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The Inheritance of Fertility Restoration in Male-sterile Wheat Carrying Cytoplasm Derived from *Triticum timopheevi*

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Summary. The inheritance of the restoration of fertility in material carrying the cytoplasm of *Triticum timopheevi* was studied in F_2, F_3 and F_4 generations. In the material used the segregation of restoration was shown to fit the hypothesis of three major, dominant, partially dominant or additive genes each of which made a different contribution to restoration but which acted cumulatively to produce the phenotypic expression observed.

From the material it was possible to extract homozygous lines carrying known combinations of these three genes which can be used as tester lines to investigate the inheritance of genes for restoration derived from other sources.

Introduction

Since the discovery of cytoplasmic male-sterility in wheat carrying *Aegilops caudata* cytoplasm (Kihara 1951) a large number of alien cytoplasms have been successfully transferred to tetraploid and hexaploid wheat, most of them being found to induce male-sterility. They are listed by Tsunewaki (1970). Genes have been found, from a number of sources, which will override the sterilizing effect of these alien cytoplasms and restore fertility to the F_1 generation. Kihara (1968) considered a number of these sources of restoration and showed that they fell into three groups. First, those where homozygosity of the restoring gene or genes is required for recovery of self fertility; secondly, those where the presence of complementary genes causes a heterotic effect for restoration in the F_1 generation which is lost on further backcrossing; and thirdly a group, where a strong restoring gene or genes, is capable of restoring fertility in single doses. In the development of a commercial hybrid wheat variety it is the restorer sources from this third group that will be of most use since these contain a gene or genes that can be simply backcrossed into a range of potential parental lines.

The restoration sources, quoted by Kihara, for the male sterility caused by the cytoplasm of *Triticum timopheevi* fell into this third group. It is thus important, for hybrid wheat research, to understand the inheritance of restoration of the *T. timopheevi* cytoplasm. Wilson (1968a) reviewed the work in this field and listed a number of sources of fertility restoration for the *T. timopheevi* cytoplasm. Other sources are listed by Zeven (1967) and Apltauerova (1968). Some of the genes from some of these restoration sources have since been assigned to specific chromosomes by other workers (Bahl, Maan and Lucken, 1970; Robertson and Curtis, 1967; Tahir and Tsunewaki, 1969; Talaat, 1969 and Zeven, 1970).

The level of self-fertility caused by these sources of restoration in F_1 plants is extremely dependent on the environment (Wilson, 1968a). Maan and Lucken (1967) using aneuploids of the variety Chinese Spring showed that it is also dependent on the number of doses of the restorer genes present. These facts have made studies of the inheritance of the genes carried by different sources of restoration extremely difficult. In his review, however, Wilson (1968a) concluded that, on the evidence to date, restoration of fertility was "possibly due to at least three genes functioning in character expression as cumulative dominants where *Rr Rr Rr* is equal to *RR RR RR*" and that "the individual gene contributions are not necessarily equal". This paper will present evidence to corroborate these conclusions.

Materials and Methods

Two lines, the male sterile (*timopheevi*)-Bison[10] and a restorer line F_3 ((*T. timopheevi* × Marquis[3]) × Bison), were supplied by Dr. R. W. Livers (Kansas State University) in the winter of 1964–65. They were crossed together and F_1 plants crossed as female to the spring wheat variety Maris Ensign (Cappelle-Desprez × Teutonen) with the objective of selecting from the F_2 population the most fertile plants for further backcrossing to the locally adapted variety Maris Ensign. This work was carried out in the glasshouse. Under these conditions selection of the highest expression of fertility proved to be very difficult since no plants were ever found to be fully restored. In the winter of 1966–67 the winter wheat variety Maris Beacon ((Hybrid 46 × Mildew resistant Cappelle-Desprez) × Professor Marchal) was crossed onto an F_2 plant from the first backcross of the restorer line to Maris Ensign. This cross, designated WMS 53, thus had the following pedigree: ((male sterile Bison × Marquis restorer) × Maris Ensign[2]) × Maris Beacon.

The intention of this cross was to initiate a backcrossing programme to produce a male-sterile line of Maris Beacon. No record was kept of the fertility status of the maternal F_2 plant since it was expected that fully male-sterile plants would segregate out in the F_1 or F_2 generations. 25 crossed seeds were set and these were grown in pots

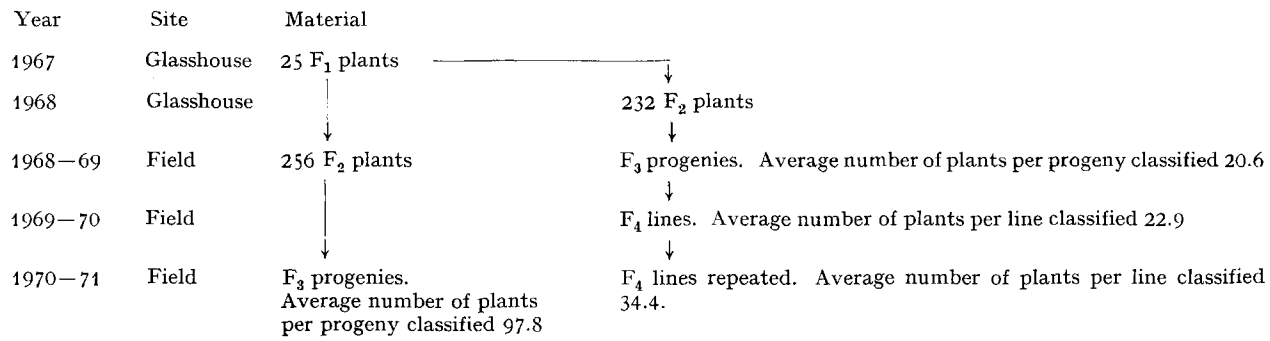

Figure 1. Derivation, site and extent of WMS 53 material grown

in the glasshouse during the summer of 1967. All 25 plants unexpectedly showed an incomplete but very high level of fertility. Maris Beacon had thus possibly contributed a gene or genes causing restoration different from those derived from the Marquis material.

An F_2 population of WMS 53 was grown in the glasshouse during the summer of 1968 and F_2, F_3 and F_4 progenies were grown in the field from winter sowings over the years 1969 to 1971 (see Fig. 1). The segregation of fertility restoration in all these generations of the cross WMS 53 was recorded.

The level of restoration was assessed for each plant by observing the anthers produced by primary tillers at anthesis. On the basis of anther type the plants were assigned to one of three classes, fully fertile or F plants, semi-sterile or *SS* plants and fully sterile or *S* plants. F plants were those where normal anthers were present in all florets; *SS* plants had normal anthers at the base of the ear but a variable number of florets at the apex of the ear that had underdeveloped male-sterile anthers; *S* plants were those which had no normal anthers.

This classification was greatly facilitated by still, warm weather and by good anther extrusion. Every progeny was looked at over a period of days. In the early stages of anthesis, when normal anthers were extruded, it was possible to classify most of the *F* plants. Over the next few days, as the florets containing male-sterile anthers began to gape, it was possible to classify the *SS* plants and finally at the end of the anthesis period, when *S* plants had all their florets gaping it was possible to classify these. As this classification was very time consuming and concentrated into the few days when the plants were at anthesis, several different people were employed to do the work on all the material. This also helped to eliminate any personal bias there may have been in making the classifications.

Results

F_2 1968—69 and F_3 1970—71

The numbers of plants in each category observed in the field F_2 generation grown in 1968—69 and in 19 F_3 progenies derived from fully fertile F_2 plants and grown in the field in 1970—71 are given in Table 1.

The SS class obviously included a range of restoration levels. At one end of the scale these plants had a few florets, always at the base of the ear, with normal anthers while the rest of the ear was male-sterile. These plants were, in fact, rare and confined to the F_2 population and certain F_3 progenies. The majority of SS plants had approximately a half to two-thirds of the ear, always at the base, with normal anthers and only the top florets were male-sterile. Thus the distinction between S and SS classes was, in most of the material, clear cut.

Table 1. *Observed segregations of fertile, semi-sterile and sterile plants in the F_2 population grown 1968—69 and in the F_3 progenies grown in 1970—71*

Material	Progeny No.	Fertile	Semi-sterile	Sterile
F_2 1968—69		151	35	70
F_3 1970—71	4	89	0	0
	5	93	0	0
	18	97	0	0
	2	44	11	0
	17	73	9	0
	11	40	0	25
	14	68	0	29
	20	54	0	18
	1	18	47	34
	3	60	22	9
	5	60	42	5
	6	81	10	13
	7	52	8	26
	8	56	8	16
	9	57	17	25
	10	33	27	18
	12	34	31	28
	16	78	13	7
	19	35	23	31

At the other end of the semi-sterility scale were plants in which only the topmost florets had male-sterile anthers. Such plants were often difficult to distinguish from F plants. As they were found much more frequently than SS plants at the other end of the scale the distinction between the F and SS classes was, in some progenies, very difficult.

The observed F_2 segregation of restoration did not fit any of the usual Mendelian ratios for one, two or three genes and seemed to be of a quantitative nature, certain genotypes above a threshold level exhibiting some degree of restoration with other genotypes above a higher threshold level exhibiting complete restoration. However it also seemed that relatively few genes might be operating since the segregations of the F_3 progenies fell into four groups. Three F_3 progenies did not segregate and consisted entirely of F plants, two consisted of F and SS plants

only, three of F and S plants only and eleven of all three classes. F plants predominated in all the segregating F_3 progenies but in the group of progenies that segregated all three classes varying ratios of SS to S plants were observed. Thus fully restored plants from the F_2 population were exhibiting a range of distinct segregation when grown on as F_3 progenies.

Attempts were made to construct a genetic model that would fit these observed data. The number of genotypes present in the F_2 generation would obviously depend on the number and kind of genes that were operating. By assuming different numbers of major genes to be operating different ranges of possible F_2 genotypes were produced. Phenotypes were then assigned to these possible F_2 genotypes so that a segregation approximating to that observed was produced. The expected F_3 segregations of those genotypes designated as fully fertile could then be worked out to see if the observed F_3 segregations were produced. By this process a model of the inheritance of restoration was produced which closely fits the observed data so far presented.

This model is presented diagrammatically in Fig. 2 and required that there were three independent loci, referred to as *A*, *B* and *C*, operating in fertility restoration. At each locus one allele, denoted by a capital letter, contributed to restoration while an alternative allele, denoted by a lower case letter, did not. If the genotype of the F_1 plants was Aa Bb Cc then there would have been 27 possible genotypes in the F_2 population. Twelve of these, numbered 1--12 in Fig. 2, were considered to give fully fertile phenotypes while eight, numbered 20—27 in Fig. 2, were considered to give fully male-sterile phenotypes. The remaining genotypes were considered to produce the semi-sterile phenotypes observed. However, in order to allow for the difficulty experienced in classifying SS plants at either end of the range four genotypes were considered to have alternative phenotypes. Thus genotypes 13 and 14 in Fig. 2 were considered to have a 50 per cent chance of classification into either the F or SS classes. Similarly genotypes 18 and 19 were considered to have a 50 per cent chance of classification into either the SS or S classes.

The 27 genotypes would have produced the phenotypes assigned to them (a) if the genes acted cumulatively to produce the phenotype; (b) if the genes made different contributions to restoration, gene *A* being more effective than gene *B* which, in turn, was more effective than gene *C*; (c) if gene *A* was fully dominant, gene *B* was partially dominant and gene *C* behaved either additively or as a partial dominant, and (d) if the combined contribution to restoration of certain combination of genes fell below minimum threshold levels for the expression of partial and complete restoration while that of others fell at the threshold levels.

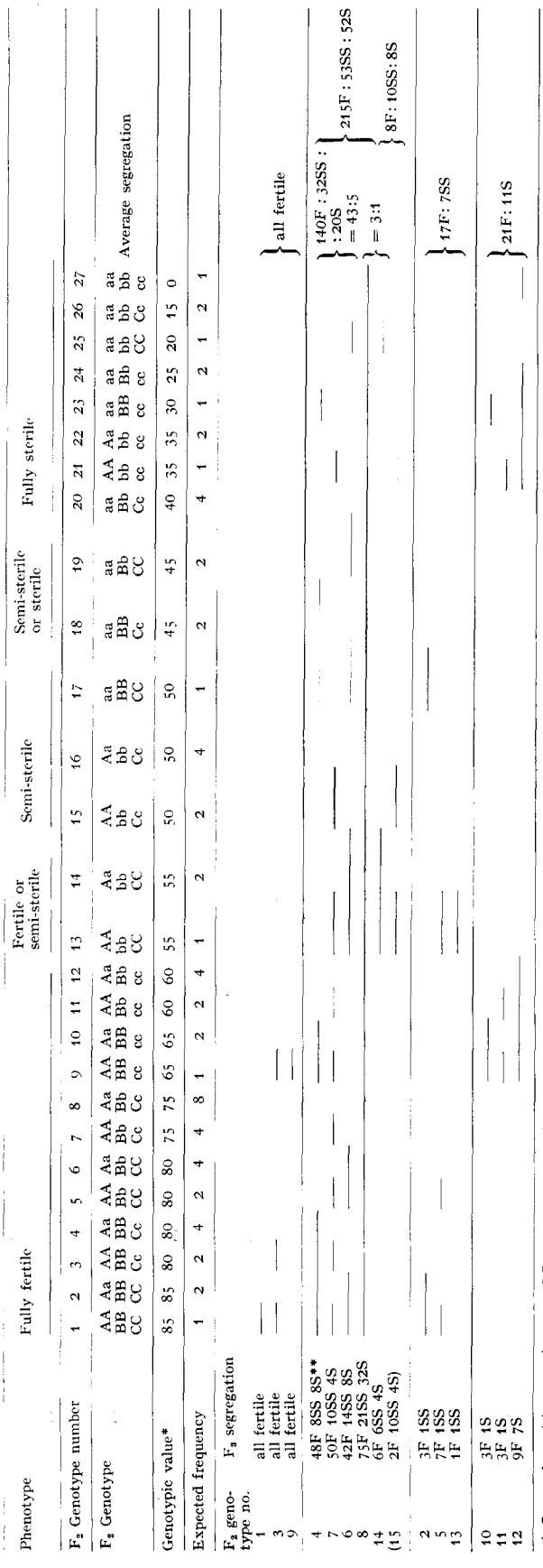

Figure 2. Hypothetical model of the inheritance of restoration in WMS 53 material

* Gene values: AA = 35, Aa = 35, BB = 30, Bb = 25, CC = 20, Cc = 15.
** F = fertile, SS = semi-sterile, S = sterile.

The continuous range of phenotypes observed in the F_2 population from fully fertile through semi-sterility to fully male-sterile suggested a continuous range of genotypic values from genotype 12 through to genotype 20. This could only occur if the contributions to restoration of the individual genes had the following relative values, $Cc = x$, $CC = x + y$, $Bb = x + 2y$, $BB = x + 3y$, $Aa + AA = x + 4y$, and where x is equal to or larger than y. As an example genotypic values are given in Fig. 2 for the situation when gene *C* is partially dominant, x and y being given the arbitrary values of 15 and 5 respectively. In this situation the threshold genotypic value for the expression of any restoration would be 45 and genotypes having a value of 40 or less would be male-sterile. The threshold between the SS and F classes would occur at 55 and genotypes having a value of 60 or more would be fully fertile.

Given this model it was possible to work out the expected segregation ratios for the F_2 population and for F_3 progenies derived from fully fertile F_2 plants and to show that the observed segregations fitted these expectations closely.

The expected F_2 segregation is 37.5F : 10.5SS : 16S or more conveniently in whole numbers 75F : 21SS : 32S. The observed F_2 segregation was not significantly different from this expectation ($\chi^2 = 1.737$, $P = 0.1 - 0.2$).

The twelve F_2 genotypes assigned fully fertile phenotypes account for 36/34 of the 37.5/64 fully fertile F_2 plants expected while a further two F_2 genotypes, number 13 and 14 in Figure 2, falling as they do on the upper threshold level, account for the remaining 1.5/64 expected fully fertile F_2 plants. Hence 14 different genotypes produce the F plants found in F_2. The expected F_3 segregation of these 14 genotypes is also given in Fig. 2. Genotypes 1, 3 and 9, produce only F plants in F_3; genotypes 4, 6, 7, 8 and 14 produce all three classes of plant, genotypes 2, 5 and 13 produce only F and SS plants and genotypes 10, 11 and 12 produce only F and S plants. Thus the four observed groups of segregations are expected in F_3.

In the model two of the five F_2 genotypes that produce all three classes of plant in F_3, number 8 and 14, produce 25 per cent S plants in F_3 progenies, their expected ratios being 75F : 21SS : 32S and 6F : 6SS : 4S respectively. In F_3 they thus segregate three plants with some restoration: one plant with no restoration.

The other three F_2 genotypes that produce all three classes of plant in F_3, numbers 4, 6 and 7, produce less than 25 per cent. S plants in F_3 progenies. Their expected F_3 segregations, 48F : 8SS : 8S, 50F : 10SS : 4S and 42F : 14SS : 8S are very similar and could not be distinguished in progenies where the number of plants classified was small. In these circumstances it would be expected that an average ratio would be found. The average F_3 ratio of these three F_2 genotypes would thus be 140F : 32SS : 20S or 43 plants with some restoration: 5 plants with no restoration.

F_3 progenies segregating all three classes should thus be divisible into two groups, those segregating 3:1 some restoration: no restoration and those segregating 43 some restoration: 5 no restoration, each group consisting of two and three further segregation ratios respectively.

Eleven F_3 progenies gave observed segregations consisting of all three classes of plant. The segregation of each progeny for plants with some restoration to plants with no restoration was tested against the expected ratios of 3:1 and 43:5. The figures for χ^2 given in Table 2 show that overall the segregations did not fit either a 3:1 of a 43:5 ratio and that the segregations were significantly heterogeneous.

Table 2. *1971 F_3 WMS 53 progenies giving three classes: fertile, semi-sterile and sterile*

Progeny No.	Some Restoration	No Restoration	χ^2 3:1	P	χ^2 43:5	P
1	65	34	4.609	*	60.732	***
3	82	9	11.080	***	0.027	NS
5	84	5	17.832	***	2.197	NS
6	91	13	8.664	**	0.483	NS
7	60	26	1.256	NS	36.199	***
8	64	16	1.067	NS	7.874	**
9	74	25	0.000	NS	23.349	***
10	60	18	0.153	NS	13.398	***
12	65	28	1.293	NS	38.638	***
16	91	7	16.667	***	1.125	NS
19	58	31	4.588	*	56.850	***
Total	794	212	8.272	**	122.436	
Heterogeneity (10 degrees of freedom			67.148	***		

NS = Not significant; * = $P < 0.05$; *** = $P < 0.001$.

Two of the progenies, numbers 1 and 19, were significantly different from both the 3:1 and the 43:5 ratios and will be considered again later. Of the other nine progenies five were not significantly different from the 3:1 ratio but were significantly different from the 43:5 ratio and four progenies were significantly different from the 3:1 ratio but were not significantly different from the 43:5 ratio.

The five progenies not significantly different from the 3:1 ratio were tested against the expected ratios 75F : 21SS : 32S and 6F : 6SS : 4S. The figures for χ^2 are given in Table 3. The five progenies fell into two distinct groups each fitting one of the expected ratios.

Similarly the four progenies not significantly different from the 43:5 ratio were tested against the three expected ratios 42F : 14SS : 8S, 48F : 8SS : 8S and 50F : 10SS : 4S. The figures for χ^2 are given in Table 4. Here two progenies distinctly fitted the 42F : 14SS : 8S ratio, one distinctly fitted the 48F : 8SS : 8S ratio and one, while not being signifi-

Table 3. *1971 F_3 Progenies giving three classes but not significantly different from ratio three some restoration to one no restoration*

Material not significantly different from	Progeny number		Fertile	Semi-sterile	Sterile	χ^2 75:21:32	P	χ^2 6:6:4	P
3 some restoration	7		52	8	26	3.638	NS	31.271	***
to	8		56	8	16	4.577	NS	39.466	***
1 no restoration	9		57	17	25	0.055	NS	21.550	***
	10		33	27	18	19.405	***	0.769	NS
	12		34	31	28	24.919	***	1.423	NS
		Total	232	91	113	7.600	*	60.994	***
		Heterogeneity				39.342	***		
75 Fertile	7, 8, 9	Total	165	33	67	3.141	NS		
to 21 semi-sterile		Heterogeneity				5.129	NS		
to 32 sterile									
6 Fertile	10, 12	Total	67	58	46			0.961	NS
to 6 semi-sterile									
to 4 sterile		Heterogeneity						1.231	NS

NS = Not significant, * = $P < 0.05$, *** = $P < 0.001$.

Table 4. *1971 F_3 WMS 53 progenies giving three classes but not significantly different from ratio 43 some restoration to 5 no restoration*

Material not significantly different from	Progeny number		Fertile	Semi-sterile	Sterile	χ^2 42:14:8	P	χ^2 48:8:8	P	χ^2 50:10:4	P
43 some restoration	3		60	22	9	0.717	NS	11.417	**	7.919	*
to	5		60	24	5	4.470	NS	15.718	***	8.687	*
5 no restoration	6		81	10	13	9.528	**	0.807	NS	8.905	*
	16		78	13	7	8.486	*	2.572	NS	0.501	NS
		Total	279	69	34	9.694	**	13.612	**	7.013	*
		Heterogeneity				16.107	**				
42 Fertile	3, 5	Total	120	46	14	4.355	NS				
to 14 semi-sterile		Heterogeneity				0.832	NS				
to 8 sterile											
48 Fertile	6, 16	Total	159	23	20			1.663	NS		
to 8 semi-sterile											
to 8 sterile		Heterogeneity						1.716	NS		

NS = Not significant, * = $P < 0.05$. ** = $P < 0.01$, *** = $P < 0.001$.

cantly different from either the 48F : 8SS : 8S or the 50F : 10SS : 4S ratio, had a much smaller χ^2 for, and hence a greater probability of fitting, the 50F : 10SS : 4S ratio. Thus progenies giving all three classes of plant were found to fit each of the five expected segregation ratios.

The three F_2 genotypes that produce only F and SS plants in F_3 progenies are expected in the model to segregate 3F : 1SS, 7F : 1SS and 1F : 1SS and their average ratio would be 17F : 7SS.

Two of the 19 F_3 progenies classified gave only F or SS plants. These were tested against the three expected ratios and their segregations and figures for χ^2 are given in Table 5. One progeny distinctly fitted the 7F : 1SS ratio and the other, while not being significantly different from either the 3F : 1SS or the 7F : 1SS ratio had a much smaller χ^2 for, and hence a greater probability of fitting, the 3F : 1SS ratio.

The three F_2 genotypes that produce only F and S plants in F_3 progenies are expected to segregate 3F : 1S, or 9F : 7S and their average ratio would be 21F : 11S.

Three of the 19 F_3 progenies classified gave only F and S plants. These were tested against the two expected ratios 3F : 1S and 9F : 7S and their segregations and figures for χ^2 are given in Table 6. Also included in Table 6 are the segregations for progenies 1 and 19 which, while giving all three classes of plant,

Table 5. *1971 F_3 WMS 53 progenies giving only fertile and semi-sterile plants*

Progeny number	Fertile	Semi-sterile	χ^2 3:1	P	χ^2 7:1	P	χ^2 1:1	P
2	44	11	0.733	NS	2.829	NS	19.800	***
17	73	9	27.333	***	0.174	NS	49.951	***

NS = Not significant, *** = $P < 0.001$

Table 6. *1971 F_3 WMS 53 progenies showing only fertile or sterile plants together with the two progenies giving all three classes but which were significantly different from the 3:1 or 43:5 some restoration to no restoration ratios*

Material	Progeny number	Fertile	Semi-sterile	Sterile	Some restoration	No restoration	χ^2 21:11	P	χ^2 9:7	P	χ^2 3:1	P
	1	18	47	34	65	34	0.000	NS	3.559	NS	4.609	*
	11	40	0	25	40	25	0.481	NS	0.731	NS	6.283	*
	14	68	0	29	68	29	0.862	NS	7.564	**	1.240	NS
	19	35	23	31	58	31	0.008	NS	2.876	NS	4.588	*
	20	54	0	18	54	18	2.805	NS	10.286	**	0.000	NS
		Total			285	137	0.683	NS	21.840	***	12.540	***
		Heterogeneity					3.572	NS				
Significantly different from 3 fertile to 1 sterile	1, 11, 19	Total			163	90	0.161	NS	6.874	**		
		Heterogeneity					0.328	NS	0.292	NS		
Not significantly different from 3 fertile to 1 sterile	14, 20	Total			122	47					0.712	NS
		Heterogeneity									0.528	NS

NS = Not significant. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

were significantly different from both the 3:1 and the 43:5 some restoration to no restoration ratios (Table 2). All five progenies were, individually and overall, not significantly different from the expected average ratio of 21 some restoration: 11 no restoration. Each progeny was not significantly different from either the 3F : 1S ratio or the 9F : 7S ratio. However, the three progenies, individually not significantly different from the 9F : 7S ratio were significantly different from this ratio overall. These three progenies included progenies 1 and 19. The presence of SS plants in these progenies and the overall difference from the 9F : 7S ratio may have been due either to misclassification or to the presence of background modifying genes, further indications of which will be given later.

The remaining three F_3 progenies of the 19 classified consisted entirely of F plants. Thus each of the 19 progenies could be fitted to one of the eleven different F_3 segregations expected. Table 7 gives the expected and observed frequency of each of these segregations. The observed frequency fitted the expected frequency very well with a χ^2 for ten degrees of freedom of 6.900 ($P = 0.7-0.8$). The expectations of the model fitted to the data presented so far were then tested against other F_2, F_3 and F_4 data not used in the construction of the model.

Table 7. *Expected and observed frequencies of segregation ratios in 1971 F_3 WMS 53 progenies*

Segregation ratio	Expected frequency	Expected frequency from 19 progenies	Observed frequency	Progeny numbers
All fertile	4	2.028	3	4, 15, 18
48F 8SS 8S	4	2.028	1	6,
50F 10SS 4S	4	2.028	1	16,
42F 14SS 8S	4	2.028	2	3, 5,
75F 21SS 32S	8	4.056	3	7, 8, 9,
6F 6SS 4S	1	0.507	2	10, 12,
3F 1SS	2	1.014	1	2
7F 1SS	2	1.014	1	17,
1F 1SS	0.5	0.254	0	—
3F 1S	4	2.028	2	14, 20
9F 7S	4	2.028	3	1, 11, 19
Total	37.5	19.013	19	

F = Fertile, SS = Semi-sterile, S = Sterile.

F_2 1968. In the glasshouse in the summer of 1968, 232 F_2 plants were classified. The fertility of normal varieties growing as controls was reduced from normal and no fully fertile plants were found among the F_2 plants. The glasshouse environment was thus effectively suppressing the expression of fertility.

The F_2 population consisted of 181 plants with some restoration and 52 plants with no restoration. This observed segregation fitted the expected F_2 segregation of 3 some restoration: 1 no restoration quite well ($\chi^2 = 0.895$, $P = 0.3-0.5$). The most fertile F_2 plants were selected for growing on as F_3 progenies.

F_3 1968–69. 106 F_3 progenies were grown on from the glasshouse F_2 plants and of these 46 segregating progenies were classified. These fell into four groups. The largest, consisting of 25 progenies, segregated all three classes with the F class predominating. A second group, consisting of 10 progenies also segregated all three classes but the SS class predominated. A third group of six progenies segregated only F and SS plants, while the fourth group of seven progenies segregated only F and S plants.

These last two groups would be expected according to the model. Since the number of plants per progeny was low the segregation of each progeny in each group was tested against the expected average segregation for that group. The segregations and figures for χ^2 for each progeny in each group are given in Table 8. Individually and overall the progenies segregating only F and SS plants fitted the expected

Table 8. *1969 F_3 WMS 53 progenies giving three classes with semi-sterile plants predominating, two classes, fertile and semi-sterile, or two classes, fertile and sterile*

Progeny number	Fertile	Semi-sterile	Sterile	χ^2 8:16:8	P	Progeny number	Fertile	Semi-sterile	χ^2 17:7	P	Progeny number	Fertile	Sterile	χ^2 21:11	P
27	7	10	4	0.905		20	18	4	1.285		13	21	6	1.768	
61	8	10	1	5.211		30	18	3	2.251		17	12	9	0.670	
65	6	13	5	0.250		41	16	5	0.292		34	15	8	0.002	
76	7	9	6	0.818		73	12	4	0.135		58	16	6	0.492	
80	2	10	4	1.500		75	14	4	0.421		59	18	5	1.627	
81	4	9	4	0.059		86	12	9	1.905		66	15	8	0.002	
82	6	13	4	0.740							100	9	7	0.623	
91	4	8	5	0.176											
98	6	8	4	0.667											
99	4	9	3	0.375											
Total	55	99	40	2.161	NS		90	29	1.325	NS		106	49	0.524	NS
Hetero-geneity				8.540	NS				4.964	NS				4.660	NS

17F : 7SS ratio. Similarly, the progenies segregating only F and S plants fitted, individually and overall, the expected 21F : 11 S ratio.

Also given in Table 8 are the segregations of those progenies that fell into the second group, those segregating all three classes with SS plants predominating. This is not the kind of ratio that would immediately be expected from the model.

In the glasshouse F_2 population that gave rise to these F_3 progenies all the selected plants were SS. Thus it is possible that some genetically SS F_2 plants were kept. If some plants having the genotype number 15 (see Fig. 2) were selected they would have an F_3 segregation of 2F : 10SS : 4S. This would be easily confused in small progenies with the expected 6F : 6SS : 4S segregation and their average segregation would be 8F : 16SS : 8S, or, in other words, three classes will SS plants predominating. Table 8 shows that the progenies from the second group fitted this average ratio of 8F : 16SS : 8S.

The major group of progenies, those consisting of all three classes with F plants predominating, are, according to the hypothesis, likely to consist of four segregations, 48F : 8SS : 8S, 50F : 10SS : 4S, 42F :

Table 9. *1969 F_3 WMS 53 progenies giving three classes; fertile, semi-sterile and sterile with the fertile class predominating*

Progeny No.	Fertile	Semi-sterile	Sterile	Some resto-ration	No resto-ration	χ^2 3:1	P	χ^2 43:5	P	χ^2 215:53:52	P
1	12	3	6	15	6	0.143	NS	7.414	**		
2	16	3	3	19	3	1.515	NS	0.244	NS		
5	9	1	4	10	4	0.095	NS	4.947	*		
9	16	3	3	19	3	1.515	NS	0.244	NS		
10	15	3	5	18	5	0.131	NS	3.159	NS		
11	16	3	4	19	4	0.711	NS	1.199	NS		
15	12	2	6	14	6	0.267	NS	8.222	**		
19	12	2	8	14	8	1.515	NS	15.868	***		
21	9	2	6	11	6	0.961	NS	11.272	***		
26	14	5	2	19	2	2.683	NS	0.018	NS		
35	14	5	2	19	2	2.683	NS	0.018	NS		
36	12	2	1	14	1	2.689	NS	0.227	NS		
42	16	6	1	22	1	5.232	*	0.908	NS		
44	19	3	2	22	2	3.556	NS	0.112	NS		
46	16	2	3	18	3	1.285	NS	0.336	NS		
52	19	2	1	21	1	4.909	*	0.813	NS		
55	15	1	7	16	7	0.363	NS	9.876	**		
57	13	5	4	19	4	0.711	NS	1.199	NS		
68	15	4	4	19	4	0.711	NS	1.199	NS		
69	11	4	3	15	3	0.667	NS	0.753	NS		
78	12	6	5	18	5	0.131	NS	3.159	NS		
84	8	6	6	14	6	0.267	NS	8.222	**		
93	11	8	1	19	1	4.267	*	0.628	NS		
95	15	5	4	20	4	0.887	NS	1.005	NS		
105	13	2	4	15	4	0.157	NS	2.304	NS		
Total	340	88	95							1.571	NS
Total				428	95	13.033	***	33.643	***		
Hetero-geneity						31.354	NS				

Table 10. *Totals of 1969 F_3 WMS 53 progenies giving three classes but predominantly fertile that were considered to fit either 3:1 or 43:5 some restoration to no restoration*

Material fitting		Fertile	Semi-sterile	Sterile	Some restoration	No restoration	χ^2 3:1	P	χ^2 43:5	P	χ^2 75:21:32	P	χ^2 140:32:20	P
3 Some restoration to 1 no restoration	Total	187	49	76							0.241	NS		
	Total				236	76	0.068	NS						
	Heterogeneity						7.649	NS						
43 Some restoration to 5 no restoration	Total	153	39	19									0.826	NS
	Total				192	19			0.451	NS				
	Heterogeneity								3.097	NS				

14SS : 8S and 75F : 21SS : 32S. The average ratio for these four segregations is 215F : 53SS : 52S. The observed segregation for the 25 progenies that fell into this major group are given in Table 9. The overall total segregation of 340F : 88SS : 95S is shown in Table 9 to be not significantly different from the expected average segregation 215F : 53SS : 52S.

As before, each progeny was tested against the two ratios 3:1 and 43:5 some restoration to no restoration (Table 9). As the plant number per progeny was small most progenies were not significantly different from either ratio. If, however, each progeny was considered to fit the ratio for which it had the smallest, nonsignificant figure for χ^2 then 15 progenies could be considered to fit the 3:1 ratio while the other ten could be considered to fit the 43:5 ratio.

The overall total segregations for these two groups of progenies are given in Table 10. According to the model the material fitting the 3:1 ratio should consist of progenies segregating 75F : 21SS : 32S, the F_2 segregation, while the material fitting the 43:5 ratio should consist of progenies segregating 48F : 8SS: 8S, 50F : 10SS : 4S or 42F : 14SS : 8S and should fit the average ratio 140F : 32SS : 20S. The figures for χ^2 given in Table 10 show that the total segregation for the 15 progenies considered to fit the 3:1 some restoration to no restoration ratio fitted the expected F_2 segregation while the total segregation of the ten progenies considered to fit the 43:5 some restoration to no restoration ratio fitted the expected average ratio 140F : 32SS : 20S.

F_4 1969—70 and 1970—71. F plants of the 1968—1969 F_3 progenies showing the simpler two class segregations were grown on in the field as F_4 families in 1969—70 and repeated in 1970—71.

The combined segregations for both years observed in the F_4 family derived from the F_3 progeny number 30 are given in Table 11. Of the eight F_4 lines that were fully classified in both years two did not segregate and the other six were not significantly different from a 3F : 1SS ratio. This pattern of segregation is consistent with that observed for progeny 30 in F_3 (Table 8), and indicates that the F_2 plant from which the F_3 progeny was derived had the genotype number 2 (see Fig. 2) Aa BB CC. Thus the non-segregating lines in the F_4 family must have been homozygous for all three genes and have had the genotype AA BB CC while the SS plants in the segregating lines must have been homozygous for only the *B* and *C* genes and have had the genotype aa BB CC.

Table 11. *Combined segregations for 1969—70 and 1970—71 of F_4 lines derived from F_3 progenies 30 and 86*

F_3 Progeny no.	F_4 Progeny no.	Fertile	Semi-sterile	χ^2 3:1	P	χ^2 1:1	P
30	2	50	0				
	4	50	0				
	1	34	6	2.133	NS		
	3	24	7	0.097	NS		
	5	42	11	0.509	NS		
	6	42	12	0.233	NS		
	7	18	11	2.587	NS		
	8	38	15	0.308	NS		
	Total	198	62	0.184	NS		
	Heterogeneity			5.673	NS		
86	1	25	34			1.372	NS
	2	37	20			5.070	*
	4	19	24			0.581	NS
	3	22	21			0.023	NS
	Total	103	99			0.079	NS
	Heterogeneity					6.967	NS

The combined segregations for both years observed in the F_4 family derived from the F_3 progeny number 86 are also given in Table 10. The four lines that were fully classified in both years all segregated and overall fitted the ratio 1F : 1SS. This segregation would be expected from F_3 plants having the threshold F_2 genotype number 13 (see Fig. 2) AA bb CC.

The segregations observed in a third F_4 family, that derived from the F_3 progeny number 17, are given in Table 12. One F_4 line did not segregate in either

Table 12. *Segregations of F_4 lines derived from F_3 progeny 17*

Year	Seg. and not seg.	Progeny number	Fertile	Semi-sterile	Sterile	Some restoration	No restoration	χ^2 3:1	P	χ^2 9:7	P
1970	Not seg.	3	20	0	0	20	0				
		1	13	0	14	13	14				
		6	12	3	9	15	9				
		10	8	0	11	8	11				
		Total				36	34	20.743	***	0.661	NS
	Segreg.	2	15	6	4	21	4				
		4	11	6	7	17	7				
		5	9	4	7	13	7				
		7	11	3	9	14	9				
		8	11	0	4	11	4				
		9	14	0	4	14	4				
		Total				90	35	0.600	NS	12.600	***
1971	Not seg.	3	39	0	0	39	0				
		1	19	0	15	19	15				
		6	18	0	18	18	18				
		10	17	2	5	19	5				
		Total				56	38	11.929	***	0.422	NS
	Segreg.	2	26	0	15	26	15				
		4	27	2	9	29	9				
		5	24	0	8	24	8				
		7	31	0	10	31	10				
		8	25	0	6	25	6				
		9	22	0	10	22	10				
		Total				157	58	0.448	NS	24.578	***
1970 & 1972	Not seg.	3	59	0	0	59	0				
		1				32	29	16.531	***	0.356	NS
		6				33	27	12.800	***	0.038	NS
		10				27	16	3.419	NS	0.747	NS
		Total				92	72	31.252	***	0.002	NS
		Heterogeneity						1.140	NS		
	Segreg.	2				47	19	0.515	NS	6.004	*
		4				46	16	0.021	NS	8.112	**
		5				37	15	0.411	NS	4.693	NS
		7				45	19	0.751	NS	5.143	NS
		8				36	10	0.261	NS	9.056	**
		9				36	14	0.240	NS	5.040	NS
		Total				247	93	1.004	NS	37.146	***
		Heterogeneity						1.120	NS		
		Overall total of seg. progenies				339	165	16.095	***	24.834	***
		Heterogeneity						16.057	*		

year. The combined segregations of the other lines for both years for plants with some restoration to plants with no segregation were tested against the ratios 3F : 1S and 9F : 7S. The figures given in Table 12 for χ^2 show that overall the total segregation did not fit either ratio and that the F_4 lines were heterogeneous. However, if, as before, each line was considered to fit the ratio for which it had the smallest non-significant figure for χ^2 then three lines fitted the 3F : 1S ratio while the other six lines fitted the 9F : 7S ratio. This pattern of segregation is consistent with that observed for progeny 17 in F_3 (Table 8) and indicates that the F_2 plant from which the F_3 progeny was derived had the genotype number 12 (see Fig. 2) Aa Bb cc. Thus the nonsegregating F_4 line must have been homozygous for only the *A* and *B* genes and have had the genotype AA BB cc.

The observed segregation into F, SS and S plants given by these F_4 lines are also given in Table 12, and show some differences between the two years. In 1970—71 seven of the lines did not segregate any SS plants while the other two gave only two SS plants each. In 1969—70, however, five of the lines gave some SS plants. If the conclusion that this F_4 family was derived from an F_2 plant having the genotype Aa Bb cc is correct, then according to the hypothesis, these SS plants would not be expected. However, in the 1970—71 F_3 progenies some SS plants were found in progenies that only fitted the 9 some restoration: 7 non-restoration ratio (Table 6).

These F_3 lines would also have been derived from F_2 plants having the genotype Aa Bb cc. The F_3 segregation of this genotype produces F plants with genotypic values that are very close to the threshold value. The presence of the unexpected SS plants may thus be due to the fact that accurate classification was difficult close to the threshold genotypic levels.

The sowing in 1969—70 was badly thinned out by attacks from wheat bulb fly and the plants classified were effectively much wider spaced than those in 1970—71. If this wide spacing had some effect on the ease of classification this might explain the differences between the two years.

Alternatively the presence of these unexpected SS plants may have been due, not to misclassification, but to the presence of background modifying genes whose effects were only noticeable in material that was segregating at or about the threshold between semi-sterile and fully fertile expressions and whose effects were more noticeable in widely spaced sowings.

Discussion

The fact that, within the SS class of plants, a range of fertility levels was observed, in which the degree of sterility always increased from the apex of the ear downwards, suggests that restoration is brought about by some substance which is translocated up the stem to the ears. Different genotypes might well produce different quantities of this substance which, in turn, might not produce restoration in any given floret unless present in a critical concentration. This critical concentration might not be so easily achieved at the top of the ear as at the base.

A hormonal explanation of restoration such as this would easily allow for both the threshold genotypic levels postulated in the hypothetical model of restoration inheritance and the different contributions postulated for the different genes.

The glasshouse environment is obviously not favourable to restoration since in 1968 no fertile plants were found in the glasshouse grown F_2 population of WMS 53. But since the segregation of plants with some restoration to plants with no restoration of this F_2 population fitted the expected 3 : 1 ratio, and since the complete range of expected F_3 segregation was found in 1968—69 in the F_3 progenies derived from the most fertile of these F_2 plants, the glasshouse environment must have affected the function of some substance produced by the genes segregating rather than affecting the expression of the genes themselves.

Genotypes that were producing only just sufficient restorer substance for restoration of the complete ear might well be more susceptible to variation in the environment or in the genetic background of the material and consequently their observed phenotypes might well be more variable than the rest. This might well be the reason for the different segregations observed, in 1969—70 and 1970—71, in the F_4 lines derived from F_3 progeny 17 and for the presence of SS plants in two of the 1970—71 F_3 lines which distinctly fitted the 9 some restoration: 7 no restoration ratio.

All the data presented can be fitted to the suggested hypothetical model of restoration inheritance of three major genes of differing effect that act cumulatively. They thus confirm Wilson's view of the mode of inheritance of restoration of *T. timopheevi* cytoplasm (Wilson, 1968a).

These data also provide evidence to confirm other suggestions made by Wilson in another review (Wilson 1968b). Wilson reported that some male-sterile lines were easier to restore than others and suggested that this was because the male-sterile lines carried a gene or genes for restoration which were inadequate on their own but which could make a contribution to restoration in any F_1 hybrid produced by crossing the male-sterile line to a restorer-line.

This situation would be possible with the genes segregating in the WMS 53 material since the suggested model of inheritance indicates that genotypes homozygous for each gene separately, i.e. AA bb cc, aa BB cc and aa bb CC all produce male-sterile phenotypes (see Fig. 2).

Wilson also presented a hypothetical explanation for genetic-environmental fertility interactions. According to this normal fertility would require more restorer genes in some environments than in others. For example, normal fertility would require three genes in some environments but might require four in environments less suited to the expression of restoration. The environment of winter sowings in the field at Cambridge must, on this criterion, be favourable to restoration since in the WMS 53 material the heterozygous three gene genotype Aa Bb Cc was always found to be fully fertile.

Wilson also suggested that F_1 hybrid varieties would be better adapted to a range of possible environments if they carried a surplus of restorer genes over that necessary for normal fertility in an average environment. This would be reasonable if different field environments suppressed to some extent the functioning of some restorer gene product in the same way as the glasshouse environment at Cambridge appears to do. It would be possible, by using the WMS 53 material to produce F_1 hybrid varieties having a surplus of restorer genes since by crossing the triple homozygote AA BB CC to, for example, the male-sterile, single gene homozygote aa BB cc an F_1 generation of the constitution Aa BB Cc would be produced which would, according to the model have a higher genotypic value than the 3 gene F_1 hybrid Aa Bb Cc.

In fact the difference between these genotypic values would probably be only small in genotype-environment interaction terms. More effective surpluses of restoring ability could presumably be produced if a fourth completely different gene could be introduced into the system.

There is some indication from the data presented here that such a fourth gene is already known. The fact that the 25 F_1 plants of WMS 53 grown in the glasshouse in 1967 showed an unexpectedly high level of restoration first indicated that Maris Beacon might be contributing a gene or genes different from those derived from the (*T. timopheevi* × Marquis[3]) × Bison restorer material. Livers (1964) reported that the segregation of an F_2 population, derived by crossing male sterile (*timopheevi*-) Bison with a restorer plant of (*T. timopheevi* × Marquis[3]), fitted a ratio of 9 fertile: 6 partially fertile: 1 sterile. Although this same Marquis derived restorer material was involved in the crossing programme from which WMS 53 was produced this segregation was never found. It is thus probable that the Marquis material carries a gene or genes for restoration different from the genes segregating in WMS 53.

This can now be tested easily since from the three F_4 families reported here it has been shown that homozygous lines carrying different combinations of the three genes *A*, *B* and *C* can be extracted. These all carry the *T. timopheevi* cytoplasm and consequently can be used as female parents in test crosses with the Marquis restorer material or, indeed, with any material suspected of carrying genes for restoration of sterility induced by *T. timopheevi* cytoplasm. The segregations observed in the F_2 generations of such test crosses would determine whether new material carried any genes different from *A*, *B* or *C*.

Now that the inheritance of three genes for restoration has been observed at Cambridge in a favourable environment which seems to be consistent from year to year it will be possible to use homozygous lines of known genotype as a tester set of lines for the evaluation of other environments and of potentially new restorer genes.

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