

KARYOTYPE ANALYSIS AND MEIOSIS IN *TRAMEA VIRGINIA* FROM HIMACHAL PRADESH

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SUMMARY

The present study was carried out on mitotic and meiotic chromosomes of *Tramea virginia* collected from Bilaspur region of Himachal Pradesh. Diploid chromosome number was found to be 25. Actual lengths of chromosomes were measured at spermatogonial metaphase. Total complement length as well as relative lengths of chromosomes were calculated. The present findings revealed the XO type of sex determination in this species.

Keywords: Holocentric chromosomes, *Tramea virginia*, dragonfly, karyotype, meiosis.

INTRODUCTION

Dragonflies constitute a well-known and widely distributed insect group belonging to the suborder Anisoptera of order Odonata. Approximately 6256 species of odonates have been recorded all over the world and about 487 species are reported from India. Of these, 276 species belonging to 8 families fall in the suborder Anisoptera (Subramanian & Babu 2017). Till now, only a few species of odonates have been studied cytogenetically due to small size of chromosomes and lack of major morphological variations in the karyotypes in this group.

Distinctive features that distinguish chromosomal complements of odonates from other groups are, holocentric chromosomes (Cumming 1964, Kiauta 1967, 1968, 1969a, b, 1975), presence of **m** chromosomes (Lefevre & McGill 1908) and XX-XO type of sex determination mechanism (Kiauta 1969a, 1975, Tyagi 1978). Haploid chromosome number in the dragonflies ranges from 3 to 19 (Kiauta 1975). Most of cytological studies pertain to family Libellulidae in which cytological data are available for 35 species (Asana & Makino 1935, Dasgupta 1957, Kiauta 1975, Tyagi 1978, Walia 2008, Walia et al. 2011). $2n = 25$ including a pair of **m** chromosomes was the modal chromosome number of this family. Variations were reported in chromosome numbers and less common were the variation in presence or absence of **m** chromosome pair. Variations in chromosome numbers arise due to fusion and fragmentation of holokinetic chromosomes.

In Himachal Pradesh, about 76 species and subspecies of dragonflies are known taxonomically (Babu 2014). Due to lack of data on the chromosomes of a dragonfly *Tramea virginia* (Rambur 1842) from Himachal Pradesh it was considered worthwhile to investigate the chromosomes of this species from this part of India.

MATERIAL AND METHODS

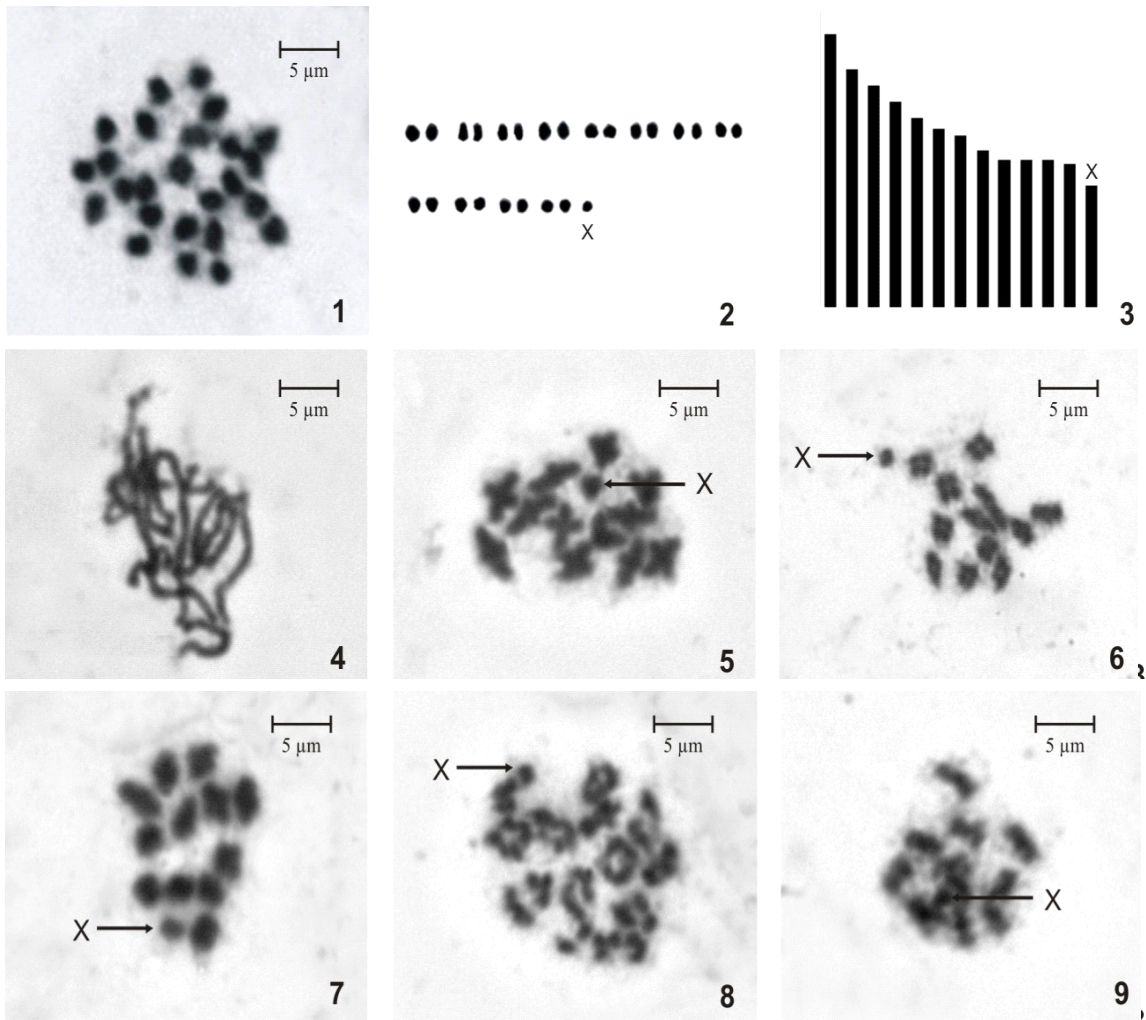
For chromosomal preparations, live male specimens of *T. virginia* were collected from Bhaged locality (31.4188°N latitude and 76.6427°E longitude and altitude 375 m above sea level) of Bilaspur district of Himachal Pradesh during April-May, 2016. Identification of individuals was done on the basis of keys provided by Fraser (1936). Germinal tissue was dissected out in normal saline solution after anaesthetizing the individuals with chloroform and pretreated with 0.7% sodium citrate solution for 30 min at room temperature. The material was fixed in 1:3 acetic acid-ethanol solution for 25–30 min and squashed in 45% acetic acid by gentle tapping and pressing the slides with cover slips in between the folds of blotting paper. Cover slips were dislodged off from the slides with a sudden jerk. The slides and cover slips were air dried for 2 or 3 d in a dust free chamber. After drying, staining was done in 2% Giemsa for 20 min. The slides were then mounted in DPX and the permanent slides were observed under binocular research microscope. Well spread chromosome complements were selected for photomicrography and chromosomal measurements. Lengths of chromosomes were measured using ocular micrometer and the total complement length was calculated. The relative lengths (percentages of total complement length) were calculated and idiogram was constructed based on relative lengths. Karyotype was prepared from photomicrographic print.

OBSERVATIONS

Chromosomes were observed at spermatogonial metaphase and meiotic stages. Spermatogonial metaphase possessed 25 chromosomes without **m** chromosomes (Fig. 1). X chromosome was morphologically similar to other autosome pairs at spermatogonial metaphase and it was the smallest element of the complement (Fig. 2). The mean length of chromosomes ranged from $1.28 \pm 0.08 \mu\text{m}$ to $2.78 \pm 0.06 \mu\text{m}$ and the total complement length was $46.33 \pm 0.62 \mu\text{m}$. The mean relative length of chromosomes ranged from 2.65 ± 0.16 to 6.00 ± 0.10 (Table 1). Idiogram showed gradual decrease in

TABLE 1: Karyotype analysis in *T. virginia*.

Chrom. pair	Mean length of chromosomes (μm) \pm S.E.	Relative length \pm S.E.
1	2.78 ± 0.06	6.00 ± 0.10
2	2.43 ± 0.06	5.23 ± 0.08
3	2.26 ± 0.06	4.87 ± 0.08
4	2.09 ± 0.06	4.50 ± 0.10
5	1.92 ± 0.04	4.14 ± 0.06
6	1.82 ± 0.05	3.93 ± 0.06
7	1.75 ± 0.05	3.77 ± 0.07
8	1.58 ± 0.04	3.42 ± 0.09
9	1.49 ± 0.00	3.22 ± 0.04
10	1.49 ± 0.00	3.22 ± 0.04
11	1.49 ± 0.00	3.22 ± 0.04
12	1.45 ± 0.02	3.14 ± 0.07
X chromosome	1.28 ± 0.08	2.65 ± 0.16



Figs 1–9: Cytology of *T. virginia*. 1–3. Karyotype. 1. Spermatogonial metaphase ($2n = 25$). 2. Karyotype. 3. Idiogram. (X, X chromosome). 4–9. Meiosis. 4. Pachytene. 5. Early diakinesis showing 12 cross shaped bivalents and rounded univalent X chromosome. 6. Diakinesis showing 12 bivalents and peripheral X chromosome. 7. Metaphase I showing 13 elements having maximum condensation. 8. Early anaphase I showing movement of chromosomes to different poles. 9. Prophase II showing characteristic 'e' shaped chromosomes. (Arrow indicates X chromosome).

chromosome length from first to eighth autosome pairs, while ninth to twelfth autosome pairs were almost of the same length (Fig. 3).

During pachytene, the chromosomes appeared as network of entangled threads (Fig. 4). At early diakinesis, 13 elements were visible out of which, 12 were bivalents and one was univalent X chromosome. Bivalents were cross shaped having single interstitial chiasma (Fig. 5). As diakinesis

advanced, bivalents became rectangular while, X chromosome remained in rounded form (Fig. 6). At metaphase I, chromosomes showed extreme condensation and the X chromosome remained at the peripheral position (Fig. 7). At early anaphase I, bivalents started separating and moving to opposite poles (Fig. 8). At prophase II, chromosomes attained characteristic ϵ shape (Fig. 9).

DISCUSSION

The diploid chromosome number in *T. virginia* was found to be 25 as reported in this species collected from Mansar lake of Jammu and Kashmir by Sharma & Durani (1995). These workers reported a pair of **m** chromosomes and precocious segregation of these chromosomes in some meiotic stages. However, in the present study, **m** chromosomes were not observed in this species. Earlier, presence as well as absence of **m** chromosomes was reported in different species of genus *Tramea*. The species such as *T. abdominalis*, *T. carolina* and *T. lacerata* were reported without a pair of **m** chromosomes (Cruden 1968, Cumming 1964) while, *T. basilaris*, *T. cophysa* and *T. limbata* (Asana & Makino 1935, Cumming 1964, Dasgupta 1957) were reported with a pair of **m** chromosomes. Kiauta (1968) stated that geographical differences in populations were the reasons for such a variation in the presence or absence of **m** chromosomes in the same species. Further, the present study revealed holokinetic nature of chromosomes in the species investigated which is in conformity with the findings of Sharma & Durani (1995). The presence of a single X chromosome observed during spermatogonial metaphase and meiotic stages in the specimens studied was suggestive of XO (σ) type sex determination mechanism in this species.

REFERENCES

- ASANA J J & MAKINO S 1935 A comparative study of the chromosomes in the Indian dragonflies *J Fac Sci Hokkaido Imp Univ Ser* **4** 67–86
- BABUR 2014 Diversity of dragonflies (Odonata) in Himachal Pradesh India *Agrion* **18** 41–47
- CRUDEN RW 1968 Chromosome numbers of some North American dragonflies (Odonata) *Can J Genet Cytol* **10** 200–214
- CUMMING R B 1964 *Cytogenetic studies in order Odonata* Ph D Thesis Univ Texas Austin
- DASGUPTA J 1957 Cytological studies of some Indian dragonflies II A study of the chromosomes during meiosis in thirty species of Indian Odonata (Insecta) *Proc Zool Soc Calcutta* **10** 1–65
- FRASER F C 1936 *Fauna of British India including Ceylon and Burma* Odonata Vol 3 Taylor & Francis London
- KIAUTA B 1967 Considerations on the evolution of chromosome complement in Odonata *Genetica* **38** 430–446
- KIAUTA B 1968 Morphology and kinetic behaviour of the odonate sex chromosome with a review of the distribution of sex determining mechanism in the order *Genen Phaenen* **12** 21–24
- KIAUTA B 1969a Sex chromosomes and sex determining mechanisms in Odonata with a review of the cytological conditions in the family Gomphidae and references to the karyotypic evolution in the order *Genetica* **40** 127–157

- KIAUTAB 1969b Autosomal fragmentations and fusions in Odonata and their evolutionary implications *Genetica* **40** 158–180
- KIAUTAB 1975 *Cytotaxonomy of dragonflies with special reference to the Nepalese fauna* Nepal Research Centre Kathmandu
- LEFEVRE G & MCGILL C 1908 The chromosomes of *Anasa tristis* and *Anax junius* *Amer J Anat* **7** 467–487
- SHARMA O P & DURANI S 1995 A study on the chromosomes of three species of dragonflies (Odonata Anisoptera) *Nat Acad Sci Lett* **18** 97–101
- SUBRAMANIAN K A & BABU R 2017 *A Checklist of Odonata (Insecta) of India Version 3* Zool Surv India Kolkata
- TYAGI B K 1978 The chromosome numbers and sex determining mechanisms newly recorded in thirteen Indian dragonflies (Odonata) *Chrom Inf Serv* **25** 5–7
- WALIA G K 2008 Comparative cytological data on twenty six species of family Libellulidae (Anisoptera Odonata) *Fraseria* **7** 77–122
- WALIA G K, KAUR H & KAUR J 2011 Karyotypic variations in the chromosome complement of *Pantala flavescens* (Fabricius) of the family Libellulidae (Anisoptera Odonata) *Cytologia* **76** 301–307

