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XENIA AND OTHER INFLUENCES FOLLOWING FERTILIZATION.*

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Occurrence of Xenia.

A great deal of confusion between near and remote influences from alien pollen has come about as the result of laxly grouping together under the topic "xenia," a miscellaneous assortment of phenomena more or less closely associated with fertilization and embryo development. Xenia is hybridization exposed. The effect of foreign pollen is made immediately apparent in the endosperm of some angiosperms. Maize has come to be the classic example for illustrating xenia, but VON RUMKER (1) in 1911, and others have called attention to its occurrence in rye. Though known best through the cultivated plants, it is possible to have xenia in any of the angiosperms in which differences exist in the color or composition of the endosperm. In many species differences really present may not be known because the endosperms are covered by other, often opaque,

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parts of the seeds. In these plants xenia would not become patent until the seeds were cut open and the endosperm tissue disclosed.

In members of the Graminaceæ, and especially in the cultivated cereal crops with their superior development of endosperm, xenia has been observed for a long time. Until recently it has been marveled at, not only by agriculturists, who probably first saw the phenomenon, but also by botanists, who noted, yet were unable to account for it. Many of the latter were unwilling to credit the possibility of an immediate effect of pollen and they attempted to explain what they saw as the result of previous hybridization.

Origin of the Name.

The word xenia, (Gr. xenios), means hospitality. When in 1881, Focke (2) first named the influence of foreign pollen, he evidently had in mind only the genial, (or xenial), relations existing between guest and host and in his own words, regarded this variation from the normal form as a present from the plant given pollen to the one taking it (Gastesbeschenk). He did not limit the extent of the influence to any special tissue or part of the plant, so we find him passing without an effort from xenia in maize, an actual case, to an effect in peas in which the seed coat and even the pod color is involved. He likewise applies the name xenia to a darkening observed in grapes, when pollen from a dark variety was transferred to a variety of lighter color. Presumably, in this case it is the pericarp that is to be understood. If the pericarp is meant the example cannot be properly classed as xenia, as will be shown later. In peas, vetches and lilies mentioned in his writings as illustrations of xenia the variations are more than likely due to a previous cross fertilization and hybridity rather than to any immediate effect of the pollen, and Focke himself so additted by taking the precaution to state that he was not sure that these citations were after all, xenia.

It is worth while noting that previous hybridity had always been the stumbling block in the paths of those whose efforts were directed towards demonstrating the existence of xenia, and it was to disprove allegations of hybridity, I mean hybridity resulting from a cross fertilization taking place at some unknown time in the past, that a number of the early experiments were planned, especially those of VILMORIN and of HILDEBRANDT in 1867, and of KORNICKE (3) in 1872.

Kornicke's investigations are of exceptional interest inasmuch as he concluded that the influence of the pollen is immediately apparent in those races of maize in which the color is in the aleurone layer. Likewise that the effect of xenia does not pass out of the endosperm or appear in any other part of the seed. He went a step further, offering objection to HILDEBRANDT'S work on the grounds that the color in the races used by HILDEBRANDT was located in the pericarp and therefore could not appear as the immediate effect of the pollen.

It is plain, therefore, that nearly ten years before the term xenia was proposed, the need of limiting it to a definite expression occuring in a definite tissue was felt. And even though FOCKE'S interpretation admitted of a wide range of examples and rather indefinite ideas of the nature of this influence, maize became the medium used for the experimental demonstration of xenia, partly due to the fact that the results were easily secured, and partly because those obtained for maize were beyond dispute. This should be remembered in connection with the more modern investigations in endosperm formation and the discovery of triple or multiple fusion.

In 1899 both DE VRIES (4) and CORRENS (5) published papers on xenia. De Vries, who used a pure strain of sweet corn onto which he crossed pollen from a strain of starch corn showed that the kernels exhibiting xenia proved to be true hybrids when grown the second year. In other words, since he was sure that hybridization had not taken place in the races he was using, xenia served as an indicator to separate those seeds which when planted would produce hybrid plants from those which would produce the original pure strain. CORRENS concluded that the influence of the foreign pollen is limited to the endosperm, affecting only the color or the composition of the reserve food materials.

Xenia and Triple Fusion.

On August 24, 1898 NAWASCHIN (6) reported for the first time before the Russian Society of Naturalists the triple fusion which takes place between the two polar nuclei and the second sperm. This one finds occasionally referred to as "double fertilization," though this name is open to serious objection since double fertilization properly describes eggs fertilized by two sperms. In triple fusion the fusion has nothing to do with the fusion of the egg and the sperm, but bodies outside the egg fuse with another sperm. Shortly after NAWASCHIN'S paper was read, GUIGNARD (7) published a complete description of triple fusion with a number of figures announcing that the process is common throughout the angiosperms generally instead of being limited to the Liliaceæ, as was first supposed. The year before this significant morphological evidence was made known, CORRENS in the paper above referred to, drew the inference that triple fusion possibly was the means of explaining xenia, though no detailed researches on the development of the endosperm had been undertaken. The relation between triple fusion and endosperm formation also was suggested. It remained for GUIGNARD (8) in 1901 to clear up all doubt as to the occurrence of the same process in maize as takes place in the formation of endosperms of other angiosperms. In a number of different species MERREL, LAND, COULTER and others have demonstrated that triple fusion is general among the angiosperms, the typical case being where two polars, one micropylar and the other antipodal, of the female gametophyte, fuse to form the definitive nucleus. It is with this nucleus that the second male cell from the pollen tube unites.

As soon as it became certain that there is a triple fusion in the formation of the endosperm, xenia was no longer a mystery but is now regarded as an occurrence as normal as seed formation itself. The writer (9) pointed out somewhere else that it is a simple matter to demonstrate xenia experimentally in definite areas on an ear of corn as desired, and was able to produce an ear, a photograph of which appears in the paper referred to, on which about one-fourth of the grains appeared blue, while the grains in near by rows were pure white. This was done by careful manipulation of the silks and the application of pollen from a race of contrasting color in the aleurone layer. The value of this experiment is twofold, for it establishes the fact that the variation is due to an effect of the pollen, since only those grains within that area in which it was desired to produce the color appeared blue, and second, because the occurrence was in harmony with the expectation it was explainable as the normal result of the chromosome mechanism. What is this mechanism that causes xenia? In the processes preceding fertilization in the angiosperms the pollen tube discharges its contents of the two male cells into the embryo sac. One of these cells reaches the egg cell and by fusing with it completes fertilization. Rapid divisions take place in the fertilized egg and the embryo is formed. In order for the endosperm to be formed, the second male nucleus must enter into a fusion also and with the definitive nucleus which has arisen as the result of the fusion of the polar nuclei. Hence the name triple fusion aptly describes this union.

Xenia and Xeniophyte Tissue.

The resulting endosperm is therefore a unique tissue in the Plant Kingdom. Since it is a fusion product, as is the embryo, it must contain more than the haploid (x, or gametophytic), number of chromosomes. On the other hand, although formed similarly to the sporophyte, it contains more than the 2x or diploid number. Since the definitive nucleus is made up of two polar nuclei, each containing a full set of chromosomes and another set is brought in by the male cell, the endosperm must have at least 3x, or a triploid number of chromosomes. However, the number is not necessarily limited to three, for in some cases adjacent cells start fusing with the polars before the male cell joins in the fusion. Because of its unparalleled origin, the endosperm is different from the sporophyte and yet, unlike the so-called "endosperm" of gymnosperms, is not female gametophyte. So TRELEASE (10) has named this tissue, which he characterizes as "neglected," the xeniophyte, for it is here and here only that xenia will be expressed if it is to appear.

The formation of the xeniophyte is so closely associated with the formation of the sporophyte, that one would expect to find them closely related to one another in the plant's life processes. This is actually the condition. They are side by side in the seed and in sprouting the growing embryo parasitically consumes the reserve store of food materials of which the xeniophyte is composed. As far as the heredity is concerned, this use which the plant is able to make of the stored food materials of the endosperm disposes of the nx chromosome number of the xeniophyte tissue, and together with that any irregularities in the expression of the contained chromosomes.

Immediate Effect of the Pollen Limited to the Endosperm.

In view of the special mechanism operative in endosperm formation, xenia has come to hold a limited meaning, a restriction in meaning that was pointed out by one far-sighted botanist at least ten years before the word came into print. Certainly then, there should be no confusion in the significance of the term with the morphological evidence as clear as we now have it, nor is there any excuse for carrying the name of a perfectly definite and limited effect over into another field where the effects are from undetermined causes. But modern textbooks are by no means clear on the subject, and even Coulter (11) makes a statement that is so ambiguous that it might entirely prevent one who did not already know from finding out what xenia in corn is, instead of being able to demonstrate it to his own satisfaction. The statement reads: "For example, when a race of white or yellow corn is crossed with pollen from a race of red corn, many of the resulting kernels are red or mottled." By red corn those varieties with red in the pericarp only are generally understood, since corn with red pericarp is commonly enough grown as field corn, and white corn crossed with pollen from this would not have a red or mottled appearance. Doubtless this is one of those small, ludicrous mistakes from which no first editions are free; there are strains of corn with reddish endosperm, no doubt, but they are not common nor would they first come to mind when "red corn" is spoken of. If the author had said blue or purple, instead of red, or colorless and colored endosperm, there could have been no misinterpretation of his meaning.

In addition to ambiguity as in the above instance, the term xenia suffers from many misuses that likewise render its meaning obscure. What is referred to here is the abuse of the word by classing as xenia influences that follow fertilization, but are not brought about by the introduction of new hereditary factors into that tissue in which the variation appears. In none of the examples that are to be cited as having been wrongfully called xenia is there a triple fusion in which the second male nucleus from the foreign pollen adds something to the fusion which causes a difference in the expression.

The Nature of Some Effects Following Fertilization.

Some of the examples that have recently been brought to the writer's attention are: 1. The common folklore of farmers that watermelons pollinated by pumpkin pollen have a lower sugar content. This matter has also been made the subject of investigation recently. 2. A report by Daniel (12) of a walnut containing a hazel nut kernel. 3. A change in the sugar content of dates due to pollination. 4. A change in the color of the shell of fowl's eggs due to the influence of the male bird.

While some of these influences are not yet fully recognized, the author will not attempt here to confirm or deny the existence or non-existence of any of them. But since they are being pushed forward for discussion, those who are interested in them should insist that they be discussed in proper terms and not confuse them with another effect the nature of which is just beginning to be understood, and the term used to describe this effect just acquiring a distinct, comprehendable meaning.

In the case of the cucurbits, the date and a number of similar examples there is an effect occurring in the fleshy pericarp at a greater or less distance from the region of fertilization. Focke's observations on grapes belong under this head. Now that we know the facts about triple fusion it is obvious that this effect cannot be xenia. Although it follows fertilization it is not so closely connected with it that it is possible for determiners brought in by the pollen to be expressing themselves. The pollen cannot produce an effect in the pericarp for the determiners expressing themselves in the pericarp belong to the preceding sporophyte generation. Exactly what causes the change is not known, but one might postulate a specific chemical reaction following a specific pollination.

This is a very different thing from xenia. In xenia there is merely the expected expression of determiners capable of expressing themselves in the endosperm. There is nothing strange about it, except that they are appearing in a new place, that is in a race or variety in which they had not previously made an appearance. If any xenia occurred in dates or watermelons it would have to be inside the seeds, not in the fleshy pericarp.

In the paper by DANIEL the supposed hybrid, indicating its hybridity in xenia, is planted and sprouts. At the critical point, one reads "malhereusement le jardinier," etc., etc. But there is no need to know what misfortunes befell the luckless gardener. Xenia could not occur in this case because neither the walnut nor the hazelnut kernel contain any endosperm. This case has a superficial resemblance to xenia, but is simply the inheritance of dominant characters in a hybrid, if indeed a hybrid kernel was really formed. It is like a cross between yellow and green peas in which the color of the cotyledons (embryo tissue) shows through the pericarp.

An anonymous discussion of the effect of the male bird on the color of the shell of the eggs of fowls and of canaries is to be found in the May, 1915, number of the JOURNAL OF HEREDITY. This discussion is interesting and no objection would be made to it if the wording of the title were similar to that suggested above. But with xenia dependent upon triple fusion for its occurrence, calling any influence of the male bird, however remarkable, by the name of xenia is unthinkable.

Many plants, if no pollination takes place, develop a cleavage plane which cuts off not only the old flower, but frequently a portion of the stem also to a considerable distance away from the region of the fertilization. If pollination is successful, no abscission layer forms. The life processes continue in the stem which remains thick and green and capable of bearing the weight of the fruit. One is compelled to consider therefore, that the inheritance of the ability to form a cleavage plane is inhibited by the developing zygote. There is an increasing metabolism following fertilization. The ability to cut off one portion of the plant from the rest is an older adaptation of the individual plant than the ability to form fruits. It is resident in the vegetative parts of the plant; it is to be found in the lowest orders. In apples and pears and many common fruits if pollination is only partially instead of completely successful and well formed seeds are not distributed evenly around the fruit, there is a tendency towards flattening on the side where the pollination was faulty. The writer will gladly show the development of the cleavage plane as exhibited by the common morning glory, Ipomoea purpurea, to anyone interested. The examples were obtained by removing the styles from unopened flowers. The flowers opened normally and remained open as usual for several hours. In from five to seven days after the time that fertilization would normally have taken place, the floral organs and all of the floral stem to within a few millimeters of the main stem of the plant dropped off. The same plant was kept growing in the greenhouse for more than a year during which time no flowers whose development was not interfered with in the manner so described, were observed to drop off. In the Chinese hibiscus, Hibiscus rosa-sinensis, a fruit that was forming apparently normally suddenly dropped off. Upon examination it was found to have seeds developing in only one locule, due to imperfect pollination. The conclusion seems warranted that the influence from the developing zygotes was not strong enough to inhibit the formation of the cleavage plane which cut off the stem about 1 cm. below the flower. It is not to be inferred that the writer believes that the developing zygote is the only influence to inhibit the formation of an abscission layer or that the lack of proper pollination the only cause of its appearance. The question is bound up with the plant's general metabolic processes, especially that of food storage.

HUME (13) found that pollination or the lack of it brought about a change in the color and texture of the fleshy material of the fruit of Diospyros kaki, the Japanese persimmon. When the seeds were evenly distributed the flesh was uniformly dark colored, but when the seeds were not evenly distributed the fruit contained both light colored astringent flesh and dark colored edible flesh. "The dark flesh particularly when it softens and becomes somewhat juicy, has a sort of gritty consistency due to the presence of short well developed fibers, while the light flesh lacks these and is smooth to the palate." Clearly then, here is an influence due to an increasing metabolism following fertilization. The effect appears in the carpellate tissue outside the region of fertilization.

Just as fertilization is not the only influence leading up to cleavage plane formation, so the developing zygote is not the only cause of the coagulation of the emulsion colloid with which the tannin is associated, or in other words, of ripening of these fruits. Lloyd (14) has observed that the entire fruit can become non-astringent while still green and on the other hand that some seedless fruits lost their astringency. The accumulation of carbon dioxide in the tissues influences non-astringency by hastening coagulation. Killing the tissues also causes softening. But while these explanations may be partially independent of seed production they are not completely so since the rate of the process of becoming non-astringent is greatly hastened in the region of a large growing seed.

Another example that is more striking in certain ways, is set forth in an interesting paper by CLOSE (15) on the immediate effect of cross pollination in apples. As a result of a certain cross he obtained a difference in the outline of the fruit and a brighter red color. "Even the fruit stems showed a variation, for out of 25 fruits taken at random from the general group, 19 had fleshy stems. Of the 23 crosses one had a fleshy stem, two slightly fleshy stems and the rest were slender." This experimental work has not, in the writer's knowledge been repeated, nor have checks been made by other observers whose results serve as a comparison with these just quoted, there is no reason to doubt this evidence though the data are meager. The point to be borne in mind is that these are influences removed some distance from the region of fertilization and not expressed as the result of any hereditary factors brought in by a male cell. That at once classes them apart from xenia.

How Are These Influences to be Described?

The writer proposes **ectogony,** (Gr., ectos, outside + **o-gony,** the fertilization product), as a convenient word for properly describing those influences which follow fertilization but are remote from it. Ectogony covers a whole field of influences that are the result of an increasing metabolism, or the effect of some chemical substance, or the response to some influence other than that brought in by hereditary factors. It would not be limited as xenia has necessarily been shown to be in the light of morphological researches, to an effect appearing only in triple fusion endosperm tissue.

There is a sharp contrast in thought between ectogony and telegony, a word derived from the same roots. Ectogony deals with real influences though scarcely intimate ones following fertilization. Telegony means the alleged influence of a father on offspring, subsequent to his own. No cases of telegony are known to exist, or to have ever existed, for in order for the thing to be possible, the father, or the growing embryo begotten by him would have to bring about a change in the unfertilized eggs, or in the uterus of the mother which would in turn affect the eggs, so that even after these eggs were fertilized from another father there would still remain some influence of the previous one.

In ectogony a change takes place outside the region of fertilization as a result of a new influence produced by the developing zygote. In xenia, on the other hand, a change that is more apparent than real is produced, since the new heredity introduced by the male nucleus consists of determiners that must express themselves in the same way that they would in the race to which they are native. There is nothing different from the normal, nothing unusual actually taking place in xenia, simply old determiners in a new place offer a surprise.

Xenia can frequently be successfully employed to separate cross pollinated from close fertilized kernels in maize, as the writer (16) recently indicated, and its practical advantages from the geneticist's standpoint are far reaching. The whole subject, however is practically unexamined by investigators to date and offers an inviting field in which much good work can be done.

Apart from the usefulness of xenia and apart from general interest in it as a fit subject for future investigation, the significance of the word has been brought into the widest botanical consideration by the introduction of the term xeniophyte. This reason is, per se, sufficient for retaining the meaning of xenia in its strict definiteness. If a distinction among influences following fertilization is to be made at all, the place to begin classifying the different effects is where the morphological evidence shows that a natural separation exists.

The opportunity is welcomed to make public acknowledgment of the gratitude felt toward Professor John H. Schaffner, of the Ohio State University, for helpful criticism and guidance during and before the time that the above paper was in preparation.

Summary.

Xenia is a phenomenon limited to the endosperm of angiosperms.

The xeniophyte, like the sporophyte, is a product of fusion. In the latter the egg nucleus and one male nucleus fuse; in the former there is fusion between the second male nucleus and the

definitive nucleus. The definitive nucleus is formed by the fusion of two nuclei from opposite poles of the female gametophyte. The fusion nuclei appear following three successive divisions of the megaspore nucleus, during which the egg is differentiated.

Ectogony is suggested as a necessary word to describe those influences which are due to the developing zygote. In xenia the variation appears as the direct result of the introduction of hereditary factors.

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