

METHODOLOGY

TWO SIMPLE METHODS FOR THE PREPARATION OF MITOTIC AND MEIOTIC CHROMOSOMES OF ORTHOPTERA

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ABSTRACT

Two alternative methods for the preparation of orthopteran chromosomes are described: the first is based on mitotic or meiotic cell suspensions in a glass tube, and the second one on cell "spreading" directly on microscope slides. The methods were used to prepare chromosomes from grasshoppers belonging to the genera *Xyleus*, *Schistocerca*, *Abracris*, *Chromacris* and *Tropidacris*. The techniques gave satisfactory and highly reproducible results.

INTRODUCTION

In most cytogenetic studies of orthopterans reported in the literature, mitotic and meiotic chromosomes are obtained using the classical squash technique. However to obtain permanent preparations through this technique it is necessary to remove the coverslip, which results in a considerable loss of cells and qualitative deterioration of material. Techniques using cell suspension were introduced by Webb *et al.* (1978) in studies on embryos of the grasshopper *Warramaba virgo*, and by Motara *et al.* (1985), who obtained good meiotic preparations from testicular material of *Aedes aegypti*.

In the present study we describe two alternative methods for the preparation of mitotic and meiotic chromosomes of adult grasshoppers, based respectively on the use of cell suspensions prepared in small Petri dishes and in centrifuge tubes, and on simple cell "spreading" on microscope slides.

Considering that the quantity and quality of chromosome material are of fundamental importance for a more detailed karyotypic analysis of orthopterans (because it allows one to study the same individual through different cytogenetic techniques) the cell spreading method permits better results, because it prevents the possible loss of material in the test tubes and pipettes, usually observed with other methods.

In the present study we examined representative samples of grasshoppers of the genera *Xyleus*, *Schistocerca*, *Abracris*, *Chromacris*, *Tropidacris*, as well as others not identified yet. The animals were collected at different localities in the state of Pernambuco, Brazil, taken to the laboratory and held in captivity.

MATERIALS AND METHODS

The two methods cited above were used for the following cytological preparations:

1. CELL SUSPENSION. The grasshoppers (males and females) were treated with 0.05 to 0.1% colchicine in insect saline solution at the proportion of 0.1 ml/5 g body weight for 6 to 8 hours. When only meiotic analysis was desired, this pretreatment step was eliminated. The preparations were obtained from gastric caecae, ovarioles (cells from the ovariole wall) and testes according to the following procedure:

a) *Dissection*. The animals were dissected in insect saline solution gastric caeca, testes and ovaries were removed, cleaned and "sectioned" in 5 ml of hypotonic 0.075 M KCl solution until a cell suspension was obtained;

b) *Hypotonic treatment and fixation*. The material was submitted to hypotonic treatment for 30 minutes at room temperature and centrifuged at 800 rpm for five minutes. The supernatant was discarded and the remaining material was fixed three times in 3:1 ethanol:acetic acid. The material was resuspended in fresh fixative, and two drops of the suspension were added to a clean slide maintained in ice water, and air dried;

c) *Staining*. For standard staining the slides were covered with 2% Giemsa in sodium phosphate buffer, pH 6.8, for six minutes. Then they were washed in distilled water and allowed to dry.

2. CELL SPREADING ON A MICROSCOPE SLIDE. As in the preceding method, when the objective was to obtain only mitotic chromosomes the animals were treated with colchicine. The material was prepared as follows.

a) *Dissection*. The animals were dissected and the testes or ovaries removed and placed in insect saline. The material was then transferred to fresh saline, the ovaries were cleaned to liberate the ovarioles and, when testes were prepared, the peritoneal membrane was removed to liberate the testicular follicles;

b) *Hypotonic treatment*. The material was transferred to a small Petri dish containing five ml of tap water for hypotonic treatment during two to three minutes;

c) *Fixation*. The material was transferred to a small dish containing five ml of fresh fixative (3:1 ethanol:acetic acid) and fixed for 30 minutes. This preparation may be stored in fixative in the freezer for long periods of time;

d) *Chromosome preparations*. One or two ovarioles or testicular follicles were placed on each clean and dry slide. If the material appeared to be somewhat rigid, a drop of 45% acetic acid was added for 30 seconds and excess acid was removed with tissue paper. The material was "sectioned" and spread on the slide using a thin glass overslip, or by cutting it with a surgical blade. Fixative was added, one drop at a time, and the slides were gently rotated until the cells were fully spread. The preparation was then immediately placed in an oven at 37°C, for five to 10 minutes, in order to evaporate the excess fixative.

e) *Staining*. For standard staining, the same procedure was used as in item "c" of the previous method.

For C banding, CMA/DAPI fluorescence or silver nitrate staining, the slides were aged at room temperature for two to three days before treatment.

RESULTS AND DISCUSSION

Both methods yielded good results for larger animals, whereas the cell "spreading" method can be used both for larger and very small species. Including the ones having small testes and ovaries, which in some cases had only eight to 16 testicular or ovarian follicles, respectively.

Another advantage of the second method lays is that one can use the entire material without the losses that frequently occur when the classical squash technique, or the suspension technique, is used. This is particularly important when it is necessary to

analyse a large number of cells per individual (e.g. for determination of chiasmata frequency) or to prepare many slides per individual for the application of different techniques (G and C banding, silver staining, fluorescence, etc.).

Figures 1 and 2 illustrate the quality of the preparations obtained by the cell suspension and cell "spreading" techniques. The methods of chromosome preparation by cell suspension used by Webb *et al.* (1978) and Motara *et al.* (1985) have many applications and provide quite satisfactory results, but their application have some limitations. The first method is based on embryo cells, a type of material which most of the time is not available, which forces the researcher to work with adult animals. The second one is based on the use of 25 to 30 testes from different individuals for a single suspension, thus preventing individual karyotypic analysis.

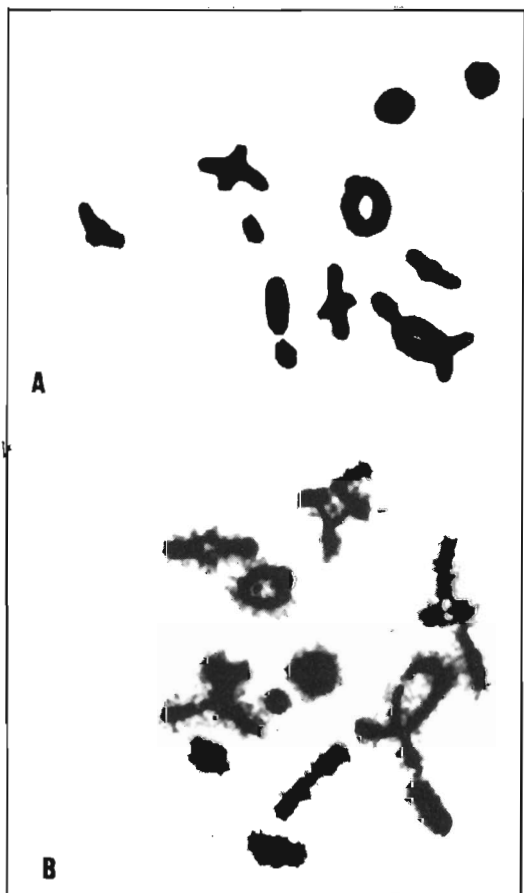


Figure 1 - (A) Methaphase I of *Abracris flavolineata*; (B) diakinesis of *Tropidacris collaris*.



Figure 2 - (A) C-banded spermatogonial metaphase of *Xyleus angulatus*; (B) C-banded diakinesis of *Tropidacris collaris*.

ACKNOWLEDGMENTS

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Fundação de Amparo à Ciência e Tecnologia (FACEPE).

I am most grateful to Dr. Marcelo Guerra and Dr. Vilna Maia for a critical reading of the manuscript and useful comments.

RESUMO

Dois métodos alternativos para preparação de cromossomos de ortópteros estão sendo descritos: o primeiro utilizando suspensão de células mitóticas e meióticas, feitas em tubos de vidro; o segundo utilizando

"espalhamento" de células, realizado diretamente sobre a lâmina de microscopia. Os métodos foram usados, em gafanhotos, representantes dos gêneros *Xyleus*, *Schistocerca*, *Abracris*, *Chromacris* e *Tropidacris* com resultados satisfatórios e com grande repetibilidade.

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(Received March 14, 1991)