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RESEARCH ARTICLE

CHROMOSOMAL STUDIES OF TWO SPIDER SPECIES OF PHOLCIDAE (ARANEAE: HAPLOGYNAE)

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ABSTRACT

Pholcidae (Haplogynae) is the most diverse family which comprises 1,241 taxonomically defined species, of which only 19 have been cytogenetically analyzed. The two pholcids species, *Crossopriza lyoni* (Blackwall 1867) and *Artema atlanta* (Walckenaer 1837) are cytogenetically studied following Conventional, and AgNO3 impregnation techniques. The karyotype revealed presence of (2n=23) 22AA+X0♂ and (2n=32) 30AA+X_1X_2♂ chromosomes in *C.lyoni* and *A. atlanta* respectively. In both species, the chromosomes were exclusively biarmed, and the sex chromosomes are the largest elements of the karyotypes. Silver nitrate stained NOR-specifications were noticed in *C.lyoni* at the telomeric regions of pairs of chromosomes (4 and 6) and sex chromosome in the complement. *A. atlanta* exhibited the NOR-specifications in 2 pairs of chromosomes in the complement.

INTRODUCTION

Spiders are one of the most interestingly diverse animal orders. The order Araneae is composed 44,906 nominate species distributed among 3935 genera and 114 families all around the world (**Platnick, 2014**). The order is divided into Mesothelae and Opisthothelae. Opisthothelae is sub divided into basic clades, namely, Mygalomorphae and Araneomorphae (**Coddington, 2005**). The latter being divided into three groups viz., basal araneomorph, haplogynes and entelegynes. Haplogyne spiders form a modestly diversified clade of araneomorphs. Karyotypes of almost 792 species of spiders belonging to 288 genera and 68 families have been studied so far (**Araujo *et al*., 2014**). An interestingly important feature of spider chromosomes is the prevalence of multiple sex chromosome polymorphisms (**Araujo *et al*., 2011**). Besides multiple sex chromosomes, spiders also include a sex chromosome pair (SCP) in which the chromosome lacks morphological differentiation (**Kral *et al*., 2011**). The prevailing SCDS was type X; but the X_1X_2 and X_1X_2Y types were also recorded for some species. The chromosome morphology was predominantly metacentric (**Bole-Gowda, 1958; Srivastava and Shukla, 1986; Sharma and Parida, 1987; Král *et al*., 2006; Araujo *et al*., 2005**).

The Family Pholcidae form a most diverse family containing about 1,241 species within 81genera projected to be of worldwide geographical dispersal (**Platnick *et al*., 2014**). Of the total number of pholcids only 19 species have been karyotypically described. Pholcid chromosomes offer as an attractive source of genetic material for cytogenetic research since karyological information gathered thus far came from only few species from Indian fauna (**Bole-Gowda. 1958; Srivastava and Shukla 1986; Parida and Sharma 1987; Sharma and Parida 1987**). Karyosystematics of pholcids reveal exhibiting a range of basal chromosome numbers for the group (2n=15 to 2n=32) (**Araujo *et al*., 2005**).The impressive part of these analyses of Pholcids has driven to an understanding that maximum spider karyotypes appeared to reflect in possession of metacentric chromosomes in the complements (**Kral *et al*., 2006; Oliveira *et al*., 2007**). An increased representation of cytogenetic data and analysis of several clades of Pholcidae, would contribute to the establishment of relations between the karyotypes found within the family.

The present study aims to characterize the chromosomes of two species of Pholcidae, *viz., Crossopriza lyoni* and *Artema atlanta* based on the karyotype, C-banding and NOR- banding profiles.

MATERIALS AND METHODS

The specimens of *C. lyoni* and *A. atlanta* were collected in natural populations from Bangalore University, Jnanabharathi campus, Bengaluru, Karnataka, India. The collected specimens were identified following the keys of Sebastian and Peter (2009). The voucher specimens preserved in 70% ethanol are deposited in the Museum of Department of Zoology, Bangalore University, Bengaluru,

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Karnataka, India. Conventional air-drying technique (**Chowdaiah and Venkatachalaiah, 1987**) with appropriate modifications was adopted for the preparation of mitotic (from gut epithelium) chromosomes for the present studies. Diluted Giemsa solution (3%) was used for conventional staining of the chromosomal preparations. Mitotic chromosomes were subjected to C- banding (**Sumner, 1972**) and NOR staining (**Howell and Black, 1980**) with minor modifications. The somatic metaphase chromosomes in the preparation of karyotype were arranged essentially based on aligning them in the decreasing order of their total length and in tow with **Levan *et al.* (1964)** principles. Chromosome preparations were observed using Zeiss Axioskop 2 plus Microscope and well spread complements were photographed.

RESULTS

Mitotic metaphase complement and karytotype of *Crossopriza lyoni*

In the karyotype of *C. lyoni* (male) (Figure 1a), all the chromosomes are metacentric and the largest chromosome was considered as the sex chromosome. The autosomal chromosomes are arranged according to the descending order of their lengths. The somatic metaphase complement of *C. lyoni* consists of 2n♂=23(22+X) (Figure 2a).

C-banding

C-banded preparations revealed discrete C-heterochromatin at the paracentromeric region of all the chromosomes in the complement. The C-staining pattern (Figure 2b) indicates that constitutive heterochromatin is not only confined to the centromeric regions but also occur in traces at telomeric regions of some chromosomal pairs.

Silver nitrate impregnation

Somatic chromosomal preparations following NOR staining revealed silver impregnation at five telomeric regions occupying the short arms the chromosomal elements of pair 4, the long arm of the chromosome elements of pair 6, and one of the arms of the metacentric X chromosome in the complement (Figure 2c). Interphase nuclei often exhibit a minimum of one and a maximum of four NOR spots (Figure 2d).

Mitotic metaphase complement and karytotype of *Artema Atlanta*

The analysis of mitotic metaphases of *A. atlanta* (male) revealed the karyotype with no apparent chromosomal size classes. Based on the chromosome, morphology the elements of the karyotype were arranged in pairs in the order of decreasing size (Figure 1b).

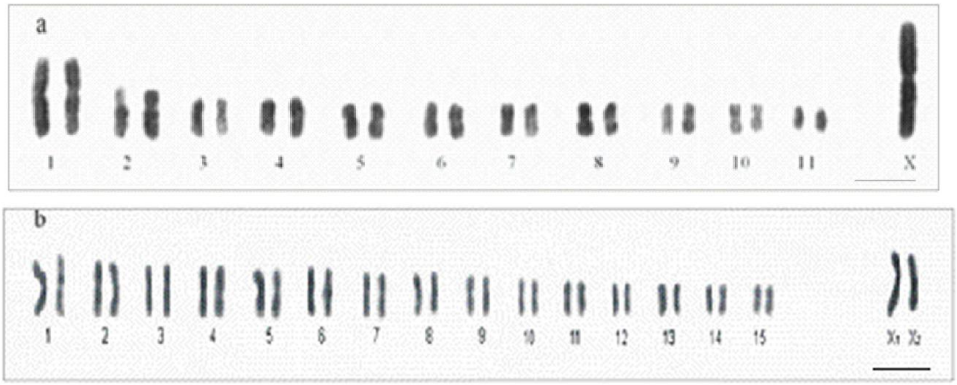

Fig. 1. Karyotypes of *Crossopriza lyoni* (a. 2n=23=22AA+X♂) and *Artema atlanta* (b. 2n=32=30AA+X_1X_2♂) species standard stained with Giemsa

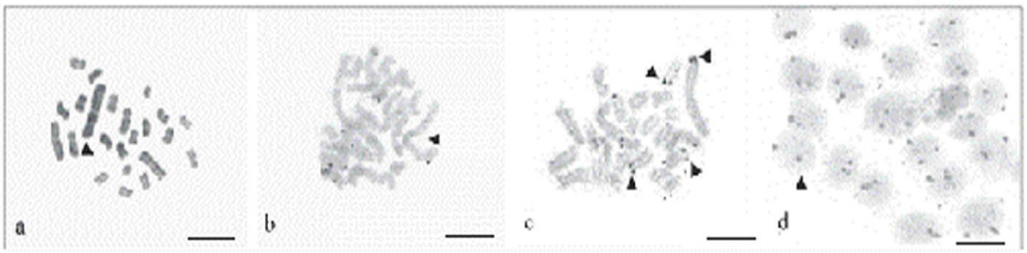

Fig. 2. *Crossopriza lyoni* (♂) - a. Somatic metaphase complement; b. C-stained somatic metaphase complement; c. Mitotic metaphase revealing NOR on 2 pairs of chromosomes and on sex chromosome; c. Interphase cells revealing NOR spots

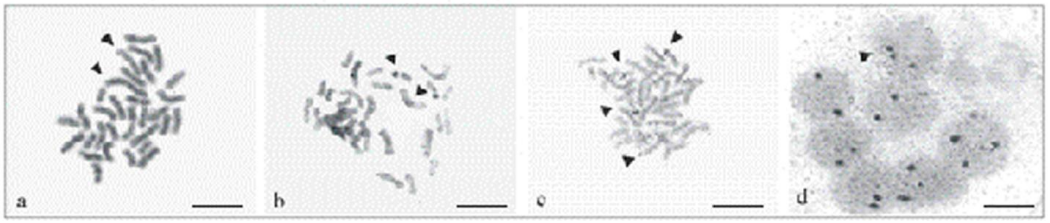

Fig. 3. *Artema atlanta* (♂); a Somatic metaphase complement; b. C-stained somatic metaphase complement; c. Mitotic metaphase revealing NOR on 2 pairs of chromosomes; f. Interphase cells revealing NOR spots. Scale 10µm

In the complement sex chromosomes were certainly identified as the largest element of the karyotype. The somatic metaphase complement of *A. atlanta* consists of 2n=32 (30+X_1X_2) (Figure 3a).

C-Banding

C-banded analysis revealed discrete C-heterochromatic regions localized at the centromeric region of all the autosomal chromosome elements in the complement. The C-staining pattern (Figure 3b) of the karyotype indicates that constitutive heterochromatin is confined heavily at the telomeric regions of the sex chromosomes.

Silver nitrate impregnation

Somatic chromosomal preparations following NOR staining revealed silver impregnation at their telomeric regions upon short arms of the two pairs of theautosomal chromosome elements (Figure 3c). Interphase nuclei often exhibit a minimum of one and a maximum of four NOR spots (Figure 3d).

DISCUSSION

Karyological information procured in respect of spiders belonging to Indian spiders belonging to the family Pholcidae is highly limited and fragmentary (**Bole-Gowda, 1958; Mittal, 1966; Parida and Sharma, 1987; Sharma and Parida, 1987; Datta and Chatterjee, 1992**). **Bole-Gowda (1958)** presented the first karyot ype for an Indian Pholcid example, *viz., C. lyoni* depicting the diploid chromosome number 2n♂=27(26AA+X). Subsequently, sporadic reports were made available based on other taxa (**Cokendolpher, 1989; Araujo *et al*., 2005; Král *et al*., 2006**). These results seem projecting a supplementary data towards the demonstration and maintaining of the chromosome number ranging from 2n=15 to 2n=32 that include variable sex chromosome composition in the species belonging to Pholcidae.

In the current study, the chromosomal data obtained for *C. lyoni* and *A. atlanta* are in corroboration with the previous reports (**Parida and Sharma, 1987**) but differs with those described by **Bole-Gowda (1958).** The karyotypes of *Agelena limbata*, *Delena Cancerides* and *Evarcha hoyi* from different populations were characterized as chromosomal races due to the centric and tandem fusion of allosomes and autosomes (**Maddison, 1982; Rowell, 1990; Tsurusaki *et al*., 1993**). *C. lyoni* also characterizes chromosomal races/ cytotypes. The XX system in *C. lyoni*, Oriental population with 2n♂=23 (**Sharma *et al*., 1959**) was derived from X_1X_2Y by steady heterochromatinization and complete degeneration of Y chromosome.

The results for *A. atlanta* was found to be 2n♂=32 (**Sharma and Parida, 1987; Parida and Sharma, 1987a**). The meta/submetacentric morphology of the chromosomes found in *A. atlanta* agree with that of the chromsomes of majority of the pholcids species karyotyped thus far, except *Pholcus crypticolens* (**Suzuki, 1954**) and *Pholcus manueli* (**Xiuzhen *et al.*, 1997a**) which exhibit acrocentric chromosome morphology. Determination of the process of karyotypic differentiation among the populations of *C.lyoni* is incomplete because of lack of substantial chromosome characterization data for the species. Additional chromosome information will provide a better understanding of the basic karyotype characteristics in the genus as well as the mechanisms of origin of the chromosomal races of *C.lyoni.* The karyotype diversity of *C.lyoni* species is due to the wide geographic distribution.

The possibility of modification of X_1X_2Y system into X system in some of the haplogyne spiders indicated that the population with 2n♂=23 could be the ancestral (**Král *et al*., 2006**). The species with biarmed and lower chromosome numbers would be the derivatives from centric fusions followed by pericentric inversions or by tandem fusions. The karyotypic differentiation of extant species may occur by the increase or decrease in the basic chromosome number. In general the constitutive heterochromatic regions are confined to the centromeric regions or at the telomeric regions in some of the chromosomes and in some cases at the intercalary regions of the chromosomes. The present results on the mitotic metaphase shows localization of the C-heterochromatin at the centromeric regions (Figure 2b) in all the chromosomes in *C.lyoni*, while inconsistently also at the telomeric regions of some of the chromosomes and sex chromosomes in *A. atlanta* (Figure 3b). C-banding staining of the chromosomes of *C.lyoni* enabled in characterizing the constitutive heterochromatic localization in the centromeric regions that may include paracentric regions for extrapolation. The presence of the C-heterochromatin at the telomeric regions indicate the role of centric fragmentation during the process of chromosome evolution in spiders (**Kral *et al*., 2006**).

NOR localization over particular set of autosomal chromosomes in the complement was found to serve as a cytological diagnostic tool. It is evident in *C. lyoni* (Figure 2c, 2d) and *A. atlanta* (Figure 3c, 3d) of the haplogyne group of spiders. It was interesting to note that those of the haplogyne spiders examined exhibited the NOR specificities over the sex chromosomal counterparts (Figure 3c) (**Araujo *et al*., 2005; Král *et al*., 2006**).

The presence of the evident NORs in the interphase in both the species suggests that the nucleolar cistrons are active in the early phase of the cell division and they tend to switch off as the divisional stage progresses. The lack of NOR specificities on the sex chromosomes of *A. atlanta*, is possibly due to chromosome rearrangements or differential activation of this region. It is not yet possible at the moment to determine a quantitative pattern of active NORs in haplogyne spiders due to the nominal number of species whose chromosomes are subjected to Silver nitrate impregnation.

Conclusion

The cytogenetic analysis of the two species of spiders belonging to Pholcidae seems to be extremely important to establish the karyotypic evolutionary trend in haplogynes. Studies on chromosome banding specificities would provide a better relevation upon the present understanding of the chromosome characteristics and karyotype evolution in pholcids (Haplogynae).

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