ALLELISM AND COMPARATIVE GENETICS OF FERTILITY RESTORATION OF CYTOPLASMICALLY POLLEN STERILE MAIZE

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THE GENETICS of restoration of pollen fertility to cytoplasmically pollen sterile maize has been the subject of widespread investigation for the past five or six years, although to date not much of the accumulated genetic data has been published. Data presented by JONES (1951), JONES and MANGELSDORF (1951) and EDWARDSON (1955) indicate that complete fertility restoration in T cytoplasm (explained below) is determined by a single dominant gene. However, unpublished data of some of the other workers in this field have indicated that in some cases other types of gene action might operate. At times conflicting reports have been given for the same fertility restorer (FR) inbred line. In the course of a series of investigations originally designed only to compare the effectiveness of fertility restoration of several FR lines, a few principles have been demonstrated which serve to explain some of the divergent results. Additionally, these experiments have indicated the probable genotypes (with regard to fertility restoration) of several FR and sterilizable (NR) lines, to an extent that it is worth while to present them as hypotheses, acknowledging that complete genetic proof of the hypotheses is not yet at hand.

RHOADES described an instance of cytoplasmically inherited pollen sterility in maize in 1931 and 1933, but little additional study was made of this phenomenon until JONES and EVERETT (1949) outlined a method which the senior author, in cooperation with DR. P. C. MANGELSDORF, had developed to utilize cytoplasmic pollen sterility for production of hybrid seed corn without detasseling. In 1950 JONES stated that some genotypes (NR) were sterile when in "sterile" cytoplasm, and others (FR) would interact with "sterile" cytoplasm in such a way as to produce normal pollen fertile plants; i.e., interaction of both nuclear and cytoplasmic factors is responsible for the cytoplasmic pollen sterile condition. The F_1 of NR $\times$ FR, in "sterile" cytoplasm, was pollen fertile. Segregation of the nuclear factors was demonstrated. JOSEPHSON and JENKINS in 1948 had shown that the interaction of nuclear and cytoplasmic factors is responsible for a type of cytoplasmic pollen sterility discovered in the inbred 33-16. JONES and MANGELSDORF in 1951 stated that many inbreds are still segregating for nuclear factors for fertility restoration. They described 3 different sources of cytoplasmic sterility, designated them "S", "T" and "B", and stated that some genotypes were sterile in one but would restore pollen fertility (more or less) to another source of cytoplasm; in other words all types of sterility-inducing cytoplasm are not necessarily identical. Backcross and F_2 data published by them, and by JONES (1951), indicated that in T cytoplasm fertility restoration by the inbred Ky21 is due to a single dominant gene and that

sterilizability of the inbreds C106 and Ky39 is due to a single recessive gene. ROGERS and EDWARDSON in 1952 stated that inbreds K55, Tx127c and TxGJ39 produced completely fertile F_1 hybrids when crossed to the pollen sterile inbred Tx203Ms (T cytoplasm). EDWARDSON (1955) after classifying 23 F_2 populations and 13 backcross populations from crosses of cytoplasmic pollen sterile × Latin American open pollinated varieties concluded that fertility restoration appeared to be due to the action of a single dominant gene, although he mentioned the possibility that in some cases it could be due to 2 factors with dominant and recessive epistasis. C106 in T cytoplasm was used as pollen sterile tester.

METHODS AND MATERIALS

Method of classifying plants for tassel fertility

It should be emphasized that all data presented herein are based on observations of the anthers of living plants during their flowering period. At the time of flowering, plants were examined every other day. Exserted anthers were classified into 3 groups: (1) Plump, normal appearing anthers with a large pore through which pollen obviously had been shed were called "fertile anthers". Random checks (made throughout the flowering period each season) with a hand microscope and with iodine stain and a compound microscope indicated that in every instance 90 to 100% of the pollen shed by these anthers looked viable (plump, filled with purple-staining granules). Nevertheless, it should be kept in mind that in this report all discussion of the "pollen fertility" of various plants is based primarily on the appearance of their anthers rather than on the appearance of the pollen in the anthers. (2) Anthers which had a pore and shed some pollen, but which were shrunken in some places and plump in others, and in general looked abnormal, were called "partially fertile anthers". Spot checks with the microscope showed that 10 to 90% of the pollen in these anthers looked viable, and that the most abnormal anthers usually had the lowest percentages of viable-appearing pollen. (3) Exserted anthers which had no pore and thus could not shed pollen and which were extremely shrunken throughout their length were called "sterile anthers". Usually, all pollen in these anthers looked sterile, although small numbers of apparently fertile pollen grains occasionally were found. All anthers which remained within the glumes (non-exserted) also were classified as "sterile anthers". Microscopic examination showed that these anthers virtually never contained viable-appearing pollen grains.

Using this anther classification as a basis, each tassel was put into one of 5 major classes, as follows:

I. No anthers exserted.

II. Anthers exserted, but all (or very nearly all) anthers are sterile.

III. Anthers exserted and in any of the following combinations: (1) some sterile, some partially fertile and some fertile (on a single tassel), or (2) only sterile and partially fertile anthers, or (3) only partially fertile and fertile anthers, or (4) only partially fertile anthers. Tassel as a whole tends to be more nearly sterile than fertile.

IV. Similar to III, but tassel as a whole tends to be more nearly fertile.

V. All (or very nearly all) exserted anthers are fertile.

Tassels falling into one of the last 4 classes were further classified (by means of rough estimates) into one of 3 sub-classes, as follows:

A. Less than $\frac{1}{3}$ of the total number of anthers on the tassel exserted.

B. $\frac{1}{3}$ to $\frac{2}{3}$ of the anthers exserted.

C. $\frac{2}{3}$ or more anthers exserted, except in the case of Class V. Sub-class C of Class V included only those plants in which virtually all of the anthers were exserted, and all other Class V plants having more than $\frac{1}{3}$ exserted anthers were placed in sub-class B. Class VC, therefore, contained all of the plants with fully fertile tassels.

The repeatability of tassel classifications according to the standards outlined above was tested by having several persons classify the same group of plants in a segregating population. It was found that in most cases a given plant was given the same (or, at the least, a closely related) classification by all persons. The most difficult classifications were those which required one to distinguish between classes III and IV.

Lines studied and types of crosses made

The experiments to be discussed here may be divided into 4 groups: (1) tests for allelic relationships among the genes responsible for the fertility restoration properties of 5 FR inbred lines; (2) a series of backcrosses involving each of these FR lines and using the NR inbred WF9 (usually in T cytoplasm) as the recurrent parent of each backcross; (3) similar to section 2, involving Ky21 (FR inbred) with NR inbreds C106, K77, K4 and SK2 (in T cytoplasm on one side and in N cytoplasm on the other side of the cross) as recurrent parents, and BH2 (FR inbred) with C106 and K4 (in both T and N cytoplasms) as recurrent parents; (4) 3-way testcrosses, involving BH2 and Ky21, with C106 and WF9 (in both T and N cytoplasms) used as sterilizable testers.

The lines studied, their characteristics with regard to cytoplasmic sterility and the institutions from which they were obtained are shown in the following summary. FR lines restore fertility; NR (non-restorer) lines are sterilizable. T cytoplasm is "sterile"; N is "normal".

T cytoplasm is indicated according to JONES' notation, by means of a superscript after the inbred pedigree. The absence of a superscript is understood to mean that the inbred genotype is in "normal" cytoplasm. T cytoplasm will be designated also as "Cyt^T", in contrast to normal cytoplasm, "Cyt^N" during discussion of general genetic hypotheses.

F_1 hybrids were made in the 10 possible combinations of the 5 FR lines. The F_1 hybrids then were crossed to $WF9^T$ and the resulting 3-way crosses were grown and classified. $WF9^T$ used as female parent for these crosses was from a sample of composited ears. A single F_1 ear (FR $\times$ FR) was used to provide the F_1 plants for each 3-way cross. Three ear progenies per 3-way cross were grown.

For the second series of tests, $WF9^T$ was backcrossed as female to each of the five possible F_1 hybrids of $WF9^T$ $\times$ FR. In several instances an equivalent of the re-

Inbred	Source of seed	Sterilizability	Cytoplasm
C106^T	Conn. Agric. Exp. Sta.	NR	T
K4^T	Conn. Agric. Exp. Sta.	NR	T
WF9^T	Conn. Agric. Exp. Sta.	NR	T
C106	Conn. Agric. Exp. Sta.	NR	N
K4	Conn. Agric. Exp. Sta.	NR	N
WF9	Conn. Agric. Exp. Sta.	NR	N
K77^T	Pioneer Hi-Bred Corn Co.	NR	T
SK2^T	Pioneer Hi-Bred Corn Co.	NR	T
K77	Pioneer Hi-Bred Corn Co.	NR	N
SK2	Pioneer Hi-Bred Corn Co.	NR	N
BH2	Pioneer Hi-Bred Corn Co.	FR	N
F5DD1	Pioneer Hi-Bred Corn Co.	FR	N
WG3	Pioneer Hi-Bred Corn Co.	FR	N
Ky21	Kentucky Agric. Exp. Sta.	FR	N
K55	Kansas Agric. Exp. Sta.	FR	N

ciprocal cross also was made, using WF9 in normal cytoplasm as pollen parent. In either instance, therefore, the backcross had T cytoplasm. Each F_1, and each backcross, was made with bulked seed (a mixture of seed from several ears of the inbred or F_1 used to make the cross) but the final backcross ears were kept separate, and were planted as individual ear progenies. Usually three progenies per backcross were planted, each progeny replicated three times. Thus, in a typical backcross, the three progenies were derived from three FR plants and six WF9^T plants (or three WF9^T and three WF9 plants, in the case of the "reciprocal" crosses). It is probable that the three FR plants were derived from three different parent ears and that the WF9^T (and WF9) plants also were from separate parent ears.

For the third series of tests, the F_1 hybrids C106^T × Ky21, K4^T × Ky21, K77^T × Ky21, SK2^T × Ky21, C106^T × BH2 and K4^T × BH2 were backcrossed, each to the normal cytoplasm form of its NR inbred. In all cases the F_1 was used as female. The backcrosses involving K77 and SK2 were made with bulked seed in the same way as described for the WF9 backcrosses. Each of the other backcrosses was made with plants from a single ear progeny of the F_1. Thus, each set of these backcross progenies tested one NR^T plant of the recurrent parent, one FR plant and several NR plants of the recurrent parent. Three ear progenies of each backcross of this third series of tests were grown in each season. Each progeny was replicated three times.

The crosses of the fourth series were made by crossing an F_1 hybrid (NR^T × FR) to a different NR inbred. The 3-way cross (C106^T × Ky21)WF9 was made with plants of the same F_1 progeny used to make the backcross (C106^T × Ky21)-C106. The 3-way (C106^T × BH2)WF9 was made with plants of the same F_1 progeny used to make the backcross (C106^T × BH2)C106. Thus, in each instance a single FR plant and a single C106^T plant have been tested in 2 types of cross. The

3-way (WF9^T × BH2)C106 was made from the same bulked sample of F_1 seed that was used to make (WF9^T × BH2)WF9, and so did not test the same BH2 and WF9^T plants (necessarily) that were used to make the backcross, but it did test a series of plants drawn from the same samples that had furnished the BH2 and WF9^T plants tested in the WF9 backcross. Two to three ear progenies per 3-way cross were grown, three replications per progeny.

No FR or partially FR plants (with respect to T cytoplasm) have ever been found by me in any of the above mentioned NR lines. Each F_1 plant that was used to make a backcross or a 3-way cross was completely fertile (tassel classification VC) at the time of crossing. Samples of the F_1 hybrids used to make each backcross were grown next to the corresponding backcross and both were classified at the same time. All populations discussed herein were grown at Johnston, Iowa during the seasons of 1953, 1954 or 1955 except for one backcross: (C106^T × BH2)C106, which also was grown near Homestead, Florida, during the winter of 1954–5.

RESULTS

Allelism testcrosses

Almost all of the plants of each allelism testcross were fully fertile, as is shown in table 1. Every cross, however, produced a few partially fertile plants, usually of tassel classification IVC or VB. In WF9^T(WG3 × BH2) and in WF9^T(WG3 × Ky21), three and one plants, respectively, were recorded as sterile. When small progenies of selfed VC plants from each progeny of each test cross were grown in 1955, about 30 to 45% of the plants in every progeny were sterile indicating that in no case was fertility of the testcross due to the absence of T cytoplasm.

Backcrosses involving five fertility restorer lines with WF9 as recurrent parent

These backcross progenies segregated in ratios of about one fertile: three sterile plants, as is shown in table 2. Most plants were either fully fertile or completely sterile. The backcross progenies involving F5DD1 had more partially fertile and fewer sterile plants than did any of the other backcrosses. Various chi square computations (tables 3, 4 and 5) were made after compositing classifications I through IIC (sterile) and IIIA through VC (fertile). Calculations of sampling chi square (table 3) showed no significant differences among replications of any backcross except WF9^T(WF9^T × Ky21), indicating that at a given location classification results usually are repeatable. Most backcross progenies gave a satisfactory fit to a 1:3 ratio, as is shown in table 4. All backcrosses involving F5DD1 deviated significantly ($P < .05$) from 1:3 but when the F5DD1 data were recalculated after putting all partially fertile plants into the "sterile" class a reasonably good fit to a 1:3 ratio was obtained for all progenies. Calculations of heterogeneity chi square indicate that: (1) all progenies of the WF9 backcrosses (except those involving F5DD1) are reasonably similar with respect to goodness of fit to a 1:3 ratio and as a population the chances are fairly good that they are segregating in a ratio of 1:3 (table 5A); (2) segregation ratios of "reciprocal" backcrosses did not differ

TABLE 1

Allelism test crosses: WF9^T crossed to F_1 hybrids of 5 fertility restorer lines

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V			
				A	B	C	A	B	C	A	B	C	A	B	C	
WF9^T(BH2 × F5DD1)	1	1954												1	34	35
	2	1954													32	32
	3	1954													33	33
WF9^T(BH2 × Ky21)	1	1954												3	30	33
	2	1954													33	33
	3	1954													37	37
WF9^T(WG3 × BH2)	1	1954													34	34
	2	1954	3									1	2		29	35
	3	1954													31	31
WF9^T(K55 × BH2)	1	1954													35	35
	2	1954									1	1		1	33	36
	3	1954											2		31	33
WF9^T(F5DD1 × Ky21)	1	1954													34	34
	2	1954												1	30	31
	3	1954												1	33	34
WF9^T(F5DD1 × WG3)	1	1954													36	36
	2	1954												1	35	36
	3	1954													31	31
WF9^T(K55 × F5DD1)	1	1954											2	2	30	34
	2	1954												2	32	34
	3	1954													38	38
WF9^T(WG3 × Ky21)	1	1954	1									4		3	24	32
	2	1954										1			33	34
	3	1954										3		2	31	36
WF9^T(Ky21 × K55)	1	1954										3		3	27	33
	2	1954													32	32
	3	1954													34	34
WF9^T(WG3 × K55)	1	1954											1		29	30
	2	1954										2			30	32
	3	1954										2		1	30	33

significantly (table 5B); and (3) segregation ratios of the same backcross in 2 different years did not differ significantly (table 5C). The BH2 backcrosses compared in table 5C are not strictly comparable since the progenies grown in 1955 were not from remnant seed of the progenies grown in 1954.

TABLE 2

Backcrosses involving five fertility restorer lines; WF9 recurrent parent

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V			
				A	B	C	A	B	C	A	B	C	A	B	C	
WF9^T(WF9^T × BH2)	315-5241-1	1954	116	1						2	2	2	1	1	36	161
	315-5241-2	1954	115								1			3	51	170
	315-5241-3	1954	227		1					2	2		1		76	309
	315-5241-4	1955	77								1				16	94
	315-5241-5	1955	70							1	1	1			18	91
	315-5241-6	1955	71				1					2			30	104
WF9^T(WF9^T × F5DD1)	G301024-7	1953	119				1		1	4	1	3	1	3	56	189
	G301024-8	1953	75				3			3	1	4	3	1	30	120
(WF9^T × F5DD1) WF9	G301050-6	1953	53							3		1	5		27	89
WF9^T(WF9^T × Ky21)	315-5243-1	1954	53									1			12	66
	315-5243-2	1954	161											2	67	230
	315-5243-1	1955	14									2			1	17
	315-5243-2	1955	13									2			6	21
(WF9^T × Ky21) WF9	425203-1	1955	76									1			12	89
	425203-2	1955	70									6			23	99
	425203-3	1955	73									3			21	97
WF9^T(WF9^T × K55)	315-5253-1	1954	51		1										16	68
	315-5253-2	1954	55												15	70
	315-5253-3	1954	50	1										1	13	65
	315-5253-4	1954	53				2								14	69
	315-5253-5	1954	47				1	1		1	1				12	63
	315-5253-6	1954	47		1				1						22	71
	315-5253-7	1954	52											2	17	71
	315-5253-8	1954	50												16	66
	315-5253-9	1954	50				2								16	68
WF9^T(WF9^T × WG3)	G301018-1	1953	75							1					20	96
(WF9^T × WG3) WF9	G302060-4	1953	34												12	46
	G302060-5	1953	20												8	28

Ky21 backcrossed with four different recurrent parents and BH2 backcrossed with two different recurrent parents

About one half of the plants in the backcross progenies involving C106, K4, K77 and SK2 were sterile (table 6). These results are in sharp contrast to the 1:3 ratios obtained for backcrosses involving WF9 (table 2). An additional difference was that many pollen shedding plants of the backcrosses involving C106, K4 and K77

TABLE 3

Chi square test for uniformity of sampling; backcrosses of five FR lines with WF9 as recurrent parent

Pedigree	Year	Number of replications	Number of classes*	DF	Sampling χ^2	P
WF9^T(WF9^T × BH2)	1954	3	2	3	.869	.83
	1955	3	2	3	3.157	.38
(WF9^T × Ky21) WF9	1955	3	2	3	1.019	.80
WF9^T(WF9^T × Ky21)	1954	2	2	2	12.880	<.01
WF9^T(WF9^T × F5DD1)	1953	3	2	3	1.887	.60
(WF9^T × F5DD1) WF9	1953	3	2	3	4.726	.19
WF9^T(WF9^T × K55)	1954	2	2	2	.026	.99
(WF9^T × WG3) WF9	1953	2	2	2	.002	>.99

* Two classes are: (1) tassel classifications I through IIC, "sterile", and (2) tassel classifications IIIA through VC, "pollen shedding."

were not completely fertile. They usually were classified as IVC. (C106^T × Ky21)-C106, however, had large numbers of IIIA, IIIB and IIIC plants. In all backcrosses class IV tassels were easily distinguished from class V tassels, and in (C106^T × Ky21)C106 the class III tassels usually were easily distinguished from class IV tassels. Occasional intergrades occurred in all backcrosses, however. Sampling chi squares (table 7A) show that with one exception there were no significant differences among replications of a backcross. Most backcross progenies gave a satisfactory fit to a 1:1 ratio as is shown in table 8. Tassel classifications were combined into "sterile" and "pollen shedding" classes prior to this computation, as was done for the WF9 backcrosses. The ratios of three backcross progenies deviate significantly ($P < .05$) from a 1:1 ratio, and two or three other progenies show a rather poor fit. With two exceptions, progenies within a backcross were not significantly heterogeneous with respect to goodness of fit to a 1:1 ratio (table 9).

It is apparent in table 6 that although progenies within a given backcross usually have similar percentages of sterile plants, they often differ from each other with respect to proportions of partially fertile:fertile plants. When the sampling chi square method is used to measure this variability (progenies, instead of replications, are treated as samples) it can be shown (table 7B) in several instances that progenies of a backcross are significantly heterogeneous ($P < .05$). In most instances the heterogeneity is due to differences in proportions of partially fertile:fertile plants. The fact that each backcross which had a high sampling chi square for progenies nevertheless had a low sampling chi square for replications is further evidence that progeny differences were based on genetic differences, for they were repeated in the same manner from replication to replication. It also shows that the classification system was reasonably reliable. One can conceive that if all differences in classification were due to random choice of a classification for each tassel, the choices could be randomized in about the same fashion from replication to replication and thus give a low sampling chi square for replications. However, if such were true these choices also should have been randomized within the progenies included in each replication, resulting in a low sampling chi square for progenies as well.

TABLE 4

Chi square tests for goodness of fit to 1 : 3 ratio; backcrosses of five FR lines, with WF9 as recurrent parent

Pedigree	Year	Ear number	Total plants	Sterile plants		χ^2 for 1:3 ratio*	P
				Obs.	Exp.		
WF9^T(WF9^T × BH2)	1954	315-5241-1	161	117	120.75	.465	.50
		315-5241-2	170	115	127.50	4.901	.03
		315-5241-3	309	228	231.75	.244	.64
	1955	315-5241-4	94	77	70.50	2.397	.13
		315-5241-5	91	70	68.25	.180	.68
		315-5241-6	104	71	78.00	2.513	.12
WF9^T(WF9^T × F5DD1)	1953	G301024-7	189	119	141.75	14.605	<.01
				133†	141.75	2.160	.16
		G201024-8	120	75	90.00	10.000	<.01
				90†	90.00	0.000	>.99
(WF9^T × F5DD1) WF9	1953	G301050-6	89	43	66.75	11.329	<.01
				62†	66.75	1.352	.25
WF9^T(WF9^T × Ky21)	1954	315-5243-1	66	53	49.50	.989	.33
		315-5243-2	230	161	172.50	3.067	.08
	1955	315-5243-1	17	14	12.75	.491	.49
		315-5243-2	21	13	15.75	1.921	.17
(WF9^T × Ky21) WF9	1955	425203-1	89	76	66.75	5.127	.03
		425203-2	99	70	74.25	.973	.33
		425203-3	97	73	72.25	.004	.95
WF9^T(WF9^T × K55)	1954	315-5253-1	68	52	51.00	.079	.78
		315-5253-2	70	55	52.50	.576	.49
		315-5253-3	65	51	48.75	.416	.53
		315-5253-4	69	53	51.75	.121	.73
		315-5253-5	63	47	47.25	.005	.94
		315-5253-6	71	48	53.25	2.071	.16
		315-5253-7	71	52	53.25	.117	.74
		315-5253-8	66	50	49.50	.020	.89
		315-5253-9	68	50	51.00	.079	.78
WF9^T(WF9^T × WG3)	1953	G301018-1	96	75	72.00	.500	.49
(WF9^T × WG3) WF9	1953	G302060-4	46	34	34.50	.029	.87
		G302060-5	28	20	21.00	.191	.67

* Calculated for two classes as follows, except where indicated otherwise: (1) "sterile", I through IIC, and (2) "pollen shedding" IIIA through VC.

† "Sterile" class includes classes I through VB. Fertile class is VC only.

F_1 hybrids of cytoplasmic sterile lines × fertility restorer lines

When samples of the F_1 hybrids used to make each backcross were grown beside the corresponding backcross populations, it was found that a few plants in each F_1 were classified as incompletely fertile (usually IVC), as shown in table 10. C106^T ×

TABLE 5

Heterogeneity chi squares for backcrosses tested against 1:3 ratio

A. Among progenies of a backcross in one year

Pedigree	Year	Number of progenies	DF	Heterogeneity χ^2	*P*
WF9^T(WF9^T × BH2)	1954	3	2	2.277	.34
	1955	3	2	5.061	.08
WF9^T(WF9^T × F5DD1)	1953	2	1	.839*	.38
WF9^T(WF9^T × Ky21)	1954	2	1	2.930	.09
	1955	2	1	2.096	.16
(WF9^T × Ky21) WF9	1955	3	2	5.588	.06
WF9^T(WF9^T × K55)	1954	9	8	3.383	.91
(WF9^T × WG3)WF9^T	1953	2	1	.325	.58
All progenies except F5DD1 crosses		25			
Total			25	27.376	.34
Pooled			1	1.083	.30
Interaction			24	26.293	.34

B. Between "reciprocal" backcrosses

Pedigree	Year	DF	Heterogeneity χ^2	*P*
WF9^T(WF9^T × F5DD1) vs. (WF9^T × F5DD1) WF9	1953	1	.230	.65
WF9^T(WF9^T × Ky21) vs. (WF9^T × Ky21) WF9	1955	1	.600	.45
WF9^T(WF9^T × WG3) vs. (WF9^T × WG3) WF9	1953	1	.592	.46

C. Between years

Pedigree	Years	DF	Heterogeneity χ^2	*P*
WF9^T(WF9^T × BH2)	1954 vs. 1955	1	1.345	.25
WF9^T(WF9^T × Ky21)	1954 vs. 1955	1	.337	.58

* "Sterile" class includes tassel classifications I through VB.

Ky21 had an especially high proportion of such plants. Anthers of even the VC plants in this F_1 did not look quite as fertile as those of other F_1 hybrids. The IVC plants were not sharply distinct from VC; rather, they represented a point in a continuous series at which one arbitrarily decided he must call the plant IVC, instead of VC. The IVC tassels in other F_1 hybrids likewise were not sharply distinct from VC; that is, they had a few too many partially fertile anthers to be called VC, but did not have nearly as many partially fertile anthers as the majority of IVC plants

TABLE 6

Backcrosses involving Ky21 and BH2 with C106, K4, K77 and SK2 as recurrent parents

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V			
				A	B	C	A	B	C	A	B	C	A	B	C	
(C106^T × Ky21)	F406049-1	1954	98	1			4	5	15	2	5	67			10	207
C106	F406049-2	1954	101				8	8	13	4	6	47			3	190
	F406049-3	1954	118				5	3	13	1	3	42			7	192
	F406049-4	1955	56				8	4	15	2		19				104
	F406049-5	1955	31				6	1	15			16			14	83
	F406049-6	1955	48				6	1	19	1		20			3	98
(K4^T × Ky21)	F407038-1	1954	95					1	10			51			34	191
K4	F407038-2	1954	114						7	3		53	1		30	208
	F407038-3	1954	93				3		8	9	1	45	2	2	34	197
(K77^T × Ky21)	F506059-10	1955	40						5	1		45			7	98
K77	F506059-11	1955	44								1	4			41	90
	F506059-12	1955	45									9			36	90
(SK2^T × Ky21)	F506062-11	1955	47												48	95
SK2	F506062-12	1955	44									1			43	88
	F506062-13	1955	45											1	53	99
(C106^T × BH2)	F405039-1	1954	102						4		2	49	1	1	38	197
C106	F405039-2	1954	106					2	4		1	56	1		36	206
	F405039-3	1954	104				1		5	1	4	52		1	34	202
	F405039-5	1955	52				1	1	13	2		23			7	99
	F405039-6	1955	56					1	4			19	1		17	98
	F405039-7	1955	41				1	1	3			23			34	103
(K4^T × BH2)	F407036-1	1954	100	1			1		5	1		45		1	53	207
K4	F407036-2	1954	102						20	1		61	1		30	215
	F407036-3	1954	103	1			1		10		3	53			27	198

TABLE 7

Chi square tests for uniformity of sampling, comparing (a) replications and (b) progenies of backcrosses involving Ky21 and BH2, with C106, K4, K77 and SK2 as recurrent parents

Pedigree	Year	Number classes	A. Comparing replications				B. Comparing progenies			
			Number reps.	DF	Sampling χ^2	P	Number progenies	DF	Sampling χ^2	P
(C106^T × Ky21) C106	1954	4***	3	9	9.849	.46	3	9	12.882	.17
	1955	4	3	9	5.986	.74	3	9	26.975	< .01
(K4^T × Ky21) K4	1954	3**	3	6	30.118	< .01	3	6	2.727	.84
(K77^T × Ky21) K77	1955	3	3	6	2.836	.85	3	6	81.710	< .01
(SK2^T × KY21) SK2	1955	2*	3	3	.184	.98	3	3	.474	.92
(C106^T × BH2) C106	1954	3	3	6	3.486	.75	3	6	.636	> .99
	1955	3	3	6	.899	.99	3	6	25.413	< .01
(K4^T × BH2) K4	1954	3	3	6	3.972	.67	3	6	16.938	< .01

* Two classes are: (1) "sterile", I through IIC; (2) "pollen shedding", IIIA through VC.

** Three classes are: (1) "sterile", I through IIC; (2) "partially fertile," IIIA through VB, (3) "fertile," VC.

*** Four classes are: (1) "sterile," I through IIC; (2) "low grade partially fertile," IIIA through IIIC; (3) "high grade partially fertile" IVA through VB; (4) "fertile" VC.

TABLE 8

Chi square tests for goodness of fit to 1:1 ratio; backcrosses involving Ky21 and BH2, with C106, K4, K77 and SK2 as recurrent parents

Pedigree	Year	Ear number	No. of plants	Sterile plants		χ^2 for 1:1 ratio*	*P*
				Obs.	Exp.		
(C106^T × Ky21) C106	1954	F406049-1	207	99	103.50	.392	.54
		F406049-2	190	101	95.00	.758	.40
		F406049-3	192	118	96.00	10.084	<.01
	1955	F406049-4	104	56	52.00	.616	.45
		F406049-5	83	31	41.50	5.314	.02
		F406049-6	98	48	49.00	.040	.85
(K4^T × Ky21) K4	1954	F407038-1	191	95	95.50	.006	.94
		F407038-2	208	114	104.00	1.924	.17
		F407038-3	197	93	98.50	.614	.45
(K77^T × Ky21) K77	1955	F506059-11	98	40	49.00	3.306	.08
		F506059-12	90	44	45.00	.004	.84
		F506059-13	90	45	45.00	.000	>.99
(SK2^T × Ky21) SK2	1955	F506062-11	95	47	47.50	.010	.93
		F506062-12	88	44	44.00	.000	>.99
		F506062-13	99	45	49.50	.818	.38
(C106^T × BH2) C106	1954	F405039-1	197	102	98.50	.228	.65
		F405039-2	206	106	103.00	.174	.68
		F405039-3	202	104	101.00	.178	.68
	1955	F405039-5	99	52	49.50	.252	.63
		F405039-6	98	56	49.00	2.000	.17
		F405039-7	103	41	51.50	4.282	.04
(K4^T × BH2) K4	1954	F407036-1	207	101	103.50	.120	.73
		F407036-2	215	102	107.50	.562	.47
		F407036-3	198	104	99.00	.506	.48

* Calculated for two classes: (1) "sterile", I through IIC; (2) "pollen shedding," IIIA through VC.

TABLE 9

Heterogeneity chi squares for backcrosses tested against 1:1 ratio

Pedigree	Number of progenies	Year	DF	Heterogeneity χ^2	*P*
(C106^T × Ky21) C106	3	1954	2	7.484	.02
		1955	2	5.180	.08
(K4^T × Ky21) K4	3	1954	2	2.436	.30
(K77^T × Ky21) K77	3	1955	2	1.912	.40
(SK2^T × Ky21) SK2	3	1955	2	.474	.79
(C106^T × BH2) C106	3	1954	2	.004	>.99
	3	1955	2	6.520	.04
(K4^T × BH2) K4	3	1954	2	1.130	.58

in their corresponding backcrosses. K4^T × Ky21 and K77^T × Ky21 had one and two sterile plants, respectively. These plants were phenotypically similar to others in the F_1 and did not appear to be outcrosses.

3-way crosses involving two NR lines

Ratios of approximately one sterile to one pollen shedding plant were obtained for all progenies of (C106^T × Ky21)WF9, (C106^T × BH2)WF9, and (WF9^T ×

TABLE 10

F_1 hybrids of cytoplasmically pollen sterile lines by fertility restorer lines, grown next to their corresponding backcross progenies

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V		
				A	B	C	A	B	C	A	B	C	A	B	C	
WF9^T × BH2	bulk	1954										65			65
		1955										5			64	69
WF9^T × F5DD1	bulk	1953												2	176	178
WF9^T × Ky21	bulk	1954													71	71
		1955										2			44	46
WF9^T × K55	bulk	1954										1			66	67
WF9^T × WG3	bulk	1953										5		1	65	71
		1954													31	31
C106^T × Ky21	1	1954										11	1	1	22	35
		1955										19			29	48
K4^T × Ky21	6	1954	1									4	1		62	68
K77^T × Ky21	bulk	1955	2									5			41	48
SK2^T × Ky21	bulk	1955													41	41
C106^T × BH2	3	1954										2		4	66	72
		1955										2			74	76
K4^T × BH2	1	1954													69	69

TABLE 11

3-Way crosses involving Ky21 and BH2

Pedigree	Ear number	Year	Number of plants per tassel classification												Total
			I	II			III			IV			V		
				A	B	C	A	B	C	A	B	C	A	B	C	
(C106^T × Ky21) WF9	F406047-1	1955	63									6			40	109
	F406047-2	1955	52									2			50	104
	F406047-3	1955	54									3			48	105
(C106^T × BH2) WF9	F405041-1	1955	40				1		1						63	105
	F405041-2	1955	46					1				1			53	101
	F405041-3	1955	49				1	1				1			44	96
(WF9^T × BH2) C106	F405043-2	1955	49				2					1		1	38	91
	F405043-3	1955	38						1			3			50	92

TABLE 12

Chi square tests for goodness of fit to 1:1 ratio; 3-way crosses involving Ky21 and BH2

Pedigree	Year	Ear number	Total plants	Sterile plants		χ^2 for 1:1 ratio*	*P*
				Obs.	Exp.		
(C106^T × Ky21) WF9	1955	F406047-1	109	63	54.50	2.652	.11
		F406047-2	104	52	52.00	0.000	>.99
		F406047-3	105	54	52.50	.086	.77
(C106^T × BH2) WF9	1955	F405041-1	105	40	52.50	5.952	.02
		F405041-2	101	46	50.50	.802	.39
		F405041-3	96	49	48.00	.042	.85
(WF9^T × BH2) C106	1955	F405043-2	91	49	45.50	.538	.47
		F405043-3	92	38	46.00	2.782	.10

* Calculated for two classes: (1) "sterile", I through IIC; (2) "pollen shedding", IIIA through VC.

TABLE 13

Heterogeneity chi squares for 3-way crosses tested against 1:1 ratio

Pedigree	Year	Number progenies	DF	Heterogeneity χ^2	*P*
(C106^T × Ky21) WF9	1955	3	2	1.480	.48
(C106^T × BH2) WF9	1955	3	2	3.406	.19
(WF9^T × BH2) C106	1955	2	1	2.878	.09

TABLE 14

(C106^T × BH2) C106 grown near Homestead, Florida during winter of 1954–5

Ear number	Number of plants per tassel classification												Total	χ^2 for 1:1 ratio	*P*
	I	II			III			IV			V				
		A	B	C	A	B	C	A	B	C	A	B	C			
F405039-1	57												76	133	2.714	.10
F405039-2	107												94	201	.840	.38
F405039-3	46												66	112	3.572	.06

BH2)C106, as is shown in table 11. Nearly all of the pollen shedding plants were fully fertile. In all but one instance progeny ratios did not deviate significantly from 1:1 (table 12). Table 13 shows that with respect to a 1:1 ratio progenies of no 3-way cross were significantly heterogeneous.

Backcross grown in Florida

Table 14 shows that when the three ear progenies of (C106^T × BH2)C106 that had been grown in Iowa in 1954 were grown in Florida in the winter of 1954–5 all pollen shedding plants were classified as VC, despite the fact that in Iowa many of

the pollen shedding plants were only partially fertile. In both locations about the same proportions (one half) of the plants were sterile. Chi square tests for goodness of fit to a 1:1 ratio of each of the progenies grown in Florida show no significant deviations from 1:1, but two progenies have low P values, due in both cases to an excess of fertile plants.

DISCUSSION

Three general conclusions can be drawn from the summaries of data presented in the previous section. In the first place, it is apparent that all five FR lines (in so far as they were sampled) have the same alleles of the major gene or genes needed to restore pollen fertility to $WF9^T$. Otherwise, segregation among gametes produced by the F_1 plants would have resulted in the presence of several sterile plants in the test cross $WF9^T$ (FR $\times$ FR). The data in table 1 do not demonstrate, however, whether or not all of the FR lines contain all of the factors needed for complete fertility restoration. It is conceivable that the WF9 genotype may include one or more genes which act in a complementary fashion with others furnished by the FR lines. Nor do the data prove that some FR lines do not have more FR factors than do other FR lines; that is, duplicate, non-allelic FR factors could exist in some lines but not in others. Such duplicates would not be detected by the type of testcross that has been made. It may be important to note that four plants (in table 1) were recorded as being sterile. Three of them were in one progeny of $WF9^T$ (WG3 $\times$ BH2). The three progenies of this cross were grown again in 1955 and no sterile plants were seen. It is possible that the plants were recorded as sterile by accident in 1954 (lost classification tag, for instance) or that they were the result of contaminating pollen grains on the $WF9^T$ ear. It also is conceivable that they could be the result of infrequent crossovers between closely linked FR factors of BH2 and WG3, or of mutation of a dominant FR factor to the recessive state. Either hypothesis needs more supporting data, however, before it can pass from the stage of conjecture. The small numbers of partially fertile plants appearing in each 3-way cross are not too unexpected since the F_1 hybrids of $WF9^T$ $\times$ FR also had a few partially fertile plants.

The second generalization pointed to by the data is that a backcross involving a given FR line (for example, Ky21) may segregate in one fashion when a given NR line (say, WF9) is used as a recurrent parent, but may segregate in an entirely different fashion when another NR line (C106, for example) is used as the recurrent parent. Additionally, if a testcross is made which uses both NR lines (($C106^T$ $\times$ Ky21)WF9, for example) a third type of segregation may result. In other words, the assumption is false that any two lines which will sterilize in T cytoplasm are therefore isogenic with respect to all FR genes. For if this were so, all three types of crosses should give identical segregations. It follows, therefore, that any statement as to the number of genes responsible for the FR action of a given FR line is true only with respect to the sterilizable line used as a tester. A different sterilizable line might easily be recessive for additional FR factors, and therefore would reveal genes which could not have been discovered with the first tester. Thus, two investigators, each using a different sterilizable inbred as a tester, could study the

genetics of the same FR line and come to opposing conclusions as to the number and action of the FR genes in the FR line.

The third general conclusion (and also a logical consequence of conclusion number two) which can be drawn from these data is that a uniformly sterilizable line can vary from plant to plant within the line with regard to some of the genes which determine the degree of fertility of pollen-shedding plants. JONES and MANGELSDORF (1951) have pointed out that certain long-time inbred lines can be shown to have some plants which are NR and other plants which are FR. It is demonstrated here that heterogeneity for FR factors can exist even in lines in which all plants are NR. Whether such heterogeneity has arisen via mutation in an originally homozygous line, or whether the original line was heterozygous for these genes cannot be determined from the data at hand. All of the lines used in this study were phenotypically uniform inbreds, maintained by selfing, but all lines had been maintained by compositing selfed ears for a period of several generations. (C106^T × BH2)C106, all the progenies of which trace back to a single BH2 and a single C106^T plant, is one of the backcrosses which gives convincing evidence for heterogeneity within a uniformly sterilizable line. In three replications of one backcross progeny, the proportions of plants in tassel classifications III:IV:V (the three pollen-shedding classes) were (per ten plants) 3:5:2, 3:6:1, and 3:4:3 (avg. 3:5:2). In a second progeny, grown beside the first, the three replications gave ratios of 1:3:6, 1:5:4, and 1:3:6 (avg. 1:4:5). Since ratios for the three replications are very similar ($P = .85$) it follows that the large difference between ratios of the progenies ($P < .01$) almost certainly is due to a genetic difference between the progenies. If one assumes that the two parent plants of the F_1 were homozygous, then the only hereditary difference between the two progenies was that different C106 plants were used as male parent, and any difference in tassel fertility between the two progenies must have been due to genetic differences in the two C106 plants. Nevertheless, all C106 plants that I have tested were completely sterile in Cyt^T.

It is possible that either the C106^T plant or the BH2 plant which were used to make the original F_1 ear could have been heterozygous for one or more factors which modify a major factor for restoration of pollen fertility. The F_1 plants thus would have been heterozygous and would have given rise to different types of backcross progenies even if all C106 male plants had been isogenic. If the C106^T plant had been heterozygous, the original contention that a sterilizable line may be heterogeneous for FR genes would still hold, for C106 is the pollen parent used to maintain C106^T. If the BH2 plant had been heterozygous, the F_1 also should have appeared heterozygous, but as was noted earlier this was not the case, for the few F_1 plants that were classified as high grade partially fertile did not differ sharply from VC, whereas most IVC plants of the backcross progenies were distinctly different from VC. At any rate, only VC F_1 plants were used to make backcrosses.

Several backcrosses, the progenies of which were shown to be heterogeneous with respect to tassel fertility, were made with seed from bulked F_1 ears and therefore tested a different FR plant in each progeny, as well as different NR^T and NR plants ((K77^T × Ky21)K77, for example). In these cases one can say that the FR, the NR, or even both of the inbreds in the backcross may have been heterogeneous

but one cannot determine exactly which line was so. The plant and ear characteristics of all progenies within each backcross were similar, so that it is virtually certain that in no instance was variation among progenies, with respect to pollen fertility, due to a mistake in pollinating.

The experiments presented in this study were not planned to give conclusive proof of the number of factors responsible for fertility restoration; nevertheless, they do indicate definite hypotheses which can be tested more completely in the future.

All of the FR lines tested appear to have two dominant complementary factors which are needed to restore full pollen fertility to WF9^T. That is, if WF9^T × Ky21 were of the genotype[1], *AaBb*, and WF9 were *aabb*, the backcross to WF9 would give one plant in four with the genotype *AaBb*, and three plants in four with only one dominant, or with none. The *AaBb* plants would be fully fertile, others would be fully sterile (in T cytoplasm). It is possible that additional complementary FR factors could be contributed by WF9. The backcross data alone cannot demonstrate whether or not this is the case. Also, the data do not prove whether or not the postulated two dominant complementary factors were present in every one of the fertile and the partially fertile backcross plants that were more or less arbitrarily put in the same class for chi square computation. In the case of F5DD1, as a matter of fact, it would seem more likely that the partially fertile plants did not contain both of the dominant complementary factors, since a good fit to a 1:3 ratio is achieved only when partially fertile plants are thrown into the "sterile" class. It may be that F5DD1 has, in addition to the two dominant complementary factors for full fertility, a gene or genes for partial fertility. Other FR lines could have weaker forms of similar genes for partial fertility, perhaps such that the potentiality of the genes was expressed in some environments and not in others. Conversely, it is conceivable that in some seasons random environmental conditions could affect by chance some, but not all, of the plants having the necessary two dominant FR genes, so that some *AaBb* plants would be fully fertile, but others would be only partially fertile. Or, it could be that in a uniformly severe season, factors in addition to *A* and *B* would be needed to maintain full pollen fertility. If these additional factors also were segregating in the backcross, some *AaBb* plants would have the proper forms of the additional factors and would remain fertile, but others would lack them and would be only partially fertile. It seems likely that in many situations both types of effect (environmental and genetic) could occur and would interact in various ways. Progeny tests of each of the several types of backcross plants must be made, therefore, to establish their specific genotypes.

Ky21 and BH2 seem to furnish one major dominant gene the presence of which is required to restore pollen fertility to all sterilizable lines tested. In addition they apparently furnish several dominant modifier genes which also must be present for full pollen fertility, but which have no effect without the dominant form of the major FR gene. These modifier genes would be different from, and in addition to the important second complementary dominant factor which only WF9 appears to lack. Some inbreds (in so far as they have been tested) appear to have more dominant

[1] Gene designations used throughout this discussion are merely convenient symbols, and are not presented as suggested permanent designations.

modifiers than others. SK2, for instance, seems to have all of them (see table 6; most of the pollen shedding plants are fully fertile); C106 seems to have few of them (see table 6; few of the pollen shedding plants are fully fertile).

As was the case with the WF9 backcrosses one cannot be sure until progeny tests have been made that the arbitrary division of tassel classes for calculating goodness of fit to 1:1 ratios was genetically correct in all cases. It even may be that some completely sterile plants (tassel classification I) had the major dominant FR gene, but lacked dominant modifiers to such an extent that in combination with a certain environment no pollen was shed. Some of the significant deviations of backcross progenies from 1:1 ratio may be due to some reason such as this. It is interesting to note that the WF9 backcrosses, all of whose progenies were usually more uniform with respect to segregation ratios, also appeared to be much less affected by modifier genes.

WF9 and C106 appear to be alike in that both lines apparently are homozygous recessive for the same major FR gene; but C106 seems to have the dominant form of the second complementary factor which in WF9 is recessive, and WF9 seems to have the dominant modifiers which in C106 are recessive. This hypothesis is supported by the 3-way cross segregations shown in table 11, as contrasted to the backcross segregations shown in tables 2 and 6. In the most simplified form, one could diagram an explanation of this as follows:

Cyt^T *AaBbCC* × Cyt^N *aaBBcc*		Genotype	Inbred
($WF9^T$ × FR) (C106)		*aabbCC*	WF9
		aaBBcc	C106
		AABBCC	FR
Cyt^T *AaBBCc*	2 fertile		
Cyt^T *AaBbCc*			
Cyt^T *aaBBCc*	2 sterile		
Cyt^T *aaBbCc*			

It is probable that most C106 plants are recessive for more than one modifier gene (*C*) but since WF9 also would be dominant for any additional modifiers the final ratio would not be changed. If the inbred lines may be heterozygous for modifiers as was postulated earlier, it, of course, would be necessary to determine gene differences for each sub-strain. And as was the case with the hypothesis for the WF9 backcrosses, the evidence presented here does not show whether or not either of the sterilizable lines could have contributed some FR factors not supplied by the FR line.

Further evidence for the hypothesis of a major dominant FR gene assisted by modifiers is given by the segregation of ($C106^T$ × BH2)C106 when grown in Florida in the winter of 1954–5. As has been pointed out by several workers, many lines and hybrids, in Cyt^T, are more fertile when grown in Florida in the winter than when grown in the Corn Belt during the summer. When samples of the same three progenies of ($C106^T$ × BH2)C106 that had been grown in Iowa in 1954 were grown near Homestead, Florida, in 1954–5, they segregated in about a 1:1 ratio much as they had in Iowa, but in contrast to the same progenies in Iowa, no partially fertile plants were observed. All plants were either completely fertile or completely sterile. It

would appear that under the conditions of 1954–5 in Florida no modifying gene action was required. The presence, in dominant form, of only the major FR gene was adequate for development of fully fertile tassels. Florida, 1954–5, therefore, might be regarded as having provided an environment in which one was unable to recognize the presence of any genes which modify the action of the major FR gene. The Iowa summer seems to reveal a number of modifiers, perhaps more in some years than others, and one can conceive of other environments which might reveal still others. One could argue plausibly that the difference between Florida and Iowa was merely a case of incomplete penetrance (in Iowa) of a single dominant gene, were it not for the fact that different NR backgrounds give different, repeatable ratios of "penetrance" of the same FR gene. (See discussion of ($C106^T \times$ BH2)C106 and of the contrast between SK2 and C106 backcross ratios.) It is pertinent to state here that for four years in succession I have noted, in the course of routine breeding programs, a contrast in segregating populations between Florida and Johnston similar to that described for ($C106^T \times$ BH2)C106, although in general the material was not as critical as that described here, and in some years the change to full fertility (in Florida) was not as complete as that described here.

As stated earlier, Jones found that ($C106^T \times$ Ky21)C106 produced only fully fertile and completely sterile plants, in a ratio of about 1:1. These results contrast sharply with the large proportions of partially fertile plants produced by all progenies of the same backcross in my experiments. The $C106^T$ and C106 I used were obtained from JONES and probably still average about the same, genetically, as his. Ky21 was obtained from the Kentucky Agricultural Experiment Station and may or may not have been identical with the strain used by JONES. However, in view of the results obtained in Florida, it seems most probable that differences in number of partially

TABLE 15

Chi square tests for goodness of fit to 3:5:8 ratio; backcrosses involving BH2 and Ky21, with C106 and K4 as recurrent parents

Pedigree	Year	Ear number	DF	χ^2 for 3:5:8*	*P*
($C106^T \times$ BH2) C106	1954	F405039-1	2	.492	.78
		F405039-2	2	.268	.88
		F405039-3	2	.498	.78
	1955	F405039-5	2	9.979	<.01
		F405039-6	2	2.139	.35
		F405039-7	2	13.861	<.01
($K4^T \times$ BH2) K4	1954	F407036-1	2	7.360	.03
		F407036-2	2	6.638	.04
		F407036-3	2	3.441	.18
($K4^T \times$ Ky21) K4	1954	F40738-1	2	.188	.91
		F40738-2	2	3.054	.22
		F40738-3	2	1.698	.44

* Calculated for three classes: (1) "sterile", tassel classifications I through IIC; (2) "partially fertile", classifications IIIA through VB; (3) "fertile", classification VC.

fertile plants were due to differences in climate between Iowa and Connecticut. The summers of 1954 and 1955 in Iowa were unusually hot and dry.

The number of recessive modifier genes which are present in lines other than SK2 and WF9 is not surmised very easily since there is no great uniformity of the proportions of fertile to partially fertile plants from progeny to progeny within a backcross. However, several backcross progenies do approximate ratios of 3 fertile:5 partially fertile:8 sterile plants. As is shown in table 15, P values are high for some of the progenies of (C106^T × BH2)C106 and of (K4^T × Ky21)K4 when they are tested for goodness of fit to this ratio. Segregations giving this ratio could occur if four dominant genes were postulated as follows:

		Pedigree	Genotype
Cyt^T *AaCcDdEe* × Cyt^N *aaccddee*		BH2	*AACCDDEE*
(C106^T × BH2)	(C106)	C106	*aaccddee*

Cyt^T *AaCcDdEe*	3 fertile
Cyt^T *AaCcDdee*	
Cyt^T *AaCcddEe*	
Cyt^T *AaCcddee*	5 partially fertile
Cyt^T *AaccDdEe*	
Cyt^T *AaccDdee*	
Cyt^T *AaccddEe*	
Cyt^T *Aaccddee*	
Cyt^T *aaCcDdEe*	8 sterile
Cyt^T *aaCcDdee*	
Cyt^T *aaCcddEe*	
Cyt^T *aaCcddee*	
Cyt^T *aaccDdEe*	
Cyt^T *aaccDdee*	
Cyt^T *aaccddEe*	
Cyt^T *aaccddee*	

This scheme would postulate that a single dominant gene (A) must be present in order to have any pollen fertility, and a second (C) plus one of two others (D or E) must also be present in the dominant form in order to have complete pollen fertility. Thus, two of the three modifier genes would be duplicates. Similar mental exercises could explain some of the other ratios obtained but are of little value here. The point in presenting this tentative explanation is to demonstrate that one can fit genetic ratios to segregations that have been obtained. The next step should be to test these hypotheses by means of the usual methods of progeny testing individual F_2 and backcross plants. It may well be that in those cases in which several modifiers are involved, an exact annotation of all of the genetic factors will not be possible, due to the numerous small differences in tassel fertility which would result and which would be hard to distinguish as separate classes. In addition, such small genetic variations might be masked by environmentally induced variability more easily than larger differences would be. The fact that small numbers of plants in NR^T × FR

hybrids are not completely fertile could also upset genetic studies, if this should mean that a few genetically VC plants sometimes will appear to be IVC. The reason for the presence of small numbers of partially fertile plants in the F_1 hybrids is not yet clear, however. Their appearance conceivably could be due to heterozygosity of one or the other of the P_1 lines, but the lack of a sharp break between class IV and class V tassel types makes it seem more likely that the F_1 hybrids merely are exhibiting a normal range of variation for a uniform genotype when exposed to a variable environment. Once again, it is apparent that individuals of each type must be progeny tested. In the case of $C106^T \times$ Ky21, (which had an unusually large proportion of partially fertile plants) it may be that the Ky21 plant used to make this cross did not have a full complement of dominant FR modifiers, at least with respect to the C106 plant with which it was crossed. WF9 appeared to supply all modifiers needed, for in the 3-way cross, $(C106^T \times$ Ky21)WF9, (tracing back to the same Ky21 plant) nearly all pollen shedding plants were fully fertile.

There is another and a very important pitfall in the study of the genetics of fertility restoration for those cases in which segregating generations produce large numbers of partially fertile tassels. This is the fact that some genotypes tend to have appreciable numbers of abnormal anthers, even in normal cytoplasm. This is especially true for some inbred lines, with certain unfavorable seasons bringing out the trait quite strongly. The abnormal anthers (and the pollen in them) are similar in appearance to anthers which are partially fertile or sterile due to the action of Cyt^T. Therefore, all segregating populations should be grown in both Cyt^T and Cyt^N in order to be sure one is not confusing two separate types of inheritance. In general, however, hybrid material, and most BC_1 and F_2 populations, do not have abnormal anthers (in Cyt^N) even though their inbred parents may have exhibited them. Among the Cyt^N lines used in the crosses reported herein, WF9 and Ky21 tended to have several sterile or weak exserted anthers, especially during unfavorably hot and dry seasons.

And, finally, all backcross tests should be made in at least two ways, so that one can compare segregation and gamete transmission both through the pollen and through the egg in Cyt^T; i.e., both $C106^T$ $(C106^T \times$ Ky21) and $(C106^T \times$ Ky21)C106 should be made. Although it has been shown that the WF9 "reciprocal" backcrosses did not appear to differ, each of the other backcrosses must be tested in the same way. It also would be well to make the type of cross: $C106^T$ (C106 $\times$ Ky21) in order to test for segregation in Cyt^N as contrasted to segregation in Cyt^T.

SUMMARY

Five inbred lines (in so far as they were sampled) were shown to have the same alleles of the dominant genes required for restoration of pollen fertility to the inbred $WF9^T$ (cytoplasmically pollen sterile WF9). The five fertility restorer (FR) lines are Ky21, K55 (Agricultural Experiment Station lines), BH2, F5DD1 and WG3 (Pioneer Hi-Bred Corn Company lines). Backcross tests of the FR lines using five different sterilizable inbreds as recurrent parents have demonstrated that the fact that two or more inbreds are equally sterile in T cytoplasm does not mean that they necessarily are isogenic for all FR genes. Therefore, any statement of the number

and location of the FR genes in a given FR line is applicable only to the specific sterilizable inbred line used as tester and further, only to the specific plants which were tested, until such time as cross comparisons between plants and between lines have been made. Additionally, the environment in which segregating populations are grown can influence the number of FR genes that are revealed. Preliminary breeding tests indicate that, at the least, two dominant complementary genes plus one or (probably) more dominant genes which modify the action of one of the dominant complementary genes are required for full pollen fertility in T cytoplasm when plants are grown in Iowa during rather hot dry summers.

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