IDENTIFICATION OF A GENETIC ELEMENT THAT CONTROLS THE ORGAN-SPECIFIC EXPRESSION OF *Adh1* IN MAIZE

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Manuscript received March 10, 1980
Revised copy received April 3, 1981

ABSTRACT

Allozyme balances serve as markers of quantitative behavior of electrophoretically distinguishable alleles. By the use of ADH Set I allozyme balances, it is demonstrated that all *Adh1-S/Adh1-F* individuals from more than 20 diverse *S/F* families exhibit a reciprocal correlation between *Adh1* quantitative behavior in two maize organs: the scutellum and primary root. Within an electrophoretic mobility class, the *Adh1* allele that is relatively underexpressed in the scutellum is relatively overexpressed in the primary root, and *vice versa*. Segregation tests prove that this "reciprocal effect" is the property of a *cis*-acting site that is closely linked to or within the *Adh1* structural gene, and it is not affected by diverse genetic backgrounds. Immunological and [^{3}H]-leucine incorporation experiments establish that *Adh1* quantitative variants differ in ADH1·ADH1 synthetic rates in the anaerobic primary root. The reciprocal-effect phenomenon suggests that the *cis*-acting loci controlling *Adh1* quantitative expression in each respective organ are at least in close proximity, or may share common DNA sequences. We discuss the possibility that the reciprocal-effect locus is a regulatory component of the *Adh1 cistron*.

AMONG the strategies used to investigate differential gene expression in higher organisms, genetical and biochemical studies on naturally occurring regulatory variants have proven particularly informative. Of special significance has been the identification of genetic elements that determine the relative expression of structural genes in a particular organ or at a specific developmental time. These genetic elements have been called temporal genes (PAIGEN and GANSCHOW 1965) and act either in *cis* or *trans* to the structural gene whose expression they affect (SCHWARTZ 1962, 1971; EFRON 1970; BOUBELIK *et al.* 1975; DICKINSON 1975, 1980; DICKINSON and CARSON 1979; PAIGEN *et al.* 1975; BREEN, LUSIS and PAIGEN 1977; ABRAHAM and DOANE 1978; LUSIS and WEST 1978). Two common features of these temporal variants are (1) their altered function seems to be specific to a particular developmental stage or organ, and (2) all were found in natural populations or laboratory lines, rather than following mutagenesis (FREELING and WOODMAN 1979).

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Naturally occurring maize alcohol dehydrogenase-1 (*Adh1* locus; ADH enzyme, E.C. 1.1.1.1) variants that specify electrophoretically distinguishable products have provided data on organ-specificity of allele action. Previous investigations on two *Adh1* electrophoretic alleles, *Adh1-1S* and *Adh1-1F* (abbrev. *1S* and *1F*) have shown that, in *1F/1S* hybrids, the *Adh1-1F* product predominates in the primary root, mesocotyl, pollen (SCHWARTZ 1971) and anaerobically induced primary roots (FREELING 1975); whereas, both are more equally expressed in the embryo (SCHWARTZ 1971). These two allelic variants specify clear differences in organ-specific expression.

Following the lead of SCHWARTZ (1971), we have quantified the balance between electrophoretically distinguishable allozymes specified by various naturally occurring *Adh1* alleles. ADH allozyme balances in *S/F* individuals were determined for two organs: the scutellum (embryonic storage organ of the kernel) and the anaerobically induced primary root. Since a scutellar slice suitable for an allozyme balance determination can be removed from a kernel without impairing germination, our allozyme balance studies can be extended to other organs of these same individuals.

Our results were quite unexpected. Quantitative differences among naturally occurring *Adh1* alleles map to the *Adh1* structural gene and act *cis* to it. Moreover, the *Adh1* allele that is relatively underexpressed in the scutellum is reciprocally overexpressed in the primary root, and *vice versa*. We do not find any relationship between ADH1·ADH1 protein differences and this *Adh1* organ-specific reciprocal expression. With these data, we argue that the quantitative *Adh1* variation measured involves regulatory components of the *Adh1* cistron.

MATERIALS AND METHODS

There are two unlinked genes specifying alcohol dehydrogenase enzymes in maize (SCHWARTZ 1966; FREELING and SCHWARTZ 1973): *Adh1* (on chromosome *1L*, SCHWARTZ 1971) and *Adh2* (on chromosome *4S*; DLOUHY and FREELING, unpublished). When both genes are expressed, three electrophoretically separable ADH dimers are produced: ADH1·ADH1 (Set I), ADH1·ADH2 (Set II) and ADH2·ADH2 (Set III). Set I is the major ADH (> 95%) in the scutellum and pollen. Subjecting seedlings to anaerobiosis results in the *de novo* synthesis of ADH enzymes in the primary root (FREELING 1973; SACHS and FREELING 1978). Thus, three ADH Set I allozymes can be visualized in electrophoretograms of scutellar extracts from *S/F* heterozygotes (Figure 1); whereas, six types of ADH enzymes will appear in anaerobic root electrophoretograms (Figure 2).

Lines and nomenclature: The lines used in these studies are listed in Table 1 according to the anodal migration rate of their ADH1·ADH1 dimers. The *Adh1* allele of each lines is designated by a number denoting the family and a letter denoting the electrophoretic genotype. *Adh1-F* isoalleles confer electrophoretically faster products than do *Adh1-S* isoalleles. The inbred *F* and inbred *S* lines developed by SCHWARTZ (1971) carry *Adh-1-1F* and *Adh1-1S*, respectively. The *Adh1-54S* allele was derived from EFRON's *Adh$_r$L*, *Adh1-S* line (EFRON 1970). Inbred *F*, inbred *S* and Efron *S* are available from this laboratory. The remaining lines were generously supplied by the Maize Genetics Cooperative.

Sample preparation: Individual scutellar slices (4 × 2 × 0.5 mm) were cut from dry kernels that were less than one year old. Each scutellar slice was macerated in a 1 × 1 cm cylindrical chamber containing 35 μl of extraction buffer: 10 mM Tris-HCl, pH 8.0 with 3 mM dithiothreitol (Calbiochem) (FREELING and SCHWARTZ (1973). A Whatman #3 filter paper (5 × 6 mm) was

TABLE 1

Adh1 *genotypes of the maize lines used in this study*

ADH1·ADH1 electrophoretic mobility	Line	*Adh1* designation
F	Inbred F	*—1F*
F	Red Pop	*—6F*
F	Maiz Chapolote	*—9F*
F	Ohio Yellow Pop	*—11F*
F	Papago Flour	*—12F*
F	South American Pop	*—13F*
F	R2	*—15F*
F	Zapalote Chico	*—21F*
F	Mo. Cob	*—24F*
F	Tama Flint	*—29F*
F	Super Gold Pop	*—33F*
F	A632	*—44F*
F	R177	*—45F*
F	Oh 43	*—48F*
S	Inbred S	*—1S*
S	Strawberry Pop	*—32S*
S	Hull-less Pop*	*—34S*
S	Tama-Flint, Knobless	*—35S*
S	W 23	*—52S*
S	Efron S	*—54S*

* Established from a line having both *Adh1-S* and *Adh1-F* alleles.

then placed into the chamber until saturated, blotted, inserted into a starch gel and subjected to electrophoresis. Each kernel was numbered and used in subsequent experiments.

Germination: Numbered kernels were soaked for 18 hr, spaced evenly on moist paper towels in covered glass trays and germinated without light at 27° and 85% humidity. After 2.5 to 3.5 days, seedlings with primary roots between 5.0 and 7.0 cm long were immersed completely under induction buffer: 5 mM Tris-HCl, pH 8.0 with 75 μg/ml chloramphenicol (FREELING and SCHWARTZ 1973). Anaerobiosis proceeded without light at 27°, using 25 ml of induction buffer per seedling.

Individual roots: Following anaerobic induction, the distal 5 cm portion of each primary root was removed, individually macerated in 1 × 1 cm cylindrical chambers containing 35 μl of extraction buffer. Again, a Whatman #3 filter paper was placed into each chamber, blotted and subjected to starch gel electrophoresis.

[^{3}H]-leucine incorporation experiments: Anaerobic induction and root extraction procedures were performed according to SACHS, FREELING and OKIMOTO (1980).

Pooled scutella: Scutellar slivers from dry kernels were macerated with mortar and pestle in appropriate volumes of extraction buffer. The brei was poured through miracloth and centrifuged at least twice to remove the lipid layer.

Pooled roots: Ten to 20 5-cm primary roots from 2.5 to 3.5-day-old seedlings were homogenized in extraction buffer (1 μg/mg root) with a mortar and pestle. The homogenate was centrifuged at 30,000 × g for 15 min and the resulting supernatant saved.

All tissue extractions took place at 4° with precooled buffers and equipment.

ADH enzyme assays and protein determinations: ADH activity was measured in a Hitachi Model 100–30 spectrophotometer, according to FREELING and SCHWARTZ (1973). A unit of ADH activity is defined as the amount of activity yielding an increase in optical density ($O.D._{340}$) of 0.001 per min. under our standard conditions. Protein was estimated by the Coomassie-blue method of BRADFORD (1976), with Bovine Serum Albumin, Fraction IV (SIGMA) used as a standard.

Starch gel electrophoresis and gel staining: Previously described (SCHWARTZ and ENDO 1966; FREELING 1973) electrophoretic and ADH staining methods have been further modified for the densitometric quantification of ADH Set I allozyme balances. The 11% w/v starch gels were stored for 4 to 10 hr at 4° before use. Three Whatman #3 filter papers (5 × 6 mm) saturated with tissue extract were inserted into each gel at a position 2 cm from the cathodal wick. Electrophoresis was carried out for 3 hr at 4° and a constant 250 volts. The gels were sliced horizontally in half in a cutting mold, which insured uniform thickness. The bottom halves were immersed in ADH-specific stain (SCHWARTZ and ENDO 1966) for two hr in the dark. After the stain was removed, the gels were stored in water overnight before densitometric inspection.

Native-SDS PAGE gels and fluorography: [^{3}H]-leucine-labeled anaerobic primary root extracts were subjected to native-SDS two-dimensional polyacrylamide gel electrophoresis by the methods of SACHS, FREELING and OKIMOTO (1980). Fluorography of dried gels was by the method of BONNER and LASKEY (1974), using Kodak SB5 film.

Two-dimensional immunoelectrophoresis: Two-dimensional immunoelectrophoresis followed the method of SCHWARTZ (1972). Referring to Figures 4 and 5, a Whatman #3 filter paper (2.2 × 0.5 cm) soaked in crude scutellar or anaerobic root extract was inserted 2 cm from the cathodal wick into slot x-y of an 11% starch gel (18 × 13 × 0.5 cm). The first electrophorectic dimension was run for 3 hr at 4° and constant 250 volts. A 5 × 0.5 cm Whatman #1 filter paper saturated with unfractionated anti-ADH antiserum and a 5 × 0.5 cm Whatman #3 filter paper soaked in *Adh1-Ct* crude scutellar extract were inserted into slots a-b and c-d, respectively. With the a-b slot parallel to the anodal wick, the starch gel was subjected to electrophoresis in the second dimension for 3½ hr at 4° and constant 250 volts. The starch gel portion flanked by the a-b and c-d slots was sliced in half and stained for ADH activity. The ADH immunoprecipitate stains blue.

ADH antiserum was collected from New Zealand white rabbits that were immunized with purified ADH1-1S·ADH1-1S enzyme. ADH1·ADH1 protein was purified from dry kernels by the procedure of KELLEY and FREELING (1980).

Densitometry: The stain intensities of the ADH Set I allozymes were determined by scanning gels in a Transidyne General 2970 integrating densitometer. The overlapping curves of the Set I profile were separated and their area established by electronic integration. The relative stain intensity of an allozyme is reported as its percent contribution to the Set I stain intensity. In statistical analysis of allozyme patterns, the relative staining intensities of a given allozyme (*e.g.*, percent S.S) were transformed into angles according to the relation:

$$\text{angle} = \arcsin \sqrt{\text{percentage}} .$$

Among *S/F* siblings, the mean percent contribution (upper 95% confidence interval) of an allozyme was calculated from ten or more samples. Since the asymmetrical confidence limits for any mean percent contribution varied less than 5%, only the upper confidence limit (L_2) is reported.

RESULTS AND CONCLUSIONS

ADH allozyme balance variants: We searched maize lines for *Adh1* relative-activity variants by using the following rationale. *Adh1/Adh1-F* individuals produce three Set I allozymes: the *S·S* and *F·F* homodimers and the *S·F* heterodimers (Figure 1). If both *Adh1* electrophoretic alleles are equally expressed and their products have equal specific activities, then a 1:2:1 (S·S:S·F:F·F) ratio of ADH allozymes will result. If, for some reason, the expression of the *Adh1* alleles are not equal, then the ADH allozyme ratios will be skewed toward those dimers that contain products of the more "active" *Adh1* allele. Thus, the relative activity of any pair of *Adh1-S* and *Adh1-F* alleles in an *S/F* heterozygote can be determined by electrophoretically separating the ADH Set I allozymes in gels and densitometrically quantifying the intensity of the three ADH activity bands.

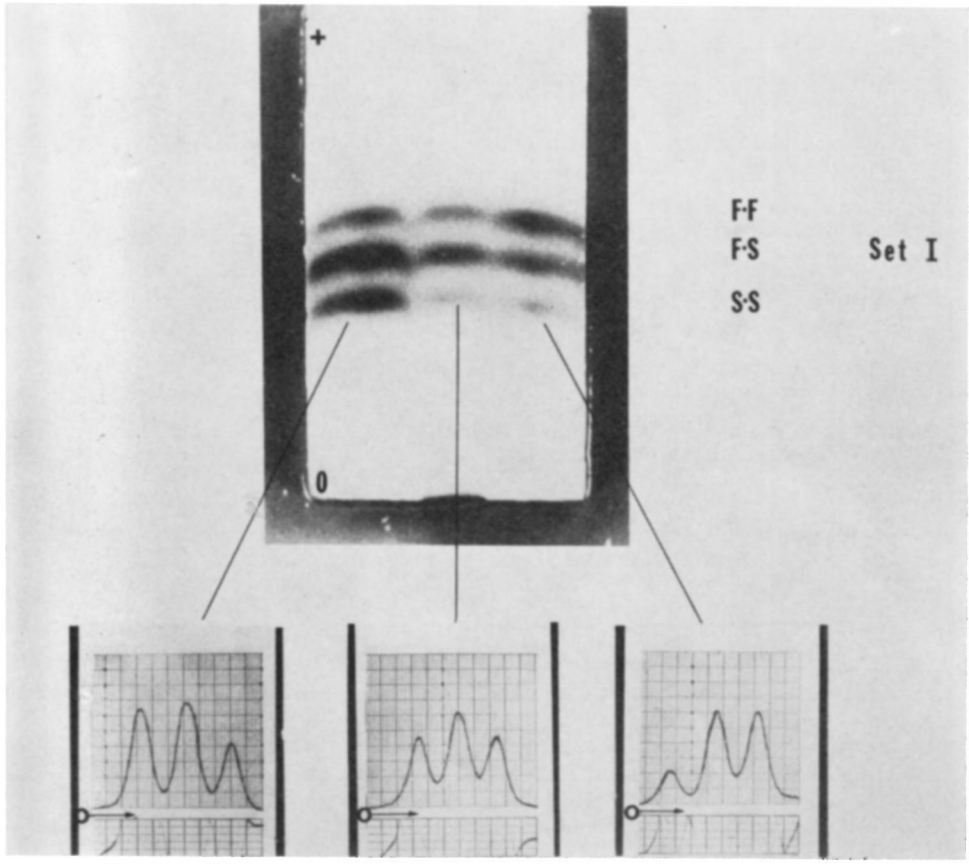

FIGURE 1.—Electrophoretograms and corresponding densitometric traces of scutellar extracts prepared separately from three F_1 *S/F* families; from left to right: *1F/1S*, *1S/33F* and *54S/33F*. The starch gel was specifically stained for ADH activity. The 0 denotes the origin and the + denotes the anode.

Before using this rationale, we needed to determine whether the densitometric quantification of ADH Set I allozyme balances was reproducible. The significance of genotype, sample preparation and gel preparation upon the variation in ADH allozyme belance was assessed in an analysis of variance experiment. Scutellar slices from inbred *F*/inbred *S* (*1F/1S*) siblings were chosen in order to minimize variation due to genotype. ADH was extracted from pooled slices and 36 individual slices. Six gel sets (six gels per set) were prepared separately. Variation between pooled and individual scutellar extracts may be attributed to both genotype and experimental error. Variation among the gel sets and within gel sets will be due to experimental error only. The results were that genotype, sample preparation and gel preparation did not have significant effects on total variation.

To further minimize variation in allozyme balances from scutellar and anaerobic root extracts, extraction conditions were adjusted to yield 1250–3500

and 1800–3500 units ADH/ml, respectively. Starch gels were left in ADH activity stain for a period of 100–180 minutes. Within this sample activity range and staining period, ADH Set I allozyme balances of scutellar or anaerobic root extracts from *1F/1S* individuals were not significantly different ($p > 0.05$) in a *t*-test. ADH allozyme balances are considered to be similar when the mean relative staining intensities of electrophoretically identical homodimers are not significantly different, *t*-test ($p > 0.05$).

The scutellar allozyme balances of various F_1, *S/F* hybrids are listed in Table 2. Three general types of balances were found (Figure 1): either the S·S homodimer stain intensity was greater than, equal to or less than F·F homodimer stain intensity. The variance of the relative staining intensities of F·F homodimers among the *S/F* families were not significantly different ($p > 0.05$) in *F* tests when compared to the percent F·F variance from the *1F/1S* family. This suggests that each family is homogeneous for a particular ADH allozyme pattern. This is not surprising considering that these *S/F* families are single-cross hybrids derived from inbred lines.

Based on these scutellar allozyme balances, there appear to be *F* alleles whose quantitative expression is less than, equal to or greater than that of *S* alleles, and

TABLE 2

ADH Set I allozyme balances in the scutellum of various Adh1-S/Adh1-F F_1 *Hybrids*

Adh1 genotype	Percentage of Set I stain intensity (± 95% confidence interval*) S·S	S·F	F·F	Sample size
1F/32S	41.32 ± 1.59	39.25 ± 1.34	19.37 ± 1.18	18
48F/1S	37.04 ± 1.52	39.42 ± 0.73	23.47 ± 1.08	18
1F/34S	37.04 ± 2.20	39.21 ± 1.17	24.36 ± 2.20	17
44F/1S	37.37 ± 1.74	41.72 ± 2.23	20.75 ± 2.37	15
1F/1S	38.85 ± 0.60	39.33 ± 0.91	21.68 ± 1.30	36
52S/1F	37.58 ± 1.10	39.30 ± 1.35	22.65 ± 1.57	18
13F/1S	37.62 ± 1.35	41.40 ± 1.10	20.89 ± 2.25	15
35S/1F	38.34 ± 1.39	38.72 ± 0.74	22.94 ± 1.79	17
29F/1S	37.28 ± 1.67	41.78 ± 1.32	22.01 ± 2.25	15
24F/1S	36.32 ± 1.54	39.64 ± 0.57	23.93 ± 1.82	18
1S/33F	30.82 ± 1.89	40.86 ± 0.51	28.24 ± 1.26	18
54S/11F	29.65 ± 1.87	43.25 ± 1.33	27.07 ± 1.20	18
1S/21F	32.05 ± 1.53	38.77 ± 0.94	29.18 ± 1.71	17
52S/33F	31.87 ± 2.03	39.20 ± 1.18	28.86 ± 1.49	15
54S/1F	27.89 ± 0.93	41.49 ± 0.72	30.53 ± 1.30	36
1S/9F	31.27 ± 1.07	40.02 ± 1.17	28.65 ± 1.25	18
6F/54S	29.65 ± 1.87	43.25 ± 1.33	27.07 ± 1.20	18
45F/1S	28.11 ± 1.62	39.49 ± 0.36	31.24 ± 1.66	17
15F/1S	27.01 ± 1.56	41.70 ± 0.53	31.59 ± 1.93	15
12F/54S	18.05 ± 2.00	39.26 ± 1.24	41.99 ± 2.23	18
54S/33F	20.25 ± 1.70	42.66 ± 1.02	37.06 ± 1.79	18

* The mean percent contribution ± 95% confidence interval of the S·S and F·F homodimers and S·F heterodimer to the Set I strain intensity was calculated from arcsine transformed percentages of individual scutella, as detailed in MATERIALS AND METHODS.

vice versa. Thus, the relative expression of an *Adh1* quantitative variant is not associated with the electrophoretic mobility of the *Adh1* gene product. The relative-activity relationships among *Adh1* alleles are shown in Table 3. In all pairwise combinations of *S* and *F* alleles constructed to date (18 *S/F* families, in addition to those in Table 2), the *Adh1* activity relationship has not been violated, irrespective of genetic background.

Reciprocal effect: After the *S/F* individuals had their scutellar allozyme balances quantified, they were germinated and subjected to a 24-hour anerobic induction treatment. Set I ADH allozyme balances from these individuals are compared in Table 4. Even though every allozyme balance was skewed towards the ADH enzymes containing ADH1-F subunits, there were at least three types of balances (Figure 2). Moreover, comparisons of anaerobic root and scutellar balances (see Table 4) revealed that the *S/F* family that showed the smallest amount of a homodimer in one organ concomitantly exhibited the greatest amount of this homodimer in the other organ, and *vice versa*. In order to define this "reciprocal effect" quantitatively, we determined the relationship between the relative number of *ADH1-S* subunit molecules (percent ADH1-S) in each organ of every *S/F* family. By assuming that any ADH1-S subunit equals any ADH1-F subunit in specific enzyme under our reaction conditions—an assumption we will soon prove correct—we calculated percent ADH1-S directly as percent S·S activity + ½ percent S·F activity. Percent ADH1-S from anaerobic primary roots calculated from Set I allozyme balances was equal to percent ADH1-S in Set II allozymes for every *S/F* family in Table 4 (data not shown). Thus, in anaerobic primary roots, *Adh2* expression does not influence ADH Set I allozyme balances. As shown in Figure 3, every *S/F* family obeys a common relationship, $r = -0.89$, between ADH allozyme balances in anaerobic roots and scutella. Among the hypotheses that can be postulated to explain this "reciprocal effect", several can be eliminated by the following studies characterizing *Adh1* quantitative behavior in both organs.

TABLE 3

Relative scutellar expression of Adh1 *alleles of diverse origin**

HIGH		LOW	
9F	*1S*	*1F*	
12F	*30S*	*6F*	
15F	*32S*	*11F*	
21F	*34S*	*13F*	
33F	*35S*	*24F*	*54S*
45F	*52S*	*29F*	
		44F	
		48F	

* The relative expression of an *Adh1* allele was determined from scutellar ADH Set I allozyme balances of various F_1 *S/F* families (Table 2). Within each group, the *Adh1* alleles are ordered according to the electrophoretic mobility of their product ADH subunits, "S" (slow) or "F" (fast).

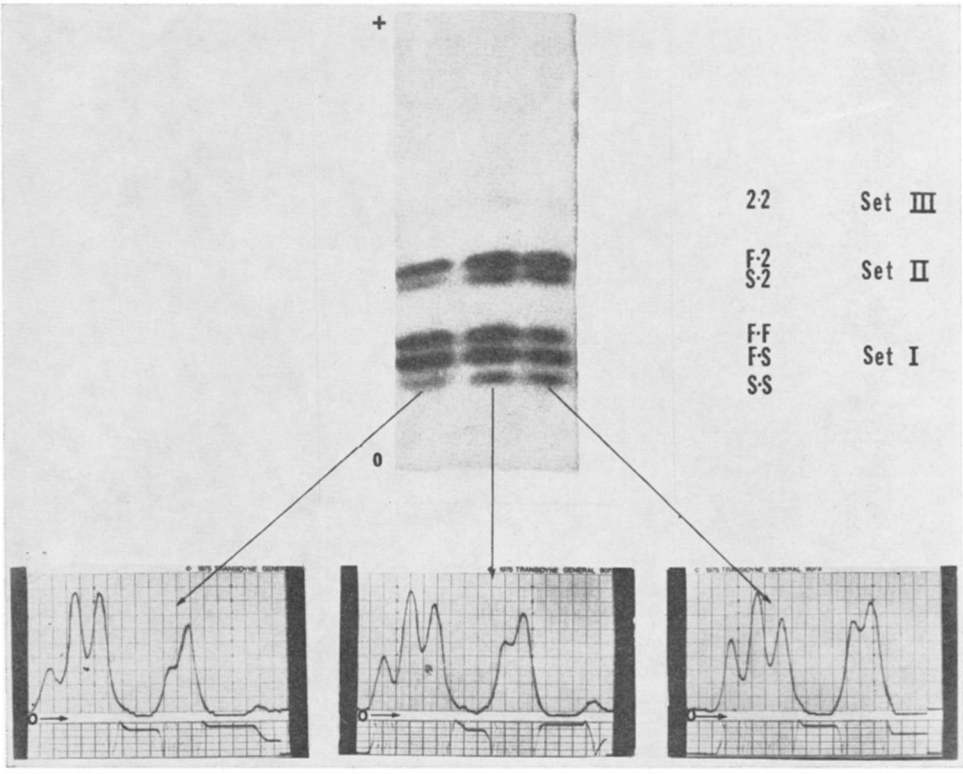

FIGURE 2.—Electrophoretograms and corresponding densitometric traces of anaerobic primary root extracts prepared separtely from three F_1 *S/F* families; from left to right: *1F/1S*, *1S/33F* and *54S/33F*. The starch gel was specifically stained fro ADH activity. The 0 denotes the origin and the + denotes the anode.

Segregation of Adh1 *quantitative expression*: Since ADH Set I allozyme ratios can be measured in *S/F* heterozygotes, our test for linkage of *Adh1* quantitative expression with the ADH1 electrophoretic mobility site (*i.e.*, the *Adh1* structural gene) is somewhat indirect. If *Adh1* quantitative expression is controlled by genetic factors tightly linked to the *Adh1* structural gene, then the *S/F* progeny generated from a cross involving an F_1 *S/F* individual should express similar ADH allozyme patterns. If, on the other hand, *Adh1* quantitative expression is controlled by loci unlinked or loosely linked to the *Adh1* structural gene, then several ADH allozyme patterns may appear among the *S/F* progeny. To distinguish between these and other possibilities, we determined the segregation of ADH allozyme patterns among *S/F* progeny generated from selected crosses (Tables 5 and 6). It is clear that *Adh1* quantitative expression in scutella and anaerobic roots segregated with the *Adh1* electrophoretic mobility site. Pairwise *t-tests* showed that, in every case, the ADH allozyme balances of *S/F* progeny were not significantly different ($p > 0.05$) from those of F_1 *S/F* families with comparable *Adh1* genotypes. Additionally, the variances of the relative staining intensities of F·F homodimers for each group of *S/F* siblings in Tables 5 and 6,

TABLE 4

ADH Set I allozyme balances in 5-cm primary roots of 24-hour anaerobically induced Adh1-S/Adh1-F F_1 *seedlings*

Adh1 genotype	Percentage of Set I stain intensity (± 95% confidence interval*) S·S	S·F	F·F	Sample size	Scutellar allozyme balance (% S·S)†
1F/32S	9.05 ± 0.45	41.04 ± 1.21	49.91 ± 2.07	28	41.32
48F/1S	10.55 ± 1.71	45.57 ± 1.43	43.38 ± 1.34	23	37.04
1F/34S	10.80 ± 1.71	42.58 ± 1.80	46.62 ± 2.08	15	37.04
44F/1S	11.18 ± 1.42	43.51 ± 1.50	45.31 ± 1.81	13	37.37
1F/1S	11.25 ± 1.56	45.00 ± 1.08	43.76 ± 1.34	27	38.85
52S/1F	11.50 ± 1.21	45.40 ± 1.08	43.10 ± 1.82	17	37.58
13F/1S	11.55 ± 1.73	43.90 ± 1.58	44.55 ± 1.73	22	37.62
35S/1F	11.81 ± 1.14	41.61 ± 1.20	46.58 ± 0.83	29	38.34
29F/1S	12.26 ± 1.53	43.83 ± 1.47	43.91 ± 1.47	19	37.28
24F/1S	12.49 ± 1.33	41.47 ± 1.50	46.04 ± 1.93	18	36.32
1S/33F	14.42 ± 1.37	45.32 ± 2.08	40.24 ± 2.21	29	30.82
54S/11F	14.47 ± 1.62	47.10 ± 1.40	38.43 ± 1.53	14	31.63
1S/21F	14.52 ± 1.72	46.22 ± 1.65	39.29 ± 2.06	22	32.05
52S/33F	14.59 ± 1.00	45.39 ± 1.05	40.02 ± 1.18	16	31.87
54S/1F	14.84 ± 0.94	48.40 ± 1.10	36.74 ± 1.32	27	27.89
1S/9F	15.65 ± 1.87	46.48 ± 2.00	37.67 ± 2.20	12	31.27
6F/54S	15.71 ± 1.21	46.44 ± 1.16	37.85 ± 1.31	12	29.65
45F/1S	16.76 ± 1.20	45.25 ± 1.05	37.99 ± 1.10	30	28.11
15F/1S	16.78 ± 2.29	46.20 ± 2.12	37.02 ± 2.33	22	27.01
12F/54S	22.08 ± 2.41	44.74 ± 2.16	33.18 ± 2.33	16	18.05
54S/33F	22.52 ± 1.65	42.88 ± 1.40	34.60 ± 1.65	18	20.25

* The mean percent contribution ± 95% confidence interval of the S·S and F·F homodimers and S·F heterodimer of the Set I strain intensity was calculated from arcsine transformed percentages of individual primary roots, as detailed in MATERIALS AND METHODS.

† From Table 2.

respectively, were not significantly different ($p > 0.05$) from those of F_1 *S/F* families as indicated by *F* tests. Further, the data in Table 6 demonstrate that ADH Set I allozyme balance variation is not associated with *Adh2* variation, since *Adh2* is unlinked to *Adh1* (FREELING and SCHWARTZ 1973). We conclude from these data that all four *Adh1* quantitative alleles tested (*1F*, *33F*, *1S* and *54S*) reflect a polymorphism at a *cis*-acting locus close to or within the *Adh1* structural gene.

A more direct test of the *cis*-acting nature of *Adh1* quantitative expression in scutella and anaerobic roots comes from the evidence presented below. Siblings generated by the cross of *1S/33F* × *Ct/Ct* were analyzed for their ADH allozyme balances. Since *Adh1-Ct* produces a dimeric product with an electrophoretic mobility greater than S·S or F·F homodimers (SCHWARTZ 1966), both *1S/Ct* and *33F/Ct* individuals will produce three electrophoretically separable ADH Set I allozymes. Assuming (proved in a later section) that these ADH allozymes have equal specific activities in scutella and anaerobic roots, respectively, the percentage contribution of an ADH1 subunit to a Set I allozyme balance can be calculated directly from allozyme activity ratios. The *cis*-acting nature of

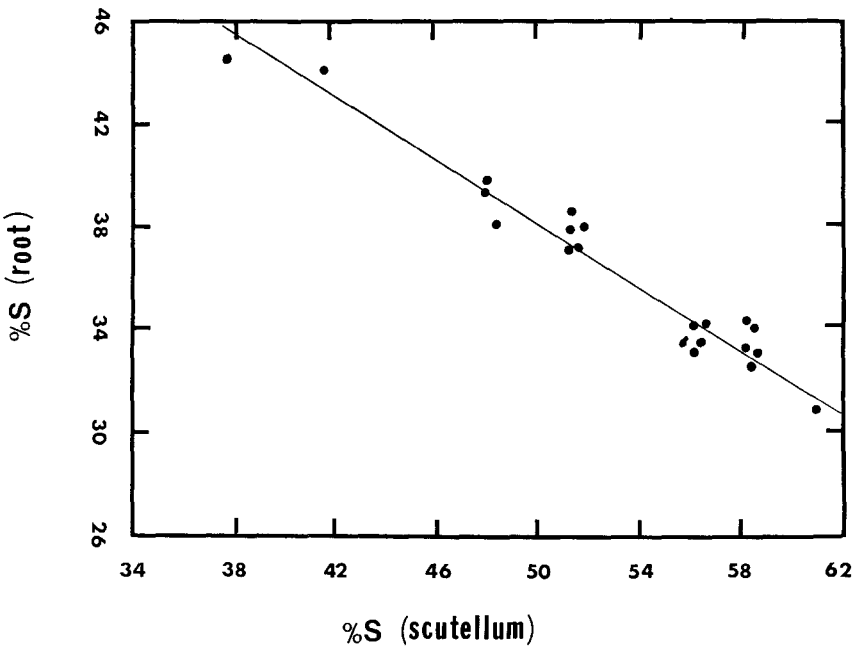

FIGURE 3.—% ADH1-S subunits in anaerobic primary roots *vs.* % ADH1-S subunits in the scutellum of various *S/F* families. % ADH1-S was calculated directly from ADH Set I allozyme activity ratios (Tables 2 and 4), by the following relationship: % ADH1-S = % S·S + ½ % S·F. Each point represents a different *S/F* family.

Adh1 quantitative expression predicts the following relationship: the ratio of ADH1-33F subunits *vs.* ADH1-Ct subunits in *33F/Ct* organs divided by ADH1-1S subunits *vs.* ADH1-Ct subunits in *1S/Ct* organs will be equal to the ratio of ADH1-33F subunits *vs.* ADH-1-1S subunits in the identical organs of *33F/1S* individuals. As shown in Table 7, the predicted and observed ratios agree remarkably well for both the scutella (1.02 *vs.* 1.05) and anaerobic root (1.77 *vs.* 1.70). These data reaffirm our conclusion that the genetic element controlling *Adh1* quantitative expression in the scutellum and anaerobic primary root behaves autonomously and acts in *cis* only. Furthermore, these results demonstrate that the quantitative expression of the *1F*, *33F*, *1S*, *54S* and *Ct* alleles is not altered (*e.g.*, paramutated) when passed through diverse genetic backgrounds.

Location of Adh1 *quantitative locus*: It was clear from the above segregation tests that there were no exceptional ADH allozyme balances among the 252 primary roots. An exceptional allozyme pattern in an *S/F* heterozygote would be expected if there were either a reciprocal recombination between a quantitative site and an electrophoretic site or a conversion of either site. Such a recombinant chromosome, when paired with either an *Adh1-S* or *Adh1-F* allele, would help to produce within the anaerobic primary root an ADH allozyme balance skewed more towards the S·S rather than the F·F homodimer. Since no aberrant allozyme balances were found, we conclude that the *cis*-acting *Adh1*

TABLE 5

Scutellar ADH Set I allozyme balances of S/F heterozygotes from selected crosses involving F_1 S/F heterozygotes

Cross	*Adh1* genotype*	Predicted % S·S†	Percentage of Set I stain intensity (± 95% confidence interval‡)			Sample size§
			S·S	S·F	F·F	
1F/1S × *1F/1F*	*1S/1F*	38.85	37.45 ± 0.97	37.85 ± 1.13	24.70 ± 1.79	24:48
1S/1S × *1F/1S*	*1S/1F*	38.85	38.89 ± 1.67	38.35 ± 0.72	22.81 ± 1.23	22:48
1S/33F × *1F/1F*	*1S/1F*	38.85	35.83 ± 1.19	41.56 ± 1.32	22.64 ± 2.23	25:54
1S/1S × *54S/1F*	*1S/1F*	38.85	35.32 ± 1.71	39.45 ± 1.27	25.29 ± 1.56	23:54
1S/33F × *33F/33F*	*1S/33F*	30.82	28.90 ± 1.89	39.95 ± 1.20	31.19 ± 1.85	29:54
1S/33F × *1S/1S*	*1S/33F*	30.82	29.20 ± 1.17	41.30 ± 0.72	29.44 ± 1.26	22:48
33F/33F × *1F/1S*	*1S/33F*	30.82	26.87 ± 2.28	42.25 ± 1.36	27.05 ± 1.34	31:54
54S/33F × *1S/1S*	*1S/33F*	30.82	31.89 ± 2.43	38.77 ± 1.16	29.83 ± 1.82	15:34
54S/1F × *1F/1F*	*54S/1F*	27.89	27.97 ± 1.10	40.43 ± 0.86	31.55 ± 0.69	52:106
54S/1F × *54S/54S*	*54S/1F*	27.89	27.43 ± 1.90	41.76 ± 0.62	30.87 ± 1.14	27:54
1F/1S × *54S/54S*	*1F/54S*	27.89	30.48 ± 2.55	42.45 ± 1.36	27.05 ± 1.34	31:54
54S/33F × *1F/1F*	*54S/1F*	27.89	27.13 ± 1.58	42.02 ± 0.91	30.85 ± 2.00	28:54
54S/33F × *33F/33F*	*54S/33F*	20.25	20.23 ± 2.01	41.42 ± 1.46	38.31 ± 1.79	23:51
54S/33F × *54S/54S*	*33F/54S*	20.25	19.09 ± 1.53	40.76 ± 0.77	40.10 ± 1.11	30:54
54S/1F × *33F/33F*	*54S/33F*	20.25	22.56 ± 1.65	40.00 ± 1.39	37.36 ± 2.55	28:54
29F/1S × *1S/1S*	*29F/1S*	37.28	38.62 ± 1.56	37.52 ± 0.81	23.85 ± 1.68	18:36
15F/1S × *1S/1S*	*15F/1S*	27.01	28.54 ± 1.12	40.47 ± 0.58	31.00 ± 0.83	24:54
45F/1S × *1S/1S*	*45F/1S*	28.11	28.32 ± 2.32	40.79 ± 1.01	31.21 ± 1.35	16:36

* *Adh1* genotype of *Adh1-S/Adh1-F* individuals only.

† % S·S value predicted if Set I allozyme balances segregate with ADH1 electrophoretic mobility.

‡ The mean percent contribution ± 95% confidence interval of the S·S and F·F homodimers and S·F heterodimer to the Set I stain intensity was calculated from arcsine transformed percentages of individual *S/F* scutella, as outlined in MATERIALS AND METHODS.

§ Sample size is reported as the number of *S/F* individuals *vs.* total sample.

TABLE 6

Anaerobic root ADH Set I allozyme balances of S/F heterozygotes obtained from selected crosses involving F_1 S/F hybrids*

Cross	*Adh1* genotype*	Predicted % S·S†	Percentage of Set I stain intensity (± 95% confidence interval‡)			Sample size§
			S·S	S·F	F·F	
1F/1S × *1F/1F*	*1S/1F*	11.25	12.34 ± 1.44	40.88 ± 1.60	40.78 ± 2.10	22
1S/1S × *1F/1S*	*1S/1F*	11.25	10.24 ± 2.43	44.10 ± 2.20	45.66 ± 2.21	13
1S/33F × *1F/1F*	*1S/1F*	11.25	12.78 ± 1.47	44.30 ± 2.59	42.92 ± 1.58	12
1S/1S × *54S/1F*	*1S/1F*	11.25	11.07 ± 1.89	43.39 ± 1.71	45.54 ± 1.93	19
1S/33F × *33F/33F*	*1S/33F*	14.42	13.83 ± 1.49	47.74 ± 1.30	38.43 ± 1.40	26
1S/33F × *1S/1S*	*33F/1S*	14.42	13.65 ± 1.95	46.90 ± 1.80	39.45 ± 1.98	11
33F/33F × *1F/1S*	*33F/1S*	14.42	13.01 ± 2.24	47.93 ± 2.00	39.06 ± 2.14	12
54S/33F × *1S/1S*	*33F/1S*	14.42	13.85 ± 1.95	45.33 ± 2.00	40.82 ± 2.53	13
54S/1F × *54S/54S*	*1F/54S*	14.84	15.33 ± 2.11	45.41 ± 1.81	39.26 ± 2.01	12
1F/1S × *54S/54S*	*1F/54S*	14.84	16.74 ± 2.25	45.06 ± 2.20	38.20 ± 2.07	15
54S/33F × *1F/1F*	*54S/1F*	14.84	15.30 ± 2.02	44.50 ± 1.70	40.20 ± 1.53	10
54S/33F × *33F/33F*	*54S/33F*	22.52	19.38 ± 2.64	47.57 ± 2.20	33.05 ± 2.26	10
54S/33F × *54S/54S*	*33F/54S*	22.52	20.22 ± 2.31	45.33 ± 1.50	34.45 ± 1.44	13
54S/1F × *33F/33F*	*54S/33F*	22.52	20.12 ± 2.04	47.18 ± 1.52	32.70 ± 1.57	17

* 2.5- to 3.5-day-old seedlings with 5-cm primary roots were anaerobically induced for 24 hours. These *S/F* individuals had been previously analyzed for their scutellar ADH allozyme balances (see Table 4).

† *Adh1-S/Adh1-F* individuals only.

‡ % S·S value predicted if ADH Set I allozyme balance segregates with ADH1 electrophoretic mobility.

§ The mean percent contribution ± 95% confidence interval of the S·S and F·F homodimers and the S·F heterodimer to the Set I strain intensity was calculaed from arcsine transformed percentages of individual primary roots, as detailed in MATERIALS AND METHODS.

TABLE 7

ADH Set I allozyme balances in two maize organs of 1S/Ct *and* 33F/Ct *individuals generated from the cross* 1S/33F × Ct/Ct

Adh1 genotype	Organ	Percentage of Set I stain intensity (+ 95% confidence interval)*			% 1S† / % Ct (A)	% 33F† / % Ct (B)	B/A‡ Calc.	B/A‡ Obs.
		% S·S	% S·Ct	% Ct·Ct				
1S/Ct	scutellum	34.60 ± 1.42	41.62 ± 1.82	23.78 ± 1.21	1.24			
	root	10.00 ± 1.62	44.91 ± 1.85	45.04 ± 2.23	0.48			
		% F·F	% F·Ct	% Ct·Ct				
33F/Ct	scutellum	34.97 ± 1.33	41.54 ± 1.09	23.49 ± 1.65		1.26		
	root	24.72 ± 1.43	42.51 ± 1.18	32.87 ± 1.15		0.85		
1S/33F	scutellum						1.02	1.05
	root						1.77	1.70

* The mean percent contribution ± 95% confidence interval of each allozyme to the Set I stain intensity was calculated from arcsine transformed percentages, as detailed in MATERIALS AND METHODS.

† The ratio of ADH1 subunits, calculated from allozyme balances (see text).

‡ The calculated B/A ratio was determined from the values reported above; the observed ratios were determined from scutellar and anaerobic root allozyme balances of *1S/33F* hybrids (see Tables 2 and 4).

quantitative locus controlling anaerobic-root quantitative expression is no farther than 0.45 map units away from the *Adh1* structural gene and might be much closer.

Estimating the location of the *Adh1* quantitative site(s) that control *Adh1* expression in the scutellum proved to be more difficult. The three types of scutellar allozyme balances (Figure 2) are not skewed greatly towards any particular homodimer. Thus, to prove that an exceptional allozyme balance among F_2 *S/F* progeny was a result of a previous recombinational event would require a strict test, such as flanking-marker exchange. Unfortunately, the lines used in this study did not have genetic markers flanking *Adh1*. Construction of such stocks might well have disrupted the *Adh1* chromosome regions that we hoped to assess.

Biochemical studies on ADH allozyme balances: The *cis*-acting nature of *Adh1* quantitative variation emphasizes the possibility that *Adh1* structural gene variation underlies the polymorphism in ADH allozyme balances in either the scutellum or anaerobic primary root. One might expect that the balances

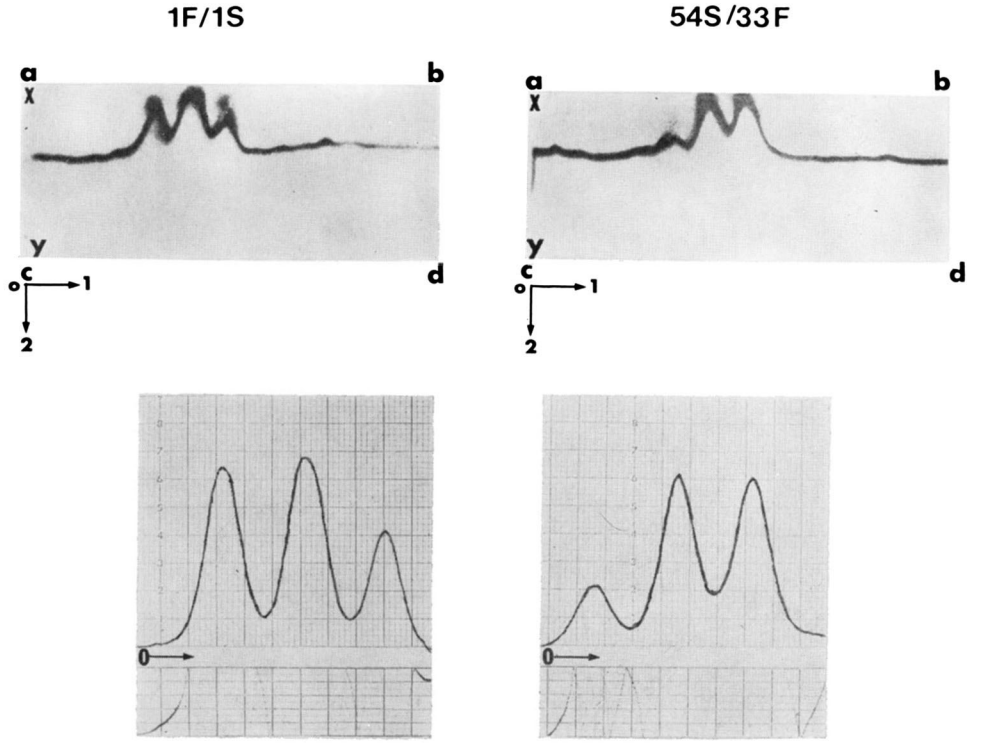

FIGURE 4.—(A) Two-dimensional immunoelectrophoretic profiles of scutellar extracts prepared from either *1F/1S* or *54S/33F* individuals. x-y marks position of the scutellar extract samples before electrophoresis in the first dimension, a-b marks the position of the ADH antiserum, and c-d marks the position of the ADH1-Ct·ADH1-Ct marker enzyme before electrophoresis in the second dimension. The arrow denotes the direction of the anode in dimensions 1 and 2. (B) Densitometric traces of electrophoretograms of scutellar extracts prepared from *1F/1S* and *54S/33F* individuals, respectively. Starch gels were stained specifically for ADH activity. The 0 denotes the origin and the + indicates the anodal direction.

result from specific activity or *in vivo* degradation rate differences between ADH1·ADH1 enzymes. The experiments testing these hypotheses were performed on two *S/F* hybrids, (*1F/1S* and *54S/33F*), with clearly different allozyme balances.

Since the precipitation peak heights in two-dimensional immunoelectrophoretic profiles correspond to the relative number of ADH enzyme molecules (SCHWARTZ 1972), the specific activities of ADH allozymes can be examined indirectly by comparing immunoelectrophoretic profiles with the densitometric traces of ADH activity gels. Comparisons of ADH allozyme balances in the scutellum and anaerobic root of *1F/1S* and *54S/33F* individuals, respectively, are shown in Figures 4 and 5. Because the immunoelectrophoretic and densitometric trances are superimposable, it can be concluded for these *S/F hybrids* that the ADH allozyme activity ratios in starch gels directly reflect the relative number of ADH1·ADH1 molecules. Furthermore, these results suggest that the ADH1·ADH1 enzymes containing the products of the *1F*, *33F*, *1S* or *54S* alleles have identical or near identical specific activities in either scutellar or anaerobic root extracts. Thus, it seems likely that these four *Adh1* quantitative variants arise from variation in a mechanism(s) that controls ADH1·ADH1 production.

The relative number of S and F subunits synthesized by *S/F* anaerobic primary roots can be determined directly by [^{3}H]-leucine incorporation experiments. (SACHS and FREELING 1978; FERL, DLOUHY and SCHWARTZ 1979). Primary roots were exposed to [^{3}H]-leucine for the last five hours of a 24-hour

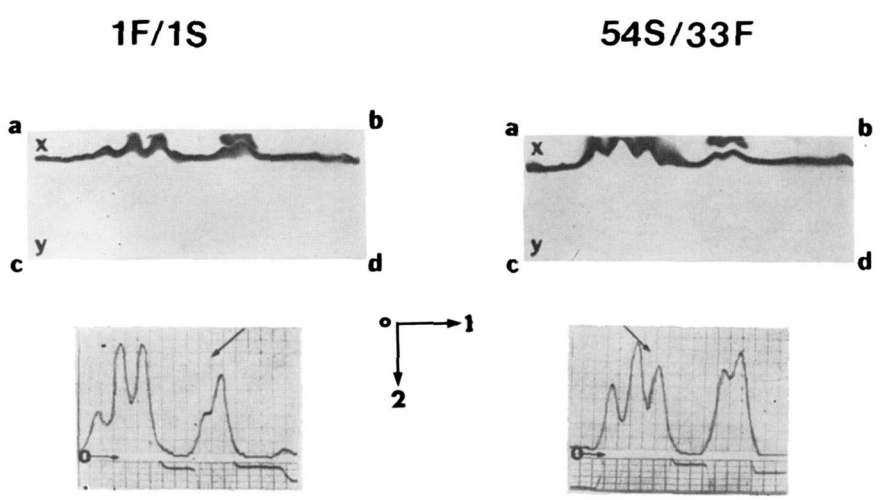

FIGURE 5.—(A) Two-dimensional immunoelectrophoretic profiles of anaerobic primary root extracts prepared from either *1F/1S* or *54S/33F* individuals. x-y marks the position of the anaerobic root extract samples before electrophoresis in the first dimension, a-b marks the position of the ADH antiserum, and c-d marks the position of the ADH1-Ct·ADH1-Ct marker enzyme before electrophoresis in the second dimension. The arrows denote the direction of the anode in dimensions 1 and 2. (B) Densitometric traces of electrophoretograms of anaerobic primary root extracts prepared from *1F/1S* and *54S/33F* individuals, respectively. The 0 denotes the origin and the + indicates the anodal direction. The gels were stained specifically for ADH activity.

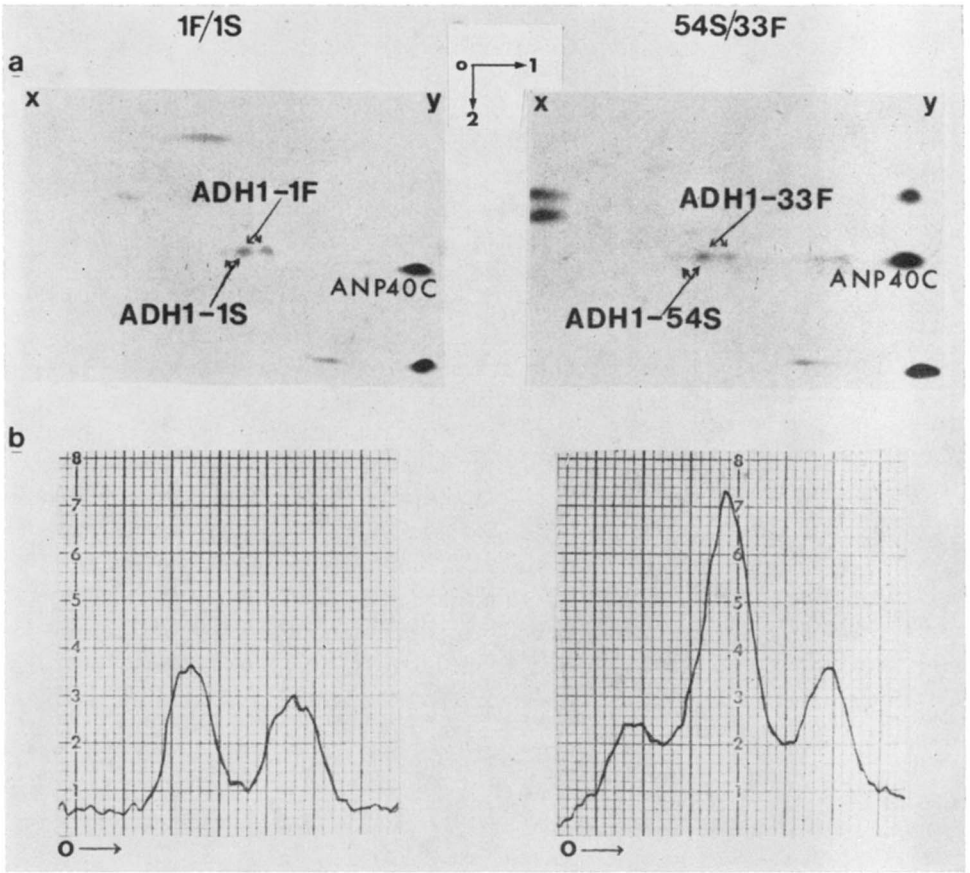

FIGURE 6.—(A) Fluorograms of native-SDS, two-dimensional gels of anaerobic root extracts prepared from either *1F/1S* or *54S/33F* primary roots. The primary roots were exposed to 100 μCi [^{3}H]- leucine for the last five hrs of a 24-hr anaerobic induction period. Only the region of the 2-D gel containing ADH1 and ADH2 polypeptides is shown here. ADH subunits are indicated along with the landmark protein ANP40C (SACHS, FREELING and OKIMOTO 1980). x-y marks the axis where the first dimension, native polyacrylamide gel was layered on top of the SDS gel. The arrows denote the direction of the anode in dimensions 1 and 2; 0 denotes the origin. (B) Densitometric traces of the respective fluorograms. Only ADH Set I allozymes shown here. The 0 denotes the origin and the + indicaes the anode in dimension one.

anaerobic induction period. Extracts from these roots were subjected to native-SDS, two-dimensional polyacrylamide gel electrophoresis. Since no other [^{3}H]-labeled polypeptides lie in the ADH region of the gel, the relative intensity of radioactively labeled ADH1 polypeptides will correlate with the relative number of ADH1 subunits synthesized during the 5-hour pulse. This assumption is further supported by the evidence that ADH1-1S and ADH1-1F polypeptides have equal numbers of leucines (KELLEY and FREELING 1980). As shown in Figure 6, there is a distinct difference between the relative intensity of [^{3}H]-leucine-labeled ADH1 subunits in *1F/1S* and *54S/33F* anaerobic roots. Densitometric traces of the fluorograms revealed that the relative radioactivity for

ADH1-S subunits was 34.3% for *1F/1S* primary roots and 46.2% for *54S/33F* primary roots. Average percent ADH1-S data calculated from allozyme activity ratios in 24 hour anaerobically induced primary roots is 33.8% and 44.0% for these respective hybrids. We conclude from these data and the immunoelectrophoretograms that, for the *1F*, *33F*, *1S* and *54S* alleles, the variation in *Adh1* quantitative expression in anaerobic primary roots is attributable to differences between ADH1·ADH1 allozyme synthetic rates.

Because we measured ADH Set I allozyme balances in dry scutella, we cannot determine directly whether the allozyme balance variation in this region arises from differences between synthesis, degradation or dimerization rates of ADH1·ADH1 enzymes. However, we do not find any correlation with the *in vitro* thermolabilities or dimerization properties of ADH1·ADH1 homodimers and the scutellar ADH allozyme balances (unpublished results). We find that F·F homodimers are more thermostable and reassociate *in vitro* more completely than S·S homodimers. These experiments were performed on crude scutellar extracts from the *1F*, *33F*, *1S* and *54S* lines. *S/F* hybrids constructed from these lines do not always display a scutellar balance skewed towards F·F homodimers. (Table 2). These data favor the differential ADH1·ADH1 synthesis argument for scutellar ADH allozyme balance variation, but obviously do not rule out other mechanisms.

The thermolability behavior of 1F·1F, 33F·33F, 1S·1S and 54S·54S enzymes points to the possibility ADH1·ADH1 degradation rate differences may account partially for differences in ADH1·ADH1 enzyme accumulation in anaerobically induced primary roots. We find that ADH activity in 5-cm primary roots from *1F/1S*, *1S/33F* and *54S/33F* seedlings increases at a zero-order rate up to and beyond 48 hours of anaerobic induction. Before and during this induction period, the ADH Set I allozyme balances do not change. These data imply that each Set I allozyme is accumulating at a zero-order rate; that is, there appears to be no detectable influence of a first-order degradation process on ADH1·ADH1 allozyme production in primary roots.

In summary, allozyme balances in the scutellum and anaerobic root of *1F/1S* and *54S/33F* individuals reflect differences between the relative number of ADH Set 1 allozyme molecules. In the anaerobic roots, the relative level of ADH1·ADH1 allozyme molecules is attributable to differences between the zero-order synthetic rates of ADH1·ADH1 enzymes. The relative level of ADH Set 1 allozymes in the scutellum is not associated with the electrophoretic mobility, *in vitro* dimerization property differences between ADH1·ADH1 allozymes. Coupled with the segregation tests, these biochemical studies provide evidence that the *Adh1* quantitative variants, *1F*, *33F*, *1S* and *54S*, differ at a *cis*-acting locus that regulates the production of ADH1·ADH1 molecules. Moreover, the reciprocally correlated ADH allozyme balances (*i.e.*,*Adh1* quantitative expression) in the scutellum and primary root suggest that the genetic elements coordinating organ-specific *Adh1* expression are at least tightly linked and may even share common DNA sequences. Since we could not separate the reciprocal-effect site and *Adh1* structural gene (*i.e.*, electrophoretic mobility), we cannot rule out the possibility that the reciprocal effect loci reside within the *Adh1* structural gene.

Developmental stability of the reciprocal effect: Before considering the possible molecular mechanisms accounting for the reciprocal effect phenomenon, it is necessary to determine whether the reciprocal effect is developmentally stable within each *S/F* individual. If *Adh1* quantitative behavior in either the scutellum or anaerobic primary root is the property of ADH1 polypeptides, then the ADH allozyme balance in each organ would be expected to be independent of one another; that is, there should be no association between the most extreme scutellar and anaerobic root allozyme balances within an *S/F* family. By an extreme allozyme balance, we mean an ADH allozyme balance (represented as percent F·F) greater or less than one standard deviation away from the mean allozyme balance of an *S/F* family. Of the 221 *S/F* individuals generated from the crosses listed in Tables 5 and 6, 22 express an extreme balance in both organs. As shown in Table 8, there is a clear association between extreme allozyme balances in each organ; the highly significant χ^2, corrected for continuity, was equal to 8.41. Each of these 22 *S/F* individuals have a unique genetic background. Thus, the inverse relationship between extreme ADH allozyme balances in the scutellum and anaerobic primary root implies that the reciprocal effect marks an extremely stable developmental process. Moreover, these data reaffirm that the organ-specific *Adh1* quantitative programs are interrelated in a strictly quantitative manner. It seems likely that ADH1 polypeptide differences do not account solely for the reciprocal-effect phenomenon.

DISCUSSION

In this report, we describe differences in the quantitative expression of *Adh1* alleles from 21 maize inbred or exotic lines. By use of ADH allozyme balances in *S/F* hybrids constructed from the inbred lines, we discovered an inverse relationship between *Adh1* quantitative behavior in the scutellum and the anaerobically induced primary root. Within the same electrophoretic mobility class, the allele with the lowest expression in the scutellum exhibits the greatest expression in the anaerobic root, and *vice versa*. We have termed this novel phenomenon the "reciprocal effect."

In order to eliminate the several formal explanations that could account for the reciprocal effect, we performed genetical and biochemical experiments on four *Adh1* quantitative variants: *1F*, *33F*, *1S* and *54S*. Segregation tests establish that

TABLE 8

Twenty-two S/F *individuals with extreme ADH allozyme balances* in both their scutellum and anaerobic primary root*

		Extreme in anaerobic primary root: high % F·F	low % F·F	Total
Extreme in scutellum	high % F·F	3	7	10
	low % F·F	10	2	12
	Total	13	9	22

* Extreme allozyme balances are greater than one standard deviation (σ) away from the mean % F·F value.

Adh1 quantitative behavior in the scutellum and anaerobic primary root is the property of a *cis*-acting site(s) within 0.45 map units of the *Adh1* structural gene. The possibility that organ-specific *Adh1* quantitative behavior is the property of ADH1·ADH1 polypeptides is not supported by our biochemical studies. First, ADH Set 1 allozyme activity ratios in both organs reflect ADH Set 1 allozyme protein ratios. Second, *Adh1* quantitative behavior in the scutellum is not associated with qualitative differences among ADH Set I allozymes (*i.e.*, electrophoretic mobility, *in vitro* thermolability and *in vitro* dimerization property). Finally, [^{3}H]-leucine incorporation experiments indicate that *Adh1* quantitative expression in anaerobic primary roots is attributable to differences between the zero-order synthesis rates among ADH allozymes.

Further proof that *Adh1* quantitative expression in anaerobic primary roots is not associated with mechanisms operating on ADH1 polypeptides *per se* comes from *in vitro* translation studies (FERL, BRENNAN and SCHWARTZ 1980; SACHS, FREELING and OKIMOTO 1980). Anaerobic primary root mRNA from *1F/1S* seedlings produces, in an *in vitro* translation system, 1S and 1F monomers equal in molecular weight to those from dissociated ADH1·ADH1 and ADH1·ADH2 active enzymes. Further, the relative intensities of F and S radioactively labeled monomers were consistent with the relative *in vivo* expression of these alleles in *F/S* anaerobic seedlings. These studies show that neither differential ADH1 processing nor ADH1 dimerization rates *in vivo* accounts for *Adh1* quantitative expression in *F/S* anaerobic primary roots.

Since all *Adh1* quantitative variants tested exhibit the reciprocal effect, it seems likely that the *1F*, *33F*, *1S* and *54S* variants are representative of the four general types of *Adh1* quantitative variants. Therefore, we conclude that differences in ADH1 polypeptide synthesis rates account for the polymorphism in *Adh1* expression in anaerobic roots of the 21 lines listed in Table 1. In the case of scutella, we can conclude only that *Adh1* quantitative behavior is most likely not due to qualitative differences between ADH1·ADH1 enzymes.

A speculation concerning the reciprocal-effect element: We have shown that the quantitative behavior of every *Adh1* allele and allele combination tested fits an organ-specific reciprocal relationship. The question of the positional relationship of *Adh1* quantitative site(s) in the scutellum and primary root is important in understanding the nature of the reciprocal effect. A reciprocal-effect mutant, *Adh1-S1951a*, has been recovered from progeny of an *Adh1* mutant originally derived from material irradiated with accelerated neon ions (FREELING and CHENG 1978; FREELING and WOODMAN 1979). It is likely that a single mutational event can alter *Adh1* quantitative behavior simultaneously in the scutellum and primary root. However, *Adh1-S1951a* expression is not proof that the reciprocal-effect site is a single locus, since it is the only recovered mutant of this nature. Nevertheless, these data provide added support to the hypothesis that the *cis*-acting loci coordinating organ-specific *Adh1* quantitative expression are, at the least, in close proximity.

Because F_1 backcrosses did not separate the reciprocal effect loci from the *Adh1* structural gene, these regulatory loci may reside within coding sequences of the *Adh1* cistron. Obviously, structural gene variants may exhibit a regulatory type

of behavior (see reviews by PAIGEN 1971; CALHOUN and HATFIELD 1975; FORGET 1978). However, we did not find a relationship between *Adh1* structural gene variation and *Adh1* quantitative polymorphism. We propose that ADH1 behavior is not responsible for the reciprocal effect. On the other hand, it is possible that DNA sequences encoding ADH1 information might specify regulatory information as well (see BOGENHAGEN, SAKONJU and BROWN 1980). Irrespective of the location of reciprocal- effect loci, our data establish that there is a regulatory genetic element of the *Adh1* cistron that coordinates organ-specific *Adh1* quantitative expression. Moreover, the extreme allozyme balance relationship suggests that *Adh1* quantitative behavior (reciprocal effect) is programmed at a determinative step prior to (or during) the establishment of scutellar and primary root cell lineages. We propose that the determinative event involves an unequal distribution of *Adh1* quantitative potential. We advance no molecular models, but predict that these regulatory *Adh1* variants will be profitably studied at the level of nucleotide sequences.

The importance of measuring regulatory variation: During the past ten years, it has been well documented by techniques that measure qualitative differences among enzymes (*e.g.*, electrophoretic mobility and heat stability of kinetic properties) that natural populations are highly polymorphic at structural gene loci. The evolutionary significance of most structural gene variation is the subject of much speculation, and it is still undetermined. One hypothesis is that regulatory rather than structural gene variation plays the predominant role in adaptive evolution (BRITTEN and DAVIDSON 1971; KING and WILSON 1975; WILSON, CARLSON and WHITE 1977). This theory has received support from evidence that regulatory genes are adaptively significant in prokaryotes (HALL 1978, and references therein). However, before the importance of regulatory variation in adaptive evolution can be adequately assessed, the extent and nature of regulatory variation within and among populations must be determined.

Several studies indicate that regulatory gene variation may be quite common in natural populations (WARD and HERBERT 1972; WARD 1975; PRAKASH 1977; MCDONALD and AYALA 1978; ABRAHAM and DOANE 1978; DICKINSON and CARSON 1979; DICKINSON 1980). This study illustrates that allozyme balances can be a powerful tool for detecting regulatory variation among diverse individuals. We have identified a genetic element that coordinates the organ specificity of *Adh1* quantitative expression. We have surveyed only six maize races: Northern Flint (*30S*), Great Plains Flint (*29F* and *35S*), Chapalote (*9F*), Zapalote Chico (*21F*), Papago Flour (*12F*) and corn-belt dents. Since there are at least 300 maize races (*cf.*, BROWN and GOODMAN 1977), we most likely have not characterized the full extent of *Adh1* regulatory variation. Whether the quantitative polymorphism at the *Adh1* cistron has any adaptive significance, such as providing a differential response to flooding stress (see MARSHALL, BROUE and PRYOR 1973; BROWN, MARSHALL and MUNDY 1976; DIEDENHOFEN 1977) remains to be determined.

JAMES C. WOODMAN was a recipient of Public Health Service predoctoral training grant, 5T32-GMO7127. This research was supported by Public Health Service grant GM21734 to M. FREELING. Special thanks goes to MARTY SACHS for performing the native-SDS, two-dimensional gels.

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Corresponding editor: R. L. PHILLIPS