Florida: Duval Co., 1.0 mi. NW Bayard, 1 ♂ (TTU 16619), 1 ♀ (TTU 16620).

Geomys pinetis mobilensis – Florida: Okaloosa Co., 0.5 mi. W county line on Hwy. 90, 1 ♀ (TTU 16630).

Geomys pinetis pinetis – Georgia: Camden Co., Kingsland, 1 ♀ (TTU 16638); Georgia: Camden Co., 5.9 mi. W St. Marys, 1 ♀ (TTU 16641).

Summary. 4 of the 5 subspecies of *Geomys pinetis* were karyotyped. All specimens examined had a diploid number of 42 and a fundamental number of 80. This karyotype was compared with the described karyotypes of other species of *Geomys* and was considered to be derived from an ancestoral form having a karyotype of about 70 acrocentric elements.

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Mitotic Activity of Endopolyploid Root Cells in *Allium cepa*

The cellular differentiation may be interpreted as a controlled process through which cells with the same genetic charge reach different protein constitutions. In this sense one of the genetic patterns responsible for cytodifferentiation is the differential duplication of the chromosome material. Both in animal and in plant development, somatic polyploidy goes with the differentiation of many cells[1–3]. In addition, polyploidization of differentiated cells is in many cases produced through an endopolyploid process[4,5]. In the course of cytological studies on *Allium cepa* meristems, marked differences of size between the cell nuclei in a same root may be observed which might indicate a polyploidization of certain cells as reported in other species of *Allium*[6].

The aim of the present study is a first approach to the problem of cytodifferentiation by endopolyploidy from diploid meristematic cells, examining the chromosome constitution of polyploid cells of *Allium cepa* roots which have been experimentally induced to divide.

Materials and methods. Allium cepa L. root meristems were used. Onion bulbs were grown in the dark at constant temperature (25 °C) with tap water renewed every 24 h and continuously aerated. Roots from several bulbs were fixed in 3:1 ethanol-acetic and the specimens were prepared by staining the squashes with acetohydrochloric orcein.

Roots were inmersed for 10 min in a solution of thymidine (Schuchardt) diluted with tap water at a concentration of 5 mg/ml and then allowed to recover in renewed tap water. Several roots from each bulb were excised at intervals of 2 h, throughout 4 h recovery from treatments. In order better to separate the metaphase chromosomes, the excised root tips were submerged in a 1 mg/ml colchicine (Sigma) solution for 1 h before fixation. The culture conditions already described were maintained throughout the experiments.

Results and discussion. In all *Allium cepa* bulbs studied, the meristematic cells from the control roots exhibited before the beginning of experiments normal chromosome constitution: $2n = 16$ chromosomes. The root-tips treated, washed and inmersed in aerated tap water, show cells with $4n$ nuclei at 3 to 5 h (after colchicine-treatment).

Most of the cells blocked in metaphase – treatment with colchicine – show the typical *Allium cepa* chromosome complement (Figure a) but a small percentage of cells of bigger size in c-metaphase with the dotation $4n = 32$, or with 16 arrangements of 4 chromatids may be observed (Figure b). These metaphases $4n$ display chromosomes forming pairs next to each other or pairs of chromosomes held together at the region adjacent to the kinetochore, which is known under the name of diplochromosomes. The c-anaphases observed in these polyploid cells consisted of groups of 4 very closely allocated chromatids, in the characteristic form of 'ski pairs'.

The existence of endopolyploid cells may be due to a process of differentiation or dedifferentiation[7], as well as the experimental induction. Concerning the experimental induction of endopolyploidy cited in the literature, one must distinguish between the induction of this state and the mitotic promotion of endopolyploid cells. So, several agents with a stimulating effect on the cell division are known: phytohemaglutinine in lymphocyte cultures[8] and a variety of growth hormones in plant systems[9,10].

Colchicine effect. Regarding the origin of polyploid observed cells, we must reject the possibility of an action of colchicine. C-mitotic agents are known to be able to induce polyploidy and even endopolyploidy[11,12], but that polyploidization is produced by the metaphase parade of the cells, causing the formation of restitution nuclei and the achievement of polyploid level with the posterior chromosome replication of these cells. In our case, the fixation of the roots at the end of the treatment with the c-mitotic agent (1 h) excludes the possibility that the metaphases $4n$ blocked by colchicine owe their ploidic level to the action of this drug.

Thymidine effect. As polyploid cells in mitosis were not observed in control roots, it seems evident that some external factor is responsible for this apparent cell-chymera in the meristematic population (cells $2n$ together with others $4n$), so the question arises whether these tetraploid cells owe their polyploid level to the thymidine-induction. The short space of time (3 h) between the treatment with thymidine and the first fixation of the roots which show $4n$ metaphases seems to discard this nucleoside as the inductor of the observed polyploidy in same point of the S period (there is a quantitative study underway of the kinetics of these cellular events).

Endoreduplicated cells. The induction of endoreduplication (interphase endopolyploidy) has been reported in

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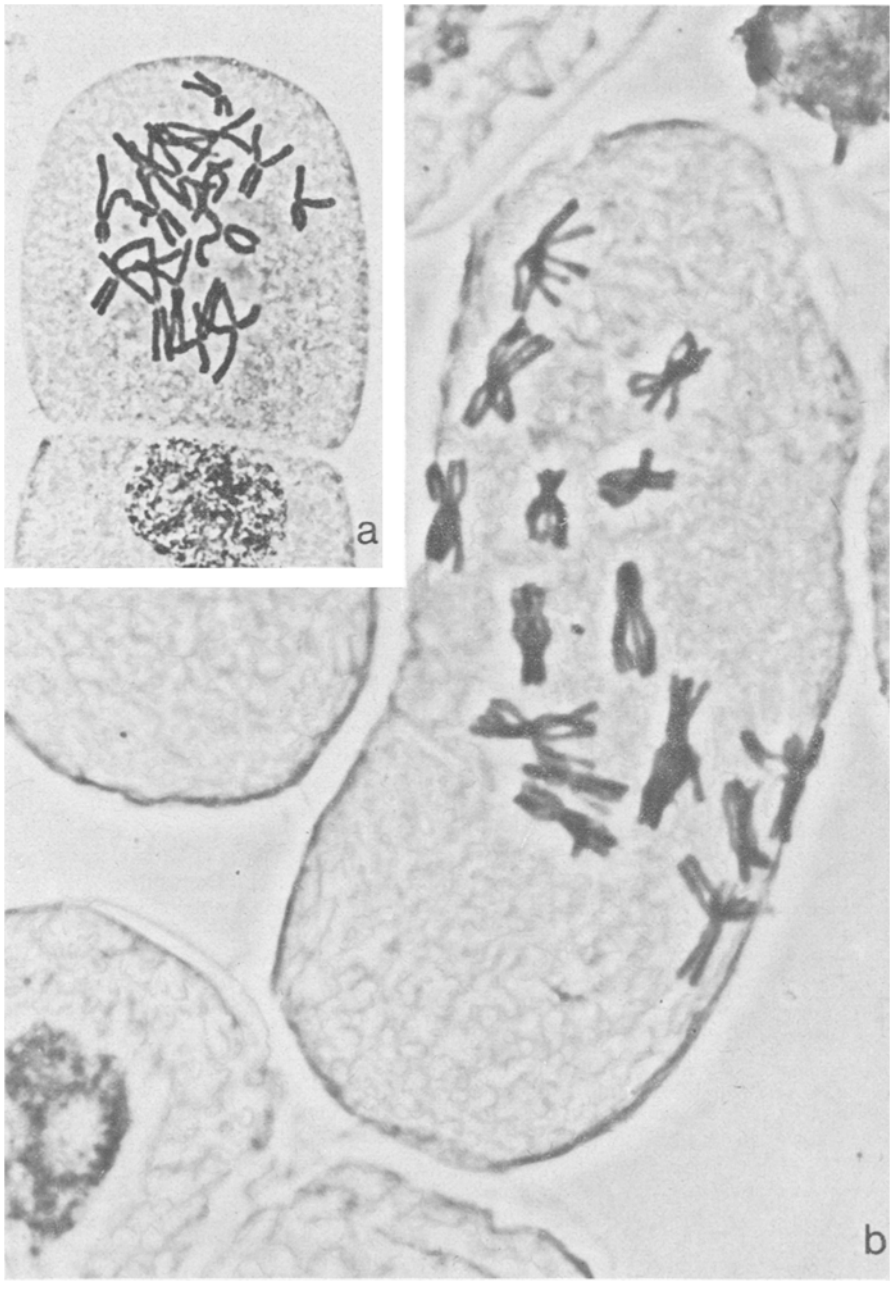

Phase contrast photomicrographs of meristematic cells from root tips of *Allium cepa* after treatment with 5 mg/ml thymidine and 1 mg/ml colchicine. a) Metaphase of diploid cell showing the normal chromosome complement. b) Polyploid metaphase with 16 arrangements of 4 chromatids (diplochromosomes).

cultured mammalian cells with mercaptoethanol[11,13] and colchicine[14], and in *Zea mays* root tips with hydroxylamine sulfate[15]. The present results indicate that the endopolyploidy observed in *Allium cepa* roots is the expression of the thymidine mitosis stimulation of naturally-endopolyploid, non-dividing cells.

Our findings confirm that thymidine, apart from its probable action on the cell cycle kinetics of meristematic population, stimulates the cell division as has been reported in mammalian cells[16]. Concerning the possible mechanism ot endopolyploidy in the cells studied, the existence of diplochromosomes leads us to postulate an endoreduplicative origin for these tetraploid cells. TSCHERMAK-WOESS[17] indicated that the root cells enter the endomitotic cycle, within a distinct region of the root, but the squash technique used did not show the position of the cells $4n$ in the *Allium cepa* roots.

Summary. Thymidine appears as the possible inductor of the mitosis in cells already differentiated, or in the process of differentiation, which normally do not enter division. The endopolyploid process, which may be the expression of chromosomal endoreduplication, seems to play an important role in *Allium cepa* cytodifferentiation.

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