DIFFERENTIAL FERTILIZATION IN THE INHERITANCE OF STEM RUST RESISTANCE IN HYBRIDS INVOLVING A COMMON WHEAT STRAIN DERIVED FROM TRITICUM TIMOPHEEVI¹

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ALTHOUGH genes governing many characters have been transferred between species, the inheritance of only a few of these genes has been studied in detail in their new genetic backgrounds. ALLARD and SHANDS (1954) reported that the stem rust resistance transferred from *Triticum timopheevi* Zhuk. to a common wheat variety C.I. 12633 was controlled in certain hybrids by duplicate dominant genes linked with a recombination value of 14.8 percent. NYQUIST (1957) found that the genes governing resistance were located on chromosome XIII. However, when still other hybrids were studied, the data did not always support the hypothesis of duplicate linked genes. Consequently, the inheritance of stem rust resistance has been studied further.

MATERIALS AND METHODS

The resistant parent C.I. 12633 used in these studies was a common wheat, *T. aestivum* L. ($n = 21$), which derived its stem rust resistance from *T. timopheevi* ($n = 14$). C.I. 12633 was developed by twice backcrossing the inter-specific hybrid 2666A2-2-15-6-3 $\times$ *T. timopheevi* to the common wheat parent, followed by selection of a single plant in the eighth selfed generation. The common wheat parent 2666A2-2-15-6-3 was itself a selection from the cross Illinois No. 1 $\times$ Chinese Spring. The susceptible parents were Ramona (C.I. 8241-1), Federation (C.I. 4734), White Federation (C.I. 4981), Chinese Spring (C.I. 6223), and 2666A2-2-15-6-3.

The stem rust studies were conducted in summer-rust nurseries in the field at Davis from 1952 to 1960. Individual plant reactions were determined from spaced-plantings (80 seeds per 16-foot row), but when only family reaction was to be determined, denser plantings were used (30-60 plants per eight-foot row). Physiologic races 11, 17, and/or 56 of stem rust, *Puccinia graminis tritici* Eriks. and Henn., were present during the test years. (Races were identified by Drs. E. C. STAKMAN and J. J. CHRISTENSEN, Cooperative Rust Laboratory, University of Minnesota, St. Paul, Minnesota.) C.I. 12633 was immune to all races present whereas all of the susceptible parents were highly susceptible. Only plants with the parental-type reactions occurred in segregating generations with the rare exception of individuals on which there were a few pustules. In 1959 plants of

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this type were more numerous. Progenies of these exceptional plants all segregated and included no exceptional plants; therefore it was concluded that the exceptional plants were fully resistant and represented a unique interaction between heterozygous individuals and the environment.

Brachytic, sterile individuals which occurred in the segregating generations of the Ramona, Federation, and White Federation hybrids were excluded in the analyses of inheritance of stem rust resistance.

Backcross F_1 plants and their progenies were observed for awns to detect possible selfing in the manual backcrossing operation. Ramona and White Federation are awnless, and 2666A2-2-15-6-3 and C.I. 12633 are awned. Awnless is dominant. White Federation was used as a tester for the 2666A2-2-15-6-3 $\times$ C.I. 12633 hybrid in order that every selfed individual, if any, could be identified. In all backcrosses no selfed individuals were positively identified.

Heading date was taken as the day when the first spike had completely emerged above the upper leaf sheath from approximately 50 percent of the plants.

Only the first and second florets in a spikelet were utilized in evaluating failure of seed set. Sterility of backcross F_1 plants was determined by examining at least 100 florets per plant. For statistical tests of differences in seed set, percentages were transformed using the arc sine angular transformation.

All homogeneity tests were conducted by the method of BRANDT and SNEDECOR as outlined by FISHER (1958) for hierarchical subdivisions.

RESULTS

F₂ ratios: The data for F_2 plants and F_3 families were combined for each hybrid or F_1 group to obtain an overall F_2 ratio as shown in Table 1. All results combined within each overall F_2 ratio were considered homogeneous for the following reasons: (1) Homogeneity was found between F_2 and F_3 data for each hybrid or F_1 group. (2) The F_2 ratios from F_1 plants grown during different seasons were homogeneous except those of Chinese Spring $\times$ C.I. 12633. For that hybrid, differences occurred between the ratios obtained from F_1 plants grown in different seasons (Table 1). For all other hybrids, however, the different seasons or environments in which the F_1 plants flowered had no influence on the percentage of susceptible plants. (3) Chi-square tests for each hybrid or seasonal F_1 group showed no heterogeneity between reciprocal crosses, among the different female plants, or among the different F_2 families from the same female plants. (4) The procedure of not classifying brachytic plants for rust reaction did not influence the ratios, since it was found that the two characters were inherited independently.

All possible comparisons or tests of heterogeneity between the ratios of different homogeneous groups or hybrids were significantly different at the one percent probability level (mostly 0.1%) except Ramona *vs.* Federation (probability two percent), Federation *vs.* White Federation (eight percent), and Chinese Spring (3) *vs.* 2666A2-2-15-6-3 (28 percent). Thus five highly significantly different F_2 ratios existed: Ramona hybrid with 26.6 percent susceptibility, White Federa-

TABLE 1

Classification of F_2 plants and F_3 families for reaction to stem rust

Hybrid	Generation	Stem rust reaction		Percent susceptibility	Percent survival of F_2 seed
		Resist. (F_2); homo. resist. and seg. (F_3)	Susc. (F_2); homo. susc. (F_3)		
Ramona $\times$ C.I. 12633	F_2	1103	392	26.2	36.4
	F_3	175	70	28.6	60.6
	Totals	1278	462	26.6	...
Federation $\times$ C.I. 12633	F_2	619	177	22.2	40.8
White Federation $\times$ C.I. 12633	F_2	2411	592	19.7	36.4
	F_3	310	64	17.1	63.0
	Totals	2721	656	19.4	...
Chinese Spring $\times$ C.I. 12633 (1)*	F_2	1412	260	15.6	42.9
	(2)† F_2	872	106	10.8	39.6
	F_3	326	46	12.4	66.7
	Totals	1198	152	11.3	...
	(3)‡ F_2	262	15	5.4	35.5
2666A2-2-15-6-3 $\times$ C.I.12633	F_2	3397	258	7.1	37.9
	F_3	597	49	7.6	80.8
	Totals	3994	307	7.1	...

* F_1 plants were grown summer 1952—from published data (NYQUIST 1957).† F_1 plants grown summer 1951.‡ F_1 plants grown spring 1952.

tion 19.4 percent, Chinese Spring (1) 15.6 percent, Chinese Spring (2) 11.3 percent, and 2666A2-2-15-6-3 7.1 percent.

F_3 generation: The among-family distribution for the F_3 generation of the different hybrids is presented in Table 2. The homozygous susceptible families in the Ramona and White Federation hybrids occasionally had one or two resistant plants which were presumed to be natural outcrosses. In each hybrid, about 50 percent of the families segregated except for the 2666A2-2-15-6-3 hybrid grown in 1952, in which only 27.2 percent of the families segregated. When another group of F_3 families of the 2666A2-2-15-6-3 hybrid was tested in 1959, about 50 percent segregated. The large deficiency of segregating families in the first group of the 2666A2-2-15-6-3 hybrid was presumably due to inadequate family sizes for the segregation ratios concerned (Tables 2 and 3). The distribution of F_3 families for all hybrids, excluding the 2666A2-2-15-6-3 (1952) group, gave 348 segregating to 384 nonsegregating which fitted a 1:1 ratio, $P = .10-.20$.

The frequency distribution of the percent susceptibility within segregating F_3 families in each hybrid is presented in Figure 1. The within-family segregation in most hybrids was similar to the F_2 ratio of the particular hybrid, although the segregating F_3 families were not homogeneous for most hybrids (Table 3). When

TABLE 2

Classification of F_3 families for stem rust reaction, mean number of plants per family in each class, and comparison between mean number of plants in different classes

Hybrid	Year grown	Rust reaction	Observed	Percent- age of total families	Mean number of plants per family	Percent survival	Comparison between mean numbers	
							Kind of comparison	t
Ramona $\times$ C.I. 12633	1953	Homo. resist.	60	24.5				
		Segregating	115	46.9				
		Homo. susc.	70	28.6				
White Federation $\times$ C.I. 12633	1952	Homo. resist.	66	31.9	60.03	37.5	Resist. vs. seg.	-0.56
		Segregating	108	52.2	61.33	38.3	Resist. vs. susc.	1.93
		Homo. susc.	33	15.9	53.91	33.7	Seg. vs. susc.	2.51*
Chinese Spring $\times$ C.I. 12633 (2)	1952	Homo. resist.	40	39.6	66.75	41.7	Resist. vs. seg.	-0.36
		Segregating	44	43.6	68.07	42.5	Resist. vs. susc.	2.23
		Homo. susc.	17	16.8	57.88	36.2	Seg. vs. susc.	2.62*
2666A2-2-15-6-3 $\times$ C.I. 12633	1952	Homo. resist.	166	66.4	57.76	36.1	Resist. vs. seg.	1.38
		Segregating	68	27.2	54.54	34.1	Resist. vs. susc.	3.30**
		Homo. susc.	16	6.4	43.81	27.4	Seg. vs. susc.	2.39*
	1959	Homo. resist.	77	43.0	69.26	21.8	Resist. vs. seg.	-1.82
		Segregating	81	45.3	73.95	23.3	Resist. vs. susc.	-1.48
		Homo. susc.	21	11.7	75.19	23.8	Seg. vs. susc.	-0.31

* Significant at the 5% level.

** Significant at the 1% level.

TABLE 3

Homogeneity tests of segregating F_3 families for their pooled ratio within different hybrids

Hybrid	Number of families	Percent susceptibility of pooled ratio	χ^2	P
White Federation $\times$ C.I. 12633	108	12.65	249.869	<.001
Chinese Spring $\times$ C.I. 12633 (2)	44	5.04	77.367	.001
2666A2-2-15-6-3 $\times$ C.I. 12633 (1952)	68	4.37	98.290	.013
2666A2-2-15-6-3 $\times$ C.I. 12633 (1959)	81	9.02	96.862	.190

each family was tested for goodness-of-fit to the F_2 ratio of its hybrid, acceptable fits (one percent level) were obtained for all families except those in the White Federation hybrid. In that hybrid 13 out of 108 families had highly significantly less susceptibility. For the 3:1 ratio, similar tests showed that several families in each hybrid did give an acceptable fit, but the distributions (Figure 1) suggested that such families simply represented the extreme among families truly segregating for something less than 25 percent susceptibility.

Comparisons between the distributions of segregating F_3 families of the Chinese Spring (2) and 2666A2-2-15-6-3 (1952) hybrids, and between the distributions of the 2666A2-2-15-6-3 (1952) and 2666A2-2-15-6-3 (1959) hybrid-groups (Figure 1) suggested again that the season in which the F_2 plants flowered influenced the ratio of their progeny. First, the similarity of the distributions for

FIGURE 1.—Frequency distribution of segregating F_3 families in different hybrids.

the Chinese Spring (2) and 2666A2-2-15-6-3 (1952) hybrids seemed to be due to the fact that the F_2 plants of both hybrids flowered and reproduced in the *same* season. This similarity in distributions suggested that there might be no basic genetic difference between any Chinese Spring and 2666A2-2-15-6-3 hybrids if the hybrid generations were grown in the same season. Unfortunately, no F_2 data were available for both hybrids from F_1 plants grown in the same season. However, it was observed that the only available data for the Chinese Spring hybrid from *spring* grown F_1 plants (Chinese Spring (3)) did not differ significantly from the 2666A2-2-15-6-3 data which were all from spring grown F_1 plants. Secondly, the difference between the distributions for the 2666A2-2-15-6-3 (1952) and 2666A2-2-15-6-3 (1959) hybrid-groups seemed to be due again primarily to the fact that the F_2 plants were grown in *different* spring seasons. This seasonal difference seemed to exist in contrast to the homogeneity of F_2 data for this hybrid from F_1 plants grown in different spring seasons. A probable subsidiary cause for the difference between distributions was the difference in genotypic viability between the two seasons as discussed below.

A test of differential genotypic viability within a hybrid was made by comparing the mean number of plants (or survival percentage) in each of the three types of F_3 families (Table 2). Statistical tests indicated that the mean number of plants in the homozygous susceptible families was significantly lower at the five percent level than the mean number in either the homozygous resistant or segregating families for all hybrids except 2666A2-2-15-6-3 (1959), where the trend was opposite but nonsignificant. Thus the susceptible plants for all hybrids had a slightly lower viability in the 1952 summer nursery. This reduced viability

would reduce the F_2 percent susceptibility in each hybrid. However, since all hybrids showed nearly the same viability pattern, it would not have affected significantly the rather large differences between ratios observed for the hybrids. It would have caused some distortion of results when segregating F_3 families were fitted to rigid expected ratios such as the 3:1. However, little, if any, distortion would have occurred when segregating families were fitted to observed F_2 ratios.

Backcross F_2 generation: The data are presented in Table 4. Where the F_1 was the female, the percent susceptibility in the backcross ranged as follows: White

TABLE 4

Classification of backcross F_2 families for reaction to stem rust in reciprocal hybrids between F_1 plants and the susceptible parent

Hybrid	Year BCF ₂ plants grown	Rust reaction		Percent suscepti- bility	Percent survival of BCF ₁ seed	1:1 ratio	
		Segre- gating	Homo. susc.			χ^2	P
F_1 ♀ × susceptible parent ♂							
(F_1 -Ramona × C.I. 12633) ♀	1955	161	118	42.3	50.5		
× Ramona	1957	41	26	38.8	81.7		
	1959	216	186	46.3	95.3		
		<u>418</u>	<u>330</u>	44.1	...	10.353	.001-.01
(F_1 -White Federation × C.I. 12633) ♀ × White Federation	1952	139	99	41.6	86.5		
	1955	124	90	42.1	35.5		
	1957	43	43	50.0	88.7		
	1959	290	200	40.8	96.1		
		<u>596</u>	<u>432</u>	42.0	...	26.163	<.001
(F_1 -2666A2-2-15-6-3 × C.I. 12633) ♀ × 2666A2-2-15-6-3	1955	66	61	48.0	40.2		
	1957	228	213	48.3	79.9		
		<u>294</u>	<u>274</u>	48.2	...		
(F_1 -2666A2-2-15-6-3 × C.I. 12633) ♀ × White Federation	1955	65	69	51.5	37.0		
		<u>359</u>	<u>343</u>	48.9	...	0.365	.50-.70
Susceptible parent ♀ × F_1 ♂							
White Federation ♀ × (F_1 -White Federation × C.I. 12633)	1955	14	25	64.1	40.6		
2666A2-2-15-6-3 ♀ × (F_1 -2666A2-1955 2-15-6-3 × C.I. 12633)	1955	162	27	14.3	20.3		
	1957	63	9	12.5	87.8		
		<u>225</u>	<u>36</u>	13.8	...		

Federation 42.0, Ramona 44.1, and 2666A2-2-15-6-3 48.9. Chi-square tests for differences gave:

White Federation (42.0) *vs.* Ramona (44.1) $\chi^2 = 0.775$, $P = .30-.50$,
 White Federation (42.0) *vs.* 2666A2-2-15-6-3 (48.9) $\chi^2 = 7.885$, $P = .001-.01$,
 Ramona (44.1) *vs.* 2666A2-2-15-6-3 (48.9) $\chi^2 = 3.275$, $P = .05-.10$.

Again significantly different ratios occurred. Since the Ramona and White Federation backcrosses appeared homogeneous, their combined ratio (42.9%) was compared with the 2666A2-2-15-6-3 ratio (48.9%) and found to be highly

significantly different, $\chi^2 = 7.221$, $P = .001-.01$. Thus two highly significant backcross ratios occurred: one represented by the Ramona and White Federation hybrids (42.9%) and the other by the 2666A2-2-15-6-3 hybrid (48.9%). Furthermore, each cross in the first group deviated highly significantly from a 1:1 ratio, whereas the 2666A2-2-15-6-3 hybrid gave a good fit to a 1:1 ratio (Table 4).

Where the F_1 was the male, adequate data existed only for the 2666A2-2-15-6-3 hybrid. For that hybrid, however, only 13.8 percent susceptibility was observed (Table 4). This was in striking contrast to the 48.9 percent susceptibility, or 1:1 ratio, observed when the same F_1 hybrid was the female.

Although no plant counts were made, the ratios within segregating backcross families for each hybrid appeared to segregate like the corresponding F_2 ratio. For the 2666A2-2-15-6-3 hybrid, in which the proportion of susceptible plants was less than in the Ramona and White Federation hybrids, it was certain that all families had much less than 25 percent susceptibility.

Association between stem rust reaction and heading time: The data are presented in Table 5. Highly significant associations were observed for all hybrids

TABLE 5

Mean heading time in April 1953 of parental rows, and F_1 and backcross F_2 families for different stem rust reactions, and tests of significance of difference in mean heading time of different stem rust classes

Parent or hybrid	Generation	Heading time				Comparison		
		Parent†	Homo. resist.	Seg.	Homo. susc.	Resist.-seg. t	Resist.-susc. t	Seg.-susc. t
Ramona × C.I. 12633	F_3	30.7‡	18.3	11.8	5.9	9.63**	18.55**	8.71**
White Federation × C.I. 12633	F_3	17.6	20.0	21.1	24.7	-1.78	- 4.55**	- 3.62**
(F_1 -White Federation × C.I.) × W.F.	BCF_2	17.6	...	17.6	21.3	...	...	-10.00**
Chinese Spring × C.I. 12633	F_3	22.3	24.4	23.7	25.9	1.74	- 1.26	- 1.98
2666A2-2-15-6-3 × C.I. 12633	F_3	28.6	20.6	22.1	32.2	-2.80**	-13.23**	-11.07**

† Heading time of susceptible parent; C.I. 12633 headed April 23.5.

‡ Ramona heading time is for March.

** Significant at the 1% level.

except Chinese Spring. By comparing the mean heading date of homozygous resistant and homozygous susceptible families, it was concluded that the plants representing the homozygous susceptible genotype derived from Ramona were about 12 days *earlier* in heading time than the plants representing the homozygous resistant genotype derived from C.I. 12633. In contrast, the plants representing the susceptible genotypes derived from the White Federation and 2666A2-2-15-6-3 parents were about five and 12 days *later*, respectively. This occurred in the White Federation hybrid despite the fact that heading in the White Federation parent itself was earlier than heading in the C.I. 12633 parent. Thus genes for heading time were linked with the genes for rust reaction.

INTERPRETATION AND DISCUSSION

The foregoing results were interpreted on the basis that one or two genes governed resistance and are considered below under the one-gene and two-gene hypotheses, respectively.

One-gene hypothesis: It postulated that (1) a single dominant gene governed rust resistance, (2) a differential fertilization gene or factor was closely linked with the rust gene or was the same as the rust gene itself, since no evidence for crossing-over existed, and (3) a different degree of differential fertilization occurred for each hybrid or seasonal F_1 group. Differential fertilization referred to any phenomenon on the male side in which certain types of male gametes effected fertilization more frequently than would be expected on a chance, Mendelian basis. This hypothesis as formulated above explained all observed results except the disturbed 1:1 backcross ratios for the Ramona and White Federation hybrids. It explained the different F_2 ratios, the among-family F_3 distribution (50 percent of the families were expected to segregate, and the remaining 50 percent to be distributed between homozygous resistant and homozygous susceptible classes, depending upon the degree of differential fertilization), the within-family segregation in the F_3 and backcross F_2 generations (similar to the F_2 ratio), and the reciprocal backcross ratios of the 2666A2-2-15-6-3 hybrid only. In fact, for the 2666A2-2-15-6-3 hybrid when the observed proportions of transmission of the two kinds of rust alleles on the female side (actually used a theoretical 50:50 ratio) and on the male side (86.2:13.8) of the F_1 were combined, the calculated expected 6.9 percent of susceptible plants fitted closely the 7.1 percent actually observed in the F_2 generation. This good agreement suggested, furthermore, that the degree of differential transmission of the two kinds of male gametes, those carrying the allele for resistance R and those carrying the allele for susceptibility r , was the same on the pistil of the F_1 hybrid itself as on the 2666A2-2-15-6-3 parent. The relative proportions of R and r male gametes which effected fertilization were estimated from the overall F_2 percentage of susceptibility for each hybrid, assuming 50 percent female transmission of the r allele or the transmission observed in the backcross when it was known to differ significantly from 50 percent (Table 6). The ratios of $R:r$ gametes showed that the pollen carrying the rust resistant allele or the chromosomal segment from *T. timopheevi* was favored in all cases except for the Ramona hybrid.

Various complementary hypotheses which might account for the disturbed 1:1 ratio of segregating to susceptible families for the Ramona and White Federation hybrids were examined. They are as follows:

(1) Natural crossing on susceptible backcross F_1 plants. This hypothesis assumed that a high degree of outcrossing occurred between *some* susceptible backcross F_1 plants and resistant plants. Consequently their progenies were classified as segregating instead of homozygous susceptible, giving a distorted 1:1 ratio. In 1959 all backcross F_1 plants were grown on a three-foot grid to eliminate or greatly reduce outcrossing. Since the results were homogeneous with previously grown material, natural crossing was not the causal factor for the disturbed 1:1 ratio.

TABLE 6

Estimated percent relative effectiveness of R and r male gametes for each significantly different hybrid or group under the hypothesis of one gene with differential fertilization, assuming the indicated percent female transmission of r allele

Hybrid	Percent female transmission of r allele	Estimated percent relative effectiveness of male gametes		Male gametic ratio R:r
		R	r	
Ramona × C.I. 12633	44.1	40	60	0.67:1
White Federation × C.I. 12633	42.0	54	46	1.16:1
Chinese Spring × C.I. 12633 (1)	50	69	31	2.22:1
Chinese Spring × C.I. 12633 (2)	50	77	23	3.44:1
2666A2-2-15-6-3 × C.I. 12633	50	86	14	6.14:1

(2) Greater elimination of *r* eggs and/or *rr* zygotes or young embryos. Various observations on the F_1 plants suggested the possibility of egg or zygotic elimination:

(a) Seed-set sterility under selfing. Sterility in seed set on the parents and different F_1 hybrids in different years is given in Table 7. All seeds present on the F_1 hybrids were normal size. Although various environmental factors influenced considerably the seed set from year to year, the minimum sterility in seed set for the Ramona and White Federation hybrids was still very high compared to that for the 2666A2-2-15-6-3 hybrid.

TABLE 7

Percentage of sterility in seed set, of pollen abortion, and of quartets with micronuclei, and heading time in days from March 31 for the parents and F_1 plants in spring nurseries of designated years

Parent or F_1 hybrid	Percent seed-set sterility*					Percent pollen abortion† 1960	Quartets-1961		
	1953	1957	1958	1959	1960		Total number of quartets	Percent quartets with micronuclei	Heading time 1960
Ramona	12.9	1.6	4.7	2.7	1.9	3018	672	2.5	11
White Federation	1.7	1.6	8.7	1.7	1.7	3099	626	0.6	21
Chinese Spring	3.7	..	..	..	..	704	..	..	..
2666A2-2-15-6-3	2.1	0.6	..	3.4	1.9	2706	322	3.9	37
C.I. 12633	1.1	1.5	..	1.1	2.4	2238	390	1.3	32
Ramona × C.I.12633	25.9	..	..	26.2	25.3	2980	1600	10.8‡	18
White Federation × C.I. 12633	34.6	..	..	41.2	39.9	3040	1794	13.1‡	22
Chinese Spring × C.I. 12633	11.8	..	..	..	..	1338	941	16.4	..
2666A2-2-15-6-3 × C.I. 12633	11.6	..	..	5.2	27.2§	3420	1562	13.3‡	28

* Boldfaced numbers represent the minimum value observed among years.

† Each percentage based on about 2000 pollen grains.

‡ Also included data from 1952 summer, and 1953 spring nurseries.

§ Spikes on shorter, later flowering culms were much more sterile than spikes on longer, earlier flowering culms.

(b) Seed-set sterility under manual backcrossing. The Ramona and White Federation F_1 hybrids also exhibited about the same level of high sterility (27.2 and 30.0 percent, respectively) when they were hand pollinated in both 1954 and 1958. Sterility of other crosses was only about ten percent.

(c) Pollen abortion. The high pollen abortion for the Ramona and White Federation hybrids shown in Table 7 suggested the possible occurrence of egg or embryo sac abortion. However, the cause of embryo sac and pollen abortion may be different; for example, the observed pollen abortion of nearly 50 percent may be due to the pollen-killing gene on chromosome X recently reported by SEARS and LOEGERING (1961).

To test for greater elimination of r eggs or rr zygotes, the backcross F_1 plants for the Ramona, White Federation, and 2666A2-2-15-6-3 hybrids were examined for seed-set sterility. The data are presented in Table 8. No significant difference occurred between the mean sterility of those plants heterozygous resistant and those susceptible for rust in the Ramona and White Federation hybrids. Thus greater elimination of r eggs or rr zygotes did not seem to occur in these two hybrids. Interestingly, however, a highly significant difference between mean sterilities occurred for the 2666A2-2-15-6-3 hybrid. In this case the opposite effect was suggested: namely, that some R eggs were eliminated either as gametes prior to fertilization or as zygotes. However, the magnitude of the difference of 1.35 percent in sterility was so small that the observed association could not have modified significantly the 48.9 percent susceptibility observed in this cross.

It should be noted here that the above tests may be biased by uncontrolled environmental conditions, since the heading time was different for heterozygous resistant and susceptible plants (Table 5). Thus in the Ramona and White Federation hybrids a true genetic difference in sterility may have been negated by a more favorable environment for seed setting on the susceptible plants than on the heterozygous resistant plants. A model of two pairs of complementary genes for gametic sterility similar to that suggested by OKA (see STEBBINS 1958) with one

TABLE 8

*Tests of the difference in mean sterility in seed set of backcross F_1 plants classified according to their rust reaction**

Hybrid	Total number of plants	Unit of measure	Seed-set sterility		Range	Difference	t	P
			Resist.	Susc.				
(F_1 -Ramona $\times$ C.I. 12633) $\varnothing \times$ Ramona	67	percent arc sine	24.42	19.24	0.00-69.81			
			27.78	22.88		4.90	1.35	.10-.20
(F_1 -White Federation $\times$ C.I. 12633) $\varnothing \times$ White Federation	86	percent arc sine	29.35	29.21	0.85-81.13			
			30.11	31.28		-1.17	-0.36	.70-.80
(F_1 -2666A2-2-15-6-3 $\times$ C.I. 12633) $\varnothing \times$ 2666A2-2-15-6-3	107	percent arc sine	2.99	1.64	0.00-12.50			
			9.08	6.01		3.07	3.71	<.001

* Backcross F_1 plants grown in 1957 spring nursery.

locus linked to the rust locus with a recombination value 29 percent would give 43 percent susceptibility in the backcross and show a difference of only 4.8 percent in seed sterility between heterozygous resistant and susceptible plants. Such a small difference could be masked by environmental effects (see Table 7 for environmental effects of seed set). Likewise, no true difference may exist for the 2666A2-2-15-6-3 hybrid, even though a significant difference was observed.

Another specific kind of elimination of *r* eggs which may have occurred was associated with the hand crossing procedure only, and would not exist or be detected under the selfing regime considered above. This hypothesis postulated that differential ripening or receptivity of the pistil to pollen at hand-pollination time occurred, and that this differential receptivity of the pistil was under female gametophytic control, as determined by the rust allele of its own embryo sac. Thus fewer *r* eggs might become fertilized, because under hand pollination, where all pistils are pollinated at the same time, those pistils carrying *r* eggs might be too young or too old. To test this hypothesis, both the sterility and rust ratios were determined for different levels within the hand-pollinated spikes of the Ramona and White Federation hybrids for the 1959 material. The number one spike level consisted of the two lowest spikelets, one on each side of the rachis (each spikelet contained only the two basal florets). The number two level constituted the next higher pair of spikelets, and so on. For each hybrid, data from the same numerical level were combined. Even though a considerable range in maturity of pistils existed vertically at pollination time among spike levels, both the sterility and rust ratios among the different spike levels were homogeneous. This fact suggested that no sterility arose from improper stage of maturity at pollination time, and that no greater elimination of *r* eggs occurred at any spike level due to the manual crossing procedures used.

(3) Lower viability of susceptible backcross F_1 plants. All of the backcross F_1 plants for the 1959 material were established in the greenhouse and transplanted to the field. Nearly complete establishment (95.7%) occurred, and differential genotypic viability, if any, was negligible.

None of the three complementary hypotheses investigated to account for the disturbed 1:1 egg ratio for the Ramona and White Federation hybrids suggested a possible explanation, except the one of greater elimination of *r* eggs and/or *rr* zygotes. However, other factors in the Mendelian process of a one-gene model such as preferential or nonrandom segregation (RHOADES 1952), or megaspore competition (DARLINGTON 1937) may be involved. LUIG (1960) has reported unequal transmission on the female side in wheat. No mechanism is known from cytological observations to account for preferential segregation. ALLARD and SHANDS (1954) have reported that various hybrids of C.I. 12633 showed no more cytological irregularity than other common wheat hybrids. Furthermore, a comparison of the proportion of quartets with micronuclei in the Ramona and White Federation hybrids with the 2666A2-2-15-6-3 hybrid evidenced no greater cytological irregularity for the former hybrids (Table 7). Thus if only one gene governed resistance, the disturbed 1:1 ratio most probably would be

related in some manner to the high level of sterility observed, or be due to preferential segregation or megaspore competition.

Two-gene hypothesis: Two dominant, linked genes governing rust resistance with different recombination values among hybrids, and with different degrees of differential fertilization for each hybrid also explained the observed data. Differential fertilization was postulated to occur and to be completely associated with each of the two rust loci, to be the same at each rust locus, and to show epistatic (duplicate dominant) interaction between the two loci.

The different degrees of linkage or recombination p between the rust genes R_1-r_1 and R_2-r_2 for the Ramona, White Federation, and 2666A2-2-15-6-3 hybrids were estimated from the backcross data only (where the F_1 was the female) and are presented in Table 9. The standard error s for each value of p was calculated by the method of maximum likelihood (ALLARD 1956).

The different degrees of differential fertilization under the above hypothesis were calculated by the following procedure. The relative proportion of male gametes effecting fertilization on an F_1 hybrid is given by

$$\frac{R_1R_2}{a(1-p)} + \frac{R_1r_2}{ap} + \frac{r_1R_2}{ap} + \frac{r_1r_2}{b(1-p)} = 1 + p(a-b) \quad (1)$$

where a = relative effectiveness of the three kinds of male gametes, R_1R_2 , R_1r_2 , or r_1R_2 , in fertilization,

b = relative effectiveness of r_1r_2 male gametes in fertilization, and

$$a + b = 1,$$

or by multiplying both sides of equation (1) by the reciprocal of its right-hand member

$$\frac{a(1-p)}{1+p(a-b)} + \frac{ap}{1+p(a-b)} + \frac{ap}{1+p(a-b)} + \frac{b(1-p)}{1+p(a-b)} = 1 \quad (2)$$

The proportion of r_1r_2 male gametes effecting fertilization on the F_1 hybrid, $(r_1r_2)_m$, is estimated from

$$(r_1r_2)_m = \frac{\text{proportion of susceptibility in } F_2}{\text{proportion of susceptibility transmitted by eggs in backcross}}, \quad (3)$$

and is given for each hybrid in Table 9. Then b is estimated from

$$\frac{b(1-p)}{1+p(a-b)} = (r_1r_2)_m \quad (4)$$

TABLE 9

Estimates of various parameters postulated under the hypothesis of two dominant linked genes with differential fertilization*

Hybrid	p	s	$(r_1r_2)_m$	Percent relative effectiveness of male gametes		Differential fertilization ratio of male gametes
				R_1R_2 , R_1r_2 , or r_1R_2	r_1r_2	
Ramona $\times$ C.I. 12633	0.118	0.036	0.602	34.3	65.7	0.52:1
White Federation $\times$ C.I. 12633	0.160	0.031	0.462	45.7	54.3	0.84:1
2666A2-2-15-6-3 $\times$ C.I. 12633	0.023	0.038	0.146	84.8	15.2	5.58:1

* See text for their meaning.

by substituting $(1-b)$ for a , the estimated value for $(r_1r_2)_m$, and the estimated value for p , assuming equal recombination for both male and female organs. The percent relative effectiveness, a and b , of the two groups of male gametes or the ratio of differential fertilization for each hybrid is given in Table 9.

Using p , a and b , the proportion of different kinds of families and the ratios within segregating families in the F_3 generation were calculated, and were found to agree satisfactorily with the observed results. Thus, this hypothesis—with different degrees of recombination between two linked genes and with differential fertilization of the same magnitude associated with each rust locus—fitted all observed data. It is recognized that this proposed hypothesis with its many variables is not the only one that could fit the observations.

Among the observed recombination values, two significantly different ones occurred, as two statistically significant backcross ratios were previously shown. They were $p = 14.19 \pm 2.35$ percent for the combined estimate from the Ramona and White Federation hybrids and $p = 2.28 \pm 3.77$ percent for the 2666A2-2-15-6-3 hybrid. The hypothesis that the 2666A2-2-15-6-3 F_1 hybrid was heterozygous for a paracentric inversion, which would reduce greatly the recombination value, was investigated. Cytological examination of 247 pollen mother cells from middle anaphase to late telophase of the first division of meiosis of the 2666A2-2-15-6-3 F_1 showed no bridge-fragment configurations. Thus no cytological evidence existed for a paracentric inversion to account for the lower recombination value in the 2666A2-2-15-6-3 hybrid. Similar cytological investigation of 121 and 132 cells of the Ramona and White Federation hybrids, respectively, also showed no bridge-fragment configurations.

Although it was not known what caused the different recombination values, some factor influencing chiasma frequency may have existed, such as temperature or some cryptic structural difference in chromosome XIII. Concerning temperature, it was noted that with rising temperatures in the spring, the heading time of different F_1 hybrids (Table 7) was negatively correlated with the percent susceptibility in the F_2 . However, its significance is possibly negated because data from F_1 plants grown in both the spring and summer, where temperature trends were opposite to the time of heading, were homogeneous. With respect to some chromosomal feature in 2666A2-2-15-6-3, C.I. 12633 and 2666A2-2-15-6-3 are likely to differ less cytologically, because they are related genealogically. It would be more reasonable to hypothesize that the parents of the Ramona and White Federation hybrids would differ more cytologically and show lower recombination values.

One vs. two genes for rust resistance: The hypothesis that one gene governed rust resistance was investigated extensively for two reasons: (1) The hypothesis was biologically simpler, and (2) it was highly suggestive from the results of the 2666A2-2-15-6-3 cross. However, to explain the observed results the one-gene model required some rather rare mechanism to account for the disturbed 1:1 egg ratio. The hypothesis of two dominant linked genes with zero and 14 percent recombination between hybrids similarly required some unlikely features to account for the different recombination values, but provided a more acceptable

explanation of the disturbed 1:1 egg ratio. The problem of distinguishing between one *versus* two relatively closely linked genes is not simple in the presence of wide ratios and somewhat variable differential fertilization. ALLARD and SHANDS (1954) reported the occurrence of an F_3 family believed to represent a repulsion phase heterozygote, but its breeding behavior was not investigated further. It may have represented only an extreme case of differential fertilization. LOEGERING and SEARS (1961) have recently shown that the common wheat variety Timstein, which obtained its stem rust resistance from *T. durum*, has only one gene for resistance instead of the two linked genes previously reported.

Variations in stability of differential fertilization: Regardless of the genic model for stem rust resistance, differential fertilization was involved in both the one-gene and two-gene hypotheses. The degree of differential fertilization within each hybrid seemed surprisingly stable and uniform even though observed under diverse environmental and genetic (F_3 families from different F_2 plants) conditions. However, the following variations were noted: (1) The Chinese Spring hybrid showed some variation among F_1 plants grown in different seasons, particularly between spring and summer seasons where environmental conditions were quite different. (2) The observed ratios of segregating F_3 families of the 2666A2-2-15-6-3 hybrid were quite different for the 1952 and 1959 materials for which the F_2 populations were grown in different spring seasons. (3) Although the segregating F_3 families for each hybrid were quite similar to the corresponding F_2 ratio, a difference between the F_2 and F_3 generations within a hybrid was commonly observed. (4) All segregating F_3 families within a hybrid were not homogeneous. The first two variations must be attributed to environmental effects, which caused different degrees of differential fertilization. Perhaps a difference in temperature existed at flowering time or during pollen-tube growth. Others (CHAO 1928; BURNHAM 1936; BEMIS 1959; and LONGLEY 1961) also have observed environmental influences on differential fertilization. The third variation is probably a genetic effect, since segregating progenies from F_1 and heterozygous F_2 plants grown concurrently were different. This genetic effect might be simply the genetic difference in mean flowering time between the F_1 and the heterozygous F_2 plants, with a concurrent environmental difference affecting the degree of differential fertilization. The fourth variation may have the same explanation, since heterozygous F_2 plants probably varied for flowering time.

Comparisons with other cases of differential fertilization: This case of differential fertilization in wheat was unlike those reported in maize by EMERSON (1934), BURNHAM (1936), RHOADES (1943, 1948, cited by TABATA, 1961), SCHWARTZ (1950), LONGLEY (1961), and DOLLINGER (1961), in rice by CHAO (1928), in lima beans by BEMIS (1959), and in barley by TABATA (1961). The main difference in this case was that alien chromosomal material was involved. The factor affecting differential fertilization presumably was carried on a short chromosomal segment transferred with the stem rust resistance from *T. timopheevi*. This situation largely accounted for other features which were different. First, the factor affecting differential fertilization was completely linked with

the character permitting its detection. Secondly, more than one abnormal level of differential fertilization was observed among the different hybrids. This was probably largely a consequence of the close linkage. A multiple allelic series as reported by SCHWARTZ (1950) for the *Ga₁* locus in maize might be put forth to explain the difference in relative transmission of the susceptible segments from different susceptible parents relative to the one resistant segment. A more likely situation, however, is that the different susceptible chromosomes show no difference in gametophytic activity, and that the resistant segment interacts differently as a male gametophyte with the background genotype (possibly heading time) of the different hybrids. It was surprising that in such a hybrid as the 2666A2-2-15-6-3, pollen carrying the alien chromosomal segment was greatly favored over pollen lacking the alien segment (Tables 6 and 9). CAMERON and MOAV (1957) also have reported a case in which pollen containing alien chromatin was favored greatly over pollen lacking the alien chromatin.

SUMMARY

When the same homozygous wheat strain C.I. 12633, which derived its stem rust resistance from *T. timopheevi*, was crossed with various susceptible varieties, different F_2 ratios were observed ranging from 26.6 to 7.1 percent susceptible plants. Differential fertilization of different degrees for the various hybrids was proposed as one of the major causes of the different ratios observed. The gene(s) causing differential fertilization was the same as or was completely linked with each of the one or two genes proposed as controlling stem rust resistance of C.I. 12633. The pollen carrying the resistance to stem rust or the alien chromosomal material from *T. timopheevi* was favored in some hybrids.

The hypothesis of one dominant gene for rust resistance with differential fertilization explained all observed results except the disturbed 1:1 egg ratio of the Ramona and White Federation hybrids for which no explanation was known. However, it was definitely established that natural crossing and differential genotypic viability were not involved. The results likewise suggested that differential elimination of eggs, zygotes, or both did not occur, but a possible bias in the results was discussed. Preferential segregation and megaspore competition were suggested as additional possibilities.

The second alternative hypothesis of two dominant linked genes with zero and 14 percent recombination for different hybrids with differential fertilization explained all observed results, but the exact mechanism suppressing recombination between the two rust loci was unknown.

An association occurred between stem rust reaction and heading time.

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