KARYOTYPE STUDY OF THE SOUTH AMERICAN FRUIT FLY, *Anastrepha fraterculus* (Wied.) IN ARGENTINA

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Abstract

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The most frequent karyotype of *Anastrepha fraterculus* in Argentina is described here on the basis of mitotic metaphase morphology. It was named "*fraterculus Arg 1*". The diploid number is 2n = 10 + XX/XY and in males it comprises five homomorphic pairs and one heteromorphic pair, the latter being the sexual pair. Samples from different populations were cytologically analyzed, and "*fraterculus Arg 1*" is present in all of them at a high frequency (about 60 %). A typical C band pattern of the X chromosome was found only in the Montecarlo (Misiones province) population.

1. INTRODUCTION

The genus *Anastrepha* is scarcely known from a taxonomic point of view, and species identification is based on the morphology of the apex of the ovipositor [1]. Male adults and inmatures are mostly undistinguishable. The most serious taxonomic problem involves *A. fraterculus* because intraspecific variation is not well understood.

Cytological studies of Brazilian [2] and Mexican populations [3] of *Anastrepha fraterculus* have demonstrated karyotypic variation. Bush [3] indicated that it was not possible to identify karyotypical differences among *A. fraterculus*, *A. mombinpraeoptans* and *A. distincta.*

Solferini and Morgante [4] investigated samples collected on various species of host fruits from different areas of Brazil. They distinguished four karyotypes, which differed in their sexual chromosomes. Karyotype 1 presented a Y/X ratio of approximately 0.5 ; C banding produced a heterochromatic block on each end of the X chromosome and a totally heterochromatic Y chromosome. Karyotype 2 differed from the former in having a longer Y chromosome, carrying a satellite on it. Karyotype 3 had a long X chromosome with a constriction which separated 1/3 of the distal portion of the chromosome; the Y chromosome being similar to that of karyotype 2. In karyotype 4, both sex chromosomes had a similar length and a secondary constriction. This work suggests that *A. fraterculus* represents a complex of sibling species. Through further investigation, karyotype 3 was later assigned to *A. sororcula* [5].

A great amount of chromosomal variation within Argentine populations of *Anastrepha fraterculus* has been detected [6] & [7].

The present work reports the existence of the reference (most frequent) karyotype (59,7%) within regional samples of *Anastrepha fraterculus* as well as a configuration which, up to now, would be a marker of Montecarlo population.

2. MATERIALS

Larvae were obtained from green and yellow fruits of guava shrubs, probably *Feijoa sellowiana* (from Castelar and Ituzaingó, Buenos Aires province) and *Psidium guayaba* (from Ituzaingó, Buenos Aires province and Tucumán province) and from *Prunus persica* collected from Montecarlo, Misiones province*. Samples of adult flies were systematically identified as *Anastrepha fraterculus* (courtesy Eng. Norma Vaccaro, INTA, Concordia).

Third instar larvae were recovered (n=210) from host-fruit pulp to obtain cytological preparations.

3. METHODS

Mitotic metaphase plates were prepared from neuroblast cells of third instar larvae. Reproductive tissue was obtained from adults one day after emergence.

3.1. Preparation of cerebral ganglia.

Larvae were dissected in a drop of 0,75 M KCl. Each cerebral ganglion was put separately on a culture slide with KCl during 5 min, changed to fresh fixing solution (1:3, glacial acetic acid, ethanol) for 6 min, and then to 45% acetic acid for 3 min. Each ganglion was transferred to a microscope slide with a drop of 45% acetic acid and squashed by striking it with a glass or plastic rod, to spread the tissue. Slides were air dried horizontally and kept in closed coplin jars for at least 15 days at room temperature. The same treatment was used for reproductive tissue.

In both cases the tissue was stained in 4% orcein solution and then squashed in lacto-propionic medium (1:1), to obtain slides.

C banding was carried out as described in Ref.[8], with the following modifications: bariumhydroxide solution was used at 27-29°C for 7 min. Chromosomes were stained with 5% Giemsa (Gurr R66) solution in phosphate buffer (pH 6.8) for 15 min.

3.2. Preparations were examined without mounting them.

A drop of immersion oil was put directly on the slide. Such preparations can be kept for six months after examination.

4. RESULTS

Karyotypical analysis showed the existence of chromosomal diversity among and within Argentine geographic populations.

The most frequent karyotype (59,7%), present within all the analyzed samples was named "*fraterculus Arg 1*" (from now on "*fArg 1*"). It is the reference wild type karyotype for

* Buenos Aires = Central region; Tucumán = North Western region; Misiones = North Eastern region.

Argentina. Its 2x = 10 + XX/XY complement, consists of five pairs of homomorphic and telocentric autosomes, an acrocentric X chromosome and a small submetacentric Y chromosome (Fig. 1). The Y chromosome is approximately 2/3 the length of the X chromosome. The autosomal pairs are almost undistinguishable one from each other, except for pair II which is characteristically the largest in the complement: X/II = 0.74. The X chromosome is generally curve shaped

It is worthwhile explaining that in populational studies the heteromorphic pair is generally associated with the sexual pair. However, in *fArg 1* this was not the case, because the same heteromorphism was observed in meiosis plates.

The C banding technique applied to *fArg 1* has revealed two opposite terminal blocks of heterochromatin on the X chromosome, one near the centromere and the other one can be marking a secondary constriction. From now on, it will be named "X_1". The small submetacentric Y chromosome showed a pericentromeric C band (Y_1). These are useful markers to identify *fArg 1* (Fig.1).

In addition to *fArg 1*, other less frequent chromosomal configurations might be shared by two or more *A. fraterculus* Argentine populations. This is also the case for the sample of peaches collected from Montecarlo, Misiones. Nevertheless the chromosomal analysis also demonstrates the existence of a distinctive karyotype. The Y_1 chromosome is approximately 1/2 the X chromosome and the ratio X/II = 0.82. C banding technique, showed that the X chromosomes carry two bands on one end (Figure 2). This particular banding of the sexual chromosome (named "X_2") was only observed among flies of the Misiones population, thus it can be considered as a marker.

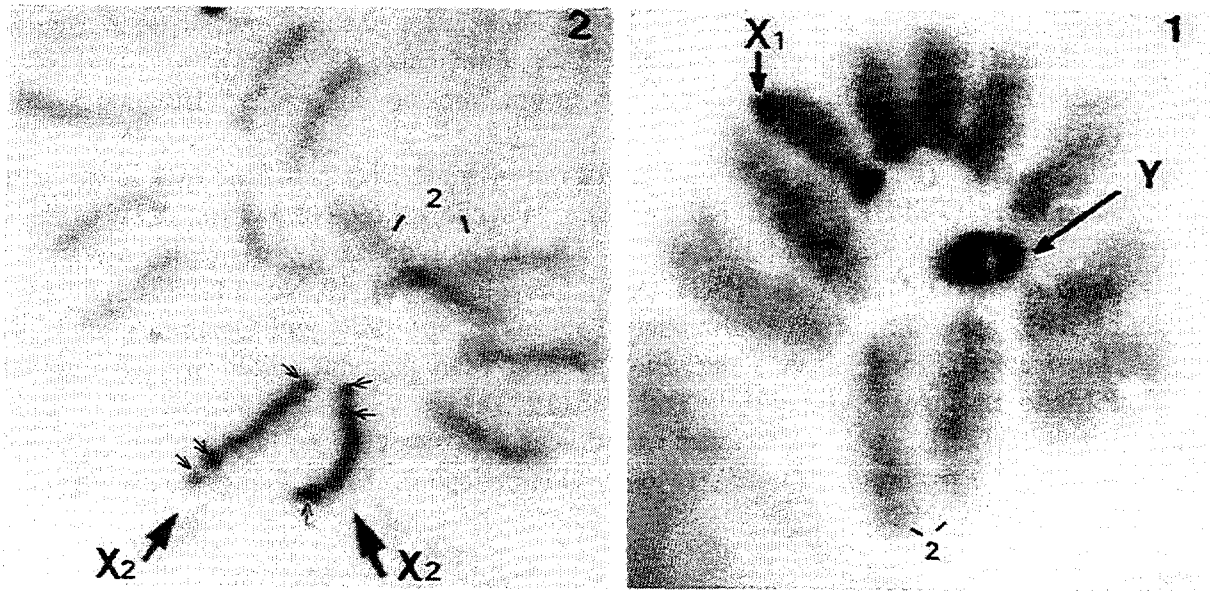

Figure 1 and 2: *(1) Mitotic metaphase of male "fraterculus Arg 1" carrying the X_1; C banding reaction. (2) Mitotic metaphase of female from Montecarlo, Misiones carrying the X_2; C banding reaction.*

5. DISCUSSION

Cytogenetic studies of *A. fraterculus* revealed that *fArg 1* is different from the karyotypes previously described by Solferini and Morgante.[4] for Brazilian populations of this insect. As discussed by Bush [3], no heteromorphism of the sexual pair was observed in Mexican populations, whereas in Brazilian specimens a heteromorphic sex pair was detected [2].

The X_1 chromosome showed the same C banding pattern as that of "karyotype 1" described by Solferini and Morgante.[4], which carries a totally heterochromatic Y-chromosome and presents a Y/X ratio of 0,5, thus differing from *fArg 1*.

The X_2 chromosome of Montecarlo, Misiones is longer than the X_1: it has an extra C band. These data are correlated with the differential ratios between Y_1/X_1 and Y_1/X_2, and between X_1/II and X_2/II.

Investigation of "*Anastrepha fraterculus complex*" needs the work and support of different groups and technologies so that results can be correlated in views to understand phylogenetic relationships.

The effort of cytologists in applying rigorous scientific techniques to visualize chromosomal differences which are probably markers of reproductivelly isolated populations will be helpful in taxonomic studies. Cytological data are a tool which supplements and strengthens the work of taxonomists based on morphological data.

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