Theoretical and Applied Genetics 43, 323—328 (1973)

Inheritance Studies with Inbred Lines of Maize having Different Activity Levels of the AP_1 Controlled Acid Phosphatase Isozymes

Y. EFRON

Department of Field and Vegetable Crops, The Hebrew University, Faculty of Agriculture, Rehovot (Israel)

Summary. The genetic control of acid phosphatase-1 (AP_1) activity in pollen of maize was studied by crossing inbred lines having different AP_1 isozymes and different activity levels of the AP_1 enzyme. Usually, the intensities of the *SS* and *FF* isozyme bands were not equal in pollen of AP_1^S/AP_1^F heterozygous F_1 hybrids, but the relative intensities of the two bands were not correlated to the activity levels of the parental lines. The AP_1^S/AP_1^S and AP_1^F/AP_1^F F_2 populations differed in their mean level of activity. Both populations showed segregation in the activity levels indicating single gene control. The intensity ratios of the *SS* and *FF* bands in the different heterozygous AP_1^S/AP_1^F F_2 plants did not segregate. The results support the competition model for gene regulation proposed by Schwartz (1971).

In a previous paper (Efron, 1971a) five distinct levels of AP_1 acid phosphatase activity were found among 23 inbred maize lines. Variation in enzyme activity in different inbred lines of maize had previously been demonstrated for a number of other enzymes (Zieserl and Hageman, 1962; Hageman *et al.*, 1967; Roose and Sarkissian, 1968; Efron, 1970a). Hybrids between lines with different activities usually show intermediate levels of activity (Hageman *et al.*, 1967).

Two major difficulties arise in an attempt to study the genetic control of the differences in specific enzyme activity: 1. the presence of other phosphatases; 2. conventional biochemical methods do not enable one to distinguish between the activities specified by each of the two parental genomes. Three allelic isozymes, AP_1^F, AP_1^I and AP_1^S, with fast, intermediate and slow migration rates, have been described for the AP_1 locus (Efron, 1970b). Allelic isozymes having different electrophoretic mobilities have been used (Efron, 1970a; 1971b) to study the genetic basis for the variation in alcohol dehydrogenase activity in maize. The limitations mentioned above were overcome in the present study by crossing inbred lines with known activity levels but having different AP_1 alleles.

Materials and Methods

Samples of mature fresh pollen of maize were studied by starch gel electrophoresis. Extract preparation, electrophoretic techniques and staining procedure have been described before (Efron, 1971a). The activity of the AP_1 controlled acid phosphatase was determined in the pollen of F_1 hybrids and of segregating F_2 plants.

Determination of the relative activity in the F_1 hybrids: The staining intensities of the isozyme bands were used as a measure of enzyme activity. Seven homozygous AP_1^S/AP_1^S inbred lines with different acid phosphatase-1 activity were crossed in almost all possible combinations with six homozygous AP_1^F/AP_1^F inbred lines representing five groups of activity (Table 1). All the F_1 hybrids were grown in the field and samples of mature fresh pollen were collected from six different plants of each hybrid and stored at −10 °C. Three different extracts from each pollen sample were electrophoresed separately and stained specifically for acid phosphatase. The ratio of the colour intensities developed at the positions of the *SS* and *FF* isozyme bands was scored visually in each gel by seven unbiased persons. The intensity of the *FF* band was always used as a reference (activity value of 1.0). The values obtained are termed relative *S* activity.

Table 1. *Mean relative intensities of the AP_1^S isozyme bands in relation to the AP_1^F isozyme bands in 33 different AP_1^S/AP_1^F hybrids*

AP_1^S/AP_1^S parental line	Group of activity+	AP_1^F/AP_1^F parental line and group of activity+					
		K-4	*C*131-*A*	*B*-53	*B*-57	*O*h-7	*B*-50
		1	2	3	3	4	5
N-6	1	3.48*	2.13	3.32*	2.23	2.61*	.55*
B-55	1	3.66*	3.43*	3.49*	3.80	2.65	1.96
B-56	2	3.00	3.20	3.00	—	3.30	1.00
Hy	3	2.85*	—	2.80	2.12*	2.50	—
E_1^S/E_1^S	3	2.02*	—	1.90*	1.91	1.79*	.49
C-103	4	.51*	—	.59	.57*	—	—
M-14	4	2.16*	—	—	1.86*	1.30*	1.12

+ Group 1 = Highest activity and Group 5 = Lowest activity
* Mean values of two reciprocal hybrids.

Determination of the AP_1 activity in segregating F_2 plants: Four different reciprocal F_1 hybrids from crosses between AP_1^S/AP_1^S lines from activity groups 1 and 3 (high and intermediate activity levels, respectively, Efron, 1971a) and AP_1^F/AP_1^F lines from activity groups 3 and 5 (intermediate and low activity levels, respectively) were selected for studies of segregating F_2 plants (Table 2). Pollen samples from all plants of each cross were first classified for their AP_1 genotypes. The AP_1^S/AP_1^S and AP_1^F/AP_1^F homozygous plants were then scored for their relative activity, as described before (Efron, 1971a). The AP_1^S/AP_1^F heterozygous plants were scored for the relative activity of the *SS* and *FF* isozyme bands as described for the F_1 hybrids.

In 17 out of the 33 hybrids, and in the four F_2 segregating populations, the two reciprocal combinations were tested separately and there were no differences between the two reciprocals. Therefore, the results were combined.

Table 2. *Segregation and goodness-of-fit in four different F_2 families in crosses involving the AP_1^S and AP_1^F alleles in the pollen of maize*

Cross	Genotypic class			X^2 (1:2:1)	$P(1\ df)$
	AP_1^S/AP_1^S	AP_1^S/AP_1^F	AP_1^F/AP_1^F		
B-55 × *B*-53	52	112	54	.20	0.95—0.90
N-6 × *B*-53	45	114	56	1.91	0.50—0.25
N-6 × *B*-50	50	113	54	.54	0.90—0.75
E_1^S/E_1^S × *B*-50	60	138	57	1.79	0.50—0.25

Results

The AP_1 controlled acid phosphatase is a dimer (Efron, 1970b). However, only the *FF* and the *SS* isozyme bands are found in the pollen of heterozygous AP_1^S/AP_1^F plants. The absence of the *FS* hybrid band indicates that the enzyme present in mature pollen is probably synthesized after tetrad formation (Efron, 1970a). In the present study, the intensity ratio of the *SS* and *FF* isozyme bands was tested in 33 different hybrids. Equal intensity of the two bands (relative *S* activity of about 1.0) was found in only three out of the 33 crosses tested (Table 1). In all other hybrids the two bands showed different intensities with a range of relative *S* activity from 0.50 to 3.80. However, the intensity ratio of the two bands was usually not correlated with the activity levels of the two homozygous parental lines (Table 1).

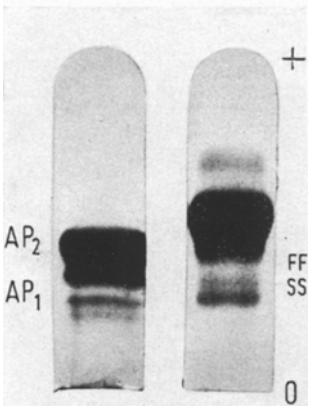

Fig. 1. Zymogram of pollen extracts from AP_1^S/AP_1^F heterozygotes from the *N*-6 × *B*-50 (left) and *N*-6 × *K*-4 (right) hybrids. Note the difference in the relative intensities of the *FF* and *SS* isozyme bands

The hybrids *N*-6 × *K*-4 (Fig. 1, right) and *N*-6 × *B*-50 (Fig. 1, left) are used to illustrate this point. Both *N*-6 and *K*-4 show a similarly high activity level (group 1) but in the hybrid *N*-6 × *K*-4 the *SS* band which was contributed by *N*-6 was about 3.48 times more intense than the *FF* band contributed by *K*-4. On the other hand, in the hybrid *N*-6 × *B*-50 the *FF* band originating from *B*-50 (lowest activity level) was twice as intense as the *SS* band contributed by *N*-6 (highest activity level).

Inbred lines of the same AP_1 genotype and the same level of activity differ in the relative contribution of their AP_1 allele. For example, the mean relative *S* activity of the six different hybrids of *N*-6 was lower (2.39) than the mean relative *S* value for the same six hybrids of *B*-55 (3.17). The difference was even greater between *C*-103 and *M*-14, both from activity group 4. The mean relative *S* values of the two comparable hybrids were 0.54 and 2.16, respectively (Table 1). In contrast to these differences, the relative activities of the *SS* and *FF* isozymes were similar when a particular line was crossed with different lines having different activity values. For example, values of 2.02, 1.90, 1.91 and 1.79 were obtained when E_1^S/E_1^S was crossed with AP_1^F lines from activity groups 1, 3, 3 and 4, respectively. Or, values of 3.66, 3.43, 3.49, 3.80 and 2.65 were found in the crosses of *B*-55 with AP_1^F lines from activity groups 1, 2, 3, 3 and 4, respectively.

Four different hybrids were selected for further studies of segregating F_2 populations. The hybrids selected were: *B*-55 × *B*-53 and *N*-6 × *B*-53, representing AP_1^S lines with a high activity level (group 1) and an AP_1^F line with intermediate activity (group 3); *N*-6 × *B*-50 (AP_1^S high × AP_1^F low) and E_1^S/E_1^S × *B*-50 (AP_1^S intermediate × AP_1^F low). In all crosses, the AP_1^S and AP_1^F alleles segregated in the normal Mendelian fashion expected from two co-dominant alleles of a single gene (Table 2). This excludes the possibility that the differences in the activity of the *SS* and *FF* isozymes in the F_1 hybrids were due to any abnormal segregation of the two alleles in the pollen grains.

The mean relative *S* activity values in the AP_1^S/AP_1^F heterozygous F_2 segregants were very similar to the mean values of the F_1 generation in all four cros-

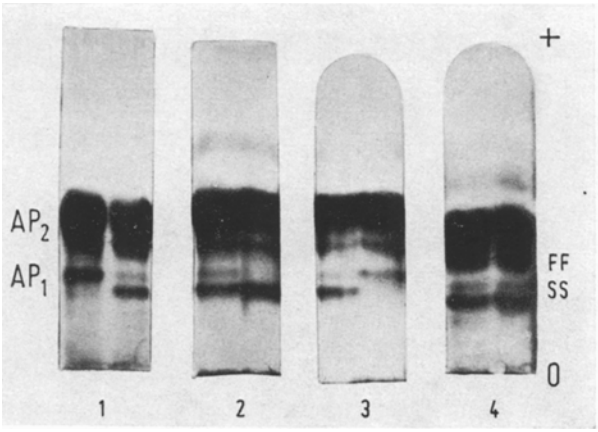

Fig. 2. Zymograms of pollen extracts from segregating F_2 plants from the cross B-55 × B-53. 1. left — AP_1^F/AP_1^F; right — AP_1^S/AP_1^F. 2. left — AP_1^S/AP_1^F; right — AP_1^S/AP_1^S. 3. left — AP_1^S/AP_1^F; right — AP_1^F/AP_1^F. 4. left — AP_1^S/AP_1^F; right — AP_1^S/AP_1^F

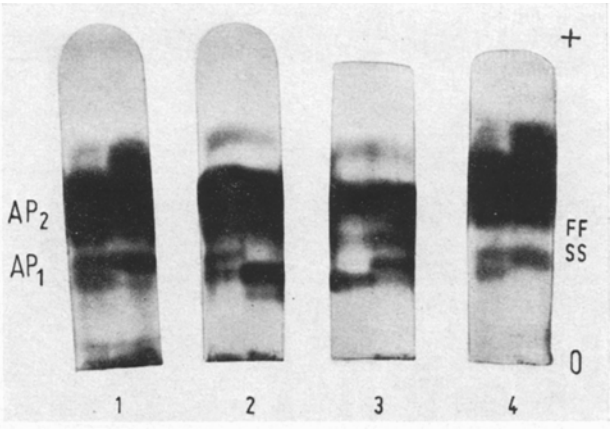

Fig. 3. Zymograms of pollen extracts from segregating F_2 plants from the cross N-6 × B-50. 1. left — AP_1^S/AP_1^F; right — AP_1^F/AP_1^F. 2. left — AP_1^S/AP_1^F; right — AP_1^S/AP_1^S. 3. left — AP_1^S/AP_1^S; right — AP_1^S/AP_1^F. 4. left — AP_1^S/AP_1^F; right — AP_1^F/AP_1^F

Table 3. *Distribution of the relative AP_1^S activity values in pollen of heterozygous AP_1^S/AP_1^F plants from four different segregating F_2 families*

Cross	Frequency (%) of plants with relative AP_1^S activity values							Mean AP_1^S relative activity	
	.29	.33	.40	.50	.66	1.0	1.5	F_2	F_1
N-6 × B-50	—	10.0	22.7	51.0	9.1	7.3	—	.51	.55
E_1^S/E_1^S × B-50	1.4	33.6	16.9	44.6	1.4	2.1	—	.44	.49
	Frequency (%) of plants with relative AP_1^S activity values								
	2.0	2.5	3.0	3.5	4.0	4.5	5.0		
B-55 × B-53	3.4	10.3	35.4	22.5	25.0	2.6	.8	3.33	3.49
N-6 × B-53	7.0	14.0	37.6	20.0	20.0	.7	.7	3.26	3.32

Table 4. *Mean, standard deviation, range and coefficient of variation of the AP_1/AP_2 values in pollen of AP_1^S/AP_1^S and AP_1^F/AP_1^F homozygous plants from four different F_2 segregating populations**

Cross+	Activity groups	Genotype	Number of plants tested	Average activity	Standard deviation	Range	C. V.
B-55 × B-53	1 × 3	AP_1^S/AP_1^S	40	.177	.051	.087—.278	43.2
		AP_1^F/AP_1^F	42	.119	.029	.076—.189	24.0
N-6 × B-53	1 × 3	AP_1^S/AP_1^S	40	.132	.036	.087—.212	27.1
		AP_1^F/AP_1^F	39	.077	.026	.042—.152	34.3
N-6 × B-50	1 × 5	AP_1^S/AP_1^S	28	.050	.021	.015—.105	42.4
		AP_1^F/AP_1^F	42	.060	.041	.018—.158	68.3
E_1^S/E_1^S × B-50	3 × 5	AP_1^S/AP_1^S	42	.037	.023	.009—.097	63.0
		AP_1^F/AP_1^F	54	.071	.035	.018—.146	48.9

* The AP_1/AP_2 values were used as a measure for the activity of the AP_1 acid phosphatase (Efron, 1971a).
\+ The results of the two reciprocal crosses were combined.

ses. Also, there was no apparent distribution of the individual plants. The majority of the plants in the B-55 × B-53 and N-6 × B-53 crosses showed relative S activities between 3.0 and 4.0. In the N-6 × B-50 and E_1^S/E_1^S × B-50 crosses, 83.7 and 95.1 percent of the populations had activity values between 0.33 and 0.50 (i.e., the FF band was two to three times more intense than the SS band) (Table 3). Moreover, when B-55 was the AP_1^F parent, the SS band was always stronger than the FF band (Fig. 2), but when B-50 was the AP_1^F parent, the FF was usually the darker band with only a few exceptions where the FF and the SS bands were of the same intensity (Fig. 3). Hence, it might

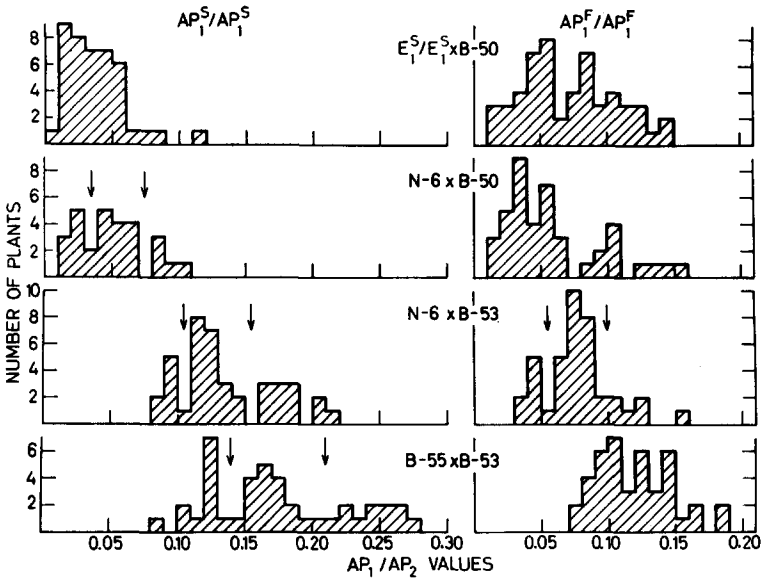

Fig. 4. Distribution of the AP_1 relative activity (AP_1/AP_2 values) in the AP_1^S/AP_1^S and AP_1^F/AP_1^F homozygous F_2 populations of four different crosses. The arrows indicate the suggested phenotypic classification where the distribution can fit the 1:2:1 ration

be concluded that the relative S activity was not a subject for segregation in the F_2 populations.

When the activity of the homozygous AP_1^S/AP_1^S and AP_1^F/AP_1^F segregants was tested (Table 4 and Fig. 4), the activity level of the AP_1 acid phosphatase clearly segregated in the F_2 populations. It is difficult to conclude from the results presented in Fig. 4, how many genes are involved in the determination of AP_1 activity. However, in some of the populations especially in the SS group, it is suggested that a single gene with two co-dominant alleles is responsible for the difference in the activity levels of the two parental lines. For example, the SS population from the N-6 $\times$ B-53 cross may be divided into three groups: values of 0.8—.11; values of .11—.15 and values of .16—.22 with a distribution of 8, 20, and 12 plants, respectively. This distribution fits well the 1:2:1 ratio expected ($P = .50—.75$). The distribution of the AP_1^S/AP_1^S populations from the crosses B-55 $\times$ B-53 and N-6 $\times$ B-50 and the AP_1^F/AP_1^F population from the N-6 $\times$ B-53 cross also fit the expected 1:2:1 ratio. In the other populations there are more plants with low activity level than would be expected from normal Mendelian segregation of two co-dominant alleles. However, even though four populations showed good fit to the 1:2:1 ratio, the suggested classification into genotypic groups (arrows in Fig. 4) is different in each population. The mean and the distribution of the populations are also different (Table 4). In the crosses of high $\times$ intermediate activity the average level of activity of the F_2 populations was higher than in the crosses of intermediate $\times$ low and high $\times$ low activity. But there was also a difference between the two populations of the high $\times$ intermediate activity. The mean level of activity in the B-55 $\times$ B-53 of both the AP_1^S and AP_1^F populations was higher than in the N-6 $\times$ B-53 cross. Also, the upper range in the first cross was higher. On the other hand, the two other crosses, $E_1^S/E_1^S \times B$-50 (intermediate $\times$ low) and N-6 $\times$ B50 (high $\times$ low), were similar to each other.

An interesting feature of the segregating populations was the difference between the homozygous AP_1^S/AP_1^S and AP_1^F/AP_1^F populations in each cross (Table 4). In the first two crosses (B-55 $\times$ B-53 and N-6 $\times$ B-53) the average activity values of the AP_1^S/AP_1^S populations were higher then the AP_1^F/AP_1^F populations. In the other two crosses (N-6 $\times$ B-50 and $E_1^S/E_1^S \times B$-50) the AP_1^F/AP_1^F populations showed the higher average activities.

Discussion

A difference in the activity of an enzyme may be qualitative (in specific activity) or quantitative (in the number of active enzyme molecules). If it is qualitative, the relative activities of the two isozyme bands in heterozygous AP_1^S/AP_1^F would be expected to be similar to the relative activities of the two parental lines. The results of this study did not show a correlation of this type, so it might be concluded that the differences in the AP_1 acid phosphatase activity in pollen of maize were not due to differences in specific activity.

Quantitative differences are expected to be due to some mechanism of genetic and/or environmental control. The fact that plants with equal intensities of the AP_1^S and AP_1^F isozyme bands were the exception and not the rule shows that the control mechanism is not a simple environmental one, and that the two alleles in the different inbred lines react differently to this control mechanism.

A possible explanation for the results obtained in this study might be based on the competition model

for regulation of gene action proposed by Schwartz (1971), viz. that the number of enzyme molecules which are synthesized in a certain tissue at a given time is controlled by the presence of a limiting factor which acts at the gene level. He showed that there was competition and that there were differences in competitive ability between alleles for the limiting factor. Following this hypothesis, it is assumed that the present inbred parents differed in the activity level of the AP_1 controlled acid phosphatase because they had different amounts of the limiting factor. Also, the AP_1 allele from different inbred lines might vary in competitive ability for the limiting factor. Thus, although both *N*-6 and *K*-4 had high activity levels, in the cross *N*-6 × *K*-4 the AP_1^S allele contributed by *N*-6 was a much better competitor than the AP_1^F allele contributed by *K*-4. On the other hand, *N*-6 had a higher level of activity than *B*-50 (groups 1 and 5, respectively) but in the *N*-6 × *B*-50 cross the AP_1^F band was more intense than the AP_1^S band. Thus, the AP_1^F allele contributed by *B*-50 is a better competitor than the AP_1^S allele contributed by *N*-6.

Both the AP_1^S and AP_1^F alleles may vary in competitive ability. Among the AP_1^S lines, *B*-55 and *C*-103 showed the highest and lowest competitive abilities, and among the AP_1^F lines *B*-50 was the best competitor while *K*-4 and *B*-53 had the lowest competitive ability.

As described before, pollen extracts from AP_1^S/AP_1^F heterozygotes showed only two bands, without hybrid enzyme formation. The absence of the hybrid enzyme shows that the enzyme is probably synthesized after haploidization and segregation of the AP_1^S and AP_1^F alleles at meiosis. Alternatively, the enzyme may be synthesized prior to haploidization but, for some reason, in this tissue dimerization is limited to identical subunits (Salthe, Chilson and Kaplan, 1965). If dimerization is limited to identical subunits and the enzyme is synthesized before haploidization, the results fit the competition model without difficulty. On the other hand, if the enzyme is synthesized after haploidization, one could argue that interallelic competition can not take place in haploid cells. However, Schwartz (1971) showed for alcohol dehydrogenase in pollen of maize that the effect of the interallelic competition for the limiting factor operates at the diploid stage and that this effect must persist into the haploid microspores, so that even in the absence of further competition the two alleles maintain their differential gene activity in enzyme synthesis.

The main features of the F_2 populations support Schwartz's hypothesis. Segregation for the amount of the limiting factor may be responsible for the segregation of activity levels of the AP_1 acid phosphatase. On the other hand, according to the limiting factor hypothesis, the relative competitive abilities of the two alleles, and therefore also the relative intensities of the bands, are expected to be constant. The results obtained in the AP_1^S/AP_1^F heterozygous F_2 plants are in good agreement with this expectation.

Using the competition model, Schwartz (1971) explained the difference in behaviour of the E_1 prime and standard alleles (Schwartz, 1964) by assuming that other non-allelic genes compete with the E_1 for its limited "activating" factor. Both esterase and phosphatase are non-specific enzymes and a number of non-allelic genes are found for each of them in maize. The assumption that other non-allelic genes compete with the AP_1 acid phosphatase for the limiting factor might explain the difference in activity between the F_2 homozygous AP_1^S/AP_1^S and AP_1^F/AP_1^F populations. If the limiting factor is distributed equally in the AP_1^S/AP_1^S and AP_1^F/AP_1^F populations and other non-allelic genes compete for the same limiting factor, the AP_1 allele with the higher competitive ability is expected to show higher average activity than the AP_1 allele with lower competitive ability. Both AP_1^S/AP_1^S lines *B*-55 and *N*-6 are better competitors than the AP_1^F/AP_1^F line *B*-53 (Table 1) and in both the *B*-55 × *B*-53 and *N*-6 × *B*-53 F_2 populations higher activity levels were found for the AP_1^S/AP_1^S than for the AP_1^F/AP_1^F homozygous plants (Table 4). On the other hand, the AP_1^F/AP_1^F line *B*-50 is a better competitor than the AP_1^S lines *N*-6 and E_1^S/E_1^S (Table 1). Accordingly, the AP_1^F/AP_1^F populations in the *N*-6 × *B*-50 and E_1^S/E_1^S × *B*-50 F_2 generation showed higher mean activity values than the AP_1^S/AP_1^S populations (Table 4). Moreover, *N*-6 is a better competitor than E_1^S/E_1^S (Table 1). When these two lines were crossed with *B*-50 the difference between the AP_1^S/AP_1^S and AP_1^F/AP_1^F F_2 populations was higher in the cross E^S/E^S × *B*-50 than in the cross *N*-6 × *B*-50.

Another possible way to explain the difference between the AP_1^S/AP_1^S and the AP_1^F/AP_1^F populations is by assuming linkage between the AP_1 gene and the gene which controls the level of activity (Efron, 1970a). This might explain the difference between the two populations in the *B*-55 × *B*-53 and *N*-6 × *B*-53 crosses. But, it would then be expected that in the other two crosses (*N*-6 × *B*-50 and E_1^S/E_L^S × *B*-50) the AP_1^S/AP_1^S populations would also show higher average activity levels than the AP_1^F/AP_1^F populations, since *N*-6 and E_1^S/E_1^S had higher activity levels than *B*-50. This was not the case, so the possibility of linkage between two genes as the only explanation for the differences between the AP_1^S/AP_1^S and the AP_1^F/AP_1^F populations must be rejected.

In the discussion above, the results were explained by the competition model proposed by Schwartz (1971) for the regulation of gene action. Only the use of a large number of crosses enable us to arrive at a certain general conclusion. Taking only one

hybrid at a time other possible explanations could be found. Therefore, if only two lines had been included in this study, a completely different conclusion might have been drawn, depending on the two lines selected.

Literature

1. Efron, Y.: Alcohol dehydrogenase in maize: Genetic control of enzyme activity. Science **170**, 751–753 (1970a). — 2. Efron, Y.: Tissue specific variation in the isozyme pattern of the AP_1 acid phosphatase in maize. Genetics **65**, 575–583 (1970b). — 3. Efron, Y.: Differences between maize inbreds in the activity level of the AP_1 controlled acid phosphatase. Biochem. Gent. **5**, 33–44 (1971a). — 4. Efron, Y.: Regulation of the activity of alcohol dehydrogenase isozymes during the development of the maize kernel. Molec. Gen. Genetics **111**, 97–102 (1971b). — 5. Hageman, R. H., Leng, E. R., Dudley, J. W.: A biochemical approach to corn breeding. Advanc. Agron. **19**, 45–86 (1967). — 6. Roose, E. E., Sarkissian, I. V.: Isocitric dehydrogenase and isocitric lyase in heterotic and nonheterotic maize (*Zea mays* L.). Crop Sci. **8**, 683–686 (1968). — 7. Salthe, S. N., Chilson, O. P., Kaplan, N. O.: Hybridization of lactic dehydrogenase *in vivo* and *in vitro*. Nature **207**, 723–726 (1965). — 8. Schwartz, D.: Tissue specificity and the control of gene action. In: Struktur und Funktion des genetischen Materials, pp. 201–203 (Erwin-Baur-Gedächtnisvorlesungen III, 1963). Berlin: Akademie-Verlag 1964. — 9. Schwartz, D.: Genetic control of alcohol dehydrogenase — a competition model for regulation of gene action. Genetics **67**, 411–435 (1971). — 10. Zieserl, J. F., Hageman, R. H.: Effect of genetic composition on nitrate reductase activity in maize. Crop Sci. **2**, 512–515 (1962).

Received March 3, 1972

Communicated by R. W. Allard

Dr. Y. Efron
Department of Cereal Crops
Israel Agricultural Research Organization
Neve-Ya'ar Experimental Station
P. O. Haifa (Israel)