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### RESEARCH ARTICLE

#### DIFFERENT TECHNIQUES USED FOR CHROMOSOMAL ANALYSIS OF THREE SPECIES OF WOLF SPIDERS (LYCOSIDAE: ARACHNIDA)

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#### Abstract

Spiders (Arthropoda: Arachnida: Araneae) are fascinating and most diverse group of air breathing chelicerate arthropods in the animal kingdom. Three different techniques namely- Spreading technique by Traut, chromosomal fixation by Imai and air drying technique are studied for chromosomal analysis of three species of wolf spiders. Chromosome data are reported for three species belonging to families Lycosidae from India. The diploid chromosomes number (2n) as determined from testicular material was 28 in all three species studied and as follows: *Hippasa agelenoides*:  $2n=28 (26+X_1X_2)$ , *Lycosa bistriata*  $2n= 28 (26+X_1X_2)$ , *Pardosa birmanica*:  $2n=28 (26+X_1X_2)$ . Sex determining mechanism is  $X_1X_20$  type with chromosomes being acrocentric in morphology. The sex elements are unequal and heteropycnotic during meiotic prophase.

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#### Introduction:-

According to Platnick (2014), the order Araneae possesses 114 families, 3935 genera, and 44,906 species. The order Araneae ranks seventh in total species diversity among all other organisms. The order Araneae consists of three primary clades, namely Mesothelae, Mygalomorphae and Araneomorphae, the last one being the phylogenetically most derived and largest group (Coddington and Levi, 1991). Mesothelae and Mygalomorphae exhibit less than 3000 species; in contrast, the Araneomorphae includes more than 41,000 species belonging to 95 families (Platnick, 2014).

Spiders are found on all continents (except Antarctica, although spider fragments have been reported there and at elevations as high as 5,000 metres (16,400 feet) in the Himalayas. Many more species occur in the tropics than in temperate regions. Though most spiders are terrestrial, one Eurasian species is aquatic and lives in slow-moving fresh water. There are a few species that live along shores or on the surface of fresh or salt water.

The abundance of the spiders can be summarized in the following few sentences of Gertsch (1949). "Spiders are among the dominant predators of any terrestrial community. When the fauna of the soil and its plants cover is analyzed, they come to light in vast numbers, in such convincing abundance that it is evident that they play a significant part in the life of every habitats."

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The family Lycosidae (wolf spiders) belongs to the superfamily Lycosoidea that is included in the Entelegyne lineage of Araneomorph spiders (Jocqué and Dippenaar-Schoeman, 2007). The lycosids occur all over the world and are very diverse with 2367 species in 116 genera described so far (Platnick, 2010).

In terms of cytogenetics, lycosids are one of the best explored families of Entelegyne spiders along with the families Araneidae and Salticidae. Despite this, there are many lycosid genera for which the karyotypes are unknown or uncertain. Suzuki (1954) classified spider karyotypes into three types based on the number of Chromosomes: (i) karyotypes with high chromosome numbers ( $2n > 46$  in males) are regarded as primitive (mesothelids, mygalomorphs), (ii) those with  $2n = 34$  (The so-called intermediate type) and (iii) those with low chromosome numbers ( $2n < 34$ ).

There are a number of tissues that can be used in cytogenetic studies of spiders, such as gonads (testes and ovaries), cerebral ganglion, and cultured blood cells, as described by Wang & Yan (2001). The gonads, especially the testes, have been found to be more suitable than other tissues for karyotype analysis in the vast majority of cytogenetic investigations. In addition to data regarding the diploid number, length, and morphology of chromosomes, analyses of chromosomes during meiosis have contributed to the identification of types of sex chromosome systems in spiders. This is very important in the case of Araneae due to the diversity of simple or multiple sex chromosome systems that have been recorded in representatives of this order.

The present study on the Arachneae species fauna throws light on study of different techniques for cytogenetic analysis of spiders for which three species of wolf spiders are considered.

### Material and Methods:-

Spiders were collected from natural habitat in Bangalore University Jnanabharathi campus, Bangalore, Karnataka, India. The species were identified by the reference to primary literature, with Spiders of India (Sebastian and Peter, 2009). The identified species belonged to the family Lycosidae namely Hippasa agelenoides (Simon, 1884), Lycosa bistriata (Gravely, 1924) and Pardosa birmanica (Simon 1884).

Majorly two methods were used to collect the spiders depending on their habitat. They are mentioned below-

#### Hand picking method:

One of the best methods to collect spiders is by hand. A soft paintbrush or cotton swab can be used to gently knock the specimen into a collecting vial. The specimens can also be carefully picked by hand. Turning over stones and logs exposes many spiders and hand collecting is the method of choice. Hippasa species were collected by this method.

#### The sweep net method:

This is one of the simplest ways to collect spiders; the ideal habitat for using sweep net is in grasslands and from flowers. While using the sweep net it was dragged back and forth across a low group of weeds and brushes a number of times with a quick, steady motion. Most of the samples of Lycosa bistriata and Pardosa birmanica were collected by this method.

#### Preservation of sample:

70% Ethyl alcohol can be used as the Standard Preservative (grain alcohol, ethanol). The locality of collection, period of collection and number of individuals were given in Table 1.

**Table 1:-** Biological data on three species of wolf spiders.

Collected species	Number of spiders	Period of collection	Locality
<b>Sub family: Lycosinae</b> 1. Hippasa agelenoides (Simon) 2. Lycosa bistriata (Gravely)	Adults - 18 Adults -12	Jan 2019- May 2019	<b>BANGLAORE UNIVERSITY, JB CAMPUS</b>
<b>Sub family: Pardosinae</b> 1. Pardosa birmanica (Simon)	Adults - 14		

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### Different Techniques for chromosomal analysis:

#### Modification of the spreading technique by Traut (1976):

All specimens collected were adult males as well as females. The following procedure was carried out:-

1. Gonads and intestine were dissected out in a glucose free physiological solution (Lockwood, 1961), hypotonized in 0.075M KCl for 12-15 minutes at room temperature (RT) and fixed in 2 batches of freshly prepared Carnoy's fixative (Ethanol : Chloroform : Glacial acetic acid-6:3:1)
2. The first batch should be kept for 10 minutes and the second batch for 20 minutes at Room Temperature.
3. A cell suspension was prepared from a piece of tissue in a drop of 60% acetic acid on a slide using a pair of tungsten needles. The slide was placed on a histological plate at 42°C and the drop was evaporated by moving with tungsten needle.
4. Slides were stained with a 5% Giemsa Solution in Sorensen Phosphate Buffer (pH-6.8) for 27 minutes at Room Temperature.
5. The slides were rinsed in distilled water.
6. Investigation of cells under an Olympus BX53 microscope and photographed by Axion vision digital camera using Cellsens Software.

#### IMAI'S, (1988) Technique:

In this technique 3 fixatives were used:-

FIXATIVE 1 was prepared in the proportion of 3:3:4 (Methanol: Acetic acid: Distilled water)

FIXATIVE 2 was prepared in the proportion of 1:1 (Methanol: Acetic acid)

FIXATIVE 3 was prepared in the proportion of 3:1 (Methanol: Acetic acid)

#### The procedure followed was mentioned below:

1. The tissues (gonads, intestines) and eggs were dissected in hypotonic solution (KCl II or KCl IV).
2. The dissected materials were incubated in humid chamber (40 minutes in KCl II or KCl IV).
3. The tissues and eggs were transferred to the slides; FIXATIVE 1 was added and kept for 10 minutes.
4. The tissues and eggs were macerated and a thin smear was made with a needle; FIXATIVE 2 was added and kept for 1 minute.
5. The slides were drained.
6. FIXATIVE 3 was added for 30 minutes.
7. The slides were drained.
8. A few drops of 60% acetic acid were added across the slide.
9. The slides were flame dried in slanting position.
10. The flame dried slides were stained with Giemsa Stain for 8 to 10 minutes.
11. The slides were washed with distilled water and dried.

#### Air Dried Technique:

The preserved specimen is dissected to extract somatic tissue (mitotic preparation) and gonads (meiotic preparation) for chromosomal investigations by "air dried technique". The modified air dried technique (Venkatachalaiah and Chowdaiah, 1987) is as follows:-

1. Dissection of the materials (gonads/digestive tract) in 1% sodium citrate solution.
2. The dissected material was treated with 2-3 drops of 0.2% colchicines and 5 ml of KCl-0.125M i.e. 0.93gm/100ml (meiotic preparation) and KCl-0.075M i.e. 0.55 gm/100 ml (mitotic preparation) was added.
3. The material was minced thoroughly with mincing scissors and treated for about 45-60 minutes in hypotonic and colchicines solutions. This cell suspension was centrifuged at 800 rpm for 5 minutes.
4. The supernatant after centrifugation was discarded and 3-5 ml of hypotonic solution was added to the filtrate; it was refluxed with Pasteur's pipette and centrifuged at 800 rpm for 5 minutes.
5. The supernatants discarded and 3-5 ml of freshly prepared fixative (3:1 methanol: acetic acid) is added. The solution was refluxed and centrifuged at 800rpm for 5 minutes. This step was repeated twice.
6. The supernatant was discarded after centrifugation and the cells are resuspended in the 3-5 ml of fixative, 3-4 drops of this suspension was dropped on to a pre-refrigerated slide and allowed to dry on the slide warmer at 25°-35°C for 2-3 minutes.
7. The slides were stained with 5% Giemsa Solution for 5 minutes and washed thoroughly in distilled water.

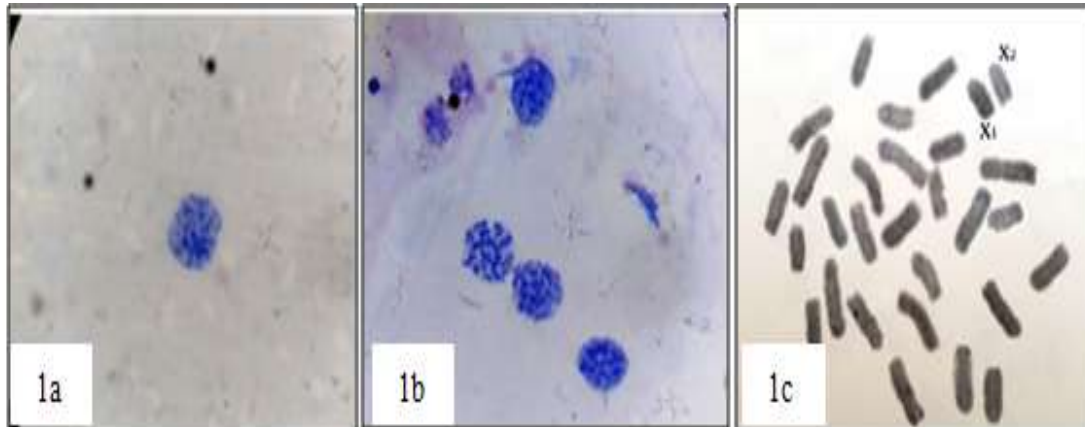
8. The air-dried, stained slides were observed under microscope for the well spread chromosomes for microphotography for further analysis.

### Results:-

#### Subfamily- Lycosinae:

##### *Hippasa agelenoides* (Simon, 1884):

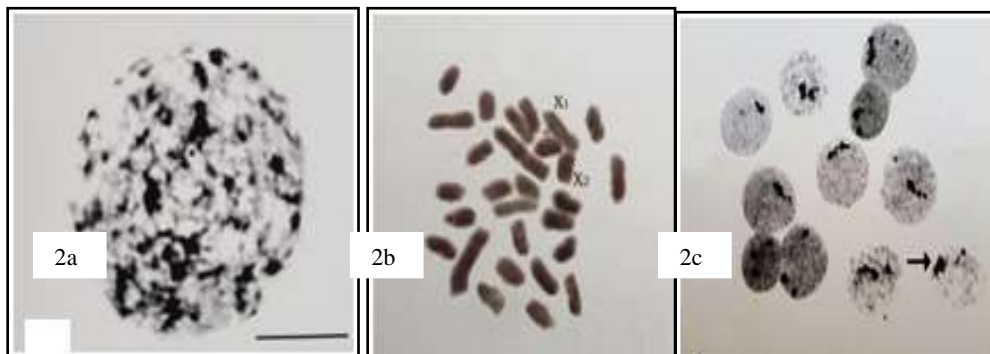
Testicular materials from 18 adult males were used for present investigation. The diploid number was determined to be 28 as observed from well spread spermatogonial metaphase plates. Chromosomes are rod shaped. The 2 X's were distinguishable in leptotene, pachytene and diplotene. The diploid number of species was with 13 autosomes and 2 clearly recognizable rod shaped elements. The best result of species came with Traut Technique (Fig.1). Sex determining mechanism was  $X_1X_20$  type with chromosomes being acrocentric in morphology.



**Fig 1:-** *Hippasa agelenoides*: Giemsa stained Testicular cells (1a and 1b) Spermatogonial prometaphase (1c) Mitotic metaphase  $2n=28$  ( $26AA+X_1X_2$ ),  $n=(13+X_1X_2)$ , well spread chromosome plate showing  $X_1X_2$ .

##### *Lycosa bistrata* (Gravely, 1924):

The diploid number of species was 28 as studied from testicular material taken from 12 male individuals. The interphase nuclei contain deeply stained irregular bodies. The X chromosomes were highly condensed, unequal in length and rod shaped. The chromosomes of species were acrocentric in shape. Sex determining mechanism was  $X_1X_20$  type. The best result of this species came with Imai technique (Fig.2).



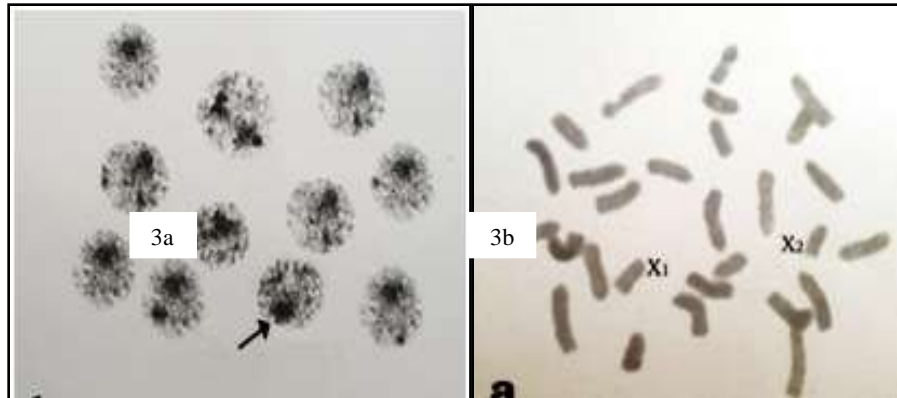
**Fig 2:-** *Lycosa bistrata*: (2a) Interphasic nuclei of male *Lycosa bistrata*. Conventionally stained, showing a conspicuous heteropycnotic positive chromatinic block (2b), Spermatogonial mitotic metaphase plate  $2n=28$  ( $26AA+X_1X_2$ ), well spread chromosome plate showing  $X_1X_2$ , (2c) subjected to silver staining, NOR bearing chromosomes were indicated.

#### Subfamily- Pardosinae:

##### *Pardosa birmanica* (Simon, 1884):

Testicular materials from 14 adult males were used for study of the species. The diploid chromosome number was  $2n=28$ . The chromosomes were acrocentric and rod shaped. The 2 X chromosomes were prominent. The haploid

chromosome number was 15 with 13 autosomes and 2 unequal parallel rod shaped X chromosomes. Sex determining mechanism was  $X_1X_2O$  type. The best result of species came with air drying technique (Fig.3).



**Fig 3:** *Pardosa birmanica*: (3a) Interphasic nuclei of male *Pardosa birmanica* (3b) Giemsa-stained Mitotic metaphase  $2n=28$  ( $26AA+X_1X_2$ ), and acrocentric chromosomes well spread chromosome plate showing  $X_1X_2$ .

### Discussion:-

The pioneering contribution on the karyotypes of Indian spiders was of Bole-Gowda (1958) and Sharma et al. (1958). Then major contributions in this field were those of Mittal (1970); Datta & Chatterjee (1983); Parida et al. (1986); Srivastava & Shukla (1986); Sharma & Parida (1987) and Datta et al. (1995). With respect to Indian spiders there are 325 cytogenetic records found till date. 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.

Cytogenetic studies of the family Lycosidae (Arachnida, Araneae) are scarce and were performed in less than 4% of the 2324 known species (Platnick, 2008). Most of the analyzed species had only telocentric or acrocentric chromosomes, which were ranged from  $2n=18$ ,  $n = 8+X_1X_2$  (male) to  $2n = 28/30$  (male/female),  $n = 13+X_1X_2$ (male). The  $2n=28/30$ , which is present in 50% of the analyzed species, is probably the modal number (basic diploid number) of the family. The sex chromosome mechanism  $X_1X_2/X_1X_2X_3X_4$  (male/female) occurs in 94% of the lycosid species and was considered as an ancestral trait in spiders. Up to now, 102 species of lycosids (including 23 species, determined only to the genus level) from 21 genera have been analysed. These data were recently summarised by Chemisquy et al. (2008).

Diploid chromosome numbers of lycosid range from  $2n = 18$  in *Lycosa* sp. (Srivastava & Shukla, 1986) to  $2n = 28$  in most other species. Datta & Chatterjee (1992) proposed that the  $XO$  system found in lycosid and uloborid spiders originated from the  $X_1X_2O$  SCS by centric fusion of the  $X_1$  and  $X_2$  chromosomes, followed by pericentric inversion or partial deletion in one of the X chromosome arms, giving rise to an acrocentric element. Alternatively, the acrocentric X chromosome could have originated from tandem fusion between the  $X_1$  and  $X_2$  chromosomes. The pioneering works describing  $XO$  SCS in spiders were those of Wallace (1900; 1905). In present study the diploid chromosome number for all three species studied is 28 with  $X_1X_2O$  as sex determining mechanism in males. In all three species *Hippasa agelenoides*, *Lycosa bistrata* and *Pardosa birmanica*  $2n=28(26+X_1X_2)$ ,  $n=13+(X_1X_2)$  with  $X_1X_2O$  as sex determining mechanism.

It is evident from cytological survey of the family that  $2n=28$  is the most frequent diploid number with 61.54% of the known species. 23.08% of the species have  $2n=26$ , 3.12% are with  $2n=24$  and 10.26% are with  $2n=22$ . Our results shows diploid number of species to be 28 with  $X_1X_2O$  as sex determining mechanism.

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