2. Nonrandom fertilization by pollen in mixtures.

Experiments have been underway to investigate selective fertilization effects involving pollen of strains with contrasting endosperm color. The varieties chosen for an investigation of the hereditary characteristics of the phenomena are Jarvis, which has yellow endosperm, and Weekley, which has white endosperm. The technique used to measure the trait is to pollinate pairs of plants of each variety which flower at the same time with mixtures of their pollen. At harvest time, 100 kernels of each ear are classified for endosperm color. Table 1, which gives the results expected if fertilization by the two kinds of pollen in the mixture is random, shows the relationship between endosperm color and mating type on each ear. Considering a pair of plants as a unit, the expected proportion of self-pollinated seed is given by the expected proportion of pure yellow seed + pure white seed as follows:

$$[p(100) + (1-p)(100)]/200 = 1/2.$$

In other words, in the absence of selective fertilization, the ratio of self:cross seed is expected to be 1:1 regardless of the relative proportion of yellow:white pollen in the mixture.

Two samples of the original varieties, Jarvis and Weekley, have been examined for possible selective fertilization effects (Table 2). In the first test, satisfactory seed set was obtained on 42 pairs of ears. The percentage of selfed kernels was 50.52% when averaged over all pairs. The heterogeneity chi-square was significant, however, which suggests that variability exists among the pairs for percentage of self seed. Nineteen pairs showed significant deviations from the expected 1:1 ratio; 9 pairs had more self seed than expected (inbreeders) and 10 had fewer than expected (outbreeders).

Out of the 42 pairs, 18 pairs were selected for additional study. Self seed of these were planted in a winter nursery in 10 plant rows, and each plant was self-pollinated to reveal misclassified seed. Segregating ears were discarded. Nonsegregating ears were shelled in bulk to give S_2 seed of each progeny. These were tested in 1967 as paired progeny. Pollinations were with mixtures of pollen of individually paired plants within each progeny pair. Seed was obtained on 16 of the 18 progeny

Table 1. Expectations of random fertilization by mixed pollen of a contrasting pair of parent plants.

Parental genotype	Number of seeds classified	Pollination by mixed pollen with "$\underline{Y}$" and "$\underline{y}$" gametes in frequencies p and 1-p, respectively			
		Endosperm		Expected number of seeds	Mating type
		Color	Genotype		
YY	100	Yellow	YYY	p(100)	Self
		Cream	YYy	1-p(100)	Cross
yy	100	Cream	yyY	p(100)	Cross
		White	yyy	1-p(100)	Self
Total No. kernels	200			200	

Table 2. Results of pollination of two samples of paired plants and their inbred progeny with mixtures of their pollen.

Year	Generation	No. pairs tested	Average percent-age of self seed	Chi-square tests*		Parent-offspring regression
				1:1	Hetero-geneity	
1966	S_0 Parents	42	50.52	N.S.	**	.293
1967	S_2 Progeny	16	54.59	*	**	
1970	S_0 Parents	25	50.34	N.S.	**	.290
1971	S_1 Progeny	18	49.74	N.S.	**	

*Goodness of fit test for 1:1 ratio of self:cross seed and the corresponding test for heterogeneous ratios among individual pairs of plants or progeny.

pairs, as indicated in Table 2, and the parent-offspring regression was estimated to be .293. The heterogeneity chi-square shows that significant differences exist among progeny pairs for percentage of self seed.

The original varieties were again evaluated in 1970 and 1971 in the same way as before. Out of the 25 pairs of plants classified, there were 6 which showed a significant deviation from 50% selfed seed; 3 gave more than 50% self seed (inbreeders) and 3 gave less than 50% self seed (outbreeders). The self progeny of all 25 pairs were tested in paired progeny rows in 1971. Of these, 18 progeny pairs could be evaluated by pollen mixtures. Those not represented were eliminated because of differences in flowering time.

Note the excellent agreement between the results of the two samples in terms of average percent selfing and in terms of parent-offspring regression.

The progeny tested in 1967 were grouped into one of three categories based upon the percentage of self seed. These are: (1) pairs with more than 50% self seed (inbreeders); (2) pairs with very nearly a 1:1 ratio of selfs:crosses (neutrals); and (3) pairs with more than 50% crossed seed (outbreeders). Seed which has been classified as self seed of each pair was planted and the progeny self-pollinated to reveal misclassification and assure purity of the yellow and white strains. This resulted in S_4 seed of 4 pairs of lines in the inbreeder class and 5 pairs of lines in the neutral class and in the outbreeder class. These S_4 lines were then inter-mated within classes and strains to form subpopulations of each variety representing each of the three categories.

The subpopulations, which were the result of one selection cycle, were evaluated in 1969 and their progeny in 1970 by the techniques already described. The responses to selection, summarized in Table 3, provide estimates of realized heritability. These estimates are of the same order of magnitude and agree very well with heritabilities indicated by parent-offspring regression (Table 2). This indicates that the response to divergent selection is essentially symmetrical and is in excellent agreement with expectations based upon parent-offspring covariances in the original populations.

Table 3. Results of divergent selection for percentage of self seed on pairs of plants pollinated with mixtures of their pollen.

Direction of selection	Average percentage of self seed		
	Selection differential	Selection response	Realized* heritability
Inbreeders	19.73	6.53	.331
Outbreeders	11.12	3.07	.276

*Computed as Selection Response/Selection Differential.

Table 4. Evaluation of subpopulations resulting from divergent and canalizing selection for percentage of self seed on pairs of plants pollinated with mixtures of their pollen.

Sub-population	Generation	No. pairs tested	Average percent-age of self seed	Chi-square tests‡		Parent-offspring regression
				1:1	Hetero-geneity	
Inbreeders	S_0 Parents	19	57.05	**	**	
	S_1 Progeny	14	53.72	**	**	.015
Neutrals	S_0 Parents	19	50.82	N.S.	**	
	S_1 Progeny	7	50.06	N.S.	N.S.	.096
Outbreeders	S_0 Parents	30	47.45	**	**	
	S_1 Progeny	26	47.78	**	**	-.166

‡Goodness of fit test for 1:1 ratio of self:cross seed and the corresponding test for heterogeneous ratios among individual pairs of plants or progeny.

Data of each of the 3 subpopulation pairs are summarized in Table 4. The 19 pairs of ears representing the inbreeder class average 57% self seed, which is a significant deviation from the 1:1 ratio of selfs:crosses. Eight pairs of the 19 pairs tested had significantly more than 50% self seed and none had significantly less than 50%. In 1970 the 14 progeny pairs averaged significantly more than 50% self seed. Heterogeneity among progeny pairs is indicated; 8 of the progeny pairs had significantly more than 50% self seed and none had significantly less than 50%.

The neutral populations averaged approximately 50% selfing in both years of the test.

The outbreeder class, represented by 30 pairs in 1969, had significantly less than 50% self seed on the average. Heterogeneity is indicated and 2 pairs of the 30 had significantly more than 50% self seed while 9 had significantly less. The progeny pairs evaluated in 1970 show essentially the same pattern, with only one pair with significantly more than 50% selfing and 10 pairs with significantly less.

These data show quite conclusively that divergent selection was effective, and that populations which show a degree of selective fertilization have been developed. The parent-offspring regressions computed within each selected population suggest that heritable variance for the trait has been exhausted. This could be due to the effectiveness of selection, but a more likely cause is inbreeding resulting from small effective populations.

In order to study the mechanism governing this trait in more detail, inbred lines are being developed to represent the two divergent classes; viz. inbreeders and outbreeders. The lines, which originated in the selection study described above, were developed as paired progeny by selection within the inbred pairs through the S_4 generation. Data for pairs of lines which were eliminated because of differential flowering time, difficulty in seed classification, or variable expression of the trait are not included (Table 5).

Table 5. Pairs of inbred lines selected for nonrandom fertilization effects of mixtures of their pollen.

Designation of inbred pairs		Percent self seed from pollen mixture				
		Generation of inbreeding				
Yellow	White	S_0	S_1	S_2	S_4	S_6
P1	P2	79.5	--	82.5	92.0	84.0
P3	P4	79.5	--	82.5	97.2	95.1
P5	P6	79.5	--	71.5	98.5	81.4
P7	P8	63.2	--	56.5	93.2	42.5
P11	P12	39.2	--	20.2	49.5	--
P19	P20	63.2	--	56.5	36.5	--
P101	P102	47.0	42.0	31.5	--	--
P103	P104	47.0	38.0	35.0	--	--

Table 6. Percentage of self seed from pollen mixtures of related pairs of inbred lines.

White inbreds	"Yellow" inbreds			Mean
	P1	P3	P5	
P2	84	92	65	80
P4	--	95	55	75
P6	64	87	81	77
Mean	74	91	67	78

Mean of diagonals = 87

Mean of off diagonals = 73

The data show that the development of pairs of lines for the inbreeder class has been more successful than for the outbreeder class. Note, however, that three of the pairs which are distinct inbreeders are descendants of the same pair of S_0 plants. The two pairs of S_2 lines which appear to be in the outbreeder class are also closely related. The rather wide fluctuations from generation to generation seen in some of the pairs, like P7-P8 for example, are probably due primarily to environmental variations although genetic sampling error may also contribute.

Since three of the inbreeder pairs are closely related, the possibility that major loci are involved will be considered in crosses with unrelated lines to be evaluated in the future. Preliminary evidence which bears upon this question was obtained by testing them in all possible pairs of contrasting endosperm color (Table 6). The diagonals of Table 6 represent the pairwise combinations in which the lines were developed. The off-diagonals represent combinations not considered in the development of the lines. The agreement between the two means shows that the selective fertilization effect is nearly as great in the unselected combinations as it is in the selected combinations, and suggests considerable genetic similarity among the inbreds of each endosperm type. The greatest difference observed involves line P3, which appears to result in significantly greater selective fertilization effects in combination with all 3 of the white lines tested.

It should be mentioned that an examination of the pairs of ears of these inbreds, where the degree of selective fertilization is much greater than in the varietal material, shows convincingly that the effect is essentially symmetrical. That is to say, in the inbreeder class for example, the yellow line discriminates against the white pollen just as strongly as the white line discriminates against the yellow pollen. In all of the cases tried, however, the two inbreds will set crossbred seed normally when the pollens are not mixed together. The pattern observed here appears to be in contrast with patterns reported for known gametophyte factors.

R. H. Moll