DOUBLE MINUTE CHROMOSOMES IN *Anastrepha fraterculus* (Wiedemann) (DIPTERA: Tephritidae): A MODEL FOR CANCER STUDIES

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ABSTRACT *Anastrepha fraterculus* (Wied.), is known as the South American Fruit Fly. In order to develop efficient control strategies, we need deep knowledge on its biology along with periodical studies on population dynamics. Citogenetic studies on natural populations of this pest fly made it possible to reveal the presence of double minute chromosomes (DMs) in several natural populations of this insect. Cytogenetic studies performed in our insect laboratory, allowed the genetic analysis through a genealogical methodology. The establishment of families made it possible the genetic studies allowing the rigorous identification, characterization and confirmation of new chromosomal variants, frequently misinterpreted when the materials analyzed only come from nature. Double minute chromosomes -considered a type of chromosomal rearrangement- are extra chromosomal gene copies. This study sought to answer: What role do these DMs chromosome play in laboratory populations of the pest derived from natural populations? How are they transmitted from one generation to another? To answer our questions, we analyzed, for 25 generations, two laboratory populations derived from Tucuman (T) and Buenos Aires (BA -tester) guava,. Data were recovered from cytological analysis of ganglia preparations revealed with H33258. The natural population from BA didn't carry DMs. Results showed DMs in flies of both laboratory populations. DMs were transmitted from parents to progenies through 25 generations, their transmission was randomized in number. DMs are the cytological expression of resistance mechanisms used by the pest as a response to environmental stress. DMs in BA strain marked the change to the laboratory environment. *A. fraterculus* is a model insect for the study of cancer.

KEYWORDS: cytological markers, environmental stress, Breakage-Fusion-Bridge, gene amplification, insect pests

Anastrepha fraterculus (Wiedemann), known as the South American Fruit fly, is considered a quarantine pest which produces significant loses to fruticulture in our country. In 1992, it was declared a National Priority Pest. To develop efficient control strategies, deep biological knowledge of the species and periodic studies of population dynamics are required. Cytogenetic studies on natural populations of the fruitfly revealed form the first time the presence of doublé minute chromosomes (DMs). Since 2003 our works developed in the insect cytogenetics laboratory allowed the genetic analysis of the materials through a genealogical management. Genetic studies of families, allow rigurous identification, confirmation and characterization of new variants, frequently misinterpreted when the analyzed materials only come from nature. DMs chromosomes, considered a type of chromosomal rearrangement, are extrachromosomal gene copies. Our working hypothesis is that DMs are the cytological expression of resistance mechanisms used by the pest in response to stress situations. The present work aims to analyze the presence and role of tiny double chromosomes in *A. fraterculus* and their transmission under controlled conditions. To this end, two laboratory populations of guava trees from Buenos Aires and Tucumán were analyzed. The natural population of Buenos Aires did not present DMs. Our results show for the first time the presence of DMs in the flies of both laboratory populations, confirming the proposed hypothesis. DMs were passed from parent to offspring through 25 generations and were random in number.

INTRODUCTION

All living organisms need the cell cycle to increase in size and/or replace dead cells.

The cell cycle consists of three distinct phases: interphase, mitosis, and cytokinesis (Figure 5). Before a eukaryotic cell can begin mitosis and divide, it must replicate its DNA, synthesize histones and other proteins associated with the DNA of the chromosomes, produce an adequate supply of organelles for the two daughter cells, and assemble the structures necessary for them to intersect. carry out mitosis and cytokinesis. These preparatory processes occur during the interphase of the cell cycle, in which, in turn, three stages are distinguished: the Gl, S and G2 phases (Curtis et. al., 2008).

The breeding of *A. fraterculus* under laboratory conditions allows genetic studies to be carried out by families, rigorously identify genetic variants (chromosomal, biochemical, molecular), confirm and characterize new mutants, frequently misinterpreted when studies are only carried out on materials that come from nature. Thus it is possible to understand the meaning of variability

The most common karyotype is composed of 5 pairs of telocentric autosomes, an acrocentric X chromosome, and a small submetacentric Y chromosome, such that 2n = 2x = 10 + XX/XY (Lisfchitz et. al., 1999) (Figure 4). The heteromorphic pair is generally associated with the pair of XX or XY sex chromosomes which remain separate during

mitotic metaphase.

During the S phase (of synthesis) the chromosomal material is duplicated. Between cell division and the S phase there are two G phases (from the English gap, interval). The first of these (G1) is a period of general growth and duplication of cytoplasmic organelles. During the second (G2), chromosome condensation begins and the assembly of structures directly associated with mitosis and cytokinesis begins. After the G2 phase, mitosis occurs, where the duplicated chromosomes are distributed between the two daughter nuclei.

a) Prophase. The centrioles begin to move towards opposite poles of the cell, the condensed chromosomes (consisting of two sister chromatids) are already visible, the nuclear envelope breaks and the formation of the mitotic spindle begins.

b) Early metaphase. Spindle polar and kinetochore fibers pull each pair of chromatids back and forth.

c) Late metaphase. Chromatid pairs line up at the equator of the cell.

d) Anaphase. The chromatids separate. The two sets of newly formed chromosomes are pushed toward opposite poles of the cell.

e) Telophase. The nuclear envelope forms around each chromosome set and the chromosomes decondense and again take on a diffuse appearance. The nucleoli reappear. The mitotic spindle becomes disorganized and the plasma membrane invaginates in a process that causes the two daughter cells to separate.

Finally, in cytokinesis, the cytoplasm divides, separating the mother cell into two identical daughter cells. When no more cells are required, they enter a state called G0, in which they leave the cell cycle and enter a period of latency (Curtis et. al., 2008).

Cells continually monitor their external environment as well as their internal physiological state and functions. In the absence of necessary nutrients or growth factors, animal cells can exit the cell cycle and enter a resting state called G0. Following growth stimulation, cells re-enter the cell cycle (Hartl & Jones, 2006).

Cells have mechanisms that respond to stress symptoms, including DNA damage, oxygen depletion, inadequate amounts of nucleoside triphosphates, and (in the case of animals) loss of intercellular adhesion. Within the cell, several key events in the cell cycle are monitored. When defects are identified, cell cycle progression stops at a checkpoint allowing time for correction and repair. The checkpoint serves to maintain the correct order of the phases of the cell cycle (Hartl & Jones, 2006).

There are three key checkpoints in the cell cycle (Figure 6):

- DNA damage checkpoint
- Checkpoint at centrosome duplication
- Checkpoint in the mitotic spindle

Failure at any checkpoint in the cell cycle results in genetic instability leading to different types of chromosomal mutations

The malfunction of the mitotic spindle can lead to aneuploidy, while an error in centrosome duplication can lead to polyploidy. Failures at the DNA damage checkpoints (Figure 8) can result in chromosomal aberrations of various types, including translocations, deletions, and amplifications of genes or chromosome regions. Amplified genes can be found as tandem repeats within a chromosome or extrachromosomal circles that lack a centromere and telomeres (Hartl & Jones, 2006).

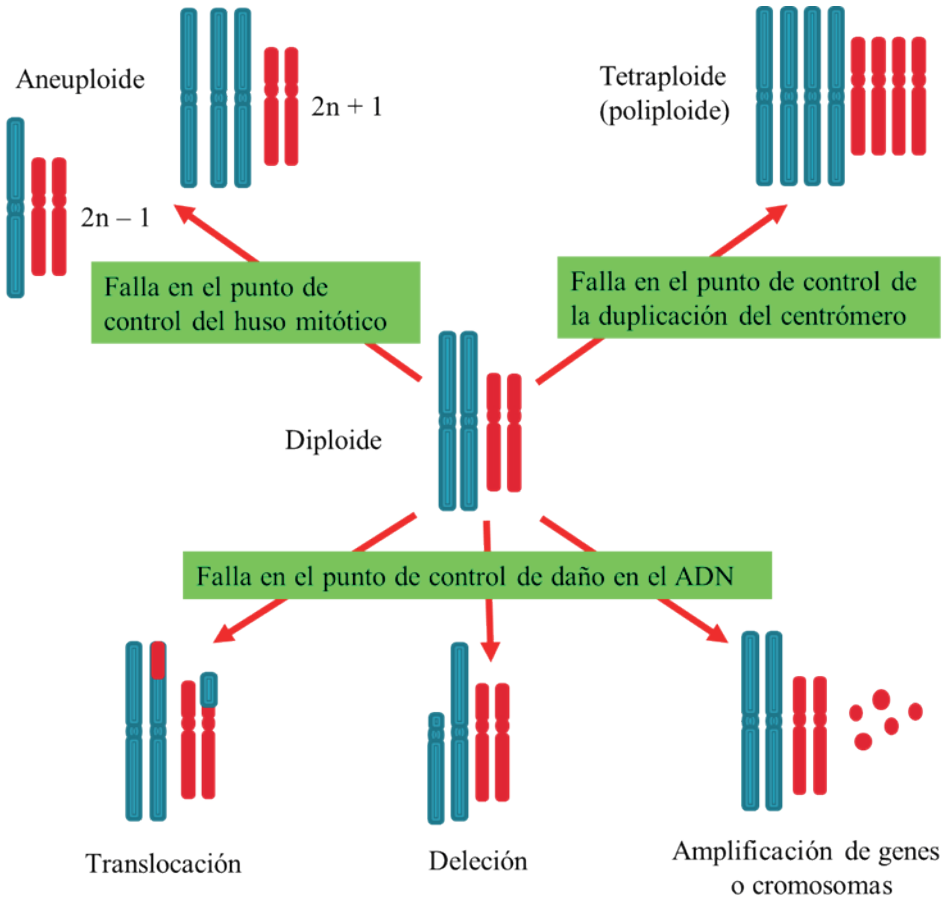

Figure 8. Contribution of failures in control points to genomic instability (Adapted from Hartl & Jones, 2006)

c. gene amplification ACTUALIZAR

DNA amplification is defined as a molecular process that results in an increase in the number of copies of a discrete chromosomal region of DNA (Mondello et. al., 2010; Mukherjee & Storici, 2012). In increase in the number of copies of a gene. There may also be an increase in the RNA and protein made from that gene. Gene amplification is common in cancer cells, and some amplified genes may cause cancer cells to grow or become

resistant to anticancer drugs. Genes may also be amplified in the laboratory for research purposes.

These types of mutations have been observed in a large number of human tumors including breast, lung, ovarian, colon cancer, leukemias, and neuroblastoma (Baskin et. al., 1981; Hahn, 1993; Foureman et. al., 1998; Schoenlein et.al., 2003, Albertson et.al., 2003, Albertson, 2006, Teicher, 2006, Sanborn et.al., 2013, Yu et.al., 2013, L'Abbate et.al., 2014, Poddighe et al., 2014).

Clinically, the amplification has prognostic and diagnostic utility and is a mechanism of acquired drug resistance (Baskin et. al., 1981; Schimke, 1984; Albertson et. al., 2003; Albertson, 2006; Teicher, 2006; Starczynski et al., 2012).

d- tiny double chromosomes (DMs)

DMs chromosomes were observed and described for the first time in *A. fraterculus* by Basso (2003) in natural populations of Argentina.

DMs and homogeneously stained regions (HRS) are two cytogenetic manifestations of gene amplification (duplication) (Cowell, 1982). They can be located as extrachromosomal elements (DMs) or within the chromosomal arm, giving rise to homogeneously stained regions (HSR) (Mondello et. al., 2010).

DMs are acentric circular fragments, without telomere, that replicate autonomously. Having no centromere, DMs segregate randomly at mitosis and may be lost during cell division unless they confer a proliferative advantage to cells, such as when they carry amplified drug resistance genes (Hahn, 1993; Mondello et. al., 2010).

The presence of DMs in cells of cancer patients is an indicator that the administered drug has ceased to be effective and should be replaced by another (Teicher, 2006).

This work aims to answer the following questions:

What role do DM chromosomes play in laboratory populations derived from natural populations? How are they transmitted from one generation to another?

Materials and Methods

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