



## SEX CHROMOSOMES OF SALTICID SPIDER *PLEXIPPUS PAYKUHLII*

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### ABSTRACT

The spermatogenetic studies of *Plexippus paykuhli* was carried from 13 adults from three habitats of the campus of D M College of Sciences, with hypotonic fixative treatments and 4% Giemsa staining. The chromosomes number in pachytene stage was confirmed to be 28 and sex determination mechanism was  $X_1X_2O$  type. Each gamete received 15 or 13 chromosomes and distribution seem to be disturbed in latter stage of the meiosis. During early stages of interphase, the two X chromosomes started as two dots and after S phase they became as larger irregular heteropycnotic body inside the nucleus. The dots were nearly jointed together and could be seen jointed till early pachytene probably at the centromeric regions. From the pachytene stage onwards close association could be seen till the metaphase I stage. The two X chromosomes were heteropycnotic throughout the meiosis I and late replicating. They were fairly joined together at the centromeric region till the early pachytene stage but seem to be separate out during the later stages.

**Key words:** *Plexippus paykuhli*, cytology, Manipur, X chromosomes, peripheral location, Heterochromatization

Spiders, the four-legged arthropods include 44,500 species (Platnick, 2014). It has three primary clades viz., Mesolothelae, Mygalomorphae and Araneomorphae, the last being the most derived and largest (Coddington and Levi, 1991). It has 207 species under 73 genera from India according to Keswani et al. (2012). From Manipur a total of 152 species belonging to 87 genera and 26 families are known (Kananbala et al., 2018). Sharma and Sharma (2014) reported 325 cytogenetic records till date in India. Of these 232 species (71.38%) have sex chromosome system of the  $X_1X_2O$  type; 48 species (12.92%) have an XO system; 39 species (12%) have an  $X_1X_2X_3O$  system; 1 species (0.3%) have sex chromosome system of the  $X_1X_2Y$  type; 4 species (1.23%) have an  $X_1X_2X_3X_4O$  system; 1 species (0.3%) has an SCS of the  $X_1X_2Y$  type. The cytology of the species of *Plexippus paykuhli* had reported only  $X_1X_2$  but not the chromosome complements. The cytogenetic studies on the family Salticidae and genera *Plexippus* are very scanty and the present study attempts this.

### MATERIALS AND METHODS

The adult males of *Plexippus paykuhli* Savigny et Audouin, were killed in refrigerator at  $-10^{\circ}\text{C}$  for 10 min and the testis were dissected in the hypotonic solution (0.075 M KCl) and kept for five minutes and crushed with tips of the needles and fixed with fixative (1:3

glacial acetic acid and methanol) and air dried. The slide was stained in 4% Giemsa stain, and observed under Opt-scope compound microscope and photographs taken with the attached camera. The species was identified according to Proszynski (2017).

### RESULTS AND DISCUSSION

Taxonomic status of *Plexippus paykuhli* was confirmed with the characters given in C.L. Koch, 1846. Cytological studies revealed the following:

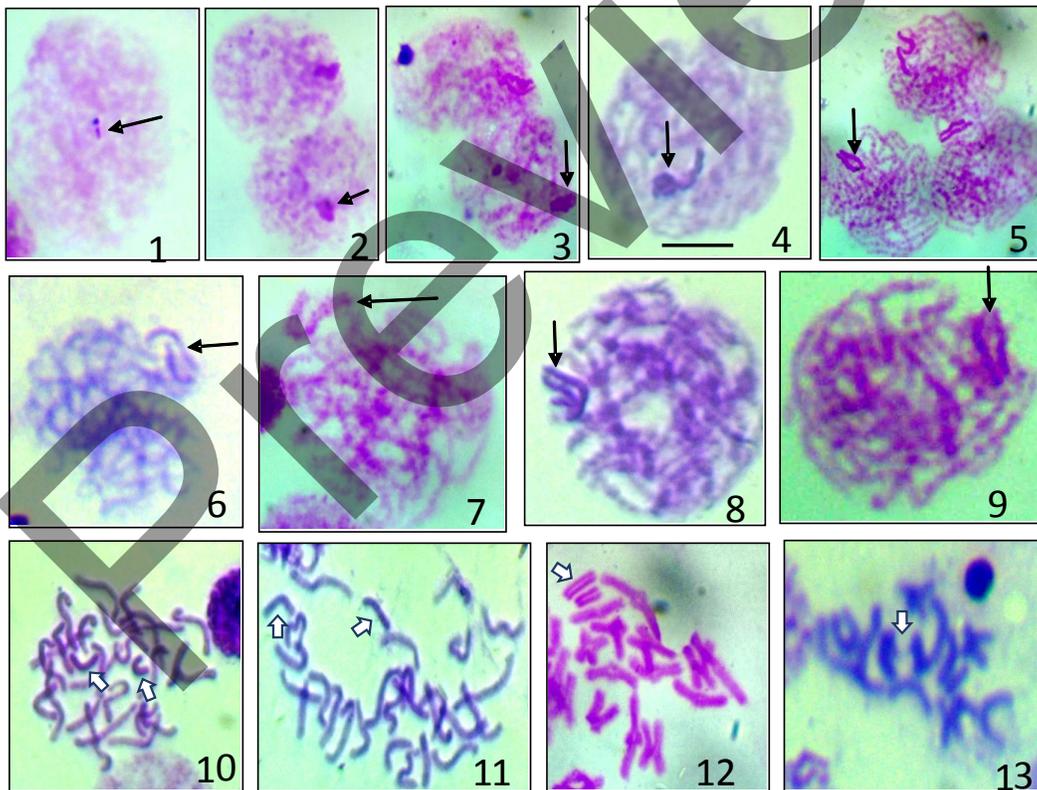
The observations on meiosis I in the spermatogenesis in adult *P. paykuhli* revealed that acetocarmine staining was not efficient. The diploid count is 28 as seen in other Salticidae (Sharma and Sharma, 2014). The distribution of the 13 or 15 chromosomes in the diakinesis or metaphase I showed that the sex determination mechanism is  $X_1X_2O$ , with karyotypic formula as  $26A+X_1X_2O$ . The observations were made from the nuclei having the sufficient staining and started from the interphase stage. Early interphase showed densely stained dots like structures representing the two X chromosomes were distinctly visible (Fig. 1). Later these dots become larger, became almost rectangular blocks which could not be recognized as two Xs (Figs. 2, 3). In the early leptotene stage the two heteropycnotic blocks became separate out and fairly separate out in

the late leptotene stage (Fig. 4- 6). The separation could be seen in the late zygotene stage (Fig. 7), the joining of the two persist again till late pachytene (Figs. 8, 9), late pachytene showed distantly located X chromosomes (Figs. 10, 11) which might be due to plane of the spreading, during diplotene, diakinesis stage they lie side to side (Fig. 12) and persisted till metaphase I (Fig. 13). The latter stage might show some interesting figures but present study focused on the meiosis I.

The two X chromosomes were always heterochromatic as reported in many 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 nonhomologous 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% of the species that have been cytogenetically examined (Aroujo et al., 2014). The peripheral localization of the sex chromosomes could be linked to heterochromatization of the sex chromosomes as proposed by Oliveira et al. (2007) that the X1X20 system could have arisen by gradual heterochromatinisation and erosion of the Y chromosome. Montgomery (1905) referred to as “heterochromosomes” or “odd-chromosomes” by Berry (1906). The nomenclature of “accessory chromosomes” was adopted by Painter (1914) and others. In a brief communication describing the X element in *Amaurobius* sp., King (1925) was the first to use the term “sex chromosome” in



Figs. 1- 13. Stages of Meiosis I in the spermatogenesis in adult *Plexippus paykuli*.

Early interphase showed densely stained dots structures representing the two X chromosomes (1, arrowed), were become larger in size (2, 3 arrowed), in the late leptotene stage the two heteropycnotic blocks became separate out (4 arrowed) and fairly separate out in the late leptotene stage (5, 6 arrowed), the two chromosomes were fairly separate out in the zygotene stage (7 arrowed), the joining of the two persist again till late Pachytene (8, 9 arrowed), late pachytene showed distantly located X chromosomes (10, 11 white arrowed), during diplotene, diakinesis stage they lie side to side (12 white arrowed) and persisted till metaphase I (13 white arrowed). Bar represents 10  $\mu$ m.

spiders. Euchromatic histone methyltransferases (EHMTs) methylate histone and non-histone proteins. Euchromatic histone methyltransferases in regulating heterochromatin anchorage to the nuclear periphery (NP) via non-histone (LMNB1) methylations, EHMTs methylate LMNB1 that associates with the H3K9me2 marked peripheral heterochromatin, Ketkar et al. (2018).

According to Platnick (2014), the order Araneae possesses 114 families, 3935 genera, and 44,906 species. However, currently, there are 791 cytogenetic records ([www.arthropodacytogenetics.bio.br/spiderdatabase](http://www.arthropodacytogenetics.bio.br/spiderdatabase)). 325 cytogenetic records are found for Indian spiders till date. Of these 232 species (71.38%) have sex chromosome system of the  $X_1X_20$  type; 48 species (12.92%) have an  $X0$  system; 39 species (12%) have an  $X_1X_2X_30$  system; 1 species (0.3%) have sex chromosome system of the  $X_1X_2Y$  type; 4 species (1.23%) have an  $X_1X_2X_3X_40$  system; 1 species (0.3%) has an SCS of the  $X_1X_2Y$  type.

The  $X_1X_20$  has been considered a plesiomorphic feature in spiders because it occurs in representatives of the phylogenetically basal family Liphistiidae (Mesothelae) (Suzuki, 1954) as in the present study and also reported by Sharma and Sharma (2014). Revell (1947) was the first to suggest that the  $X_1X_20$  SCS most likely originated from an  $X0$  system in spiders, considering the proposition of White (1940), who suggested that duplication of the X chromosome from an  $X0$  system gave rise to the multiple X chromosome systems. The present study with dissimilar X chromosomes of 7:5 in length could be explained as by Revell (1947) suggested that these X chromosomes had undergone evolutionary differentiation after originating from an  $X0$  system. Patau (1948) proposed that the  $X_1X_20$  SCS was formed by centric fission of a large X chromosome in an  $X0$  system. Due to the fact that all X chromosomes of  $X0$  systems that were registered at that time exhibited subterminal or terminal centromeres, Patau (1948) suggested that the smaller  $X_1$  and  $X_2$  chromosomes had originated from the  $X0$  system not only by simple centric fission, but through additional rearrangements such as 1) centric fragmentation and fission in the long arm terminal region followed by inversion of the long chromosome segment, resulting in a dicentric chromosome; 2) fission in the middle region of the dicentric chromosome, forming two acrocentric  $X_1$  and  $X_2$  chromosomes of similar size. Many explanations had been forwarded but need more studies on many families and genera

to accept or discard the propositions in future. The X chromosomes were seen to be connected at the centromeric region that could be the results of the homology and mechanism could be explained by nondisjunction of the X chromosomes during anaphase as Postiglioni and Brum-Zorrilla (1981) hypothesised that non-disjunction of one X chromosome of the  $X_1X_20$  system, followed by loss of homology between the X chromosomes had occurred. The two X chromosomes were heteropycnotic throughout the Meiosis I and late replicating. They were fairly joint together at the centromeric region till the early Pachytene stage but seem to be separate out during the later stages. Future works on various families and genera will give insight into the mechanism of such fascinating phenomenon.

#### ACKNOWLEDGEMENTS

The authors thank the Principal and HOD, Department of Zoology, D.M. College of Science, Imphal for providing the laboratory facilities. The people who helped to procure the spiders particularly Ishwar Haobam are acknowledged.

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(Manuscript Received: August, 2019; Revised: November, 2019;  
Accepted: November, 2019; Online Published: November, 2019)

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