

Meiotic chromosome behaviour of a newly recorded ant-like spider, *Myrmarachne melanocephala* MacLeay, 1839 from Manipur, India

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ABSTRACT: Cytological and chromosomal studies of ant-like spiders *Myrmarachne melanocephala* MacLeay, 1839, were undertaken with 12 males captured alive in the months of January to July 2023 from three habitats. The haploid count of male specimens was observed to be 13: 4 acrocentric (including X chromosome), 6 subtelocentric, 2 submetacentric, and 1 metacentric and showed XO sex determination mechanisms, so the diploid count of the species was 25 (13+12). The single sex chromosome occupied roughly 4-6 per cent of the nuclear volume prominently in the Interphase – Prophase I stage. The structure of X chromosome in interphase stage was circular-rectangular block. The peculiar shape of rod-shaped X chromosome was maintained from early pre-leptotene stage till the end of the division particularly the Prophase I. The synapsis started early from late leptotene and duration of zygotene was long enough to visualize the perfect ones in late zygotene. There were nine diplotene bivalents with interstitial chiasma.
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KEYWORDS: Araneae, Salticidae, haploid, prophase, diplotene chiasma, sex chromosomes

The genus *Myrmarachne* MacLeay, 1839 display the marvellous Batesian mimicry, (Wanless, 1978; Yamasaki and Ahmad, 2013; Yamasaki and Edwards, 2013). The genus comprised of 195 species (World Spider Catalog version 25.0) with distribution from Australia to Africa and New World (<https://en.wikipedia.org/wiki/Myrmarachne>). Their habitat consists of leaves and around trees, herbs or shrubs but needs a more intensive look. One such ant-like spider is *M. melanocephala* MacLeay, 1839, a new record from Manipur, mimicking the black ant (<https://indiabiodiversity.org/observation/show/15460659>).

Its ant model is *Tetraoponera rufonigra* Jerdon, 1851 (Kumar *et al.*, 2021). The species is distributed from Pakistan to Indonesia (Edwards and Benjamin, 2009; World Spider Catalog version 25.0) with type locality from West Bengal (https://en.wikipedia.org/wiki/Myrmarachne_melanocephala). The species was also newly recorded in Jharkhand (Kumar *et al.*, 2021). Currently cytological works on the genus are rare to find with exception to *M. laurentina* ($2n^{\sigma} = 28, X_1X_20$) (Štáhlavský *et al.*, 2020). In the present study meiotic division in *M. melanocephala* MacLeay, 1839 (Araneae, Salticidae), was analysed

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to ascertain the diploid count, chromosomal behaviour and other details. Spiders of *M. melanocephala* were collected from three different places [Khurai Konsam leikai - 24°83'68.54", 93°97'37.42" (2 males, 3 females); DM College of Science - 24°82'20.55", 93°94'15.63" (3 males, 3 females); DM Community College - 24°82'21.62", 93°94'15.96" (7 males, 5 females)] in the months of January to July, 2023 and brought to laboratory for further investigation. Identification of the species was confirmed with Prószyński (2020).

Chromosome preparation: Squashed preparation

- The abdomen of the spider was dissected and testes were removed in hypotonic solution, KCl (0.56M) along with yellowish fat and other unwanted materials and exposed to the solution for 15 minutes. Fixative (Carnoy's fluid I) comprising of 1-part glacial acetic acid and 3 parts of methanol by v/v was added and left alone for 10 minutes. The fixed testes were stained with two per cent aceto-orcein for 30 minute and softened with 45 per cent glacial acetic acid. The stained testes were covered with cover slip and squashed by applying thumb pressure after covering the slip with blotting paper (Belling, 1921). Well-spread 50 dividing cells were analysed under microscope for each stage and well spread selected cells were photograph using digital camera attached on Optscopes light microscope. The number of dividing cells in a single testis lobule was fair enough to display all the stages of meiosis division. The details of the chromosomes could be visualised as the chromosomes were fairly big in this species.

Interphase: The single sex chromosome occupied 4-6 per cent of the nuclear volume prominently in the interphase-Prophase I stages. The structure of X chromosome in interphase stage was heavily stained rectangular/circular block. The peculiar shape of rod X chromosome is maintained from early pre-leptotene stage till the end of the division particularly the Prophase I (Figs. 1A, B, C arrow indicates X chromosome).

Leptotene: The condensed pre-leptotene chromosomes were de-condensed in leptotene stage with easily distinguishable chromomeres along the whole length of the chromosomes. The typical

rod-shaped X chromosomes are always at periphery of the plate (Figs. 1D, E arrow indicates X chromosome).

Zygotene: The stages were characterised by pairing of homologous chromosomes known as synapsis. The shortened synapsed chromosomes were easily visible in most of the cell plates (Figs. 1F, G). At some point the pairing of the homologous chromosomes initiated preferably at the terminal ends while some elements showed the exposed terminals.

Pachytene: The stage was characterised by thick shortened deeply stained chromosomes with nearly hollow space most likely the remnant of synaptonemal complexes (Fig. 1H).

Diplotene: The characteristic features of the stage were the presence of the chiasma and diplotene loop of the terminal chiasma. There were nine interstitial chiasmatic chromosomes (Fig. 1I). The X chromosomes were characterised by deeply stained (heteropicnotic) rod shape and uniformly dispersed.

Diakinesis: The dispersed chromosomes were coming towards the equatorial region. Here only two interstitial chiasmata could be observed (Figs. 1J, K). The X chromosomes were nearly peripheral.

Metaphase I: The localisation of the chromosomes at the equatorial plate was complete in this stage (Fig. 1 L). The X chromosomes were nearly peripheral.

The remaining stages of meiotic divisions like Anaphase I, Telophase I and Meiotic II stages were classical as in all other organisms (Figs. 1M-R) (Šáhlavský *et al.*, 2020). According to Sharma and Sharma (2014) cytogenetics records of 325 species of Indian spiders exist. Of these 232 species (71.38%) have sex chromosome system of the X_1X_2O type; 48 species (12.92%) have XO system; 39 species (12%) have $X_1X_2X_3O$ system; 1 species (0.3%) has X_1X_2Y type; 4 species (1.23%) have $X_1X_2X_3X_4O$ system; 1 species (0.3%) has X_1X_2Y type. The present species is of XO system in accordance with (Araujo and Schneider, 2012) but *M. laurentina* ($2n\♂ = 28, X_1X_2O$) (Šáhlavský *et*

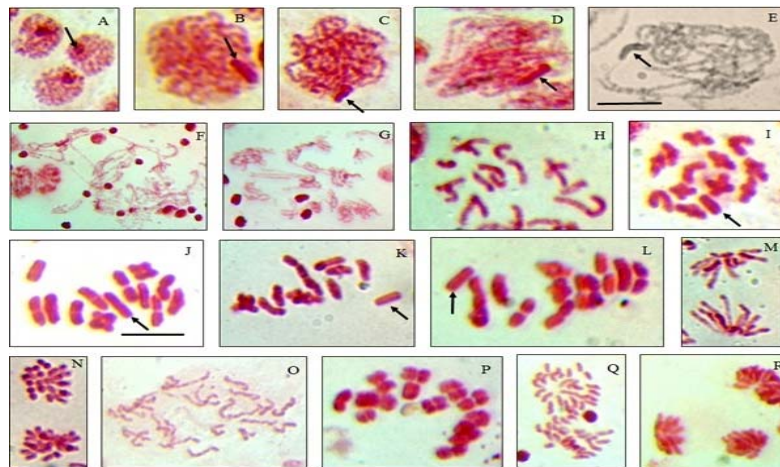


Fig. 1 Meiotic cells of the ant-like spider, *Myrmarachne melanocephala*. A–C: Interphase depicting heteropycnotic sex chromosome, D–E: Leptotene, F–G: Zygotene, H: Pachytene, I: diplotene, J–K: diakinesis, L: metaphase I. M: anaphase I, N: telophase I, O: Spermatogonial pro-metaphase/metaphase II, P: metaphase II, Q: anaphase II, R: telophase II, Scale 10 (micrometer)

al., 2020) was different from the present study.

The X0 system found in *Oxyopes* (metacentric), *Myrmarachne*, *Misumena* and *Xysticus* (acrocentric) could be derived from X_1X_20 system (Hackman, 1948) in two ways - 1. The metacentric X of the X0 system could have been derived by centric fusion between X_1 and X_2 chromosomes. This mechanism was also employed by several authors (Araujo and Schneider, 2012) to explain the origin of the X0 Sex Chromosomes System (SCS), which involves a metacentric X, in many spider groups. The acrocentric X of the X0 system could have originated through gradual elimination of one X chromosome of the X_1X_20 SCS, as suggested by Suzuki (1952, 1954). The author put forth this proposition based on the fact that some thomisid species with an X_1X_20 system presented gradual differences between the lengths of X_1 and X_2 chromosomes (with both showing the same, slightly different or markedly different sizes). Furthermore, some species even exhibited an X0 system, suggesting that elimination of one X of the X_1X_20 system had taken place in the course of evolution.

It seems that the meiotic Prophase I is lengthy and the zygotene stage is perfect in displaying the homologous pairing or synapsis. It is both terminal and interstitial (Figs. 1E, F, G). As there is no reference for comparing, the haploid count of male specimens with 13 evidences from the diplotene-

metaphase I count with four acrocentric (including X chromosome), six submetacentric, two submetacentric, and one metacentric, is open for further confirmation from the mitotic cells. The diploid count of 25 (13+12) for the species is open for any suggestions too.

The behaviour of the X chromosomes that is peripheral localisation seems to be universal as heteropycnotic particularly in spiders. According to White (1940), the term heteropycnosis was introduced to describe the different levels of condensation and staining that certain chromosomes exhibit in the course of mitosis and/or meiosis. This heteropycnotic pattern can be positive or negative, and it is related to a high or low degree of chromosome condensation, respectively. Manifestation of heteropycnosis is commonly visualised in the sex chromosomes, especially in male meiotic cells; the high level of chromosome condensation in these cells seems to prevent recombination between non-homologous regions of heteromorphic sex chromosomes (McKee and Handel, 1993). In spider spermatogenesis, a heteropycnotic pattern of the sex chromosomes has been recorded for roughly 25 per cent of the species that have been cytogenetically examined, which belong to different suborders (Mygalomorphae and Araneomorphae) and families (Araujo and Schneider, 2012). Regardless of the type of sex

chromosome system, 95 per cent of these spider species showed positively heteropycnotic sex chromosomes in premeiotic interphase and prophase I nuclei (Figs. 1A-C, E-G, I-M) and occasionally, also in metaphase II cells. In late meiotic stages, the sex chromosomes usually appeared to be isopycnotic (Araujo and Schneider, 2012).

The diploid count of the male *M. melanocephala* is 25 (13+12) with X0 system. The haploid karyotype of the species is 4 acrocentric (including X chromosome), 6 subtelocentric, 2 submetacentric, and 1 metacentric. The X chromosome is always peripherally localised and the prophase I is long enough to see the zygotene synapsis and have nine interstitial diplotene elements.

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