Meiosis in *Theobroma cacao*[1]

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ABSTRACT

In this study of *Theobroma cacao* no aneuploids were found, all cells studied contained twenty chromosomes. Meiosis proceeds regularly, with pachytene being the stage of longest duration, interphase I or prophase II the shortest. Pairing appears to be bivalent with an occasional pre-metaphase I disjunction to account for the two or four univalents observed. One nucleolus is observed per cell, usually associated with one of the bivalents or two univalents. No multivalents have been observed in the clones studied by us. Disjunction is regular, the chromosomes being divided equally between the two poles in anaphase I. Cell wall formation does not occur at telophase I so that the two nuclei produced at this stage reside within the same cell. The second meiotic division appears to be normal also. Cell walls develop after the tetrad stage of telophase II, leading, eventually, to the four microspores.

COMPENDIO

En este estudio sobre *Theobroma cacao* todas las células estudiadas tenían 20 cromosomas; no se observaron células aneuploides. El proceso de meiosis se desarrolló normalmente, en el que el estadío de paquitene fue el de mayor duración; interfase I o profase II fue la de menor duración. El apareamiento de cromosomas parece ser bivalente pero, ocasionalmente, debido a la separación de cromosomas en la prometafase I, se puede observar la formación de dos o cuatro univalentes. Se observó un nucleolo por célula, el cual está asociado a uno de los bivalentes o a dos univalentes. No se encontraron multivalentes en los clones estudiados. La separación de cromosomas ocurre de manera regular, los cromosomas están igualmente divididos entre los dos polos en la anafase I. La formación de la pared celular no ocurre durante la telofase I, de manera que los dos núcleos producidos en este estadío permanecen en la misma célula. La segunda división meiótica parece transcurrir normalmente. Las paredes celulares se desarrollan después del estadio de tétradas de la telofase II para, eventualmente, producir cuatro microsporas.

INTRODUCTION

Although researchers have observed meiosis during their cytological studies of *Theobroma cacao* (2, 3, 5, 6, 7), clear photomicrographs of meiotic chromosomes have not been published. Therefore, in the course of our cytological studies, we have compiled a series of photomicrographs illustrating the stages of meiosis during pollen development in *T. cacao*.

MATERIALS AND METHODS

Mature trees of *T. cacao* were cultivated in 22-gallon containers in a greenhouse at The Pennsylvania State University. Three different clones were studied. Depending upon the specific tree, or the meiotic stage desired, flower buds 1.5 to 2.2 mm in length were collected between 7 to 8 am, fixed in a modified Carnoy's solution (1:1:3-chloroform: glacial acetic acid: 95% ethanol) for 24 h at 4°C, then stored at 4°C in 70% ethanol until needed. Anthers were excised, hydrolyzed in 1N HCl for 15 min at 60°C, washed in three changes of distilled water, then squashed in iron-propiocarmine/hematoxylin (1) or aceto-orcein stains. Both stains worked equally well. If no staining was desired (for example, where one wishes to observe the chromosomes using phase contrast techniques, followed by further treatment of the unstained chromosomes) the hydrolyzed anthers were squashed in a glycerine/45% acetic acid solution (1 drop of glycerine per ml 45% acetic acid). Preparations were observed at 1200X using a Leitz Dialux 20 Binocular Microscope equipped with

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Zernike phase contrast or Smith interference contrast optics The best results were obtained with the phase contrast system Photomicrographs were taken through the microscope utilizing Kodak Plus-X Pan or Ektachrome 200 films

Observations

The reduction of the somatic complement of chromosomes to the haploid state during meiosis is crucial to the production of gametes in plants and animals In plants, meiosis occurs in the developing anthers or ovaries, giving rise, ultimately, to the pollen grains and egg sac, respectively The process of meiosis in *T cacao* reduces the somatic chromosome number of 20 to the gametic chromosome number of 10

Although the process of meiosis in continuous, various stages have been recognized; most of which will be illustrated and described herein

Leptotene (not shown) – Leptotene is the observable beginning of meiosis The chromosomes in the nucleus of the pollen mother-cell begin to shorten, thicken and become visible at high magnifications The nucleolus may also be seen within the nucleus

Zygotene (Fig 1) – Homologous chromatin strands or chromosomes beging to pair (synapse) Careful study of these strands in the figure shows strands of two different thicknesses The thinner ones have not yet synapsed, the thicker ones have

Pachytene (Fig 2) – The chromosomes have competely synapsed and appear to be single threads They continue to condense and thicken In *T cacao*, pachytene appears to be the stage of longest duration The relative duration of pachytene was determined empirically, in that the largest number of various bud sizes showed pachytene, while the other stages had more restrictive ranges

Late Diplotene/Early Diakinesis (Fig 3) – In diplotene, the chromosomes are very condensed, therefore chromosome associations can be observed As the paired chromosomes pass through diplotene, they begin to separate, but are held together at various points called chiasmata Opeke and Jacob (6) as well as Carletto (2) have reported multivalents in *T cacao* When multivalents are present, they may be observed in the diplotene, diakinesis, and metaphase I stages as groups of three or four chromosomes attached by chiasmata However, in all of the cells in diplotene, diakinesis or metaphase I which we have studied (over 400 cells), we have observed no multivalent chromosome associations, but usually bivalents and occasionally closely associated pairs of univalents Davie (3) reported finding only bivalents at diplotene and metaphase I In diakinesis the chromosomes contract further, separate even move from one another than in diplotene, but are still held together, usually at the ends of the chromosomes, by their chiasmata During diakinesis, the nucleolus disappears In Fig 3, we plainly see nine pairs of chrosomes (bivalents) and two closely associated univalents (arrow) These univalents may result from a pre-metaphase separation (disjunction) of a bivalent, a process known for other plant taxa (4) We have observed two or four univalents in diplotene, diakinesis of metaphase I cells As is usually the case, one of the bivalents is in intimate contact with the nucleolus

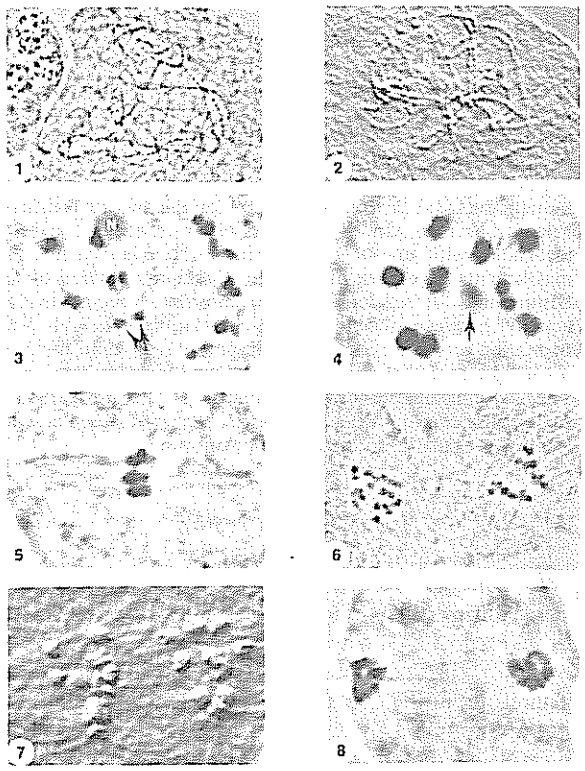


Fig 1. Zygotene 1856X. PC 2. Pachytene 1745X. IC. 3 Late diplotene/early diakinesis 2540X PC. 4 Late diakinesis 3230X. PC 5. Metaphase I. 2436X. PC. 6. Early anaphase I 1365X PC 7. Early anaphase I. 2605X. IC. 8. Late anaphase I 2800X PC. 9. Telophase I. 1629X. PC. 10. Interphase I. 1653X. PC. 11. Prophase II. 1042X. PC. 12. Metaphase II. 1375X PC. 13. Early anaphase II. 2030X. PC. 14. Telophase II. 1044X. PC. 15 Tetrad state. 830X. PC. 16 Microspores. 991X. IC

PC = specimen photographed through Zernike phase contrast optics. optics
IC = specimen photographed through Smith interference contrast optics
N = nucleolus

Late Diakinesis (Fig. 4) – The chromosomes have reached their maximum state of contraction and thickness. The nucleolus has disappeared. Ten bivalents are present in Fig. 4. One of the bivalents (arrow) is slightly out of the plane of the others and so is slightly blurred.

Metaphase I (Fig. 5) – The condensed chromosomes line up on the metaphase plate across the center of the cell, one chromosome of each bivalent on either side of the plate. Each chromosome is attached to a spindle fiber.

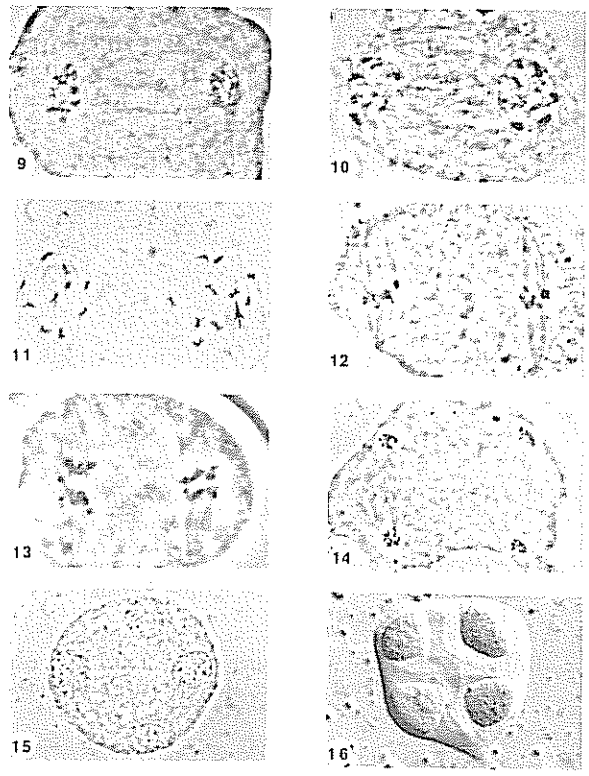
Anaphase I (Figs. 6, 7, 8) – Anaphase I is the stage in which the reduction of the somatic chromosome number takes place. The chromosomes of the bivalentes disjoin (separate), each homologous chromosome being guided to an opposite pole of the cell by its attached spindle microtubule. Two cells are illustrated in Fig. 6. The chromosomes in the cell on the left are beginning to separate, while those in the cell on the right have already completely separated and are moving toward the two poles of the cell. Disjunction in *T. cacao* is regular, in that one half of the chromosomes (ten in *T. cacao)* migrate to each pole (Fig. 7). In Fig. 8, late anaphase, the chromosomes have almost reached the two poles.

Telophase I (Fig. 9) – Telophase I begins when the chromosomes moving in anaphase I have reached the poles of the cell. A nuclear membrane is reconstituted around the chromosomes at each pole. In many plants, a cell wall now develops between the two nuclei; however, in *T. cacao*, the cell wall is not formed, and the two nuclei reside within the same cell.

Interphase I (Fig. 10) – Interphase I is a very brief stage separating the first division of meiosis from the second one; we found it in only one bud of the nearly 800 buds of *T. cacao* examined. In interphase I the chromosomes are once agains diffuse, but usually not as diffuse as in the nucleus of the pollen mother cell before meiosis began.

Prophase II (Fig. 11) – Prophase II is the beginning of the second division of meiosis. The membranes of the two nuclei disappear, while the two groups of recondensing chromosomes appear. One can count ten chromosomes, the gametic chromosome number of *T. cacao*, in each group. Chromatids, the duplicated arms of the chromosomes, are also visible (arrow).

Metaphase II (Fig. 12) – in metaphase II the chromosomes of the two nuclei line up on their corresponding metaphase plate. As in metaphase I, the chromosomes are attached to spindle fibers.



Anaphase II (Fig. 13) – Unlike anaphase I, where the two homologous chromosome were separated and migrated toward the poles, in anaphase II the 10 individual chromosomes at each metaphase plate divide lengtwise, and each group of 10 chromosomes is pulled toward a pole in the cell. Fig. 13 is of an early anaphase II.

Telophase II through to the Microspores (Figs. 14, 15, 16) – Telophase II begins with the four groups of 10 chromosomes each arriving at the poles (Fig. 14). New nuclear membranes are formed around these chromosome groups to give rise to the tetrad (Fig. 15). The chromosomes within these nuclei become diffuse and difficult to see. Cell walls are now formed and four separate microspores, in a tetrahedral arrangement, are observed within the wall of the original pollen mother cell (Fig. 16).

Although the process of meiosis is now terminated, the individual microspores undergo further development and eventually form the pollen grains (7).

CONCLUSIONS

No aneuploids were observed in this study, all cells studied contained 20 chromosomes. Meiosis proceeds regularly in *Theobroma cacao*, with pachytene being

the state of longest duration, interphase I or prophase II the shortest. Pairing appears to be bivalent with an occasional pre-metaphase I disjunction to account for the two or four univalents observed. One nucleolus is observed per cell, usually associated with one of the bivalents. No multivalents have been observed in the clones studied by us. Disjunction is regular, the chromosomes being divided equally between the two poles in anaphase I. Cell wall formation does not occur at telophase I so that the two nuclei produced at this stage reside within the same cell. The second meiotic division appears to be normal also. Cell walls develop after the tetrad stage of telophase II, to eventually produce the four microspores.

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