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## Somatic Reduction in Cycads

**Abstract.** *Recurrent somatic reduction is a normal ontogenetic process in apogeotropic roots of cycads, which develop into dichotomously branching coralloid masses. The reduced cells make up part of a ring of differentiated cortical tissue lying midway between the pericycle and the epidermis; they serve as fillers among the large cells and become charged with slime. The differentiated tissue is colonized by a species of blue-green algae.*

Reduction in numbers of chromosomes by dividing somatic cells is a cytological occurrence found occasionally in vascular plants; it is a naturally recurring process in *Allium cepa* L. and *Rhoeo discolor* L. (1, 2), and among hybrids of *Rubus* species (3); it can be induced and accelerated by treatments with chemicals (2, 4). In *Rubus*, somatically reduced cells give rise to phenotypically dissimilar primocanes and to mosaic patterns and checks in leaves (3); in sorghum they give rise to haploid mutants (5).

Somatic reduction seemed to play no significant role in cell differentiation and function; consequently it ceased to evoke interest among plant scientists as a phenomenon warranting continuing study. Cycads provide an exception to the concept of an insig-

nificant role for natural, recurrent somatic reduction, which is found in certain roots that develop into massive coralloid structures. Chamberlain (6) described these roots as follows:

All cycads have remarkable apogeotropic roots. These grow up instead of down, branch dichotomously and profusely, forming coralloid masses above the ground. The vascular structure is about the same as normal roots, but bacteria, or "bacteriodes," get in very near the tip and cause some distortion, which seems to prepare the way for the entrance of a blue-green alga, *Anabaena*. The alga multiplies rapidly, so that there is a blue-green zone midway between the vascular cylinder and the epidermis. While the zone is usually only one cell wide, the cells are so enlarged radially that the zone is readily visible to the naked eye. The tubercles are almost universal in seedlings and are much more prevalent in the greenhouse than in the field.

Meyer (7) reported that the endophytes of cycads nourish themselves at least partly on slime that is secreted by cortical cells into the intercellular spaces in the region of differentiation.

I first chanced upon somatic reduction in *Cycas revoluta* Thunb. ( $2n = 22$ ). The discovery led me to investigate *C. circinalis* L. ( $2n = 22$ ), *Ceratozamia mexicana* Brongn. ( $2n = 16$ ), *Dioon spinulosum* Dyer ( $2n = 18$ ), *Encephalartos horridus* Lehm. ( $2n = 18$ ), *Macrozamia moorei* F. Muell. ( $2n = 16$ ), and *Zamia floridana* A. DC. ( $2n = 16$ ); recurrent somatic reduction is an ontogenetic characteristic common to all.

In view of Chamberlain's statement that all cycads have coralloid apogeotropic roots inhabited by algae, and of my finding that somatic reduction occurs in the several species examined cytologically, one may rationalize that the cytological features seen in these species probably occur in all members of the Cycadaceae.

The cytological phenomena described herein were seen during studies of excised root tips collected and squashed in aceto-orcein by use of normal procedures. In order to make certain that the phenomena seen really do exist and are not artifacts induced by treatment or squashing, sectioned roots were stained with crystal violet by Randolph's procedure (8). This material was not amenable to photography, however, because seldom can all chromosomes be found on the same plane of focus. The fact that the walls of reduced cells are fragile, tending to rupture under the pressure of squashing, accounts for the appearance of chromosomes in fragments of cytoplasm in

some of the photographs (see Fig. 1).

All the photographs (Fig. 1) used for illustrating various features of somatic reduction are of *C. revoluta*, but identical features occur in the other species of cycads examined. Tubercles developing on roots of a young offset of *C. revoluta* are shown in Fig. 1A. The midcortical zone of cells, which is inhabited by the blue-green alga *Anabaena cycadeae* Reinke, is clearly evident in Fig. 1B. Cells in this zone, undergoing reduction, as well as those remaining diploid but becoming large and thin-walled, divide largely along longitudinal lines. There are, however, enough divisions along radial and lateral lines to maintain the zone's width at one or two cells and its position at mid-cortex. Cells interior and exterior to it follow the usual pattern of mitosis and differentiate into typical cortical tissue.

The chromosome complement, a mitotic metaphase, and a large cell in the differentiated zone are shown in Fig. 1, C-E. Somatic reduction begins with a typical metaphase in a diploid initial, like the one in Fig. 1D. Instead of dividing equationally, however, the chromosomes assort into two haploid groups (Fig. 1F). After cytokinesis, the reduced groups may organize nuclei of metabolic cells, or, after a brief interphase, may undergo further reduction (Fig. 1, G-I). Again, metabolic nuclei may be organized (Fig. 1J), or further reduction may occur (Fig. 1K). Reduction can continue to the point where nuclei are organized containing only two chromosomes (Fig. 1L) and sometimes only a single chromosome. The end products are groups of small linear cells interspersed among the larger diploid cells (Fig. 1, M-O).

*Cycas revoluta* and *C. circinalis* have 22 somatic chromosomes, so the first reduction distributes 11 chromosomes to the two daughter cells. Since 11 is an odd number, one daughter cell receives five chromosomes, the other six chromosomes coming from the second reduction (Fig. 1I). The third reduction results in 3:2 and 3:3 distributions, respectively, and final reduction results in nuclei having only one or two chromosomes. In species having 18 chromosomes, such as *D. spinulosum* and *E. horridus*, the first reduction results in daughter cells having nine chromosomes each; assortment thereafter, is uneven because of the odd number, just as it is in *C. revoluta* and *C. circinalis*. In species having 16 chromosomes, such as *Ceratozamia*

*mexicana*, *M. moorei*, and *Z. floridana*, successive reductions result in equal numbers.

Somatically reduced cells are small, with dense cytoplasm and thin, fragile walls (Fig. 1O); they serve as fillers among the large thin-walled cells in the midcortical layer of differentiated tissue. In fact it is they that become charged with slime rather than the in-

tercellular spaces as Meyer suggested (7). I studied the phenomena described in the apical meristems and in regions of cell differentiation and elongation only; I did not follow development beyond the point at which the alga invades the differentiated layer of cortical tissue.

One point is clear: somatic reduction is not induced by the alga; it is

a natural ontogenetic process culminating in the development of slime cells that provide the environment suitable for colonization by the alga. Chamberlain (6) reported the development of coralloid structures from apogeotropic roots in the absence of *Anabaena*, noting that they do not become as massive and fleshy as those that are colonized.

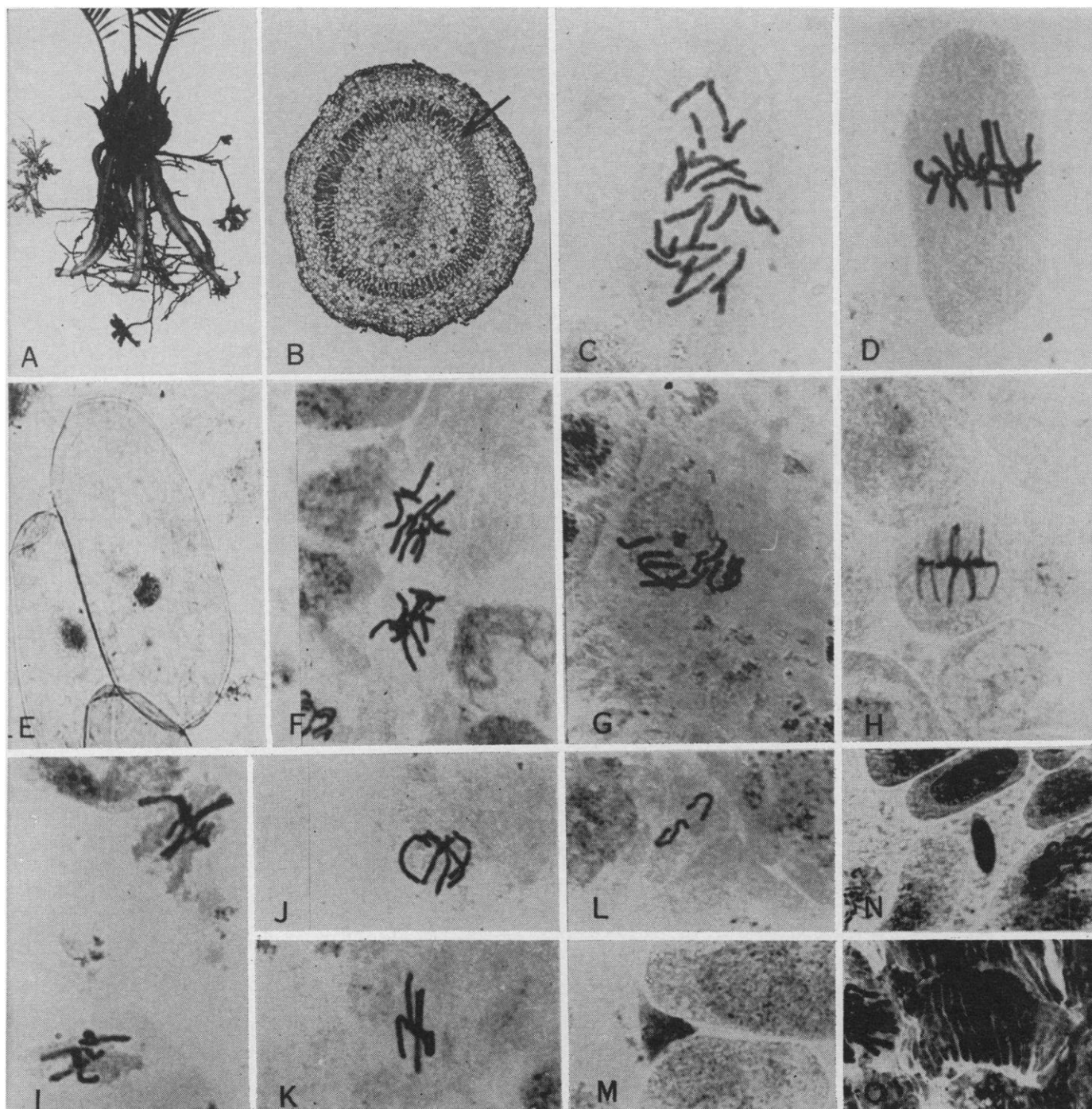


Fig. 1. Somatic reduction in *Cycas revoluta* ( $2n = 22$ ). (A) Coralloid tubercles partly consisting of somatically reduced cells developing on apogeotropic roots. (B) Transsection of apogeotropic root, showing differentiated midcortical zone, site of somatically reduced cells (indicated by arrow). (C) Squashed metaphase cell showing 22 chromosomes. (D) Equatorial view of metaphase. (E) Large diploid cell in midcortical differentiated zone. (F) Chromosomes assorted into two haploid groups of 11 each. (G) Prophase in haploid cell. (H) Equatorial view of metaphase in haploid cell. (I) Assortment of chromosomes of haploid cell into a group of six (top) and a group of five (bottom). (J) Six chromosomes organizing a nucleus. (K) Equatorial view of metaphase with six chromosomes. (L) Reduced cell with two chromosomes. (M and N) Reduced cells among diploid cells. (O) Bands of reduced cells among diploid cells.

The literature provides no answer to the question of what the function of the coralloid root may be, apart from its serving as a place of habitation for the alga. One may suppose the function to be storage of food and water, since it is highly charged with both. The significance, if any, of the relation between alga and plant remains a matter for conjecture.

Tissues inhabited by organisms in certain species of plants (for example, in root nodules of legumes) consist of polyploid cells (8). The existence of somatic reduction as a normal ontogenetic process, participating in development of a functional tissue in a plant, may be unique, however.

A significant aspect of somatic reduction is the ability of cells having reduced numbers of chromosomes to organize metabolic nuclei and function normally.

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### Hexokinase Isozymes in Human Erythrocytes

In 1966 Eaton, Brewer, and Tashian (1) reporting from this laboratory described seven isozymes of hexokinase in hemolyzates from adult human bloods. Subsequently, Holmes, Malone, Winegrad, and Oski (2) published on this subject, including studies of an association between a particular type of hexokinase isozyme and fetal hemoglobin. The method used by Holmes *et al.* as adapted from Katzen and Schimke (3) was considerably different from that which we had used in our studies, and resulted in a demonstration of two bands of activity in

hemolyzates of adult humans, rather than seven. In commenting upon these differences in isozyme patterns in the two studies, Holmes *et al.* suggested that in our system additional, presumably artifactual, bands were being produced in the course of preparing the hemolyzates or during electrophoresis. In the absence of any data, this does not seem to be any more likely, and is perhaps less likely, than the possibility that the method employed by Holmes *et al.* is failing to resolve all of the isozymes actually present. We have studied this problem by reinvestigating the conditions of assay in both systems.

We have used our method (1) as published except for two modifications, which, although they do not affect the number of isozymes, do increase the strength of the bands and make resolution sharper. These modifications are an increase in the concentration of adenosine triphosphate (ATP) in the developing solution from  $2.4 \times 10^{-4}M$  to  $2.4 \times 10^{-3}M$ , and an increase in the concentration of magnesium chloride from  $10^{-3}M$  to  $10^{-2}M$ .

Our initial comparative studies were carried out with the method of Holmes *et al.* (2). Subsequently, two modifications were introduced. The amount of nicotinamide-adenine dinucleotide phosphate (NADP), an expensive reagent, used in the method of Holmes *et al.* is in excess by at least tenfold (over \$30 per 100 ml of staining solution). NADP concentration of  $5 \times 10^{-4}M$  rather than  $5 \times 10^{-3}M$  was employed without affecting results. (We use  $1.2 \times 10^{-4}M$  in our method.) One other modification in the method of Holmes *et al.* was adopted to avoid a partial encroachment of hemoglobin bands on the hexokinase patterns, namely, the gel buffer was pH 8.2, rather than 8.6. These modifications were compared with the original method in detail, and we were certain that the basic results and conclusions from the comparative study were unaffected by the modifications.

Holmes *et al.* list four differences between their technique and the one we have used as possible causes of the difference in results. Referring to our system they say, "Major differences include a preliminary extraction of the hemolyzate with toluene, the absence of 2-mercaptoethanol from the hemolyzate and gel buffers, the use of tris-boric acid gel buffer, and the use of an electric field of 8 volt/cm."

We have been able to demonstrate that, with both systems, extraction of the hemolyzate with toluene does not affect the number of isozymes. Such extraction does increase the sharpness of the bands, probably because it rids the hemolyzate of lipids and stromal components. We have also shown that the presence or absence of 2-mercaptoethanol does not affect the number of bands in either system. Also, it does not appear that the use of tris-boric acid gel buffer rather than the barbital-EDTA (ethylene diaminetetraacetic acid) buffer used by Holmes is crucial, since we have been able to resolve five or six bands of activity while using the barbital-EDTA buffer of Holmes *et al.* (see below). The barbital-EDTA buffer (normally used in hemoglobin electrophoresis) is considerably poorer, however, in providing band separation, and hence, band resolution. Concerning the use of an electric field of 8 volt/cm rather than the 6 volt/cm recommended by Holmes *et al.*, it is true that the lower voltage does not resolve seven isozymes unless the electrophoresis is allowed to run for an inordinately long period, in which case, resolution is poor. A voltage of 8 volt/cm is not unusual for starch-gel electrophoresis, particularly when the electrophoresis is carried out in a cold room, and we are aware of only one possible harmful effect of greater voltage, that is, heating of the gel. Carrying out the electrophoresis at 4°C eliminates this as a problem, and we are certain that voltage differences are playing no role in producing extra bands, although it does affect the resolution of bands by affecting the amount of migration.

The most important difference between the two methods appears to be one not mentioned by Holmes *et al.*, and involves the type of tetrazolium dye used for the histochemical identification of the enzyme. We have used MTT-tetrazolium, whereas Holmes *et al.* used nitro blue tetrazolium. In comparative studies of both methods, the MTT dye consistently resolved more bands of activity than the nitro blue dye. In hemolyzates from human adult blood, six isozymes of the red cells were uniformly and reproducibly detected with, basically, the technique of Holmes *et al.*, but with the use of MTT-tetrazolium as the staining dye and a slightly higher voltage.

Our principal conclusion then is that the method of Holmes *et al.* does not resolve all of the hexokinase isozyme