Rev. Brasil. Genet. 14, 3, 653-660 (1991)
(Brazil. J. Genetics)

HAPLOID KARYOTYPE ANALYSIS OF *Chelymorpha variabilis* BOHEMAN (COLEOPTERA CHRYSOMELIDAE) WITH MICROSPREADING TECHNIQUES

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ABSTRACT

The independence of nucleolus and sex chromosomes Xyp in the coleoptera *Chelymorpha variabilis* Boheman has been demonstrated in a previous paper.

We have now applied microspreading and optical techniques to spermatocytes of this species. Results from TEM techniques are also presented. Haploid karyotypes showed the presence of a nucleolar organizer region in the 5th pachytene bivalent. Sex chromosome axes of X and Y were always found unpaired without any substance surrounding them, quite different from the typical Xyp that has been observed, Postiglioni and Brum-Zorrilla, (Genetica 77: 137-141, 1988). The ease of access to spermatocytes for karyotyping suggests the use of microspreading techniques to study the haploid karyotype of insect chromosomes. Our findings support the non-chiasmatic association of X and Y sex chromosomes.

INTRODUCTION

It is known that NORs in invertebrate mitotic chromosomes are difficult to find with conventional silver techniques (Goodpasture and Bloom, 1975; Howell, 1977).

A new perspective in the analysis of mitotic and meiotic chromosomes was opened with the development of the microspreading technique. This method facilitates the analysis of features such as: location of nucleolus, association of sex chromosomes, visualization of core axes, identification of chromosome rearrange-

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ments, and haploid karyotype construction (Moses *et al.*, 1977; Solari, 1980). However, in coleopteran species, these techniques have been rarely applied (Wise *et al.*, 1982). Some studies have been made on *Chelymorpha variabilis* Boheman (Coleoptera) (Postiglioni and Brum-Zorrilla, 1988). It was demonstrated, with acridine orange and silver stain, that the nucleolar material of the NOR meiotic region is not connected with the sex chromosomes Xyp to form the typical "parachute" found in Coleoptera. A dense substance was found inside them, which was denominated lumen, (Smith and Virkki, 1978). A similar feature has recently been found between the X-Y chromosomes in a species of *Disprepes* from Puerto Rico (Virkki *et al.*, in manuscript). The lumen, in this species, gave a positive reaction when it was treated with cytochemical techniques. Therefore this dense material is apparently a non histone protein.

The objective of this paper was to complete our previous research by analyzing the haploid karyotype of *C. variabilis*. The autosomal bivalent that carries the NOR's X and Y axes was identified with more assurance by using microspreading techniques.

MATERIAL AND METHODS

Larvae and eggs of *C. variabilis* Boh. were collected on vines of *Convolvulus arvensis* (Convolvulaceae). They were bred in the laboratory during one year at 24°C. The material was put into 200 cc. glass vials which were lined with absorbent paper and covered with nylon gauze (Vaio and Postiglioni, 1974).

The food was changed three days a week. Pachytene spermatocytes were observed two days after emergence of the imago. This was the best period to obtain large amounts of pachytene.

Spreading Techniques

Spermatocytes were spread with a mixture of Fletcher's (1979) and Solari's (1980) methods, according to the following description: the testes of each male, prepared in a squash preparation, were placed in TCM 199 (Parker) medium, disaggregated with a micropipette one by one, under a stereomicroscope to form a cell suspension. One small drop (0.02 ml) was spread in 0.5% NaCl, in a watch glass 1.5 cm in diameter and the nuclei were covered with a clean slide. The material was immediately fixed in 4% paraformaldehyde plus 0.03% SDS, at pH 8.0, for 10 minutes. Afterwards it was cleaned in 0.4 Kodak Photoflo at pH 8.0 (30 sec.) and air-dried.

The spread preparations were stained in a 70% solution of $AgNO_3$ and incubated in a water-bath during two hours at 60°C (Fletcher, 1979). Afterwards, good spread pachytenes were selected and the measure of the chromosome axes was obtained. The mean values of Relative lengths (R.L.) Absolute Lengths (A.L.), Centromeric Index (C.I.) and Reciprocal Arm Ration (R) of the chromosomes axes were analyzed with a sonic X-Y plane digitizer, interfaced to a DEC-PDP-11 with an accuracy of 0.02 cm. Nine pachytene spreadings were measured (Moses *et al*., 1977).

RESULTS

Chelymorpha variabilis (Boheman) has the typical meioformula 10^{II} + Xyp of the Polyphaga group. Metacentric chromosomes showed a secondary constriction in the 5th pair (Vaio and Postiglioni, 1974). The meiotic chromosomes, treated with silver stain showed, in the pachytene stage, two yellow brown masses (n) located at both sides of an autosomal bivalent (Figure 1a). An electron microscopical study of spermatocytes also showed the existence of a synaptonemal complex (SC) between the two dense chromatic masses (n). They corresponded to nucleolar material (Figure 1b). The pachytene stage was found 48 hours after the imago appeared. In this stage, we observed ten autosomic bivalent axes and two small axes which were clearly differentiated from the rest. They were interpreted as sex chromosomes X and Y, due to their short length and because they were always unpaired. They also showed strong positive silver staining, which contrasted with the other elements, appearing as dense and thick axes.

Each bivalent had a notable prominence along its length (arrows) (Figure 2). We assume that such structures could have corresponded to the kinetochore, as has been described in the mouse (Fletcher, 1979).

Some bivalents also showed small knobs in the telomeric regions. A characteristic nucleolar mass (n) was observed near the telomere of the short arm in bivalent number 5. A classification of chromosome bivalents was made by using means of Relative Length (R.L.) and Centromeric Index (C.I.), according to Levan *et al*.'s (1964) nomenclature.

Table I illustrates these results. The analysis of these tables showed a range of variation in the C.I. of 0.39 to 0.42. The values of C.I. equaled 0.40 for the axes numbers 1, 2, 8 and 10, 0.41 for the axes 3, 5, 7 and 12, and C.I. = 0.42 for the bivalent numbers 6, 9 and 11. Only bivalent number 4 had a C.I. = 0.39.

Taking into account these values, we could construct the haploid karyotype by ordering the elements in decreasing size. The range in arm ratio had indicated that all chromosomes were metacentric. A microspreading of the same early pachytene with all bivalents and both sex chromosome axes is shown (Figure 2b).

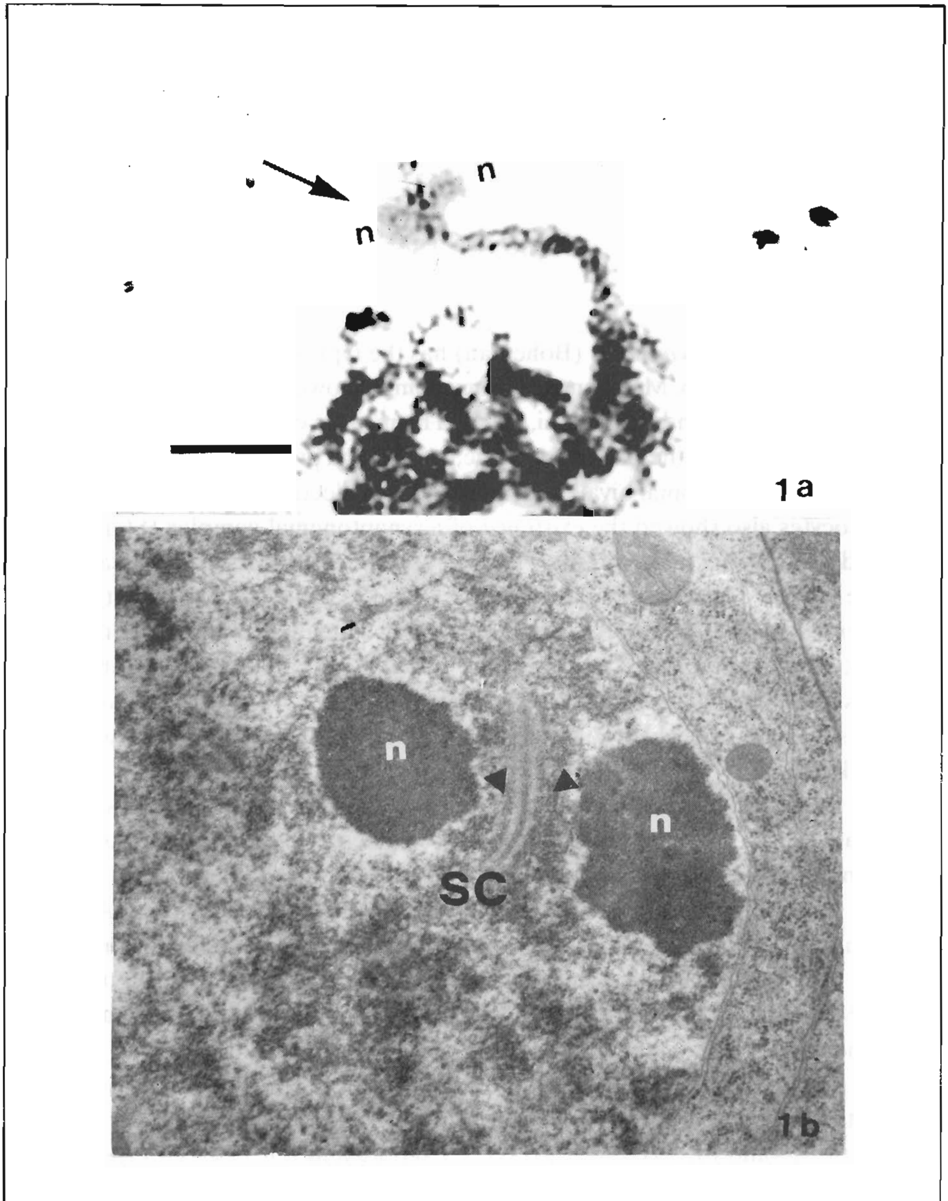

Figure 1 - (a) Pachytene nucleus of *C. variabilis* Boheman. Nucleolar masses (n) associated to autosomic bivalent stained with $AgNO_3$ (70%); (b) Synaptonemal complex (S.C.). Dense chromatinic masses (n) at both sides of S.C. are observed.

Figure 2 - (a) Haploid karyotype of *C. variabilis* stained with $AgNO_3$ (70%). Bivalent number 5 shows nucleolar mass (n); (b) *C. variabilis* pachytene spermatocyte nucleus prepared with the spreading procedure and stained with $AgNO_3$ (70%). n = nucleolus. X and Y sex chromosome axes Bar = 10 μm.

Table I - Quantitative characteristics of haploid axes of *C. variabilis*. Relative length (R.L.) (%); centromeric index (C.I.) and reciprocal arm ratio (R), and morphology (M) in pachytene spermatocytes.

Axes	Relative Length ± 0.05	Centromeric Index ± 0.02	R	M
1	13.95	0.40	1.62	m
2	11.73	0.40	1.54	m
3	10.77	0.41	1.52	m
4	9.81	0.39	1.65	m
5	9.33	0.41	1.45	m
6	8.86	0.42	1.41	m
7	8.27	0.41	1.53	m
8	7.57	0.40	1.61	m
9	6.34	0.42	1.40	m
10	5.34	0.40	1.58	m
11	4.26	0.42	1.45	m
12	3.74	0.41	1.46	m

n = 9 pachytene nucleus; m = metacentric.

DISCUSSION

In a previous paper the non-relationship between autosomic nucleolus and sex chromosome system Xyp in *C. variabilis* was demonstrated (Postiglioni and Brum-Zorrilla, 1988).

The use of specific techniques to determine monocatenarian nucleic acid (Stocker and Lisanti, 1972) and argentic techniques (Howell, 1977; Hofgartner *et al.*, 1979) has provided a good methodology to solve the problem of the association of the Xyp in coleoptera chromosomes, (Postiglioni and Brum-Zorrilla, 1988).

Our results confirm these concepts. Two visible masses of nucleolar material are shown in the subterminal region of the 5th. bivalent, while the sex chromosomes are separated as can be observed in the haploid karyotype.

When microspreading is applied to human spermatocytes, which have been treated with SDS, chromatin is normally unstained, nuclear envelope remmants are not apparent, and the nucleoli stand out as a strongly stained mass associated with an autosomic bivalent (Solari, 1980). Similar results have been obtained with our material. Both sex chromosomes were found separated from each other in pachytene when microspreading was used. Lack of nucleolus was observed in both chromosomes. Chromatin density is also clearly different in the nucleolus and the sex chromosomes. Wettstein (1981) using TEM techniques, described the sex chromosomes Xyp of *C. variabilis* as a condensed and light chromatin which forms a compact body that lies against the nuclear membrane in a diffuse stage. This author also suggested the existence of other mechanisms in meiosis to solve the problems of pairing zones, when the genetical homology and S.C. mediating structure were not present. The discovery of a lack of pairing axes X and Y through the use of microspreading techniques, has reinforced the idea about the terminal non-homologous associations as a manner of maintaining the typical Xyp of these species during meiotic prophase. These suggestions are in accordance with the results obtained from the positive heterochromatic blocks that we have found in terminal position in this species (Postiglioni and Brum-Zorrilla, 1988).

Differentiation of sex chromosomes X_1, X_2 and Y_1 with microspreading technique have been observed in boll weevil spermatocytes (Wise *et al*., 1982). Nucleolar substance was localized in some bivalents of this species. A relationship between nucleolus and sex chromosome was not found. These facts agree with our findings in *C. variabilis* because the identification of sexual axes was more clearly observed in our material.

According to our results the finding of an important number of pachytene spermatocytes, the construction of an haploid karyotype, the location of nucleolus in the 5th. bivalent, and the identification of unpaired axes X and Y, we could suggest that microspreading techniques applied to Coleoptera spermatocytes is a feasible way to resolve problems such as structure and associations of insect chromosomes.

ACKNOWLEDGMENTS

We wish to thank Dr. R. Wettstein for his valuable help, for performing the electron microscopy study and for furnishing the microphotograph (Figure 1b).

RESUMO

A independência do nucléolo e dos cromossomos sexuais Xyp no coleóptero *Chelymorpha variabilis* Boheman foi demonstrada em trabalho anterior. No presente trabalho foram aplicadas técnicas ópticas e de "microspreading" para espermatócitos desta espécie. Resultados de técnicas TEM também são apresentados. Cariótipos haplóides também mostraram a presença de uma região organizadora do nucléolo no 5º paquíteno bivalente. Os eixos dos cromossomos sexuais X e Y foram sempre encontrados não-pareados e sem nenhuma substância em torno dos mesmos, diferindo do Xyp típico já observado, Postiglioni and Brum-Zorrilla (Genetica 77: 137-141, 1988). A facilidade de acesso aos espermatócitos

para cariotipagem sugere o uso de técnicas de "microspreading" para estudar o cariótipo haplóide dos cromossomos dos insetos. Nossos resultados apoiam a associação "non-chiasmatic" dos cromossomos sexuais X e Y.

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(Received November 22, 1989)