MACROSPOROGENESIS AND EMBRYO-SAC DEVELOPMENT IN EUCHLAENA MEXICANA AND ZEA MAYS

By D. C. Cooper

Assistant professor of genetics, Wisconsin Agricultural Experiment station

INTRODUCTION

Studies of macrosporogenesis and the development of the macrogametophyte in the Maydeae are almost wholly limited to corn (Zea mays L.), and little is known concerning these stages in the life history of other members of this tribe. True (12), Guignard (7), and Pindexter (9) described the ovules of corn, observed double fertilization, and briefly outlined the early stages in the development of the embryo. Weatherwax (13, 14, 15) and Miller (8) studied the details of macrosporogenesis and embryo-sac development. Later Weatherwax (17) compared the development of the endosperm of Coix (Coix lacryma-jobi) with that of corn. More recently Randolph (10) has presented an account of the developmental morphology of the caryopsis of maize in which embryogeny, endosperm formation and structure of the pericarp are fully described. The detailed cytological studies of chromosome morphology in teosinte (Euchlaena mexicana Schrad.) and teosinte-corn hybrids (Beadle (2, 3); Emerson and Beadle (6); Arnason (1)), as well as the genetical analyses of such hybrids, warrant a comparative study of macrosporogenesis and embryo-sac development in the two parents and the hybrid.

MATERIALS AND METHODS

The annual type of Florida teosinte was used in the present investigation. This variety, at the latitude of Madison, Wis. (43°), does not come into flower under field conditions until late in the fall. In order to force the plants into flower so as to make the desired crosses with corn, the seeds were planted in 8-inch pots in the greenhouse about the middle of April. The pots were taken to the field about May 15, the precise date depending upon the weather, and sunk into the ground so that the tops of the pots were flush with the level of the soil. A short-day treatment such as was described by Emerson (5) was begun at this time. This was accomplished by placing barrels over the plants at 5 o'clock each afternoon and removing them at 7 o'clock the next morning. This practice was continued until the plants began to blossom (about July 15), when the barrels were removed entirely. Plants treated in this manner flowered abundantly and set a good crop of seed. Reciprocal hybrids between yellow dent corn and teosinte were obtained.

Pistillate spikelets of teosinte and of corn of various ages were collected during the summer of 1934, dipped in Carnoy’s fluid for a 1

1 Received for publication Apr. 10, 1937; issued October 1937. Paper from the Department of Botany and the Department of Genetics (no. 211), Wisconsin Agricultural Experiment Station.

2 The writer desires to express his appreciation for support received from the Wisconsin alumni research fund during the period of these investigations.

3 Reference is made by number (italic) to Literature Cited, p. 550.

Journal of Agricultural Research, Washington, D. C.

Vol. 55, No. 7  
Oct. 1, 1937  
Key no. Wis.-90

22709—37—5 (539)
short time (one-half to 1 minute), and transferred directly to Kar-
pechenko’s modification of Nawaschin’s fluid. The material was
allowed to remain in the latter fluid from 36 to 48 hours, then washed,
dehydrated, and embedded in 52° paraffin in the usual manner, cedar
oil being used as the clearing agent. After embedding, longitudinal
sections were cut at thicknesses varying from 12μ to 20μ. The older
material was cut thicker in order to obtain the embryo sac in as few
sections as possible. The sections were mounted serially, stained
in dilute Delafield’s haematoxylin, and counterstained in safranine.
This combination is valuable because of the clear differentiation of the
cytoplasm, spindle, and forming cell plate.

OBSERVATIONS

EUCHLAENA MEXICANA

Development of the Ovule

The single ovule first appears as an erect, rounded protuberance at
the base of the carpel, later becoming more or less conical in shape.
An apical hypodermal cell becomes differentiated as the primary
archesporial cell while the ovule is in an upright position. This cell
(fig. 1, A, a) differs from its neighbors in being conspicuously larger,
with a somewhat denser cytoplasm and a larger nucleus (fig. 1, A).
Shortly after the differentiation of the primary archesporial cell the
integuments develop as outgrowths of the epidermis near the base of
the ovule on the side away from the central axis of the pistillate spike.
The inner integument (i) is the first to appear and shortly after
its initiation and just below it the primordium of the outer in-
tegument starts development. The entire ovule grows more rapidly
on the side on which the integuments first appear so that it bends
toward the main axis of the spike (fig. 1, D). The bending takes
place in the region of the origin of the integuments and continues
until the mature ovule assumes an amphianatropous position, i. e.,
it is a type intermediate between the amphitropous and the an-
tropous forms but approaches the latter (fig. 2, L).

The inner integument, which remains two layers of cells in thick-
ness, except at the apex in the mature stages, grows so that it has
reached a level even with the tip of the ovule by the time the four
spores are formed as a result of macrosporogenesis (fig. 2, E). The
ovule at this time is so bent that the longitudinal axis of the row of
spores makes an angle of approximately 45° to the pedicel. The integ-
ument continues to elongate, growing slightly beyond the apex of
the nucellus and forming a very short micropyle (fig. 2, L). The apical
portion in the region of the micropyle becomes four or five layers of
cells in thickness by the time the ovule is mature. The outer integu-
ment likewise remains two layers of cells in thickness except at the
apex, where it may be three or four cells in thickness. It does not
develop sufficiently to cover more than two-thirds of the ovule.

The archesporial cell becomes the macrospore mother cell without
further division and remains adjacent to the epidermal layer of the
ovule until the nucleus shows advancing stages of the meiotic pro-
phases (fig. 1, B, C). At later stages the cells of the epidermis divide
to form three or four layers of nucellar tissue beyond the spore mother
cell (fig. 1, D, E, F).
FIGURE 1.—Euchlaena mexicana: A, Young ovule; a, archesporial cell; i, first evidence of integument formation; X 425.  
B, Portion of nucellus with developing archesporial cell; X 425.  
C, Archesporial cell or macrospore mother cell at the onset of meiosis, X 425.  
D, Young ovule with developing integuments. Nucleus of macrospore mother cell at a late spireme stage, X 425.  
E, Portion of nucellus with macrospore mother cell. Nucleus at diplonema, X 425.  
F, Same as E, nucleus at diakinesis; X 425.  
G, Nucleus of macrospore mother cell, diakinesis; X 1,625.  
H, Macropore mother cell, heterotypic equatorial plate stage; X 850.  
(Drawings made with camera lucida at table level.)
FIGURE 2.—Euchlaena mexicana: A, Interkinesis, nuclei preparing to divide; × 850. B, Homoeotypic division; × 850. C and D, Tetrad showing arrangement of spores; × 425. E, Longitudinal section through pistillate flower, ovule contains a tetrad of spores; × 45. F, Portion of ovule with two-nucleate embryo sac and 3 disintegrating spores; × 425. G, Four-nucleate embryo sac, cell-plate formation; × 425. H, Portion of ovule with four-nucleate embryo sac, later stage of cell-plate formation; × 425. I, Three-celled, eight-nucleate embryo sac showing cell-plate formation; × 425. J, Seven-celled, eight-nucleate embryo sac; × 425. K, Embryo sac showing increase of antipodal cells and the beginning of starch formation; × 425. L, Ovule with maturing embryo sac, shows development of integuments; × 45. (Drawings made with camera lucida at table level.)
The macrospore mother cell is about twice as long as wide at the onset of meiosis (fig. 1, B, C). It is somewhat flattened at both ends and wider in the apical portion than at the base. The nucleus, which may be either near the apical end of the cell or in the midregion, passes through the phases characteristic of the heterotypic division (fig. 1, C to H). Ten pairs of chromosomes are present at diakinesis (fig. 1, F), one of which is closely associated with the nucleolus (fig. 1, G). The chromosomes at this stage vary in length so that the longest pair is approximately twice as long as the shortest. The spore mother cell increases in size during the heterotypic prophase so that it is fully three times as long as wide at diakinesis.

The cytoplasm of the spore mother cell remains finely vacuolate while the nucleus is passing through the prophase. Toward the end of diakinesis, however, the cytoplasm at the ends of the cell becomes more vacuolate and a denser zone appears immediately surrounding the nucleus (fig. 1, F). The heterotypic spindle, whose axis is parallel to the longitudinal axis of the cell, is surrounded by a dense layer of cytoplasm (fig. 1, H). At the conclusion of the heterotypic division the spore mother cell is unequally divided by a cell plate, the apical (micropylar) daughter cell being about half as long as the basal (chalazal) cell. A thick wall is laid down by the chalazal cell at its micropylar end during the interim between the two meiotic divisions. The micropylar cell remains thin-walled (fig. 2, A).

The axis of the homoeotypic spindle in the chalazal daughter is cell longitudinal; the dense layer of cytoplasm immediately surrounding the spindle is not as apparent as in the preceding division (fig. 2, B). This cell likewise divides unequally, and a thick wall is formed on the micropylar end of the basal cell (fig. 2, C, D). The axis of the spindle in the micropylar cell formed as a result of the first meiotic division may be either longitudinal, transverse, or at an oblique angle to the long axis of the ovule. The nuclear division in this cell lags somewhat behind that in the chalazal cell (fig. 2, B). Two thin-walled daughter cells of approximately equal size are formed (fig. 2, C, D). The four daughter cells resulting from the meiotic divisions thus consist of one elongate chalazal cell which becomes the functional spore and three cells nearly equal in size which ultimately disintegrate. Not more than one spore tetrad was observed in any ovule (fig. 2, E).

**Development of the Embryo Sac**

The cytoplasm of the functional macrospore becomes highly vacuolate toward the chalazal end (fig. 2, C, D), and ultimately a large vacuole is formed in this region. Shortly after the first nuclear division in the formation of the gametophyte a second vacuole is formed between the two nuclei (fig. 2, F). This vacuole becomes much larger than the first, and as a result the nuclei are widely separated. Both daughter nuclei now divide and a distinct cell plate is formed across each spindle (fig. 2, G). The embryo sac is thus divided into three regions, namely, a large central region containing two nuclei (one daughter nucleus from each pair) and a large central vacuole (fig. 2, H); a chalazal region containing one nucleus and a basal vacuole; and an apical region with one nucleus and finely vacuolate cytoplasm.
FIGURE 3.—Euchlaena mexicana: A, Embryo sac showing starch in egg and endosperm mother cell; X 425. B, Apical portion of embryo sac at time of fertilization; tip of pollen tube (pt) lies beneath fertilized egg; pn, polar nuclei fusing; sn, male gamete nucleus fusing with polar nuclei; en, egg nucleus; sn, male gamete nucleus fusing with egg nucleus; syn, synergids; m, micropyle; X 425. C, Embryo sac shortly after fertilization showing zygote and two-nucleate endosperm; X 175. D, Embryo sac with a two-celled embryo and accompanying suspensor cell. A multinucleate endosperm is present; X 175. E, Longitudinal section through endosperm and young embryo with multicellular suspensor(s). Note persistent antipodals and synergids; X 175. F, Same as E. Later stage showing development of endosperm; X 175. G, A few endosperm cells from the antipodal region. Highly vacuolate cytoplasm with little evidence of storage materials; X 425. H, A few cells of the endosperm lying adjacent to the embryo showing dense cytoplasm and abundant storage materials; X 425. (Drawings made with camera lucida at table level.)
The cell plates formed across the spindles of the second division become increasingly distinct as additional cell plates are formed across the spindles of the third division (fig. 2, I). The apical region delimited by the second division divides to form the two synergids. The basal region divides to form two antipodals, one in which the cytoplasm is finely vacuolate and one having a large basal vacuole. The nucleus in the apical portion of the central region divides and a cell plate is formed, cutting off an egg with dense cytoplasm and leaving one nucleus in the central region. Thus the egg nucleus is the sister of one of the polar nuclei. The basal nucleus of the central region likewise divides, and a cell plate cuts off a third antipodal cell, leaving one daughter nucleus in the central region. The large middle cell now contains two nuclei separated by a central vacuole. This vacuole soon disintegrates into a number of smaller ones and the two polar nuclei move to the midregion of the cell, ultimately coming to lie closely adjacent in the vicinity of the egg apparatus (fig. 2, J). These nuclei are embedded in a central strand of cytoplasm that extends from the antipodals to the apex of the cell. The seven-celled, eight-nucleate embryo sac now present is approximately three times as long as, and correspondingly wider than, the functional macrospore before the division of its nucleus.

The embryo sac grows to twice its original length (fig. 3, A). During its period of growth the antipodal cells divide, reaching a number of 30 or more. These cells have distinct walls and are usually uninucleate, but occasionally a cell with two to four nuclei is present. The nuclei stain heavily and the cytoplasm is finely vacuolate.

The egg apparatus consists of three cells, the egg and two synergids. All of these are somewhat pear-shaped, the smaller ends extending toward the micropyle. The cells increase greatly in size during the final growth period of the embryo sac. The nucleus of each synergid lies in the midregion of the cell just above a large basal vacuole. The apical cytoplasm stains heavily and a conspicuous filiform apparatus is present at the micropylar end of each synergid (fig. 3, A).

The egg nucleus is embedded in dense cytoplasm in the midportion of the cell. The cytoplasm except in the vicinity of the nucleus is vacuolate, and a large vacuole occupies the micropylar end of the egg. The primary endosperm cell of the embryo sac remains binucleate. In its cytoplasm, as the cell matures, many small and several large vacuoles are formed. The two polar nuclei, lying in the apical portion of the cell, are in close contact. Many starch grains are present both in this cell and in the egg; there appear to be none in the synergids and antipodals. No starch is to be seen in the newly formed seven-celled embryo sac (fig. 2, J). As the sac develops, however, starch grains are formed in the dense cytoplasm immediately surrounding the polar nuclei and in that about the egg nucleus (fig. 2, K). The egg enlarges greatly just prior to fertilization, so that it extends into the embryo sac for some distance beyond the basal ends of the synergids.

**Fertilization and Development of the Embryo**

Fertilization takes place between 15 and 20 hours after pollination. The pollen tube enters the embryo sac between the synergids; usually neither of these is disorganized at this time (fig. 3, B, C). One of the
male nuclei (sn) fuses with the egg nucleus (en), and the other (sn') fuses with the two polar nuclei (pn). Both fusions are shown in figure 3, B. The end of the pollen tube (pt) can be seen in the micro-pyle and extending between the apical cells of the nucellus. Its rounded tip lies beneath the zygote. The tube is constricted in the region where it is surrounded by the apices of the synergids and zygote. The dense cytoplasm of the primary endosperm cell becomes re-organized at or shortly after fertilization so that it forms a thin layer lining the periphery, a large vacuole occupying the central region of the cell. It is difficult to analyze the details of the zygote nucleus because of the many starch grains present in the immediately adjacent dense cytoplasm. Much starch is present also in the endosperm cytoplasm, especially in the region of the fusing nuclei. Division of the endosperm nucleus occurs almost immediately after fertilization; two and often four nuclei are formed before the complete disintegration of the remnants of the pollen tube.

Four to eight nuclei are present in the endosperm before the zygote divides; by the time a four-celled embryo is formed 25 to 30 endosperm nuclei lie in the thin peripheral layer of dense cytoplasm (fig. 3, D). Soon cell walls are formed between the endosperm nuclei, and large highly vacuolate cells are formed. Those endosperm cells in the neighborhood of the embryo now divide rapidly so that many small cells are present in this region, whereas the endosperm cells in the region of the antipodals are large and highly vacuolate. The endosperm contains many cells by the time the embryo reaches the stage shown in figure 3, E, in which the suspensor (s) is well differentiated. The endosperm develops at the expense of the nucellus, which in time is completely destroyed with the exception of the epidermis, which becomes a part of the seed coat. From this time on growth of the embryo is particularly active in two directions, toward the antipodals, which form a basal cap, and toward the nucellus at a right angle to the longitudinal axis of the embryo.

In material collected 5 days after pollination, the outgrowth of the endosperm directly opposite the embryo is more pronounced and the whole mass of endosperm has greatly increased in size. The peripheral layer of endosperm cells at this stage is conspicuous because of a differential staining reaction. Very little storage material is observed in the endosperm (fig. 3, A), except in those cells immediately adjacent to the embryo. The cytoplasm of these cells is dense and stains heavily and in some cells starch grains are present (fig. 3, H). The synergids are usually both intact although somewhat shrunken. They lie immediately beneath the suspensor shown in figure 3, F. The antipodal cells are well formed at this stage and show no signs of disintegration.

zea mays

The ovule of corn develops in a manner similar to that described for teosinte. The form at maturity is likewise similar. A hypodermal cell in the apical region becomes the primary archesporial cell (fig. 4, A). This cell does not divide to form a primary parietal and primary sporogenous cell but functions as a macrospore mother cell. The longitudinal axis of the heterotypic spindle is parallel with the long axis of the cell (fig. 4, B). After the first meiotic division two unequal daughter cells are formed, the chalazal cell being about twice

as long as the micropylar one. A thick wall is formed at the apex of the chalazal cell immediately after this division.

During the homoeotypic division the longitudinal axis of the spindle in the chalazal cell is approximately parallel to the long axis of the cell, whereas the axis of the spindle in the micropylar cell is usually at an angle (fig. 4, C, D). Nuclear and cell division in the chalazal cell usually precede division in the micropylar cell. The chalazal cell divides unequally and the innermost is the larger of the two daughter cells (fig. 4, E). A thick wall is formed at the apex of the innermost cell. The micropylar cell divides so that the two daughter cells are approximately equal in size. Of the four spores now present, the innermost and largest becomes the functional spore (fig. 4, E). The other three, approximately equal in size, ultimately disintegrate.

The nucleus of the functional macrospore divides (fig. 4, F). The daughter nuclei pass to opposite ends of the sac and then divide. Cell plates are laid down across the spindles of this second division in such a way that a three-celled embryo sac is formed consisting of a uninucleate cell at each end and a large central binucleate cell (fig. 4, G). By the time the embryo sac has reached this stage the three nonfunctional spores are in an advanced stage of disintegration.

The embryo sac is fully twice as long at the time of the third division as was the functional macrospore. The sac has likewise increased in diameter in the micropylar region, the diameter here being three times that of the spores. In consequence, the embryo sac is somewhat pear-shaped at the time of the third division (fig. 4, H, I, J). Cell plates are laid down across the spindles of the third division in such a way that a seven-celled, eight-nucleate embryo sac being formed. The egg nucleus is a sister of one of the polar nuclei. Later the antipodal cells increase in number (30 to 40 or more) by division and a large amount of starch is stored in the egg and the primary endosperm cell. Many of the antipodal cells contain two or more nuclei each. This antipodal tissue remains as a well-developed structure in almost mature grains of corn which were collected 25 days after pollination.

**ZEA MAYS × EUCHLAENA MEXICANA**

The process of macrosporogenesis in *Zea mays × Euchlaena mexicana* is essentially similar to that found in both parents. The primary archesporial cell becomes the spore mother cell (fig. 4, K). Four spores are formed as the result of the two meiotic divisions, the two toward the chalaza having thick walls at their micropylar ends (fig. 4, L). The innermost and largest spore becomes the macrospore and the others disintegrate. The developing macrogametophyte passes through stages similar to those described for teosinte (fig. 4, M to P). Conspicuous cell plates form across the spindles of the second division (fig. 4, N). The sac is now three-celled and four-nucleate (fig. 4, O). All four nuclei divide (fig. 4, P), cell-plate formation ensues, and an eight-nucleate, seven-celled embryo sac is the result. The antipodal cells divide so that 30 to 40 are present in the mature gametophyte. Starch grains are formed in the egg and in the primary endosperm cell. The starch is particularly abundant in the neighborhood of the egg nucleus and of the fusion nuclei.
DISCUSSION

The ovules of teosinte, corn, and the hybrid between them are amphianatropous at the time of maturity. True (12) described the ovule of corn as being campylotropous; Miller (8) and Randolph (10) found it to be of a modified campylotropous type; Weatherwax (13) reported it as anatropous. However, his figure of the ovule of *Coix* shortly after fertilization is similar to the ovules of *Euchlaena* and *Zea* as herein described.

The primary archesporial cell in the three forms studied functions directly as the sporogenous cell without further division. Weatherwax (15) described a periclinal division of the archesporial cell of corn forming a parietal cell and the macrospore mother cell. No cell wall is formed, however, according to him, and the parietal cell is “immediately consumed.” The writer has examined a large number of preparations containing many stages in the development of the archesporial cell in order to find evidences of such a division. During the early stages of development of the ovule the nucellar cells adjacent to the archesporial cell divide periclinally in the manner shown in figure 4, A, but no evidence of a division of the archesporial cell was observed prior to the first meiotic division.

The spore mother cell divides to form four spores. The thick walls at the micropylar ends of the two innermost of the four spores are particularly conspicuous in teosinte and in the corn-teosinte hybrid. These walls take a deep purple color when stained with Delafield’s haematoxylin and a bright pink color when the iodine-gentian violet combination is used. In no case observed were only three spores formed because of a failure of the completion of the homoeotypic division in the micropylar cell as described by Weatherwax (15).

The chalazal spore functions as a macrospore and the three apical spores disintegrate. Miller (8) reported that all four spores enter into the formation of the embryo sac. On the other hand, Weatherwax (15) found, as the writer has, that the chalazal spore alone continues development and the other three become disorganized. Brink (4), in a plant heterozygous for the waxy type of starch, found approximately a 1–1 segregation of the two types of starch in the embryo sac. This evidence excludes the possibility of all four macrospores entering into the formation of a macrogametophyte.

The development of the embryo sac is interesting because of the cell-plate formation across the spindles of the second division. In the material stained with Delafield’s haematoxylin the plates are clearly differentiated. This fact, in addition to the position of the spindles of the third division, substantiates the observation of Weatherwax that one of the polar nuclei is a sister of the egg nucleus. Figure 2, I, showing cell-plate formation after the third nuclear division in teosinte, shows that the synergids on the one hand, and the egg nucleus and the upper polar nucleus on the other, represent sister nuclei. Schnarf (11) considers this as probably being the case in most, if not all angiosperms.

During the period of further maturation of the macrogametophyte the antipodal cells increase in number by division until 30 to 40 or more are present. These cells are of approximately the same size in teosinte and corn. Those of *Coix* (17) are fewer in number, much larger, and more highly vacuolate. This antipodal tissue remains
intact for a long period after fertilization, forming a cap at the apex of the endosperm. Material of teosinte 5 days after pollination shows well-developed antipodal cells as do also corn kernels collected 25 days after pollination. Weatherwax (16) and Randolph (10) found evidence of the presence of antipodal tissue just opposite the dent in fully matured corn kernels.

SUMMARY

Ovule development, macrosporogenesis, and the formation of the macrogametophyte are essentially similar in the annual variety of Florida teosinte, yellow dent corn, and a corn-teosinte hybrid.

Just before the appearance of the primordia of the integuments, a single hypodermal cell of the nucellus is differentiated as a primary archesporial cell. This cell functions as a macrogametophyte mother cell.

The macrogametophyte mother cell by two divisions produces a row of four macrospores. The chalazal spore becomes the embryo-sac mother cell and the three micropylar spores disintegrate. After the second nuclear division in the embryo sac, cell plates are formed across the spindles, producing a three-celled embryo sac. A third nuclear division, followed by cell division leads to the formation of an eight-nucleate, seven-celled embryo sac. The two synergid nuclei are sister nuclei; so are the egg nucleus and one polar nucleus.

The antipodal cells continue to divide during the course of maturation and growth of the embryo sac so that 30 to 40 cells or more are formed. These persist as a definite tissue in the developing seed. During the later stages of the maturation of the embryo sac starch is stored in the egg and the primary endosperm cell.

The ovule at the time of fertilization is amphianatropous in form. Fertilization occurs in teosinte between 15 and 20 hours after pollination.

The synergids are not disorganized as a result of the entrance of the pollen tube. They persist in the region of the micropyle for 4 to 5 days and then disintegrate.

LITERATURE CITED


Oct. 1, 1937 *Macrosporogenesis in Euchlaena mexicana and Zea mays* 551

(6) ——— and Beadle, G. W.

(7) Guignard, L.
1901. LA DOUBLE FÉCONDATION DANS LE MAIS. Jour. Bot. 15: [37]–50.

(8) Miller, E. C.

(9) Poindexter, C. C.
1903. THE DEVELOPMENT OF THE SPIKELET AND GRAIN OF CORN. Ohio Nat. 4: 3–9, illus.

(10) Randolph, L. F.

(11) Schnarf, K.

(12) True, R. H.

(13) Weatherwax, P.

(14) ———

(15) ———

(16) ———

(17) ———