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Karyotypic architecture of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae: Chrysomelinae) from Kurukshetra, Haryana, India

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ABSTRACT

This study examined the chromosomal structure and behavior of *Zygogramma bicolorata*, a species belonging to the subfamily Chrysomelinae within the family Chrysomelidae. Traditional Giemsa staining was employed for morphological characterization, while C-banding was used to detect constitutive heterochromatin. Karyotype analysis revealed a diploid chromosome number of 2n = 24 and a meioformula of 11AA + Xyp. Most chromosomes were either metacentric or submetacentric, whereas the Y chromosome was acrocentric. Within the subfamily Chrysomelinae, both increases and decreases in chromosome number were observed, likely resulting from centric fission and centric fusion events, respectively. But in genus *Zygogramma* only increase in chromosome number suggests the involvement of Robertsian centric fission in the generation of karyotypic diversity.

INTRODUCTION

The order Coleoptera, through numerous speciation events, has become the most species-rich group among insects, exhibiting the greatest diversity in species. As early as 1968, Arnett documented approximately 350,000 Coleopteran species. A decade later, Smith and Virkki (1978) compiled chromosomal data for 2,160 species, subspecies, and parthenogenetic forms across 45 families, based on studies conducted up to 1975. In the four decades since, there has been substantial progress in our understanding of chromosomal cytology within this order. Nevertheless, despite its extraordinary species richness, cytogenetic research utilizing specific banding techniques remains relatively limited. The family Chrysomelidae, in particular, has been the focus of extensive chromosomal studies. A review by Petitpierre et al. (1988) reported cytogenetic data for 759 species; about 3% of the family primarily using conventional staining methods. Members of this family display a broad range of karyotypic variation and have established both the upper and lower bounds of chromosome numbers observed within Coleoptera. Previous studies revealed the diploid number of species *Zygogramma bicolorata*, but the present investigation has reported the centromeric position of chromosomes, sex chromosomal mechanism and behavior of the chromosomes during different stages of meiosis and mitosis.

Materials and Methods

Collection:

Adult male specimens of *Zygogramma bicolorata* were collected from the Kurukshetra University Campus in Kurukshetra, Haryana, India. The geographical coordinates of the collection site are 29°57'20.635" N latitude and 76°49'18.235" E longitude.

Slide Preparation:

Beetles were sacrificed in a 0.56% potassium chloride (KCl) solution to extract testicular tissue. The testes were then treated with 0.001% colchicine for 20 minutes to arrest cell division, followed by hypotonic treatment in 1% sodium citrate solution for another 20 minutes. The tissue was subsequently fixed in a 1:3 mixture of acetic acid and methanol for 30 minutes. Slides were prepared using the standard air-drying technique.

Staining Procedure:

Chromosomal behavior and structure were studied using the staining method described by Yadav and Lyapunova (1983). The testicular tissue was placed in 2–3 drops of 50% glacial acetic acid on grease-free, ethanol-cleaned slides. It was then macerated with a fine needle, air-dried, and stained with 2% Giemsa solution.

Photography:

Photomicrographs of at least 10 well-spread metaphase plates were taken using an Olympus C-7070 digital compact camera. These images were analyzed to determine chromosome number, morphology, and karyomorphometric characteristics. Chromosomes were classified according to centromere position into metacentric (m), submetacentric (sm), subtelocentric (st), and telocentric (t) categories, following the criteria of Levan et al. (1964).

Analysis:

Karyomorphometric analysis was conducted on 10 metaphase plates showing distinct centromeres and intact, non-overlapping

chromosomes, based on the methodology outlined by Yadav and Lyapunova (1983). Measurements included the percentage relative length of each chromosome by using the formula given below.

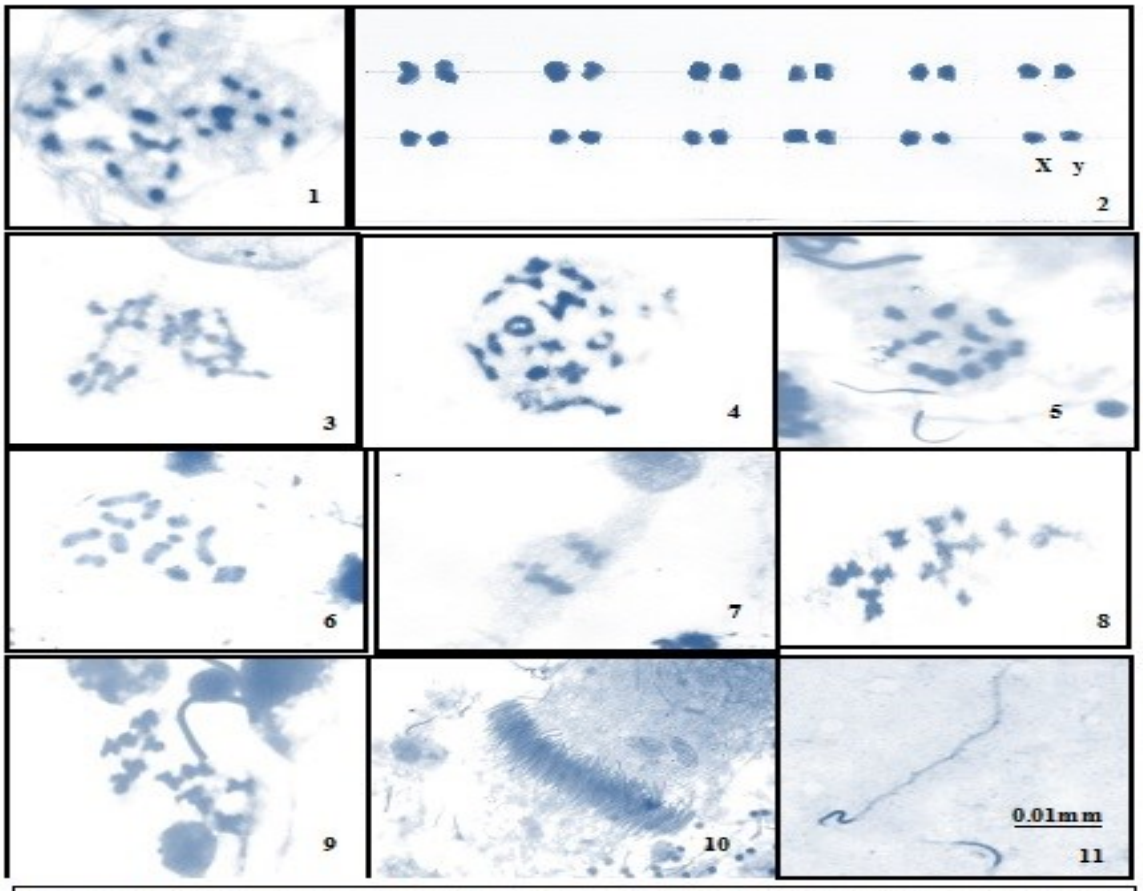
$$Percentage\ Relative\ length\ of\ chromosome = \frac{\text{Length of individual chromosome}}{\text{Total length of chromosomes in one set}} X\ 100$$

Additionally, C-bands were determined using the methods given by Sumner (1972) to reveal the constitutive heterochramatin in chromosomes at different stages.

Results and Discussion

Spermatogonial metaphase analysis confirmed a diploid chromosome number of 24 (Fig. 1). The karyotype includes eight pairs of metacentric autosomes (pairs 1–8) and three pairs of submetacentric autosomes (pairs 9–11), along with a metacentric X chromosome and an acrocentric y chromosome (Fig. 2). The percentage relative length of autosomes ranged from 5.89% to 11.54%, while the X and Y chromosomes measured 5.84% and 5.53%, respectively (Fig. 12).

At the pachytene stage, autosomal bivalents exhibited a characteristic woolly appearance (Fig. 3), and the sex chromosomes were positioned peripherally at the polarized end of the nucleus. During diakinesis, the presence of seven rod-shaped, two ring-shaped, and two cross-shaped bivalents, along with a highly condensed sex vesicle was observed (Fig. 4). At metaphase I, two ring-shaped and nine dumbbell-shaped autosomal bivalents were noted, accompanied by a sex chromosome complex forming a parachute-like Xyp configuration (Fig. 5). C-banded metaphase I spread revealed the presence of two heterochromatic regions in each autosomal bivalent and a single heterochromatic region in the sex pseudovivalent (Fig. 6). The meiotic formula for this species is represented as 11AA + Xyp. In late anaphase I, autosomes and sex chromosomes segregated and migrated toward opposite poles, depicting dot like sex chromosomes lagging behind the autosomes (Fig. 7). Following the first meiotic division, two distinct types of metaphase II plates were observed: 11A + X (Fig. 8) and 11A + y (Fig. 9). Chromatid separation provided clear visibility of chromosome morphology, consistent with observations made at spermatogonial metaphase. On average, each sperm bundle contained approximately 110 spermatozoa (Fig. 10). Spermatozoa exhibited long tails and hooked heads (Fig. 11).



Zygogramma bicolorata Pallister Fig. 1:Spermatogonial metaphase; Fig. 2: Karyotype; Fig. 3: Pachytene; Fig. 4: Diakinesis; Fig. 5: Metaphase-I; Fig. 6 Metaphase-I (C-Banded); Fig. 7: Anaphase-I; Fig. 8: Metaphase- II with X; Fig. 9: Metaphase-II with y; Fig. 10: Sperm Bundle; Fig. 11: Sperms

The haploid number of chromosomes in Chrysomellidae varies from 4 in *Homoschema nigroventre* (Virkki and Purcell 1985) to 32 in *Disonycha bicarinata* (Vidal 1984). A range of haploid numbers from 5 to 29 was observed in subfamily Chrysomellinae with cytological data in 222 species of 37 genera belonging to 9 tribes of Chrysomellinae was on record till date (Petitpierre *et al*. 1988). Only 3 species of the genus *Zygogramma* belonging to the tribe Doryphorina were presented with diploid number and meioformula till date : *Zygogramma bigenera* Stal with meioformula 11+X0 (Virkki, 1964); *Z. bicolorata* Pallister with diploid number 24 and meioformula 11+Xyp (Yadav *et al*. 1992a) and *Z. tetragramma* Klug with diploid number 27 and meioformula 13+X (Vidal, 1984).

The mean chiasmata frequency per bivalent was 1.1, with a terminalization coefficient of 1.0. A frequency of 1.1 means that, on average, each bivalent forms slightly more than one crossover. This is typical for many organisms where at least one crossover per bivalent is necessary for proper chromosome segregation. The presence of more than >1 crossover suggests a moderate level of recombination, which increases genetic diversity. The fact that it is slightly more than 1 implies that some bivalents may have multiple chiasmata, while others have just one. A value of terminalization coefficient 1 means that all chiasmata are terminal they have moved to the ends of the

chromosomes. This is typically seen at diakinesis, where the chiasmata have fully terminalized in preparation for metaphase-I. Full terminalization ensures correct alignment and segregation of chromosomes. Recombination is occurring consistently, with most chromosomes undergoing at least one crossover. By diakinesis, these crossover points have completely moved to the ends of the chromosomes, which is a normal part of meiotic progression. This indicates normal meiotic behavior with effective recombination and chromosome segregation mechanics. The similar conditions were also encountered in other families of Coleoptera (Kaur 2019, 2022; Kaur and Yadav 2011, 2014, 2018, 2021, 2024)

During the course of present investigations, cytogenetic data of *Zygograma bicolorata* confirmed the earlier reports given by Yadav (1971), Yadav and Pillai (1977) and Yadav *et al*. (1992b). *Zygogramma bicolorata,* possess haploid number 12 is the most prevalent condition exhibited by 35 species of tribe Chrysomelina with Xyp and XO sex determining mechanism (Steven 1906, 1909, Robertson 1966, Albizu 1968,Vidal 1984, Petitpierre *et al*. 1988 and present report). Basic diploid number 20 of order Coleoptera was not found in tribes Chrysomelina (Petitpierre *et al*. 1988). The detailed karyotypic analysis of *Zygogramma bicolorata* presented here reveals chromosomes of both metacentric and submetacentric types, suggesting an evolutionary trend towards the consistent recombination.

Subfamily Chrysomellinae, showed both increase and decrease of chromosome number which involve the centric fission and centric fusion respectively. Chromosomal formula 11+Xyp in *Zygogramma bicolorata* agreed with reports given by Yadav (1971) and Dasgupta (1973), but other two species of this genus possess XO sex chromosome mechanism, which suggested that the centric fission if followed by erosion of y chromosome. Xyp system, typical of Coleoptera, is commonest sex chromosome mechanism including *Zygogramma bicolorata* with 11AA+Xyp, in subfamily Chrysomelinae. Family Chrysomellidae is a 'heterogenous taxonomic assemblage' and on the basis of cytological studies many authors favour the award of full familial status to a number of subfamilies (Virkki 1964, Smith 1970).

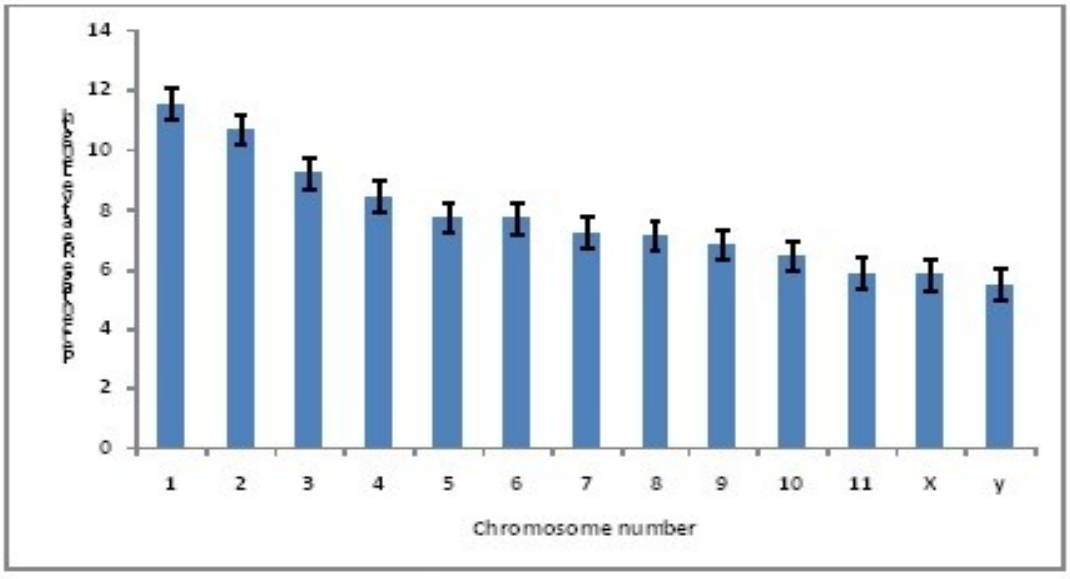


Fig. 12: Percentage relative length of chromosomes of *Zygogramma bicolorata*

Regarding the evolution of higher Chrysomelids, Crowson (1960) considered them polyphyletic in origin having developed in the Aulacoscelinae- Chrysomelinae and Eumolpinae- Galerucinae lines. Virkki (1964) reports a tendency for increase of the autosome number, probably by centric fission in the first developmental line, as in *Aulacophora feviecollis* of subfamily Galerucinae (Yadav, 1978) only exception being some species of *Trimarcha* (Petitpierre 1970, 1973), a very primitive genus with 2n= 20. The second developmental line looks cytologically more complicated, however, two main trends are observable, a decrease as well as increase in chromosome number.

Variations in the centromeric positions of chromosomes among different genera within the same subfamily indicate a degree of karyotypic diversity. This variability was attributed to various chromosomal rearrangements, including autosome–autosome and X–autosome fusions, pericentric inversions, Y chromosome loss, and chromosomal fissions. Such rearrangements are also key contributors to the overall karyotypic diversity observed within the Chrysomellidae family. Applying differential and molecular cytogenetic methods to members of this group could help to identify distinct chromosomal markers, thereby enhancing our understanding of chromosomal differentiation across various tribes and genra of Chrysomellidae.

Conflict of Interest

There aren't any disclosed conflicts of interest.

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