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Karyotypable somatic metaphases obtained in *Agrotis spinifera* (Noctuidae, Lepidoptera) by application of *in vitro* colchicine air drying technique

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Abstract

A modified technique of *in vitro* colchicine treatment has been applied to the somatic chromosomes of a lepidopteran species, *Agrotis spinifera*. The aim to obtain highly elongated karyotypable elements has been achieved from the brain ganglia metaphase chromosomes of male and female revealing 2*n*=62 in both the sexes which were further confirmed by diakinesis and metaphase I in male meiotic prophase chromosomes. Further, female heterogamety with ZW:ZZ sex mechanism has been evidenced on the basis of somatic karyotypes prepared from brain ganglia, chromosomal slides by using air drying Giemsa staining procedure in contrast to the usual isodiametric chromosomes obtained by conventional method of acetolactic orcein squash preparation. This is the first report of identification of sex mechanism in the species, which can be used for further investigation by application of G- and C-banding techniques.

Keywords: *karyotypes, somatic metaphases, differential staining, sex mechanism, Lepidoptera.*

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Introduction

Though genetical data of Lepidoptera is sufficient (Robinson, 1971), cytological proofs of their sex chromosomes and nature of centromeres are scanty (Suomalainen, 1969, 1971; Bigger, 1975, 1976; Kawazoe, 1992; Saitoh, 1989; Izumi and Seto, 1995; Sahni, 1997; Rishi *et al*., 1997, 1999, 2000, 2001). There has been a great difficulty in discerning the homologous pairs of autosomes and the heterogametic sex chromosomes from the metaphase plates because the karyotypable elongated metaphase elements could not be obtained from somatic metaphases by earlier conventional method of acetolactic orcein squash preparation (Maeki, 1981; Rishi and Rishi, 1985, 1990; Dhawan, 2016).

Earlier technical difficulties included:

- isodiametric morphology of chromosomes
- non karyotypable elements
- tendency of chromosomes to clump together at metaphase
- sex chromosomes forming a bivalent not recognizable by heteromorphism
- inadequacy of techniques

Female heterogamety in *Agrotis spinifera* is a new report to cytology

Materials and Methods

Different instar larvae of *Agrotis spinifera* were collected from the host plant *Helianthus annus* in the months of April and May from Kurukshetra. Some of them were fed to maturity in the laboratory. Prepupal brain ganglia of males and females were dissected out in 0.75% sodium chloride solution containing colchicine (0.01%) and kept for 45 minutes. These tissues were then treated with 1% sodium citrate solution for 15 minutes and fixed in methanol-acetic acid (3:1) for 30 minutes. Tissues were then treated in a drop of 45% acetic acid and spread on clean slides for **air drying**. Slides were stained in 2% Giemsa. Chromosome counts were made from 50-70 metaphases in each male and female specimen from more than twenty larvae. All micrographs

were taken with Olympus PM6 photomicrograph attachment at an initial magnification of x500.

Observations and Results

The karyotypic details of male and female Giemsa stained and G-banded somatic metaphases and their karyotypes are as under:

Somatic Metaphases:

Female 2n = 62 (Fig.1)
Male 2n = 62 (Fig.3)

Somatic Karyotypes:

Female (Fig.2)

3 pairs of metacentrics
1 pairs of subtelocentrics
3 pairs of subtelocentrics
23 pairs of acrocentrics
1 pair of hetromorphic sex chromosome(ZW) comprising a largest acrocentric W and a second largest submetacentric Z being smaller than W but still larger than the first pair of autosomes.

Male (Fig.4)

3 pairs of metacentrics
1 pairs of subtelocentrics
3 pairs of subtelocentrics
23 pairs of acrocentrics
1 pair of sex chromosomes (ZZ) larger than the first pair of autosomes.

Chromosomal Sex Mechanism: ZW:ZZ

Chromosome Formula

Female =6m+3sm+6st+47a
Male =6m+2sm+6st+48a

Fundamental Number (FN)

Female =71
Male =70

Morphometric Data of Female Somatic Karyotype:

Actual mean length of largest chromosome =2.255um
Actual mean length of smallest chromosome =0.989um
Relative length of the largest chromosome =4.425
Relative length of smallest chromosome =1.941
Ratio of largest to smallest chromosome =2.280
Total mean haploid length =50.954um

Morphometric Data of Male Somatic Karyotype:

Actual mean length of largest chromosome =1.850um
Actual mean length of smallest chromosome =0.650um
Relative length of the largest chromosome =4.722
Relative length of smallest chromosome =1.659
Ratio of largest to smallest chromosome =2.846
Total mean haploid length =39.179um

Spermatogonial Metaphase (Fig.5)**:** 2*n*=62

Polyploidy (Fig.6):

Polyploidy is very common among the spermatogonial metaphases from larval testes. Figure 6 shows a highly polyploid nucleus under the effect of colchicine treatment. Almost all the elements reveal clear splitting of the chromatids and the location of primary constrictions.

Male Meiotic Stages:

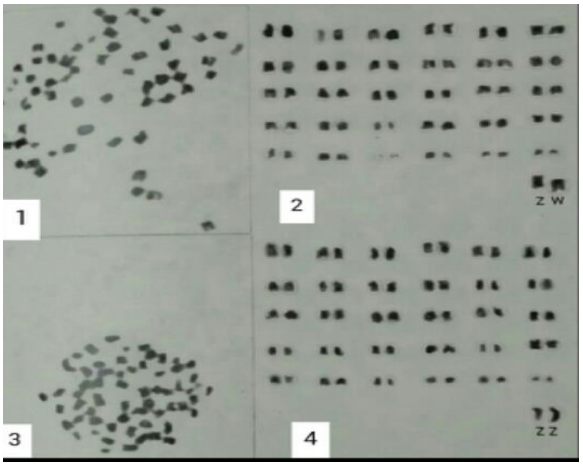
Diakinesis (Fig.7) reveals discrete chiasmata in each of the individual bivalents. The chiasma frequency per bivalent varies from 1 to 2. Metaphase I (Fig.9) proceeds with condensation of all the 31 bivalents. Dumbbell shaped bivalents are very clearly seen in the side view of prometaphase I (Fig.8). Metaphase II (Fig.10) reveals 31 univalents and confirms the haploid number of complement to be n=31.

Sex Chromatin (Fig.11)**:**

A single darkly stained sex chromatin body found in the somatic interphase nucleus from brain cells of female *A. spinifera* depict the female to be the heterogametic sex with sex chromatin representing the W chromosome in the resting nucleus.

Discussion

Agrotis spinifera (2*n*=62) belongs to the family Noctuidae of insect order Lepidoptera. The most frequently occurring diploid number of this family is 2*n*=62 which is clearly shown by the histogram given by Sahni (1997) depicting the frequency distribution of haploid chromosome numbers in 74 species of Noctuidae. Our present reports of female heterogamety obtained by modified method of *in vitro* colchine treatment is in karyotypic consonance of the family Noctuidae (Rishi *et al.*, 2001). Sex chromosome mechanism with ZW:ZZ sex chromosome, has been confirmed from the karyotypable elements obtained from the male and female somatic metaphases. Female heterogamety has further been confirmed on the basis of heteropycnotic body



Figures1: Somatic metaphase chromosomes from brain cells of *Agrotis spinifera* female showing splitting of the chromatids(2*n*=62); 2: Karyotype prepared from fig 1; 3: Somatic metaphase chromosomes from brain cells of *Agrotis spinifera* male showing splitting of the chromatids; 4: Karyotype prepared from fig 3.

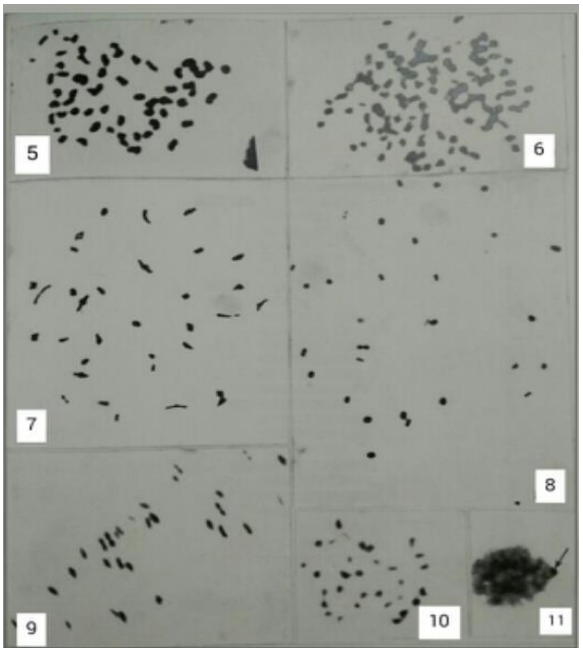


Figure 5: Spermatogonial mitotic metaphase of *Agrotis spinifera*; 6: Polyploid spermatogonial nucleus with most of the elements showing splitting of the chromatids; 7: Diakinesis of the male meiotic prophase; 8: Prometaphase I, n=31 (side view); 9: Metaphase I (n=31); 10: Metaphase II; 11: Female interphase nucleus with distinct heteropycnotic sex chromatin body

found in more than 70% of the somatic interphase nuclei of brain cells of female larvae. Occurrence of sex chromatin in the interphase nuclei of the heterogametic females of Lepidoptera has been found to correspond to the heteropycnotic W or Y chromosome (Marec and Traut, 1994; Rishi *et al.*, 1997). Recent molecular insights revealed the evolution of W chromosome in 3 species of Lepidoptera (Dalikova *et al.*, 2017) using comparative genomic hybridisation. Presently reported ZW:ZZ sex mechanism has been authenticated in a pyralid moth *Sylepta multilinealis* and a lemon butterfly, *Papilio demoleus* by successful application of a differential staining technique of G-banding (Dhawan, 2016).

Karyotypic evolution and sex determining mechanisms have been described in 29 orders of insects (Blackmon *et al.*, 2017). Both types of kinetic organisations-holokinetic as well as monokinetic have been claimed for Lepidoptera chromosomes (Bigger 1975, 1976). Our earlier reports of localized centromeres in *Ergolis merione* , *Papilio demoleus* and *Sylepta multilinealis* (Rishi *et al*. 1997, 2000; Dhawan 2016) are in consonance with the monokinetic organization of mitotic chromosomes of different species of *Pieris* (Bigger, 1976) and electron microscopic study of *Ephestia* and *Trichoplusia* chromosomes (Gassner and Klemetson, 1974). Present results of advanced spermatogonial metaphases revealed contracted chromosomes with a small proportion of obviously holokinetic chromosomes as evidenced by Bigger (1975) who suggested that early metaphase exhibit a monocentric type of organization but as metaphase proceeds, influence of primary centromere is either lost or superseded by the combined influence of the rest of the centromere. This fits into the idea of Traut (1986) who claimed the dual nature of Kinetochore organisation in Lepidoptera. The present discovery of distinct localised centromeres in early somatic metaphases of a noctuid moth, *Agrotis spinifera*, is thus extremely worth further investigation particularly with regard to the theories proposed in order to explain karyotypic evolution in Lepidoptera.

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