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The Male Meiotic Cycle in the Genus *Eucalyptus*

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Plates V-VI

This investigation was undertaken originally to determine the chromosome number of various eucalypts. Observations of the course of meiosis have been made in the pollen mother cells of eight species, and, as no work seems to have been carried out on meiosis in *Eucalyptus* previously, it seemed desirable to place the main results of the investigation on record in view of the important place which the genus occupies in our flora. The account of the meiotic cycle given should be reasonably typical of the process in Tasmanian forms at least.

MATERIAL AND TECHNIQUE

The collection of buds at the appropriate stage of development was made usually on a clear sunny day between noon and 4 p.m. The anthers were squeezed from the young buds into a tube containing fixative, which was then partially exhausted with a vacuum pump, in order to remove air from the tissues, and so ensure rapid fixation. The small size of the buds in some species made this process long and tedious.

The fixatives employed were (*a*) an alcohol, acetic acid, ferric hydrate mixture, (*b*) Navashin's modification of Karpechenko's fluid, and (*c*) Helly's liquid, a mercury bichloride fixative used as a check against artifacts which might be due to acetic acid fixation.

The first fixative was used in all preliminary work, for which it was admirably suited, as the light, slightly waxy anthers sank in it immediately, and were rapidly fixed. After fixation for two or three hours the anthers were washed in absolute alcohol, and were then ready for clearing and paraffin infiltration. Despite the apparently drastic nature of this fixative, the resulting preparations were

remarkably good, and compared very favourably with material fixed in Navashin and Helly. These latter fixatives were relied on for all fine detail in the cytological structures.

Sections about 20 μ in thickness were cut by the paraffin method, and stained in gentian violet, haematoxylin, or sulphurous fuchsine (Feulgen). Photographic records have been made extensively during the investigation, as it is considered that the camera provides the best means of illustration, whenever it is applicable.

Chromosome Number

The chromosome number has been determined for *E. globulus*[1], *E. Johnstoni*[1], *E. linearis*, *E. pauciflora*, *E. viminalis*, *E. obliqua*, *E. salicifolia*, and *E. cordata*, which are fairly representative of the main types of Tasmanian eucalypts. In each of these species the haploid number has been found to be eleven.

The stage best suited to the purpose of counting the chromosomes is that of late diakinesis, which is particularly conspicuous in most of the material examined. The chromosomes at this juncture are compact, well-separated bodies dispersed throughout the nucleus (Plate V, figs. 1 and 2). This has been used as a standard stage for counting. In metaphase I and anaphase I the chromosomes are smaller and more closely crowded, often showing a tendency to clump, an appearance doubtless exaggerated by fixation. Many well-figured plates have been examined nevertheless, and photographs of some are appended. The counts have, of course, been confirmed at practically every subsequent stage. For example, the telophase II nuclei shown in Plate VI, figures 6-7, are from an anther in which it was possible to make many unambiguous counts, in spite of the fact that this stage is particularly difficult. Prophase II (Plate VI, fig. 3) includes one phase in which good counts can be made.

The Meiotic Cycle

The course of meiosis is of the type associated with a vesicular[2] nucleus. The second prophase appears to follow an unusual course. A detailed account of those phases which appear quite normal will not be given, but typical photographs are shown. The prophase I stages from leptotene to diplotene have not been examined in great detail, but there is nothing to suggest deviation from the normal here. It is hoped to examine the chromosome figures and chiasmata at some later time.

Metaphase I chromosomes appear as spheroidal bodies, and this, together with early anaphase figures, suggests complete terminalization of chiasmata. In metaphase I and anaphase I plates in *E. globulus* there is evidence of secondary pairing (Plate V, fig. 3, and

[1] See References (p. 44). [2] See References (p. 44).

cf. fig. 6). Owing to variability in the metaphase arrangements which have been observed, nothing further can be said about the pairing tendency yet, but there are many cells exhibiting chromosome patterns which make the conclusion difficult to resist.

The most interesting feature of the meiotic cycle is a very characteristic appearance which occurs during prophase II (Plate VI, figs. 2-3). In this, the chromosomes appear as well-figured bivalents, distributed in each nucleus over that half of the nuclear boundary which is nearest to the centre of the cell. When viewed from the poles of the cell they are seen to exhibit two saucer-like distributions, whose convex surfaces are towards each other.

The course of the cycle from anaphase I to metaphase II is briefly as follows:—

The formation of an interphase nucleus from the anaphase I plate commences with the appearance of nuclear sap, and hence the delimination of a nuclear boundary, accompanied by the movement of the chromosomes out towards this boundary. The amount of chromatin in the chromosomes decreases, and a single nucleolus is formed. The organization of the interphase nucleus is carried out very completely, particularly in *E. obliqua* (Plate V, fig. 8).

In early prophase II the chromosomes reappear, being distributed throughout the nucleus, which, by this time, has increased considerably in diameter (Plate V, fig. 9; Plate VI, fig. 1). The form of the chromosomes is difficult to ascertain here, but becomes clearer as the prophase continues. The later stages are characterized by a movement of the chromosomes in each nucleus towards the sister nucleus, culminating in their arrangement on the nuclear boundaries as two saucer-shaped distributions, the convex faces being towards each other. These are seen in lateral view in Plate VI, fig. 2, and in polar view in fig. 3. The two chromatids of each bivalent are attached to each other near their centres, presumably the region of the centromere, their distal parts being generally widely separated. The nucleolus has disapeared by this time, although occasional cells were found in which it had persisted to this stage (Plate VI, fig. 4, cell A).

Following this, the chromosomes condense into a compact form, the mutual repulsion of the chromatids in each bivalent having ceased apparently, while the nuclear-cytoplasmic interface disappears, and the chromosomes pass to their metaphase positions on the two spindles. The cell would appear to be in an unstable state at the time of the disappearance of the nuclear boundary, as then there is a marked tendency for the chromosomes to become aggregated. Fixation apparently causes a collapse of the matrix in which they are distributed. Judging from the infrequency of the occurrence of clearly defined chromosomes on plates in metaphase II, this phase must be passed through very rapidly.

Anaphase II and telophase II are quite normal.

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REFERENCES

[1] McAULAY, A. L., CRUICKSHANK, F. D., and BRETT, R. G., 1936.—*Nature*, 138; 550.
[2] MANTON, T., 1935.—*Proc. Roy. Soc., Series B*, Vol. 118; 522.

PLATE V

FIG. 1.—Cells of *E. obliqua* in diakinesis. In one cell eleven tetrads are just discernible. A nucleolus is present. × 900. Helly, gentian violet.

FIG. 2.—Cell of *E. Johnstoni* in late diakinesis. The nucleolus has disappeared. The photograph was made in two exposures superimposed on the same negative. × 1000. Alcohol-acetic, gentian violet.

FIG. 3.—Metaphase I. in *E. globulus*. Note evidence of secondary pairing and cf. fig. 6. × 2000. Helly, gentian violet.

FIG. 4.—Metaphase I. in *E. Johnstoni*. × 2000. Alcohol-acetic, gentian violet.

FIG. 5.—Early anaphase I. in *E. globulus*. × 2000. Helly, gentian violet.

FIG. 6.—Anaphase I. *E. globulus*. × 2000. Helly, gentian violet.

FIG. 7.—Late anaphase I. *E. globulus*. × 2000. Helly, gentian violet.

FIG. 8.—Late telophase I. *E. obliqua*. × 2000. Helly, gentian violet.

FIG. 9.—Early stage in prophase II. in *E. obliqua*. × 2000. Helly, gentian violet.

PLATE VI

FIG. 1.—Later stage in prophase II. *E. obliqua.* × 2000. Helly, gentian violet.

FIG. 2.—Prophase II. *E. globulus.* 'Saucer' stage in lateral view. × 2000. Helly, gentian violet.

FIG. 3.—Prophase II. *E. globulus.* 'Saucer' stage seen from above, showing structure of bivalents. × 2500. Helly, gentian violet.

FIG. 4.—Cells of *E. globulus* in various stages of prophase II. Cell 'A' has almost reached the 'saucer' stage, nucleoli still present; cell 'B' is that shown in fig. 2; cell 'C' shows an oblique view of the 'saucer' stage, both nuclei being visible because of low magnification; cell 'D' shows chromosomes clumped approaching metaphase II. × 900.

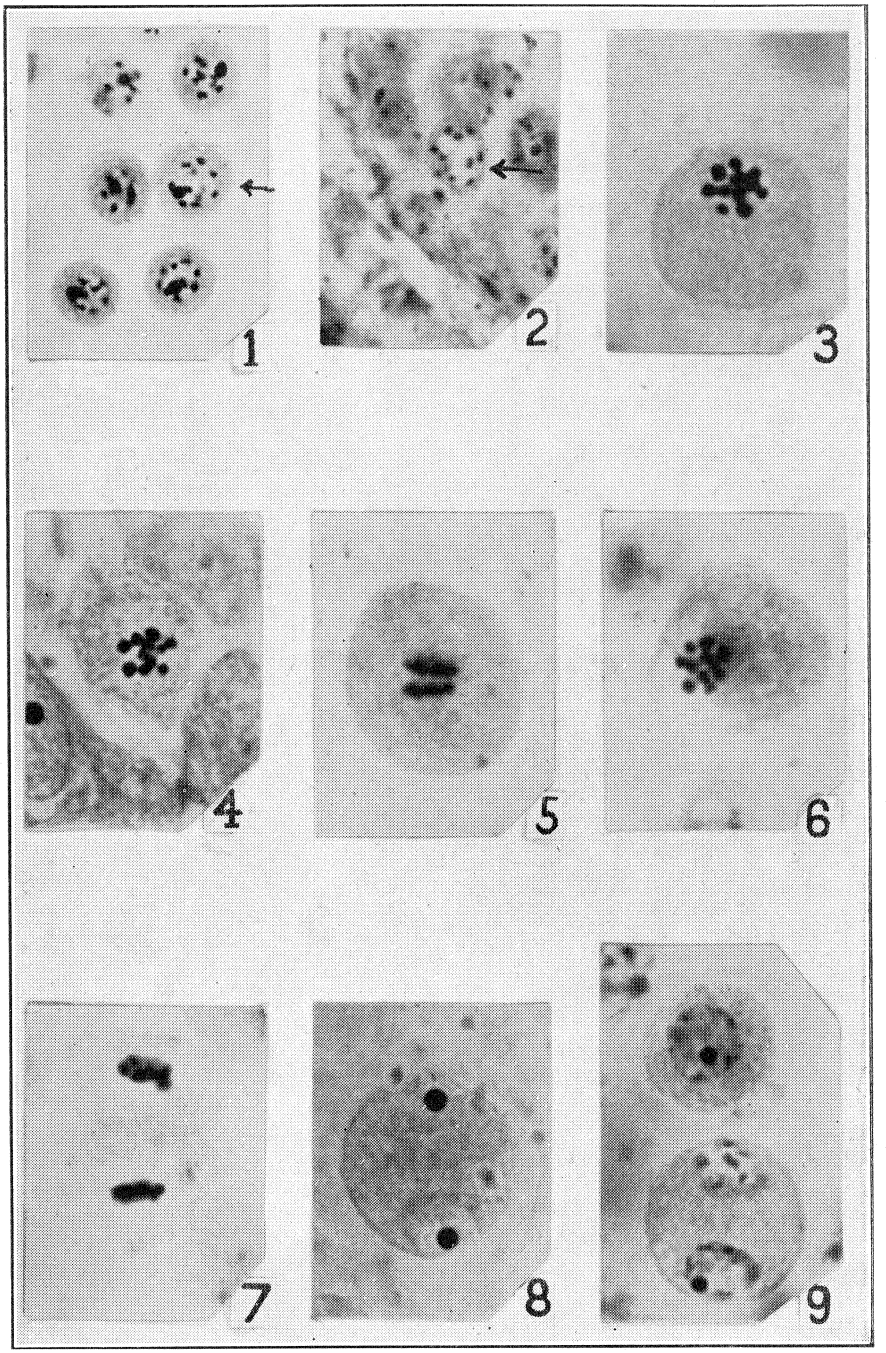
FIG. 5.—Metaphase II. *E. globulus.* × 900. Helly, gentian violet.

FIG. 6.—Cell in telophase II. in *E. obliqua.* × 2000. Helly, gentian violet.

FIG. 7.—The fourth nucleus of the cell shown in fig. 6. Eight chromosomes are shown on the periphery; the other three, one of which is just visible, are arranged close to the nucleolus. × 2000.

FIG. 8.—Later stage in telophase II. *E. globulus.* × 2000. Helly, gentian violet.

FIG. 9.—Completion of telophase, showing daughter cells fully formed within wall of parent cell. *E. globulus.* × 2000.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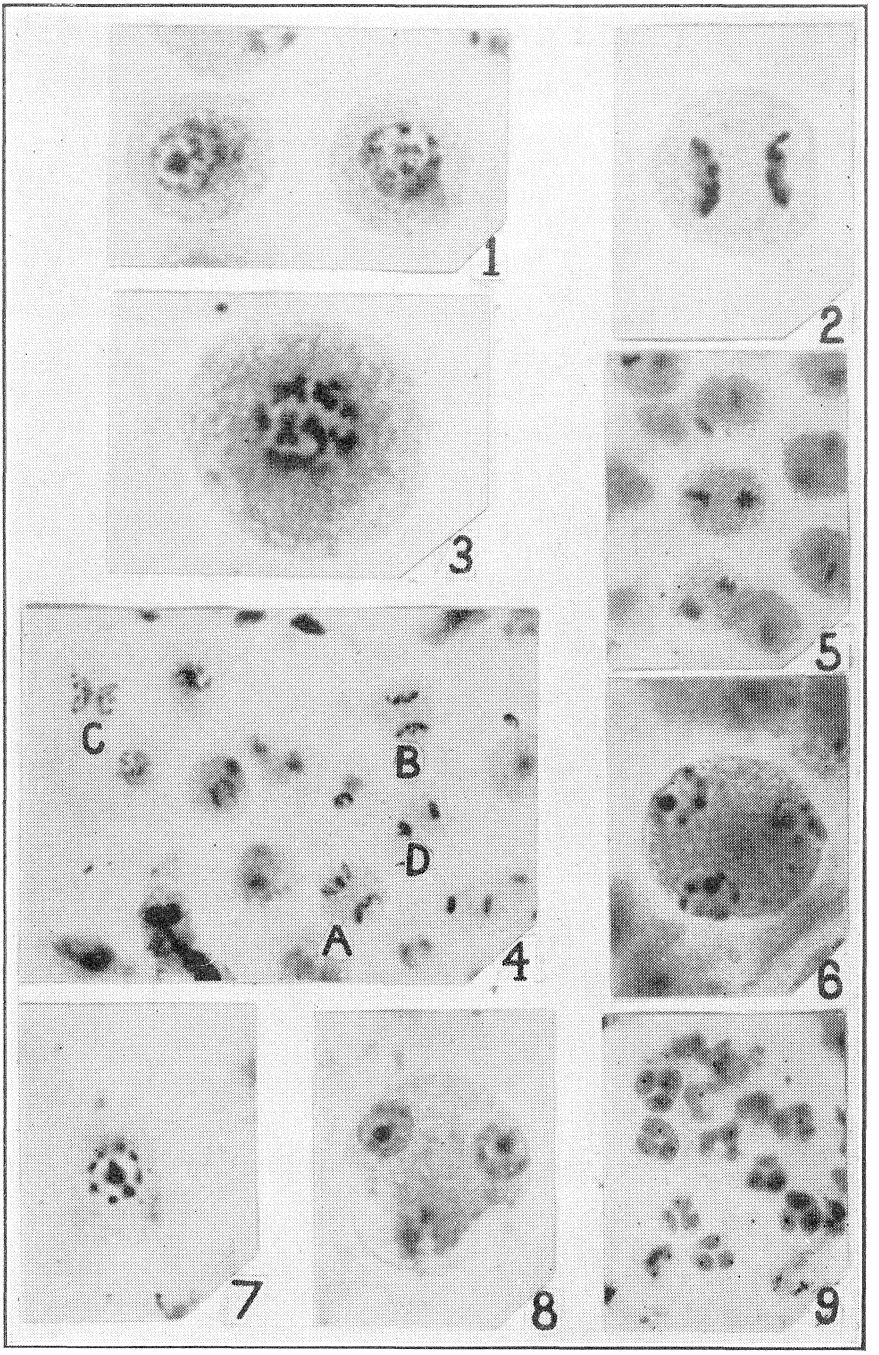
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