

THE NATURE AND EXTENT OF HETERO-FERTILIZATION IN MAIZE

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INTRODUCTION

The term hetero-fertilization has been applied (SPRAGUE 1929) to the process resulting in those exceptional cases in which the embryo and endosperm differ genetically. It has been suggested that these exceptions may occur because (a) the egg and polar nuclei are of different genetic constitution and fuse with identical sperms or, conversely, (b) the egg and polar nuclei are of the same genotype but at syngamy fuse with sperms having unlike genotypes. These conditions in maize may be brought about in various ways. The persistence and functioning of the four megaspores, mutations, non-disjunction of one or more chromosome pairs when the generative nucleus divides to form the sperms, or the functioning of unlike sperms from two pollen grains might result in hetero-fertilized kernels. A brief resumé of the cytological happenings prior to and during syngamy will facilitate the explanation of this phenomenon.

GAMETOGENESIS AND FERTILIZATION

Gametogenesis and fertilization in maize have been described in detail by MILLER (1919) and WEATHERWAX (1919). MILLER concluded that all four of the megaspores persisted as in the *Lilium* type of embryo sac. WEATHERWAX, previously of the same opinion, states in his 1919 paper

that "only the chalazal one of the four megaspore potentialities persists." The embryo sac at the time of fertilization shows the egg decidedly alveolar in appearance. The synergids are considerably longer than the egg and may show signs of disintegration before fertilization occurs. The antipodals have undergone very rapid division from the eight cell stage and may present the appearance of a definite tissue. The polar nuclei are imbedded in a strand of cytoplasm connecting the egg and antipodals. They retain their identity until fertilization takes place.

In the anther, after formation of microspores, the microspore nucleus divides equationally giving rise to a vegetative and a generative nucleus. Before anthesis the generative nucleus divides again to produce two crescent shaped sperms. Pollen grains falling on the silk germinate, sending out pollen tubes which grow down the styles. Only a single tube reaches the embryo sac, which it penetrates, usually causing the destruction of one of the synergids. The two sperms are discharged into the embryo sac where one fuses with the egg to give rise to the scutellum and the embryo proper. The second sperm fuses with the two polar nuclei. This final triple fusion product gives rise to the endosperm of which the aleurone layer is a part. Genetic investigations so far reported for maize are in conformity with these findings.

INHERITANCE OF SCUTELLUM COLOR

Purple and red scutellum color has been shown to be rather complex in its mode of inheritance (SPRAGUE 1932). Before purple or red color can develop in the scutellum, the fundamental aleurone factors A , C , R , and i must be present. The P_r , p_r factor pair which differentiates purple and red aleurone has a similar effect on scutellum color. The development of scutellum color is also dependent upon at least five other factors which interact in the following manner: S_1 must always be present in a dominant condition. Any two of the three factors S_2 , S_3 , and S_4 , must likewise be present in a dominant condition. Finally, the factor pair s_5 must be present in a homozygous recessive condition. Such a system of interaction permits the existence of homozygous colored scutellum strains of four different genotypes. In several cases in this paper the genotypic identity of a particular true breeding strain has not been determined. The factor complex resulting in colored scutellum in these instances is designated S_x , or, if the strain in question has colorless scutellum, s_x .

THE PHENOMENON OF HETERO-FERTILIZATION

Seeds with white aleurone normally have no purple or red scutellum color and, when selfed, either produced only white aleurone kernels or

kernels segregating in ratios characteristic of the action of an inhibitory factor. In 1925 and 1926, however, a few kernels were found with colorless aleurone and colored scutellums (figure 1). When these kernels were planted and the progeny selfed, the resulting ears showed segregation for both scutellum and aleurone color (figure 2). This was unexpected since the parent seeds had colorless aleurone and the subsequent segregation for aleurone color did not indicate the presence of an inhibitory factor. Thus, the kernels with colored scutellums and colorless aleurone were peculiar in two respects. Colored scutellums were present with colorless aleurone and the progeny of these colorless aleurone seeds segregated for aleurone color in subsequent generations characteristically like that previ-

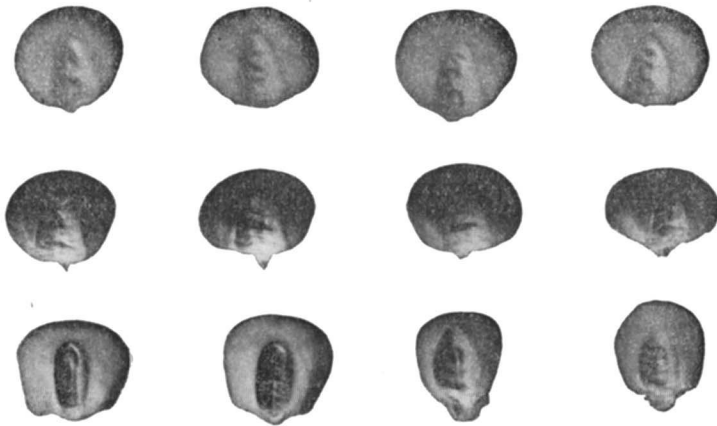


FIGURE 1.—Normal kernels with colorless aleurone, colorless scutellums (top row), colored aleurone, colored scutellums (middle row), and hetero-fertilized kernels with colorless aleurone and colored scutellums (lower row).

ously reported only from hybrid colored seeds. The aberrant seeds just described have been called hetero-fertilized since the embryo and endosperm are of different phenotypes.

It should perhaps be emphasized that the phenomenon of hetero-fertilization is not confined to progenies segregating for aleurone and scutellum coloration. Considerable evidence is available which indicates that hetero-fertilization occurs occasionally in all corn. Hetero-fertilization has been observed by Professor R. A. EMERSON of CORNELL UNIVERSITY and Mr. F. D. RICHEY of the UNITED STATES DEPARTMENT OF AGRICULTURE. In neither case were there large numbers of such seeds, and because their material was not favorable for a study of this condition, their observations were never reported.

The relation between aleurone and scutellum factors, however, provides an ideal situation for detecting this phenomenon. Advantage has been taken of the ease of identifying hetero-fertilized kernels having colorless aleurone and colored scutellums to investigate their occurrence. Kernels having colored aleurone and colorless scutellums likewise may be hetero-fertilized, but, as they cannot be identified without obtaining selfed

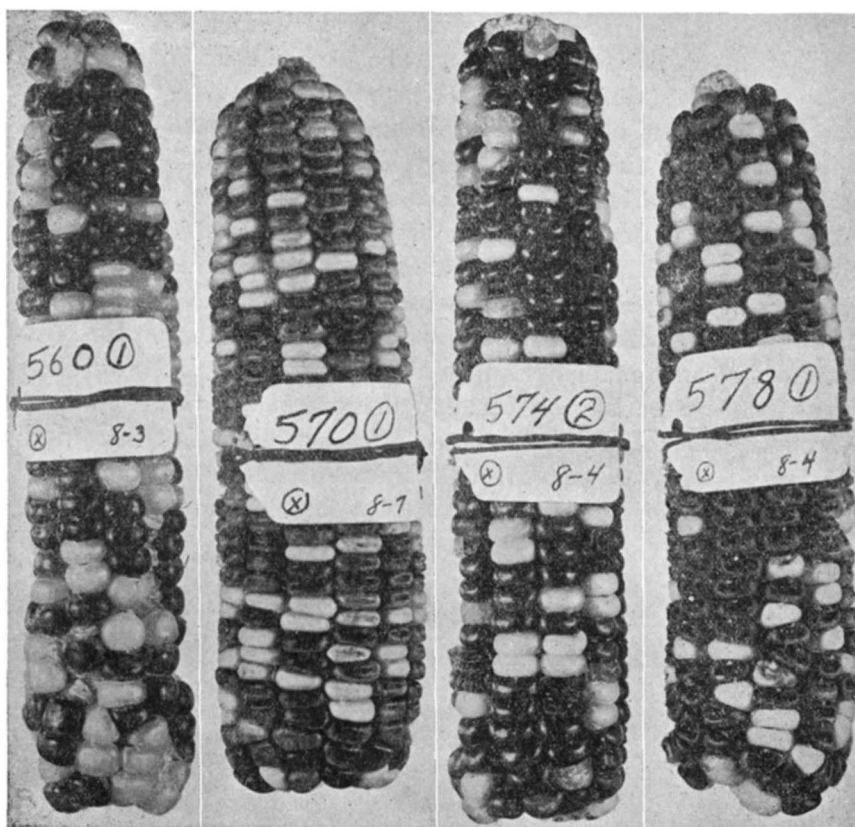


FIGURE 2.—Selfed ears obtained from the hetero-fertilized kernels shown in figure 1.

progeny, they have not been used in this study further than to establish the fact that they occur approximately as frequently as their complementary type.

GENETIC TESTS OF THE NATURE OF HETERO-FERTILIZATION

Genetic tests have been made of three of the four hypotheses mentioned as possible causes of hetero-fertilization. The results of these tests indicate

that at least the majority of all hetero-fertilized seeds are due to the functioning of genotypically unlike sperms from two pollen grains.

Among the selfed progeny of the first hetero-fertilized seeds studied was one ear which had a high percentage of these aberrant seeds. A high hetero-fertilized stock from this ear has been used in the tests reported here.

Persistence of the four megaspores

Cytologic and genetic evidence so far reported is in agreement in indicating that three of the four megaspores die. Since hetero-fertilization ordinarily does not occur with a high frequency, and since the number of young embryo sacs observed by cytologists must necessarily have been limited, there remains the possibility that hetero-fertilization is due to the persistence and functioning of the megaspore tetrad. The production of hetero-fertilized seeds due to the persistence of the four megaspores in an embryo sac of either the *Lilium* or *Smilacina* type would depend on the genotypes of the spores and their subsequent rôle in the formation of the egg and fusion nuclei. If the four spores persisted and the eight nuclei functioned at random, plants from a high hetero-fertilization stock heterozygous for the essential scutellum and aleurone factors would be expected to produce some hetero-fertilized seeds when pollinated with a multiple recessive stock. Representative data from such crosses are presented in table 1. No hetero-fertilized seeds have been obtained in these tests.

TABLE 1
Normal and hetero-fertilized kernels on ears of the cross AACcRr S_z × AcR s_z.*

PEDIGREE NUMBER	COLORED ALEURONE	COLORLESS ALEURONE	HETERO-FERTILIZATION
632-1×493-2	138	146	0
-2×493-2	142	149	0
-3×493-2	146	152	0
-4×493-2	102	95	0
-5×493-2	147	143	0
-6×493-2	88	85	0
Total	763	770	0

* *S_z* denotes the complex required for colored scutellums, *s_z* that for colorless scutellums, but with the particular combination of factors in question unidentified.

Mutation

The relative mutation rates of the aleurone factors *A*, *C* and *R* have been studied by STADLER (1930). Of the three genes, *R* was shown to mutate most frequently, one mutation occurring for every 2500 gametes tested.

Mutations of the scutellum factors have not been observed but probably are of no more frequent occurrence than those of the aleurone series. Since hetero-fertilized seeds appear with approximately equal frequency in progenies segregating for each of the three aleurone factors, and since hetero-fertilization occurs about thirty times as frequently as mutation at the *R* locus, mutation of an aleurone factor or factors does not offer a satisfactory explanation of this phenomenon.

Non-disjunction

The records for aleurone color in 65 selfed progenies grown from hetero-fertilized seeds (colorless aleurone and colored scutellum) are presented in table 2. The segregations serve as a test of non-disjunction as the causal agency of hetero-fertilization. To effect hetero-fertilization, non-disjunction of one of the chromosome pairs carrying aleurone factors must take place at the equational division of the generative nucleus. For illustration we may assume that the II chromosome is involved and that the generative nucleus in question carries *R*. Non-disjunction would result in sperms of two types $n+1$ (*RR*) and $n-1$ (*O*). To obtain a hetero-fertilized seed the $n+1$ sperm must fuse with an egg having the factor *r*. The embryo would then be *RRr* and the endosperm *rrO*. The selfed progeny of such a seed should segregate in a ratio of 35 colored to 1 colorless aleurone individual if there were no selection against $n+1$ gametes.

In a study of extra chromosome individuals derived from a triploid, McCLINTOCK (1929) has shown that $n+1$ ♂ gametes seldom function in competition. In addition, there appeared to be some selection against $n+1$ ♀ gametes since equal numbers of $2n$ and $2n+1$ plants were not obtained from backcrosses. The data presented indicated that approximately 70 percent of the $n+1$ ♀ gametes functioned. With the complete elimination of $n+1$ ♂ gametes and a 30 percent elimination of $n+1$ ♀ gametes, the theoretical ratio approximates 14 to 1 for the duplex condition and 2 to 1 for the simplex.

In table 2 among the ears showing a single factor segregation for aleurone color, there are four ears whose Dev./P.E. exceeds 3, due to deficiencies in the colorless aleurone class. The observed ratio for these four ears taken as a group is 4.43 to 1. While these segregations are undoubtedly aberrant, it seems clear that the deviations obtained cannot be considered as random fluctuations from the expected 14 to 1. In the same group are two progenies whose ratios approximate the expected 2 to 1 for the simplex trisome. The observed ratio for colored to colorless aleurone in these two progenies (570-1 and 574-2) is 2.13 to 1.

TABLE 2 (continued)

PEDIGREE	ALEURONE			SCUTELLUM				HETERO-FERTILIZED KERNELS
	COLORED	COLORLESS	DEV./P.E.	COLORED	COLORLESS	RATIO	DEV./P.E.	
	9:7 ratio for aleurone color							
341-2	45	32	0.57	24	21	9:7	0.59	0
342-1	275	182	2.51	218	57	3:1	2.43	0
558-1	132	130	2.84	122	10	15:1	0.93	2
-2	119	95	0.28	113	6	15:1	0.80	8
560-1	137	105	0.17	73	64	9:7	1.04	0
-3	121	109	1.65	60	61	27:37	2.44	0
-4	106	74	1.06	56	50	9:7	1.05	1
563-2	37	25	0.81	30	7	3:1	1.26	0
-3	128	109	1.03	92	36	3:1	1.21	2
564-1	127	90	1.00	99	28	3:1	1.14	0
565-2	146	126	1.27	108	38	3:1	0.42	0
568-1	137	115	0.89	106	31	3:1	0.95	0
569-4	101	86	0.91	33	68	27:37	2.85	0
571-2	224	182	0.65	116	108	9:7	2.00	0
579-1	27	22	0.25	27	0	1
-2	206	165	0.42	193	13	15:1	0.06	1
581-1	99	77	0.00	99	0	29
582-1	147	112	0.24	106	41	3:1	1.20	0
-2	128	101	0.16	118	10	15:1	1.08	0
-3	88	72	0.47	56	32	9:7	2.07	0
-4	137	97	1.05	107	30	3:1	1.24	0
597-1	108	89	0.60	49	59	27:37	0.99	0
Total 9:7	2775	2195	0.14					44

27:37 ratios for aleurone color

569-1	122	180	0.93	62	60	9:7	1.79	0
-6	30	31	1.64	14	16	27:37	0.76	0
579-3	21	23	1.10	20	1	15:1	0.41	0
581-3	173	200	2.44	158	15	15:1	1.95	23
595-1	133	187	0.34	133	0	2
718-2	21	28	0.15	15	6	3:1	0.56	14
Total 27:37	500	649	0.43					39

Among the ears exhibiting a 9:7 or 27:37 segregation for aleurone coloration, the deviations of the individual ears and of the groups are well within the limits of random sampling. These results indicate that non-disjunction may occasionally result in hetero-fertilized seeds but is inadequate to account for the phenomenon in general.

Sperms from two pollen grains

Selfed ears were selected from the high hetero-fertilization stock which were homozygous for the factors necessary for colored scutellum with the exception of the aleurone factor *R* which was segregating. Colorless aleurone, colorless scutellum segregates from these progenies were grown and pollinated with a pollen mixture composed of approximately equal parts by volume of its own (*r*) and sib (*R*) pollen. If hetero-fertilization is due to the functioning of unlike sperms from two pollen grains, an occasional seed with colored scutellum and colorless aleurone is to be expected. Some of the results from mixed pollinations of this type are presented in table 3. Hetero-fertilized seeds are seen to occur very frequently. These results indicate that the usual cause of hetero-fertilization is the functioning of sperms from two pollen grains of unlike genotypes.

TABLE 3

Numbers of normal and hetero-fertilized kernels on ears resulting from the cross $ACrS_x \times \begin{cases} ACrS_x \\ + \\ ACrS_x \end{cases}$

PEDIGREE NUMBER	TOTAL KERNELS		HETERO-FERTILIZED KERNELS
	<i>R</i>	<i>r</i>	
993-6 × 993+ 994	10	10	1
-10 × 993+ 994	8	21	8
6786-6 × 6787+6790	75	60	29
7830-2 × 7830+7829	107	60	18
-2½ × 7830+7829	53	72	3
-3 × 7830+7833	38	88	12
7834-1 × 7834+7833	34	83	28
Totals	325	394	99

Is there some protective mechanism in maize which ordinarily limits the functioning male nuclei to those derived from a single pollen tube? In animal eggs this protective mechanism is a heavy membrane which develops around the egg after fertilization. No such structure has been reported in the higher plants and even if one were universally present it would not interfere with the process of hetero-fertilization. The usual protective mechanism in plants must be sought in the embryo sac and its enveloping tissues or in the style. No evidence is available at present which may distinguish between these possibilities.

THE EXTENT OF HETERO-FERTILIZATION

Hetero-fertilization seems to be widespread, and occurs rather frequently. Since such kernels cannot be detected in most material without recourse to their selfed progeny, unusual behavior due to this phenomenon has doubtless frequently been ascribed to a faulty classification of the parent seed. The frequencies of hetero-fertilized kernels with colorless aleurone, and colored scutellums among the colorless aleurone class in progenies segregating for aleurone and scutellum color were 1 in 50 and 1 in 81 for all such progenies grown in 1928 and 1929, respectively. Only the colorless aleurone class was used in these calculations since it is the only one in which the phenomenon can be detected by direct observation.

The above results suggest that hetero-fertilization occurred more frequently in 1928 than in 1929. This difference, however, is due to the inclusion of excess progenies from the high hetero-fertilization stock in 1928. When these are omitted from the averages, the frequency becomes 1 in 76 which is in excellent agreement with the 1929 data. Within the high hetero-fertilization stock the phenomenon occurs much more frequently as shown by the data in table 3. Among the 394 colorless aleurone kernels there occurred 99 cases of hetero-fertilization or 1 such case for every 4 seeds obtained.

Among progenies segregating in a 3 to 1 ratio for aleurone color, only a small proportion of the possible hetero-fertilizations will produce kernels with colorless aleurone and colored scutellums. The theoretical proportion of such kernels increases with the number of aleurone factor pairs heterozygous. In table 2, hetero-fertilized kernels with colorless aleurone and colored scutellums occurred with a frequency of 1 in 60 for the 3 to 1 group, 1 in 49 for the 9 to 7 group, and 1 in 17 for the 27 to 37 group. Using the frequency found for the 3 to 1 group as a basis for calculation and assuming the factors necessary for colored scutellums to be present equally, the expected frequencies are 1 in 60, 1 in 34, and 1 in 20, respectively. The agreement is fair. Apparently hetero-fertilized kernels occur approximately in the proportions expected from the frequencies of the possible unlike sperm combinations which may cause them.

SUMMARY

An aberrant condition is described for maize in which the endosperm and embryo are of different phenotypes. The process by which this is accomplished is called hetero-fertilization.

Genetic tests indicate that hetero-fertilization is due to the fusion of the egg and of the polar nuclei with sperms of unlike genotypes.

Non-disjunction may play a minor rôle in producing these genotypically different sperms.

Results from mixed pollinations indicate that the genotypic differences are usually due to the participation in the fertilization process of sperms from more than one pollen grain.

Hetero-fertilization occurs with a frequency of approximately 1 seed in 80 in normal cultures and 1 in 4 in the high hetero-fertilization stock.

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