Cytological Studies on Chironja[1]

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INTRODUCTION

A new type of citrus fruit was found early in 1956 by the senior author in the interior mountainous region of Puerto Rico, in an area where a mixed population of orange (*Citrus sinensis*) and grapefruit (*Citrus paradisi*) was under cultivation. The fruit was named *chironja* because its morphological characters resembled those of the orange (*china*)[3] in some respects and the grapefruit (*toronja*) in others (1).

The chironja is a versatile fruit which has the advantage of being utilized in three different ways as a fresh table fruit: 1, Cut in half and eaten with a spoon as a grapefruit; 2, easily peeled and sections eaten like a tangerine; and 3, squeezed as an orange for juice. The fruit has a high vitamin C content and is low in acid. Experiments have indicated that it is easily processed and that it has excellent keeping qualities.

A commercial chironja orchard has been established in the northwest region of Puerto Rico. The great number of inquiries concerning the chironja received from citrus areas of the continental United States, Hawaii, Mexico and various countries of South America and Europe is evidence that widespread interest exists in the possibilities of propagating this new fruit on a large scale. In view of such interest, and the vast commercial potential of the chironja, the genetic origin of this fruit has become of technical interest. Preliminary cytological studies thus were carried out to determine the number of chromosomes, as well as their structural changes, if any.

This investigation comprised the study of somatic chromosomes and details of the meiotic process. Special attention also was given to the analysis of a few pachytene cells during meiosis.

MATERIALS AND METHODS

For the study of somatic chromosomes the modified leaf-tip squash method developed by Shambulingappa (4) was followed. Young leaf-tips were fixed in a mixture of three parts of alcohol and one part of propionic acid, the latter having previously been saturated with ferric acetate. The

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[3] "China" is a Puerto Rican localism for orange.

material was left in the fixative for at least 5 hours at a temperature of 16° to 18° C. After this time, the leaf-tips were transferred to a watch glass containing a mixture of nine parts of 0.75-percent acetocarmine and one part of 1 N HCl. The material was heated over a spirit lamp until the solution began to boil. The material was then cooled and washed with pure acetocarmine three or four times and finally left in the acetocarmine solution for 1 hour. When the material was sufficiently stained, a small piece from the tip was placed on a clean slide and squashed in a drop of 0.5-percent acetocarmine under a cover glass.

Detailed meiotic studies were made of the pollen mother cells. For these meiotic studies the simple propiono-carmine smear technique developed by Swaminathan et al. (5) was followed. The analysis of the slides was carried out under a Leitz-Ortholux microscope equipped with phase contrast. All microphotographs were made from fresh slides using a green filter.

OBSERVATIONS

Examination of the leaf-tip squashes revealed that the number of somatic chromosomes of this fruit is 18 (fig. 1). The chromosomes are generally short and vary in size gradually. The length of the shortest pair of chromosomes is less than half the length of the longest pair, with the others falling between these two extremes. The centromeric position is generally median or submedian except in the shortest pair where it is subterminal with an arm ratio of 3:1.

Analysis of a few pachytene cells where chromosomes were reasonably spread out showed non-pairing regions in one or two bivalents (fig. 2). Occasionally a quadrivalent in the form of a cross was also observed at pachytene stage. Mostly bivalents were seen at diakinesis. However, approximately 4 to 5 percent of the cells contained two univalents and 2 to 3 percent had ring-type quadrivalents (fig. 3). Rarely two nucleoli were also observed at this stage (fig. 4). Metaphase I revealed some abnormalities such as presence of 2 or 4 univalents in about 6 to 8 percent of the cells (fig. 5), and rare occurrence of a quadrivalent. In addition, one important observation was made at metaphase I, i.e., the presence of a well-marked heteromorphic bivalent in 60 to 65 percent of the cells (fig. 6) and nine regular bivalents (fig. 7). At anaphase I, abnormalities such as lagging of chromosomes and chromatin bridge without any fragments were noticed in about 8 to 10 percent of the cells (fig. 8). Likewise, during the second meiotic division, abnormalities such as non-orientation of the chromosomes at metaphase II and lagging of chromosomes at anaphase II were seen. Occasionally micronuclei occurred at the tetrad stage.

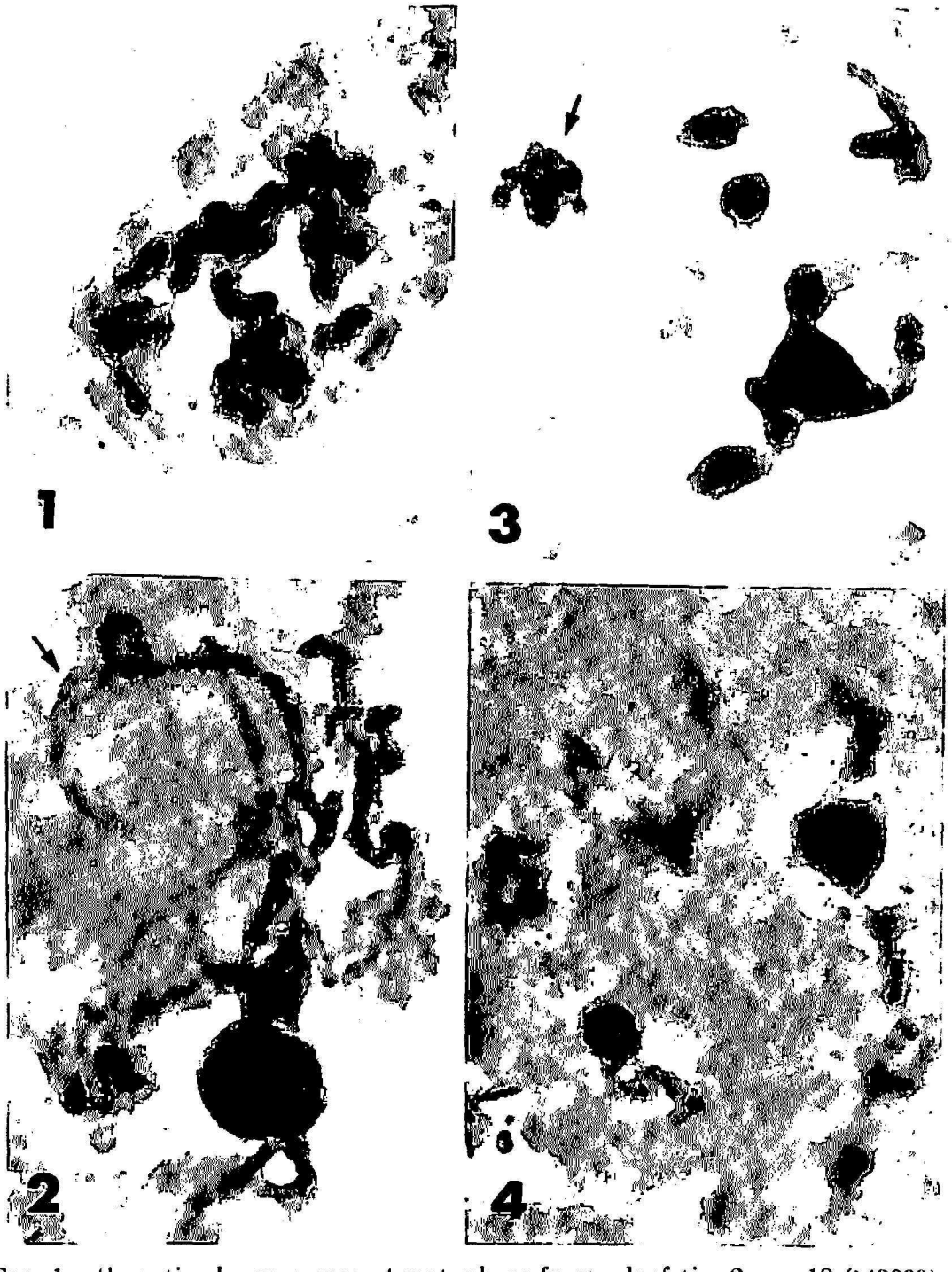

FIG. 1.—Somatic chromosomes at metaphase from a leaf-tip, $2n = 18$ (×3000).

FIG. 2.—Pollen mother cell showing non-pairing region in a bivalent at pachytene (×3000).

FIG. 3.—Diakinesis with $7_{II} + 1_{IV}$ (×1500).

FIG. 4.—Diakinesis showing two nucleoli (×1500).

DISCUSSION

It is believed that most species of *Citrus* are the result of bud mutation or hybridization (6). It is well known that *Citrus* species intercross very easily. Swingle (6) reported several instances of such natural hybrids.

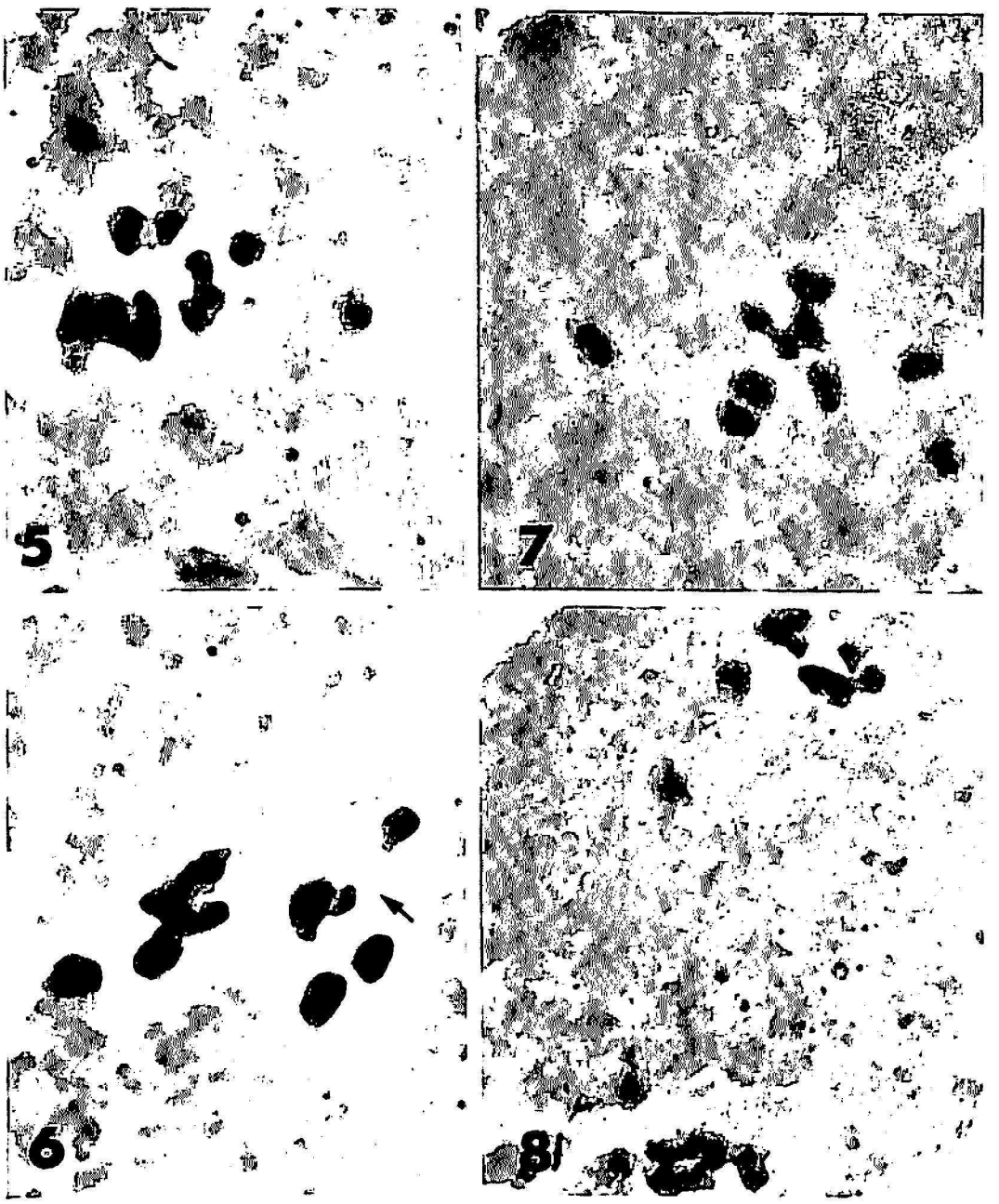

FIG. 5.—Metaphase I with 3 to 4 univalents (×2000).
FIG. 6.—Metaphase I showing a heteromorphic bivalent (×2000).
FIG. 7.—Metaphase I with regular 9 bivalents (×2000).
FIG. 8.—Anaphase I with lagging chromosomes (×1500).

Many of these hybrids produce seeds that apparently are normal and produce plants true to their type. In chironja, as in many other citrus plants, seeds also are produced through nucellar embryony (1).

Virkki (7) observed mostly bivalents at diakinesis and metaphase I during meiosis. His studies, however, were restricted to a limited number of cells. The meiotic studies reported here indicate that the genetic constitution of chironja is not homozygous. The presence of non-pairing regions in one or two bivalents at pachytene, occurrence of univalents and

quadrivalents at diakinesis and metaphase I, presence of a well-marked heteromorphic bivalent at metaphase I and a chromatin bridge at anaphase I are factors that strongly indicate chironja might have originated as a hybrid. No doubt, some of the abnormalities mentioned above have also been reported in other types of citrus (2,3). The existence of such meiotic abnormalities could be attributed to: 1, hybrid origin, in which two progenitors differ genetically, and 2, spontaneous changes in the chromosomes which will be perpetuated through vegetative propagation.

The authors' opinion that chironja may have arisen as a hybrid is based on circumstantial evidence and the comparative external morphological characters between chironja and the supposed parents, viz., orange (*C. sinensis*) and grapefruit (*C. paradisi*). These two species have been under cultivation and planted close to one another in Puerto Rico for many years in the area in which the chironja was discovered.

The material used in the present investigation came from a mixed population of the species mentioned above. It has been observed that the shape of the leaf and the fruit of the chironja closely resemble those of the grapefruit. The flower morphology of chironja is intermediate between the two supposed parents.

Due to limited information, and because of the production of seeds through nucellar embryony, it cannot be said that the progenitors of chironja are orange and grapefruit. Available data, however, suggests hybridization.

SUMMARY

Studies on somatic chromosomes in leaf-tips and meiosis in pollen mother cells were carried out in chironja, a new citrus fruit discovered in Puerto Rico. Meiotic abnormalities such as non-pairing regions at pachytene, occurrence of univalents and quadrivalents at diakinesis and metaphase I and presence of a well-marked heteromorphic bivalent at metaphase I were recorded. Based on the data gathered through meiotic studies, together with morphological and other circumstantial evidence, it appears that chironja may have arisen as a hybrid.

RESUMEN

Se realizaron estudios de los cromosomas somáticos de los ápices foliares y de la meiosis en las células madres del polen de la chironja, una nueva fruta cítrica descubierta en Puerto Rico. Se anotaron las siguientes anormalidades meióticas: regiones no apareadas en la etapa paquiténica, incidencia de univalentes y cuadrivalentes durante la diacinesis, y la presencia de un bivalente heteromórfico bien marcado durante la metafase I. Los datos obtenidos mediante los estudios meióticos, así como la evidencia morfológica y la circunstancial, nos hacen creer que la chironja haya surgido como un híbrido.

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