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Una técnica alternativa para el cariotipado mitótico en saltamontes: bandeo C y Fluorescente en *Adimantus ornatissimus* (Orthoptera: Acrididae)

An alternative technique for mitotic grasshoppers karyotyping: Fluorescent and C-banding in Adimantus ornatissimus (Orthoptera: Acrididae)

Elio R. Castillo, Alberto Taffarel, Dardo A. Martí

Resumen

Los ortópteros son el material de preferencia para estudios citogenéticos debido al tamaño de sus cromosomas y a su bajo número diploide, sin embargo existen dificultades para obtener cromosomas mitóticos de alta calidad para tinciones diferenciales, siguiendo un protocolo fácil y reproducible. Con la intención de optimizar la calidad de los cromosomas mitóticos y minimizar el material no cromosómico en las preparaciones, se han utilizado diferentes procedimientos y tejidos. Los preparados mitóticos se realizan macerando ciegos gástricos y posteriormente se utiliza hielo seco, CO_2 a alta presión o nitrógeno líquido para remover el cubreobjetos. En el presente trabajo describimos una técnica que optimiza la visualización y la caracterización morfológica de los cromosomas mitóticos y facilita la aplicación de los procedimientos de tinción diferencial. Como material citológico de referencia analizamos a *Adimantus ornatissimus* (Burmaister 1838), la especie posee un 2n=23/24 ♂/♀, y un sistema de determinación sexual X0/XX.

Palabras clave: ciegos gástricos; mitosis; cromosomas; Orthoptera; técnica de frotis.

Abstract

Orthopterans are a preferred material for cytogenetic studies because of their large chromosome size and low diploid number; however a difficulty is to maximize a high quality mitotic chromosomes obtention and the choice of an easy and reproducible protocol. Different procedures and tissues were used to optimize mitotic chromosome observation with conventional staining, preserving, as much as possible chromosome morphology, and minimizing the non-chromosomal background material. Grasshopper mitotic metaphases preparations are usually obtained from squashed female gastric caeca and posterior exposure of slides to dry ice, CO_2 flow, or liquid nitrogen to remove coverslips. Grasshopper embryos are also used for cytological analyses. As a reference cytological material, we analyzed Adimantus ornatissimus (Burmeister 1838) chromosomes. This specie showed 2n=23/24 ♂/♀ and a X0/XX sex chromosome system. We describe the frotis technique which optimizes the visualization and characterization of mitotic chromosome morphology and facilitates differential staining procedures.

Key words: gastric caecum, mitosis, chrosomomes, Orthoptera, frotis technique.

Introduction

Species of Orthoptera are distributed over a wide range of habitats. However, as it happens in other groups of animals, a significant amount of species included in the order are confined to the Neotropical region, and only a few to cold latitudes [10]. Most of Neotropical grasshopper diversity is represented by Acridoidea [29], which include the family Acrididae, the most widely studied at the cytogenetic level [10, 29].

Grasshoppers are an ideal material for basic and population cytogenetic studies because of large chromosome size, low chromosome number, and exceptionally clear meiosis [10]. Used as experimental material, they contributed significantly to the knowledge of chromosome behavior in mitosis and meiosis including pairing, synapsis, chiasma formation and recombination, and to the study of numerical and structural mutations and chromosomal polymorphisms [3, 5, 10, 13, 14, 19, 22, 26].

Since many decades Acridoids cytogenetic analyses have been performed and based on male individuals due the easiness of meiotic chromosome preparations. On the other hand, female meiosis presents many difficulties for chromosome study [8, 15].

An additional difficulty is to choose an adequate protocol to maximize the obtention of high quality mi-

totic chromosomes. Different procedures and tissues are used to optimize mitotic chromosome observation with conventional staining preserving, as much as possible, chromosome morphology, and minimizing the background material, producing slides with well-spread and complete metaphases. Moreover, mitotic preparations have to be usually manipulated for differential staining, including fluorochromes [25]. Generally, mitotic chromosome preparations of grasshoppers are obtained by squashing female gastric caeca after incubation with a mitotic poison [12, 17, 18, 24]. Slides are then exposed to dry ice, CO_2 flow or liquid nitrogen immersion to remove the coverslip [7, 21]. Mitotic cytological preparations are also obtained through squashing of females ovarioles [7, 17, 20].

Grasshopper embryos are also cultured for mitotic chromosome obtention [1, 11, 25]. However, additional care must be taken when the protocol is developed, like extractions of embryos from the pods and in the first steps of breeding [1, 11].

We present an improved, alternative and easy technique that facilitates and optimizes the visualization and characterization of mitotic chromosome morphology in grasshoppers. The protocol was adapted from a technique used in vertebrates from intestinal tissue [23], and modified for orthopteran species in our laboratory (see below) mainly using less works solutions and replacing the intestinal suspension dropping by the frotis procedure.

Materials and methods

Test species. Eight adult females of *Adimantus ornatissimus* (Burmeister 1838) (Orthoptera, Acrididae) were studied at eight localities of Misiones province, Argentina (Fig. 1).

Mitotic preparations

Mitotic chromosomes were obtained from intestinal cells of gastric caecum through a new air-drying technique adapted by us to orthopteran, from a protocol used in vertebrates. The same is as follow:

1. Females were injected at the level of the fifth abdominal segment with 0.05% colchicine in insect saline (ClNa 0.9%) for 4-5 hours.
2. After colchicine exposure, gastric caeca were dissected and immersed in a KCl 0.75% hypotonic solution for 45-55 minutes.
3. Before definitive fixation in 3:1 methanol: glacial acetic acid, tissues were passed through a battery of fixative solution to remove hypotonic solution's remains, and then stored at 4ºC.
4. A small portion of gastric caecum is ruptured in 45% acetic acid. The final solution is resuspended twice using a Pasteur pipette during a period of 12 to 15 minutes.
5. Chromosomal preparations have to be performed under an airfoil chemical fume hood, equipped with an air extractor, to prevent vapor inhalation.

Figure 1. Female habitus of *Adimantus ornatissimus* from Moconá, San Pedro, Misiones, Argentina.

6. A drop of the suspension is then placed on one end of a warm slide, from which the material is extended towards the opposite side, with the aid of another glass slide (frotis). The last step is repeated from one side of the slide to the other until no liquid is left.
7. Finally, slides are air-dried at room temperature.

Staining. For solid staining phosphate buffered 5% Giemsa is used. For sequential staining, C banding [27], and C-DAPI banding [2] destaining must be performed. After observation and photomicrography of mitotic metaphases, immersion oil is removed from slides by three successive immersions in pure Xylene, washed in distilled water, and air dried at room temperature. Then, for Giemsa removal, slides are immersed in 3:1 methanol:glacial acetic acid. Double staining with CMA3/DAPI was done as described by Schweizer *et al* (1983) [28].

Results and discussion

The first record of *A. ornatissimus* males and females chromosome number was proposed by Mesa *et al* (1982) [16] but cell draw or photomicrography has never been showed or described. We observed a 2n=23 diploid number in males [16], and our results agree with the female chromosome set which shows 2n=24 (FN=24) telocentric chromosomes (Fig. 2 a). This karyotypic constitution is common in females of Acrididae [5, 16]. Based on chromosome size measurements three distinct groups were distinguished: three pairs of large chromosomes (L1-L3), five medium sized (M4-M8), three small (S9-S11), and the sex chromosomes which is similar in size to M4.

In most cytogenetic studies, using grasshoppers as experimental organisms, males are more frequently studied, because testes dissection is easy (with basic anatomical knowledge), and a lot of meiotic chromosome preparations per male individual can be obtained, with an elevated percentage of cells in different meiotic phases [9]. Male meiotic preparations don´t present major difficulties and reveal relevant details of chromosome morphology, bivalents configurations, different condensation patterns (as happens in sex chromosomes at post-pachytene phases), chromosome segregation, nucleolar cycle, and also recombination sites, represented by chiasmata.

A radically different situation occurs with female meiosis, where a systematic and laborious protocol needs to be followed, from the initial capture of females through the correct dissection of the egg, to the obtention of a single first metaphase per egg. Gravid females have to be fed and oviposition has to be stimulated. When oviposition is going to take place, eggs are extracted and the micropilar region of the primary oocyte is dissected out and gently squashed [15]. The yield of the technique is extremely low.

On the other hand, mitotic chromosome information is relevant to determine the chromosome number and cytological characteristics like constitutive heterochromatin and NORs [4, 6, 25]. Thus, in females, mitosis is the main source of chromosomal information and to that end, a number of different tissues (e.g. caeca, ovaries and embryos) and techniques are usually employed [4, 6, 25]. However, some procedures like embryo culture are laborious, requiring a lot of time and cares [4]; others do not yield good quality chromosomes.

The technique described in this paper maximizes the percentage (ca. 90%) of well-spread, high quality and complete metaphases in each preparation. In the same way, slide preparation involves less time, equipment, cost and space in comparison with other protocols, for example, when the coverslips have to be removed with ice dry, liquid nitrogen or CO_2 [7, 21]. One disadvantage of liquid nitrogen, dry ice or CO_2 procedures is the lost of material when the coverslips is removed (personal observation). In most of the cases, part of the preparation remains in the slide and part in the coverslips. Although use of siliconized coverslips prevents the loss of material in simple chromosome squashes, the protocol requires more time compared with our technique. Another difficulty of this protocol is the distribution of chromosomes in different focal planes (personal observation).

With the alternative frotis procedure the totality of intestinal material and also the complete slide surface is used. In standard squash caeca preparations, the material is confined to a limited area. Our procedure allows complete spread cells with chromosomes in one focal plane (Fig. 2 a, b, c, d, e). As happens with other procedures, mitotic metaphases trated with Giemsa, can be used in differential staining techniques after slides decoloration took place (Fig. 2 c, d, e).

The technique proposed in this paper for orthopteran mitotic chromosome preparations combines high quality, perfect reproducibility of the results and processing speed, with minimum laboratory conditions dispensing the use of dry ice or mechanical procedures, normally used in coverslips removal.

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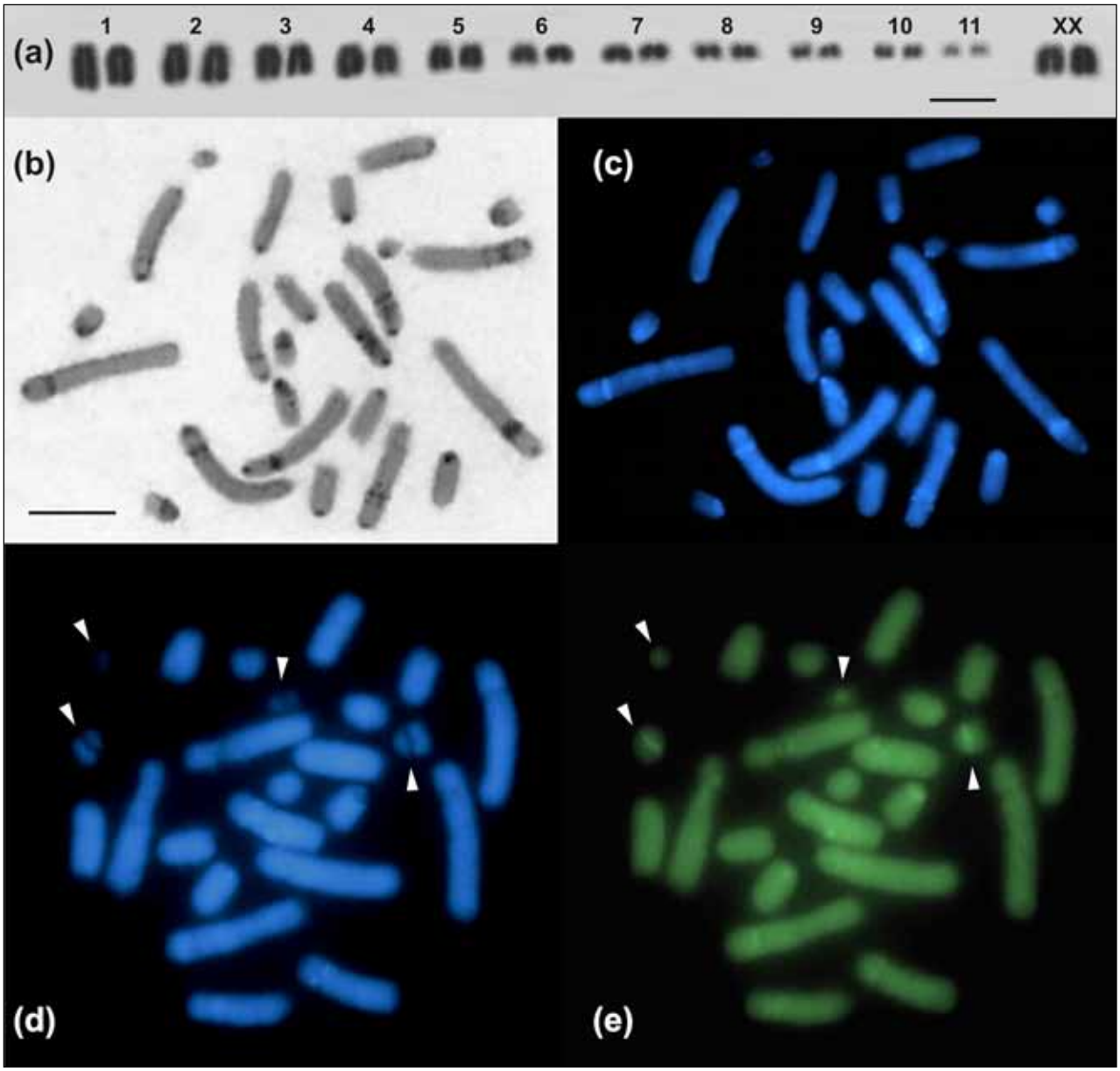

Figure 2. Female mitotic chromosomes of Adimantus ornatissimus. (a) Karyotype with Giemsa staining, (b) C-banding; constitutive heterchomatin patterns are dark stained and (c) sequential C-DAPI banding. DAPI/CMA3 staining, arrowheads signaling negative DAPI heterochromatic blocks in (d) and chromomycin-positive sites in (e). Bar = 10 µm.

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• Elio Rodrigo Castillo[1,2,3]
Licenciado en Genética de la UNaM. Becario de CONICET-CEDIT y realiza el Doctorado Ciencias Biológicas en la Universidad Nacional de Córdoba. Desempeña actividades de docencia en la Cátedra de Genética Evolutiva de la F.C.E.Q.yN. (UNaM) con el cargo de Ayudante de Primera. Correo electrónico: castillo.eliorodrigo@gmail.com

• Alberto Taffarel[1]
Se encuentra desarrollando su tesis de grado de la carrera Licenciatura en Genética, bajo la dirección del Dr. Dardo Martí, en el Laboratorio de Genética Evolutiva de la UNaM. Correo electrónico: radova@gmail.com

• Dardo Andrea Martí[1,2]
Dr. en Ciencias Biológicas de la Universidad Nacional de Córdoba. El Dr. Martí es Investigador Adjunto de CONICET. Asimismo, se encuentra bajo su dirección el Laboratorio de Genética Evolutiva de la F.C.E.Q.yN. (UNaM) y es docente de la cátedra de Genética Evolutiva desempeñando el cargo de Profesor Adjunto Regular. Correo electrónico: darmarti@fceqyn.unam.edu.ar

1. Universidad Nacional de Misiones, Facultad de Ciencias Exactas, Químicas y Naturales, Laboratorio de Genética Evolutiva. Félix de Azara 1552. CP. 3300 Posadas, Misiones, Argentina.
2. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Rivadavia 1917, 1033. Ciudad Autónoma de Buenos Aires. Argentina.
3. Comité Ejecutivo de Desarrollo e Innovación Tecnológica (CEDIT).