GENETIC CONTROL OF MALATE DEHYDROGENASE ISOZYMES IN MAIZE[1]

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ABSTRACT

At least six nuclear loci are responsible for the genetic control of malate dehydrogenase (L-malate: NAD oxidoreductase; EC 1.1.1.37; MDH) in coleoptiles of maize. Three independently segregating loci (*Mdh1, Mdh2, Mdh3*) govern the production of MDH isozymes resistant to inactivation by ascorbic acid and found largely or solely in the mitochondria. A rare recessive allele found at a fourth nuclear locus (*mmm*) causes increased electrophoretic mobility of the MDH isozymes governed by the *Mdh1*, *Mdh2* and *Mdh3* loci.——Two loci (*Mdh4, Mdh5*) govern MDH isozymes that are selectively inactivated by homogenization in an ascorbic acid solution and that appear to be nonmitochondrial (soluble). *Mdh4* and *Mdh5* segregate independently of each other and independently of *Mdh1*, *Mdh2* and *Mdh3*. However, there is close linkage between the migration modifier and *Mdh4*.——Multiple alleles have been found for all of the *Mdh* loci except the migration modifier, and electrophoretically "null" or near "null" alleles (as expressed in standardized sections of maize coleoptile) have been found for all loci except *Mdh4*. Duplicate inheritance commonly occurs for *Mdh1* and *Mdh2* and also for *Mdh4* and *Mdh5*.——Inter- and intragenic heterodimers are formed between subunits specified by the three loci governing the mitochondrial MDH isozymes. The same is true of the alleles and nonalleles at the two loci governing the soluble variants. No such heterodimers are formed by interactions between mitochondrial and soluble MDH isozymes.

WE are currently making an isozyme survey of the races of maize of Latin America (STUBER, GOODMAN and JOHNSON 1977; GOODMAN 1978). Our ultimate purpose is to compare racial groupings based upon gene frequencies with previously obtained racial groupings based on morphology. We also hope to be able to compare our results with the results of a similar survey based on chromosome knob patterns (MCCLINTOCK 1978; MCCLINTOCK, KATO and BLUMENSCHEIN 1980).

In attempting to apply standard methods to the wide range of variation found

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among the races of maize, we have found that either modifications of electrophoretic methods or reanalyses of the genetic mechanisms have been required for some isozymes. For MDH, additional alleles and loci have been discovered, and some previous hypotheses (YANG and SCANDALIOS 1975b; YANG, SORENSON and SCANDALIOS 1977) have been modified. Our results are based upon segregation ratios rather than comparative biochemical analyses, but NEWTON (1979b) is currently undertaking further studies of maize MDH. Her studies, which complement those reported here, include a proposal for a coherent set of labels for the loci and their known alleles constructed in accordance with the code adopted by maize research workers (COE and NEUFFER 1977). Here we follow her revised nomenclature.

MATERIALS AND METHODS

About 25,000 maize plants have been surveyed, including 156 racial collections or composites from Mexico, Central America and Venezuela; inbred lines and many of their F_1, F_2 and testcross derivatives from the southeastern open-pollinated varieties, Jarvis Golden Prolific and Indian Chief; several commercial and experimental single-cross hybrids; many widely used commercial inbred lines (ZUBER 1975; HENDERSON 1976) and their F_1, F_2 and testcross derivatives; trisomic stocks (and materials derived from them) from Dr. R. J. Lambert of the Maize Genetics Cooperation Stock Center; and a number of F_1, F_2, testcross and backcross progeny derived from individual plants with newly discovered variants. All crosses involved pairs of individually pedigreed plants. The majority of our results are based upon crosses among locally adapted inbred lines.

All analyses were made on 1.2 cm sections of coleoptiles taken from plants grown in darkness at 22° for about five days. (The coleoptile of the plant can be used for genetic analysis without sacrificing the plant itself.) Each section was homogenized with 10 to 20 μl of a solution containing 16.7% sucrose and 8.3% sodium ascorbate in a 400 μl disposable microcentrifuge tube (immersed in an ice bath) using a power-driver Delrin (an acetal resin plastic) pestle. (Substitution of ascorbic acid for sodium ascorbate eliminates certain bands, including the bands of YANG and SCANDALIOS' 1974 soluble fraction.) Homogenized material was centrifuged for four min in a refrigerated Beckman/Spinco No. 152 Microfuge and stored at —60° for later electrophoresis. (Fresh material, homogenized in either medium, yielded results indistinguishable from materials frozen in this manner.) Filter paper (Whatman No. 2) wicks, 2 mm × 11 mm, were used to absorb samples of the thawed supernatant for insertion into a slit (2.9 cm and 3.2 cm from the edges of the pH 5.0 and pH 5.7 gels, respectively) in a starch gel.

Gels were made in acrylic molds 1 cm deep and 18 cm wide. With a gel mold 13 cm long, we used 345 ml of a pH 5.0 L-histidine (0.004 M in the gels, 0.05 M in the electrode trays), citric acid (0.002 M in the gels, 0.024 M in the electrode trays) buffer added to 44.7 g of starch (22.35 g each of Electrostarch Lot 371 and Connaught Lot 323-3); 7.5 W of constant power (SCHAFFER and JOHNSON 1973) was delivered to the horizontal gels for 7.5 hours at 2°. With a gel mold 18.5 cm long, we used 500 ml of a pH 5.7 L-histidine (0.009 M in the gels, 0.065 M in the electrode trays), citric acid (0.003 M in the gels, 0.019 M in the electrode trays) buffer added to 64.8 g of the starch described above; 17 W of constant power was applied for 7.25 hours at 2°. (All pH measurements were made at 25°.)

After electrophoresis, enzyme activity was revealed by immersion of a gel slice in 50 ml of 0.1 M Tris-HCl buffer (pH 9.1), 100 mg malic acid (neutralized with NaOH), 1.25 to 20 mg β-nicotinamide-adenine dinucleotide (NAD), 10 mg nitro-blue tetrazolium (NBT), 1.25 mg phenazine methosulfate (PMS), and 3.75 ml H_2O at 38° for about one hr. Gels were then rinsed and stored in water until scored and photographed. They may be fixed in a solution of methanol:water:glacial acetic acid (5:5:1 ratio by volume), wrapped in Saran Wrap, a thin plastic sheeting, and stored indefinitely.

RESULTS

The solution of sucrose and sodium ascorbate (at pH 7.38) that we routinely used for homogenization allows the expression of products of all *Mdh* loci that will be discussed. Substitution of ascorbic acid for sodium ascorbate in the homogenization solution lowers the pH to 2.13 and selectively eliminates certain bands, so that the products of only three loci are observed: *Mdh1*, *Mdh2* and *Mdh3*. The ascorbic acid solution causes little apparent change in the electrophoretically observable products of these three loci, but it does increase their resolution because of the elimination of overlapping bands resulting from other *Mdh* loci.

At the *Mdh1*, *Mdh2* and *Mdh3* loci, several alleles are identified alphabetically and numerically, so that each allele at each locus has a unique designation, *e.g.*, *Mdh1-A1*, *Mdh1-A6*, . . . ; *Mdh2-B3*, *Mdh2-B5*, . . . ; *Mdh3-C16*, *Mdh3-C18*, ... The enzymes are apparently dimers, as evidenced by the presence of intermediate (hybrid) zones of enzyme activity in zymograms of heterozygotes (Figure 1). There is also heterodimer formation by the combination of products of nonallelic MDH isozymes (Figure 2). Thus, in a single plant, these three loci alone can generate a total of 21 bands: nine resulting from two homodimers plus a heterodimer per locus; and 12 resulting from four possible heterodimers between each set of two loci, with three such sets of two loci (1,2; 1,3; 2,3). Clearly with multiple alleles at each locus, the total number of MDH bands possible is quite large. We have not observed all possible allelic combinations, but have studied the segregation of each allele found.

Electrophoretically "null" alleles are found rarely at each of the three loci; in homogenates of coleoptile tissue, these usually fail to produce hybrid bands, although at least some of the "nulls" for *Mdh3* appear to have detectable but faint activity and produce very weak heterodimer patterns. It is likely that at least some of these "nulls" do have MDH activity in some tissues. Several commercial inbred lines are homozygous for "null" alleles at either *Mdh1* (line H25) or *Mdh2* (lines Ky21 and 4Co82). We have produced viable plants simultaneously homozygous for "nulls" at both *Mdh1* and *Mdh2* and at both *Mdh2* and *Mdh3*, but have yet to produce "nulls" simultaneously homozygous at all three loci. Lines B8 and M49 appear to be among the few materials in which the *Mdh2* allele does not result in a detectable homodimeric product, but does, however, interact with other alleles of *Mdh2* and alleles of *Mdh1* and *Mdh3* to produce heterodimers with apparently normal activities. Whether the absence of a homodimer is due to the allele itself or to a closely linked regulatory or inhibiting locus is not known. A very similar, if not identical, allele at the *Mdh2* locus has been found by NEWTON (1979b).

Distances of migration from the origin toward the anode for the isozyme bands corresponding to the nonnull alleles at each locus are shown in Table 1 for both the pH 5.0 and pH 5.7 systems. The relative position of the various hybrid bands thus far observed are presented in tables filed in the GENETICS editorial office.

Many of the *Mdh* alleles coding for the isozymes that are not resistant to the ascorbic acid treatment have been found rarely, and then often in unadapted

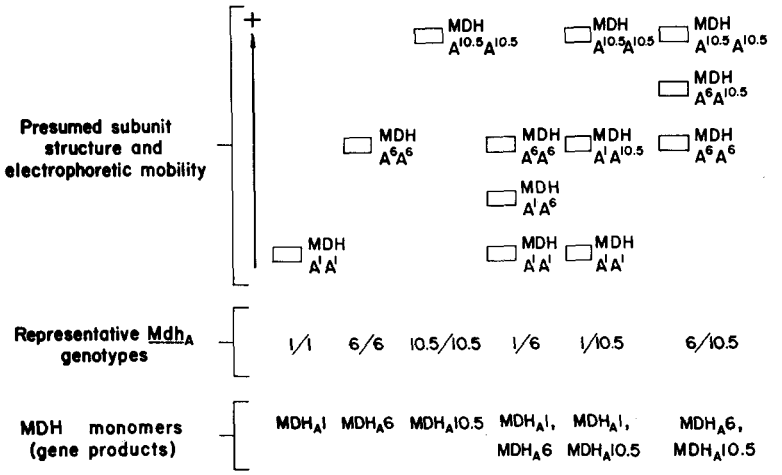

FIGURE 1.—Schematic diagram of representative electrophoretic migration for a single mitochondrial *Mdh* locus in maize.

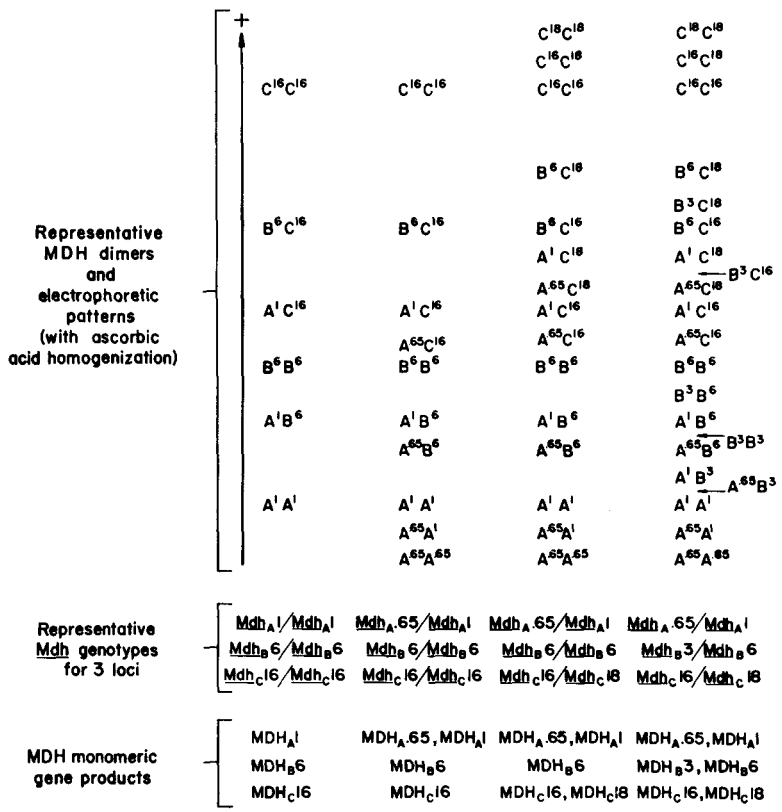

FIGURE 2.—Schematic diagram of the electrophoretic migration positions for representative isomeric products of *Mdh1*, *Mdh2* and *Mdh3*.

TABLE 1

Anodal mobilities of the non-"null" MDH allelic isozymes in maize coleoptile tissue homogenized in ascorbic acid solution

Locus	Allele	Distance from origin (mm) pH 5.0 gel	pH 5.7 gel
	A0.05	—	26.4 (43.0)*
	A0.1	—	31.5†
	A0.65	9.2	46.7
	A1	14.2	52.5
Mdh1	*A1.5*	15.7	54.0
	A3.5	21.0	63.5
	A6	33.4	76.1
	A9	47.5	86.1
	A10.5	54.1	95.1
	B0.03	4.1	24.2
	B0.3	—	38.4
	B3	18.0	60.0
	B3.5	20.0	63.0
	B4.5	‡	‡
Mdh2	*B5*	25.4	69.1
	B5.5	26.5	70.6
	B5m	25.3	71.8
	B5.6	28.8	76.1
	B6	33.4	76.1
	B7.7	40.7	84.0
	C11.5	64.0	97.0
	C16	80.4	110.1
Mdh3	*C16.3*	80.7	111.9
	C16.9	87.4	119.3
	C18	91.7	130.1

* Two monomeric gene products (plus a hybrid dimer) result from this allele (see DISCUSSION).
† Several additional isomers result from this allele (see DISCUSSION).
‡ Under these gel conditions, no homodimer is expressed by this allele in maize coleoptiles.

material. Figure 3 presents a schematic diagram of the type of banding patterns found for the additional isozymes. Two loci, *Mdh4* and *Mdh5*, govern the inheritance of these bands. Multiple alleles are found at each locus (Table 2): *Mdh4-D8, Mdh4-D12*, . . . ; *Mdh-5E12, Mdh5-E15*, ... In this case also, the MDH enzymes are apparently dimers (Figure 3). Again there is heterodimer formation by the combination of products of nonallelic MDH isozymes. We have, however, detected no evidence of heterodimer formation between any product of *Mdh1* or *Mdh2* or *Mdh3* with any product of *Mdh4* or *Mdh5*.

We have yet to discover any "null" alleles of *Mdh4*, but Tx325 and Tx303, commercial inbred lines, are "null" for *Mdh5*. Other materials may also be "null" for *Mdh5*, but *Mdh4-D12* is almost universal, and in its presence *Mdh5-E12* cannot be distinguished from *Mdh5-E"null"* without testcrossing. Migration distances for the enzymes corresponding to the non-"null" alleles of *Mdh4* and

TABLE 2

Anodal mobilities of the additional non-"null" allelic MDH isozymes in maize coleoptile tissue homogenized with sodium ascorbate solution

Locus	Allele	Distance from origin (mm) pH 5.0 gel	pH 5.7 gel
Mdh4	*D8*	40.3	84.3
	D8.5	44.3	86.3
	D10.5	53.5	93.3
	D12	60.4	97.1
	D14.5	72.2	106.5
Mdh5	*E12*	60.4	97.1
	E15	73.8	107.6
	E16	79.1	109.1
	E16.5	82.2	114.7
	E16.7	83.8	116.1

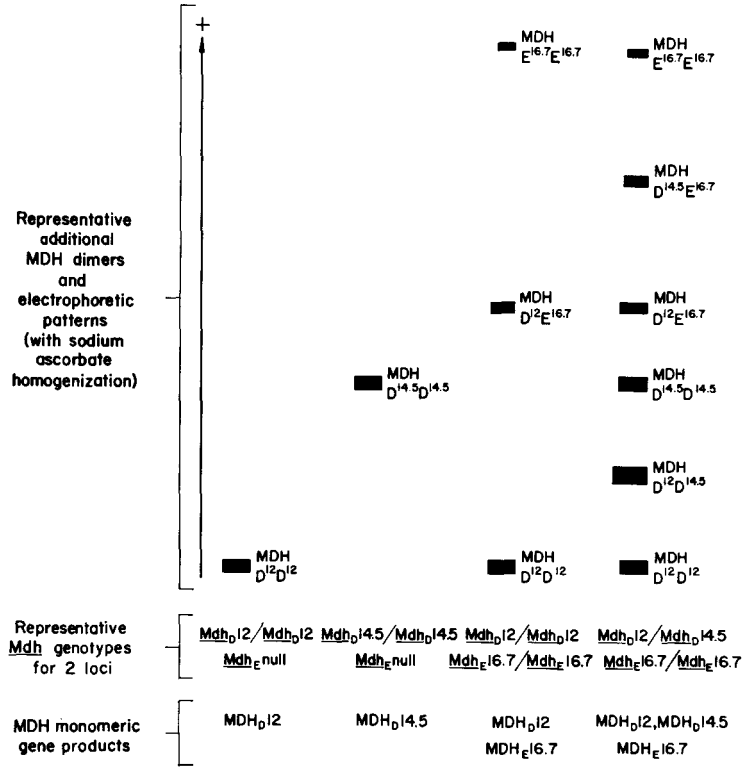

FIGURE 3.—Schematic diagram of the electrophoretic mobilities for representative isomeric products of *Mdh4* and *Mdh5*.

Mdh5 are presented in Table 2, and the corresponding distances for the hybrid enzymes thus far studied are in tables filed in the Editorial Office. Although these distances are affected by gel-to-gel variation, the heterodimers of *Mdh4-D8.5* do have highly unusual migration patterns, with the positions of the hybrid bands often deviating very substantially from the usual rule of half the distance between the homodimers.

Figure 4 shows a zymogram consisting of inbred material, a heterozygous control, and two heterogeneous samples of mitochondrial isolates (SHAH and LEVINGS 1974) from the laboratory of C. S. LEVINGS. All material was prepared with sodium ascorbate. The darkest band (near the top of the gel) represents the product of *Mdh4-D12* (and possibly *Mdh5-E12*). It is virtually absent in the raw mitochondrial preparation from LEVINGS (samples 25 and 26), whereas the products of *Mdh1*, *Mdh2* and *Mdh3* show little, if any, loss of activity. We have tentatively concluded that *Mdh1*, *Mdh2* and *Mdh3*, which interact genetically and whose products show little effect from the ascorbic acid treatment, are responsible for the production of the mitochondrial MDH isozymes. Similarly, we hypothesize that *Mdh4* and *Mdh5* correspond to YANG and SCANDALIOS' (1974) soluble variants. Figure 5 shows the inbred materials of Figure 4 as they appear after the ascorbic acid treatment. The controls and LEVINGS' mitochondrial preparation were treated as for Figure 4.

A sixth locus, *mmm*, affects the migration patterns of the homodimers and

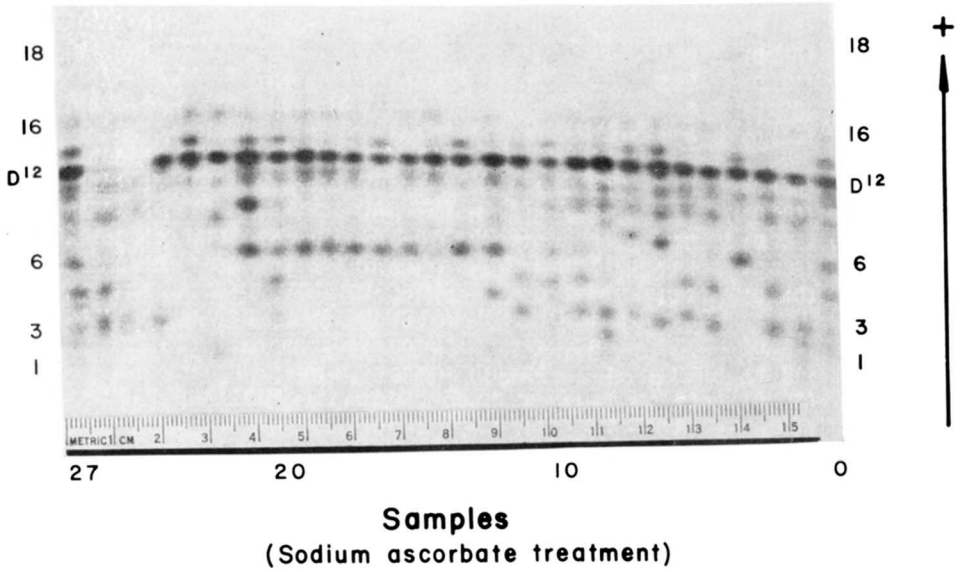

FIGURE 4.—Zymogram (pH 5.7 gel) of several inbred lines of maize (samples 1–9, 11–19, 21–24), heterozygous controls (samples 0, 10, 20, 27), and two mitochondrial preparations from C. S. LEVINGS' laboratory (samples 25 and 26). Bands 1, 3, 16, and 18 are homodimers specified by *Mdh1*, *Mdh2*, *Mdh3* and *Mdh3*, respectively. Those lines with a lightly staining band 6 are homozygous for *Mdh1-A6*, but not *Mdh2-B6*, which specifies a characteristically darkly staining band 6 homodimer.

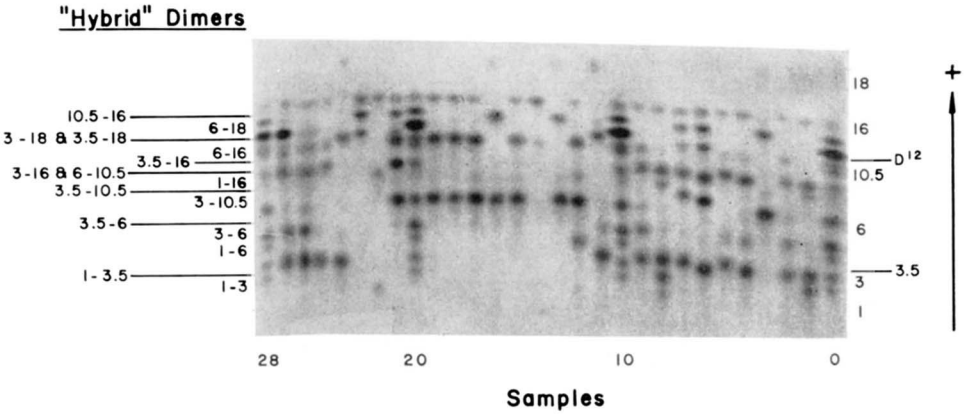

FIGURE 5.—Zymogram (pH 5.7 gel) of the same materials in samples 0 to 26 as in Figure 4, but with the inbred materials prepared with ascorbic acid. Sample 27 represents the initial material from which the mitochondrial preparations (samples 25 and 26) were derived. Sample 28 is a control. Bands 3.5 and 10.5 are homodimers specified by *Mdh2-B3.5* and *Mdh1-A10.5*, respectively.

heterodimers of *Mdh1*, *Mdh2* and *Mdh3*. A rare allele at this locus (*mmm*) is found in several inbred lines (Ky228, Mc5NT, Mo24W, W629A) as well as in several Latin American collections. The rare allele is easily detectable only in the homozygous condition, when it causes all the homodimers and heterodimers that we have studied for *Mdh1*, *Mdh2* and *Mdh3* to migrate slightly further. There is no detectable effect on the products of *Mdh4* or *Mdh5* and the effects when *mmm* is heterozygous with its alternative allele, *Mmm*, are barely discernable.

Many data for these six loci were from locally available materials, including the Latin American collections surveyed. For *Mdh4*, we have used mostly alleles derived from exotic stocks and the inbred line Tx325. For the other loci, our data often came from crosses involving "randomly" inbred lines from the Jarvis and Indian Chief varieties, which have been widely used in the quantitative genetics program at N. C. State University. These lines were provided by DR. R. H. MOLL and are designated herein by the prefix "NCar". More commonly available and more agronomically desirable lines are listed in Table 3, which includes a reasonably complete assortment of readily available *Mdh* genotypes. We obtained inbred lines, whenever possible, from the experiment stations at which they originated (HENDERSON 1976); certain lines, including B8, B9A and W64A, show considerable source-to-source variation.

Table 4 presents the summary chi-square tests for independent assortment and the chi-square tests of expected segregation ratios for each pair of loci. The tabular matter documenting these segregation ratios, including the observed and expected numbers of individuals in each category are too extensive to include here, but are on file with the Editor, along with the pedigrees of the crosses studied. In many cases, we studied the presence or absence of a homodimer, rather than follow both alleles at a locus. There were several reasons for this. *Mdh1-A6* and *Mdh2-B6* are both very common, and their homodimers migrate to the same

TABLE 4

Summaries of linkage tests between all pairs of Mdh1, Mdh2, Mdh3, Mdh4, Mdh5 *and* mmm

Loci *Mdh*	Alleles	Family Type*	Family Size	Independence† χ^2	Independence† d.f.	Segregation ratio‡ χ^2	Segregation ratio‡ d.f.
1,2	*A1* or *A0.1,B3*	C,F	449	0.15	1	0.39	3
1,2	*A1,B3*	R,F	219	0.01	1	4.34	3
1,3	*A1,C18*	R,F	162	0.89	2	2.01	5
1,4	*A1* or *A6,D12*	C,F	123	0.14	2	11.70	5
1,4	*A1,D14.5*	R,T	72	2.83	1	5.44	3
1,5	*A1,E15*	C,F	157	0.70	1	4.30	3
1,5	*A1,E15*	R,F	152	0.11	1	1.96	3
1,5	*A1,E15*	R,F	82	0.54	2	2.19	5
1,mmm	*A1,mmm*	C,T	48	0.50	1	1.83	3
1,mmm	*A1,mmm*	R,T	48	0.02	1	3.33	3
2,3	*B3* or *B6,C18*	C,F	186	4.56	2	6.56	5
2,4	*B3,D12*	C,F	115	4.03	2	14.31	5
2,4	*B3,D12/D14.5*	F	61	1.09	2	4.82	5
2,4	*B3,D8*	C,F	72	0.83	4	8.44	8
2,4	*B3* or *B6,D14.5*	R,T	104	1.87	1	7.69	3
2,5	*B3,E15*	C,F	327	0.02	1	1.61	3
2,5	*B3,E15*	R,F	140	0.43	1	0.61	3
2,5	*B6,E15*	R,T	90	0.09	1	1.47	3
2,mmm	*B3,mmm*	R,T	96	0.17	1	0.58	3
3,4	*C16,D12* or *D14.5*	C,F	284	4.24	4	7.83	8
3,5	*C16,E15*	C,F	47	0.35	2	2.47	5
3,mmm	*C18,mmm*	R,T	24	0.07	1	3.33	3
4,5	*D8,E15* or *E16.5*	R,F	145	0.09	2	4.08	5
4,mmm	*D14.5,mmm*	R,T	120	108.41	1	109.27	3
5,mmm	*E15,mmm*	R,T	24	0.17	1	0.33	3

* C = Coupling; R = Repulsion; F = F_2; T = Testcross.

† $P(\chi_1^2 \geq 3.84) = 0.05$; $P(\chi_2^2 \geq 5.99) = 0.05$; $P(\chi_4^2 \geq 9.49) = 0.05$.

‡ χ_3^2 tests 9:3:3:1 ratio for F_2's, 1:1:1:1 for testcrosses;
χ_5^2 tests 6:3:3:2:1:1 ratio, χ_8^2 tests 4:2:2:2:2:1:1:1 ratio;
$P(\chi_3^2 \geq 7.82) = 0.05$; $P(\chi_5^2 \geq 11.07) = 0.05$; $P(\chi_8^2 \geq 15.51) = 0.05$.

to locate *Mdh2* to chromosome *6*. NEWTON (1979b) further localized it to the long arm of *6* and, since it does not seem to be linked to *Ep* or *6-Pgd1*, two tightly linked loci near *Y*, it must be close to the end of the long arm of *6* (WEISSINGER, STUBER and GOODMAN 1979). *Mdh4* and *mmm* have been located to the long arm of chromosome *1* by NEWTON (1979a), and our data suggest they are about 25 to 30 map units from *Pgm1-Adh1-Phi*, a group of isozyme markers which themselves span no more than 20 map units (WEISSINGER, STUBER and GOODMAN 1979). The order of these isozyme loci appears to be *Mdh4* : *mmm* : *Pgm1* : *Adh1* : *Phi*.

The pooled F_2 and testcross data shown in Tables 5 and 6 suggest that the F_2 segregation at each locus fits the usual 3:1 ratio and that the corresponding testcross ratio is 1:1.

TABLE 5

Pooled F_2 data for individual Mdh *alleles at each locus*

Locus	Allele	No. of families	Present	Absent	Total	χ_1^2(3:1)
	A0.05	2	13 (12.0)*	3 (4.0)	16	0.33‡
	A0.1	2	33 (32.2)	10 (10.8)	43	0.07
Mdh1	*A0.65*	1	13 (12.0)	3 (4.0)	16	0.33
	A1	17	629 (621.8)	200 (207.2)	829	0.34
	A10.5	1	38 (36.0)	10 (12.0)	48	0.44
	Totals		726 (714.0)	226 (238.0)	952	0.81
	B0.03	1	11 (9.0)	1 (3.0)	12	1.78
	B0.3	3	28 (26.2)	7 (8.8)	35	0.47
Mdh2	*B3*	24	735 (725.2)	232 (241.8)	967	0.52
	B3.5	3	83 (79.5)	23 (26.5)	106	0.62
	B5.5	2	15 (12.0)	1 (4.0)	16	3.00
	B7.7	1	17 (18.0)	7 (6.0)	24	0.22
	Totals		889 (870.0)	271 (290.0)	1160	1.66
	C11.5	1	15 (17.3)	8 (5.8)	23	1.17
Mdh3	*C16.3*	1	14 (12.0)	2 (4.0)	16	1.33
	C18	4	301 (290.2)	86 (96.8)	387	1.59
	Totals		330 (319.5)	96 (106.5)	426	1.38
	D8	7	161 (154.5)	45 (51.5)	206	1.09
	D8.5	2	34 (33.0)	10 (11.0)	44	0.12
Mdh4	*D10.5*	1	6 (7.5)	4 (2.5)	10	1.20
	D12	6	257 (246.8)	72 (82.2)	329	1.70
	D14.5	4	159 (168.0)	65 (56.0)	224	1.93
	Totals		617 (609.8)	196 (203.2)	813	0.34
	E15	12	395 (396.0)	133 (132.0)	528	0.01
Mdh5	*E16*	1	11 (12.0)	5 (4.0)	16	0.33
	E16.5	2	80 (78.8)	25 (26.2)	105	0.08
	Totals		486 (486.8)	163 (162.2)	649	0.00

Locus	Allele	No. of families	Absent or heterozygous	Homozygous	Total	χ_1^2
Mdh1	*A1.5*	1	11 (12.0)	5 (4.0)	16	0.33
	Anull	3	93 (87.8)	24 (29.2)	117	1.26
Mdh2	*Bnull*	5	114 (120.0)	46 (40.0)	160	1.20
Mdh3	*Cnull*	2	32 (33.0)	12 (11.0)	44	0.12
Mdh5	*Enull*	1	25 (24.0)	7 (8.0)	32	0.17

* Expected values for a 3:1 ratio shown in parentheses.
† Expected values for a 1:2:1 ratio shown in brackets.
‡ $P(\chi_1^2 \geq 3.84) = 0.05$; $P(\chi_1^2 \geq 2.71) = 0.10$; $P(\chi_2^2 \geq 5.99) = 0.05$; $P(\chi_2^2 \geq 4.61) = 0.10$.

TABLE 6

Pooled testcross data for individual Mdh *alleles at each locus in maize*

Locus	Allele	No. of families	Present	Absent	Total	χ_1^2(1:1)
	A0.05	9	103 (90.5)*	78 (90.5)	181	3.45†
	A0.1	5	45 (44.5)	44 (44.5)	89	0.01
	A1	8	116 (110.0)	104 (110.0)	220	0.65
Mdh1	*A3.5*	1	4 (6.0)	8 (6.0)	12	1.33
	A6	2	27 (24.0)	21 (24.0)	48	0.75
	A9	1	7 (5.5)	4 (5.5)	11	0.82
	A10.5	2	25 (24.0)	23 (24.0)	48	0.08
	Totals		327 (304.5)	282 (304.5)	609	3.33
	B0.03	2	13 (11.0)	9 (11.0)	22	0.73
	B3	16	182 (183.5)	185 (183.5)	367	0.02
	B3.5	9	104 (99.5)	95 (99.5)	199	0.41
	B4.5	1	3 (5.5)	8 (5.5)	11	2.27
	B5	2	14 (12.0)	10 (12.0)	24	0.67
Mdh2	*B5m*	1	10 (12.0)	14 (12.0)	24	0.67
	B5.6	1	11 (11.5)	12 (11.5)	23	0.04
	B5.5	2	12 (11.0)	10 (11.0)	22	0.18
	B6	5	64 (72.0)	80 (72.0)	144	1.78
	B7.7	1	7 (6.0)	5 (6.0)	12	0.33
	Totals		420 (424.0)	428 (424.0)	848	0.08
	C11.5	2	8 (9.0)	10 (9.0)	18	0.22
Mdh3	*C16*	3	23 (21.0)	19 (21.0)	42	0.38
	C16.9	4	15 (14.5)	14 (14.5)	29	0.03
	C18	6	51 (47.0)	43 (47.0)	94	0.68
	Totals		97 (91.5)	86 (91.5)	183	0.66
	D8	1	7 (8.0)	9 (8.0)	16	0.25
Mdh4	*D8.5*	5	33 (30.0)	27 (30.0)	60	0.60
	D10.5	5	17 (20.5)	24 (20.5)	41	1.20
	D14.5	10	206 (192.0)	178 (192.0)	384	2.04
	Totals		263 (250.5)	238 (250.5)	501	1.25
	E12	1	6 (6.0)	6 (6.0)	12	0.00
	E15	2	61 (57.0)	53 (57.0)	114	0.56
Mdh5	*E16*	4	36 (38.0)	40 (38.0)	76	0.21
	E16.5	6	37 (40.0)	43 (40.0)	80	0.45
	E16.7	5	32 (31.0)	30 (31.0)	62	0.06
	Totals		172 (172.0)	172 (172.0)	344	0.00
mmm	*Mmm*	7	115 (108.0)	101 (108.0)	216	0.91

* Expected values for a 1:1 present:absent ratio shown in parentheses.
† $P(\chi_1^2 \geq 3.84) = 0.05$; $P(\chi_1^2 \geq 2.71) = 0.10$.

DISCUSSION

The malate dehydrogenase isozymes in maize have been studied by YANG and SCANDALIOS (1974, 1975a,b) and YANG, SORENSON and SCANDALIOS (1977). We have reached somewhat different hypotheses as to the genetic mechanisms involved as a result of surveying a wider range of materials and by achieving somewhat better separation and resolution. By comparing their results with ours, using the same materials, we have been able to determine how most of the isozyme bands correspond (Table 7). They (YANG and SCANDALIOS 1975b) established that the *Mdh1* and *Mdh2* loci (*m-Mdh*^{s} and *m-Mdh*^{s} in their original terminology; *mdh4* and *mdh2* in YANG, SORENSON and SCANDALIOS 1977) are independent, but proposed that the heterodimers resulting from the interaction of these two loci with *Mdh3-C16* (see Figure 6) were instead the products of loci (*mdh3* and *mdh1*, respectively) linked to *Mdh1* and *Mdh2*. In addition, their *m-Mdh*^{s} band included several variants, including *Mdh1-A6*, *Mdh2-B6*,

TABLE 7

Apparent correspondence between the mitochondrial MDH isozymes studied by YANG *and* SCANDALIOS *(1974) and* YANG, SORENSON *and* SCANDALIOS *(1977) and bands described in this report*

YANG and SCANDALIOS' (1974) band position	Interpretation of YANG *et al.* (1977)	Distance from origin of pH 5.0 gel (mm)	Interpretation in this study
m-MDH[1]	Allele *mdh1–m1* or *mdh3–m1*	56.1	Heterodimer of *Mdh1–A6* or *Mdh2–B6* and *Mdh3–C16*
m-MDH[2]	Allele *mdh1–m2*	50.5	Heterodimer of *Mdh2–B3.5* and *Mdh3–C16*
		47.5	Heterodimer of *Mdh2–B3* and *Mdh3–C16*
m-MDH[3]	Allele *mdh2–m3* or *mdh4–m3* or some other cause	44.2	Heterodimer of *Mdh1–A1* and *Mdh3–C16* or other heterodimers (see DISCUSSION)
		33.4	Allele *Mdh1–A6* or *Mdh2–B6*
m-MDH[4]	Heterodimer of m-MDH[3] and m-MDH[5]	27.2	Heterodimer of *Mdh1–A6* or *Mdh2–B6* and *Mdh2–B3.5*
		25.4	Heterodimer of *Mdh1–A6* or *Mdh1–B6* and *Mdh2–B3*
m-MDH[5]	Allele *mdh2–m5*	22.0	Heterodimer of *Mdh1–A6* or *Mdh2–B6* and *Mdh1–A1*
		20.0	Allele *Mdh2–B3.5*
		18.5	Allele *Mdh2–B3*
m-MDH[6]	——	16.5	Heterodimer of *Mdh1–A1* and *Mdh2–B3.5*
		15.8	Heterodimer of *Mdh1–A1* and *Mdh2–B3*
m-MDH[7]	——	14.2	Allele *Mdh1–A1*

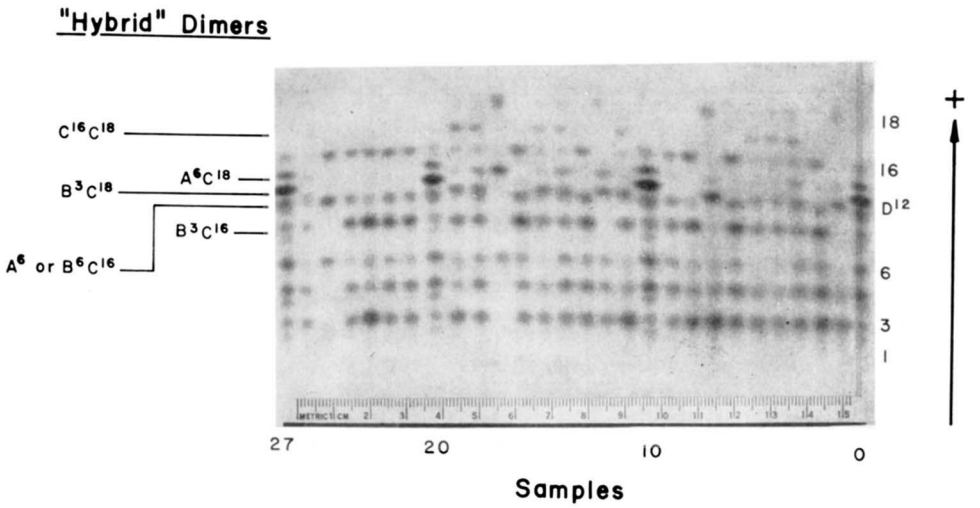

FIGURE 6.—Zymogram (pH 5.7 gel) of an F_2 of a maize plant with genotype *Mdh1-A6/A6*; *Mdh2-B3/B6*; *Mdh3-C16/C18*. Samples 0, 10, 20 and 27 are controls prepared with sodium ascorbate. Samples 1 to 9, 11 to 19, and 21 to 26 are F_2 plants prepared with ascorbic acid.

and heterodimers between products of *Mdh1-A1* and *Mdh3-C16* (and possibly of *Mdh1-A1*, *Mdh1-A10.5*, and of *Mdh1-A10.5*, *Mdh2-B3*).

When *Mdh3-C16* is homozygous (the usual condition), as in plants 2, 6, 8, 9, 13, 16, and 21–25 of Figure 6, all plants having *Mdh1-A6* or *Mdh2-B6* and *Mdh2-B3* have hybrid bands formed by heterodimers of *Mdh1-A6* or *Mdh2-B6* with *Mdh3-C16* and *Mdh2-B3* with *Mdh3-C16*. With *Mdh3-C18* homozygous (plants 1, 7, 12, and 17 of Figure 6), the corresponding hybrid bands, the results of heterodimers of *Mdh1-A6* or *Mdh2-B6* with *Mdh3-C18* and *Mdh2-B3* with *Mdh3-C18*, migrate further than do the heterodimers resulting from *Mdh3-C16*. Compare plant 17 with plant 25, and plant 7 with plant 8. The former comparison involves *Mdh3-C16 vs. Mdh3-C18* in the presence of *Mdh1-A6* and *Mdh2-B6*, and the latter comparison involves *Mdh3-C16 vs. Mdh3-C18* in the presence of *Mdh1-A6* and *Mdh2-B3*.

In Figure 5, samples 11 and 13 differ by one allele. Sample 11 has *Mdh2-B3*, whereas sample 13 has *Mdh2-B6*. Both are homozygous for *Mdh1-A6* and *Mdh3-C18*. Note the correlated change in the darkness of the bands from heterodimers formed between *Mdh1-A6* and *Mdh3-C18* and between *Mdh2-B6* and *Mdh3-C18* with the darkness of the "parental" homodimers. A similar comparison can be made between samples 14 and 15, which differ by the presence of *Mdh1-A6* in sample 14, but both *Mdh1-A6* and *Mdh2-B6* in sample 15; in this case the heterodimers involve *Mdh3-C16*. Similar correlations between the intensities of the "parental" homodimers and their heterodimeric products can be seen for the heterodimers of *Mdh2-B3* and *Mdh3-C16* in Figure 6. With less heterogeneous materials, such correlations are easily demonstrated for each possible set of heterodimers and homodimers. [*Mdh3* has now been localized to the long arm of

chromosome 3 by NEWTON (1979b), who has shown it to be within 5 map units of *sh2* (GOODMAN, NEWTON and STUBER 1979).]

In several positions on the gels, the presence of a band can be determined by more than one allele or pair of alleles. However, with a few exceptions, both alleles at *Mdh2*, *Mdh3* and *Mdh4* can be scored without testcrossing. The exceptions include inability to distinguish between a homozygote and a heterozygote when one allele is a "null". Often, both alleles of *Mdh1* can also be scored, but we cannot determine whether *Mdh1-A6* is present when *Mdh2-B6* (a much darker staining form) is present. The data obtained thus far suggest that "null" alleles for *Mdh1* are rare; we have found them in only one collection of the race Tehua from Mexico and in the inbred line H25. Similarly, without testcrossing, we cannot determine whether *Mdh5-E12* is present in the presence of *Mdh4-D12*, a much more darkly staining variant.

While our results can be duplicated at much higher pH values (pH 6.5 to 7.0), there is considerable loss of separation and resolution when that is done. For example, with a common tris-citrate (pH 7.0) buffer system, homodimers specified by *Mdh3-C16*, *Mdh4-D12* and *Mdh5-E12* migrate to the same position (NEWTON 1979b). Similar pH values to ours have been used for the purification of MDH enzymes (YANG and SCANDALIOS 1974).

Several bands that occasionally occur on our gels appear to be a result of post-translational alteration of products of single genes. Several homodimers of *Mdh4* consistently have a trailing band of lighter-staining material. This is most apparent for *Mdh4-D12*, for which the trailing band appears to correspond to the soluble-2 form of YANG and SCANDALIOS (1974), and for *Mdh4-D8* and *Mdh4-D14.5*. To a lesser extent, a slightly faster migrating band often accompanies the homodimeric gene product of *Mdh2-B6* in homozygotes. One allele of *Mdh1* appears to result ultimately in two products, which in turn form a heterodimer. Each of the two products of *Mdh1-A.05* forms heterodimers with subunits specified by *Mdh2* and *Mdh3* as well. Although this could represent a duplication, we have seen no evidence of recombination among almost 200 testcross progeny. Another allele at the same locus, *Mdh1-A.1*, results in a series of numerous enzymatic products; we are now transferring that allele to a genetic stock null for *Mdh2* and *Mdh3* in an attempt to determine the exact nature of the phenomenon. The bands resulting from the addition of *Mdh5-E16.5* into genotypes homozygous for *Mdh4-D12* segregate clearly and distinctly, while *Mdh5-E16.5* appears to result in a series of lightly staining isomers in the presence of *Mdh4-D14.5*. Each of these phenomena appears to be unrelated to gel pH. In each case, we are transferring the particular allele involved from unadapted material to appropriate, adapted genetic stocks for further study.

LITERATURE CITED

COE, E. H., JR. and M. G. NEUFFER, 1977 The genetics of corn. pp. 111–223. In: *Corn and Corn Improvement*. Edited by G. F. SPRAGUE. American Society of Agronomy, Inc., Madison, Wisconsin.

GOODMAN, M. M., 1978 A brief survey of the races of maize and current attempts to infer racial relationships. pp. 143–158. In: *Maize Breeding and Genetics.* Edited by D. B. WALDEN. John Wiley and Sons, New York.

GOODMAN, M. M., K. NEWTON and C. W. STUBER, 1979 Inheritance of malate dehydrogenase isozymes in maize. Genetics **91:** s41.

HENDERSON, C. B., 1976 Maize Research and Breeders Manual, No. VIII. Illinois Foundation Seeds, Inc. Champaign, Illinois.

MCCLINTOCK, B., 1978 The significance of chromosome constitutions in tracing the origin and migration of races of maize in the Americas. pp. 159–184. In: *Maize Breeding and Genetics.* Edited by D. B. WALDEN. John Wiley and Sons, New York.

MCCLINTOCK, B., T. A. KATO Y. and A. BLUMENSCHEIN, 1980 Chromosome constitution of races of maize. Their significance for interpreting relationships among races and strains in the Americas. Colegio de Post-Graduados, Sec. Agric. y Recursos Hidráulicos, Chapingo, Mexico.

NEWTON, K. J., 1979a A gene which alters the electrophoretic mobilities of maize mitochondrial malate dehydrogenase isozymes. Genetics **91:** s88–89. ——, 1979b Genetic analyses of MDH: An operational model. Maize Genetics Cooperation News Letter **53:** 16–24.

SCHAFFER, H. E. and F. M. JOHNSON, 1973 Constant (optimum) power electrophoresis. Analytical Biochem. **51:** 577–583.

SHAH, D. M. and C. S. LEVINGS, III, 1974 Mitochondrial DNA from maize hybrids with normal and Texas cytoplasms. Crop. Sci. **14:** 852–853.

STUBER, C. W., M. M. GOODMAN and F. M. JOHNSON, 1977 Genetic control and racial variation of β-glucosidase isozymes in maize (*Zea mays* L.) Biochem. Genet. **15:** 383–394.

WEISSINGER, H. H., C. W. STUBER and M. M. GOODMAN, 1979 Linkage relationships between isozyme loci in maize. Genetics **91:** s136.

YANG, N. S. and J. G. SCANDALIOS, 1974 Purification and biochemical properties of genetically defined malate dehydrogenase in maize. Arch. Biochem. Biophys. **161:** 335–353.

YANG, N. S. and J. G. SCANDALIOS, 1975a Cytoplasmic synthesis of soluble and mitochondrial malate dehydrogenase isozymes in maize. Arch. Biochem. Biophys. **171:** 575–585. ——, 1975b De novo synthesis and developmental control of the multiple gene-controlled malate dehydrogenase isozymes in maize scutella. Biochem. Biophys. Acta **384:** 293–306.

YANG, N. S., J. C. SORENSON and J. G. SCANDALIOS, 1977 Genetic control of mitochondrial malate dehydrogenases: Evidence for duplicated chromosome segments. Proc. Natl. Acad. Sci. U.S. **74:** 310–314.

ZUBER, M. S., 1975 Corn germ plasm base in the U.S.—is it narrowing, widening, or static. Annu. Corn Sorghum Res. Conf. Proc. **30:** 277–286.

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