

Differential distribution of
constitutive heterochromatin in two species
of brown spider: *Loxosceles intermedia* and *L. laeta*
(Aranae, Sicariidae), from the metropolitan region
of Curitiba, PR (Brazil)

Distribuição diferencial
da heterocromatina constitutiva em duas espécies
da aranha marrom: *Loxosceles intermedia* e *L. laeta*
(Araneae, Sicariidae) da região metropolitana de
Curitiba, PR (Brasil)

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Spiders of the genus *Loxosceles* range about 15 mm in body length and have long and thin legs (FISCHER, 1996). Exhibit nocturnal and sedentary habits and inhabit quiet places, such as tiles, brick holes, wall cracks, underneath barks, and caves. Under favorable conditions, they can also be found frequently in homes, under blankets, clothes, inside shoes, behind wall pictures and in other similar

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places. The genus *Loxosceles* includes a high number of species found worldwide. In South America, the estimation for number of species varies between 16 (BÜCHERL, 1960) and 30 (GERTSH, 1967). Seven species of *Loxosceles* are found in Brazil: *L. adelaida*, *L. amazonica*, *L. gaúcho*, *L. hisurta*, *L. intermedia* and *L. laeta* (GERTSCH, 1967). The last two species are common in southern region of the country, especially in Curitiba and its surroundings (MANFREDINI *et al.*, 1993).

Loxosceles species are commonly known as “brown spiders”, due to its color. In the epidemiologic aspect, Curitiba has a wide dissemination of brown spiders, with predominance of *L. intermedia* (MANFREDINI *et al.*, 1993; COUTINHO, 1996).

In this city, the “Loxoscelism” [the term describing lesions and reactions induced by bites of these spiders], represents an increasing problem of public health. Up to 3,000 accidents were notified by the health service institutions along the year of 2002 (VETTORELLO, 2002) (Fig. 1). The great majority of the accidents occurs when the spider is squeezed against the body of the victim.

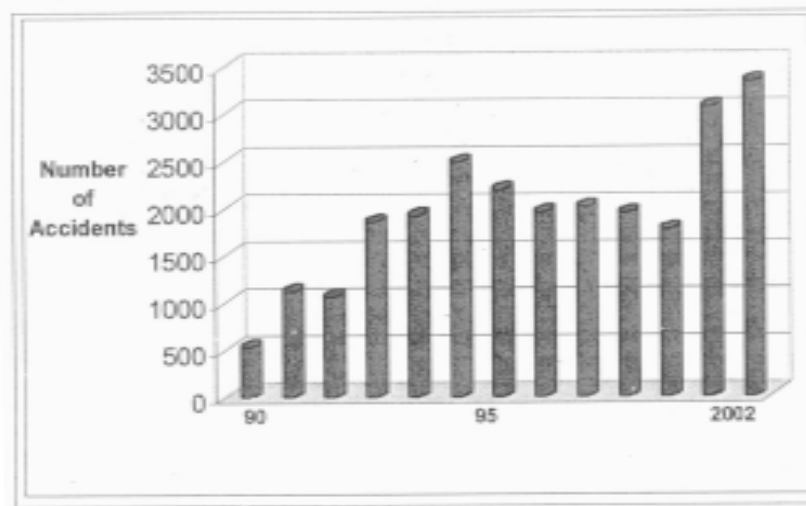


Fig. 1. Epidemic profile of the accidents with *Loxosceles* sp in Curitiba, Paraná – Brazil (1993-2002). [Reference: 1990-1992 — Secretaria de Estado de Saúde-PR (SESA – PR); 1993-2002 — Centro de Epidemiologia-Secretaria Municipal de Saúde (SMS) — Curitiba, PR]

Although these spiders do not show any aggressive behavior, their venom may induce severe disorders on the human body (ROSENFELD *et al.*, 1957; CARDOSO *et al.*, 1990; VEIGA *et al.*, 2001). The venom causes hemorrhage and dermonecrosis at the site of bite (Fig. 2), but other systemic effects also occur such as haemolysis, thrombocytopenia, disseminated intravascular coagulation and renal failure (DENNY *et al.*, 1964; COUTINHO, 1996; VEIGA *et al.*, 1999; MONTEIRO *et al.*, 2002).

In some cases, when the ulcerations produced by brown spider venom are extensive, plastic surgery has been recommended for the compromised dermonecrotic areas (BARBARO *et al.*, 1992).

Cytogenetic studies in spiders of genus *Loxosceles* are rare. In *L. rufipes* and *L. rufescens*, the same diploid number ($2n=20$) was found (BEÇAK & BEÇAK, 1960; DIAS & SAÈZ, 1965, 1966). These two species correspond probably to *L. gaucho* and *L. laeta*, respec-



Fig. 2. Dermonecrotic lesion characteristic of the accident with *Loxosceles* spiders . [Picture gently yielded by Dr^a Marlene Entres.]

tively, in the current nomenclature. In addition, the diploid number of *L. reclusa* was described by TUGMON *et al.* (1990): $2n=18$, X_1X_2 in males and $2n=20$, $X_1X_1X_2X_2$ in females. A difference between the diploid number of males and females was also found in *L. laeta* from Lima (Peru), which showed $2n=23$ in males and $2n=24$ in females (SILVA, 1988). The same karyotypes were observed in *L. laeta* occurring in São Paulo (Brazil) (OLIVEIRA, 1998), with a multiple chromosome sex determination system of the type $X_1X_2Y/X_1X_1X_2X_2$. *L. gauch*o showed a similar karyotype, with the same sex determination system (OLIVEIRA *et al.*, 1996; OLIVEIRA, 1998).

In this study, the karyotypes of *L. intermedia* and *L. laeta*, through conventional and C banding techniques, in mitotic and meiotic chromosomes, are analyzed in order to contribute to a better understanding of the chromosomal diversity and reproductive biology of this group.

MATERIAL AND METHODS

Adult individuals of *L. intermedia* and *L. laeta* were collected in houses in Curitiba (PR, Brazil) or provided by the *Laboratório Interdisciplinar de Pesquisa em Animais Peçonhentos* (LIPAPE) (Setor de Ciências Biológicas, UFPR, Curitiba, PR). Cytological preparations were obtained from testis of adult males (meiotic and pre-meiotic cells) and from embryos (mitotic cells), according to WEBB *et al.* (1978). Chromosomes were stained with Giemsa for analysis of diploid number and chromosomal morphology. C-banding was carried out according to SUMNER (1972). Slides were analyzed, and the best preparations were selected. The photomicrographs were taken in a ZEISS microscope.

RESULTS

Pre-meiotic cells of *Loxosceles intermedia* showed 23 chromosomes in metaphase, with 10 homomorphic pairs and three heteromorphic chromosomes, which corresponded to X_1 , X_2 and Y (Fig. 3). This conclusion was confirmed by the presence of 12 homomorphic pairs in embryonic cells. All the chromosomes of the complement were biarmed, including the Y, which the smallest chromo-

some of the complement and its morphology is submetacentric.

The karyotype of *L. laeta* was very similar to the one described for males of *L. intermedia* (Fig. 4). The analysis of cells in diakinesis-metaphase I in spermatocytes (MI), in both species, revealed the presence of 10 autosomic bivalents and a sexual trivalent (Fig. 5). These findings confirmed the occurrence of a multiple chromosome sex system in both species. The Y chromosome is clearly visible. Figures 5a and 5b show the associations between X_1 , X_2 and Y in *L. intermedia* and *L. laeta*, respectively. Pairing occur through the terminal region of X_1 , X_2 and Y (Fig. 5c).

C banding analysis revealed different patterns in the two species. Hence, while conspicuous blocks of pericentromeric constitutive heterochromatin were observed in only a few pairs of *L. intermedia* chromosomes (Fig. 6a-c), they are found in all the chromosomes of *L. laeta* (Fig. 6b-d). In both species, these blocks are found in X_1 and X_2 , while the Y chromosome is almost totally heterochromatic. Figures 6c and 6d show the C banding patterns in pre-meiotic cells of *L. intermedia* and *L. laeta*, respectively.

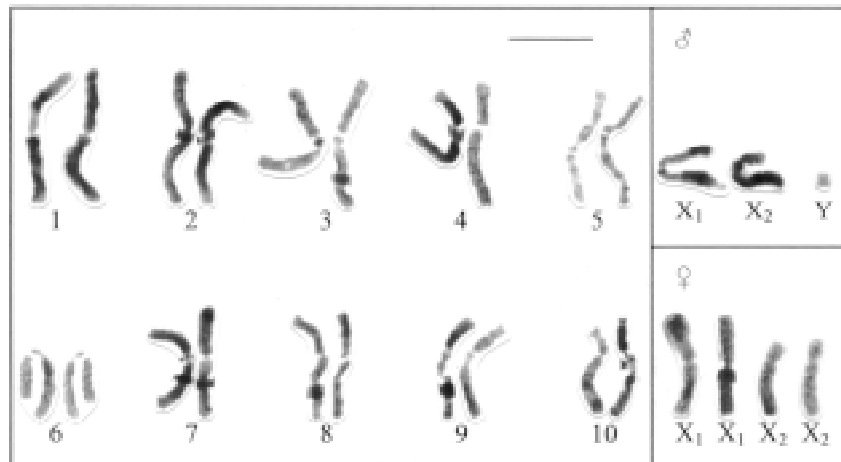


Fig. 3. Karyogram of *L. intermedia* standing out the sex chromosomes X_1X_2Y (male) and $X_1X_1X_2X_2$ (female). [Bar=10 μ m.]

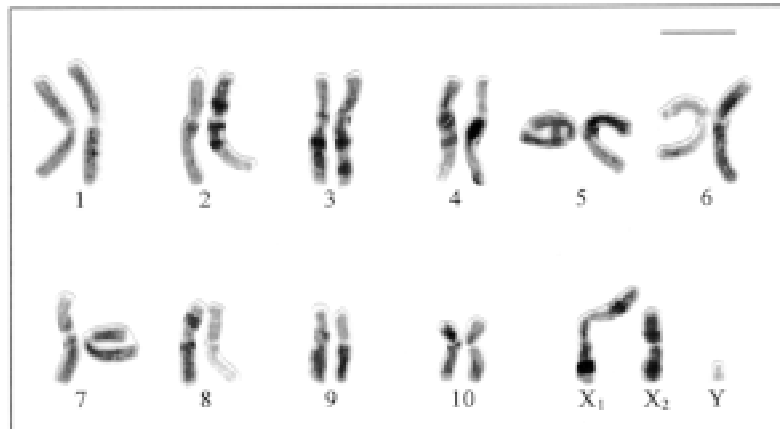


Fig. 4. Karyogram of adult male of *L. laeta*. [Bar = 10 μ m]

DISCUSSION

Comparative cytogenetic analysis between males of *L. laeta* and *L. intermedia*, showed similar karyotypes, composed of biarmed autosomes and three heteromorphic chromosomes, which correspond to X₁, X₂ and Y. In both species, the Y was the smallest chromosome of the complement. On the other hand, 12 pairs of homomorphic chromosomes were observed in embryonic cells of *L. intermedia*, probably corresponding to a female karyotype. Hence, the chromosome sex determination system revealed to be multiple, X₁X₂Y/X₁X₁X₂X₂/in both species.

Our data confirm previous studies in *L. intermedia* and *L. laeta* (SILVA, 1988, OLIVEIRA, 1998). However, these studies only inferred the female karyotype. Hence, this study describes the karyotype of females of *L. intermedia* for the first time.

The karyotypes of the two species analyzed were very similar to the one of *L. gaucho*, concerning the diploid number and the sex chromosome determination system. However, *L. gaucho* had two pairs of acrocentric chromosomes (OLIVEIRA *et al.*, 1996, OLIVEIRA, 1998). Probably, this difference is the result of pericentric inversions.

From around 38,000 species of spiders (order Aranae) described

so far, only 1 % have been analyzed cytogenetically, with chromosomal morphology, diploid number and sex chromosome determination systems described (OLIVEIRA, 1998). In the majority of these karyotypes (around 72 %), the sex chromosome determination system found corresponds to the type $X_1X_1X_2X_2/X_1X_2Y$. Variations found in other systems occur due to a variation in the number of X chromosomes, which can evolve as products of translocation events

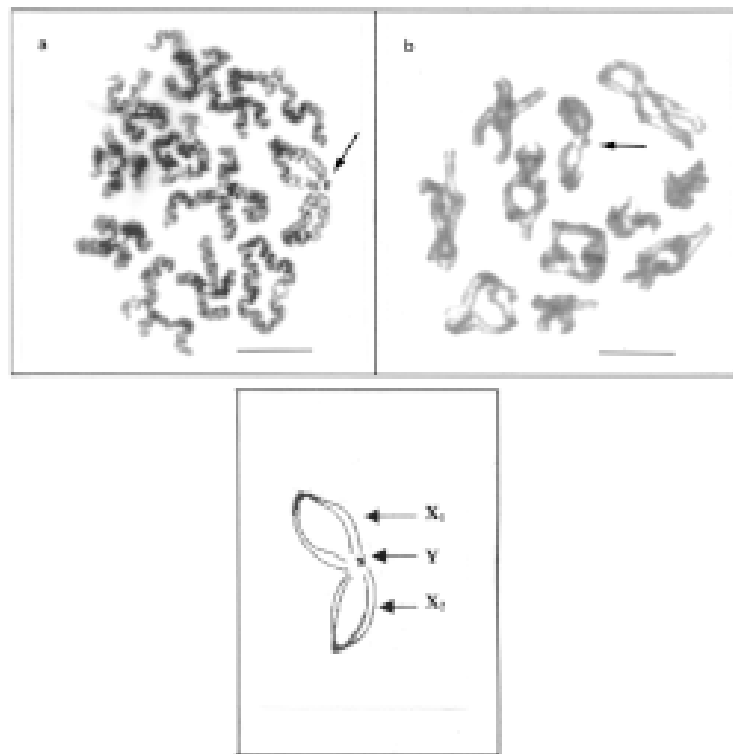


Fig. 5. Primary spermatocyte at diakinesis-metaphase-I: a) *L. intermedia*; b) *L. laeta* and c) Schematic diagram of sex trivalent. Arrow shows the sex trivalents in both species. [Bar=10 μ m]

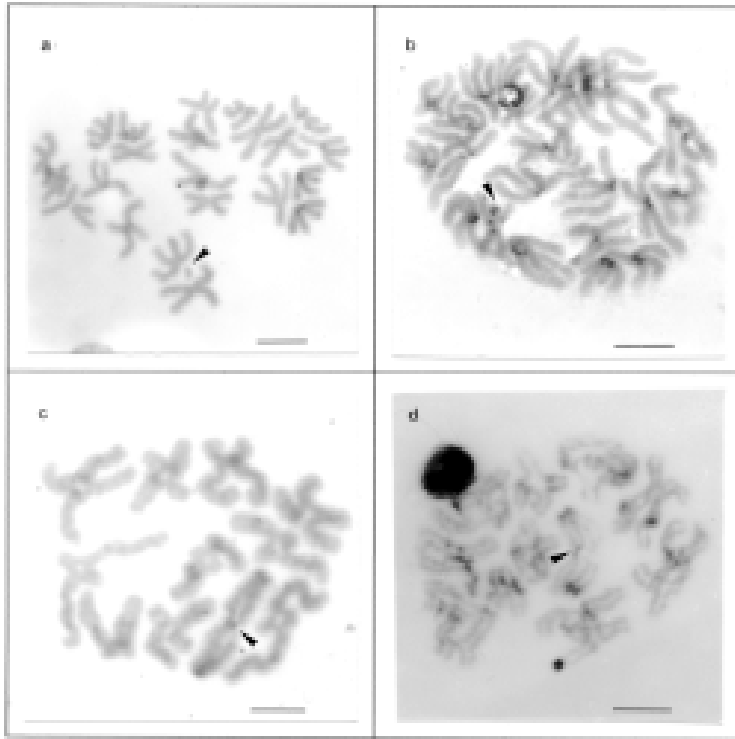


Fig. 6. C- banding: a) metaphase of *L. intermedia*; b) metaphase of *L. laeta*; c) diakinesis-metaphase-I of *L. intermedia* and d) diakinesis- metaphase-I of *L. laeta*. Arrow shows the Y chromosome. [Bar=10 μ m]

between the X and autosomes. Moreover, the number of X chromosomes in males corresponds to half of the number found in females. In the karyotypes of *Loxosceles* species (which correspond to less than 1 % of the karyotypes analyzed so far), males presenting a Y chromosome are described in three of the four species analyzed: *L. intermedia*, *L. laeta* and *L. gaucho*, while *L. reclusa* with X_1X_2 in males and $X_1X_1X_2X_2$ in females (OLIVEIRA, 1998). These data suggest that the system $X_1X_2Y/X_1X_1X_2X_2$ derived from a sys-

tem $X_1X_2/X_1X_1X_2X_2$, found in most of the analyzed species of spiders, is probably due to the mechanisms involving the original X and autosomes, such as translocations.

This study presents, for the first time, the results of C-banding technique in spider chromosomes. The patterns of distribution of constitutive heterochromatin found in *L. intermedia* and *L. laeta* were clearly different, being an important cytological difference distinguishing them karyotypically. In both species, the Y chromosome is conspicuously marked. The submetacentric morphology and small size of this chromosome suggest a common evolutionary history of these two species.

The findings described in this study, concerning important chromosomal characteristics, demonstrate the importance of applying karyological studies in other species of spiders, which can reveal differential characters to improve taxonomic identification of taxons in this group.

SUMMARY

The genus *Loxosceles* includes cosmopolitan small spiders, which have nocturnal and non-aggressive habits. Two species of brown spiders are found in the metropolitan area of Curitiba: *L. intermedia* and *L. laeta*. The first one is more abundant, and responsible for many accidents (Loxoscelism). The aim of this study is to analyze cytogenetically these spiders, through conventional (Giemsa) and c-banding techniques in pre-meiotic, meiotic and embryonic cells. The specimens were collected in houses or kindly provided by *Laboratório Interdisciplinar de Pesquisa em Animais Peçonhentos* (LIPAPE). Cytological preparations were obtained from embryonic cells or from testis of adult specimens. The data showed that both species are karyotypically similar, and had different diploid numbers in males and females ($2n=23$ and $2n=24$, respectively). This difference is the result of a multiple sex chromosome system ($X_1X_2Y/X_1X_1X_2X_2$). The use of c-banding technique revealed that a few chromosomes of *L. intermedia* showed pericentromeric blocks of

constitutive heterochromatin, including gonosomes X_1 and X_2 . On the other hand, all the chromosomes of *L. laeta* showed conspicuous pericentromeric C-positive segments. In both species, the Y chromosome revealed to be almost totally heterochromatic. This study described the result of the use of c-banding technique for the first time in *Loxosceles*, which revealed an important karyotypical distinction between *L. intermedia* and *L. laeta*.

KEY WORDS: *Loxosceles*, brown spider, karyotype, c-banding.

RÉSUMÉ

Les araignées du genre *Loxosceles* sont petites, présentent une distribution cosmopolite, ont des habits nocturnes et ne sont pas agressives. Dans la région métropolitaine de Curitiba, on y trouve deux espèces de l'araignée marron: *L. intermedia* et *L. laeta*. La première c'est la plus abondante et responsable, dans les dernières années par des nombreux accidents connus sous le nom de loxoscelisme. Le but de cet article est celui d'étudier la cytogénétique de ces araignées, par moyen des colorants ordinaires dont le Giemsa et par le technique du bandage C en utilisant des cellules C, des cellules pré-meyotiques, meytiques et embryonnaires. Des échantillons ont été soit obtenues dans des maisons particulières, soit fournies par le *Laboratório Interdisciplinar de Pesquisa em Animais Peçonhentos* (LIPAPE), et les préparations cytologiques obtenues des testicules des adultes ou des cellules embryonnaires. Les résultats montrent que les deux espèces sont similaires du point de vue caryotype et se caractérisent par avoir un numéro diploïde différencié entre les sexes : $2n=23$ dans les mâles et 24 dans les femelles. Cette différence dans le numéro diploïde est due au système chromosomique de détermination sexuelle – multiple du type X_1X_2Y (mâles)/ $X_1X_1X_2X_2$ (femelles). L'utilisation du bandage C a montré que chez *L. intermedia* pas beaucoup de chromosomes présentent la bande pericentrométrique, y compris les sexuels X_1 et X_2 . Par contre, chez *L. laeta*, tous les chromosomes du génome présentent des bandes pericentrométriques visibles. Dans toutes les deux, le chromosome Y s'est montré presque hétérochromatique. Cet type

de bandage, en plus d'inédite chez *Loxosceles*, s'est montré aussi important dans la séparation caryotypique entre *L. intermedia* et *L. laeta*.

MOTS CLÉS: *Loxosceles*, araignée-marron, caryotype, bandage C

RESUMO

As aranhas do gênero *Loxosceles* são pequenas, apresentam distribuição cosmopolita, hábitos noturnos e não agressivos. Na região metropolitana de Curitiba, são encontradas duas espécies de aranha marrom: *L. intermedia* e *L. laeta*. A primeira é a mais abundante e responsável, nos últimos anos, por inúmeros acidentes denominados de Loxoscelismo. O presente trabalho tem como objetivo estudar citogeneticamente estas aranhas, através de técnicas de coloração comum (Giemsa) e de bandeamento C, em células pré-meióticas, meióticas e embrionárias. Exemplares foram coletados em domicílios ou fornecidos pelo Laboratório Interdisciplinar de Pesquisa em Animais Peçonhentos (LIPAPE). As preparações citológicas foram obtidas a partir de testículos de aranhas adultas ou de células embrionárias. Os dados mostram que as duas espécies são cariotipicamente similares e caracterizam-se por apresentar um número diplóide diferenciado entre os sexos: $2n=23$ nos machos e 24 nas fêmeas. Esta diferença é devida ao sistema cromossômico de determinação sexual múltiplo, do tipo X_1X_2Y (machos) e $X_1X_1X_2X_2$ (fêmeas). A aplicação da técnica de bandeamento C revelou que em *L. intermedia* poucos cromossomos apresentam banda pericentromérica, incluindo os sexuais, X_1 e X_2 , ao passo que em *L. laeta*, diferentemente, todos os cromossomos do genoma apresentaram bandas pericentroméricas conspícuas. Em ambas as espécies, o cromossomo Y mostrou-se quase que totalmente heterocromático. Este tipo de bandeamento, além de ser inédito em *Loxosceles*, revelou ser uma importante característica na diferenciação cariotípica entre *L. intermedia* e *L. laeta*.

PALAVRAS CHAVE: *Loxosceles*, aranha-marrom, cariótipo, bandeamento C.

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