THE EMBRYO SAC OF *FRITILLARIA LILIACEA*.

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(Communicated by Prof. P. Maheshwari, F.N.I.)

(*Received November 18, 1949; read January 2, 1950.*)

INTRODUCTION.

Fritillaria liliacea belongs to the family Liliaceae, tribe Lilioideae. Three species of the genus, *F. persica* (Bambacioni, 1928*a*), *F. imperialis* (Lenoir, 1934) and *F. pudica* (Sax, 1916; Eunus, 1950), have so far been studied. It was in *F. persica* that a 1+3 arrangement of the megaspore nuclei and the so-called 'Carano-Bambacioni Effect' (see Maheshwari, 1946) were first discovered by Bambacioni (1928*a*). After the primary four-nucleate stage, there is a secondary four-nucleate stage in which the two micropylar nuclei are haploid and the two chalazal nuclei are triploid. In the next stage the basal nucleus remains undivided resulting in a seven-nucleate embryo sac with only two antipodal cells.

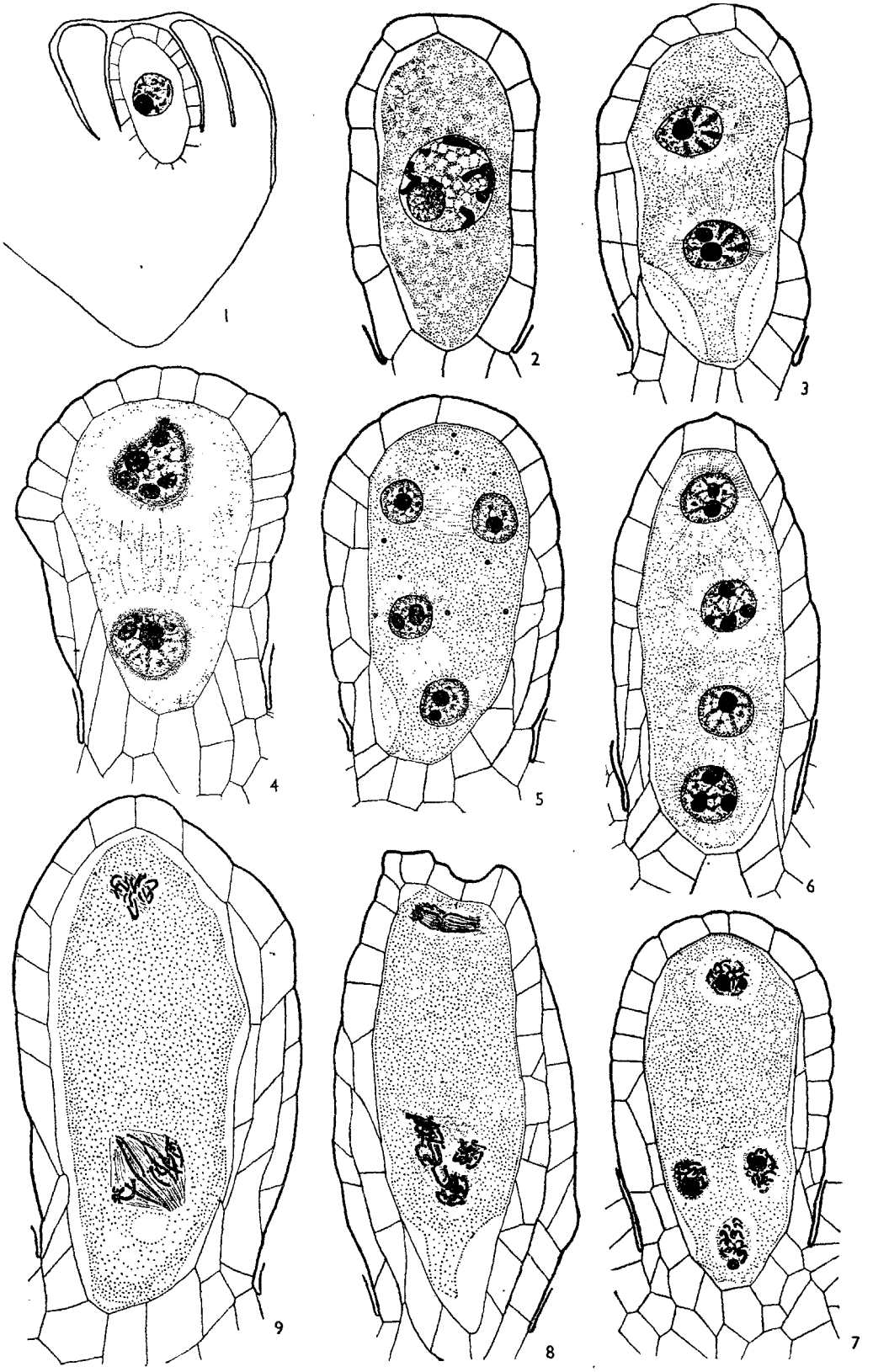
MATERIAL AND METHODS.

The material used for this investigation was obtained from Mrs. M. S. Cave (University of California) by Prof. P. Maheshwari and very kindly passed on to me for study. Sections were cut 15μ thick and stained in Heidenhain's iron-alum haematoxylin.

OBSERVATIONS.

The trilocular ovary has a large number of anatropous, bitegmic ovules arranged in two rows on an axile placenta. The integuments are well advanced even at the megaspore mother cell stage (Fig. 1). The outer integument is three- to four- layered. The inner is two- to three- layered but later it becomes four cells thick at the micropylar end.

A single hypodermal archesporial cell differentiates in the nucellus. This functions directly as the megaspore mother cell without cutting off any wall cell. Its cytoplasm is finely granular, dense, and devoid of any conspicuous vacuoles (Fig. 2). Following the first reduction division one daughter nucleus migrates to the micropylar end and the other to the chalazal end without any wall formation between them (Figs. 3, 4). Each nucleus has several prominent nucleoli. The second reduction division produces the four free megaspore nuclei arranged diagonally (Fig. 5) or in a linear row (Fig. 6). In slightly older ovules only one megaspore nucleus is seen at the micropylar end and three at the chalazal end thus producing a 1+3 arrangement (Fig. 7). All the four megaspore nuclei divide simultaneously (Fig. 8), but the spindles of three chalazal nuclei fuse to form a large common spindle (Fig. 9) which may be situated diagonally (Fig. 10) or at right angles to the long axis of the embryo sac. At the end of the division there is seen a secondary four-nucleate stage with two small haploid nuclei at the micropylar end and two large triploid nuclei at the chalazal end (Figs. 10, 11). Earlier investigators (Sargant, 1896; Mottier, 1897) assumed the larger size of the two chalazal nuclei as probably due to their own growth because this condition was found only in the larger embryo sacs.



TEXT-FIGS. 1–9.—(See p. 89 for Explanation).

The two chalazal nuclei elongate and become slightly flattened, while remnants of the spindle fibres can be identified between them for a long time. Vacuolation begins in the embryo sac at about this stage (Figs. 10, 11). In *Lilium philippinense* (Santos, 1937), the condition is similar, but in most other plants showing the Fritillaria type of development vacuolation seems to begin much earlier. As examples may be cited *Tulipa* (Bellows and Bamford, 1941), *Tamarix ericoides* (Sharma, 1939), *Piper longum* (Joshi, 1944), *Clintonia* (Walker, 1944; Smith, 1943), *Erythronium dens canis* (Hruby, 1934) and *Erythronium tuolumnense* (Cave, 1942). Joshi's (1940) figures of *Gagea fascicularis*, showing vacuolation only at the mature embryo sac stage, seem to be rather unusual in this respect. In *G. graminifolia*, studied by Romanov (1936), vacuolation is clearly seen at the time of the third division in the embryo sac prior to the secondary four-nucleate stage.

At the time of the fourth and the last nuclear division a vacuole appears in the upper part which gradually enlarges and occupies the central part of the embryo sac separating the micropylar and the chalazal groups of nuclei. Only the two micropylar nuclei and the upper triploid nucleus take part in this division (Fig. 12) producing a 7-nucleate embryo sac with four nuclei at the micropylar end and three at the chalazal. The basal chalazal nucleus shows signs of early degeneration.

The four micropylar nuclei give rise to the egg apparatus and the upper polar nucleus. Of the three chalazal nuclei, the uppermost, which is the largest, functions as the lower polar nucleus and the remaining two as the antipodal cells (Fig. 13). The three cells of the egg apparatus are more or less symmetrically arranged. The largest of the three functions as the egg. Neither the synergids nor the egg showed any prominent vacuolation.

Abnormalities.

A few abnormal embryo sacs were noticed which are briefly described below.

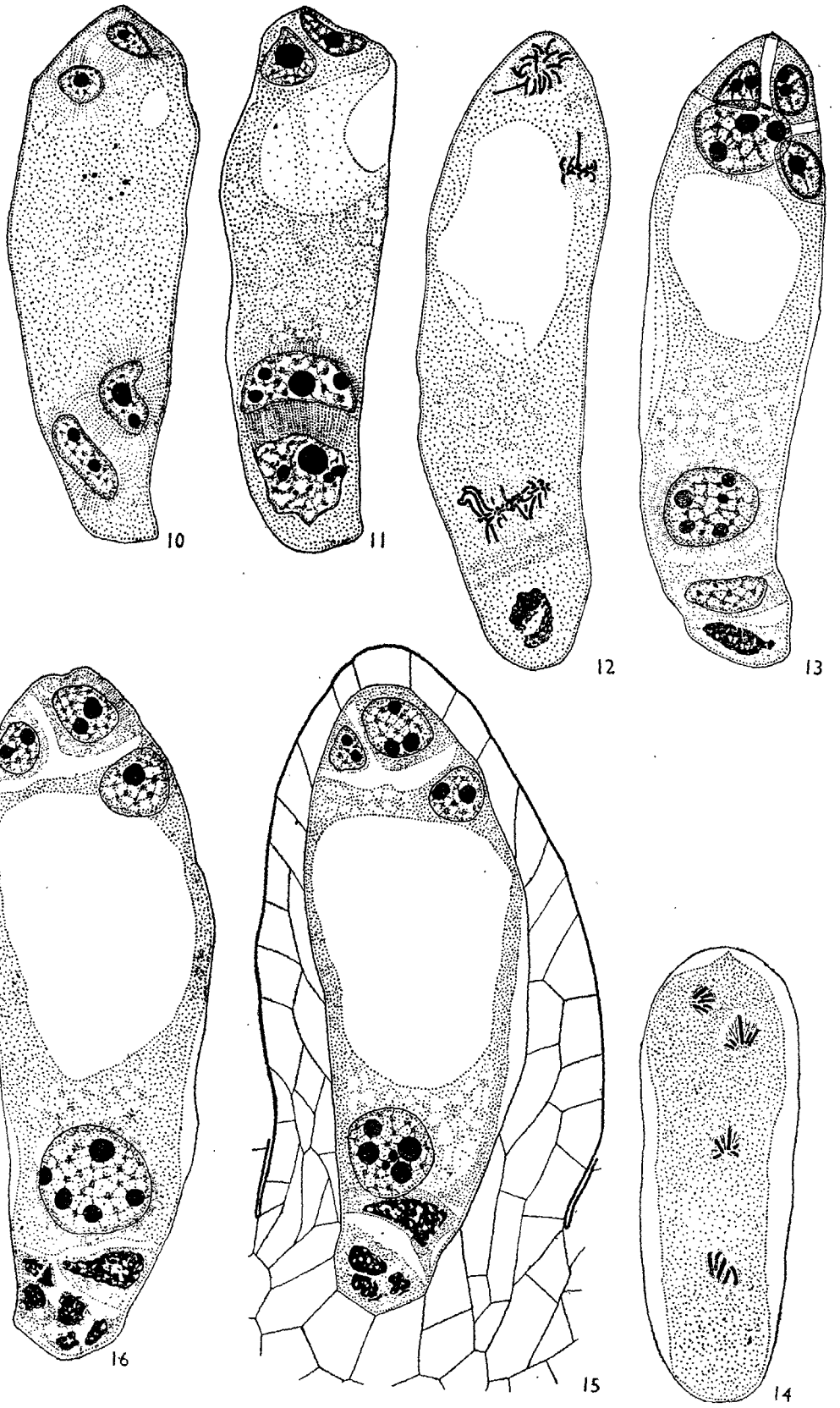
In one case all the four megaspore nuclei of the primary four-nucleate stage showed signs of early degeneration while in some others only one, two or three nuclei were degenerating.

One embryo sac, of a smaller size than the others, showed all the four megaspore nuclei in division (Fig. 14) indicating an Adoxa type of development. This is very important because it indicates that there is no fixed mode of development in *Fritillaria*. Such a condition has also been recorded in *Erythronium* (see Maheshwari, 1946) and some other genera with tetrasporic embryo sacs.

Sometimes the egg apparatus may consist of only a single synergid and the egg, the resulting embryo sacs being 6-nucleate (Figs. 15, 16). From Sax's (1916) Figs. 2 and 3 of *Fritillaria pudica* and Joshi's (1944) Fig. 23 of *Piper longum* also it appears that sometimes only one synergid may be formed instead of two. Although Sax mentions that 'in practically all cases one of the synergids is destroyed by the entering pollen tube', yet there seems to be no trace of the degenerating synergid in his Figs. 2 and 3. It is possible that at the time of the fourth nuclear division one of the micropylar nuclei also fails to divide in addition to the basal chalazal nucleus.

Explanation of Figures (on p. 88).

Figs. 1–9. Early stages in the development of the embryo sac. Fig. 1. L.s. ovule at megaspore mother cell stage, × 206. Fig. 2. Megaspore mother cell in meiotic prophase, × 432. Fig. 3. Same, late telophase, × 432. Fig. 4. Two nuclei arising after the first reduction division; remnants of the spindle fibres are still visible, × 432. Fig. 5. Late telophase of the second reduction division, × 432. Fig. 6. Four free megaspore nuclei arranged in a linear row, × 432. Fig. 7. Typical 1+3 arrangement of the megaspore nuclei, × 432. Fig. 8. Third nuclear division showing fusion of the three chalazal spindles, × 432. Fig. 9. Same, more advanced stage, × 432.



TEXT-FIGS. 10–16.—(See p. 91 for Explanation).

Some embryo sacs were observed with the synergids or the entire egg apparatus in process of degeneration before the entry of the pollen tube.

Frequently, the nucleus of the basal antipodal cell fragments (Fig. 15) into as many as 6 or 7 nuclei resulting in the formation of supernumerary antipodal cells (Fig. 16). Such a condition has not been reported previously in *Fritillaria* but was seen in several embryo sacs of *F. liliacea*. Early degeneration sets in, however, and the antipodal cells appear as structureless darkly staining masses surrounded with shrunken cytoplasm.

Summary.

1. The trilocular ovary has numerous anatropous, bitegmic ovules in two longitudinal rows on an axile placenta.

2. The hypodermal archesporial cell functions directly as the megaspore mother cell which undergoes the usual reduction divisions and produces the primary four-nucleate stage.

3. Following a 1+3 stage all the megaspore nuclei divide simultaneously but the three chalazal spindles unite to form a large common spindle resulting in a secondary four-nucleate embryo sac.

4. One more division occurs, the basal triploid nucleus usually remaining undivided.

5. The mature embryo sac is 7-nucleate and consists of four haploid cells (the egg, two synergids and the upper polar) and three triploid cells (the two antipodal cells and the lower polar nucleus). There is no sharp distinction between the synergids and the egg. All the nuclei of the embryo sac show several nucleoli in each.

6. In one case all the four megaspore nuclei were seen dividing independently indicating an Adoxa type of development. Sometimes only one synergid was seen in place of two. Frequently, the basal antipodal cell fragments and gives rise to supernumerary antipodal cells.

Acknowledgments.

It is a great pleasure to acknowledge my indebtedness to Dr. B. M. Johri under whose guidance the work was carried out. I am indebted to Prof. P. Maheshwari for providing the material and going through the manuscript and to Dr. B. Singh of the B.R. College, Agra, for his advice and encouragement.

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Explanation of Figures (on p. 90).

Figs. 10–16. Later stages in the development of the embryo sac. Figs. 10-11. Secondary four-nucleate stages, ×432. Fig. 12. Fourth nuclear division in which the lowest chalazal nucleus has failed to divide, ×432. Fig. 13. Mature seven-nucleate embryo sac, ×432. Fig. 14. An abnormal case in which all the four free megaspore nuclei are dividing independently, ×432. Fig. 15. Embryo sac showing a single synergid, egg, two polar nuclei and two antipodal cells of which the basal shows three nuclei, probably formed by a fragmentation of the original nucleus, ×432. Fig. 16. Same, the basal cell has divided into six fragments, ×432.

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