Karyological studies on Uruguayan spiders II. Sex chromosomes in spiders of the genus Lycosa (Araneae-Lycosidae)

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Abstract

Different sex-determining mechanisms (♂: XO; X_1X_2O; $X_1X_2X_3O$) were studied in species of the Lycosidae family, named for the purpose of description as follows: *Lycosa* sp.$_1$ (*malitiosa* group), *Lycosa thorelli* Keyserling, *Lycosa* sp.$_2$ (*thorelli* group) and *Lycosa* sp.$_3$. While *L.* sp.$_1$ (*malitiosa* group) shows a single metacentric sex-chromosome, *L. thorelli* and *L.* sp.$_3$ present a multiple system (X_1X_2O). A new system ($X_1X_2X_3O$) was found in *L.* sp.$_2$ (*thorelli* group) (coexisting with *L. thorelli* in the same habitat). Its particular behaviour during meiotic prophase is discussed. The sex-chromosome lengths were compared in the statistical analysis. An anova test with two unequal classes (Group A = sex-chromosomes from X_1X_2O species; Group B = sex-chromosomes from $X_1X_2X_3O$ species) and the additional component variance were used for quantitative comparisons. A non-disjunction phenomenon in the second male meiosis is postulated as the origin of specimens with an $X_1X_2X_3O$ sex-determining mechanism.

Introduction

Up to date 240 species of spiders, belonging to the group Arachneida have been studied cytologically by many researchers (Wallace, 1909; Revell, 1947; Patau, 1948; Bole-Gowda, 1950, 1952, 1958; Suzuki & Okada, 1950; Suzuki, 1954). All of them – except for 38 species of the suborder *Arachnomorphae* – have a multiple X_1X_2O mechanism in the male and an $X_1X_1X_2X_2$ in the female. Of the 38 species, 26 possess a single X in the male and the remaining 12 species present a multiple sex mechanism of the type $X_1X_2X_3O$ (White, 1973, 1978).

Early studies on the chromosomes of *Lycosidae* spiders (Montgomery, 1905; Painter, 1914; Hard, 1939; Hackman, 1948; Suzuki, 1954; Mittal, 1963) demonstrated the presence of an X_1X_2O sex-determining mechanism in the male of this family. More recently, Diaz & Saez (1965, 1966) and Brum-Zorrilla & Cazenave (1974), found the X_1X_2O (male) system as the most frequent in Uruguayan *Lycosidae* species; *Lycosa nordenskjöldii*, which has a simple XO system, is an exception within this group (Diaz & Saez, 1965). The variation of the sex chromosome found by cytological observation of *Lycosa* sp.$_1$ (*malitiosa* group); *Lycosa thorelli* Keyserling; *Lycosa* sp.$_2$ (*thorelli* group) and *Lycosa* sp.$_3$, was analysed by statistical tests permitting formulation of a hypothesis of sex chromosome evolution in these spiders.

Material and methods

Specimens

Four species, arranged in two systematic groups of spiders were studied.

- The *malitiosa* group. Within this group the species studied are named for the purpose of description as *Lycosa* sp.$_1$.
- The *thorelli* group. Within this group *Lycosa*

Genetica 56, 47–53 (1981). 0016-6707/81/0561-0047/$1.40.

thorelli Keyserling and *Lycosa* sp.$_2$ were studied. In addition, another species of the genus *Lycosa,* not included within the preceding groups was studied and will be called *Lycosa* sp.$_3$. This species showed a black abdomen and would presumably be related to *Lycosa poliostoma* (Capocasale, personal communication).

The source and number of specimens of *L.* sp.$_1$ (*malitiosa* group) *L. thorelli* Keyserling, *L.* sp.$_2$ (*thorelli* group), and *L.* sp.$_3$ are shown in Table 1. The sex chromosome nomenclature proposed by White (1973) was used in the table. We wish to point out that the specimens of *L.* sp.$_2$ (*thorelli* group) were found in the same population as those of *L. thorelli.*

Tissues

Adult male specimens of the four different species were employed. Testicular tubules were treated according to the methods used in a previous paper (Brum-Zorrilla & Postiglioni, 1980).

Statistical analysis

Eighteen sexchromosomes (6 cells) from *L.* sp.$_2$ (*thorelli* group, $X_1X_2X_3O$) and 30 sexchromosomes (15 cells) from *L. thorelli* and *L.* sp.$_3$ (X_1X_2O) were selected for the statistical analysis.

In all cases the sex-chromosomes were measured in diakinetic cells using the differentiating characteristics observed during this stage: X_1X_2 sex-chromosomes presented a parallel disposition and a positive heteropycnosis. The absence of chiasmata was taken into account for $X_1X_2X_3$ sex-chromosome identification.

As no morphological differences were observed among the sex-chromosomes of the three studied species, and banding techniques were not successful in this material, for our quantitative comparisons we used their lengths. Sex-chromosome total lengths were measured on enlarged prints (×7500) by means of a planimeter (accuracy: 0.1 μm).

For the statistical analysis, each X chromosome was independently measured and they were divided into two groups: (A) the X chromosomes from species with X_1X_2O sex mechanism (*L. thorelli* and *L.* sp.$_3$) and (B) those from *L.* sp.$_2$ (*thorelli* group, $X_1X_2X_3O$).

The anova test with two classes for unequal samples and the additional variance component were applied as comparison methods.

Results

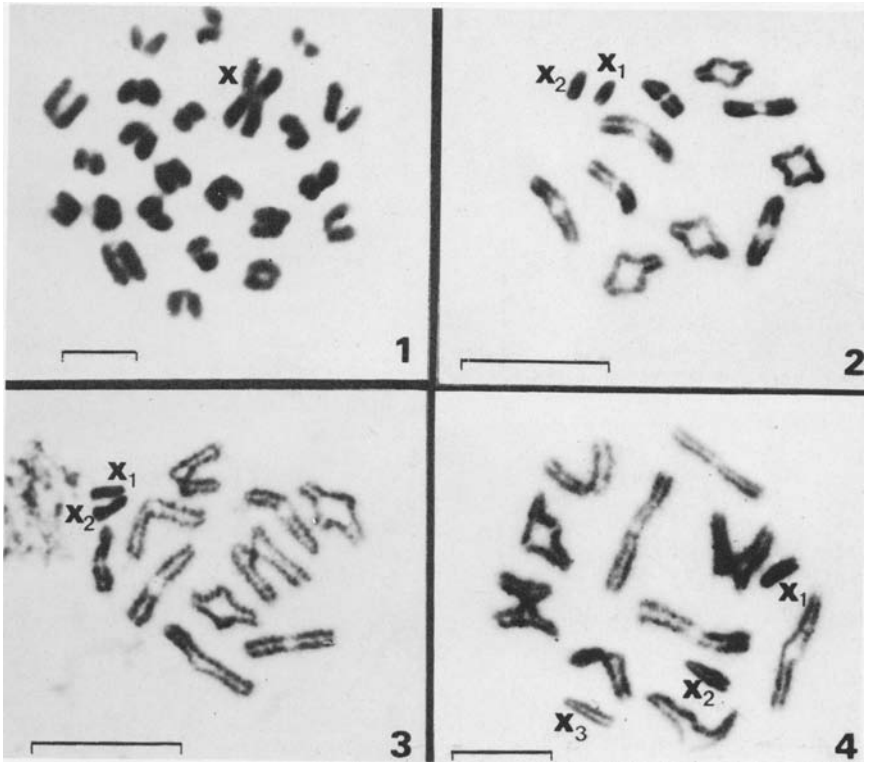
Cytological observations

Diploid chromosome numbers of *L.* sp.$_1$ (*malitiosa* group), *L. thorelli, L.* sp.$_2$ (*thorelli* group) and *L.* sp.$_3$ are presented in Table 1. The chromosomes of the four mentioned spider species are telocentric (autosomes and X chromosomes), except for the single sex chromosome of *L.* sp.$_1$ (*malitiosa* group) which is a metacentric element (Fig. 1). Terminal and interstitial chiasmata were observed only at late diakinesis of the studied species (Figs 2, 3, 4). In *L. thorelli* and *L.* sp.$_3$ the sex chromosomes (X_1X_2) are easily recognized during the meiotic prophase by their positive heteropycnosis (Figs 2, 3).

In the case of *L.* sp.$_2$ (*thorelli* group) the sex chromosomes ($X_1X_2X_3$) showed a similar degree of condensation to the autosome bivalents (Fig. 4),

Table 1. Source and chromosome number of specimens collected.

Species	Locality	Number of specimens	Diploid number
Lycosa sp.$_1$ (*malitiosa* group)	Canelones (Uruguay)	3	22 + XO
Lycosa thorelli Keyserling	Montevideo (Uruguay)	15	20 + X_1X_2O
Lycosa sp.$_2$ (*thorelli* group)	Montevideo (Uruguay)	6	20 + $X_1X_2X_3O$
Lycosa sp.$_3$	Montevideo (Uruguay)	18	20 + X_1X_2O



Figs 1–4. Sex chromosomes of *Lycosa* species: (1) Spermatogonial metaphase of *Lycosa* sp.$_1$ (*malitiosa* group);– (2-4) Diakinesis: (2) *L. thorelli*, (3) *L.* sp.$_3$, (4) *L.* sp.$_2$ (*thorelli* group). Bars represent 10 μm.

but could be recognized by the absence of chiasmata. The sex chromosomes $X_1X_2X_3$ show a peculiar behaviour during the meiotic prophase. At early prophase (Fig. 5) the three elements are associated end-to-end in a ring-like configuration. One of them is more eu-pycnotic and shows a low degree of condensation. As meiosis advances, repulsion takes place between them (Fig. 6) and one of the three members begins to separate while the other two remain associated at zygonema (Fig. 7). At pachynema the isolated sex chromosome reaches condensation in advance of the others (Fig. 8) and the three elements always appear independently located among the autosomes at diakinesis (Fig. 4).

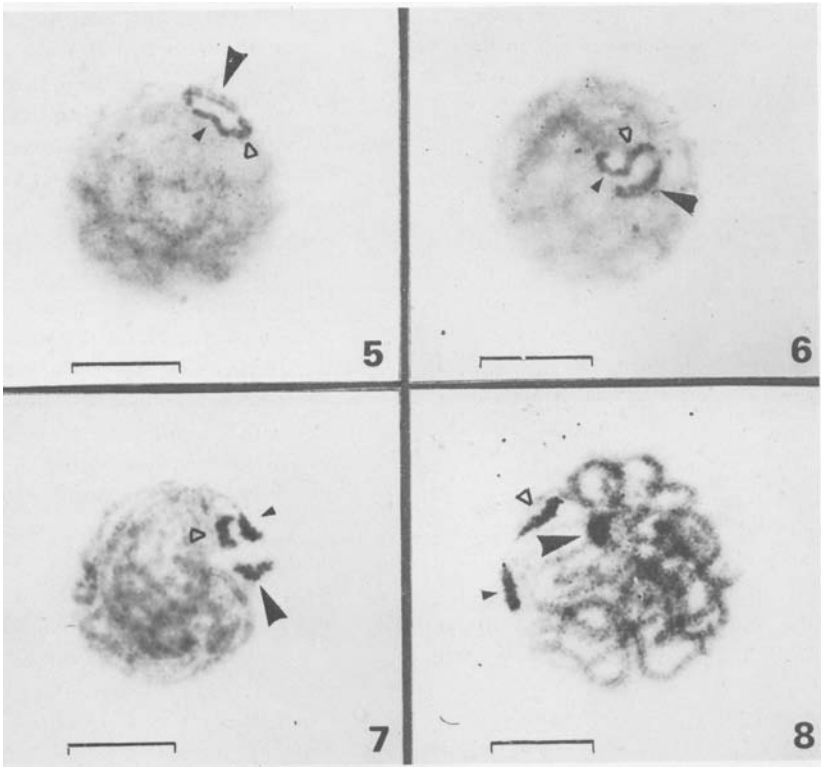
Statistical analysis

As both species – *L. thorelli* and *L.* sp.$_3$ – present similar sex-determining systems they were considered together for the statistical analysis. The result of the anova test applied to the comparison between this group (A) and the X chromosomes from *L.* sp.$_3$ (*thorelli* group) (group B) showed a high F value (9.52). Therefore the null hypothesis must be rejected ($p < 0.005$) (Table 2).

The intergroup variation represented 27.47% of the total observed variance. On the contrary, the variation found within each group corresponded to 72.53%.

Discussion

Multiple sex chromosomes $X_1X_2X_3$ have been studied in a total of eight species belonging to the family *Sparassidae* and *Angelenidae* by several authors (Sokolska, 1925; Revell, 1947; Hackman, 1948; Suzuki & Okada, 1950; Bole-Gowda, 1952). This mechanism is considered to be the less frequent among those occurring in spiders. The present research demonstrated, for the first time, its occurrence in the family *Lycosidae*. Suzuki (1950) found this sex determining mechanism in *Heteropoda venatoria* (Sparassidae, 19^{II} +



Figs 5–8. Early prophase of *Lycosa* sp.$_2$ (*thorelli* group): (5) Leptonema; – (6) middle leptonema; – (7) zygonema; – (8) pachynema. Arrows indicate sex-chromosomes (see discussion for sex-chromosome identifications). Bars represent 10 μm.

Table 2. Analysis of variance for two unequal classes (X_1X_2, $X_1X_2X_3$).

Source of variation	Degrees of freedom	Sum of squares	Mean of squares	F
Between samples	1	223.336	223.336	9.52
Within samples	46	1079.185	23.4605	
Total	47	1302.521		

$F_{.005(1,46)} = 4.08$
$n_A = 30$
$n_B = 18$

$X_1X_2X_3O$) but its behaviour during meiotic prophase clearly differs from *L.* sp.$_2$ (*thorelli group*) (*Lycosidae*, $10^{II} + X_1X_2X_3O$). While in the species studied by Suzuki only one element of the 3 X's takes a ring-like configuration, in *L.* sp.$_2$ (*thorelli* group) the three sex chromosomes are associated end-to-end to form a ring. The independence of the three X's in our material, shown in diakinesis, was not found by Suzuki (1950) who observed that the longest of the three elements is the one lying apart while the other two maintain a close, parallel, position.

According to our observations the two sex chromosomes existing in Uruguayan *Lycosidae* spiders (X_1X_2 of *L. thorelli* and *L.* sp.$_3$) are highly condensed and adopt a parallel disposition. However, $X_1X_2X_3$ of *L.* sp.$_2$ (*thorelli* group) appear elongated and lie independently among the autosomes during late meiotic prophase. Development studies at the ultrastructural level revealed the presence of a 'Junction Lamina' between X_1X_2 sex-chromosomes of *L. malitiosa* (Benavente & Wettstein, 1977). This would imply that both elements have developed a structural characteristic which keeps them closely associated up to late diplotene. However, small bundles of fibrils among the non-homologous sex chromosomes were observed in one species with three X's (*Tegenaria domestica*, Angelenidae) by Benavente & Wettstein (1978). A correlated study at the optic and ultrastructural level would be necessary for an adequate

comparison of the structure and behaviour of sex chromosomes in our species. Such studies in *L.* sp.$_2$ (*thorelli* group) may help to clarify this point.

Suzuki (1954) tried to explain the possible relationship between the origin and evolution of the three sex-determining mechanisms found up to the present in spiders (XO, X_1X_2O, $X_1X_2X_3O$), on the basis of several hypotheses formulated by other authors (Patau, 1948; Bole-Gowda, 1952). Robertsonian rearrangements (Robertson, 1916) – either centric fusion or reciprocal translocation – and also inversions of metacentric or acrocentric sex chromosomes are involved in their hypothesis.

The results obtained by the application of improved cytological techniques in *Lycosidae* spiders showed that telocentric chromosomes predominate over acrocentric ones. On the other hand the morphological uniformity found in the autosomes and sex chromosomes could support the concept of the great conservatism and stability of the karyotype in this family (Brum-Zorrilla & Postiglioni, 1980).

The karyotypic ortho-selection found in spiders (White, 1973, 1975) leads us to suggest that fusion/fission rearrangements, or duplication with subsequent loss of homology (White, 1973) rather than inversions would be the cause of sex chromosome evolution in these spiders. White (1978) emphasized that centric or tandem fusions between X_1X_2 sex chromosomes have rarely occurred to form a metacentric sex chromosome, therefore a strong barrier must exist to the establishment of such rearrangements. It would imply that the X_1X_2 sex chromosome mechanism of spiders has been maintained since their Paleozoic origins.

According to the information available at present on different sex determining systems in spiders, our results may be interpreted as follows:

(1) The XO system found in the few specimens of *L.* sp. (*malitiosa* group) could have originated by a centric fusion of telocentric X chromosomes or alternatively may have existed as an ancestral system of this type.

(2) The sex chromosomes of *L. thorelli* and *L.* sp.$_3$ turned out to be X_1X_2 in the male. If both facts: terminal position of the centromere and length of sex chromosomes are taken into consideration, either non-disjunction or duplication of a single telocentric X chromosome – both phenomena with a subsequent loss of homology – could be postulated as giving origin to this sexdetermining mechanism. White (1973) suggested duplication as a mechanism of speciation in other groups of spiders. However, the nondisjunction mechanism, which will be discussed later for the origin of the $X_1X_2X_3$ sex-mechanism, could be also postulated as the origin of the X_1X_2 sex-system in spiders. But the origin by a centric fission from species with a metacentric X or the existence of X_1X_2 sex chromosomes as an ancestral mechanism can not be ruled out.

(3) Several hypotheses have been formulated to explain the origin of multiple sex chromosomes $X_1X_2X_3$ (Suzuki, 1954; White, 1973). White (1973) suggested that X_3 would be originated by autoduplication of a small chromosome fragment derived from the X_1 or the X_2 sex chromosome. However, the different chromosomal rearrangements that have been postulated by the authors mentioned, are not based on statistical analysis.

According to the results of our statistical test, the null hypothesis should be rejected. Therefore, both groups of measurements should be considered as different. This hypothesis implies that if a species with 3 X's evolves from an ancestral species with only two sex-chromosomes – taking for granted that these two elements would not suffer changes in their length during the whole process – the observed variation would be the result of the presence of the new sex chromosome. This result would support the hypothesis presented by White (1973). But as shown in Table 2, the value of the mean of squares between samples is considerably higher than the corresponding mean of squares within samples and the existence of an additional component of variance must be taken into consideration. 72.53% of the total observed variance corresponds to the intragroup variance. Such a result leads us to conclude that the F value obtained does not indicate a real difference between both samples.

If the latter hypothesis is correct, minor differences between both ancestral X chromosome lengths would be responsible for the observed results. In this case the non-disjunction mechanism could be postulated as the most probable mechanism in the evolution of the sex-chromosomes of these spiders. Then, two different chromosomal

segregations could be produced. A single failure during the second meiotic division of the male involving either X_1 or X_2 would lead to the formation of three types of gametes: $X_1X_1X_2$, X_2O and OO or alternatively $X_1X_2X_2$, X_1O and OO. Gametes with one X chromosome would be obviously able to form progeny with 3 X's. As previously described, one of the sex chromosomes has a particular behaviour during the meiotic prophase. It shows an earlier condensation and isolation at pachynema, while the remaining elongated chromosomes are still associated by a thin filament.

The X_1X_2O sex chromosome mechanism has been considered as an ancestral form, so both sex chromosomes may have suffered independent mutations during the course of evolution. If one of them was involved in a recent non-disjunction event with subsequent loss of homology originating the few spiders with 3 X's, the affected chromosome and the new one would denote a similar behaviour in their meiotic prophase, while the other ancestral chromosome (either X_1 or X_2), would maintain its individuality. Even though at present we do not have enough information to rule out any of the alternatives presented above, we are inclined to postulate a non-disjunction phenomenon during the second male meiotic division followed by a subsequent loss of homology, as the mechanism which may have produced this particular chromosome rearrangement in the few specimens of spiders with a $X_1X_2X_3O$ sex system observed in the population of *L. thorelli*.

Additional cytological studies of this group of arthropods would help us to confirm the origin of this sex-determining mechanism in other spiders.

Acknowledgements

The authors wish to thank Prof. Roberto M. Capocasale and Prof. Fernando Costa, from the Division of Experimental Zoology of our Institute (IIBCE) for providing the specimens as well as their systematic classification. We are also grateful to Dr. Fernando Nieto from the Centro Latinoamericano de Perinatologia for his statistical guidance, Dr. J. Roberto Sotelo and Dr. Horacio Cardoso from our Institute, for their valuable discussions and critical reading.

This work was supported by 'Programa Regional de Desarrollo Cientifico y Tecnológico de la Organización de Estados Americanos' (OAS) and Ministerio de Educación y Cultura. Montevideo, Uruguay.

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Received 30.11.1979 Accepted 15.11.1980