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On the influence of artificial pollinations on the kernel characters of maize

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Introduction

The nature of sexual fertilization is one of the most important and basic problems in biology, especially in genetics. Recently, the distinctive recognition and interpretation on the mechanism of sexual fertilization have been developed under the theory of "Michurinism" genetics chiefly in U.S.S.R., based upon the widespread experimental results with peculiar techniques of pollination in higher plants. The mixed pollination or multiparental pollination has been found to provide the unexpected genetical effects on the Mendelian characters in F_1 and later generations (GLUSHCHENKO '57, KOCARJAN '48, MEDVEDEVA '55a, '57, '58 and ZAHAROVA '55).

SCHELOKOVA ('54, '56) pointed out the facts that the selectivity of gametes in feritilization was sometimes obtained by the application of mixtured pollen or supplementary pollination in several plant species, and that the degree and nature of selectivity were variable with the materials employed and the modes of pollination. The female own pollen was indicated to have such a stimulant effect to the progenies as to increase the frequency

of hybrids by addition of it (JAKOVUK '54 and KONONKOV '58).

GAVRILOVA ('56) showed with pea the suppressed effects on female own character in bud pollination. Similarly the aged pollen was indicated to produce extreme deviations from normal ratios of F_2 segregation in tomato and pea (AIZENSTAT '54). According to TER-AVANESJAN ('49), TER-AVANESJAN *et al.* ('50) and GUREVIC ('50) the hybrid progenies having the respective dominant characters of two pollen parents were secured by the application of variable quantities of pollen in certain mixturing combinations. Furthermore the restricted pollination was found sometimes to indicate the shattering effect of the hereditary characters in the offsprings (MEDVEDEVA '55b, TER-AVANESJAN '46, '50, '54, and TER-AVANESJAN *et al* '59), and also to produce the maternal type of offsprings (AIZENSTAT '53, and TER-AVANESJAN *et al* '59).

Thus, although the fundamental nature of sexual fertilization might be the fusion of a male with a female sexual cell, there are accumulated the data showing that the process of fertilization may be more complicated phenomenon and that there are a number of conditions affecting the processes, that is, the quantity or the age of male sexual cells, the age of female floweres including the positions of them on plant, the possible mutual interaction among the male and femal gametes, and the various modes of pollination procedures.

Some of the workers have presented the distinct facts against the influence of multiparental pollination on the hereditary characters by the progeny analysis (AVRATOSCUKOVA *et al* '56, EVERETT *et al* '58, OKSIJUK '55 and ZACHOW '58) and also by the cytoembryological observation (KOSTRJUKOVA *et al* '56 and OKSIJUK *et al* '57).

Experiments have been projected in the author's laboratory since 1955 and at the start different kinds of plant species, *Mirabilis*, *Petunia*, pangee, sweet pea, *Portulaca*, *Antirrhinum*, *Salvia* and maize were employed by several members of the laboratory. Here is the report on the results obtained with the maize kernels of which are distinguished by simple Mendelian characters of the endosperm.

The author has intended to ascertain in the present experiment whether the facts reported by Michurinists are acceptable as the general phenomenon or not, and then whether the usual conception on the process of sexual fertilization is to be altered or not. As will be presented and discussed later, it has been found that the irregular and unusual characters of progenies in F_1 and later generations were produced by some of the pollination treatments.

Materials and Methods

Several varieties and strains of maize with different endosperm characters were kindly supplied by Hokkaido Agriculture Experimental Station in 1955 and 1956. In 1955, plants of 7 varieties were grown in the field and some of them were used in the experiments. Three inbred strains and one variety were additively introduced in the experiment in the next year, and experiments have been followed with these materials chiefly since that time. They had the kernels of following characters:

Tsuki-hin 76, "E-1", inbred, colorless (white) sugary.

Tsuki-hin 220, "Span cross", inbred, colored (yellow) sugary.

Tsuki-hin 186, "p 39", inbred, colored (yellow) sugary.

Tsuki-hin 226 "Chusei-siro", variety, coloreless (white) flinty.

It was found from the preliminary test that the genetical behaviours of kernel characters of these varieties and inbreds were regular and the hereditary factors involved were supposed to be homozygous for each character.

The experiments were carried out using several kinds of pollination procedures in order to obtain any influence on the resulting progeny. In the earlier time the effect of two more different types of pollen in mixture was chiefly inspected, and later the ages of pollen became the object of examination. Detailed methods in the experiment will be described in each case.

The hybrid kernels produced by different pollination procedures were mostly colored sugary and colorless flinty, but intermediate types of the color and texture were frequently produced. In F_2 and later generations, the segregation frequencies of color characters derived from colored sugary hybrid kernels and also of texture characters from flinty phenotypes of hybrid kernels were respectively evaluated with χ^2-test on the one hand for the family total and on the other hand within each of the family totals.

I. Multiparental pollination with fresh pollen

The pollen of two paternal forms one of which had the characters of colored sugary endosperm and the other of the colorless flinty one was mixtured and applied to the maternal plants of colorless and sugary kernel. The mixturing procedure of the pollen grains was worked out carefully protecting the foreign pollen from a contamination and the pollen was applied immediately to silks previously bagged. Totally 53 plants were pollinated with mixtured pollen for 5 seasons, and each ear produced the hybrid kernels segregating the colorless flinty and colored sugary with variable

TABLE 1. The segregation frequencies of F_2 family totals of each of F_1 ears derived from multiparental pollination with fresh pollen.

Phenotypes of F_1 individual	Nos. of dominant kernels	Nos. of recessive kernels	% of[a] dominant phenotype	Sum of χ^2 values from individual family	D. F. (Nos. of family)
colored sugary	440	142	75.6%	0.655	2
	93	36	72.1	0.581	1
	2203	768	74.2	14.450	11
	327	97	77.1	5.526	8
	1803	563	76.2	3.951	6
	666	237	73.8	2.023	3
	1075	306	77.8*	7.053	4
	677	224	75.1	3.269	4
	1383	448	75.5	7.703	2
	520	167	75.7	3.370	2
	1291	437	74.7	2.987	6
	1333	445	75.0	7.263	6
	1061	356	74.9	3.482	5
	603	215	73.7	1.273	3
	167	88	65.5**	12.300**	1
	366	130	73.8	0.941	2
	3669	1179	75.7	21.056	18
	310	120	71.9	5.144	2
	386	130	74.8	3.395	2
	138	434	75.5	3.927	6
Total	19711	6523	75.1	110.349	98
colorless flinty	124	30	80.5	2.502	1
	1540	526	74.5	5.526	8
	3684	1279	74.1	26.355*	16
	913	328	73.6	2.583	5
	1436	461	75.7	6.317	6
	1686	603	73.7	14.846	8
	1279	433	74.7	4.803	5
	2788	952	74.5	7.634	12
	3354	1193	73.8*	11.389	13
	1390	374	78.8**	45.480**	6
	699	255	75.6	4.154	3
	1220	428	74.0	3.725	6
	1806	569	76.0	12.089	9
	2305	720	76.2	7.045	10
	1339	436	75.4	5,215	7
	1714	555	75.5	2.972	8
	1714	511	77.0*	23.502**	9
	1031	337	75.4	2.563	4
	3408	1093	75.7	30.929	20
	2351	784	75.0	54.068**	16
	3174	1065	74.9	19.364	16
	3976	1188	77.0**	39.737**	19
	3018	920	76.6*	19.627	16
	3017	957	76.0	11.528	16
Total	48966	15967	75.4*	364.245**	239

* and ** denote the significant deviations from expected segregations at the level of 5% and 1% respectively.

a) respective χ^2 values of individual family total were not presented in the table. Similar indications and abbreviation were also applied in the other tables.

numbers. Both types of kernels were expected from original single crosses but frequently intermediate types of color and texture were secured. Sometimes a few unexpected kernels (colored flinty or colorless sugary) were obtained in these F_1 ears (Table 12).

Table 1 indicated F_2 segregation frequencies of 44 family totals from some of colored sugary and colorless flinty individuals in each of F_1 ears. In progenies of sugary F_1 kernels all segregated the colored and colorless sugary kernels in almost expected frequency (totally 75.1% of dominance), except 2 family totals. On the other hand in the flinty progenies, 5 of 24 family totals provided the significantly unusual F_2 segregations for flinty and sugary textures, where 4 of them were found to be in excess of the dominant character and the rest were of the recessive character reversely (Table 10). Furthermore it was pointed out that 5 of family totals wers also found to be those composed of the significantly heterogenous and deviated families. And these abnormalities of the segregation were remarkably presented in the grand totals of all families, in which the significantly high frequencies of the dominance (75.4%) and highly significant χ^2 value of total of 239 families were obtained. From these results the F_2 progenies at least from the flinty F_1 kernels were found apparently to be derived not from the homogenous F_1 individuals, all of which were nevertheless raised from similar pollination procedure.

Some of the dominant segregants of F_2 progenies segregated normally or abnormally were again self pollinated and all of the segregation frequencies of F_3 family totals from each of F_2 ears were presented in Table 2. Some of the family totals still provided the deviated segregation and variable and heterogenous constitutions of the families. The significantly excess frequencies of dominant segregants and also significantly heterogenous family totals were totally indicated in the progenies from abnormally segregated families only from the flinty individuals, as well as in each of the grand totals of F_3 progenies with high significance even from normally segregated sugary and flinty F_2 segregants. Thus the F_2 segregants of sugary and flinty phenotypes examined were clearly indicated respectively to be the kernels that were not of genetically uniform characters as a whole.

Only the flinty kernels of the families that indicated the significantly abnormal segregations in F_3 progenies were again examined in F_4 generations (Table 3). The significantly excess dominance of the grand total (76.2% of dominance) and some of highly significant heterogeneity within the family total were still presented, though a sufficient number of the segregants was not obtained.

TABLE 2. The segregation frequencies of F_3 family totals from normally and abnormally segregated F_2 progenies by multiparental pollination with fresh pollen

Segregation of F_2 family	Phenotypes of F_2 individual	Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	Sum of χ^2 values from individual family	D. F. (Nos. of family)
normal	colored sugary	1634	492	76.9*%	5.693	6
		569	191	74.9	0.564	3
		213	51	80.7*	4.545	1
		563	156	78.3*	10.025*	3
		1316	274	82.8**	101.958**	4
		1358	412	76.7	9.963	6
		349	102	77.4	2.240	2
	Total	6002	1678	78.2**	134.985**	25
	colorless flinty	1165	292	80.0**	85.312**	6
		1032	304	77.2	14.761*	6
		1886	574	76.7	13.942	8
		113	47	70.6	1.633	1
		183	78	70.1	3.332	1
		1738	561	75.6	7.410	9
		1513	510	74.8	9.514	7
		1204	420	74.1	3.113	7
		229	67	77.4	0.883	1
		1543	499	75.6	6.657	7
		704	277	71.8*	5.952	3
		826	304	73.1	4.097	3
		2075	663	75.8	6.315	9
		1383	436	76.0	12.410	7
		2237	647	77.6**	96.249**	10
		810	246	76.7	6.163	4
		951	338	73.8	2.093	5
		1424	463	75.5	6.046	7
		587	182	76.3	2.348	3
		476	156	75.3	3.687	3
		1115	353	76.0	3.574	6
		660	196	77.1	4.771	4
	Total	23854	7613	75.8**	300.252**	117
Abnormal	colored sugary	1358	412	76.7	9.963	6
		650	211	75.5	0.733	3
		536	190	73.8	5.479	3
	Total	2544	813	75.8	16.175	12
	Colorless flinty	2497	863	74.3	17.124	10
		592	198	74.9	0.347	3
		739	109	87.1**	70.206**	3
		1042	331	75.9	8.591	5
		1055	377	73.7	3.152	5
		667	192	77.6	7.109	3
	Total	6592	2070	76.1*	106.529**	29
Totals of normal and abnormal families	Colored sugary	8546	2491	77.4**	151.163**	37
	Colorless flinty	30446	9683	75.9*	406.781**	146

TABLE 3. The segregation frequencies of F_4 family totals of F_3 flinty segregants from multiparental pollination with fresh pollen

Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	Sum of χ^2 values from individual family	D. F. (Nos. of family)
1115	366	75.3%	5.598	6
120	44	73.2	0.293	1
1188	402	74.7	3.293	5
385	128	75.0	1.549	3
256	97	72.5	1.157	1
1415	514	73.4**	15.983*	7
441	152	74.4	1.152	3
678	228	74.8	2.858	4
790	177	81.7**	23.604**	4
298	99	75.1	0.371	2
531	58	90.1**	72.577**	2
157	103	60.4**	29.621**	1
421	71	85.6**	94.670**	2
Total 7795	2439	76.2**	252.726**	41

The nature and extent of the abnormal segregation were presented in Table 10, in which all of the family totals deviated significantly from the ordinary segregation were rearranged according to the different pollination procedures. A tendency for exceeding the dominant phenotypes by the mixtured pollination was interestingly indicated in F_2 and also in F_3 or later generations. Moreover, similar deviation was strongly observed in the individual transmissions of the segregant, for example, 3 families, the segregation frequencies of which were 152 : 27, 302 : 53, and 285 : 29 respectively, were produced by the selfing of the dominant kernels with flinty character in one of the abnormal F_2 families (359 : 51). And some of the dominant segregants from 285 such kernels were retested in the next season and again very deviated families (301 : 29, 230 : 29) were obtained. However, in the individual family, the segregation abnormalities were expressed variably in the progenies, for example, ordinarily segregating families or abnormal families of the excess dominant or of the excess recessive characters were not always produced from the kernels of similary segregated parental families.

In addition to the abnormalities of the segregation, sometimes the distinguishable colored character (frequently pale-colored) appeared in the colorless flinty and colorless sugary kernels of F_1 and also in later generations. The coloration of the endosperm in this case was generally very unstable

and the colored phenotype sometimes disappeared even in the progenies from such colored kernel of F_1 or F_2 and later generations. It appeared, however, that the colored character generally tended to be transmitted more strongly with the passes of generations.

As already pointed out there are several similar cases reported by U.S.S.R. researchers with different kinds of plant species including present material. As a remarkably interesting fact, they have repeatedly mentioned the occurrence of double dominant types of F_1 hybrid forms as an effect of two pollinators in mixtured application, in tomato (TURBIN & BOGDANOVA '49,) in maize wheat, *Mirabilis* and tomato (GLUSCHENKO '57).

II. Multiparental pollination with stored pollen

From the above pollination procedure it was found that, in F_1 and also in F_2 or later generations, some of abnormal types of hybrid kernel and unexpected segregation frequencies were produced by mixtured application of the fresh pollen. In order to detect the possibility of the more extreme influence on the resulting phenotypes of kernel, pollen grans were collected at the room in the morning from the tassels which had already been cut and bottled on the previous evening and were stored for appropriate periads in a dessicator at room temperature at first and later in a refrigerator. The storing periods were variable in the first experiment from 20 to 126 hours and 7 ears with viable kernels were obtained as a result of twenty or more of mixtured pollinations. Generally the numbers of the resulting ears with viable kernels and also of the total kernels on an ear were reduced with the application of aged pollen. But the appropriate storage treatments including the storage in a refrigerator were found to produce the sufficient results with a relatively large number of viable kernels.

188 ears with viable kernels, segregating the colorless flinty and colored sugary F_1 phenotypes, were totally obtained by the mixtured application of the stored pollen for 6 years. As indicated in Table 12, a relatively large number of F_1 ears with unexpected kernels, a few of the colored flinty and a more or less large number of colorless sugary, always appeared direccly from these pollinations.

In F_2 progenies which were optionally derived from the F_1 kernels, 5 of 27 family totals from sugary F_1 phenotype and 2 of 24 family totals from flinty F_1 phenotype provided respectively the significantly abnormal ratios of segregation, and also the significant heterogeneities within family total were indicated with 1 in sugary progeny and 3 in flinty progeny (Table

TABLE 4. The segregation frequencies of F_2 family totals of each of F_1 ears derived from multiparental pollination with stored pollen

Phenotypes of F_1 individual	Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	Sum of χ^2 values from individual family	D. F. (nos. of family)
colored sugary	347	104	76.9%	0.905	1
	208	72	74.3	0.067	1
	190	64	74.8	0.005	1
	283	77	78.6	2.504	1
	1012	322	75.9	2.540	5
	1117	365	75.4	8.840	7
	704	206	77.4	7.472	4
	1343	391	77.5*	11.300	8
	1020	340	75.0	5.206	5
	1249	404	75.6	6.533	6
	885	276	76.2	2.370	5
	630	200	75.9	3.360	4
	431	148	74.4	1.070	2
	449	121	78.8*	5.267	2
	1562	560	73.6	3.600	6
	2417	861	73.7	13.583	9
	3726	1256	74.8	10.729	14
	912	305	74.9	0.014	4
	1369	465	74.6	7.408	7
	2597	934	73.5*	11.981	11
	2665	864	75.5	20.945*	11
	1662	574	74.3	2.446	7
	2541	781	76.5*	11.795	9
	2073	714	74.4	10.085	7
	508	152	77.0	1.515	2
	1519	446	77.3*	11.709	6
	249	78	76.1	0.229	1
Total	33668	11080	75.2	163.486	146
colorless flinty	1679	630	72.7*	32.816**	11
	1345	471	74.1	4.139	9
	1298	468	73.5	17.609*	8
	1065	353	75.1	9.326	7
	2163	694	75.7	7.470	11
	2781	864	76.3	18.728	14
	1568	525	75.0	21.692*	8
	2014	663	75.2	10.504	10
	2803	974	74.2	11.979	12
	541	185	74.5	0.562	4
	786	246	76.2	3.024	3
	2135	659	76.4	4.295	9
	1683	582	74.3	6.744	9
	726	237	75.4	1.796	3
	1091	334	76.6	8.580	7
	112	46	70.9	1.426	1
	831	281	74.7	5.975	5
	669	200	77.0	3.879	3
	266	85	75.8	0.115	1
	198	58	77.3	0.750	1
	722	231	75.8	0.763	3
	529	161	76.7	1.574	3
	563	154	78.5*	5.293	3
	285	114	71.4	2.714	1
Total	27853	9215	75.1	181.753*	146

TABLE 5. The segregation frequencies of some of F_2 family totals from multiparental pollination with stored pollen, according to the storage periods

Storage periods	Phenotypes of F_1 individual	Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	Sum of χ^2 values from individual family	D. F. (nos. of family)
one day	colored sugary	1012	322	75.9%	2.540	5
		1249	404	75.6	6.533	6
		1020	340	75.0	5.201	5
		1117	365	75.4	8.840	7
		704	206	77.4	7.472	4
		1343	391	77.5*	11.300	8
		630	200	75.9	3.360	4
		249	78	76.1	0.229	1
	Total	7324	2306	76.1*	45.475	40
	colorless flinty	1345	471	74.1	4.139	9
		1298	468	73.5	17.609*	8
		1568	525	75.0	21.692**	8
		1065	353	75.1	9.326	7
		2163	694	75.7	7.470	11
		2681	864	75.6	18.728	14
		722	231	75.8	0.763	3
		529	161	76.7	1.574	3
		285	114	71.4	2.714	1
		563	154	78.5*	5.293	3
	Total	12159	4085	74.9	89.308	67
2 days	colored sugary	449	121	78.8*	5.267	2
		885	276	76.2	2.370	5
	Total	1334	397	77.1	7.637	7
3 days	colored sugary	2665	864	75.5	20.945*	11
		1662	574	74.3	2.446	7
		2541	781	76.5*	11.795	9
		2073	714	74.4	10.085	7
		508	152	77.0	1.515	2
	Total	9449	3085	75.4	46.786	36
	colorless flinty	669	200	77.0	3.879	3
		266	85	75.8	0.115	1
		198	58	77.3	0.750	1
	Total	1133	343	76.8	4.744	5
4 days	colored sugary	2417	861	73.7	13.583	9
		3726	1256	74.8	10.729	14
		912	305	74.9	0.014	4
		1369	465	74.6	7.408	7
		2597	934	73.5*	11.981	11
		1519	446	77.3*	11.709	6
	Total	12540	4267	74.3	55.424	51
	colorless flinty	1683	582	74.3	6.744	9
		1091	334	76.6	8.580	7
		112	46	70.9	1.426	1
		831	281	74.7	5.975	5
	Total	3717	1243	74.9	22.725	22
5 days	colorless flinty	726	237	75.4	1.796	3
6 days	colored sugary	1562	560	73.6	3.600	6
	colorless flinty	2014	663	75.2	10.504	10
		2803	974	74.2	11.977	12
		541	185	74.5	0.562	4
		786	246	76.2	3.024	3
		2135	659	76.4	4.295	9
	Total	8279	2727	75.2	30.362	38
Total of sugary phenotype		32209	10615	75.2	158.922	140
Total of flinty phenotype		26014	8635	75.1	138.585	135

4). Contrary to the expectation, it was found that none of abnormal segregations of F_2 progeny were obtained with grand totals from each of sugary and flinty F_1 individuals, except the significant χ^2 total of all the families from the flinty F_1 individual.

As a result it appeared that the procedures of mixtured application with stored pollen provided the segregated families with almost usual manner in which, however, most of the significantly segregated family totals showed the exceeding frequencies of dominant segregants (Table 10).

Table 5 indicated F_2 segregation of the respective family totals from the results for 2 seasons of experiment according to the storage periods. In this case several of the family totals segregated significantly or heterogeneously, but in the grand totals from each procedure, significantly excess segregants of dominant kernels were only produced in the progenies of the sugary phenotype with the treament of storing the pollen for one day. Influence of the pollen storage was scarcely indicated in the results of segregation frequencies of F_2 progeny.

The F_3 progenies from some of F_2 segregants of dominant phenotype, segregated normally and abnormally, were indicated in Table 6, in which 13 and 11 family totals from respective ears with the kernels of sugary and flinty F_1 textures were provided. Almost none of significant segregations were still observed in the totals and grand totals of the families from both of the sugary and flinty kernels, irrespective of the segregation behavious in the previous generation.

The examination of the genetical behavious of the unexpected kernels appearing in F_1 and also in F_2 progenies was similarly made arbitrarily, and the colorless sugary always segregated none of the other kernels than colorless sugary ones with a few of pale colored sugary segregants in a few of progenies.

Sometimes phenotypically a few of very pale colored flinty kernels appeared in F_1, F_2 and F_3 progenies, some of which still transmitted the color character and others restored the original colorless phenotype in succeeding progenies respectively. Mixtured effect with aged pollen apparently appeared in the frequencies of aberrant family total with abnormal segregation and in the abnormally colored phenotype with less extent as compared with the case of the fresh pollen.

A few of the hybrid kernels were obtained by the single cross pollination of colorless sugary plants with the aged pollen of colored sugary character. All F_1 hybrid kernels were of the colored sugary with a relatively large number of the colorless sugary kernel (Table 12). As indicated in

TABLE 6. The segregation frequencies of F_3 family totals from normally and abnormally segregated F_2 progenies by multiparental pollination with stored pollen

Segregation of F_2 family	Phenotypes of F_2 individual	Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	Sum of χ^2 values from individual family	D. F. (Nos. of family)
normal	colored sugary	977	311	75.9%	9.424	5
		722	245	74.7	1.114	5
		546	161	77.2	1.926	4
		555	194	74.1	2.040	3
		743	268	73.5	5.560	5
		1045	296	77.9*	13.856*	6
		935	278	77.1	4.389	5
		292	85	77.5	1.579	2
		735	250	74.6	0.106	3
		522	183	74.0	0.023	2
	Total	7072	2271	75.7	40.017	40
	colorless flinty	387	156	71.3*	4.028	2
		402	112	78.2	3.117	2
		328	96	77.4	1.937	2
		901	272	76.8	6.177	6
		381	139	73.3	3.876	3
		501	180	73.6	2.469	3
	Total	2900	955	75.2	21.604	18
abnormal	colored sugary	1106	381	74.4	3.657	7
		1030	343	75.0	0.940	6
		888	273	76.5	3.311	5
	Total	3024	997	75.2	7.908	18
	colorless flinty	788	238	76.8	3.659	4
		931	350	72.7	11.720	6
		643	204	75.9	5.041	4
		241	69	77.7	2.639	2
		579	207	73.7	7.789	4
		656	221	74.8	4.030	4
	Total	3838	1289	74 9	34.878	24
Total of sugary phenotype		10096	3268	75.5	47.925	58
Total of flinty phenotype		6738	2244	75.0	56.482	42

Table 7, from each of these F_1 kernels derived from the application of pollen stored for one day and for 5 days, calculatable 3 family totals were obtained respectively and none of significantly segregated family totals were observed. Only one family total from such a normally segregated F_2 family presented highly excess numbers of dominant segregant (80.9%) and also significant heterogeneity was obtained in 4 F_3 family totals.

TABLE 7. The segregation frequencies of F_2 family totals of sugary kernels from single cross F_1 progenies by multiparental pollination with stored pollen

Storage periods	Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	Sum of χ^2 values from individual family	D. F. (Nos. of family)
one day	370	127	74.4%	0.081	1
	230	89	72.1	1.431	1
	554	191	74.4	0.200	2
Total	1154	407	73.9	1.712	4
5 days	133	37	78.2	0.949	1
	175	55	76.1	0.145	1
	295	120	71.1	3.394	1
Total	603	212	74.0	4.488	3
Total of both storage treatments	1757	619	73.9	6.200	7

Though the respective numbers of ear and also kernel on an ear were decreased, it would undoubtedly be assumed that the aged pollen should affect the characters of hybrid kernel as indicated by several U.S.S.R. workers, who mentioned that the dominant characters of male form were greatly suppressed by the pollen storage (AIZENSTAT '54, SOKOLOVA *et al* '57 and STRAUMAL '55). In the present experiment, it might be considered that the mixtured application was not so important a factor but any direct physiological influence on the pollen must appear rather in the development of resulting kernel characters by storage. This consideration would presumably be supported by the results obtained in single cross pollination with stored pollen.

III. Supplementary pllination

In the preliminary experiment the plants having the double recessive endosperm characters of kernel were alternately pollinated by the pollen of two different forms with distinguishable endosperm characters, in which the time intervals of the 2nd pollination after the 1st application were variable from 30 to 180 minutes. From the results of these applications, it was found that the resulting F_1 kernels were of colorless flinty or colored sugary characters with a few of maternal colorless sugary. In the next year the pollination was tried with the pollen stored for about one day as the 1st pollinator, and after about one day the 2nd pollinator was applied. In

TABLE 8. The segregation frequencies of F_2 family totals of each of F_1 ears derived from supplementary pollination

Orders of pollination[1)]	Phenotypes of F_1 individual	Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	Sum of χ^2 values from individual family	D. F. (nos. of family)
WS×YS×WF	colored sugary	2048	633	76.4%	8.418	7
		3514	1138	75.5	24.376*	14
		1297	433	75.0	12.697*	6
		854	316	73.0	2.517	4
		3173	1042	75.3	15.956	11
		2373	779	75.3	13.832	10
		1159	373	75.7	1.621	5
		752	268	73.7	3.521	3
		98	31	76.0	0.065	1
		1162	386	75.1	10.014	6
		406	112	78.4	3.153	1
		1264	389	76.5	15.473**	5
	Total	18098	5900	75.4	111.643**	73
	colorless flinty	1457	479	75.3	8.777	6
		1759	657	72.8*	15.006*	7
		1609	622	72.1**	17.583*	7
		3429	1225	73.7*	21.137	15
		2805	972	74.3	25.043*	13
		1706	591	74.9	3.291	8
		3084	1130	72.7**	24.194*	13
		1122	435	72.1**	8.105	5
		1124	396	73.9	2.568	4
		2606	873	74.9	7.764	19
		1072	334	76.2	10.704	5
		1670	542	75.5	3.007	7
		1779	629	73.9	9.434	6
		2933	966	75.2	8.391	11
		2380	807	74.7	27.485**	9
	Total	31127	10668	74.5*	192.489**	125
WS×WF×YS	colored sugary	1034	348	74.8	5.146	7
		755	295	71.9*	8.003	5
		320	82	79.6*	4.541	1
		745	250	74.9	0.187	3
	Total	2854	975	74.5	17.877	15
	colorless flinty	1490	539	73.4	9.508	8
		1131	488	69.9**	27.326**	6
		1548	527	74.6	6.179	8
		3987	1401	74.0	27.724*	16
		3307	1163	74.0	23.097	14
		2186	732	74.9	14.211	10
		2244	1234	64.5**	46.638**	15
		2588	891	74.4	12.009	11
		4314	1406	75.4	26.280	16
		2426	868	73.6	28.817**	9
		929	300	75.6	1.528	4
	Total	26150	9549	73.3**	223.317**	117
Total of sugary phenotype		20952	6875	75.3	129.520**	88
Total of flinty phenotype		57377	20217	73.9**	410.503**	241

1) WS, YS and WF indicate the plants with the characters of colorless sugary, colored sugary and colorless flinty kernel respectively. Similar indications were used in the other tables.

the first treatment the 1st pollinator was the plant of colored sugary and the 2nd one was of colorless flinty. In the 2nd treatment contrarily the pollinators were applied with reverse order.

All of the F_1 ears obtained segregated the original colored sugary and colorless flinty kernels with variable numbers and as indicated in Table 12 a relatively large number of unexpected colorless sugary kernels sometimes appeared, 26 grains in 7 ears from the 1st treatment and 36 in 6 ears from

TABLE 9. The segregation frequencies of F_3 family totals from normally and abnormally segregated F_2 segregants by supplementary pollination

Segregation of F_2 family	Phenotypes of F_2 individual	Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	Sum of χ^2 values from individual family	D. F. (nos. of family)
normal	colored sugary	1276	365	77.8**%	11.113*	4
		618	217	74.0	0.436	2
	Total	1894	582	76.5	11.549	6
	colorless flinty	1112	387	74.2	6.217	6
		318	85	78.9	5.630	2
		712	226	75.9	3.717	4
		1899	622	75.3	17.508*	9
		613	220	73.6	2.520	3
		652	233	73.7	2.797	3
	Total	5306	1773	75.0	38.389	27
abnormal	colored sugary	352	110	76.2	0.349	1
		303	84	78.3	2.240	1
		1114	379	74.6	2.530	4
	Total	1769	573	75.5	5.119	6
	colorless flinty	499	133	79.0*	5.826	2
		1709	600	74.0	30.391**	7
		1993	610	76.6	15.960	9
		1516	534	74.0	6.634	6
		1321	458	74.3	4.200	6
		581	176	76.8	1.294	2
		466	160	74.4	0.986	2
		251	93	73.0	2.439	2
		159	83	65.7**	11.157	1
		447	151	74.7	0.319	2
		486	145	77.0	4 929	2
		934	326	74.1	3.623	4
		1316	459	74.1	21.440**	5
	Total	11678	3728	75.8*	109.198**	50
Total of sugary phenotype		3663	1155	76.0	55.057	39
Total of flinty phenotype		16984	5501	75.5	147.587**	77

the 2nd treatment.

Examining the genetical behaviours of these F_1 kernels, some of them were arbitrarily selfed and 27 family totals from the 1st treatment and 15 from the 2nd treatment were totally obtained. As indicated in Table 8, highly significant deviations from expected segregations were observed in many of the family totals, as well as in the grand totals of all the families especially from flinty F_1 individuals. Segregated ratios from these treatments were totally 74.5% and 73.3% of the dominance in respective grand totals derived from flinty individuals, and the tendency for reducing the dominant segregants was evidently indicated in each of the aberrant family totals as in Table 10, irrespective of the treatments. In addition to the highly abnormal segregations, the significant heterogeneity within family total was remarkably presented in many of them from both of the sugary and flinty F_1 individuals, as well as in the grand totals of all the families.

In F_3 progenies from some of dominant segregants from the families segregated normally or irregularly, the significant deviations of segregation were still observed with less extent than that of F_2 progenies (Table 9).

The colored (pale-colored) kernels of segregants to be sorted out from colorless kernels were always observed in F_2 and F_3 progenies with high frequency, and the behaviours of such colored character were as unstable as in the case by mixtured pollination with fresh pollen.

Discussion

Interpreting some irregular transmission of hereditary characters, Mendelian geneticists have used to develop the consideration based upon the experimental results that these irregularities were derived not from the deviated nature of fertilization mechanism itself but from the various abnormal phenomena accompanying additievly before or after the usual fusion of sexual cells. They were mutations, chromosomal irregularities, the lethals or the selective fertilization including incompatibility or sterility which were all expected to be controlled by certain kinds of hereditary unit involved in haploid or diploid conditions.

The selective fertilization was at first noted by CORRENS ('01) with maize, and since that time a large scale of experiments has widely been carried out by several workers chiefly as to the kernel characters of different varieties or strains of corn (BRINK '25, COULTER '25, JONES '22, '24, EMERSON '34, KEMPTON '27, KIESELBACH *et al* '26 and MANGELSDORF *et al* '26). Although it was accepted that this phenomenon was controlled by "Gametophyte factors" during the course of experiments (BEMIS '59, EMERSON '34 and

NELSON '52), still other influence than that of the genic control on the process of fertilization or on the hereditary behaviours of resulting zygotes was sometimes pointed out by several authors with some of the pollination treatments. For example, BOND ('27) showed with pea a distinctive tendency, toward an excess of the recessive characters in F_2 progenies derived from bud and restricted pollinations of a F_1 hybrid. The position of seeds or pods was also found to be the factor affecting the segregations of the progeny. Similar tendency was indicated by BRINK ('25) and KEMPTON ('27) with the dessication or storage treatment of the pollen grains of corn.

It is pointed out that the mixtured application of different pollen was already carried out by several workers for different aims of experiments (CURTIS *et al* '56, LENG *al et* '51, MARSHAL '10, and MARTHER '47). Jones ('20) applied the mixtured pollen of several genetically different plants of maize and tomato and found a tendency for preferring the female own pollen. Though the selectiv fertilization in the case by Jones was suggested later by EMERSON ('34) to be also explained by the factor, Ga, it is interesting that 30 of illegitimate seeds, the character of which Jones has not described, occurred in the total 63,000 seeds as a result of the possibly alleged contaminated pollination.

It is an especially interesting phenomenon that not only the process of fertilization progressed in a usual way but sometimes more than one pollen grain was concerned in the formation of the one kernel in maize. Sprague ('32) found with maize the frequent unusual progenies of kernel having the different phenotypical colorations between aleurone and scutellum. The possible causes of "hetero-fertilization", the process of fertilization resulting in the different phenotypical embryo and endosperm, were analysed and indicated with the mixtured pollination that the genotypic differences are usually due to the participation in the fertilization process of sperms from more than one pollen grain. Hetero-fertilization found to occur with the frequencies of approximately 1 in 80 seeds in normal culture and 1 in 4 in the high hetero-fertilization stock. He described the nature and extent of such fertilization as follows:

"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.", and still more "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." Later, similar hetero-fertilization was reported by ROMAN ('48) with maize having interchanged chromosomes, and he found that unusual fertilization occurred 8 in a total 666 seeds in one interchange case and 3 among 496 in another one, though no more consideration was given by him to this astonishing fact.

In the present experiment, several unexpected results were obtained by different pollinations. In these abnormals, one of the extreme cases was the appearance of a few kernel of colored and flinty types. Colored flinty kernels were obtained not only in the F_1 progenies by the multiparental pollination with fresh and stored pollen but also by the selfing of F_1 kernels derived from the multiparental and supplementary application, and none of them were produced in the F_1 families by supplementary pollination and the F_2 progenies from multiparental pollination with stored pollen (Table 12).

On the other hand as already pointed out the frequent and somewhat detective appearances of the pale-colored kernels were observed in all of the sugary and flinty progenies from artificial pollinations, except in those from the multiparental application with stored pollen. Though relatively unstable in expression, this color character was observed to be transmitted to the progenies in not a few case, as if it was detected to be original colored kernel.

The appropriate explanation for the appearance of these kernels would not be given sufficiently based upon the factorial assumption, except for the faulty techniques including contaminated pollination, though these possibilities were never ruled out especially for a few colored flinty kernel. Some of these colored kernels were examined with the segregation of succeeding generation and most of them were found to produce unexpected irregular ratios of segregants. The appearance of the kernels with both types of dominant male characters must be regarded as consistent with the reports by several authors in U.S.S.R.

The white and sugary kernels, female type of characters, were the other irregular type of hybrid kernel. These abnormals were also raised variably from all of the experimental procedures applied, and no correlation with pollination modes would seem to exist, but it would again be noted that they were never produced by ordinary single cross pollination. At first these kernels were considered to be the results of faulty pollination by female own pollen, but later it was found by repeated treatments of different pollinations that they were possibly derived from the different kinds of phenomena other than those from the simply faulty techniques. As in-

TABLE 10. The segregation frequencies of significantly aberrant family totals in the different generations from three experimental pollinations

Modes of pollination	Genera-tions	Phenotypes of selfed individual	(a) Family totals in excess of dominant phenotype			(b) Family totals in excess of recessive phenotype		
			Total nos. of kernel obtained	% of dominant phenotype	Nos. of family	Total nos. of kernel obtained	% of dominant phenotype	Nos. of family
1) Multiparental, with fresh pollen	F_2	sugary	1381	77.8%	4	255	65.5%	1
		flinty	1764 2225 5164 3938	78.8 77.0 77.0 76.6	6 9 19 16	4547	73.8	13
		total	13091	77.1	50	4547	73.8	13
	F_3	sugary	2126 264 719 1590	76.9 80.7 78.3 82.8	6 1 3 4			
		total	4699	79.3	14			
		flinty	1457 848 2884	80.0 87.1 77.6	6 3 10	981	71.8	3
		total	5189	79.8	19	981	71.8	3
	F_4	flinty	967 589 492	81.7 90.1 85.6	4 2 2	1929 260	73.4 60.4	7 1
		total	2048	85.1	8	2189	71.8	8
2) Multiparental, with stored pollen	F_2	sugary	1734 570 3322 1965	77.5 78.8 76.5 77.3	8 2 9 6	3531	73.5	11
		total	7591	77.1	25	3531	73.5	11
		flinty	717	78.5	3	2309	72.7	11
	F_3	sugary	1341	77.9	6			
		flinty				543	71.3	2
3) supplementary, a) WS×YS×WF	F_2	flinty				2416 2231 4654 4214 1557	72.8 72.1 73.7 72.7 72.1	7 7 15 13 5
		total				15072	73.0	52
b) WS×WF×YS	F_2	sugary	402	79.6	1	1050	71.9	5
		flinty				1619 3478	69.9 64.5	8 15
		total				5097	66.2	23
	F_3	sugary	1641	77.8	4			
		flinty	632	79.0	2	242	65.7	1

dicated previously these kernels were secured also in F_1 progenies of only single cross pollination with the pollen of colored sugary kernel, though none of them appeared again in the F_2 progenies. The influence of storing the pollen would thus be presented with the tendency for producing the unexpected progenies having maternal type of characters. Above facts were considered to correspond with the results obtained by AIZENSTAT ('54) and others.

No other unexpected kernels were obtained by these different experimental procudures but interestingly in relatively most of the progenies in F_2 and later generations the segregation behaviours were certainly curious.

Table 10 represented all of the aberrant family totals derived from different pollinations. It would be found from these results that as a whole most of the family totals provided the high frequencies of dominant segregants in F_2 and F_3 progenies from multiparental application of fresh and aged pollen. On the other hand in the F_2 progenies from supplementary application irregularities were expressed reversely by the high frequencies of the recessive characters irrespective of the pollination orders, though in this pollination only a few of significantly segregated family totals were produced from F_1 kernels of colored sugry phenotype. Moreover, relatively high frequencies of family totals in which individual families segregated heterogenously were observed in F_2 and F_3 progenies mainly from flinty phenotype by the procedure of supplementary application, and also in the F_2 progenies from flinty individual by the mixtured pollination with fresh pollen (Tables 1, 2, 3, 8 and 9). Some of the significantly heterogenous family totals were accompanied by the family totals of significantly aberrant segregations in the previous generation, and others were observed in the family totals segregated normally.

A summary of these irregular segregations in F_2 and F_3 progenies from three pollination procedures was expressed in Table 11. The frequencies of the abnormalities of segregation were observed in F_2 progenies to be almost 20% or more of the previous F_1 individuals from different pollination, and it was found that in the mixtured application with fresh pollen the aberrant progenies from flinty F_1 individuals were more frequent than those from sugary F_1 individuals, but reversely the decreased frequencies of them were observed in the progenies by the application of stored pollen.

In the F_3 generations similar frequency of irregular family total (23.7%) as in F_2 generation were obtained in the progenies from mixtured application of fresh pollen, and relatively decreased frequencies of aberrant family totals were observed in the progenies from both sugary and flinty F^2 iindviduals

TABLE 11. The frequencies of aberrant family totals in F_2 and F_3 generations that segregated significantly and heterogenously, derived from different experimental pollinations

Modes of pollination	Phenotypes of F_1 individual	Nos. of family total			% of aberrant family total	% of dominant phenotype of all families	Sum of χ^2 values of all families	D. F (nos. of family)
		aberrant	normal	total				
F_2 generations								
multiparental, with fresh pollen	sugary	2	18	20	10.0%	75.1%	110.349	98
	flinty	7	14	24	29.2	75.4*	364.245**	239
	total	9	32	44	20.5			
multiparental, with stored pollen	sugary	6	21	27	22.2	75.2	163.487	146
	flinty	4	20	24	16.7	75.1	181.753*	146
	total	10	41	51	19.6			
supplementary 1) WS×YS×WF	sugary	3	9	12	25.0	75.4	111.643**	73
	flinty	7	8	15	46.7	74.5*	192.489**	125
	total	10	17	27	38.5			
2) WS×WF×YS	sugary	2	2	4	50.0	74.5	17.887	15
	flinty	4	7	11	36.4	73.3**	223.317**	116
	total	6	9	15	40.0			
3) Grand total	sugary	5	11	16	31.3	75.3	129.520**	88
	flinty	11	15	26	42.3	73.9**	410.503**	241
	total	16	26	42	38.1			
single cross, with stored pollen	sugary	0	6	6	0.0	73.9	6.200	7
F_3 generations								
multiparental, with fresh pollen	sugary	4	6	10	40.0	77.4**	151.433**	37
	flinty	5	23	28	17.9	75.9*	406.781**	174
	total	9	29	38	23.7			
multiparental, with stored pollen	sugary	1	12	13	7.7	75.7	47.925	58
	flinty	1	11	12	8.3	75.0	56.482	42
	total	2	23	25	8.0			
supplementary	sugary	1	4	5	20.0	76.0	55.057	39
	flinty	5	14	19	26.3	75.5	147.587**	77
	total	6	18	24	25.0			

by the application of stored pollen, although in the former case the frequencies appeared as reversly in the family totals from sugary and flinty individuals as in the case with F_2 generations. On the other hand, from the supplementary pollintion it was indicated that as a whole relatively high frequencies of abnormal family total and also more or less decreased frequencies of them were observed in F_2 and F_3 generations respectively.

It would possibly be pointed out that in F_2 and later generations abnormal segregation was generally observed in the family totals and in the grand totals of all the families produced by the different pollinations. These curious results were again indicated in the individual family as indicated in Table 13, where, for example, extremely high frequencies of dominant seg-

TABLE 12. Total numbers of F_1 ears obtained by different experimental pollinations, and those of unexpectable kernels in F_1 and F_2 progenies

Modes of pollination	Nos. of F_1 ears obtained	Unexpectable irregular kernels				
		Generations	Colorless sugary		Colored flinty	
			Nos. of kernel	Nos. of ears segregated kernels	Nos. of kernel	Nos. of ears segregated kernels
multiparental, with fresh pollen	53	F_1	45	11	1	1
		F_2	—	—	14	5
multiparental, with stored pollen						
1) 1 day stored	34	F_1	11	6	—	—
2) 2 days stored	33	"	111	2	—	—
3) 3 "	39	"	84	5	2	2
4) 4 "	36	"	21	6	1	1
5) 5 "	29	"	40	9	2	1
6) 6 "	12	"	1	1	—	—
7) 7 "	2	"	—	—	—	—
8) 8 "	2	"	—	—	—	—
9) 10 "	1	"	—	—	—	—
total	188		268	29	5	4
single cross with stored pollen						
1) 1 day stored	2	F_1	20	1	—	—
2) 4 days stored	5	"	10	3	—	—
3) 5 "	5	"	13	2	—	—
total	12		43	6	—	—
supplementary						
1) WS×YS×WF	21	F_1	26	7	—	—
		F_2	—	—	28	13
2) WS×WF×YS	12	F_1	36	6	—	—
total	33		62	13	28	13

regants (99% or more) and also of recessive segregants (32% or more) were observed in the F_2 or later progenies mostly from flinty indivdual.

It would apparently be understood that from above indications the progenies derived from the different artificial pollination procedures were not necessarily uniform and relatively most of the individuals were unstable in the expression of their genetical characters. On the other hand relatively a few of ordinarily singly crossed progenies were produced as a control and almost desirable frequencies of expected F_2 ratio were obtained, the results of which were not presented.

Conclusively it would safely be emphasized that some of experimental procedures tried would evidently affect the characters of resulting hybrid

TABLE 13. The extremely irregular frequencies of segregation of some of the individual families in the different generations derived from different experimental pollinations

Modes of pollination	Generations	Phenotypes of selfed individual	Nos. of dominant kernel	Nos. of recessive kernel	% of dominant phenotype	χ^2 values
multiparental, with fresh pollen	F_2	flinty	359	51	87.6%	34.501
		"	262	47	84.8	15.794
		"	152	26	85.4	10.255
	F_3	flinty	332	16	95.4	77.257
		"	154	19	89.0	18.130
		"	250	1	99.6	81.021
		"	220	129	63.0	26.637
		"	180	32	84.9	11.094
		"	302	53	85.1	19.201
		"	285	29	90.8	41.618
		"	280	3	98.9	86.503
	F_4	flinty	301	29	91.2	46.259
		"	230	29	88.8	26.318
		"	157	103	60.4	29.621
multiparental, with stored pollen	F_2	flinty	98	59	62.4	13.251
	F_4	"	259	2	99.2	81.748
supplementary	F_2	flinty	308	146	67.8	12.408
		"	243	120	66.9	12.570
		"	249	120	67.5	11.130
		"	148	22	87.1	13.184
		"	230	115	66.7	12.778
		"	245	50	83.1	10.198
		"	133	79	62.7	17.006
		sugary	376	85	81.6	10.586
		"	421	98	81.1	10.359
single cross with stored pollen	F_2	sugary	274	55	83.3	12.038

kernels in F_1 and also in later generations. As already pointed out these unusual results were impossible to be interpretd by the possible segregation and combination of hereditary units under the established mechanism of sexual fertilization except by the experimental errors. Now when hereditary characters were supposed to correlate with their hereditary units in the behaviour from parent to progeny, it would apparently be considered that important modification must occur in the ordinary process of fertilization and formation of the resulting kernels. Concerning these considerations, the data obtained by SPRAGUE ('32) and ROMAN ('48) may be very interesting.

GLUSHCHENKO ('57) developed from his multiparental pollination experi-

ment the conception that not only the process of fertilization was essentially fusion of egg and sperm nuclei but also the other supernumerary sperm nuclei are united with those of maternal tissues surrounding the embryo sac, supplying different source of nutrition to the developing embryo and endosperm. Contrary to the observation by MILLER ('19) and others, somewhat different features of the fertilization process were reported, for example, by ELLEGORN *et al* ('49). They observed with *Amaryllis* species that the contents of several pollen tubes were discharged to an embryo sac and the nuclei of somatic cells at micropylar end of the nucellus may also be fertilized with other sperms after usual double fertilization. They indicated from these results that male cytoplasm introduced into the embryo sac would affect the physical and chemcial conditions of it in proportion to the actual amount of these cytoplasms. It would seem to be significant that the embryo was wholly surrounded not only by female tissue but also by tissues fertilized with the sperms of paternal plants. Similar observations were also carried out by several workers (GAVRILOVA '53 and ZAIKOVSKAJA '52, '54), and the following effective facts were pointed out. At first, some of pollination methods, multiparental, supplementary and unlimmited pollination intensitied the physiological processes of fertilization, whereas such a pollination as self or restricted one reversely suppressed those processes due to the reduced mutual interaction among sexual cells, and this defective influence was improved by the former pollinations (SCEDRINA '59, USTINOVA '54 and VASSILEVA-DRJANOSKA '54), and secondly, the numbers of pollen tubes introduced were variable with the different kinds of parental varieties employed (RUMI *et al* '55).

ARIYASU ('59) cultured the pollen grains of five different species, and found the increased percentages of germination and accelerated growth rate of pollen tubes in proportion to the numbers of pollen grains in each group of culture. Similar phenomenon was demonstrated by BORRIS *et al* ('55), GOLBINSKY ('46) and SAITAN ('52) including an impeded effect in some combinations. The accelerated or inhibited effects in pollen culture will evidently show the mutual interaction among pollen grains of different forms. It would be possible to consider that such interaction may also occur among the pistil and pollen tubes or ovary and sperm nuclei etc. (BRITIKOV '54 and MODILEVSKY '50).

POLYAKOV ('55) observed the fertilization process by the pollination of pollen labelled with P^{32} and S^{35} on tobacco, wheat, rye and maize. He pointed out that the fertilization consisted not only of basic double one but also of a supplementary process by supernumerary pollen tubes, demonstrating the

possibility of influence of the contents of supplementary tubes on the developing embryo. He obtained the rough calculation of pollen tubes transmitted radioactive materials to one ovule ranging from 5 in rye and wheat to 2.2–2.8 in maize in pollination with labelled pollen only. In one of *Nicotiana* species, he also found that the ovule fertilized with the mixture of its own unlabelled pollen and labelled pollen of a species incompatible with the former, contained radioactive isotopes, suggesting that the isotopes gained entry as a result of physiological reaction between the components of the pollen mixture. Later he ('57) still reported the continued experiment with cotton and maize, where he obtained the great quantity of isotopes in the seed produced by supplementary pollination with labelled pollen of other variety in the time intervals 6, 12 and 24 hrs, after selfing. These results would evidently indicate that many pollen tubes were introduced into an ovule supplementary. Similar experiments were also reported by GÖRIG ('60) and POLYAKOV *et al* ('56).

The results obtained by the observation of different pollinations with labelled or unlabelled pollen seem to support strongly the following explanation of unexpected phenomenon in F_1 and later generations. It would be able to consider that the fertilization was a very complicated process in which more than two sperms would take part in penetrating directly into embryo sac or by fusing with the cells of surrounding maternal tissues, affecting the developing embryo and endosperm with the supply of the special materials. Not only the mechanical fusion of main sexual nuclei was the essential nature of sexual fertilization, but also considerably mutual interactions among both sexual materials through the metabolic system might occur as a result of fertilization. As well-known, LYSENKO ('54) explained the sexual fertilization as one of metabolic processes in which equivalent sexual cells were assimilated to one another. Gamete cells are regarded to be one of the livings with their own biochemical systems, and so when they are fused, it will be possible that the profound mutual interaction occurs through these systems, creating finally the new form of systems in the resulting zygotes.

The characters of endosperm in maize are originated from the fusion of polar nuclei with one of sperm nuclei, known as "Xenia". The heterofertilization described previously may possibly explain partly the unusual characters of F_1 kernels, especially the occurrence of unreasonable characters in F_2 and later progenies as a result of different genotypic constitutions of embryo and endosperm cells in an embryo sac. All of unusual results in the present experiment, however, will not be interpreted sufficiently by

hetero-fertilization, based upon the transmission of Mendelian units.

Appearance of double dominant and also of the frequent pale-colored characters in the different generations may be the results produced by the immediate effects of two types of sperm cell after mutually affecting to express any metabolic system producing the dominant characters, or by the additive effects of supplementary sperms fused with surrounding somatic cells on the developmental processes of the endosperm. When the embryos or the materials necessary for the development of the resulting embryos are affected in this way, it may be expected that these influences are transmitted to their progenies.

White sugary type of unexpected kernels may be somewhat different from above situation and bearing in mind the case with the storing effect of the pollen, it is possible to suppose that the sufficient metabolic function to produce the dominant characters may be reversely suppressed by unknown mechanism through similar mutual interactions of sexual cells.

It will now be difficult to explain sufficiently for the frequent appearances of the families segregated significantly in F_2 and later generations, which were apparently derived from the different artificial pollination procedures.

The general features of these abnormal and unexpected segregations are as follows: (a) Even in the families total of which was evaluated as a regularly segregated one, several significantly aberrant families were sometimes appeared. (b) The segregants exceeding the expected frequencies were not always constant as dominant or recessive kernels even in a family total detected to be significant, nevertheless as a whole somewhat a high degree of correlation was observed between the natures of irregular segregation and experimental procedures (Table 10). (c) The degrees of aberrant segregation were extremely variable in each of the individual family irrespective of the segregated characters. (d) Highly significant aberrations from expected frequency were observed in most of the grand totals of all the families in F_2 and later generations, in which the high degrees of heterogeneity within grand totals of them were also frequently observed. (e) The transmission behaviours of these irregularities were relatively not uniform, as in the case with the coloration, and the significantly aberrant families were not always provided from the dominant kernels derived from such abnormal families, and vice versa. (f) The frequencies of aberrant family totals were almost alike in F_2 generation irrespective of the modes of pollination, in which more or less high frequencies were obtained from supplementary pollination, but in F_3 generation highly decreased frequencies were indicated in the progenies from mixtured application of stored pollen

and also from supplementary pollination. (g) The degrees of aberrant segregation and also the frequencies of aberrant family total were not always higher in the progenies from flinty segregants (segregation of texture) than in those from sugary segregants (segregation of color). Moreover, in F_2 progenies from supplementary pollination, the frequencies of aberrant family total from the sugary F_1 individuals were about half of those from the flinty individuals when the sugary pollen was applied as the 1st pollinator and reversely higher frequencies were obtained in the family total from sugary F_1 kernels when the sugary pollen was applied as the 2nd pollinator (Table 11).

The explanation based upon the behaviours of gametophyte factors might be possible partly for these aberrant segregations, but with some of appropriate reasons including the above notes this possibility was safely ruled out as a plausible explanation, though sufficient experimental designs were not properly adopted to this problem.

As presented by KLJUCAREVA ('57) who suggested the effect of surrounding maternal tissues fertilized with supplementary male nuclei as a condition analogous to the grafting, if artificially different coditions were given to these physiological processes the resulting new livings will be affected to an extent varying from the slight change of physiological conditions which is not detected visibly, to the visible modification or occurrence of unusual characters by the direct influence or by the accumulation of a slight effect. These effective conditions, however, will be delicate and inconstant, and yet the livings may generally be considered to have a conservative resistance to a change of environmental conditions through evolutionary processes. These considerations seem to explain the relatively unstable and variable occurrences of unusual offspring and also the aberrant segregations of progeny. These conceptions are only hypothetical and it is necessary for the more exact explanation to obtain the accurate knowledge about the developmental mechanism of hereditary characters or the physiological nature of characters in the transmission processes.

Summary

1) The experiments with different methods of artificial pollinations, multiparental pollinations with fresh and aged pollen and supplementary pollination, have been carried out with several varieties and inbred strains of maize.

2) A few hybrid kernels with colored and flinty characters, dominant types of both pollinators, were produced with the different experimental procedures

in F_1 and later generations. And at the same time the frequent appearances of colored (frequently pale-colored) kernels were observed in the families from colorless individuals in different generations.

3) A relatively large number of colorless sugary kernels, maternal recessive characters, were similary produced with the different pollination procedures, especially with the stored treatments.

4) In addition to the appearance of these unexpected individuals, more or less extreme irregularities were frequently observed in the segregation frequencies of F_2 and later generations, the nature of which was somewhat unstable and variable in the individual families but as a whole a certain relation was observed between the phenotypes of segregant with deviated frequencies and the experimental procedures.

5) Any plausible explanalion would not sufficiently be provided for the irregular results based upon the factorial hypothesis. On the other hand data were discussed with the new consideration on the mechanism of sexual fertilization as a plausible explanation, which was derived from the cytological and genetical data obtained by many workers chiefly in U.S.S.R.

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Literature cited

AIZENSTAT JA. S. '53, *Uspehi Sovre. Biologii,* **35**: 271–290. —— '54, *Izv. Akad. Nauk SSSR,* **4**: 24–58. ARIYASU T. '59, *Bot. Mag. Tokyo,* **72**: 473–476. AVRATOSCUKOVA, N., V. FERJANCIKOVA, G. KRIVSKA-SVEREPORA, Z. NOVAKOVA and E. REZNICKOVA, '56, *Preslia* **28**: 250–261. BEMIS W. P. '59, *Genetics* **44**: 555–562. Bond C. J. '27, *J. Genet.* **17**: 269–281. BORRIS H. and H. KROLOP '55, *Naturwissen.* **42**: 301–302. BRINK R. A. '25, *Genetics* **10**: 359–394. BRITIKOV E. A. '54, *Trud. Inst. Fiziol. Rasten, K. A., Timirijazev* **8**: 3–58. CORRENS C. '01, *Bibl. Bot.* **53**: 1–111. COULTER M. C. '25, *Bot. Gaz.* **79**: 28–44. CURTIS J. J., A. M. BRUNSON, J. E. HUBBARD and F. R. EARLE '56, *Agron. J.* **48**: 551–555. ELLEGORN JA. E. and V. V. SVETOZAROVA '49, *Botan. Zurnal* **34**: 569–581. EMERSON R. A. '34, *Genetics* **19**: 137–156. EVERETT H. L., D. L. UMALL and I. S. SANTONS '58, *Philipp. Agric.* **42**: 6–11. GAVRILOVA O. M. '53, *Izv. Akad. Nauk. SSSR.* No. **6**: 37–45. —— '56, *Vsesoju. Naucno-issledo. Inst. Saharnoi Svekly, Moskva* 318–324. GLUSHCHENKO I. E. '57, *Agrob.* No. 1: 3–18. GOLUBINSKY I. N. '46, *C. R. (Doklady) Acad. URSS* **53**: 71–74. GÖRIG H. '60, *Naturwissen.* **47**: 142. GUREVIC L. I. '50, *Dokl. Akad. Nauk SSSR* **70**: 707–710. JAKOVUK A. S. '54, *Agrob.* No. 3: 116–121. JONES D. F. '20, *Biol. Bull.* **38**: 251–289. —— '22, *Biol.. Bull.* **43**: 167–174. —— '24, *Proc. Nat. Acad. Sci.* **10**: 218–221. KEMPTON J. H. '27, *J. Agr. Res.* **35**: 39–74. KIESSELBACH T. A. and N. F. PETERSON '26, *Genetics* **11**: 407–422. KLJUCAREVA M. V. '57, *Z. Obshch. Biol. Moskva* **18**: 288–297. KOCARJAN E. G. '48, *Agrob.* No. 5: 70–77. KONONKOV P. F '58, *Dokl. Vsesojuzn. Akad. Sel'skohozjajstv. Nauk Lenin.* No. 1: 16–19. KOSTRJUKOVA K. Ju. and F. S. GURECKAJA 56, *Z. Obshch. Biol., Moskva* **17**: 23–31. LENG E. R., F. R. EARLE and J. J. CURTIS '51, *Cereal Chem.* **28**: 479–482. LYSENKO T. D. '54, *Moscow.* MANGELSDORF P. C. and D. F. JONES '26, *Genetics* **11**: 423–455. MARSHALL F. H. A. '10, *London.* MARTHER K. '47, *Heredity* **1**: 175–186. MEDVEDEVA G. B. '55a, *Izv. Akad. Nauk SSSR.* No. 3: 97–119. —— '55b, *Trud. Inst. Genet.* **22**: 83–94. —— '57, *Z. Obshch. Biol., Moskva* **18**: 217–227. —— '58, *Trud. Inst. Genet.* **24**: 138–149. MILLER E. C. '19, *J. Agr. Res.* **18**: 255–266. MODILEVSKY JA. S. '50, *Izv. Akad. Nauk SSSR.* No. 2: 23–37. NELSON O. G. '52, *Genetics* **37**: 101–124. OKSIJUK P. F. '55, *Bot. Z. Kijv.* **12**: 44–51. OKSIJUK P. F. and M. I. HUDJAK '57, *Ukrajin bot. Z.* **14**: 71–77. POLYAKOV I. M. '55, *Conf. Acad. Sci. USSR. On the Peaceful Uses of Atomic Energy July 1–5, 1955, Session of Div. Biol. Sci. U.S. Atom. En. Commis. Wash.1956*: 139–144. —— '57, *Selek. i. Semeno.* No. **3**: 14–22. POLYAKOV I. M., A. N. DMITRIEVA and A. F. ZDRILJKO '56, *Z. Obshch. Biol., Moskva* **17**: 321–334. ROMAN H. '48, *Pro. Nat. Acad. Sci. USA.* **34**: 36–42. RUMI V. and N. VLASOVA '55, *Hlopkovodstvo* No. 10: 16–19. SAITAN I. M. '52, *Agrob.* No. 5: 108–111. SCEDRINA R. N. '59, *Agrob.* No. 2: 193–197. SCELOKOVA Z. I. '54, *Z. Obshch. Biol. Moskva* **15**: 109–114. —— '56, *Izv. Akad. Nauk SSSR.* No. 4: 3–14. SOKOLOVA E. P. and V. S. ZALNIN '57, *Dokl. Akad. Nuak SSSR.* **114**: 429–430. SPRAGUE G. F. '32, *Genetics* **17**: 358–368. STRAUMAL B. '55, *Hlopkovodstvo* **9**: 12–17. TER-AVANESJAN D. V. '46, *Agrob.* No. **3**: 71–77. —— '49, *Agrob.* No. 4: 105–109. —— '50, *Dokl. Akad. Nauk SSSR.* **74**: 619–622. —— '54, *Dokl. Akad. Seljskohozjaistv. Nauk Lenin* **2**: 31–35. TER-AVANESJAN D. V. and L. I. GUREVIC '50, *Trud. Priklad. Bot.*

Genet. Selekc. **28**: 88–96. TER-AVANESJAN D. V. and F. G. NIGMATULIN '59, *Selek. i. Semeno.* No. 1: 71–73. TURBIN N. V. and E. N. BOGDANOVA '49, *Izv. Akad. Nauk. SSSR.* No. 4: 432–454. USTINOVA E. I. '54, *Izv. Akad. Nauk. SSSR. Ser. Biol.* No. 2: 66–85. VASILEVA-DRJANOSKA O. A. '54, *Izv. Akad. Nauk SSSR. Ser. Biol.* No. 2: 49–73. ZACHOW F. '58, *Züchter* **28**: 241–251. ZAHAROVA G. M. '55, *Izv. Akad. Nauk SSSR.* No. 1: 32–44. ZAIKOVSKAJA N. E. '52, *Izv. Akad. Nauk SSSR. Ser. Biol.* No. 4: 68–78. —— '54 *Izv. Akad. Nauk SSSR.* **3**: 79–86.

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