

Karyological studies of five species of Lepidoptera

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Cytogenetic studies making use of *in vitro* injection of colchicine and conventional Giemsa staining have been carried out on five species of Lepidoptera (butterflies and moths) belonging to three different families. Chromosomal preparations were made from brain ganglia and testis by using NaCl-acetic Carnoy-air drying method. The chromosomes bore typical dot like or elongated structures. Karyotype of the mitotic metaphase chromosomes on the basis of size and morphology showed $2n=60$ in *D. chrysippus* (Linn.), $2n=54$ in *E. merione* (Cram.) and $2n=62$ in *D. obliqua* (Wlk.), *E. vitella* (Stoll.) and *S. mauritia* (Boisd.). Moreover, different meiotic stages from testis of these five species also confirmed their diploid number.

Key words : Lepidoptera, Colchicine, Karyotype, Mitotic, Meiotic, Testis.

INTRODUCTION

Karyological analysis of Lepidoptera has been a difficult task due to small dot-like chromosomes of similar sizes. On account of inadequate techniques in early work, sex chromosomes could not be clearly differentiated from the autosomes in a majority of the species investigated in this group. In respect to Indian Lepidoptera, only meagre data are available, namely, 8 by Gupta and Narang (1980); 30 by Rishi (1973); 45 by Mohanty and Nayak (1983); 31 by Kaur (1988) and 7 by Sharma and Bajwa (1992,1995a,b). However, the cytological data so far available, including those from neighbouring Nepal do not give satisfactory information to elucidate the cytotaxonomic relationships among lepidopteran species. Therefore, more chromosomal investigations should be done in various taxonomic groups of Lepidoptera.

In this report, the chromosomes of five species of Lepidoptera belonging to three different families were investigated with *in vitro* colchicine treatment established by Rishi *et al.* (1997).

MATERIALS AND METHODS

Different instar larvae of the five species of Lepidoptera

were collected from their respective host plants (Table 1) growing in the vicinity of Jammu University campus during April to July, 2008. Male and female specimens were fed to maturity in the laboratory. Brain ganglia and testes were processed for chromosome analysis following *in vitro* colchicine treatment (Rishi *et al.*, 1997). After this, the preparations were made by the usual NaCl-acetic Carnoy-air drying method and stained with 2% Giemsa solution.

RESULTS AND DISCUSSION

The cells of brain ganglia of both male and female sexes as well as the male gonads yielded satisfactory results. Early metaphase plates from the brain tissue of female insects showed dot like chromosomes. Sex chromosomes could not be clearly identified in some species. Makee and Tafesh (2006) showed that sex heterochromatin could be used as sex determination and cytogenetic marker to identify sex chromosomes. Chromosomal observations on the five species of Lepidoptera dealt within the present investigation is summarized in Table 1.

As far as family Nymphalidae is concerned about 435 species are cytologically known but only 12 Indian species including the 2 species *viz.* *Danaus chrysippus*

Table 1 : Karyotypic data on the five species of presently analysed Lepidoptera

Sr. No.	Species	Host plant	Diploid chromosome number (2n)	Haploid chromosome number (n)	Sex chromosome mechanism
1.	<i>Danaus chrysippus</i>	<i>Calotropis procera</i>	60	30	ZZ:-
2.	<i>Ergolis merione</i>	<i>Ricinus communis</i>	54	27	ZZ:ZW
3.	<i>Diacrisia oblique</i>	<i>Raphanus sativus</i>	62	31	ZZ:ZW
4.	<i>Earis vitella</i>	<i>Abelmoschus esculentus</i>	62	31	Unidentified
5.	<i>Spodoptera mauritia</i>	<i>Brassica oleracea</i>	62	31	Unidentified

and *Ergolis merione*, recorded in the present observation have been cytologically investigated.

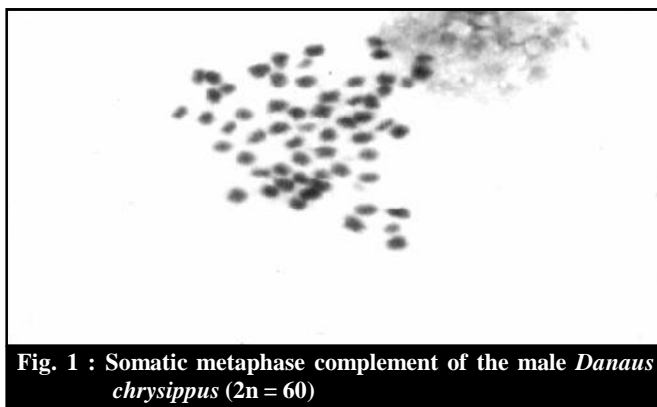
A survey of the literature in the family Arctiidae reveals that only 34 species including *Diacrisia obliqua*, investigated presently are known cytologically. The modal number was found to be $n=31$. The diploid number of 62 chromosomes reported during the present investigations from male mitotic metaphases of *D. obliqua* confirms the earlier reports of Rishi and Rishi (1981) and Malik (1988). Family Arctiidae, in general, does not indicate any significant variation in the chromosome numbers in the various cytologically known forms so far.

Family Noctuidae, a large family of moths is cytologically known by comparatively a fewer number of species. About 74 species of moths have so far been analysed cytologically. During the present investigation, only two species *viz.*, *Earis vitella* and *Spodoptera mauritia* have been worked out.

The main cytological features of the five species are described as under:

Danaus chrysippus :

Somatic metaphase (Fig. 1) complement comprised



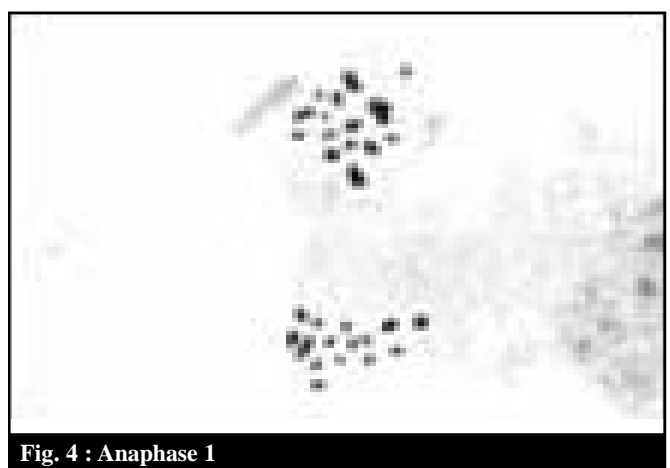
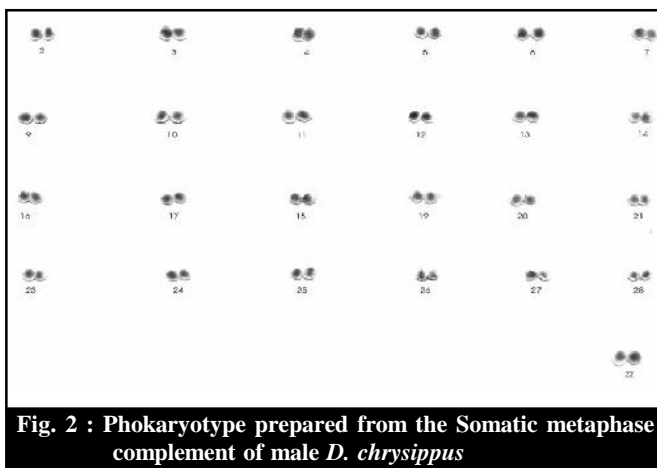
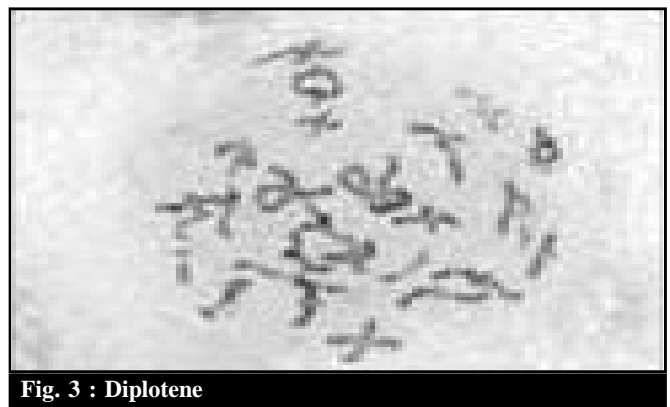
60 chromosomes in the diploid set ($2n=60$). Chromosomes were rod shaped in appearance. Somatic karyotype prepared from the somatic metaphase complement of male *D. chrysippus* (Fig. 2) possessed 29 pairs of moderate to small sized chromosomes and one pair of homomorphic sex chromosomes (ZZ) which comprised the largest chromosome of the complement. The different meiotic stages are shown in Fig. 3 and 4.

Ergolis merione :

Somatic metaphase complement of both male and female revealed 54 chromosomes in the diploid set ($2n=54$).

Male, $2n=54$ (Fig. 5) and Female, $2n=54$ (Fig. 7)

Somatic karyotype of male (Fig. 6) comprised 26 pairs of moderate to small sized chromosomes and one pair of sex chromosomes (ZZ) comprising the largest chromosomes of the complement. Somatic karyotype of female (Fig. 8) possessed 26 pairs of moderate to small sized chromosomes and one pair of heteromorphic sex chromosomes (ZW) comprising the largest 'Z' and second smallest 'W' chromosome. The different meiotic stages are shown in Fig. 9 and 10.



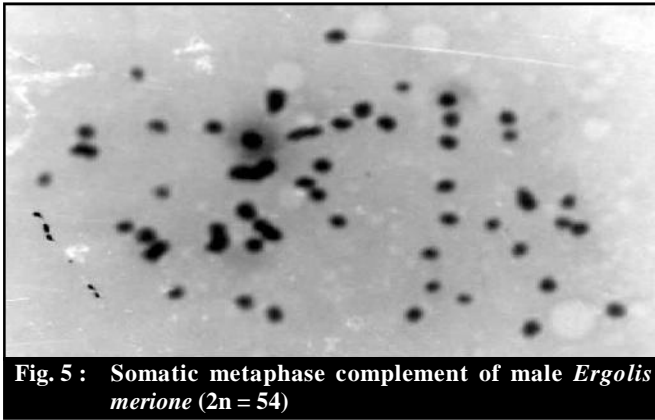


Fig. 5 : Somatic metaphase complement of male *Ergolis merione* ($2n = 54$)

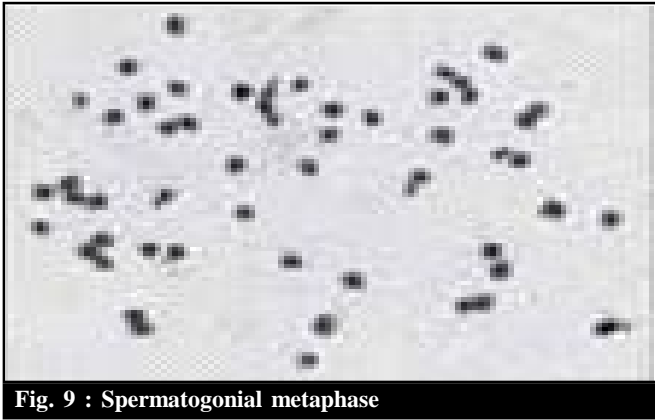


Fig. 9 : Spermatogonial metaphase

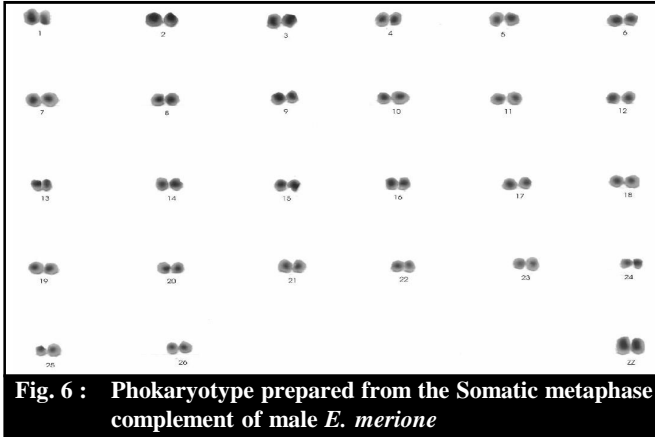


Fig. 6 : Phokaryotype prepared from the Somatic metaphase complement of male *E. merione*

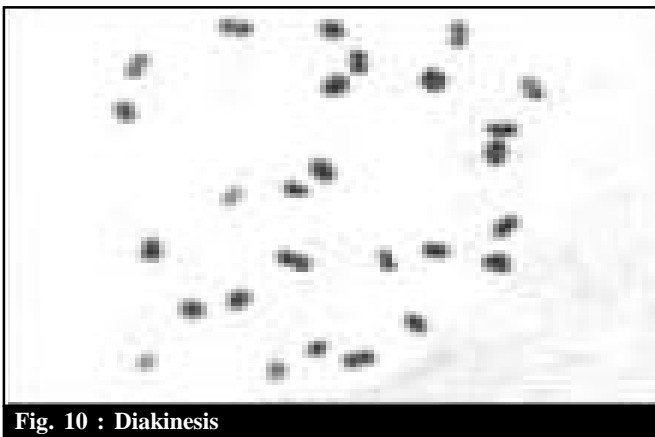


Fig. 10 : Diakinesis

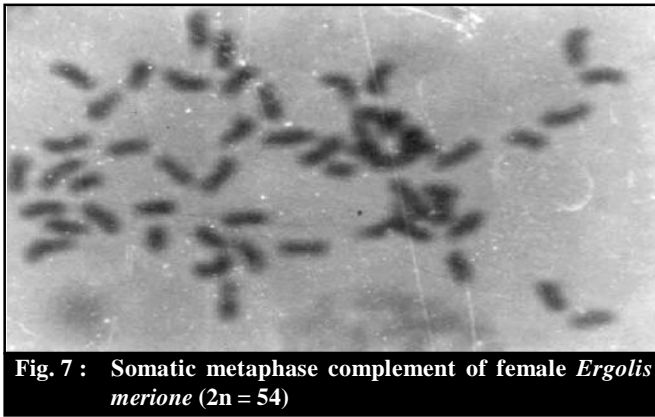


Fig. 7 : Somatic metaphase complement of female *Ergolis merione* ($2n = 54$)

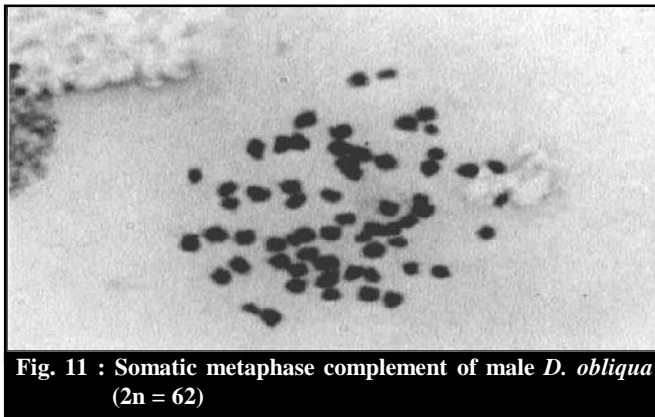


Fig. 11 : Somatic metaphase complement of male *D. obliqua* ($2n = 62$)

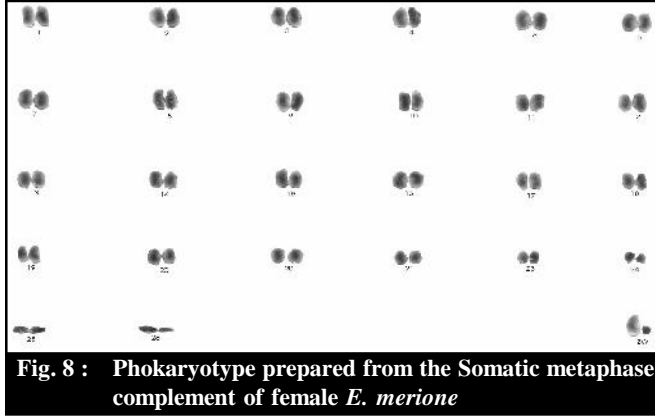


Fig. 8 : Phokaryotype prepared from the Somatic metaphase complement of female *E. merione*

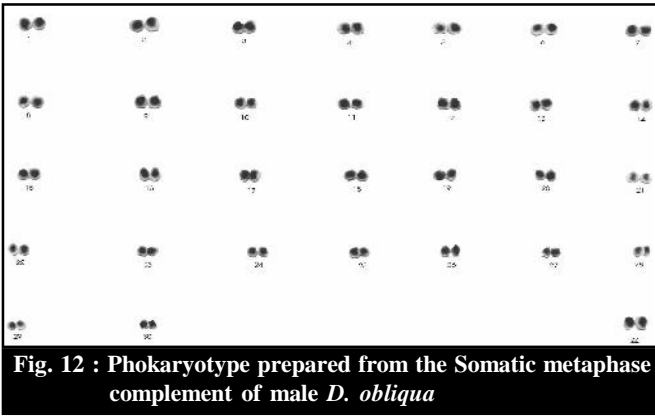


Fig. 12 : Phokaryotype prepared from the Somatic metaphase complement of male *D. obliqua*

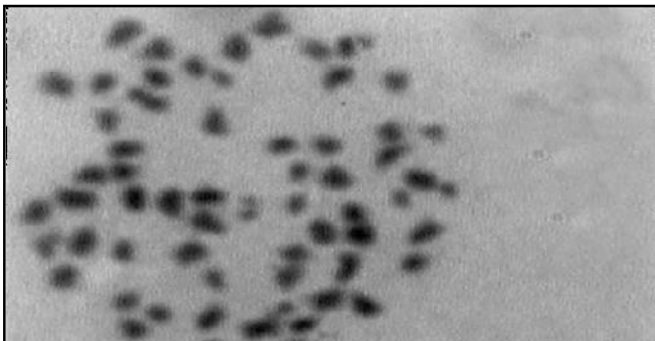


Fig. 13 : Somatic metaphase complement of fe female *D. obliqua* ($2n = 62$)

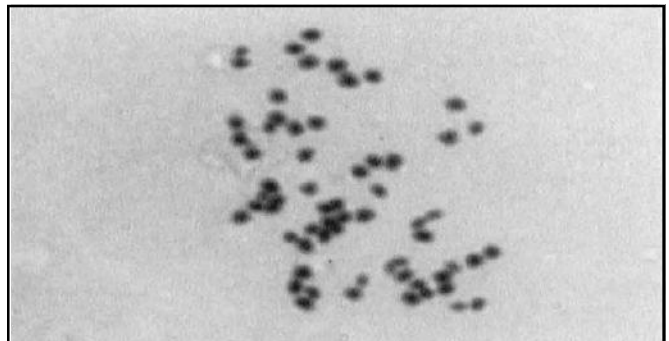


Fig. 17 : Somatic metaphase complement of male *E. Vitella* ($2n = 62$)

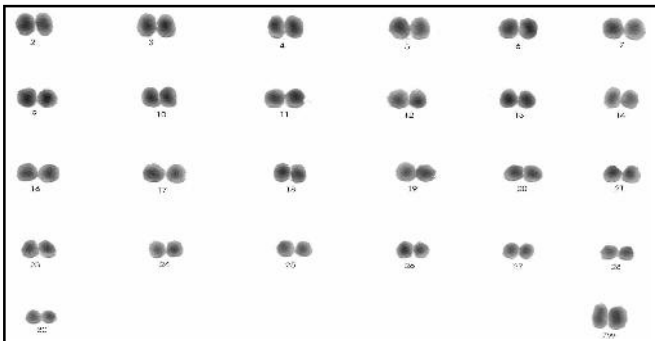


Fig. 14 : Phokaryotype prepared from the Somatic metaphase complement of female *D. obliqua*

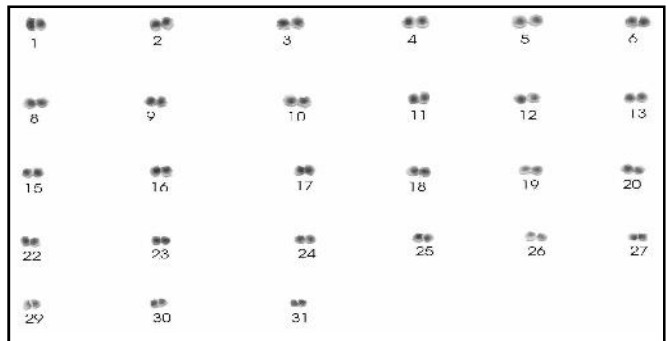


Fig. 18 : Photokaryotype prepared from the somatic metaphase complement of male *E. vitella*.

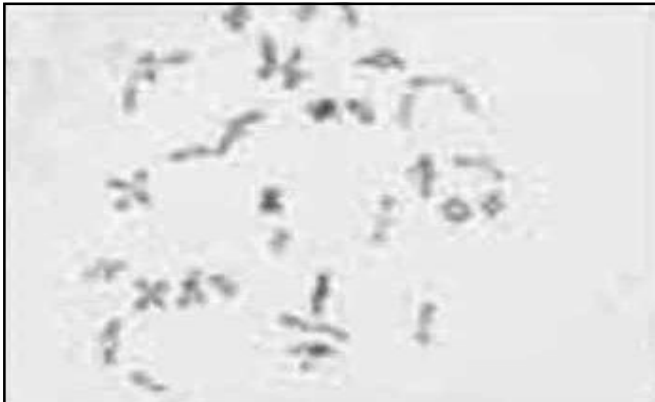


Fig. 15 : Diplotene



Fig. 19 : Diplotene



Fig. 16 : Diplotene

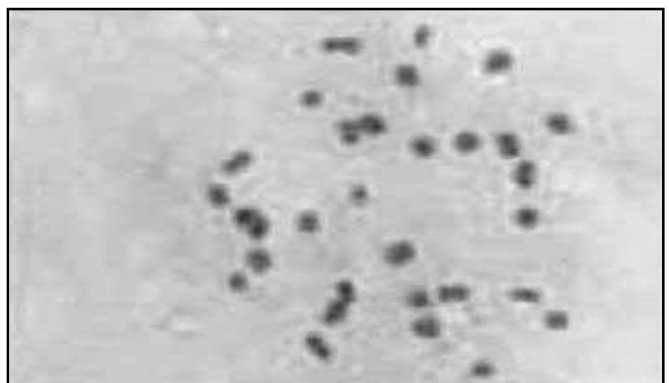
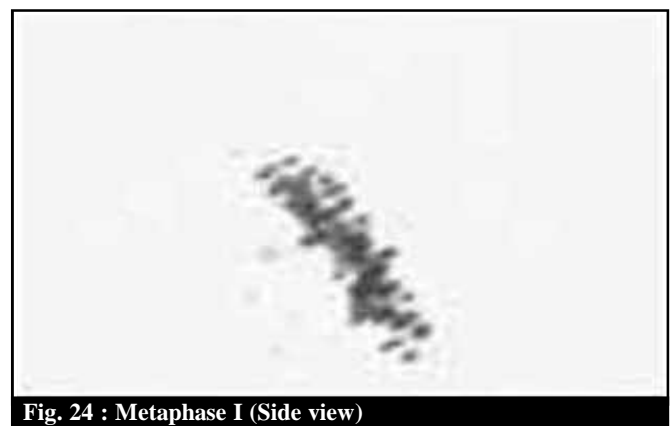
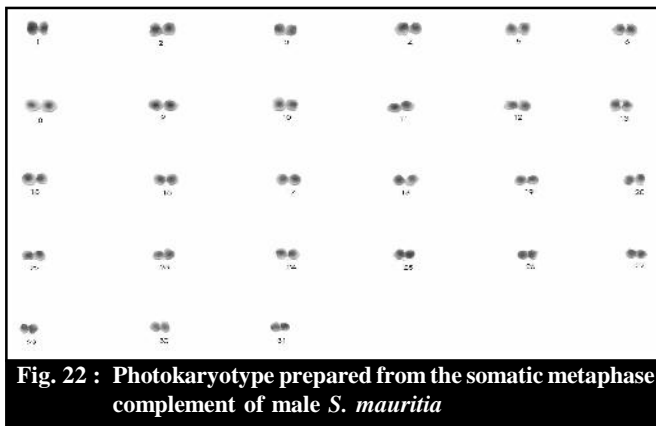
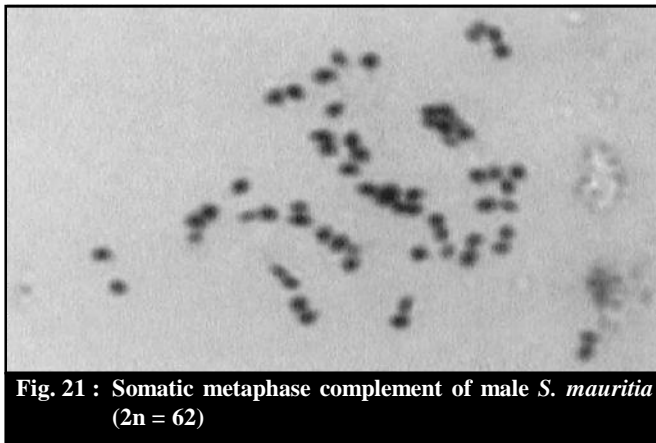


Fig. 20 : Diakinesis



Diacrisia obliqua :

Somatic metaphase complement possessed 62 chromosomes ($2n=62$). All chromosomes are dot like in appearance.

Male, $2n=62$ (Fig. 11) and Female, $2n=62$ (Fig. 13)

Somatic karyotype prepared from somatic metaphase complement of male (Fig. 12) possessed 30 pairs of moderate to small sized chromosomes and one pair of moderate to small sized chromosomes and one pair of homomorphic sex chromosomes (ZZ) which comprised the largest chromosome of the complement. Somatic karyotype of female (Fig. 14) consisted of 30 pairs of moderately to considerably small sized elements. Sex chromosomes were fairly distinct in size; 'Z' was the largest chromosome and 'W' was the second largest chromosome. The different meiotic stages are shown in Fig. 15 and 16.

Earis vitella :

Somatic metaphase complement (Fig. 17) revealed $2n=62$. Somatic karyotype of male (Fig. 18) revealed 31 pairs of moderate to small sized chromosome. Chromosome pairs gradually become shorter from the longest. The different meiotic stages are shown in Fig. 19 and 20.

Spodoptera mauritia :

Somatic metaphase (Fig. 21) complement exhibited 62 number of chromosomes ($2n=62$). Somatic karyotype prepared from the somatic metaphase complement of male (Fig. 22) exhibited 31 pairs of moderate to small sized chromosomes. The different meiotic stages are shown in Fig. 23 and 24.

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