XENIA AND THE ENDOSPERM OF ANGIOSPERMS

E. M. EAST

As is well known, the term "xenia" was proposed by FOCKE to describe any effect of pollen of another race upon the tissue of a seed plant apart from that initiating the formation of an embryo. As it has been exceedingly questionable whether any such effect beyond a chemical irritation ever occurs, the word has come to be applied to the appearance of the F_1 hybrid endosperm produced by the fusion of the second male nucleus with the so-called endosperm nucleus of the embryo sac, when its characters are different from those exhibited by the mother plant after self-fertilization.

Since the fact of this fusion was proved cytologically by GUIGNARD (10) and NAWASCHIN (11), data on this type of xenia have interested botanists because of the differences of opinion existing concerning the phylogenetic significance of the angiosperm endosperm.

The most detailed observations on xenia have been those on maize, since numerous maize varieties exist with differences in endosperm characters. The behavior of the following factors in heredity is known from the researches of DEVRIES (5), CORRENS (2), WEBBER (12), EAST and HAYES (7), and EMERSON (9). In addition, EAST (6) has found good indications of at least three additional factors that modify the expression of the red and the purple aleurone colors.

Factor	Action
S	Causing full development of starch grains
Y_1	Causing yellow color throughout endosperm
Y_2	Similar to Y_1 but not allelomorphic to it
C	Basic color factor necessary for color in aleurone cells
R	Present with C gives red color in aleurone cells
P	Present with R and C gives purple color in aleurone cells
I	Inhibits aleurone color when present with RC or PRC

Observations on crosses wherein these characters have been concerned have made it possible to formulate the following law regarding xenia:

> When two races differ in a single visible endosperm character in which dominance is complete, xenia occurs only when the dominant parent is the male; when they differ in a single visible endosperm character in which dominance is incomplete or in two characters both of which are necessary for the development of the visible difference, xenia occurs when either is the male.

It is evident that such a statement can be true only if the two male nuclei always carry the same hereditary factors and if a male nucleus always enters into the formation of the endosperm. The first requirement has been satisfied in every experiment thus far recorded; the second requirement will now be considered.

In particular cases where xenia has followed the crossing of races differing in endosperm color, aleurone color, or ability to mature starch grains, the seeds are not uniform in appearance. One may be half starchy and half wrinkled; another may be half yellow and half colorless; still another may have half of the aleurone cells red or purple and the other half colorless. Examples of this kind are rarely found, although it is a common thing to find seeds with a mottled appearance affecting only the aleurone colors.

CORRENS and WEBBER suggested independently that in these cases the male nucleus may fail to unite with the fusion nucleus and each divide independently, forming either the half-and-half seeds or those which are mottled. WEBBER also suggested, as an alternate hypothesis, the fusion of the male nucleus with one of the polar nuclei, the other polar nucleus remaining independent and dividing.

EAST and HAYES have shown that CORRENS and WEBBER were dealing here with two phenomena. The seeds that are mottled become so only from the development or non-development of color in the aleurone cells. They merely exhibit irregularity of Mendelian dominance, since in some crosses practically all seeds heterozygous for one of the factors producing aleurone color are mottled, although homozygotes are fully colored. Furthermore, the mottling does not extend to the color or other character of the deeper endosperm tissue in case the parental varieties had such differences, which necessarily would be the condition if the endosperm had been formed according to either of WEBBER'S independent development hypotheses. This criticism has also been made independently by EMERSON (**8**).

The other cases, where the endosperm is divided more or less equally into two types, remain to be explained. The hypothesis of independent development of the male nucleus seems improbable if one may judge from relevant cytological data on both animals and plants. The second hypothesis is very plausible. As a third possibility, EAST and HAYES have suggested ordinary "endosperm fertilization" with subsequent vegetative segregation similar to that occurring in bud sports. This could be proved, according to them, if among the F_1 seeds of a cross between parents differing in two allelomorphic pairs, individuals should be found in which the parental characters were combined differently. No such cases have been recorded.

The difficulty of deciding between the first and the second hypothesis of WEBBER lies in the fact that individuals of this kind are very rare, and when they have been found the investigator has not been able to say which particular endosperm character was carried by the male cell and which by the female cell. This was because they have occurred in selfed hybrids where both pollen and egg cells were segregating various Mendelian factors. In the experiments now to be described, this difficulty has been overcome.

The red color in the aleurone cells of maize is due to the interaction of two factors that may be represented by the letters *C* and *R;* this color may be changed to purple by the presence of a third factor *P*. Red is *RC* and purple is *PRC*, therefore, although it must be understood both that other factors which have never been lost in any variety may enter into the combination, and that other factors which have been lost in certain varieties may affect the development of color.

Six homozygous white varieties may exist with the following zygotic formulae: *PPRRcc*, *PPrrcc*, *PPrrCC*, *ppRRcc*, *pprrCC*, and *pprrcc*. Any cross between these varieties of such a nature that *R* and *C* or *P*, *R*, and *C* are brought together results in the red or the purple color respectively.

Among the selfed maize ears that had been produced in the course of the writer's experiments were a number giving red wrinkled and white wrinkled seeds in the ratio of 3:1. These white seeds must have either the formula *ppRRcc* or *pprrCC*.

White seeds from three such ears were planted in isolated plots and used as male parents on the flowers of plants arising from *white* seeds found on selfed ears of 13 other families. A number of these families had the proper formulae to produce color, and about 60,000 red or purple seeds were produced. There were all-purple ears and all-red ears in several families. Other combinations gave purple and white seeds or red and white seeds in the ratio of 1:1. How this came about is clear if one assumes either of the formulae given above for the male parent. Suppose the male parent had the formula *ppRRcc:* a family with the formula *pprrCC* gives all-red ears, while one with the formula *pprrCc* gives ears with red and white seeds in the 1:1 ratio; a family with the formula *PPrrCC* gives all-purple ears, while one with the formula *PprrCC* or *PPrrCc* gives ears with purple and white seeds in the 1:1 ratio.

Considering first only the all-purple and the all-red ears, one must conclude that the fusion of the "endosperm nucleus" and the second male nucleus always occurs. If it did not occur, white seeds would result, because a factor from each parent is essential for the production of color.

Among these 60,000 seeds, 6 were found that showed the half-and-half condition; that is, color had developed on one side and not on the other. They were typical illustrations of the phenomenon which WEBBER'S two hypotheses were devised to explain. They occurred in only 0.01 per cent of the fertilizations, but in spite of their rarity they show that WEBBER'S first hypothesis, assuming independent development of the male nucleus, is untenable, since independent development of the paternal and the maternal nuclei could produce no color. No decision can be made between WEBBER'S second hypothesis—fusion of the male nucleus with one polar nucleus and independent development of the other—and the hypothesis of vegetative segregation after partial development. The bilateral symmetry of the halves of the seeds with and without color favors WEBBER'S idea; at the same time, it must be pointed out that the frequency of the occurrence is not too great to compare favorably with the frequency with which "bud sports" originate. Though it would afford some satisfaction, a precise explanation of these rare aberrations is not a necessary requisite to several conclusions indicated by the experiments. It is evident that in the varieties of maize used, a paternal and a maternal nucleus carrying the same hereditary factors as are borne by the true gametes—in the case of the 7 factors investigated—always fuse in the formation of the endosperm. For this reason geneticists investigating maize have been correct in treating the endosperm as if it were an embryo. The endosperm characters have behaved exactly like plant characters. Two white varieties of sweet peas may carry factors both of which are necessary for the production of color. When they are crossed, color develops. Color develops in maize in a quite similar manner when the two complementary factors are carried by the "endosperm nucleus" and the second male nucleus. Nevertheless, one should keep in mind that the problem is complicated. COLLINS (1) found a white ear of maize in a yellow variety that behaved as if its seeds were crossed with the yellow. He interpreted the phenomenon as a mutation showing reversal of dominance, although the data on succeeding generations corroborated those obtained by previous investigators in which yellow was partially or completely dominant. It is not unlikely, however, that COLLINS merely happened upon a plant from white seed in which the male nucleus did not enter into the formation of the endosperm, although other interpretations are possible. This may seem like an odd statement after having shown that the two nuclei always fuse, but it is made advisedly. In most varieties of maize the two nuclei do appear always to fuse, but HAYES is now working out the details in a cross in which a Mexican starchy corn is one of the parents where the nuclei appear never to fuse. In other words, it seems that there may be varieties of maize in which endosperm formation is the opposite of that just described, and within each category *no change to the other has been found.* But may not such a change occur?

Whether or not the last suggestion ever proves to be true, it seems to me that from the data now collected one is entitled to discuss angiosperm endosperm formation from the viewpoint of experimental genetics.

The endosperm of the gymnosperms is essentially vegetative tissue of the female gametophyte. It results from continuous cell formation originating with the germination of the megaspore, although fertilization occurs during the process. From the time of HOFMEISTER the morphological character of the endosperm of angiosperms was considered to be the same as that of the gymnosperms until the double fertilization was discovered. This fact gave rise to the idea that the angiosperm endosperm might be a sporophytic rather than a gametophytic structure, its nature being that of a monstrous embryo, or possibly that it is a composite tissue neither gametophytic nor sporophytic.

Most botanists, however, have held with STRASBURGER to the original idea that the endosperm is gametophytic. STRASBURGER concluded that the second fusion is not a true act of fertilization uniting the parental qualities and forming an embryo, but a vegetative fusion acting merely as a stimulus to growth. Miss SARGENT, however, believes that it is a degenerate embryo, the monstrous character being caused by the interference of the antipodal nucleus having a vegetative character and an indefinite and usually redundant number of chromosomes in the act.

The difficulty in the situation appears to be the obscurity of the phylogenetic history of the fusion of the two nuclei in the embryo sac and the subsequent fusion with the second male nucleus. The problem is further complicated by the irregularity of endosperm formation in various species. Although triple fusion appears to occur in the majority of angiosperms, the following important general variations have been noted. In addition to these general variations many minor deviations have been found (COULTER and CHAMBERLAIN 4). (1) Vegetative endosperm formation may take place in a similar manner to that occurring in gymnosperms. This may occur without fertilization, or before or after fertilization. Usually the endosperm tissue is formed from the descendants of the antipodal cells, but the chalazal nucleus may degenerate and the endosperm be formed from the micropylar polar nucleus. (2) The polar nuclei may not fuse, but divide independently. (3) Fusion may include many cells.

Furthermore, endosperm formation may be initiated by free nuclear division, or the sac may be divided into two parts by a cell wall after the first division. Even when the latter phenomenon occurs, endosperm tissue may be formed in both chambers, although usually division proceeds only in the micropylar chamber.

These general cytological data being given, how do the facts from pedigree cultures bear upon the problem?

Just how much weight should be given to data from only one species when discussing the morphological significance of the endosperm is questionable. But in maize it is evident that STRASBURGER'S distinction between vegetative and generative fertilization will not hold. Cytological work on other species does not bear out Miss SARGENT'S conception, since endosperms form quite regularly without the interference of the antipodal vegetative (?) nucleus. If the perfectly regular manner in which the above-mentioned endosperm characters of maize are transmitted is considered apart from other facts, there appears to be no escape from the conclusion that the endosperm is sporophytic in character. But there is another way of looking at the matter that makes the view of COULTER seem more probable.

COULTER (3) has concluded that conditions in the embryo sac favor fusions of any number or kind of free nuclei—an indefinite process without a necessary phylogeny that results in a growth which is practically gametophytic. It is not dependent upon a male nucleus, a polar nucleus, or even a reduction division.

The experimental evidence accords perfectly with this view. The superficial endosperm characters are indeed transmitted regularly when a male nucleus takes part in the fusion, but there is no reason for believing that the remaining maternal nuclei carry *all* the characters borne by the egg because *these* characters are the same in the nuclei concerned. The egg must usually have an organization somewhat different from that of the other maternal nuclei; although it is recognized that other nuclei sometimes function as eggs. It is likely that a differentiation has ensued which makes a particular nucleus an egg, and that it is not wholly a matter of position. The general belief in the vegetative character of the antipodal cells of the embryo sac is an admission that they have not received *all* the properties retained by other four cells. It is not very heretical, therefore, to assume that the cell that becomes the egg is different from its associates. Botanists hesitate to assume the differentiation during ontogeny admitted by zoologists. They desire to believe that most plant cells can reproduce the whole plant. But this is a belief and not a fact, and until it becomes a fact it is well to recognize this plausible alternative in considering matters such as periclinal and sectorial chimeras as well as endosperms.

HARVARD UNIVERSITY

LITERATURE CITED

1. COLLINS, G. N., Heredity of a maize variation. U.S. Dept. Agr. Bur. Plant Ind. Bull. 272. pp. 7–23. 1913.
2. CORRENS, C., Bastarde zwischen Maisrassen mit besonderer Berücksichtigung der Xenien. Bibliotheca Botanica **53**:1–161. 1901.
3. COULTER, J. M., The endosperm of angiosperms. BOT. GAZ. **51**:380–385. 1911.
4. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of angiosperms. pp. x+348. New York. 1909.
5. DEVRIES, H., Sur la fécondation hybride de l'albumen. Compt. Rend. Acad. Sci. **129**:973–975. 1899.
6. EAST, E. M., The Mendelian notation as a description of physiological facts. Amer. Nat. **46**:633–655. 1912.
7. EAST, E. M., and HAYES, H. K., Inheritance in maize. Conn. Agr. Exp. Sta. Bull. 167. pp. 1–141. 1911.
8. EMERSON, R. A., Inheritance of color in the seeds of the common bean, *Phaseolus vulgaris*. Ann. Rep. Neb. Agr. Exp. Sta. **22**:67–101. 1909.
9. ———, Aleurone colors in F_2 in a cross between non-colored varieties of maize. Amer. Nat. **46**:612–615. 1912.
10. GUIGNARD, L., Sur les anthérozoides et la double copulation sexuelle chez les végétaux angiospermes. Rev. Gén. Botanique **11**:129–135. 1899.
11. NAWASCHIN, S., Resultäte einer Revision des Befruchtungsvorgangs bei *Lilium Martagon* und *Fritillaria tenella*. Bull. Acad. Imp. Sci. St. Pétersbourg **9**: no. 4. 1899.
12. WEBBER, H. J., Xenia, or the immediate effect of pollen in maize. U.S. Dept. Agr., Div. Veg. Phys. and Path. **22**:1–44. 1900.