International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 30-01-2023, Accepted: 14-02-2023, Published: 02-03-2023
Volume 8, Issue 3, 2023, Page No. 13-16

G-banded karyotypes of *Ascotis imparta* (Lepidoptera, Geometridae) revealing ZW: ZZ sex mechanism

Dr. Geetanjli Sahni
Department of Zoology, Arya PG College, Panipat, Haryana, India

Abstract
Modified method of Trypsin-EDTA G-banding was applied to the somatic metaphase chromosomes of a lepidopteran species, *Ascotis imparta* belonging to family geometridae. Fifth instar larvae were captured from economically important plant *Ricinus communis* (castor) near Brahmsarover, Kurukshetra. First the elongated karyotypable elements could be secured by application of *in vitro* colchicine treatment as the mitotic division was successfully arrested at metaphase stage. Both male and female karyotypes from somatic metaphases of brain ganglia revealed 2*n*=62. Although Giemsa-stained preparations confirmed female heterogamety with ZW: ZZ sex mechanism, this was authenticated by modified G-banding technique confirming ZZ to be the largest pair still longer than the first autosomal pair; W being comparatively smaller in between the size of 2nd and 3rd autosomal pairs. Results were compiled on the basis of Morphometric data analysis of somatic karyotypes (2*n*=62) including mean total length (um) and relative length percentage (RL%) of individual G-banded chromosomal elements in both the sexes. This is the first report of differential staining technique applied to a geometrid Lepidopteran species *A. imparta* and is worth further investigation in the field of cytogenetics.

Keywords: giemsa stained preparations, differential staining, somatic karyotypes, female heterogamety

Introduction

Differential staining technique of G-banding produces a series of consistent landmarks throughout the length of elongated metaphase chromosomes (Dhawan,2018) [3]. This allows not only the recognition of individual chromosomes in a genome but also the identification of specific segments of individual chromosomes. Pattern and trends of karyotypic evolution in many lepidopteran families are poorly studied (Lukhtanov,2014) [6]. A survey of chromosomes numbers of 32 different species of butterflies and moths (Sahni,1997) [8, 10] and analysis of our published data of 7 species of noctuid moths (Rishi *et al*, 2001) [9] demonstrate that the majority of lepidopterans preserve the haploid chromosome number *n*=31 which is definitely the ancestral number for the order Lepidoptera at whole and for the family geometridae to which the present species belongs. Sahni,1997 [8, 10] compiled the chromosomal investigations of 102 species of geometrid loopers depiciting *n*=31 to be most common haploid number but the cytological information on the sex chromosomes of this family is very scanty. Though female heterogamety has been described in most of the lepidopteran species (Dhawan, 2018) [3] based on modified *in vitro* colchicine air drying technique and karyotypic preparations of mitotic chromosomes, the heteromorphic ZW pair has not been described on the basis differential staining techniques (Sahni, 2022) [11].The present report of ZW:ZZ mechanism in a geometrid looper *Ascotis imparta* based on G-banded somatic karyotypes prepared from male and female brain ganglia of 3rd instar and prepupal larvae is new to cytology.

Materials and methods

Third instar lavae of *Ascotis imparta* were collected from the host plant *Ricinus communis* (Arind plant) in the month of December-January from Kurukshetra. Some of them were fed to maturity to attain prepupal stages. Brain ganglia of male and female larvae and testes were processed for chromosome analysis following modified *in vitro* colchicine air drying technique (Dhawan,2018) [3]. Some of the slides were stained in 2% Giemsa for meiotic preparations. Slides for G banding were further incubated in 2*SSC at 60°C for 1 hour and treated in Trypsin- EDTA solution for 5-7 minutes. After differentiating in phosphate buffer (pH 6.8), the slides were air dried. These were cleared in xylene and mounted in DPX. These were then subjected to photomicrography at 100X under oil immersion with Olympus microsocope. Morphometric measurements of chromosome cut out from photomicrographs were done with the help pf vernier calliper (0.01 mm). Finally, karyotypes were prepared after calculating centrometric indices, relative length percentage and arm ratio (Sahni,2022) [11].

Observations and results

Somatic Metaphase

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

Somatic karyotype

Female (Fig. 2)

6 pairs of long autosomes

21 pairs of moderate-sized autosomes

3 pairs of small autosomes, shorter than half the length of the largest autosomes

1 pair of heteromorphic sex chromosomes (ZW) consisting of a largest

Z and a comparatively smaller W chromosome (in between the size of 2nd and 3rd autosomal pairs).

Pair nos. 6, 11, 15, 20 and 27 are satellite chromosomes

Male (Fig. 4)
6 pairs of long autosomes
21 pairs of moderate-sized autosomes
3 pairs of small autosomes, shorter than half the length of the largest autosomes
1 pair of sex chromosomes (ZZ) comprising the largest pair still longer than the first autosomal pair.
Pair nos. 6, 11, 15, 20 and 27 are satellite chromosomes

Chromosomal Sex Mechanism: ZW:ZZ

Morphometric data of female somatic karyotype
Actual mean length of the largest chromosome = 3.303µm
Actual mean length of the smallest chromosome = 1.020 µm
Relative length of the largest chromosome. =5.021
Relative length of the smallest chromosome. = 1.551
Ratio of largest to smallest chromosome =3.326
Total mean Haploid length = 65.775 µm

Morphometric data of male somatic karyotype
Actual mean length of the largest chromosome. =7.838µm
Actual mean length of the smallest chromosome =1.458µm
Relative length of the largest chromosome. = 5.757
Relative length of the smallest chromosome =1.071
Ratio of largest to smallest chromosome = 5.377
Total mean Haploid length =136.136 µm

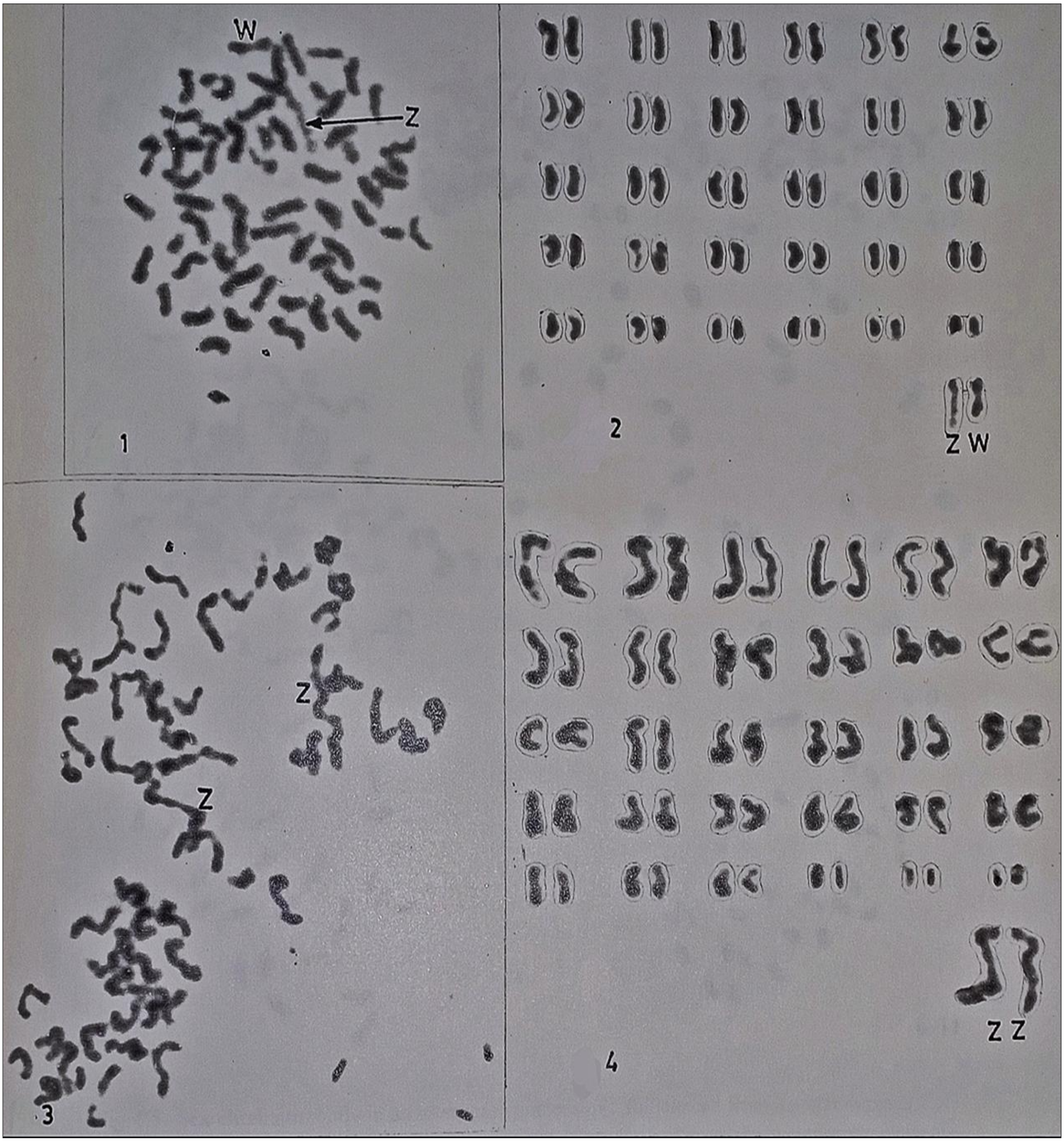

Fig 1: Somatic metaphase from brain cells of female *Ascotis imparta* (2*n*=62) **Fig.2:** Karyotype prepared from metaphase figure 1 **Fig 3:** Somatic prometaphase from brain cells of male *A. imparta* (2*n*=62) **Fig 4:** Karyotype prepared from metaphase figure 3

G-banding

Digestion of the chromosomes by proteolytic enzyme, trypsin followed by staining with buffered Giemsa lead to the differentiation of chromosomes into light and dark bands of different intensities throughout the length of the chromosomes. G-banded somatic prometaphase chromosomes of male *A. imparta* (Fig.3) show variable number of bands that are found to be constant and specific for each homologous pair. The largest pair of sex chromosomes, ZZ (Fig.4) clearly shows 5 dark bands alternating with 4 light bands. W chromosome of the hereterogametic female sex chromosome pair ZW shows a large centromeric peculiar G-negative zone which defines the primary constriction region clearly. Among the largest 6 autosomal pairs, pair nos. 1 and 4 shows clear 4 light bands alternating with 5 dark bands including the terminal dark G-positive bands. The sixth, eleventh, fifteenth, twentieth, and twenty seventh pairs of autosomes reveal a small subterminal interband region lacking in stain. These are the unique G-negative zones and facilitate the detection of satellite like structures distal to the secondary constrictions.

Discussion

Of the five families of superfamily Geometroidea, it is only for three that the cytological data are available. Though there are 102 cytologically known species of the family Geometridae, the present reports of successful application of G-banding technique is new to cytology. The meiotic chromosomal observations from prepupal testis confirm the most frequently occurring haploid number to be n=31 as reported by Sahni (1997) [8, 10] in 31 species of these geometrid loopers. But many congeneric forms of *Biston* (Malan, 1918) [7] and *Cidaria* (Suomalainen 1965) exhibit widely different haploid numbers varying from *n*=12 to 56.

In the presently studied genus *Ascotis,* one of the species *A. selenaria* worked out by Scheepens and Wysoki (1985) [13] has a haploid number of 31 chromosomes. Another species *Ascotis selenaria cretacea* studied by Saitoh (1959) [12] has *n*=34, still another congeneric form *Ascotis imparta* (present study) reveals 31 chromosomes at the first meiotic metaphase. Thus, the family Geometridae furnishes a good example of the distribution of variable chromosome numbers in Lepidoptera. G- banded Karyotypes of the presently investigated geometrid, *Ascotis imparta* shows three groups of autosomal pairs composed of long moderate sized and short elements, similar to that reported in case of *Planociampa antipala* (Kawazoe, 1992) [5]. Sex chromosomes are also comparable, ZZ being larger than the largest pair of autosomes with fairly distinct constrictions at the subterminal regions. The ill-stained regions at the middle of autosomal pair number 26 in *Descoreba simplex* (Kawazoe, 1992) [5] are similar to that of unique G-negative zones in the autosomal pair numbers 6,11,15,20,27 of the presently investigated *Ascotis imparata*. These are actually the secondary constrictions which tend to detach the satellite like structures distal to them. This species worked out during the present study is entirely new to karyology.

Present reports of ZW: ZZ mechanism based on modified differential staining procedure of Trypsin EDTA G-banding are in confirmation with our earlier publications of female heterogamety of a noctuid moth, *Agrotis spinifera* (Dhawan,2018) and a papilionid butterfly, *Papillo demoleus* (Rishi *et al*.,1997) [8].

The dark G-positive bands revealed by G-banded somatic metaphases from male and female brain ganglia of the present looper *Ascotis imparta* are found to be constant and specific in 30 homologous autosomal pairs. These results are concordant with my recent reports of chromosomes pairs having the interband regions which lack stain at the site of secondary constrictions (Dhawan, 2016) [2]. These definitely depict their satellite structure which would otherwise have not been possibly identified from earlier staining of giemsa or acetolactic orcein squash preparation.

GTG (G- banding using Trypsin and Giemsa stain) technique revealed prominent and characteristic G-bands in terminal regions of ZZ pair in male somatic karyotypes of noctuid moth, *Sylepta multilinealis* (Dhawan,2016) [2]. The reports of Z being the largest sex element in both the sexes with a subterminal constriction have been confirmed by application of further modified Trypsin-EDTA method of G-banding in present species of a geometrid moth *Ascotis imparta* along with confirmation of ZW: ZZ female heterogamety, W depicting a centric constriction with monocentric organisation depicted by *in vitro* colchicine air drying technique. Study of chromosome polymorphism probably resulting from chromosome fragmentation (Brown *et.al*,1993) [1] and claiming that the lepidopteran genome might have a division into AT and GC rich isochores would be the new prospects if such studies of differential staining are carried out in lepidopteran species in future. Moreover, there are further prospects of preparation of chromosome maps in economically important lepidopteran pests with further standardization of G-banding techniques.

Conclusion

G-banded somatic karyotypes have been reported for the first time in family Geometridae of Lepidoptera. The metaphase chromosomes from brain ganglia of male and female geometrid moth, *Ascotis imparta* have been subjected to differential staining technique of modified G-banding and these reports of ZW: ZZ female heterogametic from G-banded somatic karyotypes are new to cytology. These have the future prospects of reporting adaptive rearrangements of chromosomal segments related to mimicry, allowing “rapid evolution as a result of escaping predator through new colour morphs. especially loopers which resembles a stick or twig to match the surroundings”. This least explored family Geometridae requires intensive karyological studies with respect to karyotypic evolution.

References

1. Brown KS Jr, Thomas CE, Peter JE, Esko S. Evolutionary pattern in chromosome numbers in neotropical Lepidoptera: I. Chromosomes of the *Heliconiini* (Family Nymphalidae: Subfamily Nymphalinae). *Hereditas*,1993:117(2):109-125.
2. Dhawan G. Female heterogamety revealed by application of differential staining technique in a species of Lepidoptera. International Journal of Applied and Natural Sciences,2016:5(4):127-132.
3. Dhawan G. Karyotypable somatic metaphases obtained in *Agrotis spinifera* (Noctuidae, Lepidoptera) by application of *in vitro* colchicine air drying technique.

Halters. ISSN:0973-1555 Print, ISSN:2348-7372 Online,2018:9:176-179.

4. Hejnickova M, Dalikova M, Potocky P, Tammarce T, Trehubenko M, Kubickova S, *et al*. Degenerated, undifferentiated, Rearranged, Lost: High Variability of Sex Chromosomes in Geometridae (Lepidoptera) Identified by Sex Chromatin, 2021. Cells. 10,2230. http://doi.org/10.3390/cells10092230
5. Kawazoe A. Chromosomes of some univoltine moths in the cold season (3). Chromo. Inf. Serv,1992:53:27-30.
6. Lukhtanov VA. Chromosome number evolution in skippers (Lepidoptera, Hesperiidae). Comp Cytogenet,2014:8(4):275-291.
7. Malan DE. Ergebnisse anatomischer Untersuchungen an Standfusschen Lepidopteren Bastards. Mitt. Entomologia. Zurich,1918:4: 201-260.
8. Rishi S, Sahni G, Rishi KK. Female heterogamety, localized centeromeres in *Papilio demoleus* Linn. (Lepidoptera, Papilionidae). Chromosome Science,1997:1:109-113.
9. Rishi S, Sahni G, Rishi KK. Karyotypes of seven species of Indian Noctuid moths (Lepidoptera, Noctuidae). Chromosome Science,2001:5:39-46.
10. Sahni G. 'Chromosomal studies in some Indian Lepidoptera'. Ph.D thesis submitted to K.U., Kurukshetra, Haryana, 1997.
11. Sahni G. Karyotypic study in a pyralid moth species belonging to order Lepidoptera: an insect pest borer of the brinjal. Proc. Zool. Soc. India, 2022:21(1):79-83.
12. Saitoh K. The chromosomes of some species of Moths. Jap. J. Genet,1959:34:84-87.
13. Scheepens MHM, Wysoki M. Testicular development, spermatogenesis and chromosomes of *Boarmia selenaria* Schiff. (Lepidoptera: Geometridae). International Journal of Invertebrate Reproduction and Development,1985:8:337-348.
14. Suomalainen E. On the chromosomes of the geometrid moth genus Cidaria. Chromosoma (Berl.),1965:16:166-184.