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FEMALE HETEROGAMETY REVEALED BY APPLICATION OF DIFFERENTIAL STAINING TECHNIQUE IN A SPECIES OF LEPIDOPTERA

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ABSTRACT

The mitotic metaphases in males and females of Sylepta multilinealis Guen. were studied using in vitro colchicine treatment and differential staining technique of G-banding. Karyotypes prepared from G-banded female somatic metaphases of brain ganglia revealed 2n=62 with heteromorphic sex chromosome pair ZW, while the male karyotypes (2n=62) showed a homomorphic ZZ sex chromosome pair. Z was distinctly recognized as the largest element in the female somatic karyotypes. Pair nos. 1, 3, 8, and 10 had dark G- positive bands at their telomeric ends. Many pairs revealed dark bands and light interbands of different intensity throughout their lengths. Autosomal pair numbers 14, 23 and 28 revealed interband regions lacking in stain at the site of secondary constriction there by depicting their satellite like structures which were otherwise not clear from the normal Giemsa stained preparations. G-banding data for sex chromosomes of Lepidoptera are quite scarce. The sex mechanism study in present species is new to cytology as this is the first report of female heterogamety in S. multilinealis. The existence of sex chromosomes, thus represents a derived exceptional condition in Lepidoptera and its appearance in different systems seems to indicate a recurrent evolutionary trend worth further investigation.

KEYWORDS: Lepidopteran Chromosomes, Female Heterogamety, Differential Staining, Somatic Karyotypes, G-Banding

INTRODUCTION

Genetically there have been sufficient data about the female heterogamety in Lepidoptera (Robinson, 1971) but cytological proofs are relatively scanty (Suomalainen, 1969, 1971; Bigger, 1975, 1976; Maeki, 1981; Rishi and Rishi, 1985, 1990; Kawazoe, 1992; Saitoh, 1989; Izumi and Seto, 1995; Sahni, 1997; Rishi *et. al*., 1997, 1999, 2000a, 2001). This is mainly due to the fact that the sex chromosomes of most lepidopteran species can hardly be distinguished from the autosomes in the metaphase plates, because their size and shape are usually the same. Further the Z and W chromosomes of most lepidopteran species are of the same size, forming a bivalent that is not recognizable by heteromorphism either. Some of the latest studies (Maeki and Miyawaki, 1987; Maeki *et. al.* 1990; Kawazoe, 1992; Rishi *et. al.*, 2001) revealed that X (or Z) and its counterpart Y (or W) occurred in many lepidopteran species. Almost all types of sex chromosomes have been described in Lepidoptera with female heterogamety. The earlier workers have described the lepidopteran chromosomes as holocentric or having diffused centromeres (Bauer, 1967; Barry *et. al.*, 1967; Murakami and Imai, 1974; Suomalainen, 1953, 1969; Trentini and Marini, 1986; Virkki, 1963 and others). However, in several species of Lepidoptera, monocentric chromosomes have been shown by the application of better methodology (Bigger, 1975, 1976; Rishi and Rishi, 1990; Rishi *et. al.*, 1997, 1999, 2000, 2001). The present investigations deal with chromosome numbers and female heterogamety in *Sylepta multilinealis* by analysis of G-banded chromosome preparations obtained from brain ganglia of both the sexes.

MATERIAL AND METHODS

Different instar larvae of *Sylepta multilinealis* were collected from the host plant *Vitis veinifera* in the months of July- August. 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. G-banding was processed by ASG Technique (Sumner *et. al.*, 1971) and Trypsin treatment method modified after Sea bright, 1971. Chromosome counts were made from 50-70 metaphases in each male and female specimen from more than ten larvae.

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 (Figure 1)

Male 2n = 62 (Figure 3)

SOMATIC KARYOTYPES

Female (Figure 2)

18 pairs of moderately to considerably long autosomes.

10 pairs of small autosomes, approximately half of the length of the largest element.

2 pairs of very small isodiametric chromosomes.

Karyotype (Figure 2) prepared from female somatic metaphase (Figure 1) shows female to be heterogametic sex with sex chromosomes placed at the last. Z is the largest element in the diploid set and W is the second largest element.

Male (Figure. 4)

Karyotype (Figure 4) prepared from Figure 3 shows the largest sex chromosome

pair ZZ. Male is homogametic sex.

18 pairs of moderately to considerably long autosomes.

10 pairs of small autosomes, approximately half the length of the largest element.

2 pairs of very small isodiametric chromosomes.

G-BANDING

Female (Figures 5, 6)

G- Banded somatic prometaphase chromosomes from brain cells of female *Sylepta multlinealis* clearly show a number of dark and light bands along their lengths. Pair numbers 1, 3, 8and 10 have dark G-positive bands at one of their

telomeric ends. The second largest sex chromosome, W is totally darkly stained. The largest sex chromosome Z shows 5 dark and 4 light bands. Pair numbers 2, 6 and 12 have three distinct dark alternating with two light bands. Pair numbers 3, 5, 6, 11 and 27 show dark bands at one of their knobbed ends. Pair number 28 shows a light stained interband region. Pair number 14, 23 and 28 of autosomal chromosomes reveal interband regions lacking in stain at the site of secondary constriction thereby depicting their satellite like structures which are otherwise not clear from the normal Giemsa stained preparations.

Male (Figures 7, 8)

Much elongated somatic prometaphase chromosomes from brain cells of male *Sylepta multilinealis* responded very well to GTG (G- banding using Trypsin and Giemsa stain) technique. Prominent and characteristic G-bands are found on the terminal regions of the Z chromosomes. Pair numbers 5, 8, 23, 28 and 30 show telomeric G positive bands as found in the response to G banding. Pair numbers 7, 10, 17, 20, 23 and 28 show a large light stained interband region in each of the chromosome pair. Centromeric region of pair numbers 22 and 26 of autosomes show a positive response to G banding. Rest of the autosomes show clear light and dark bands of different intensities throughout the length of chromosomes The bands are found to be constant and specific for each homologous pair. (Figure 8)

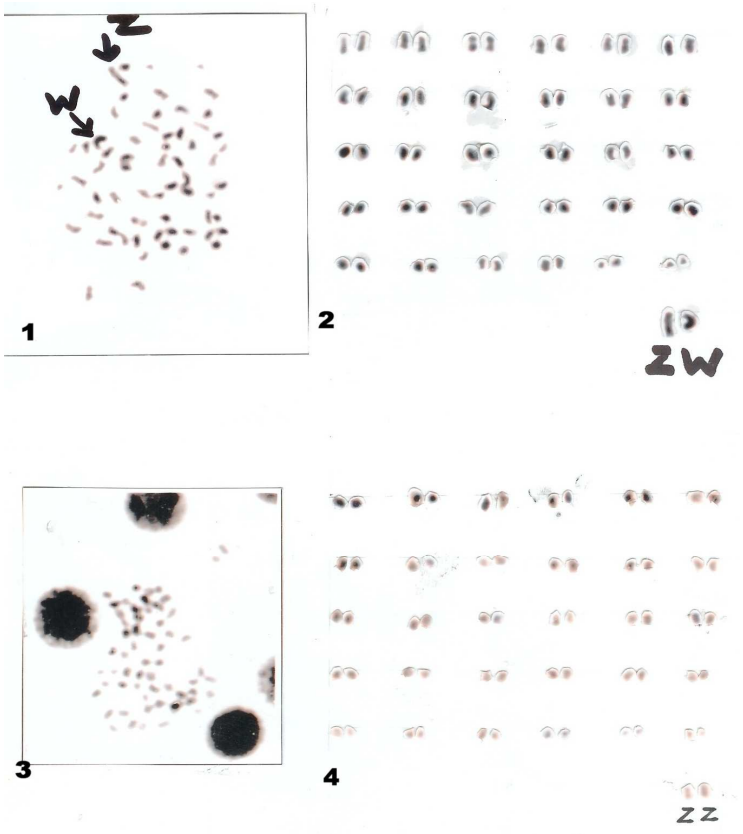

Figure 1	Somatic Metaphase from Brain Ganglia Cell of Female *Sylepta Multilinealis* (2n=62). Arrow Indicates the Largest Z and the Second Largest W Chromosomes.
Figure 2	Karyotype Prepared from Figure 1.
Figure 3	Somatic Metaphase from Brain Cell of Male *Sylepta Multilinealis* (2n=62). Arrow Indicates the Largest Z Chromosomes.
Figure 4	Karyotype Prepared from Figure 3.

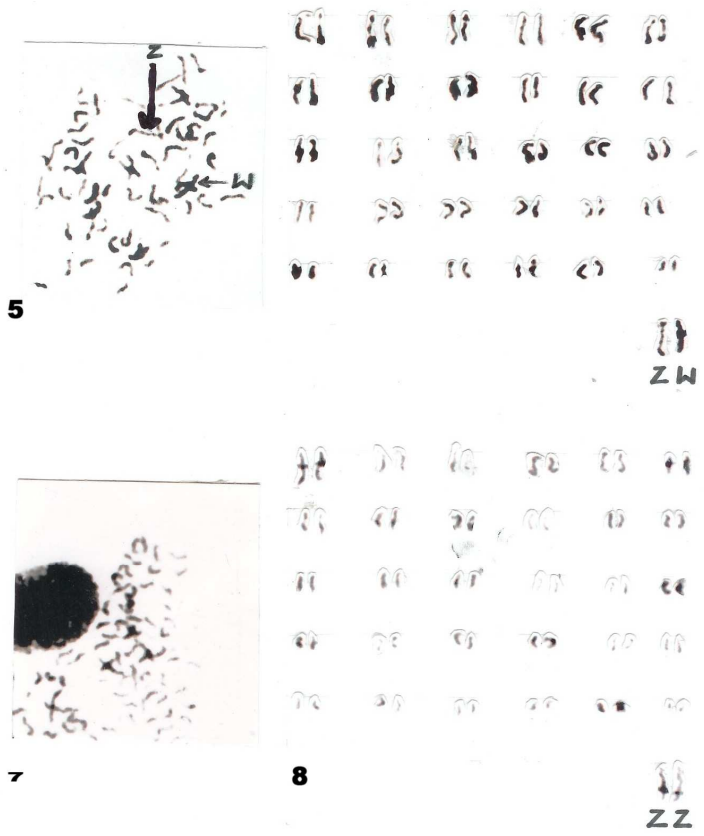

Figure 5	G- Banded Somatic Prometaphase from Brain Cell of Female *Sylepta Multilinealis.* The Largest Element Z Clearly Shows 5 Dark Bands.
Figure 6	Karyotype Prepared from Figure 5.
Figure 7	G- Banded Somatic Prometaphase from Brain Cell of Male *Sylepta Multilinealis*..
Figure 8	Karyotype Prepared from Figure 7.

DISCUSSIONS

The reports of G-banding are very scanty in lepidopteran species (Sahni, 1997). Positive G-bands have been reported by Bigger, 1975, 1976; Maeki *et. al*., 1990; Rishi and Rishi, 1990; and Rishi *et. al*, 1997. Bigger, 1975, 1976 reported female heterogamety on the basis of heteromorphic sex chromosome pair(XY) present in the G-banded karyotypes of female mitotic metaphases in Pierid species. Our earlier reports of G-banding in *Papilio demoleus* (Rishi *et. al.*, 1997) described female heterogamety on the basis of G-banded karyotypes revealing maximum number of G-bands seen per chromosome showing different sizes and staining intensities. Maeki *et. al.*, 1990 observed one or two elongated chromosomes which took the sixth pair position in the G-banded karyotypes of female *Graphium sarpedon*. He assumed that, in Lepidoptera, such elongated chromosomes having constrictions tend to readily detach a satellite from the rest and that the elements designated as supernumerary chromosomes correspond to these satellites in such origin.

During the present investigations, G-banding has been successfully applied to mitotic chromosomes of a pyralid moth, *Sylepta multilinealis* which are the first reports of female heterogamety in this species. These are in confirmation with our earlier publication of ZW: ZZ mechanism in seven species of Indian noctuid moths (Rishi *et. al.*, 2001). On the basis of G-banded somatic prometaphases, Z was distinctly recognized as the largest element with five dark and four light bands while W could be recognized as the second largest totally dark stained element. The bands were found to be constant

and specific for each homologous pair as reported by Bigger, 1975, 1976. Pair numbers 14, 23 and 28 of autosomal chromosomes revealed the features similar to that of *Graphium sarpedon* studied by Maeki *et. al*., 1990. These are certainly the chromosomes having the interband regions lacking in stain at the site of secondary constrictions thereby depicting their satellite like structure, which are otherwise not clear from the normal Giemsa stained preparations.

Though the female heterogamety with different sex mechanisms of ZW: ZZ (Rishi *et. al*., 2000); $AA^{W}Z$: ZZ (Rishi *et. al.*, 1999) and ZZ: ZO (Rishi and Rishi, 1985) have been reported by us in recent publications, present reports of G-banding in *S. multilinealis* are new to cytology. Positive G-bands obtained in the presently analyzed species indicate that its genome might have a division into AT- and GC- rich isochors. The bands in some of the autosomes are, however, too little contrasted. W, the sex chromosome, does not reveal clear alternate bands. Admittedly, the G-bands in the lepidopteran species have not yet been well differentiated though the preliminary study indicates that there are prospects for production of chromosome maps in economically important species with further standardization of banding techniques in Lepidoptera.

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