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Cytological Studies on three Species of Indian Spiders
(Araneae: Pholcidae, Hersiliidae, Lycosidae)

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Abstract

Spiders (Arthropoda: Arachnida: Araneae) are fascinating and most diverse group of air breathing chelicerate arthropods in the animal kingdom. Their distribution is worldwide except Antartica and occupies every ecological niche available except of air and sea colonization. An improved air-dried method was to prepare spider chromosomes. Chromosome data are reported for 3 species belonging to families Pholcidae, Hersiliidae and Lycosidae from India. The number of diploid chromosomes (2n) in both males and females and sex determining mechanism of each species were determined as follows: *Crossopriza lyoni*: 2n=23 (XO) male, *Crossopriza lyoni*: 2n=24 (XX) female, *Hersilia savignyi*: 2n=30 (X_1X_2O) male, *Hersilia savignyi*: 2n=32 ($X_1X_2X_3X_4$) female, *Hippasa agelenoides*: 2n=28 (X_1X_2O) male, *Hippasa agelenoides*: 2n=30 ($X_1X_2X_3X_4$) female. During the first meiotic division 11, 14, 13 autosomal bivalents were found in *Crossopriza lyoni*, *Hersilia savignyi* and *Hippasa agelenoides* respectively. *Crossopriza lyoni* have metacentric and submetacentric type of chromosomes. In *Hersilia savignyi* chromosomes are acrocentric and rod shaped in *Hippasa agelenoides*.

Key words: Spiders, Cytological studies, Sex determining mechanism, Pholcidae, Hersiliidae, Lycosidae

Introduction

The order Araneae is divided into two suborders, Mesothelae and Opisthothelae. The later is divided into infra order Mygalomorphae and Araneomorphae. The last infraorder is by far the most diverse and is divided into three groups: basal araneomorphs, haplogynae that groups spider with simple female genitalia and Entelegynae that includes spiders with more complex female genitalia (Coddington and Levi [1]). According to Platnick[2], the order Araneae possesses 114 families, 3935 genera and 44,906 species. However currently there are 791 cytogenetic records in spiders from the world. (www.arthropodacytogenetics.bio.br/spiderdatabase). Out of these, 2299 spider species belonging to 552 genera and 67 families are reported from South East Asia. Of the 552 genera, 49 (9%) are monotypic, represented by single species and 65 genera (12%) are endemic to one or more South Asian countries. About 1830 species (80%) are endemic to South Asia. The most comprehensive description on spiders of 1066 species has been listed from fewer than 43 families, covering a number of species from various families distributed in different parts of India and also recorded 200 species from Burma and Srilanka (Tikader [3]).

Spiders represents a great diversity in male diploid numbers, which range from 7 (Suzuki[4]) to 128 (Kral et al. [5]). Karyotypes of 771 spider species belonging to 65 families have been reported up to now, majority of which is concerning Araneomorph spiders (Korinkova and Kral [6]; Araujo et al.[7]).

The evolution of multiple sex chromosomes in spiders is complicated and many hypotheses have been proposed (Aviles [8].Another remarkable characteristic of spider chromosomes frequently shown is the presence of multiple sex chromosomes (White [9]). In spiders, 3 major types of sex

determining mechanism have been reported, i.e. XO, X_1X_2O and $X_1X_2X_3O$ types. All these types may have evolved from a remote XO-type ancestor by centric fragmentation accompanying inversion following breakage. The $X_1X_2X_3O$ type was derived from the X_1X_2O type by the same process described above and the modern XO-type evolved by the gradual elimination of one of the 2 X`s from the X_1X_2O type. However, the X_1X_2O type seems to be the most primitive in present day spiders as stated by Suzuki [4]). Both chromosomes number and sex determining mechanism are important in the study of spiders phylogeny and mode of chromosome evolution.

The goal of the present research is to characterize each spider species by means of cytogenetic observations in order to evaluate each into the respective level of phylogeny. Karyotype information can be helpful in establishing the evolutionary relationships between species and for differentiating species that are otherwise similar.

Material and methods

Spiders were collected from natural habitat in Bangalore University Jnanabharathi campus, Bangalore, Karnataka, India. The specimens were identified following the keys of Sebastian and Peter [10]). The voucher specimen were preserved in 70% ethanol and deposited in Museum of Department of Zoology, Bangalore University, Bengaluru, Karnataka, India.

An Improved air-dried method (Luykx [11]) with some minor modifications was followed for preparing spider chromosomes. The spider was anesthetized and abdomen was cut open on wax plate using fine scissors. Gonads and gut epithelium were removed, placed on slides and soaked in 2 drops of 0.075M potassium chloride in a humid chamber for about 35min. Any excessive solution was removed from the slide and the tissues were fixed by adding few drops of fixative I (methyl alcohol: glacial acetic acid: water=3:3:4, by volume) across the inclined slide. With the slide lying flat, gonads were immediately macerated with fine needles and then 15 drops of fixative II (methyl alcohol: glacial acetic acid =1:1, by volume) were added to the tissues. This fixative was allowed to stand for 15s, the slide was drained and placed in coupling jar containing fixative III (methyl alcohol: glacial acetic acid =3:1, by volume) for 30 min. The slide was removed and several drops of glacial acetic acid were added across the inclined slide after which the slide was flame dried. Chromosomes were stained by adding 10-15 drops of 5% Giemsa solution and were rinsed with distilled water. Mitotic chromosomes were subjected to C- banding (Sumner [12]) and NOR staining (Howell and Black, [13]) with minor modifications. Chromosome preparations were observed using Zeiss Axioskop 2 plus microscope and well spread complements were photographed.

Results

1 PHOLCIDAE

I. *Crossopriza lyoni* :- Mitotic metaphase cells of *Crossopiza Lyoni* showed a diploid number 2n = 23(22AA + XO) in males and 2n= 24(22AA + XX) in females with sex chromosomes system of XO/XX type. The chromosomes are meta and submetacentric type. The autosomes pairs gradually decrease in size and the sex chromosome are extremely large. In females Pachytene cells presented 11 synapsed autosomal bivalents and two highly condensed and heavily stained univalent sex chromosomes. In the late stages of the prophase I, the X chromosome also revealed a higher degree of condensation in relation to the autosomes.

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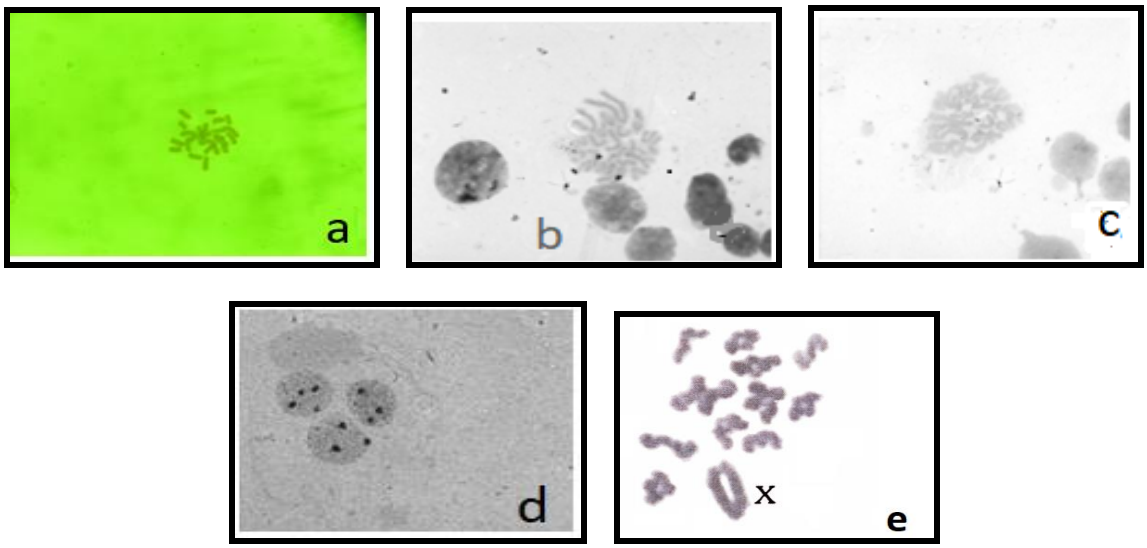

Fig.1 (a-e) ***Crossopriza lyoni*** **:** Gonadal mitotic metaphases from adult *Crossopriza lyoni* individuals. Conventionally Stained, male with 2n=23 and female with 2n=24. (a) metaphase complement (b, c) showing C bands (d) submitted to silver staining, NOR bearing chromosomes are indicated (e) Spermatocytes of adult. *Crossopriza lyoni* specimens in conventional staining-meiocyte in Prophase I with 2n=11 bivalents +X, showing the presence of metacentric and submetacentric chromosomes.

2 HERSILIDAE

I. *Hersilia savignyi :-* The diploid set of *Hersilia savignyi* has 30 chromosomes in mitotic preparations. Mitotic metaphase cells of *Hersilia savignyi* submitted to standard staining with Giemsa showed the diploid number 2n= 30 (28AA + X_1X_2) in males which were consistent with the sex chromosome system X_1X_2 O type. During prophase stages of meiotic division, sex chromosomes are more condensed and heavily stained than the autosomes. There are 14 bivalent autosomes and 2 sex chromosomes during diakinesis stage, indicating X_1X_2 O sex chromosome system. C-Banding reveals that chromosomes are heavily stained in acrocentric region. The cells impregnated with silver nitrate shows the presence of many heterochromatic blocks in the interphase nuclei.

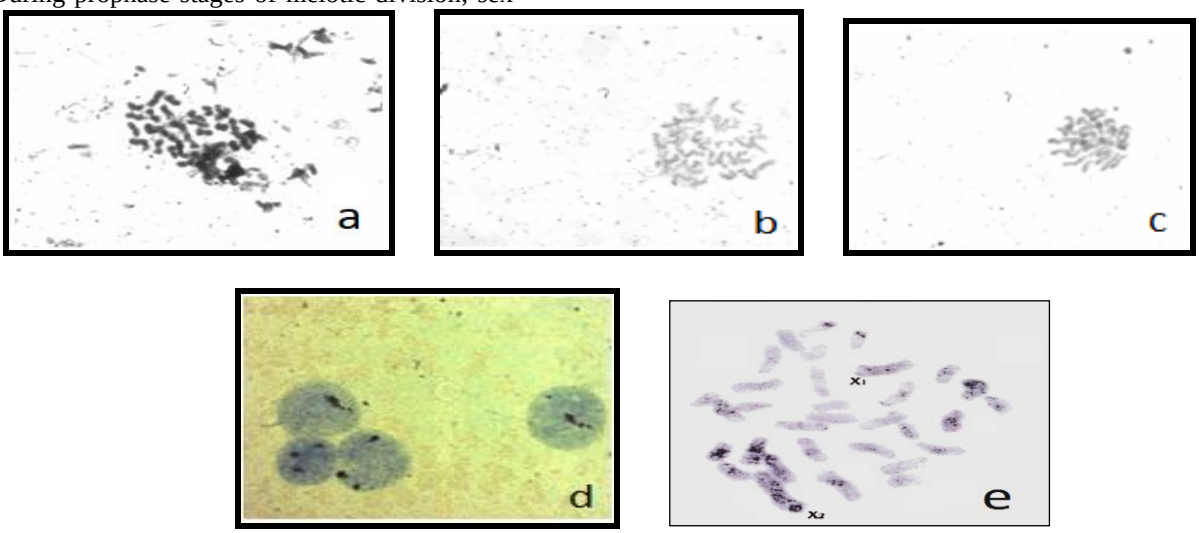

Fig.2 (a-e) *Hersilia savignyi* :- Testicular cells of *Hersilia savignyi* (a) Mitotic metaphase 2n=30 (28AA + X_1X_2) and acrocentric chromosomes (b, c) C banding (d)submitted to silver staining, NOR bearing chromosomes are indicated (e)well spread chromosome plate showing X_1X_2. Giemsa-stained

3. LYCOSIDAE

I *Hippasa aegelinoides* :- The diploid chromosome mumber was determined to be 2n=28 (26AA+ X_1X_2) in males as observed from well spread spermatogonial metaphase plates. All chromosomes are rod shaped with the sex determination system of X_1X_2 O type . Size difference is observed in the complements. The haploid number is 15 with 13 autosomes and two recognizable unequal rod shaped X elements. These X elements are not clearly distinguishable in spermatogonial metaphase but become conspicuous in subsequent stages of meiosis.

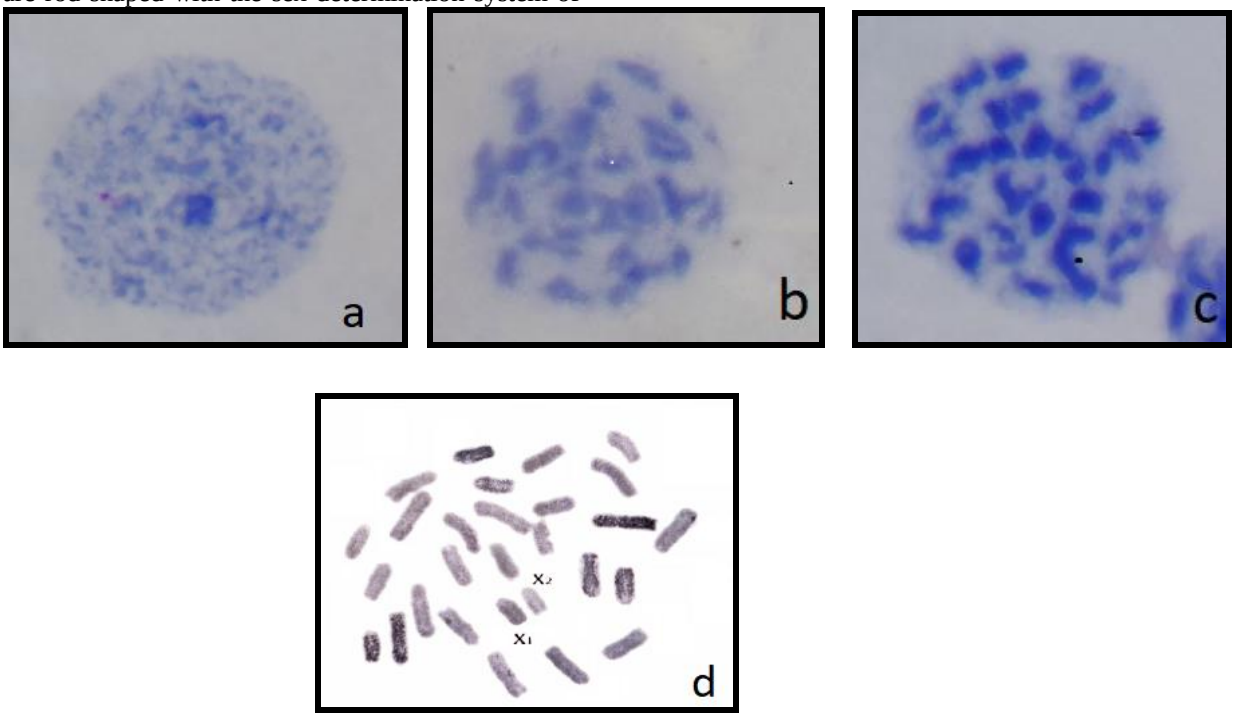

Fig.3 (a-d) *Hippasa aegelinoides* :- (a) Interphasic nuclei of male *Hippasa* species. Conventionally stained, showing a conspicuous heteropycnotic positive chromatinic block (b, c) leptotene, Two heavily stained sex chromosomes in the margins of leptotene. (d) Spermatogonial mitotic metaphase plate 2n = 28, showing the univalent sex chromosomes X_1X_2.

Discussion

Cytogenetic analysis of Indian spider species has added considerably to our knowledge about chromosomes in spiders and karyotypic evolution (Bole-Gowda [14]; Sharma et al [15]; Srivastava and Shukla [16]; Parida and Sharma [17]; Datta and Chatterjee [18]). Bole-Gowda [14] presented the first karyotype for Indian Pholcid example, viz; C.lyoni depicting the diploid chromosome number 2n=27 (26AA+X) in males. *Crossopriza lyoni* chromosomal analysis showed that the diploid number 2n=23 (22AA+X) in males predominantly meta and sub metacentric chromosomes by (Oliveira et al [19]) which matches with our study results. Presence of many heterochromatic blocks reveals different gene expression during course of development. The previously studied pholcid species have diploid number between 2n=15 and 2n=32 with meta and sub metacentric chromosomes (Cokendolpher [20]; Araujo et al [21]; Kr'al et al [22]).

Great amount of work has been done for Indian pholcids (Bole-Gowda [14]). He asserted that X_1X_2O SCS originated from XO system in the ancestor of spiders by fission in the middle of the X chromosome producing an acentric chromosome segment that translocated to a super numerary centric fragment. He also analyzed the chromosome of *Hersilia savignyi* and found that diploid chromosome number is 2n= 30 and SCS X1X2 with all acrocentric chromosomes. Sharma et al [15] recorded 2n=32 (15 II +X1X2) with metacentric autosomes and acrocentric sex chromosomes.

Hersilia savignyi males show chromosome diploid number of 30 and sex chromosome system of X_1X_2 type with all acrocentric chromosomes (Srivastava and Shukla [16]; Parida and Sharma [23]; Sharma and Parida [24]). Our present study also fits in with the above diploid chromosome number for the species. According to previous studies, the diploid chromosome number in lycosidae varies from 22 to 28 (Bole-Gowda [14]; Sharma and Tandon [25]) among which 28 is observed in majority of species. The sex chromosomes are unequal in length. Datta and Chatterjee [26, 27] proposed X_1X_2 O system is found in lycosids. Our study found the diploid chromosome number in males of *Hippasa aegelinoides* to be 28 with the sex determination system of X_1X_2 O type which fits to some extent with the previous data. *Crossopriza lyoni* chromosomal analysis showed that the diploid number 2n=23 (22AA+X) and 2n=24 (22AA+XX) in males and females respectively with sex chromosome system of XO/XX type. In *Hersilia savignyi* 2n= 30 (28AA + X_1X_2) in males and in *Hippasa* 2n=28 (26AA+ X_1X_2) in males which were consistent with X_1X_2 O sex chromosome system. These Karyological data are useful in explaining karyotypic evolution, sex chromosomes system and meiosis in araneomorph spiders.

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