

GENIC ANALYSIS FOR THE STERILITY OF HYBRIDS  
BETWEEN DISTANTLY RELATED VARIETIES  
OF CULTIVATED RICE\*

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The rice plant has numerous varieties distributed over a wide area, ranging from tropical to temperate countries, and is wonderfully rich in varietal variations. The  $F_1$  plants between distantly related varieties often show semi-sterility. Based on this hybrid sterility, the varieties were classified first by Kato, Kosaka & Hara (1928) into the sub-species 'Indica' and 'Japonica'. But lately doubts have arisen about this classification in view of the fact that the interrelation of varieties in hybrid sterility was too complicated to divide the varieties into two distinct groups (Terao & Mizushima, 1939). The present writer (Oka, 1953c, 1954a) has investigated the tendency of various characters to associate and the relationship of character association to hybrid sterility. He has put forward the view that rice varieties can be classified into two groups, 'Continental' and 'Insular', so named in accordance with geographical distribution, but largely corresponding to the 'Indica' and 'Japonica' types, respectively. The 'Insular' group can be further sub-divided into the 'Tropical' and 'Temperate' groups.

Thus hybrid sterility has been a problem of wide interest among workers on this plant. Although the mechanism of sterility has not yet been fully understood, it is known that sterility is characterized by the following features:

- (1) Crossing between distant varieties is as easy as between nearly related ones, and the  $F_1$  plants grow normally.
- (2) The  $F_1$  plants show no disturbance in chromosome pairing, even in a cross with the highest sterility.
- (3) Micro- and macro-spores begin to deteriorate at a definite stage of development after the meiosis. According to the writer's observation, the deterioration begins in microspores after the so-called 'second contracting stage', and in macrospores after the first division of the embryosac nucleus.
- (4) The degree of sterility depends on the combination of parental varieties, and if measured by the percentage of good pollen, it is not affected much by environmental conditions.
- (5) The percentage of seed setting is generally similar to the percentage of good pollen, but in rare cases significantly higher or lower than the latter.
- (6) Reciprocal crosses show no significant difference in the percentage of good pollen of  $F_1$  plants.

These facts suggest that sterility is not due to the failure of chromosome pairing, but may be due to some genotypic unbalance brought about by a recombination of chromosomes.

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In other words, in the cross of  $A_1B_1$  and  $A_2B_2$ , gametes carrying  $A_1B_2$  and  $A_2B_1$  may deteriorate. The outcome of the experiments set out in this paper will demonstrate that these  $A_1, B_1$ , etc., are not the whole chromosome, but are Mendelian factors, and under the assumption that they are duplicate genes various phenomena can be successfully explained.

THE HYPOTHESIS OF GAMETIC-DEVELOPMENT GENES AND EVIDENCE SUPPORTING IT

We may assume that a gene  $X$  which works in the gamete as a development maintainer is duplicated, and the loci are on different chromosomes. When two strains  $X_1x_2$  and  $x_1X_2$  are crossed, the  $F_1$  will produce double-recessive gametes with the frequency of 25%. Fertility will then come down to 75%. Various degrees of sterility appearing in inter-varietal hybrids may be explained by assuming that a number of such mechanisms interact. These hypothetical duplicate genes for gametic development will be called 'Gametic-Development Genes' or 'G.D. genes'.

The  $F_2$  between distantly related varieties of rice usually show a continuous array of intergrades in fertility. But when two related varieties,  $A$  and  $B$ , differing in fertility in the hybrids with the third variety  $C$ , are crossed in the manner  $(A \times B) \times C$ , the offspring may show a discontinuous segregation for fertility, because of the relative simplicity of genic differences between  $A$  and  $B$ . The writer has observed several such crosses, using strictly pure strains as materials. A few examples in which the assumption of genes could be justified by progeny tests will be presented in the following sections.

(1) *The first experiment: 563 × (219 × 221)*

Both of the varieties 219 and 221 are native to the Philippines and have characteristics of the 'Tropical-Insular group'. The former is non-glutinous, while the latter is glutinous. 563 is a Japanese glutinous variety belonging to the 'Temperate-Insular group'. The  $F_1$  of  $563 \times 219$  is semi-sterile, pollen fertility being about 65%, while those of  $563 \times 221$  and  $219 \times 221$  are nearly completely fertile.

A plant of the variety 563 was pollinated with the pollen of the  $F_1$  of  $219 \times 221$ . Table I (the upper part) shows the variation in pollen fertility found in the offspring of this cross.

Table 1. *Segregation of fertile and semi-sterile plants in some crosses of (A × B) × C type*

Cross	% of good pollen															No. of plants		
	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25		20	15
563 × (219 × 221)																		
Homozygotes*	3	3	3	.	.	.	1	2	2	1	.	.	.	.	.	.	.	15
	(9)			(6)														
Heterozygotes*	1	2	2	1	.	.	.	1	.	2	3	4	1	.	.	.	.	17
	(6)				(11)													
(108 × 143) × 563	.	1	3	8	8	6	7	4	3	8	9	5	16	12	9	5	.	104
					I				II				III					
563 × (108 × 143)	.	.	.	.	2	1	3	1	.	2	4	3	1	4	5	2	2	30
					(37)				(25)				(42)					
	(7)							(9)				(14)						

\* For the glutinous gene, + : gl.

As a matter of course, segregation of the hetero- and homozygotes for the glutinous gene is seen in Table 1 in a 1:1 ratio. It is further found in Table 1 that in both of these classes fertile plants (95-80%) and semi-sterile plants (65-35%) can be distinguished clearly. The ratio of fertile to semi-sterile plants may be judged to be 1:1 as a whole, but fertile plants are more numerous than semi-sterile ones among the heterozygotes for the glutinous gene, while semi-sterile are more numerous than fertile plants among the homozygotes. In addition to this, in semi-sterile plants heterozygous for the glutinous gene, more than 70% of good pollen grains were found to be glutinous.

These phenomena may be easily understood if we assume that a set of G.D. genes  $X_1$  and  $X_2$  are concerned, and either of them is linked with the glutinous gene  $gl$ . Under this assumption, the genotypes of the parental varieties may be judged to be as follows:

Variety 219:  $x_1 - + X_2$ ,

Variety 221:  $X_1 - gl X_2$ ,

Variety 563:  $X_1 - gl x_2$  (- shows linkage).

Let the recombination value between  $X_1$  and  $gl$  or  $x_1$  and  $+_1$  be  $p$ , the  $F_1$  of  $219 \times 221$  will have the following gametic series, and they will produce four kinds of plants when hybridized with 563, as shown below.

Genotype of $F_1$ gamete	When hybridized with 563			Exp. no.	Obs. no.
	Fertility	Glutinous gene	Frequency		
(a) $x_1 - + X_2$	Semi-sterile	Heterozygous	$\frac{1}{2}(1-p)$	10	11
(b) $x_1 - gl X_2$	Semi-sterile	Homozygous	$\frac{1}{2}p$	6	6
(c) $X_1 - + X_2$	Fertile	Heterozygous	$\frac{1}{2}p$	6	6
(d) $X_1 - gl X_2$	Fertile	Homozygous	$\frac{1}{2}(1-p)$	10	9

The value of  $p$  will be estimated to be 12/32 or 0.375, and a good agreement is found between the observed and expected numbers. It may also be understood that in the semi-sterile heterozygotes (a), since non-glutinous gametes tend to deteriorate due to the linkage with  $x_1$ , glutinous pollen grains increase against non-glutinous ones.

In order to test the validity of these assumptions for G.D. genes, the variety 563 was crossed with each of 42  $F_2$  plants of  $219 \times 221$ , and variations in pollen fertility in the descendant 42 lines were observed. The data were compared with expectations based on the above assumption of G.D. genes, by the following methods (details are given in Oka, 1953a).

First, according to the genotypes of the parental strains assumed, it will be expected that the  $F_2$  of  $219 \times 221$  segregates into ten different genotypes. From the formulas showing frequency of those  $F_2$  genotypes, the frequencies of the four kinds of plants in the whole population of  $563 \times F_2$  were formulated. They were related to the observed numbers and the recombination value  $p$  was estimated to be 0.208 by maximum likelihood calculation. The expected numbers from this value of  $p$  fitted well to the observed numbers.

Secondly, regarding each line of the  $563 \times F_2$ , the numbers of the four kinds of plants were compared with the numbers to be expected if the given line carries each of the ten  $F_2$  genotypes. Then, the probability that the observed numbers would be brought about from each genotype was calculated for each line. It was transformed into the probability that the given line carries each genotype, by using Bayes' theorem. Then, for each genotype, the probabilities found in respective lines were summed up, and were regarded as an estimate of the actual segregation ratio of the  $F_2$  genotypes. From this genotypic ratio, the recombination value was computed again by the maximum likelihood method.

$p=0.210$ , a value quite consistent with the former value  $0.208$ , was found, and the estimated genotypic ratio (sum of probabilities) gave a good fit to the ratio expected from this recombination value. These progeny tests may prove the validity of the former assumptions of parental genotypes.

It was recognized, however, that in the  $563 \times F_2$  population, semi-sterile plants could be sub-divided into two classes, with pollen fertilities of  $65-55\%$  and  $50-35\%$ . The latter class had more plants than the former. For this phenomenon, it may be assumed that the second set of G.D. genes  $Y_1$  and  $Y_2$  are concerned, in addition to the first set  $X_1$  and  $X_2$ , and  $Y_1$  is linked with  $X_1$ . (If this linkage is not assumed, the class with  $65-55\%$  fertility must have more plants than the class with  $50-35\%$  fertility.) Under this assumption, the genotypes of the parental varieties will be considered as follows:

$$219: + - x_1 - y_1 X_2 Y_2,$$

$$221: gl - X_1 - Y_1 X_2 Y_2,$$

$$563: gl - X_1 - Y_1 x_2 y_2.$$

Denoting the recombination values of  $gl - X_1$  and  $X_1 - Y_1$  by  $q$  and  $r$ , respectively, the same computations were carried out again as was done when considering one set of G.D. genes. As a result, it was found that  $q=0.178$  and  $r=0.068$ . Expectations based on these values agreed well with the data.  $q + \frac{1}{2}r=0.212$  approaches the former value of  $p$ ,  $0.208$  or  $0.210$ ; it may then be inferred that in the former computations where one set of G.D. genes was considered, the lumped effect of the two sets has been dealt with.

The varieties used, 219 and 221, differ from each other in that the former is semi-sterile, while the latter is fertile, in the hybrid with 563. The results of crossing experiments stated in this section may be valid enough to show that this difference between 219 and 221 is attributable to certain differences in G.D. genes, which are duplicate genes for gametic development and behave in accordance with Mendel's law.

(2) *The second experiment; reciprocal crosses between 108 × 143 and 563*

108 (Pei-ku) and 143 (Liu-t'ou-tzu) are Formosan native varieties of first-crop nature (insensitive to day-length), belonging to the 'Continental group'. In the hybrid with 563, 108 shows a high degree of hybrid sterility, pollen fertility being  $34\%$ , while 143 is nearly fertile ( $90\%$ ). Reciprocal crosses between the  $F_1$  of  $108 \times 143$  and 563 showed variations in pollen fertility as given in Table I (lower part). It may be recognized in Table I that the offspring of  $(108 \times 143) \times 563$  segregates into three classes with different fertilities,  $90-60$ ,  $55-40$  and  $35-15\%$ , and the class with intermediate fertility has the lowest frequency. In the same manner as in  $219 \times 221$ , this variation may be explained by two sets of G.D. genes,  $X_1, X_2$  and  $Y_1, Y_2$ , between which two pairs belonging to different sets, say,  $X_1$  and  $Y_1$ , are linked. The parental varieties may then be assumed to have the following genotypes for the G.D. genes

$$108: x_1 - y_1 X_2 Y_2,$$

$$143: X_1 - Y_1 X_2 Y_2,$$

$$563: X_1 - Y_1 x_2 y_2.$$

From the segregation ratio of the three classes,  $37:25:42$ , the recombination value between  $X_1$  and  $Y_1$  was found to be  $0.24$ . In this cross, fertilities of semi-sterile plants due

to one set and two sets of G.D. genes (classes II and III in Table 1) will be expected to be 0.75 and  $0.625 - \frac{1}{8}p = 0.595$ , respectively, if that of the fertile plants is 1.0. The fertilities actually found for these three classes were considerably lower; the difference may be accounted for by considering the effects of other genes for fertility and environmental agencies.

In the reciprocal cross,  $563 \times (108 \times 143)$ , it was found that fertile plants were markedly small in number. The simplest explanation for this phenomenon will be to assume that pollen with double-dominant combinations of G.D. genes ( $X_1$  and  $X_2$  or  $Y_1$  and  $Y_2$ ) has a low fertilizing capacity. Allowing the fertilizing capacity of double-dominant pollen to be  $1 - s$ , the value  $s = 0.26$  will be found from the segregation ratio, 7:9:14.

The above-mentioned phenomenon appeared to prevail generally among different crosses. The present situation is that it seems difficult to determine whether this tendency is due to the direct effect of G.D. genes or to the effect of other linked genes. The former assumption may be taken for granted until genes for such a certation are demonstrated. It may be worth noting that in the hybrid between two strains possessing different combinations of G.D. genes, say,  $X_1x_2 \times x_1X_2$ , the tendency to restrict a recombination of chromosomes results both from the deterioration of gametes carrying  $x_1x_2$  and from the lowering of the fertilizing capacity of pollen carrying  $X_1X_2$ .

In the same manner as in the first experiment, progeny tests were conducted in order to test the validity of the assumptions for G.D. genes. Each of 36  $F_2$  plants of  $108 \times 143$  was pollinated with the pollen of 563, and variations in pollen fertility in the descendant lines were examined. On the other hand, expectations were derived from the frequencies of genotypes to be produced in the  $F_2$  of  $108 \times 143$ , which were computed from the former assumptions for G.D. genes (details are given in Oka, 1953b).

First, the frequencies of semi-sterile plants due to one set and two sets of G.D. genes (denoted by  $x$  and  $y$  respectively) in the whole population of  $F_2 \times 563$  were expected from the values  $p = 0.24$  and  $s = 0.26$ . The values  $x = 0.300$  and  $y = 0.406$  were found. Denoting the mean fertilities of the three classes (I, II and III in Table 1) and of the whole population by  $m$ ,  $m - d_1$ ,  $m - d_2$  and  $M$ , respectively, and variance of fertility in the whole population by  $V$ , while variance due to other agencies than the assumed G.D. genes is denoted by  $R$ , then we have the following formulas:

$$M = m - d_1x - d_2y,$$

$$V = d_1^2x(1-s) + d_2^2y(1-y) - 2d_1d_2xy + R.$$

Substituting  $d_1 = 1 - 0.75 = 0.25$  and  $d_2 = 1 - 0.595 = 0.405$ , the values of  $m$  and  $R$  can be known from the values of  $M$ ,  $V$ ,  $x$  and  $y$ . The frequency distribution of pollen fertility was calculated from these values, assuming that variations due to other agencies than the G.D. genes follow a normal distribution. The calculated frequency distribution showed a good agreement with the observed distribution. In contrast to this, having repeated the same calculation under the assumption that pollen with a double-dominant combination of G.D. genes has the same fertilizing capacity as single-dominant pollen (i.e.  $s = 0$ ), the calculated distribution was found to deviate significantly from the observed one.

Secondly, distributions of pollen fertility in lines possessing respective genotypes were calculated separately. By comparing those calculated distributions with the actual datum in each line, the probabilities that the given line carries respective genotypes were estimated in the same manner as in the first experiment. Summing them up, the probabilities

that respective genotypes had occurred in the  $F_2$  of 108 × 143 were estimated. They gave a good fit to the frequencies of those genotypes to be expected under the former assumptions for the G.D. genes. The results of these calculations may prove that the inheritance of hybrid sterility in this cross can be fully explained by the hypothesis of G.D. genes.

(3) *Change of segregation ratio due to the gametic-development genes*

The gametic output from a set of G.D. genes will be

$$\frac{1}{3}X_1X_2 : \frac{1}{3}X_1x_2 : \frac{1}{3}x_1X_2.$$

If we let the fertilizing capacity of double-dominant pollen be  $1 - s$ , then the  $F_2$  genotypic segregation will be expected as follows:

$$\frac{1}{9}(1-s) \frac{X_1X_2}{X_1X_2} : \frac{2}{9} - \frac{1}{9}s \frac{X_1X_2}{X_1x_2} : \frac{2}{9} - \frac{1}{9}s \frac{X_1X_2}{x_1X_2} : \frac{1}{9} \frac{X_1x_2}{X_1x_2} : \frac{1}{9} \frac{x_1X_2}{x_1X_2} : \frac{2}{9} \frac{X_1x_2}{X_1x_2}.$$

When a gene  $A:a$  is linked with a pair of G.D. genes and the recombination value is  $p$ , the  $F_1$  gametic ratio of  $A:a$  will be  $1+p:2-p$  (repulsion) or  $2-p:1+p$  (coupling), and the  $F_2$  will segregate as shown in Table 2.

Table 2. *The modified  $F_2$  ratio of  $AA:Aa:aa$  by the effect of a set of G.D. genes*

Repulsion	...	$AA$	$Aa$	$aa$
Coupling	...	$aa$	$Aa$	$AA$
Fertile		$(1+3p^2) - sp(1+p)$	$(2+6p-6p^2) - s(1+2p-2p^2)$	$(4-6p+3p^2) - s(1-p)(2-p)$
Semi-sterile		$2p(1-p)$	$2(1-2p+2p^2)$	$2p(1-p)$
Total		$(1+p)^2 - sp(1+p)$	$2(1+p)(2-p) - s(1+2p-2p^2)$	$(2-p)^2 - s(1-p)(2-p)$

Table 2 shows that the ratio of  $AA:Aa:aa$  may vary from 1:2:1 to 1:4:4 ( $s=0$ ) or to 1:3:2 ( $s=1$ ), and the heterozygotes for  $A:a$  have more semi-sterile plants than the homozygotes. It will then be expected that phenotypically dominant plants have a lower mean fertility and a larger variance than recessive ones. The same tendency will also be expected in back-crosses.

Denoting the frequencies of semi-sterile plants in phenotypically dominant and recessive classes by  $x_D$  and  $x_R$ , respectively, the difference between the two classes in mean fertility and in variance will be written as follows:

$$\text{Difference in mean} \quad -d(x_D - x_R),$$

$$\text{Difference in variance} \quad d^2(x_D - x_R)(1 - x_D - x_R).$$

(In these formulas,  $d$  stands for the difference between fertile plants and semi-sterile plants in fertility, and may be assumed to be 0.25.) By using these formulas, the values of  $x_D$  and  $x_R$ , and therefore the values of  $p$  and  $s$  may be estimated from observed means and variances of fertility.

Regarding several mono-genic characters,  $F_2$  segregation ratios were observed with a number of crosses whose  $F_1$  showed semi-sterility. Those segregation ratios often appeared to deviate from the 3:1 ratio and in most cases the above-stated expectation was satisfied. Table 3 gives two examples for the apiculus coloration gene  $C:c$ . The parental varieties 108 (Pei-ku, Formosa) and I (70<sup>a</sup> som cau, Indo-China) belong to the 'Indica' or 'Continental' group, and are colourless at the apiculus. Both 318 (Boegi inda) and 325 (Kani-

ranga) are Indonesian varieties, belonging to the 'Tropical-Insular' group, and have a purple colour at the apiculus. Table 3 shows that in both crosses colourless plants have occurred in excess of the expected numbers based on the 3:1 ratio, and have a higher mean fertility and a smaller variance than the coloured plants. The modification of the  $F_2$  ratios may then be considered to be due to the effect of G.D. genes linked with  $C:c$ . The values of  $x_D$  and  $x_R$  were estimated from the data in Table 3, assuming that one set (in  $108 \times 318$ ) or two sets (in  $1 \times 325$ ) of G.D. genes were concerned. The frequency distributions of fertility in coloured and colourless classes were then calculated. Good fits were found between the observed and calculated distributions. Similar tests were found to be successful in cases of back-cross experiments regarding the glutinous endosperm gene.

Table 3. Variations in fertility among coloured and colourless  $F_2$  plants

Cross	Colour at apiculus	% of seed setting											No. of plants	Exp. no. (3:1)	Mean fertility	Variance
		100	90	80	70	60	50	40	30	20	10	0				
$108 \times 318$	+	4	5	9	8	18	6	10	7	3	2	1	73	81.8	0.576	0.0544
(75%)*	-	.	8	7	8	3	3	2	3	2	.	.	37	27.2	0.660	0.0470
$1 \times 325$	+	.	6	12	21	9	21	21	8	4	.	.	94	117.0	0.485	0.0356
(60%)*	-	.	9	18	17	6	6	3	2	1	.	.	62	39.0	0.520	0.0275

\* Percentage of good pollen in  $F_1$ .Table 4. Relation between  $F_1$  fertility and the deviation of  $F_2$  ratio from the expected 1:2:1 ratio

$\chi^2$ value in $F_2$	% of good pollen in $F_1$										No. of crosses
	95	85	75	65	55	45	35	25	15	5	
0-2	3	1	.	.	.	.	.	.	.	4	4
2-4	.	1	.	.	1	.	.	.	.	.	2
4-8	2	1	2	2	2	.	.	.	1	10	
8-16	1	2	.	1	.	.	1	.	.	5	
16-32	2	.	.	1	.	.	3	.	.	6	

It was found that in hybrids between distantly related varieties of rice, the apiculus coloration gene  $C$ , as well as the glutinous gene  $gl$ , generally tended to decrease in the  $F_2$ , while the phenol-reaction gene  $Ph:ph$  was in most crosses not much affected by the gametic selection due to G.D. genes. Regarding the glutinous gene, it was found, as shown in Table 4, that the lower the  $F_1$  fertility the larger the deviation from the 1:2:1 ratio in the  $F_2$ .

## (4) Restriction on gene recombination due to the gametic-development genes

Let us consider a case in which two independent genes  $A:a$  and  $B:b$  are linked respectively with two pairs of G.D. genes  $X_1:x_1$  and  $x_2:X_2$  which belong to the same set. For instance, when the  $F_1$  genotype of a cross is  $\frac{A-x_1b}{a-X_1B}$ , if the linkages are tight, gametes carrying  $AB$  will be lost, because they carry the double-recessive combination of G.D. genes. Thus, one of two possible combinations is lost. In addition to this, pollen with the double-dominant combination of the G.D. genes may have a low fertilizing capacity, so that the other recombination  $ab$  will be reduced. The parental combinations,  $Ab$  and  $aB$ , may then have high relative frequencies, and the segregation ratio may appear as if it were due to the repulsion linkage between  $A$  and  $B$ .

In this case, denoting the recombination values between  $A$  and  $x_1$  and between  $B$  and  $x_2$  by  $p$  and  $q$ , respectively, the  $F_1$  gametic series and the frequencies of  $F_2$  phenotypes will be expected as follows:

	$F_1$ gamete	$F_2$ phenotypes (when $1 - s = 0.5$ )
$AB$	$\frac{1}{2}(p + q - pq)$	$\frac{1}{16}(4 + 6p + 6q - p^2 - q^2 - 4pq - p^2q - pq^2 + 3p^2q^2)$
$Ab$	$\frac{1}{2}(1 - q + pq)$	$\frac{1}{16}(5 - p - 6q + q^2 + 4pq + p^2q + pq^2 - 3p^2q^2)$
$aB$	$\frac{1}{2}(1 - p + pq)$	$\frac{1}{16}(5 - 6p - q + p^2 + 4pq + p^2q + pq^2 - 3p^2q^2)$
$ab$	$\frac{1}{2}(1 - pq)$	$\frac{1}{16}(1 + p + q - 4pq - p^2q - pq^2 + 3p^2q^2)$

Having looked for cases falling under this expectation, a few examples were obtained. Table 5 gives the segregation ratio for apiculus colour ( $C:c$ ) and phenol-reaction ( $Ph:ph$ ) found in the  $F_2$  of  $414 \times 325$ . 414 (P.T.B. 10) is an Indian variety belonging to the 'Continental group'; it is colourless at the apiculus and has positive phenol-reaction. In contrast to this, 325 (Kaniranga) is an Indonesian variety belonging to the 'Tropical-Insular group', showing purple coloration at the apiculus and with negative phenol-reaction. The genes  $C:c$  and  $Ph:ph$  are independent. However, as the data in Table 5 show, the parental combinations (colourless-positive and coloured-negative) were excessive and recombinations were deficient in comparison with the 9:3:3:1 ratio. The  $F_1$  showed semi-sterility, pollen fertility being about 60%. The  $F_2$  plants with dominant phenotypes for either character tended to have lower fertility than those with recessive phenotypes. It may then be inferred that both  $C:c$  and  $Ph:ph$  are influenced by G.D. genes in this cross.

Table 5. *A modified  $F_2$  ratio for apiculus colour and phenol-reaction, showing a restriction on the recombination of independent genes*

Apiculus	...	Coloured		Colourless		
$Ph$ -reaction	...	+	-	+	-	Total
Observed no.		96	49	69	13	227
Expected no. 9:3:3:1		127.8	42.5	42.5	14.2	$\chi^2 = 25.54 (P < 0.01)$
Considering G.D. genes		92.8	49.3	65.1	19.3	$\chi^2 = 2.94 (P > 0.20)$

It was then assumed that  $C:c$  and  $Ph:ph$  are linked respectively with G.D. genes  $x_1:X_1$  and  $X_2:x_2$  which belong to the same set. By using the above-given formulas, the recombination values of  $C-x_1$  and of  $Ph-X_2$  (denoted by  $p$  and  $q$  respectively), were estimated by the maximum likelihood method. It was found that  $p = 0.086$  and  $q = 0.305$ . As shown in Table 5, the observed numbers were found to fit well the expected numbers based on these values. This experiment may serve as an example to show that the recombination of independent genes really produces restriction due to the effect of G.D. genes.

(5) *Polygenic nature of the gametic-development genes*

The two examples of analysis of G.D. genes stated in the first two sections are the simplest cases in which fertile and semi-sterile classes segregated distinctly. From them the behaviour of sterility can be successfully explained by assuming one set or two sets of G.D. genes. However, having repeated experiments of the same design with different strains, some of them showed several frequency peaks in the range of variation in pollen fertility, and it seemed that several sets of G.D. genes should be assumed to explain the variation. The crossing experiment given below,  $(143 \times 160) \times 563$ , is an example of such cases.



Both 143 (Liu-t'ou-tzu) and 160 (An-ka-chu) are Formosan native varieties belonging to the 'Continental group'. As stated in previous sections, 563 is a Japanese native variety belonging to the 'Temperate-Insular group'. 143 is non-glutinous, while 160 and 563 are glutinous. The  $F_1$  of  $563 \times 160$  shows a high degree of sterility, pollen fertility being about 15%, while the  $F_1$  of  $563 \times 143$  is nearly fertile. The variation in pollen fertility in the progeny of  $(143 \times 160) \times 563$  was as given in Table 6. There were at least five frequency peaks and among plants with low fertilities, homozygotes for the glutinous gene were more numerous than the heterozygotes. Various assumptions of genes were put to the test for explaining this frequency distribution. The following assumption, where five sets of G.D. genes partly linked with one another were considered, was the most successful one.

Table 6. Observed and expected frequency distributions of pollen fertility in  $(143 \times 160) \times 563$  and in the  $F_2$  of  $219 \times 218$

Cross	% of good pollen																									
	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20		15								
$(143 \times 160) \times 563$	Obs.	1	1	2	6	10	3	1	4	5	2	10	4	2	1	2	1	55 $\chi^2=8.675$ ( $P>0.75$ )								
	Exp.	2.62		2.71	2.11	5.37	6.29	2.72	2.63	5.21	4.54	5.05	7.14	4.70	2.18	0.87	0.19		0.01							
	Obs.	0	2	2	5	8	6	3	8	5	4	6	8	1	6	6	2									
$gl^l$	Exp.	1.11		1.44	1.94	3.55	5.34	5.74	4.67	4.95	5.52	6.43	7.37	7.91	6.77	4.85	2.31	0.95	72 $\chi^2=16.55$ ( $P>0.25$ )							
	Obs.	2	6	2	4	6	1	1	6	3	2	5	1	2	1	1										
	Exp.	5.68		13			14.10			10			14.15			8		7.05		3		1.81		0.27		
$219 \times 218$	Obs.	2	6	2	4	6	1	1	6	3	2	5	1	2	1	1	43 $\chi^2=5.98$ ( $P>0.30$ )									
	Exp.	8		13			14.10			10			14.15			8		7.05		3		1.81		0.27		
	Exp.	5.68		13			14.10			10			14.15			8		7.05		3		1.81		0.27		

$$160: gl - x_1 - Y_1 \quad z_1 - u_1 - v_1 \quad X_2 \ Y_2 \ Z_2 \ U_2 \ V_2,$$

$$143: + - X_1 - y_1 \quad Z_1 - U_1 - V_1 \quad X_2 \ Y_2 \ Z_2 \ U_2 \ V_2,$$

$$563: gl - X_1 - Y_1 \quad Z_1 - U_1 - V_1 \quad x_2 \ y_2 \ z_2 \ u_2 \ v_2.$$

On the basis of this assumption, frequencies of plants with different pollen fertilities were formulated, and the recombination values were estimated. Then, in the same manner as in the case of  $(108 \times 143) \times 563$ , mean fertilities and variances were calculated and were compared with the observed values. The observed distributions agreed well with the calculated distributions, as seen in Table 6. This experiment has no progeny test; the assumption of genotypes bearing so many genes cannot be a reliable one. Nevertheless, this experiment may indicate that the parental varieties differ from each other in a number of sets of G.D. genes, and some of them are linked with one another.

In these experiments in which the  $F_1$  of two related varieties has been crossed with the third variety, relatively distant from the former two, the variation in pollen fertility in the offspring usually show discontinuities, because the genic difference between the former two strains might be relatively simple. On the other hand, the  $F_2$ 's between distant varieties usually show a continuous array of intergrades in regard to fertility variation, from which direct analysis of individual genes is difficult. Having observed a number of  $F_2$ 's, however, an exceptional case was found, as given in Table 6 (the lower part), in which the variation had several frequency peaks and appeared to be discontinuous. In this case, the parental varieties, 219 and 218, are native to the Philippines, and have

characteristics of the 'Tropical-Insular group', but they differ markedly from each other in various morphological and physiological respects. Their  $F_1$  was highly sterile, pollen fertility being about 10%. In the  $F_2$ , the frequency peaks were found at 95-90, 75-70, 55, 40 and 30%. These fertilities seem to correspond to the fertility series, 1.0, 0.75, 0.75<sup>2</sup>,

0.75<sup>k</sup>, which is expected when  $k$  independent sets of G.D. genes segregate. The observed distribution was found to fit well the expected distribution if five independent sets of G.D. genes were assumed.

The outcome of crossing-experiments as stated in this section seems to suggest that the G.D. gene sets are rather numerous between distantly related varieties of rice. The continuous array of fertility usually appearing in the  $F_2$  or later generations may be due to segregation of those G.D. gene sets, though some other modifiers of fertility may also be concerned. Since they are numerous and control fertility, the G.D. gene sets may be regarded as polygenes. Biometrical-genetic methods may then be needed for analysing the variations in fertility in the  $F_2$  and later generations. Studies on this aspect will be reported elsewhere.

The results of genic analyses stated in the above sections may indicate that the hypothesis of G.D. genes can successfully explain the genetic behaviour of intervarietal hybrid sterility and other related phenomena found in rice. If we do not like to assume that the G.D. genes are duplicate genes, we may consider them as 'complementary gametic lethals'. According to this view, however, it must be assumed further that in a cross of, say,  $A_1B_1 \times A_2B_2$ , gametes carrying  $A_1B_2$  and  $A_2B_1$  differ in function; and to explain the origin of such complementary genes will also be difficult. In contrast, the assumption that they are duplicate genes seems to provide a simpler explanation for various phenomena.

#### (6) *Genes, other than G.D. genes, affecting hybrid sterility*

It may be inferred from the following facts that not only the G.D. genes, but some other genes are responsible for the sterility resulting from crosses between distant varieties of rice. First, according to the G.D. gene hypothesis, a fertile plant should produce only fertile plants in the progeny, and a semi-sterile plant should produce fertile plants and semi-sterile ones whose fertility is not lower than the parent. Actually, this expectation is often betrayed. Secondly, if a gene for a certain character is linked with a G.D. gene in a hybrid, phenotypically dominant segregants should have lower mean fertility than the recessive ones. However, the reversed situation can also be found. The simplest explanation for these phenomena may be to assume that genes which work in the zygote and maintain fertility are duplicated, and the double-recessive combination brings about sterility through some physiological effect. Such duplicate genes for fertility, if present, may have nothing to do with the  $F_1$  sterility, but may produce sterile plants in the  $F_2$  and later generations. Analysis of such duplicate genes will be reported in another paper.

#### SPECULATION ON THE NATURE OF GENETIC DIFFERENCES BETWEEN DISTANTLY RELATED VARIETIES OF RICE

It is known that hybrids between some related species in *Primula* and other genera are sterile, but show no disturbance in chromosome pairing. Stebbins (1950) considered that in such cases the chromosomes of parental species bear minute structural differences, and called them 'cryptic structural hybrids'. He pointed out further that in such cases tetra-

ploids of the hybrids generally show high fertilities, since in tetraploids chromosomes are not recombined due to selective pairing. In accordance with this view, Prof. Stebbins kindly wrote a letter to the writer suggesting that the G.D. genes might be small chromosome segments, possibly translocations.

The tetraploids of intervarietal hybrids of rice generally have higher fertility and a smaller number of tetravalent chromosomes than their parental autotetraploid strains. However, it was observed by the writer (Oka, 1954 *b-d*, 1955) that in tetraploid rice, fertility differed markedly according to the genotype, and tended to be high in vigorously growing strains. Hybrids between tetraploid strains were generally vigorous in growth, and the degree of hybrid vigour was significantly correlated to the percentage of seed setting. In the  $F_2$  and later generations, the 'fertile tetraploid hybrid' produced many sterile plants, and the mean fertility of the hybrid population fell down in successive generations, though plants with high fertilities were selected in every generation. It was found, further, that the  $F_2$  ratios for several monogenic characters were between 35:1 (random chromosome pairing) and 20.8:1 (random chromatid pairing) which were to be expected in autotetraploids. These facts seem to indicate that in tetraploid hybrids between distant varieties of rice, the chromosomes derived from the same parent do not tend to pair selectively. Should this view be correct, they cannot be said to be allotetraploids (Oka, 1955). Similar cases are known in tetraploid hybrids between some races or species of *Zea* and *Lycopersicum* (Randolph, 1943; Lesley & Lesley, 1953). It may be inferred that in these cases the rise of fertility in a hybrid is not necessarily due to the selective pairing of chromosomes.

It has been shown in this paper that hybrid sterility between rice varieties may be due to the G.D. genes, which are duplicate genes for gametic development. In addition to these G.D. genes, various kinds of duplicate genes seem to be present in rice. Then the question arises, how would genes have been duplicated in rice? In general, duplication of genes results either from the doubling of chromosomes or from translocations. Taking the latter view, the multiplicity of the G.D. gene sets, as pointed out in this paper, can be explained only by assuming that many small translocations have occurred and have been accumulated in the chromosomes. It should then be expected that in tetraploid hybrids the chromosomes pair selectively. The writer's observation on tetraploid rice does not, however, fit this expectation. On the other hand, the view that the duplicate genes would have been created by the doubling of chromosomes appears to be more reasonable. Based on the secondary association of meiotic chromosomes and the association of chromosomes in haploid plants, it has been suggested that the rice plant may in itself be a secondary balanced polyploid (Sakai, 1935). We have no conclusive criterion to evaluate the relative importance of genic and chromosomal agencies taking part in racial differentiation. However, if we take the secondary polyploidy hypothesis, the origin of G.D. genes may be easily understood as due to the doubling of chromosomes that would have occurred in the ancient history of this plant.

#### THE ROLE OF DUPLICATE GENES IN PHYLOGENETIC DIFFERENTIATION

It has been pointed out in this paper that when two independent genes are linked respectively with two pairs of G.D. genes belonging to the same set, recombination of those two independent genes is restricted to some extent. As expected from this phenomenon, it

was actually found that when a hybrid between distantly related rice varieties was propagated without selection, genotypes similar to the parental varieties tended to increase in successive generations. The results of observations on this aspect will be reported elsewhere; in this paper, the effect of gene duplication on racial differentiation will be discussed.

In mainly self-fertilized plants, genic changes, if unfavourable for reproduction or growth, may be eliminated from the population rapidly. However, if genes are duplicated, a recessive mutation at one locus may be compensated by the same gene at the other locus. Thus many genic changes may be accumulated in the population and may have a chance to be combined with one another to create new adaptive genotypes.

The other contribution of gene duplication to racial differentiation may be its recombination restricting effect in hybrids. If a number of the genes necessary for reproduction or growth are duplicated and are distributed on different chromosomes, plants with a recombination of those chromosomes may be more or less disadvantageous in a hybrid between strains with those genes in different combinations. The recombined genotypes may then tend to be eliminated through gametic or zygotic selection, and the hybrid population may have a tendency to increase its genotypes similar to the parental strains. In mainly self-fertilized plants, differences in duplicate genes may thus work as an isolating mechanism promoting racial differentiation.

Hybrid sterility and restriction on gene recombination similar to those found in rice have been known in hybrids between some tetraploid species of cultivated cotton (Stephens, 1949). In this case, participation of duplicate genes may reasonably be expected, since the parental species are tetraploids.

In accordance with these considerations, it may be inferred that the origin of wonderfully rich variations in rice is to be traced back to the doubling of chromosomes in the remote ancestor of this plant, and the formation of the mechanism of hybrid sterility, due to gene duplication, would have pre-destined the present status of differentiation of variety groups.

#### SUMMARY

1. The hybrid sterility between distantly related varieties of cultivated rice can be explained by duplicate genes which work in the gamete as development maintainers (Gametic Development Genes, or G.D. genes). Hybrids between varieties with those genes in different combinations may produce double recessive gametes which deteriorate at a certain stage of development.

2. For analysing these G.D. genes, crossing experiments of an  $(A \times B) \times C$  type were used, where  $A$  and  $B$  are closely related varieties, but differ in fertility in the hybrid with the third variety  $C$ . In one case, it was found that a G.D. gene was linked with the glutinous gene  $gl$  with a certain recombination value.

3. When a gene is linked with a G.D. gene, causing sterility in a hybrid, its segregation ratio changes, due to gametic selection in a definite manner. This phenomenon was investigated theoretically and experimentally.

4. When two independent genes are linked respectively with two pairs of G.D. genes belonging to the same set (the two pairs have the same effect but are located on different chromosomes), recombination of the former genes is restricted to some extent. This phenomenon was demonstrated in the  $F_2$  of some varietal crosses.

5. It was pointed out that the G.D. gene sets segregating in a hybrid between distantly related varieties of rice are usually numerous, and produce a continuous array of intergrades in fertility in the  $F_2$  and later generations. The G.D. gene sets may be said to act as polygenes.

6. In addition to the G.D. genes, other duplicate genes for fertility seem to be present. The origin of gene duplication was discussed. Since it was found that in tetraploid hybrids between distantly related varieties of rice, the chromosomes derived from the same parent did not tend to pair selectively, the gene duplication in rice was considered to be due more probably to the doubling of chromosomes, as suggested by the hypothesis that rice is a secondary balanced polyploid, than to numerous small translocations.

7. When varieties with those duplicate genes in different combinations are hybridized, since chromosome recombination tends to bring about unfavourable effects, genotypes similar to the parental strains will increase in the hybrid population. It was pointed out that this tendency might work as an isolating mechanism in mainly self-fertilized plants.

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