

METHODOLOGY

Evaluation of techniques for C and ASG banding of the mitotic chromosomes of *Anastrepha* species (Diptera, Tephritidae)

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ABSTRACT

Methods previously described by Canovai *et al.* (*Caryologia* 47: 241-247, 1994) which produced C and ASG bands in mitotic chromosomes of *Ceratitis capitata* were applied to the chromosomes of several *Anastrepha* species. Metaphase plate yield was substantially increased by use of imaginal disks together with cerebral ganglia. The C-bands were quite prominent allowing the resolution of tiny blocks of heterochromatin. The ASG method produced G-like banded chromosomes, which permitted recognition of each individual chromosome. These simple techniques do not require special equipment and may be valuable for karyotype variability studies in fruit flies and other Diptera.

INTRODUCTION

The difficulty in producing bands in insect mitotic chromosomes is generally accepted; however, C-bands and some bands produced by fluorescent techniques (Bedo, 1986; Gatti *et al.*, 1976) are exceptions. Insect chromosomes seem to resist to techniques that produce G-bands (Holmquist, 1987, 1990). However, one simple method which produces G-like bands, the ASG technique, was successfully applied in fruit fly *Ceratitis capitata* chromosomes (Canovai *et al.*, 1994). In addition to these studies, the authors have prepared metaphase plates of *C. capitata* using the C-banding technique. The methods of Canovai *et al.* (1994) differ from those previously used to study tephritid chromosomes (Bedo, 1986; Solferini and Morgante, 1987, 1990). We have obtained reliable results with both of Canovai's techniques in several *Anastrepha* species (Selivon, 1996). In the present study we would like to

call attention to these simple methods, especially ASG banding, which does not require special equipment and may be helpful in studies of karyotype variability in dipteran flies.

MATERIAL AND METHODS

Fruit fly species used in the present analysis, *Anastrepha fraterculus*, *A. serpentina*, *A. grandis*, *A. sororcula*, were maintained in the laboratory according to standard methods (Selivon, 1996). The techniques for chromosome preparation and staining will be described in Results and Discussion.

RESULTS AND DISCUSSION

Chromosome preparation

Canovai *et al.* (1994) prepared *C. capitata* chromosomes from cerebral ganglia of 3rd-instar larvae. We

have done the same, but we used the eye/wing imaginal disks together. Since the inclusion of the disks substantially raised the number of metaphase plates *per* slide, the treatment with colchicine could be omitted. The following steps were carried out as recommended by Canovai *et al.* (1994). Their protocol is given below. Commentaries from our own experience are included in parentheses:

1. Cerebral ganglia (and imaginal disks in our preparations) from 3rd-instar larvae were dissected in 0.9% NaCl (or insect ringer), and incubated in 10^{-4} M colchicine (omitted in most of our preparations) for 90 min;

2. The material was transferred to 2% sodium citrate for 4 min and then fixed in methanol/acetic acid 3:1 solution, for 30 min (minimum). The material can be used immediately or stored in fixative for several months, if maintained at -20°C ;

3. Slides were made by dispersing cells in a drop of 60% acetic acid and air dried (dispersion is critical; ganglia or disks must be fully disrupted with two sharp needles, and the acetic acid drop must be enough for the cells to float free but not too much to slow evaporation time).

4. The preparations were either "aged" for 3 h at 60°C or stored for about a week at 25°C (longer storage reduced, but not abolished, band yield).

C-banding technique

The technique described by Canovai *et al.* (1994) for C-banding was used without modifications: the slides were immersed in 0.2 N HCl, at room temperature for 10 min, rinsed in distilled water and transferred to a saturated solution of barium hydroxide at 50°C for 2 min. The slides were rinsed in acid water (distilled water with a few drops of acetic acid) and transferred to 2 x SSC (0.3 M NaCl; 0.03 M trisodium citrate) at 60°C for 30 min. After rinsing abundantly in distilled water, the material was stained with 5% Giemsa in Sorensen's buffer, pH 7, for 90 min (the length of staining is critical since overstaining must be avoided; in our experiments, 40 to 60 min was sufficient).

The C-bands obtained in *Anastrepha* species were quite prominent, as exemplified in Figure 1 with *A. fraterculus* and *A. serpentina* chromosomes, allowing the recognition of tiny blocks of heterochromatin.

ASG banding method

ASG banding is produced when aged slides are incubated in 2 x SSC for 30 to 40 min at room temperature, rinsed in distilled water and stained with

4% Giemsa in Sorensen's citrate buffer, pH 7, for 90 min, according to Canovai *et al.* (1994). They found that incubation time for *C. capitata* chromosomes was critical. After a short incubation, 30 to 33 min, G-bands appeared. In some metaphase and pre-metaphase plates, the chromosomes exhibited a series of stained blocks interspersed with despiralized regions of the chromatin. In more condensed metaphases, the chromosomes showed a ladder-like succession of bands similar to G-bands in other organisms. According to the authors, the pre-metaphase plates were subject to variations, and idiogram reconstructions could be made only from the more condensed chromosomes. Following a longer incubation, about 37 min, they found two types of bands: metaphases with C-banded chromosomes and others exhibiting G-like bands.

We used this technique on chromosomes from several *Anastrepha* species and obtained reliable results as shown by the *A. fraterculus* chromosomes in Figure 2. Some differences from Canovai *et al.* (1994) were nonetheless observed. The differential aspects of bands they found for the chromosomes of *C. capitata* after short/long incubation were not so clearly obtained in *Anastrepha* species. We found practically no differences after incubation for 30 to 40 min. Autosome banding in pre-metaphase was always of poor quality, although the X-chromosomes showed well-defined bands (Figure 2A). The treatment produced G-like bands in most of the metaphases per preparation, even in plates showing different degrees of chromosome condensation (Figure 2B, C, D). G-like banding allowed recognition of individual chromosomes (Figure 2D). Longer incubation produced several C-banded metaphases

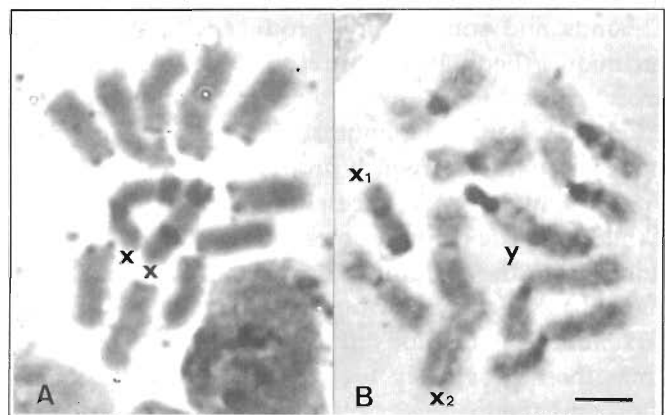


Figure 1 - Mitotic chromosomes of a female *Anastrepha fraterculus* (A) and of a male *A. serpentina* (B), prepared by the C-banding technique. In *A. fraterculus* (A), the two C-bands which occur in the X-chromosomes are clearly seen (Selivon, 1996). In *A. serpentina* (B), the several C-bands are very well marked. The species has sex chromosomes of the type X1X1X2X2 (females) and X1X2Y (males), and the chromosomes are identified according to Solferini and Morgante (1987, 1990). Scale in B represents 10 μm and is valid for both figures.

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RESUMO

Técnicas descritas anteriormente por Canovai *et al.* (*Caryologia* 47: 241-247, 1994), que produzem bandas C e ASG nos cromossomos mitóticos de *Ceratitits capitata*, foram testadas com os cromossomos de várias espécies de *Anastrepha*. O número de metáfases analisáveis aumentou significativamente quando, além dos gânglios cerebrais, discos imaginários foram utilizados nas preparações. O bandamento tipo C produzido foi bastante conspícuo, permitindo inclusive a resolução de pequenos blocos de heterocromatina. O método ASG produziu uma fração significativa de metáfases cujos cromossomos apresentavam um nítido bandamento do tipo G, que permitiu diferenciar cada cromossomo do cariótipo. Essas técnicas simples, aplicadas sem a necessidade de equipamento especial, podem ser úteis para estudos de variabilidade cariotípica intra ou inter-específica.

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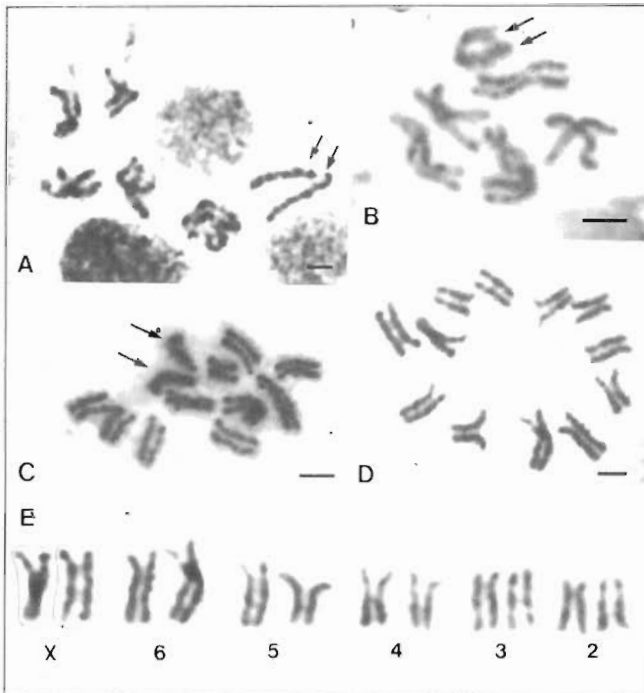


Figure 2 - Chromosomes of *A. fraterculus* after ASG banding. (A) A pre-metaphase showing the poorly resolved banding of the autosomes but nicely banded X-chromosomes (arrows). In B, C and D, metaphases are shown with evident G-like banding; arrows point out the X-chromosomes. In E, the chromosomes of plate D are arranged in decreasing order and show band correspondence for each homologue pair. Scales represent 10 μ m.

similar to the description of Canovai *et al.* (1994), for *C. capitata* chromosomes. We modified incubation time, saline concentration and temperature without any significant improvement.

CONCLUSIONS

The technique of Canovai *et al.* (1994), used with imaginal disks, is impressive for the following reasons: simplicity, excellent chromosome preservation (since squashing is avoided) and high yield of properly stained and banded metaphases. The technique facilitates the formerly difficult task of preparing C and more specifically G-like bands in insect mitotic chromosomes. ASG bands may be useful in the recognition of chromosome variability intra or interspecifically, even without special equipment.

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