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THE KARYOTYPE OF THE GRASSHOPPER *Spathalium helios* REHN 1918. (ORTHOPTERA, ACRIDOIDEA, OMMEXECHIDAE)

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ABSTRACT

The karyotype of the species *Spathalium helios* is described and compared with that of closely related *S. audouini*. The similarity between the chromosomal sex-determining mechanism of the two, suggests a common origin by means of a single X-autosome fusion.

The difference in the centromere position of the Y chromosome reinforces arguments in favor of specific status in *S. helios*, previously questioned because of a great morphological variability in *S. audouini*.

INTRODUCTION

The ommexechids are a small group of neotropical grasshoppers, well characterized by external morphology and male genitalia. Less, than fifty described species are clustered in a dozen genera. Originating in the Andino-Patagonian region, the ommexechids radiated to most of the rest of South America.

Chromosome studies of 15 of 18 species have shown a pericentric inversion in the longest pair of autosomes. The inversion changed the original acrocentric chromosome into a submetacentric one, which according to Mesa (1963) and Mesa and Ferreira (1977) was the result of a single ancestral event that led to all the present ommexechids. At least one species in each of the twelve known genera has been cytologically analyzed by the above authors. The submetacentric position of the centromere reverted independently to the original subterminal in two species, *Clarazella bimaculata* and *Pachyossa signata*. In a third species, *Conometopus sulcaticollis*, the submetacentric chromosome split into two acrocentric (or

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telocentric) chromosomes, raising the chromosome number to 25 in the male and 26 in the female for the first time in Acridoidea.

Ronderos (1972) reduced the genus *Spathalium* from thirteen to three species: *S. audouini* (Blanchard), *S. helios* Rehn and *S. liebermanni* Ronderos. *S. audouini* occurs from the southern part of the Argentinean province of Buenos Aires, to the northern part of the Brazilian State of Rio Grande do Sul (Tainhas, a locality mentioned by Ronderos (1972), is not in the State of São Paulo but in Rio Grande do Sul), and from the central and northern Argentinean provinces to the Atlantic coast. *S. liebermanni* has only been collected in Misiones, Argentina, and *S. helios* was described by Rehn in 1918 from a single female collected in 1911 in Franca (São Paulo, Brazil), the male being unknown.

So far, only *S. audouini* has been studied cytologically. According to Mesa (1963) and Mesa and Ferreira (1977), the species is $2n = 22$ in the male, the longest pair of autosomes being submetacentric and the male sex-determining mechanism of the neo XY type, with both X and Y metacentrics.

In his 1972 revision, Ronderos doubted the validity of Rehn's species *S. helios*, since only a single female specimen was available and because of the great variability of external characters in the closely related species *S. audouini*.

The recent discovery of more specimens (one adult female and one male nymph) of *S. helios* in São Paulo state, gave us the opportunity to compare karyotypes of both species and thus contribute to a better understanding of their taxonomic status.

MATERIAL AND METHODS

The specimens studied were collected in the following localities: *S. audouini*, five (No. 3011, 3025, 3049, 3093 and 3107), from Uruguay, Montevideo, Cerro de Montevideo, 5-III-64; one (No. 2165) from Montevideo, Punta Espinillo, 10-XII-62 and another (No. 3094), collected 5-III-64; one (1564) from Uruguay, Treinta y Tres, Santa Clara de Olimar, 16-I-60; one (1464) from Uruguay, Tacuarembó, Punta Arroyo Laureles, 26-XI-59; one (1411), from Uruguay without precise locality. *S. helios*, a single last instar nymph collected in Brasil, São Paulo, 9 km. W. of Itirapina, 17-X-89 (6001).

Slides from *S. audouini* were prepared by the Feulgen method with 12 min. hydrolysis. The only specimen of *S. helios* was pretreated with 0.05% colchicine during three hours and the testes placed in a hypotonic solution (1% KCl) during three minutes before fixation. The staining was done by squahing the material in a drop of 0.5% lacto-acetic orcein. In both species the fixative was Carnoy I.

C-banding was obtained as follows: colchicine-treated testes were squashed in a drop of 45% acetic acid and the slides dried at room temperature. After aging five days, the slides were washed in 0.1 N HCl for 15 min., and incubated in saturated barium hydroxide solution ($Ba(OH)_2$) for seven seconds, then rinsed in distilled water and air-dried. The slides were then incubated in 2XSSC for 1 hour at 60°C and stained with 3% Giemsa solution.

RESULTS

The chromosome number of *S. helios* is 2n = 22 (ô), as shown in the colchicine-treated spermatogonial metaphase of Figure 1E. The longest pair of bivalents is formed by submetacentric chromosomes, with an arm relation of 3:2. The following four long pairs decrease gradually in length. Three medium-sized and two very small pairs complete the autosomal complement. Except for the first pair, all autosomes are acrocentric, with minute small arms, although only the fourth pair shows them clearly (Figure 1E).

The sex chromosomes are neo XY, with the X metacentric and the Y acrocentric (Figures 1C and 1E). The XR arm presents a secondary constriction in the proximal region (Figure 1E).

During diplotene (Figure 1A), the longest pair shows in general three chiasmata, the following four pairs two chiasmata and the medium and small pairs, a single chiasma. The XL arm is strongly heterochromatic during the first prophase, while XR is euchromatic (Figure 1D). The Y chromosome appears entirely heterochromatic, but not so intensively as the XL arm. A single distal chiasma connects the XR arm with the Y chromosome, the pairing being limited to a short distal segment.

The chromosome number of the closely related species *S. audouini* was mentioned (Mesa, 1963 and Mesa and Ferreira, 1977) but the chromosomes were not shown. Our Figure 1B is from this species and shows a karyotype like *S. helios* but with the Y chromosome metacentric instead of acrocentric.

With C-banding techniques, heterochromatic blocks are detected in the centromeric region of the autosomes, while the sex chromosomes do not show any banding (Figure 1F). Slight telomeric blocks are also observed in the 7th and 8th pairs. The species did not show any response to silver NOR impregnation.

DISCUSSION

The species *S. audouini* and *S. helios* have closely related karyotypes, with 2n = 22 in the males and undoubtely also in the females, though females have not

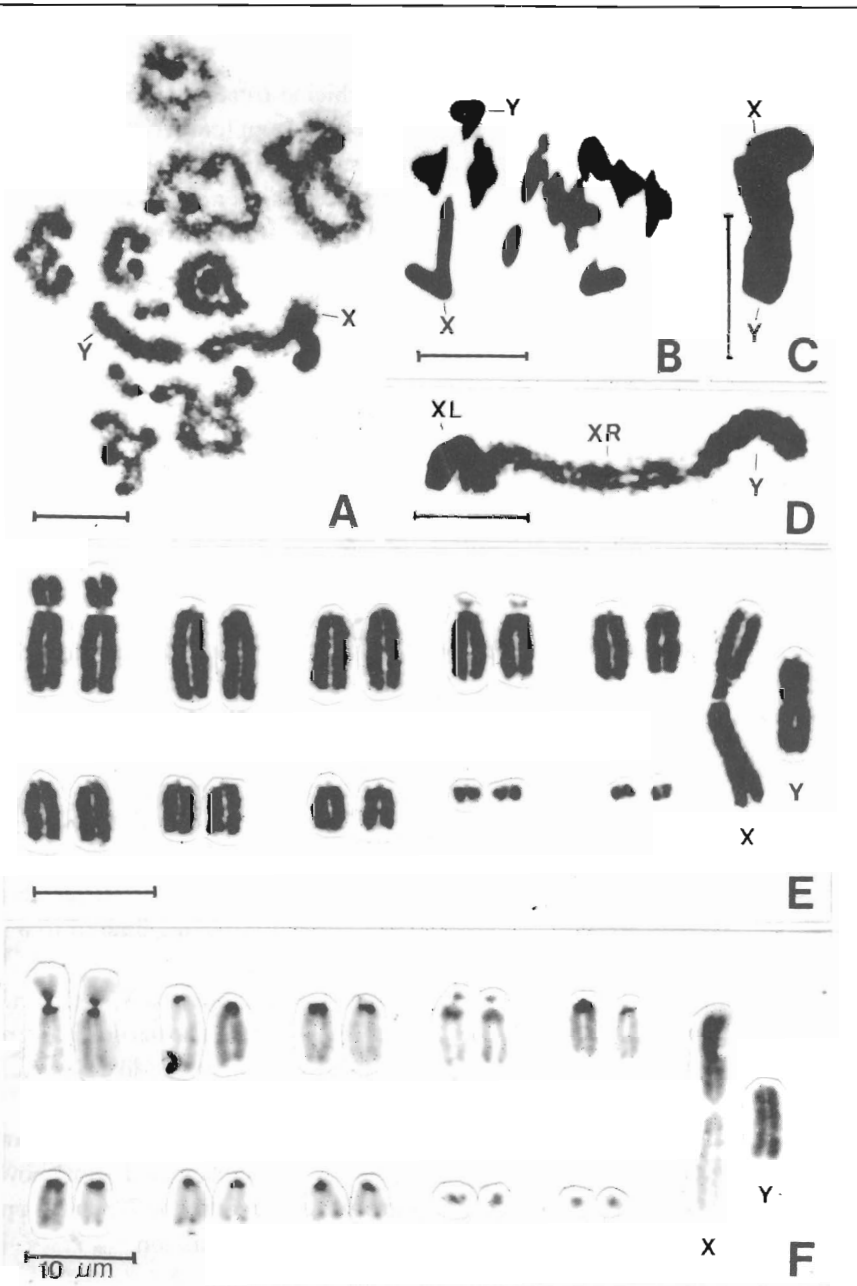

Figure 1 - (A) *S. helios* - diplotene; (B) *S. audouini* - first metaphase; (C) *S. helios* - chromosomes X and Y during MI; (D) *S. helios* chromosomes X and Y during diplotene; (E) *S. helios* - spermatogonial metaphase, pretreated with 0.05% colchicine; (F) *S. helios* - spermatogonial metaphase, showing C-banding. (Scale - 10 μm for all figures).

been studied. The long submetacentric pair of autosomes in the majority of ommexechids are present in both species. The autosomal pairs two to eight decrease gradually in size and pairs nine and ten are very small. Both species also share the neo XY♂-XX♀ sex determining mechanism, but they diverge in the shape of the Y chromosome, which is acrocentric in *S. helios* and metacentric in *S. audouini*. The degree of evolution of the neo-XY system in *S. helios* shows that the X-autosome centric fusion that gave origin to this mechanism is not recent. In fact, while the XR arm of the X remains euchromatic, the Y chromosome is already clearly heterochromatic during first prophase, and also, the XR-Y pairing is restricted to the tip end of those elements.

It is quite probable that the XY mechanism present in *S. audouini* and *S. helios* originated in a single centric fusion that took place in a relatively recent ancestor of both species. The mediocentric position of the centromere in the Y chromosome of *S. audouini* is certainly a derived condition, due to a pericentric inversion in an otherwise acrocentric Y. Whether this inversion has spread to the whole geographical range of the species or has only a restricted occurrence cannot be determined yet, since only specimens from four Uruguayan localities were studied. Furthermore, as the Y chromosome never pairs with itself and pairs with XR only in a minute distal segment, a pattern of geographic mosaicism of different inversions can be expected, due to evolutionary isolation of Y. Further studies from more localities in the species range, could elucidate the point.

For the moment, the presence of the inversion in specimens of *S. audouini* is another character in addition to differences in external morphology mentioned by Ronderos (1972) and reinforces the specific status of both species.

Future studies in the species *S. liebermanni* would help to determine whether it shares the X-autosome fusion with the other two.

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RESUMO

O presente trabalho descreve o cariótipo de *Spathalium helios* (Orthoptera) e o compara com o de *S. audouini*. Ambas espécies estão estreitamente relacionadas e a similaridade no mecanismo de determinação sexual das duas, sugere uma origem comum do mesmo, por meio de uma única fusão

ancestral X-autossomo. A diferença na posição do centrômero do cromossomo Y nas duas espécies reforça os argumentos a favor do status específico para *S. helios*, questionado devido à grande variabilidade morfológica entre espécimens de *S. audouini*.

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