lightly stained having recombination nodules. The late pachytene (fig 9) shows well spread chromosomes which are slightly more condensed. The points of chiasmata can be easily seen, the chromosomes are shortened and darkly stained (fig 10). These are not countable due to its small size and high number. In fig 11, the chromosomes are condensed and form ring like structures showing terminalisation of chaismata. The chromosomes are dark, small and numerous at this stage marked with further condensation and not countable. This stage is the diakinesis stage of Prophase I. In metaphase stage, chromosomes lying at the equatorial plane are clearly seen in the side view as shown in fig. 12. Early anaphase with parallel alignment of chromosomes is seen in fig. 13 and the chromosomes are seen pulled to opposite poles. The point of attachment is not detectable due to holocentric nature of the chromosomes.

Table 1:- Karyo-morphometric data of *Bombyx mori* L. from its spermatogonial metaphase complement, 2n=56.

Chromosome Pair Number	Mean Total Length (µ)	Total Complement length percentage (TCL%)	Relative Length Percentage (RL%)
1	7.50	5.666	100
2	7.05	5.326	94.00
3	6.85	5.175	91.33
4	6.00	4.533	80.00
5	5.90	4.457	78.66
6	5.55	4.193	74.00
7	5.30	4.004	70.66
8	5.15	3.891	68.66
9	5.10	3.853	68.00
10	5.05	3.815	67.33
11	5.00	3.777	66.66
12	4.95	3.740	66.00
13	4.90	3.702	65.33
14	4.70	3.551	62.66
15	4.65	3.513	62.00
16	4.55	3.437	60.66
17	4.45	3.362	59.33

18	4.40	3.324	58.66
19	4.30	3.248	57.33
20	4.10	3.097	54.66
21	4.05	3.060	54.00
22	4.00	3.022	53.33
23	3.90	2.946	52.00
24	3.70	2.795	49.33
25	3.15	2.386	42.00
26	3.05	2.304	40.66
27	3.00	2.266	40.00
28	2.05	1.548	27.33

Karyo-morphometric analysis of karyotype: -

Actual mean length of the largest chromosome = 7.5 µ
Actual mean length of the smallest chromosome = 2.05 µ
Relative Length % of the largest chromosome = 100%
Relative Length % of the smallest chromosome = 27.33
Ratio of the largest to smallest chromosome = 3.658
Total complement length of haploid set = 132.35 µ

Fig 1:- Rearing of silkworm larvae in rearing cage.

Fig 2:- V instar larvae taken for cytological preparations.

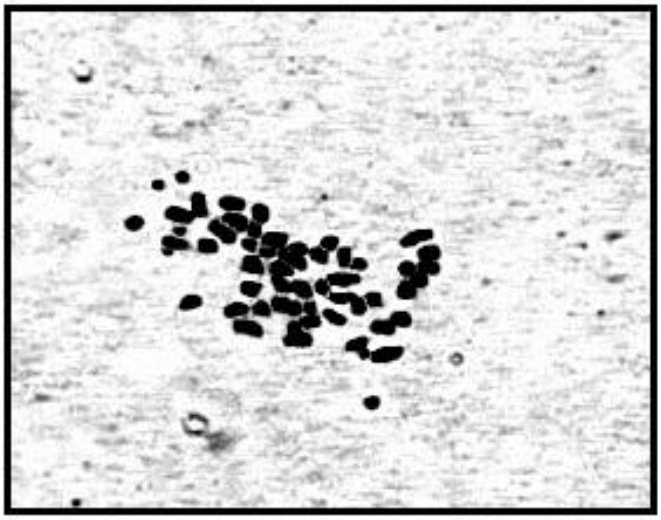


Fig.3

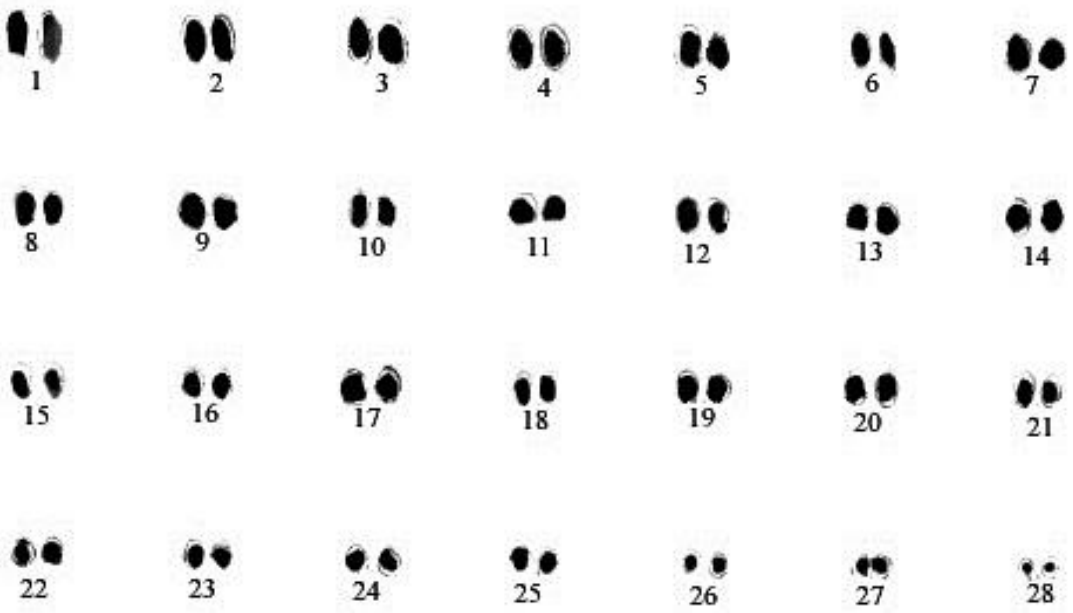
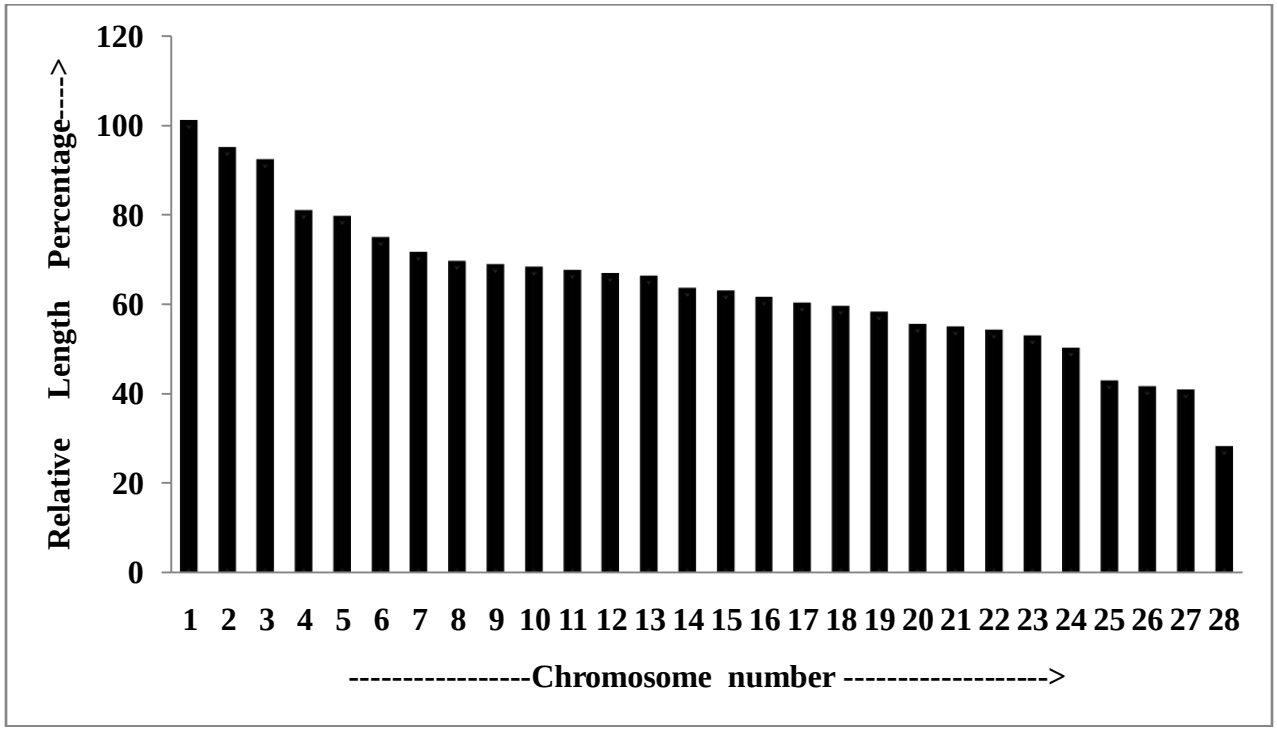


Fig.4

Fig 3:- Spermatogonial Metaphase of *Bombyx mori* L. with 2n=56.
Fig 4:- Karyotype prepared from the meiotic metaphase complement.



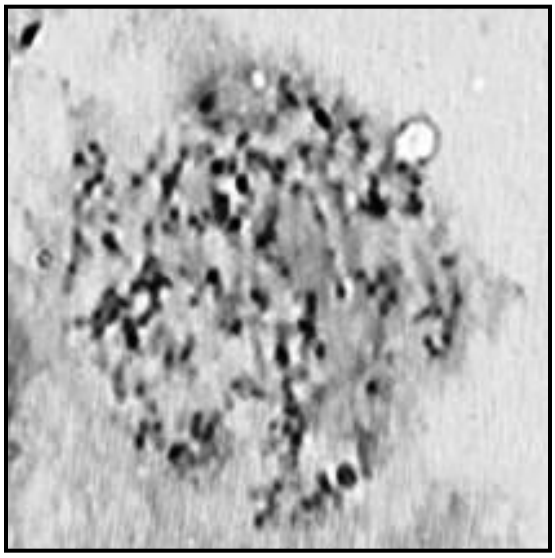


Fig 6:- Leptotene

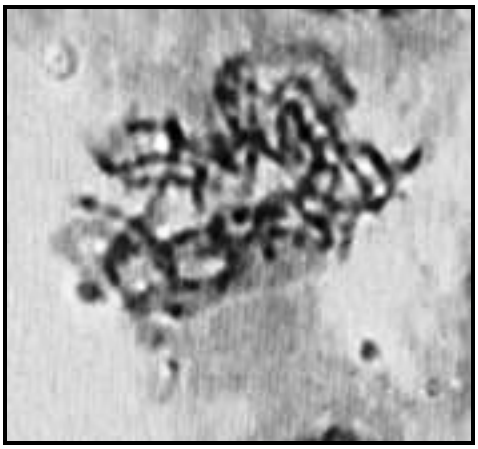


Fig 7:- Zygotene

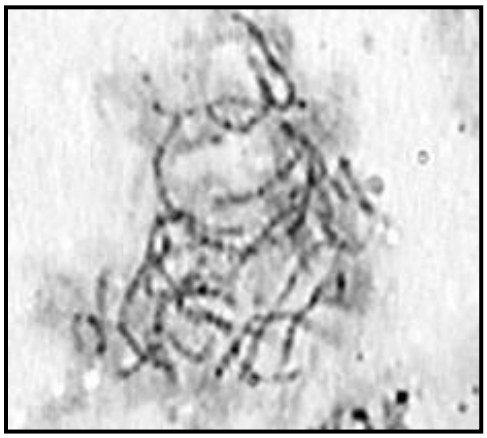


Fig 8:- Early pachytene

Fig. 9:- Late pachytene

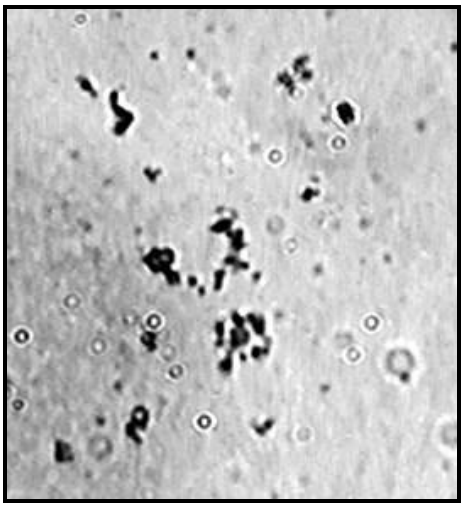


Fig. 10:- Diplotene

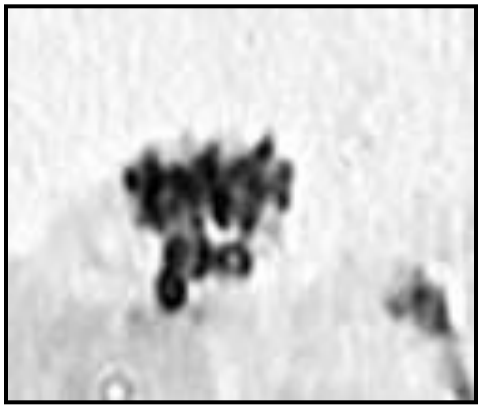


Fig 11:- Diakinesis

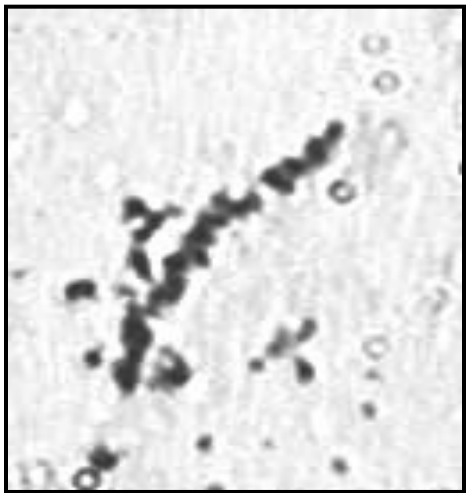


Fig 12:- Metaphase (side view)

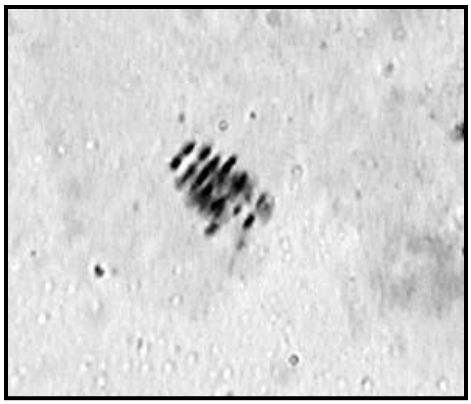


Fig 13:- Early Anaphase

Disscussion:-

The Lepidoptera have a great range in chromosome number than any other group of animals. (n=7 to n=220). In case of mulberry silkworm, *Bombyx mori* L. the diploid chromosome number has been reported for the first time by Toyama (1894) to be 2n=28, which was later reported to be a haploid number and was a result of old microscopy techniques and large number and small size of the chromosomes. Kawaguchi (1928) reported the diploid chromosomal number to be 2n=56 and haploid set to be 28 chromosomes. Further, Yoshido *et al* (2005) prepared the *Bombyx mori* karyotype using genetically mapped *Bombyx mori* bacterial artificial chromosomes as probes and assigned already established genetic linkage groups and the correct orientation in the chromosomes. The present study done on *Bombyx mori L.* under the subtropical conditions of Jammu region further established the diploid chromosome number to be 2n=56 in mulberry silkworm as reported by Kawaguchi (1928), Kaur (1988) and Yoshido *et al* (2005).

The chromosomes in lepidopterans are holocentric with a presence of multiple and diffused kinetochores. In holocentric chromosomes, the attachment region of spindle is spread throughout the chromosome. Holokinetic chromosomal organization in Lepidoptera was deduced from the missing primary constrictions and a parallel separation in mitotic anaphase (Murakami and Imai, 1974), viability of chromosome fragments (Maeki 1981), high rate of viability of X-ray induced translocations (Bauer, 1967), high doses of X-ray necessary to induce sterility and chromosome fragmentation and fusion in evolution giving rise to highly different chromosome numbers in related species.(Suomalainen,1953). Similar findings have been observed in the meiotic prophase stages like pachytene, metaphase-I, diplotene, diakinesis and anaphase stages. Murakami and Imai (1974) provided cytological evidence for holocentric chromosomes in *Bombyx mori* and *Bombyx mandarina* , Friedlander and Wahraman (1970) demonstrated some electron micrographs of silkworm chromosomes in which microtubules penetrate all along the polar faces of the chromosomes as in case of *Ostrinia nubilalis* and concluded that silkworm chromosome lacks a localized centromere.

Chromosome behavior in *B.mori* is essentially same in both the sexes from initial pairing at zygotene and formation of synaptonemal complex but the process of crossing over and chaismata formation are limited to males only, and the females of *Bombyx mori* undergo achiasmatic meiosis and the pachytene chromosomes are synapsed side to side until metaphase-I, as studied by Sturtevant(1915), Tanaka(1913), Traut(1976) and Rasmussen *et al* (1977). In the present investigation, all the meiotic stages viz., recombination nodules at early pachytene, chaismata at diplotene and diakinesis and similar 28 chromosome pair suggests that the samples studied were males thus the sex can be determined by using cytological techniques in *Bombyx mori.*

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