ORIGINAL RESEARCH PAPER

Agricultural Science

CHROMOSOMAL STUDY OF TWO ARANEAE: ARANEIDAE SPIDERS FROM AKOLA MAHARASHTRA

KEY WORDS: Araneae, Karyotype, Akola, acrocentric chromosomes, Cyrtophora, Neoscona

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ABSTRACT

Spiders (Araneae) appear to be the best studied order, of the class Arachnida concerning Chromosomal aspects. They exhibit great diversity in diploid chromosome numbers. Compared with others Araneidae spiders, chromosomes of the Araneae: spider are less studied. In this study, chromosomes of two Araneidae spiders *Cyrtophora citricola* (Forskål, 1775) and *Neoscona theisi* (Walckenaer, 1841) were observed and studied based on the samples collected from Akola region. The chromosome diploid number and the sex chromosomal system in both the spider species found $2n=(22+X_1X_1X_2X_2)$ and $2n=(22+X_1X_2)$ respectively. Both the spider species shows telocentric chromosomes.

INTRODUCTION

Chromosomal characters have been widely used in studies of the taxonomy and population genetics of spiders. Concepts of spider karyotype evolution are based mostly on advanced and most diversified clade, the entelegyne lineage of araneomorph spiders. Araneidae, a family of the suborder Araneomorphae, comprises 176 genera distributed worldwide (world spider catalogue, 2019). In India it is represented by 28 genera and 163 species (Seema keshwani, *et al.,* 2012). Araneae is one of the best studied order of the class Arachnida concerning cytogenetic study. The 823(~1.7%) species of the 47,272 taxonomically known spiders are cytogenetically studied from all over the world. The notable features of karyotype is the predominance of unusual multiple X chromosome systems such as X_1X_2/ $X_1X_1X_2X_2$, often assigned as X_1X_20, where indicates the absence of Y chromosome (Kral *et al.*, 2006). The $X_1X_2X_30$, $X_1X_2X_3X_40$ and X0 derived from the original sex chromosome system X_1X_20. Karyotype study of spiders is useful for determination of the cytogenetic relationship regarding evolution among the species.

The chromosomes shows G-bands that are rich with Adenine and thymine (A-T) gives dark band and the heterochromatic region of chromosome are rich with Guanine and Cytosine (G-C) gives light band and stained with Giemsa stain. most of the G-bands of chromosome are located from centromere to the telomere in both the spider species studied. All the chromosomes in spider species showed different banding pattern. Little attention has been paid to the Spiders with reference to their chromosomal study so far, particularly due to their ugliness and anchoretic lifestyle. Hence the present study is carried out to explore chromosomes and genetic aspects in neoscona spiders abundantly found in study area. Here the spider smooth muscle of cephalothoraxes region were harvested and used for the explants culture system. The cells were used to obtain karyotype of the spider species. The homologous chromosome shows similar banding pattern that helped to obtain karyotype in both the spider species. The outcome of the present study will be of use to prepare the database of chromosomal pattern in spiders from India.

MATERIALS AND METHODS

Spiders were collected for the present study in and around Akola district during February 2014 to January 2018. For cytogenetic preparation, the method of Webb *et al.,* (1978), described for embryos, was followed with some modification. The female spider was disinfected with the 70% ethyl alcohol for 5 minutes. The spider was dissected and muscles tissues were removed out from cephalothorax region by using SSC sodium citrate pH 7. The tissue was washed 2-3 times in the RPMI medium to remove fat bodies. The tissue was taken into few drops of media and broken with sterile bled to get single cells. This cell suspension containing very small tissues explants were transferred to the culture T25 cm^2 flasks by using sterile dropper and spread over the surface of the culture flasks. Then feeded with 0.5 ml of RPMI medium and transferred to CO_2 incubator at 37°C for 1-2 days. After 1-2 days the cells were feeded with 5 ml of fresh growth medium. For karyotype preparation 0.1 ml of colchicine was added to the flask 1 hr before harvesting of the cells. Then the 1.5 – 2 ml of trypsin EDTA 1 X 100 was added to the culture flask and kept it for incubation for 2 mins. The cells in the flask were aspired gently with the help of pipette and transferred the solution to the centrifuge tube. The solution was centrifuged at 800- 1000 rpm for 8 mins. The supernatant was discarded and pellet was broken by tapping gently with fingers. The hypotonic solution was added into the tube. The tightly covered tubes were kept into the water bath at 37° C for 15 mins. Then the 8-10 drops of fixative was added and mixed gently. Again added 3 ml of fixative and mixed gently. Then again the tube was centrifuged at 800 rpm for 10 mins. The supernatant was discarded and again 5 ml of fixative added and centrifuged at 800 rpm for 10 mins. The process was replaced thrice until the clear pellet was not obtained. Finally the tubes were tightly closed and kept into the refrigerator 25 mins. The cell suspension was dropped onto clean slides in such a way that the drops should not be overlapped, air-dried and stained with Giemsa 3% for 10-15 min then rinsed in distilled water to remove excess Giemsa stain. The best metaphase pictures were photographed with a digital camera system attached to the microscope. The chromosome groups were determined on the slides and their films were photographed. For G-banding, the slides were treated with PBS for 15 min at room temperature. After slides were rinsed distilled water, the slides were treated with 0.1 % trypsin for 1 min. Slides were air dried and stained with 3 % Giemsa. Finally the slides were prepared by mounting with DPX for chromosome counts and karyotyping.

The karyotypes were constructed by pairing chromosomes by using size, banding pattern and centromere position from

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metaphase plate. The centromere positions of all chromosomes were analyzed to understand the morphology according to the method given by Levan *et al*., (1964). The total length of chromosome (TLC) was considered as c and the length of long arm (LA) and short arm (SA) were considered as l and s, respectively, then the position of a centromere can be calculated as a ratio (r) of long arm (l) to short arm (s). When the value of the ratio (r) was in between 1.0-1.7, the chromosome was designated as metacentric and centromere was considered as medium type. When the value was in between 1.7-3.0, the centromere was considered as median type and chromosome was designated as sub metacentric. The ratio in between 3.0-7.0 indicates the chromosome as sub telocentric with centromere close to one end of the arm and it was designated as sub telocentric. The ratio in between 7.0-∞ considered as a terminal centromere with centromere located at the one corner and the chromosome has designated as telocentric.

RESULTS AND DISCUSSION

The spider smooth muscle (cephalothorax) explants were harvested and used for the explant culture system. The culture flasks were observed after every week. The cells of *cyrtophora citricola* (**Forskål**, 1775) get proliferate and tend to stay together. The cells grow slowly and become elongated. The cells were elongated and arranged in parallel with adjacent cells (fig 1-A) by the first week. The cells of *Neoscona theisi* (Walckenaer, 1841) were elongated in shape and tapered end, when settled down at the substratum started to grow by the first week (Fig 1-B). In some areas in the culture the cells appear like spindle shaped.

These cells were used for cytogenetic study after colchicine treatment. The mitotic metaphase of *Cyrtophora citricola* (Forskål, 1775) after standard staining with Giemsa showed the diploid number 2n=26 and the sex determining chromosomal system of the X_1X_2/ $X_1X_1X_2X_2$ type and the autosomes shows telocentric morphology (Table 1). The 22 autosomes ranges from 0.7 to 1 and 4 sex chromosomes of approximately same size i.e. 0.6 cm (table.1). The chromosome length of all the autosomes ranges from 0.7 cm to 1 cm. The mitotic metaphase of *Neoscona theisi* (Walckenaer, 1841) after standard staining with Giemsa showed the diploid number 2n=24 and the sex determining chromosomal system of the XO/ XX type and the autosomes show telocentric morphology (Table 2). The sex chromosomes of approximately same size i.e. 1.6 cm and shows telocentric morphology (table. 2). The chromosome length of all the autosomes ranges from 0.8 cm to 1.1cm. We found that the G-bands are located from centromere to the telomere of each chromosome from both the spider species studied. The chromosomes in both spider species shows different banding pattern. The homologous chromosomes shows similar banding pattern that helped to prepare karyotype in both the species.

The cytogenetic study is very important to know the basic chromosomes number and sex determination system in the spider. Araneae is one of the best studied order of the class Arachnida concerning cytogenetic study. The chromosomes of the majority of the species studied consist of acrocentric chromosomes only (Tugmon et al., 1990). Bi-armed chromosomes dominate only in karyotypes of some groups, namely the family Dictynidae (Kra´ L, 1995) and in the haplogyne lineage of araneomorph spiders (Cokendolpher, 1989; Rodri´Guez Gil et al., 2002, Kra´ L et al., 2004). Cytogenetic studies on the majority of females of Araneidae have similar characteristics. The majority of female shows X1X2 sex chromosome system and show telocentric chromosomes. As a result, in this study we have studied on two spider species of genus Cyrtophora (Simon 1864) and Neoscona (Simon, 1864) from Akola district cytogenetically. The Cyrtophora citricola (Forskål, 1775) investigated by examining Karyological characters. It consist of 22 autosome showed telocentric chromosomes and the sex chromosome system is X1X1X2X2. The Neoscona theisi (Walckenaer, 1841) investigated by examining Karyological characters. It consist of 22 autosomes showed telocentric chromosomes and the sex chromosome system is X1X2. We found that the G-bands are located from centromere to the telomere of each chromosome from both the spider species studied. Studies of this genus of spiders are important for the understanding of karyotype evolution in spiders, since these animals are supposed to be one of the most primitive groups of arthropodans. Moreover, obtained results might also reveal pathways of evolution within Indian spiers.

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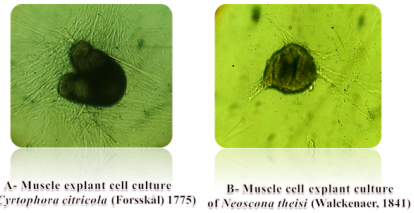

Fig. 1 Muscle explant culture of spider *Cyrtophora citricola* (Forsskal 1775) and *Neoscona theisi* (Walckenaer, 1841).

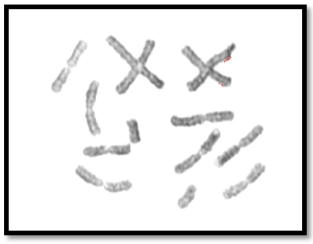

Fig 2. Metaphase plate of *Cyrtophora citricola* (Forsskål, 1775)

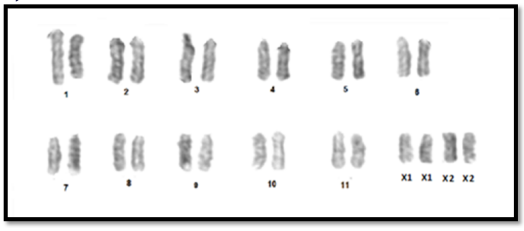

Fig 3. Karyotype of *Cyrtophora citricola* (Forsskål, 1775)

Table 1. Karyological data of *Cyrtophora citricola* (Forsskål, 1775)

Pair no.	TLC (cm)	LA (cm)	SA (cm)	LA/SA (cm)	Type
1	1	1	-	∞	T
2	1	1	-	∞	T
3	0.9	0.9	-	∞	T
4	0.8	0.8	-	∞	T
5	0.8	0.8	-	∞	T
6	0.8	0.8	-	∞	T
7	0.8	0.8	-	∞	T
8	0.8	0.8	-	∞	T
9	0.8	0.8	-	∞	T
10	0.8	0.8	-	∞	T
11	0.7	0.7	-	∞	T
X_1	0.6	0.6	-	∞	T
X_2	0.6	0.6	-	∞	T

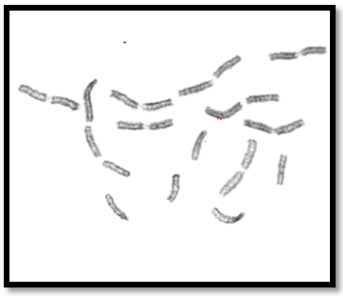

Fig 4. Metaphase plate of *Neoscona theisi* (Walckenaer, 1841)

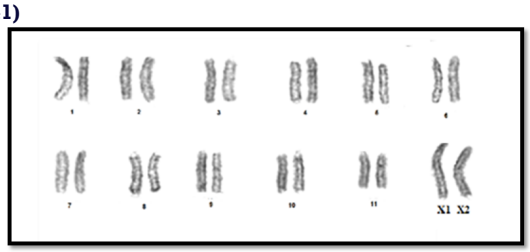

Fig. 5 Karyotype of *Neoscona theisi* (Walckenaer, 1841)

Table 2. Karyological data of *Neoscona theisi* (Walck en aer, 1841)

Pair no.	TLC (cm)	LA(cm)	SA(cm)	LA/SA	Type
1	1.1	1.1	-	∞	T
2	1	1	-	∞	T
3	1	1	-	∞	T
4	1	1	-	∞	T
5	1	1	-	∞	T
11	1	1	-	∞	T
7	1	1	-	∞	T
8	0.9	0.9	-	∞	T
9	0.9	0.9	-	∞	T
10	0.8	0.8	-	∞	T
11	0.8	0.8	-	∞	T
X_1	1.6	1.6	-	∞	T
X_2	1.6	1.6	-	∞	T

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