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Karyotypic Variations in the Chromosome Complement of *Pantala flavescens* (Fabricius) of the Family Libellulidae (Anisoptera: Odonata)

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Summary Male germ cell complement of *Pantala flavescens* belonging to the family Libellulidae has been investigated. Specimens were collected from the surrounding area of the Harike wetlands in the Punjab state of India. The species possesses $2n$ ♂=23m as the diploid chromosome number, which is less than the type number, $2n$ ♂=25m, of the family. In the chromosome complement, one autosomal bivalent is extraordinarily large due to the fusion of 2 autosomes and is responsible for the reduction in chromosome number. Precocious segregation of the m bivalent has also been noticed in some meiotic cells. This type of variations in the chromosome number and behaviour of m chromosomes indicate that the species is under the process of karyotypic evolution.

Key words *Pantala flavescens*, Libellulidae, Odonata, Chromosome number, Autosomal fusions, m chromosomes.

Libellulidae is one of the largest families, with greatest number of species, in the suborder Anisoptera. From a cytological point of view, this family is well studied compared to other families; cytogenetical data pertains to 270 species, which is less than one third of the known libellulid species. In India, 470 species are taxonomically known, and out of these 85 species, belonging to 39 genera, are of the family Libellulidae. Karyological data is available on approximately 35 libellulid species (Oguma and Asana 1932, Makino 1935, Asana and Makino 1935, Kichijo 1942, Ray Chaudhuri and Dasgupta 1949, Dasgupta 1957, Kiauta 1975, Tyagi 1978a, b, 1982, 1986, Yadav 1979, Thomas and Prasad 1981, 1984, 1986, Sandhu and Walia 1994a, b, 1995, Sharma and Durani 1995, Walia and Sandhu 1998, 2002, Walia, 2008a, b). The modal chromosome number of the family Libellulidae is n=13 (90% of the species), and a pair of m chromosomes are very frequently present with XO–XX type sex determination. Sandhu and Walia (1994a, b) and Walia and Sandhu (1998) gave an account on the female karyotypes of 11 libellulid species along with male complement, $2n$ (♂)=25m and $2n$ (♀)=26m, which confirms the XO–XX type sex determination.

In Odonata, fragmentation and fusion of the chromosomes are responsible for the variation in chromosome number, which is quite frequent in the holocentric chromosomes. Autosomal fragmentations are found to be the only way to increase the chromosome number and are responsible for the increase in the recombination index. Autosomal fusions are responsible for the decrease in the chromosome number of the secondary complement and occur in 2 ways: autosome–autosome fusion, which results in a decrease in the chromosome number and autosome-sex chromosome fusion, which results in neo-XY sex determination. Both types of autosomal fusions are observed in the family Libellulidae (Oguma 1930, Cumming 1964, Kiauta 1969a, b, 1972, 1975, 1979, Ferreira *et al.* 1979, Kiauta 1983, Agopian and Mola 1988). Interestingly, both fragmentations and fusions reported in the single libellulid species *Neurothemis tullia tullia* with chromosome complement

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2*n*=28+neoXY by Ray Chaudhuri and Dasgupta (1949). Mola *et al.,* (1999) also described a novel sex determining system in *Microthyria ungulata* with the chromosome complement $n=10+X_1X_2Y$ for the first time in the family Libellulidae. In the present study, fusion of 2 autosome pairs has also been observed in *Pantala flavescens*, which resulted in the decrease in chromosome number from 2*n*=25m to 2*n*=23m.

Precocious disjunction of bivalents rarely occurs in the meiotic cycle of Odonata. In the family Libellulidae, Kiauta and Van Brink (1978) observed precocious segregation for m bivalent in *Perithemis tenera*. Boyes *et al.* (1980) illustrated the precocious segregation of X chromosome in *Orthetrum julia falsum* and suggested that this species seemed to be genetically an unstabilized taxon. In the present study, precocious segregation of m bivalent in some meiotic cells has also been noticed in *Pantala flavescens*.

Materials and methods

Adult males were collected from the surrounding areas of the Harike wetlands in the Punjab state of India during pre-monsoon and post-monsoon seasons of the year 2009. Live specimens were dissected in 0.67% saline solution in the field and testes were taken out. The slides were prepared by air drying technique and kept in a refrigerator for further cytogenetical parameters like conventional staining, C-banding and silver-nitrate staining. For the conventional staining, slides were stained in the Carbol fuchsin as suggested by Carr and Walker (1961). For the induction of C-bands, the technique suggested by Sumner (1972) was followed. Silver nitrate staining was done by following the method suggested by Howell and Black (1980). Slides prepared for the different cytogenetical parameters were studied and well-spread meiotic plates were microphotographed. The results were used to study the structure, the behaviour of chromosomes, and the location of constitutive heterochromatin and Nucleolar Organiser Regions in the species.

Results

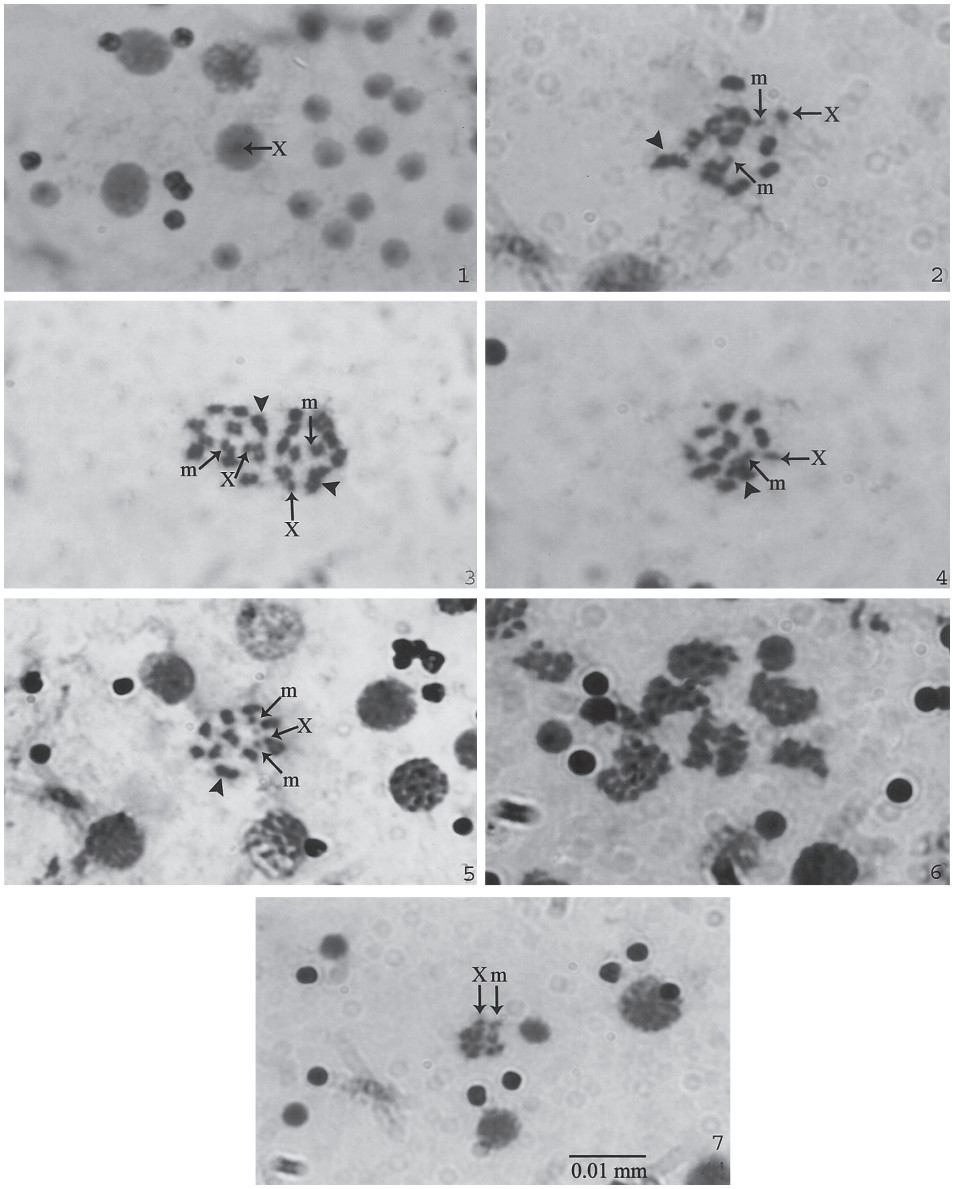
Conventional staining

The spermatogonial metaphase stage has not been observed during the present study. In interphase, only the X chromosome is discernible due to its heterochromatic nature (Fig. 1). During diakinesis, 12 elements are present, showing rectangular and rod shape structures due to the single terminal chiasma per bivalent. Autosomal bivalents also include the m bivalent, while the X chromosome is univalent. The m bivalent has been precociously divided in 1 plate (Fig. 2), while in other plate it acts as the normal bivalent (Fig. 3). The X chromosome mostly lies at the peripheral position. The largest autosomal bivalent is also present in both the plates, which is formed by the fusion of 2 autosomes (shown by arrow).

At metaphase I, chromosomes appear rod-shaped due to the condensation and terminalisation of chiasmata. 2 types of plates have been observed; in one plate, the X chromosome occupies the peripheral position, while the m bivalent is present at the inner side (Fig. 4). The other plate shows precocious segregation of the m bivalent and both the X chromosome and m bivalent lie closely inside the plate (Fig. 5). The largest autosomal bivalent is also easily distinguishable in both plates (shown by arrow). During anaphase I, the chromosomes are moving towards the opposite poles, while the X chromosome is not differentiated (Fig. 6). Metaphase II reveals 12 elements, which are half the size of metaphase I chromosomes. The X and m chromosomes lie at the periphery position (Fig. 7).

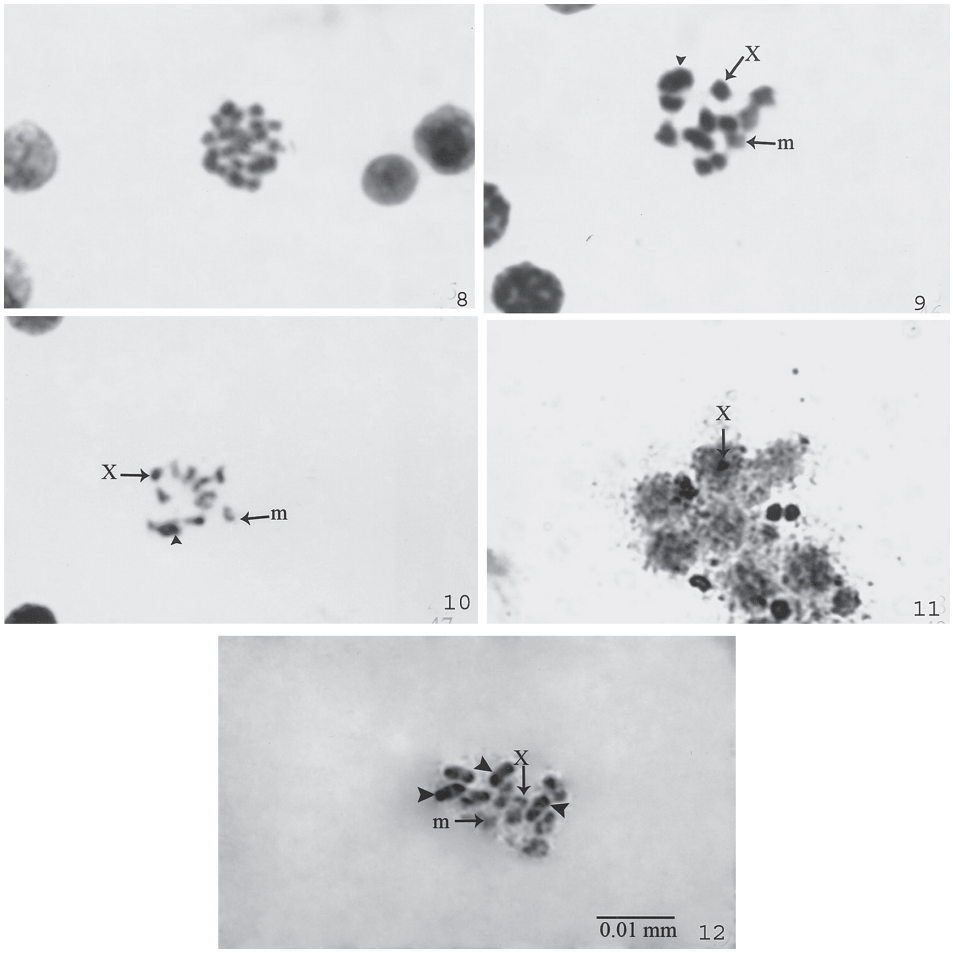
C-banding

In spermatogonial metaphase, 12 chromosomes show C-positive heterochromatin throughout



Figs. 1–7. 1. Interphase. 2. Diakinesis (showing precocious segregation of m bivalent). 3. Diakinesis (largest autosomal bivalent shown by arrow head). 4. Metaphase I (largest autosomal bivalent shown by arrow head). 5. Metaphase I (showing precocious segregation of m bivalent). 6. Anaphase I. 7. Metaphase II.

the length, while the remaining chromosomes reveal less C-positive regions (Fig. 8). At metaphase I, 9 autosomal bivalents including the X univalent are C-positive for the entire length, while 3 autosomal bivalents show light terminal C-bands and the m bivalent is C-negative. However, the largest bivalent is distinct and highly heterochromatic (shown by arrow) (Fig. 9). During metaphase II, the X chromosome and largest autosome (shown by arrow) are C-positive for the entire length, whereas 3 autosomes show terminal C-positive bands in the diakinesis and the remaining chromosomes including the m chromosome are C-negative (Fig. 10).



Figs. 8–12. 8. Spermatogonial metaphase. 9. Metaphase I (largest autosomal bivalent shown by arrow head). 10. Metaphase II (largest autosomal bivalent shown by arrow head). 11. Interphase. 12. Metaphase I (NOR bands on one side or both the sides of autosomal bivalent shown by arrow head).

Silver nitrate staining

Silver nitrate staining reveals darkly stained X chromosomes in the interphase stage (Fig. 11). Metaphase I shows dark NOR bands in 6 autosomal bivalents at their terminal ends (shown by arrow head), while the X chromosome is also showing C-shape NOR band (Fig. 12).

Discussion

Cytologically, the family Libellulidae is well studied due to the wide distribution of libellulids in all the geographical regions. In India, chromosomal data is available on approximately 35 species (Oguma and Asana 1932, Makino 1935, Asana and Makino 1935, Kichijo 1942, Ray Chaudhuri and Dasgupta 1949, Dasgupta 1957, Kiauta 1975, Tyagi 1978a, b, 1982, 1986, Yadav 1979, Thomas and Prasad 1981, 1984, 1986, Sandhu and Walia 1994a, b, 1995, Sharma and Durani 1995, Walia and Sandhu 1998, 2002, Walia 2008a, b). Most of these species possess 2*n*=25m as the diploid chromosome number, which is the type number of the family.

Varation in chromosome number due to the fragmentation and fusion of chromosomes has

also been reported in the family. Autosomal fusions are responsible for the decrease in chromosome numbers in the secondary complement and occur in 2 ways: autosome–autosome fusion, which results in a decrease in the chromosome number, and autosome-sex chromosome fusion, which results in neo-XY sex determination. Both types of autosomal fusions have been reported in the following species of the family Libellulidae *viz.*, *Perithemis lais*, 2*n*=17, (Cumming 1964); *Orthetrum brachiale*, *n*=11 and *Sympetrum eroticum*, *n*=12 (Kiauta 1969b); *Sympetrum frequence*, *n*=12 (Oguma 1930, Kiauta 1969a); *Trithemis aurora*, *n*=10+neo-XY (Kiauta 1975); *Erythrodiplax connata connata* and *Erythrodiplax media*, *n*=11m (Kiauta 1972, Ferreira *et al.* 1979); *Dythemis cannacricoides*, *n*=11 and *Oligoclade monosticha*, *n*=12m (Ferreira *et al.*, 1979); *Oligoclade amphinanne*, *n*=12m; *Dythemis williamsoni*, *n*=11+neo-XY, 12+neo-XY, 13+XO; *Orthemis aequilibris* 2*n*=12, *n*=6+neo-XY; *Orthemis ferrugina*, 2*n*=25m+XO, 23+XO, *n*=12m+XO, 11m+neo-XY, 10+neo-XY (Kiauta 1979); *Crocothemis servilia mariannae*, 2*n*=24+neo-XY (Kiauta 1983) and *Microthyria fasciata*, *n*=12+neo-XY (Agopian and Mola 1988).

Interestingly, both fragmentations and fusions were observed in the single libellulid species *Neurothemis tullia tullia* by Ray Chaudhuri and Dasgupta (1949). They noticed 2*n*=28+neoXY as the diploid number but could not explain the process of karyotypic evolution in it, while Kiauta (1968) proposed as an explanation the schematic evolution of sex determination and numerical increase of chromosome complement in the species. Mola *et al.* (1999) described a novel sex-determining system in *Microthyria ungulata* with chromosome complement $n=10+X_1X_2Y$ for the first time in the family Libellulidae. They suggested that this system was derived by the fusion of the X chromosome with an autosome and the resulting formation of a trivalent during meiosis. The X_1X_2Y sex chromosome system originated through these 2 fusion events, Firstly, the free X chromosome fused to an autosome, giving rise to neo-XY (2*n*=24, *n*=11+neo-XY). Later, the 'neo Y' fused to one member of another autosome pair, originating a new large chromosome, referred to as the 'Y' chromosome, located at the middle position in the trivalent and inherited to male progeny. The 'neo X' is a medium-sized chromosome of the trivalent, referred to as X_1 (original X). Finally, the autosome not involved in fusion co-segregate with X_1 and is considered as X_2. Both X_1 and X_2 are inherited to female progeny. In the present study, *Pantala flavescens* possesses a reduced chromosome number, 2*n*=23m, from the type number, 2*n*=25m, of the family. In the chromosome complement, 1 autosomal bivalent is extraordinarily large due to the fusion of 2 autosomes, which is responsible for the reduction in chromosome number.

Precocious disjunction of bivalents rarely occurs in the meiotic cycle of Odonata. In the family Libellulidae, Kiauta and Van Brink (1978) observed precocious segregation for the m bivalent in *Perithemis tenera*. Boyes *et al.* (1980) illustrated the precocious segregation of X chromosomes in *Orthetrum julia falsum* and suggested that this species seemed to be genetically an unstabilized taxon. In the present study, precocious segregation of the m bivalent in some meiotic cells has also been noticed in *Pantala flavescens*.

A few reports on the C-banding are also available (Thomas and Prasad 1986, Frankovic and Jurecic 1988, Prasad and Thomas 1992, Walia 2006, 2008c). In these earlier reports, C-bands are always present at the terminal ends of all the chromosomes, while during the present investigation, they show variation in the distribution of constitutive heterochromatin. In the C-banding, 9 autosomal bivalents, including the largest bivalent and the X chromosome, are C-positive for the entire length, while 3 autosomal bivalents show terminal bands and the m bivalent is C-negative. The variation in the C-banding patterns could be due to the condensation of bivalents and staining intensity of the chromosomes.

Silver nitrate staining has been done for the first time in the species. The X chromosome and 6 autosomal bivalents possess small dark NOR bands at the terminal ends on one side or both sides of the bivalents, which show association with Nucleolar Organiser Regions.

The chromosome complement of *Pantala flavescens* species shows genotypic unstability due

to the fusion of 2 autosome pairs and precocious segregation of the m bivalent during the meiosis. These observations indicate that the process of karyotypic evolution for the chromosome number and the m chromosome is ongoing in this species.

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