LINKAGE RELATIONSHIPS OF 19 ENZYME LOCI IN MAIZE[1]

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ABSTRACT

Linkage relationships of 19 enzyme loci have been examined. The chromosomal locations of eight of these loci are formally reported for the first time in this paper. These localizations should assist in the construction of additional useful chromosome marker stocks, especially since several of these enzyme loci lie in regions that were previously poorly mapped. Six loci are on the long arm of chromosome *1*. The arrangement is (centromere)—*Mdh4-mmm-Pgm1-Adh1-Phi-Gdh1*, with about 46% recombination between *Mdh4* and *Gdh1*.——Linkage studies with *a2* and *pr* have resulted in the localization of four enzyme genes to chromosome 5 with arrangement *Pgm2-Mdh5-Got3-a2-*(centromere)*-pr-Got2*. *Pgm2* lies approximately 35 map units distal to *a2* in a previously unmapped region of the short arm of 5, beyond ameiotic.——Approximately 23% recombination was observed between *Mdh4* and *Pgm1* on chromosome *1*, while 17% recombination occurred between *Mdh5* and *Pgm2* on chromosome 5. Similarly, linkages between *Idh1* and *Mdh1*, about 22 map units apart on chromosome *8*, and between *Mdh2* and *Idh2*, less than 5 map units apart on chromosome *6*, were observed. Thus, segments of chromosomes *1* and 5 and segments of *6* and *8* may represent duplications on nonhomologous chromosomes.

IN surveying Latin American races of maize (*Zea mays* L.) for enzyme polymorphism, we have attempted to map the enzyme loci that we are studying. Knowledge of the chromosomal locations of enzyme loci should contribute, not only to the construction of more useful chromosomal marker stocks, but also, in the case of multilocus enzymes, to our knowledge about the distribution of duplications on homologous and nonhomologous chromosomes. Studies of linkage disequilibrium within and among populations also depend upon a knowledge of the linkage relations among the loci studied. Furthermore, very few genes have previously been mapped to several relatively large segments of the maize genome; for example, chromosome eight. Here, we report linkage involving six enzyme loci on the long arm of chromosome *1*, four on chromosome 5, four on

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chromosome *6*, two on chromosome *8*, one on chromosome *3* and two whose localization is incomplete. With the possible exceptions of endopeptidase (EP) and phosphohexose isomerase (PHI), the isozyme systems we have used in these analyses are specified by two or more loci.

Alcohol dehydrogenase (ADH) is coded for by two unlinked loci, *Adh1* and *Adh2* (FREELING and SCHWARTZ 1973). The *Adh1* gene is responsible for most of the ADH activity and is located in the long arm of chromosome *1*, approximately 1.5 map units from *lw* (SCHWARTZ 1971). *Adh2* is linked to *su* in the short arm of chromosome *4* (FREELING and CHENG 1978; DLOUHY 1979). ADH is active as a dimer and forms ADH1·ADH2 intergenic heterodimers.

Endopeptidase (EP) is coded for by a single major structural locus localized to chromosome *6* (NIELSEN and SCANDALIOS 1974).

Glutamate-oxaloacetate transaminase (GOT): Isozymes of this dimeric enzyme are known to be associated with different subcellular compartments (MACDONALD and BREWBAKER 1972; SCANDALIOS, SORENSON and OTT 1975). *Got1* specifies allozymes that are associated with the glyoxysomes; *Got2* specifies forms active in the cytoplasm, while *Got3* encodes mitochondrial GOT allozymes (SCANDALIOS, SORENSON and OTT 1975). Interallelic heterodimers are produced as the result of interaction between alleles at each locus, but no intergenic heterodimers are formed. *Got1*, *Got2* and *Got3* were shown earlier to be, at most, loosely linked (STUBER and GOODMAN 1979a).

Glutamic dehydrogenase (GDH): The inheritance of GDH is poorly understood, but thought to be governed by two loci (MISHARIN *et al.* 1979; SUKHORZHEVSKAYA 1979), one of which has been localized to *1L* by PRYOR (1979).

Isocitrate dehydrogenase (IDH) is coded for by two unlinked loci, *Idh1* and *Idh2*, which interact to produce both interallelic and intergenic heterodimers (STUBER and GOODMAN 1980a).

Malate dehydrogenase (MDH): Five major structural loci have been identified in this system (GOODMAN *et al.* 1978, 1980; NEWTON 1979b, 1980; NEWTON and SCHWARTZ 1980). Duplicate genes, *Mdh4* and *Mdh5*, encode MDH isozymes active in the cytoplasm of the cell. *Mdh4* was located proximal to *Adh1* in the long arm of chromosome *1* and *Mdh5* was located distal to *a2* in the short arm of chromosome *5* (NEWTON 1979a; NEWTON and SCHWARTZ 1980); both localizations have been repeated by MCMILLIN and SCANDALIOS (1980a,b). The MDH isozymes compartmentalized in the mitochondria are specified by three nuclear genes: *Mdh1* is on chromosome *8* (NEWTON and SCHWARTZ 1980), *Mdh3* is in the distal region of the long arm of chromosome *3* (GOODMAN, NEWTON and STUBER 1979; NEWTON 1979b) and *Mdh2* has been located to the distal region of the long arm of chromosome *6* by means of trisomic analysis (GOODMAN *et al.* 1978, 1980), *B-A* translocations (NEWTON 1979b; NEWTON and SCHWARTZ 1980) and linkage studies (MCMILLIN, ROUPAKIAS and SCANDALIOS 1979). *MDH* is also active as a dimer. Subunits of isozymes that occupy the same subcellular compartment readily interdimerize, but heterodimers between soluble and mitochondrial MDH isozymes are not observed. A sixth locus, *Mmm*, affects the electrophoretic mobilities of the mitochondrial MDH isozymes (GOODMAN

et al. 1978, 1980; NEWTON 1979a,b; NEWTON and SCHWARTZ 1980). Close linkage has been observed between *Mdh4* and *Mmm* (GOODMAN *et al.* 1978, 1980), and *Mmm* was located to *1L* using cytogenetic criteria and linkage to *Adh1* (NEWTON 1979a,b; NEWTON and SCHWARTZ 1980).

Malic enzyme (ME): PUPILLO and BOSSI (1979) have reported two different forms of malic enzyme in maize, but our studies have been limited to a single locus that is expressed in the coleoptile.

Phosphoglucomutase (PGM) is encoded by two independently segregating loci, *Pgm1* and *Pgm2*, located on chromosomes *1* and *5*, respectively (GOODMAN, STUBER and NEWTON 1980). With our buffer systems, each allele at each locus appears to produce a pair of isozymes, with no apparent interaction of loci or alleles (STUBER and GOODMAN 1979b, and unpublished).

6-Phosphogluconate dehydrogenase (6-PGD) is encoded by two unlinked loci, *Pgd1* and *Pgd2* (STUBER and GOODMAN 1980b). *Pgd1* has been localized to chromosome *6* about 5 map units from *Ep* (WEISSINGER, STUBER and GOODMAN 1979). Interallelic and intergenic heterodimers are formed.

Phosphohexose isomerase (PHI) appears to be coded for by a single locus, *Phi*, on chromosome *1* (WEISSINGER, STUBER and GOODMAN 1979).

MATERIALS AND METHODS

We have reported procedures for homogenizing coleoptiles and preparing 2 of the 4 types of starch gels (L-histidine-citrate, pH 5.0 and pH 5.7) used for these analyses (STUBER, GOODMAN and JOHNSON 1977; GOODMAN *et al* 1980). The third gel system was a widely used Tris-citrate and lithium hydroxide-boric acid buffer system (SCANDALIOS 1969). The fourth gel used a L-histidine-citrate buffer at pH 6.5. The latter was prepared in a manner similar to the pH 5.7 gels; however, 0.065 M L-histidine and 0.007 M citric acid were used for the electrode buffer, which was diluted 1:3 (3 parts H_2O) for the gel buffer. The pH 6.5 gel was run at 15.6 watts of constant power (SCHAFFER and JOHNSON 1973) for 6¾ hr at 2°. In each case, the gel slices were incubated in their staining solutions at 38° for about an hour. The procedures used for each enzyme have been adapted from those published in several sources, such as SCANDALIOS (1969); SHAW and PRASAD (1970); SELANDER *et al.* (1971); and SCHAAL and ANDERSON (1974), and are as follows:

Alcohol dehydrogenase (ADH): Electrophoresis was in a commonly used Tris-citrate and lithium hydroxide-boric acid buffer system. The ADH activity solution contains 25 ml of 0.1 M Tris-HCl buffer (pH 8.5), 0.5 ml ethanol, 10 mg β-nicotinamide adenine dinucleotide (NAD), 3 mg phenazine methosulfate (PMS), 10 mg nitro blue tetrazolium (NBT) and 2 ml H_2O.

Endopeptidase (EP): The electrophoretic conditions were the same as for ADH. The staining procedure was adapted from MELVILLE and SCANDALIOS (1972).

Glutamate-oxaloacetate transaminase (GOT): The electrophoretic conditions were the same as for ADH. GOT activity was detected using 0.25 mg Fast Blue BB salt in 0.25 ml H_2O mixed with 9.1 mg α-ketoglutaric acid, 33.3 mg L-aspartic acid, 125 mg polyvinylpyrrolidone (PVP-40), 12.5 mg disodium salt of ethylenediamine tetra-acetic acid (EDTA), 355 mg Na_2PO_4 and 50 ml H_2O.

Glutamic dehydrogenase (GDH): The electrophoretic conditions were the same as for ADH. The staining solution was 50 ml of 0.1 M Tris-HCl buffer (pH 8.5), 150 mg L-Glutamic acid, 20 mg NAD, 5 mg PMS, 15 mg NBT and 3.5 ml H_2O.

Isocitrate dehydrogenase (IDH): Electrophoresis was in the pH 6.5 histidine-citric acid buffer. The staining procedure for IDH involved an agar overlay. To 15 ml of a 0.1 M Tris-HCl buffer (pH 9.1) we added 150 mg DL-isocitric acid, 15 mg nicotinamide adenine dinucleotide

phosphate (NADP), 10 mg NBT, 0.5 mg PMS and 2.6 ml H_2O. That solution was mixed with a cooled (20°) agar solution consisting of 200 mg epiagar, 300 mg PVP-40 in 1 ml H_2O and 15 ml of 0.1 M Tris-HCl, pH 9.1 buffer.

Malate dehydrogenase (MDH): Electrophoresis was in both the pH 5.0 and pH 5.7 histidine-citric acid buffer systems. The staining procedures for MDH were those of GOODMAN *et al.* (1980).

Malic enzyme (ME): Electrophoresis was in the pH 5.7 histidine-citric acid buffer. The staining solution was 50 ml of 0.1 M Tris-HCl buffer (pH 8.5), 25 mg DL-malic acid, 50 mg $MgCl_2$, 13 mg NADP, 10 mg NBT, 1 mg PMS and 3.5 ml H_2O.

Phosphoglucomutase (PGM): Electrophoresis was in the pH 5.0 and pH 6.5 histidine-citrate buffer systems. PGM activity was revealed with 50 ml of 0.1 M Tris-HCl buffer (pH 8.5), 250 mg glucose-1-phosphate, 25 mg EDTA, 100 mg $MgCl_2$, 5 mg NADP, 40 units glucose-6-phosphate dehydrogenase (G-6PD), 7.5 mg MTT (thiazoyl blue), 1 mg PMS and 3.2 ml H_2O.

6-Phosphogluconate dehydrogenase (6-PGD): Electrophoresis was in the pH 5.7 and pH 6.5 histidine-citrate buffer systems. The staining procedure is identical to that of PHI (below), but with the addition of 10 mg of the trisodium salt of 6-phosphogluconic acid in 0.5 ml H_2O. The result is a gel slice that reveals both PHI and 6-PGD bands.

Phosphohexose isomerase (PHI): Electrophoresis was in the pH 6.5 histidine-citric acid buffer. The PHI stain consisted of 50 ml of 0.05 M Tris-HCl buffer (pH 8.0), 50 mg fructose-6-phosphate, 50 mg $MgCl_2$, 5 mg NADP, 10 units G-6PD, 5 mg MTT, 1.5 mg PMS and 3 ml H_2O.

Individual plants chosen from standard public inbred lines were used to map five loci on chromosome *1*. C. G. PONELEIT, M. S. ZUBER, A. J. BOCKHOLT and J. H. LONNQUIST provided Ky228, Mo24W, Tx325 and W629A, respectively. Except for *mmm*, the recessive allele of the modifier of the mitochondrial MDH enzymes (GOODMAN *et al.* 1980; NEWTON 1979a), Ky228 and W629A have the common variants for each locus (*viz.*, *Mdh4-D12*, *Pgm1-A9*, *Adh1-4*, *Phi-4*). For loci reported here, the Mo24W used was identical to Ky228 and W629A, but carried a slow variant at *Pgm1*, while the Tx325 used had a fast-migrating variant at *Mdh4*, a slow-migrating ADH isozyme and a fast variant at *Phi* (Tx325 segregated for several loci, including *Adh1* and

TABLE 1

Numbers of recombinant and parental chromosome types for the 5-point testcross of Mdh4–mmm–Pgm1–Adh1–Phi *on chromosome* 1 *of maize*

Region 1 (*Mdh4* – *mmm*) Region 2 (*mmm* – *Pgm1*) Region 3 (*Pgm1* – *Adh1*) Region 4 (*Adh1* – *Phi*)

Cross tested*: $\frac{D14.5 - Mmm - A9 \quad - S - 2}{D12 \quad - mmm - A16 - F - 4} \left(\frac{\text{Tx325}}{\text{Mo24W}}\right) \times D12 - mmm - A9 - F - 4$

	Parental combinations†		Single recombinants‡				Double recombinants‡				Totals
Region:	0	1	2	3	4	1,4	2,4	2,3	3,4	
	112	10	34	11	26	0	1	1	0	195
	96	4	32	8	19	2	1	0	1	163
Totals	208	14	66	19	45	2	2	1	1	358
	Recombination %:				4.4	19.0	5.8		13.7	
	± S.E.				± 1.1	± 2.1	± 1.2		± 1.8	

* Pooled data from three families; allele symbols listed from left to right correspond to loci spanning Regions 1 to 4 above. Locus abbreviations omitted to conserve space.

† Upper row is Tx325 parental type and lower row is Mo24W parental type.

‡ Each entry in the upper row begins with the upper left allele listed under "Cross tested" (*viz.*, *Mdh4–D14.5*). Each entry in the lower row begins with the lower leftmost allele (*viz.*, *Mdh4–D12*).

Phi). The single cross Mo24W × Tx325 was testcrossed to Ky228 and W629A. Three separate families were studied: (Mo24W × Tx325) × Ky228; (Mo24W × Tx325) × W629A; and W629A × (Mo24W × Tx325); the pooled testcross data for 358 plants are presented in Table 1, since the differences between families were small and all three families shared the same (Mo24W × Tx325) parental plant.

The pedigrees of the testcross and F_2 families localizing *Pgm2*, *Mdh5*, *Got2* and *Got3* to chromosome 5, and the pedigrees of the testcross families used to study linkage between *Idh2* and *Mdh2* on chromsome *6*, linkage between *Idh1* and *Mdh1* on chromosome *8*, and linkage between *Got1* and *Me* were more complex, usually involving both inbred lines and selected plants from racial or genetic stocks. To more explicitly localize *Mdh3*, known to be closely linked to *sh2* on chromosome *3* (GOODMAN, NEWTON and STUBER 1979; NEWTON 1979b; NEWTON and SCHWARTZ 1980), a 3-point testcross was constructed using the marker stock, *a-p, et.*

TABLE 2

Numbers of recombinant and parental chromosome types among three multiple-point testcrosses in maize

Cross 1: Region 1 (*Pgm2* – *a2*), Region 2 (*a2* – *pr*), Region 3 (*pr* – *Got2*)

Cross tested*: $\frac{B4 - A2 - Pr - M2}{B3 - a2 - pr - M4} \times B4 - a2 - pr - M4$

Cross 2: Region 1 (*Mdh5* – *a2*), Region 2 (*a2* – *pr*)

Cross tested*: $\frac{E15 - A2 - Pr}{E12 - a2 - pr} \times E12 - a2 - pr$

Cross 3: Region 1 (*Adh1* – *Phi*), Region 2 (*Phi* – *Gdh1*)

Cross tested*: $\frac{F - 4 - I}{S - 5 - F} \times F - 4 - F$

	Parental combinations	Single recombinants			Double recombinants			Triple recombinants	Totals
Region:	0	1	2	3	1,2	1,3	2,3	1,2,3	
Cross 1:	55†	18	25	25	15	11	10	3	162
Cross 2:	43†	10	20	—	2	—	—	—	75
Cross 3:‡	43	4	2	—	0	—	—	—	96
	40	4	2	—	1	—	—	—	

Recombination % ± S.E.

Cross 1: *Pgm2*—29.0 ± 3.6—*a2*—32.7 ± 3.7—*pr*—30.2 ± 3.6—*Got2* (Chromosome 5).
Cross 2: *Mdh5*—16.0 ± 4.2—*a2*—29.3 ± 5.3—*pr* (Chromosome 5).
Cross 3: *Adh1*—9.4 ± 3.0—*Phi*—5.2 ± 2.3—*Gdh1* (Chromosome *1*).

* See footnotes of Table 1 for explanation of data display.
† Only results for colored (*A2/a2*) kernels are reported here, since *Pr/pr* and *pr/pr* cannot be distinguished in the presence of *a2/a2*.
‡ First line of Cross 3 corresponds to *Adh1-F/F* types; second *line* of Cross 3 corresponds to *Adh1-F/S* types.

RESULTS

The actual numbers of detected recombinants among five chromosome *1* loci are listed in Table 1. Linkage between *Adh1*, *Phi* and *Gdh1* is presented separately in Table 2. The arrangement of the enzyme loci studied on chromosome *1* is presented in Figure 1. Tables 2 and 3 present the recombination between the various loci studied on chromosome *5*. The arrangement of the loci studied on

TABLE 3

Data supporting linkage between several isozyme loci on chromosome 5 of maize

Loci	Cross tested*	Testcross data: Parental types†	Testcross data: Recombinant types‡	Totals	Percent recombination
Pgm2 – a2§	*B3 – a2* / *B4 – A2*	74 / 115	47 / 33	269	29.7 ± 2.8
Pgm2 – Mdh5	*B4 – E15* / *B8 – E12*	37 / 53	7 / 10	155	16.7 ± 3.0
	B4 – E12 / *B8 – E15*	20 / 20	6 / 2		
Mdh5 – a2§	*E12 – a2* / *E15 – A2*	125 / 72	13 / 31	241	18.3 ± 2.5
Mdh5 – Got3	*E12 – U4* / *E15 – U8*	53 / 55	13 / 9	130	16.9 ± 3.3
a2 – Got2§	*a2 – M4* / *A2 – M2*	56 / 86	76 / 51	269	47.2 ± 3.0
pr – Got2	*pr – M4* / *Pr – M2*	40 / 73	13 / 36	162	30.2 ± 3.6

F_2 data

Alleles* 1 3 / 2 4	Loci	Observed ratios‖	Totals	Percent recombination
B4 U6 / *B8 U4*	*Pgm2, Got3*	3 : 12 : 20 / 14 : 38 : 12 / 18 : 13 : 3	133	27.2 ± 3.3
U4 M4 / *U6 M7*	*Got3, Got2*	4 : 15 : 15 / 32 : 40 : 23 / 13 : 21 : 5	168	40.5 ± 3.3

* See Table 1 footnotes for further explanation of data display.

† In same order as listed under "Cross tested."

‡ In same order as listed for the alleles of the first locus under "Cross tested"; *i.e.* 33 *B4 – a2* recombinants were observed.

§ Not all seed was analyzed; equal numbers of colorless (*a2/a2*) and colored (*A2/a2*) kernels were not chosen.

‖ If genes were unlinked, ratios of 1:2:1/2:4:2/1:2:1 would be expected. Ratio ordering corresponds to

$$\frac{13}{13} : \frac{13}{23} : \frac{23}{23} : \frac{13}{14} : \frac{13}{24} : \frac{23}{24} : \frac{14}{14} : \frac{14}{24} : \frac{24}{24}.$$

TABLE 4

Testcross data demonstrating linkage between Idh1 *and* Mdh1, *between* Idh2 *and* Mdh2 *and between* Got1 *and* Me

Loci	Cross tested*	Parental types†	Recombinant types‡	Totals	Percent recombination
Idh1 – Mdh1	*A4 – A6* / *A6 – A1*	41	13	160	22.5 ± 3.3
		58	16		
	A4 – A10.5 / *A6 – A1*	15	4		
		10	3		
Idh2 – Mdh2	*B4 – B3* / *B6 – Bnu11*	25	2	355	1.1 ± 0.6
		35	0		
	B4 – B6 / *B6 – Bnu11*	16	1		
		7	0		
	B4 – B3 / *B6 – B6*	145	1		
		123	0		
Got1 – Me	*L4 – F* / *L6 – R*	39	2	87	2.3 ± 1.6
		46	0		

* See Table 1 footnotes for explanation of data display.
† In same order as listed under "Cross tested."
‡ In same order as listed for the alleles of the first locus under "Cross tested."

chromosome 5 is presented in Figure 2. The recombination data between *Idh1* and *Mdh1* (on chromosome *6*), between *Idh2* and *Mdh2* (on chromosome *8*) and between *Got1* and *Me* (localization uncertain) are presented in Table 4. Table 5 presents the recombination between *Mdh3*, *A* and *Et* (on chromosome 3).

TABLE 5

Recombination data for Mdh3, A *and* Et *on chromosome* 3 *of maize*

Region 1 (*Mdh3* – *A*) Region 2 (*A* – *Et*)

Cross tested*: $\frac{C18 \quad A \quad Et}{C16 \quad a\text{–}p \quad et} \times C16\ a\text{–}p\ et$

	Parental combinations	Single recombinants		Double recombinants	Total
Region:	0	1	2	1,2	96
C18 types	37	6	5	0	
C16 types	39	2	6	1	

Recombination % ± S.E.
Mdh3—9.4 ± 3.0—*A*—12.5 ± 3.4—*Et*

* See Table 1 footnotes for explanation of data display.

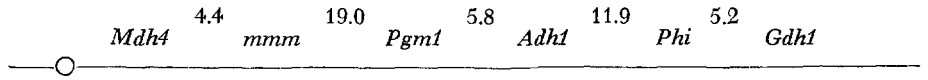

FIGURE 1.—The arrangement of six enzyme loci on chromosome *1* of maize expressed as percent recombination.

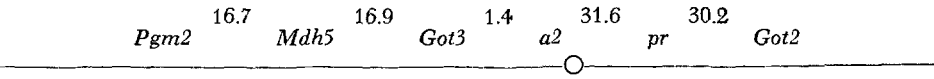

FIGURE 2.—Suggested arrangement of four enzyme loci relative to two aleurone markers on chromosome 5 of maize, expressed as percent observed recombination. *Pgm2*, *Mdh5* and *Got3* lie distal to *a2* in the short arm, while *Got2* is located distal to *pr* in the long arm. Note that the *Got3-a2* distance was estimated indirectly as the difference between *Mdh5-Got3* and *Mdh5-a2*, not from a three-point testcross.

DISCUSSION

The use of isozyme markers in maize for systematic, population and developmental studies would be enhanced if the chromosome locations of the markers were known. Linked groups of polymorphic loci are of special interest to both population and developmental geneticists; while in multilocus systems, we have the possibility of characterizing more exactly the roles of duplications on nonhomologous chromosomes. Studies of gene duplication in our allopolyploid crop plants have played a major role in unravelling their evolution (PICKERSGILL and HEISER 1976), and it is likely that evolutionary studies of multiple locus enzyme systems will substantially extend our knowledge of gene duplications in maize.

Earlier studies (NEWTON 1979a,b; GOODMAN *et al.* 1980; NEWTON and SCHWARTZ 1980) established that *Mdh4* and *mmm* are linked to *Adh1* on the long arm of chromosome *1* (SCHWARTZ 1971). The arrangement of these loci relative to the centromere was known from studies with *B-A* translocations (NEWTON and SCHWARTZ 1980). Additional analyses demonstrated that both *Pgm1* and *Phi* belong to the same linkage group (WEISSINGER, STUBER and GOODMAN 1979). Here, we report rather precise recombination fractions for all five loci from a large 5-point testcross. During the course of these studies, PRYOR (1979) reported the localization of *Gdh1* distal to *Adh1*. Linkage between *Phi* and *Gdh1* (Table 2) is consistent with his report.

A linkage group of enzyme loci on chromosome *1* made it possible to look for duplication of chromosomal segments, since several of the enzymes (ADH, GDH, MDH, PGM) have two or more loci with similar functions. With *Adh1*, *Mdh4* and *Pgm1* localized to chromosome *1* and with *Adh2* localized to the short arm of chromosome *4*, linked to *su* (FREELING and CHENG 1978; DLOUHY 1979), we began to check for possible duplicate linkage groups (limited knowledge of the genetics of GDH did not permit use of *Gdh2*). *Adh2* did not appear to be in the same linkage group as *Pgm2* or *Mdh5*; however, *Pgm2* and *Mdh5* did show linkage with each other, with about 17% ± 3% recombination (Table 3). *Mdh5* was subsequently mapped to the short arm of chromosome 5, approximately 18 recombination units distal to *a2* (NEWTON and SCHWARTZ 1980). Further linkage studies between *Mdh5*, *Pgm2*, *a2*, *Pr*, *Got2* and *Got3* established that *Pgm2*

is located distal to both *Mdh5* and *a2* in a previously unmapped region of the short arm of 5, beyond ameiotic. *Got3* maps between *Mdh5* and *a2*, while *Got2* is distal to *Pr* in the long arm, because it is not closely linked to *a2* or *Got3*. F_2 segregation data (Table 3) for *Pgm2* and *Got3* are consistent with the following order of the loci: *Pgm2-Mdh5-Got3-a2*. Our estimates of F_2 recombination values for the F_2 populations were derived using Maximum Likelihood procedures; the standard errors were calculated using the formula presented by ALLARD (1956). Although the recombination frequencies (23% *vs.* 17%) do differ between *Mdh4* : *Pgm1* and *Mdh5* : *Pgm2*, the similarity tends to support the hypothesis of chromosome segment duplication.

In our studies of IDH and MDH isozymes, we observed that unique *Idh2* and *Mdh2* variants always segregated together in small testcross families. However, the two loci are distinct since (1) some families that segregate for *Idh2* are fixed for *Mdh2*; (2) some families that segregate for *Mdh2* are fixed for *Idh2*; (3) plants having extreme variants for *Mdh2* often carry only the most common *Idh2* allele; (4) three testcross families totaling 355 plants produced four recombinants (Table 4); (5) the gene products of *Idh2* and *Mdh2* migrate to different positions on both pH 5.7 and pH 6.5 gels; and (6) concentrations of antibody prepared against purified *Mdh2* homodimers sufficient to eliminate detectable mitochondrial MDH activity (NEWTON and SCHWARTZ 1980; NEWTON, unpublished) show no cross-reaction with IDH.

From trisomic studies (GOODMAN *et al.* 1978, 1980) and *B-A* translocation studies (NEWTON 1979b), we knew that *Mdh2* was on the long arm of chromosome *6*. Linkage studies with *Ep* (near yellow, *y*) and *Pgd1*, which is closely linked to *Ep*, established that *Idh2* and *Mdh2* segregated independently of both *Ep* and *Pgd1*; therefore, *Idh2* and *Mdh2* must lie in the distal portion of the long arm of chromosome *6* (WEISSINGER, STUBER and GOODMAN 1979). More recently, MCMILLIN, ROUPAKIAS and SCANDALIOS (1979) have indicated that *Mdh2* lies about 12 map units beyond *py*, which had been the most distal, well-mapped locus on the long arm of *6* (COE and NEUFFER 1977).

Linkage between *Idh2* and *Mdh2* immediately suggested the possibility of linkage between *Idh1* and *Mdh1*. In fact, *Idh1* was found to be linked (about 23% recombination) to *Mdh1* (Table 3), which has been located to chromosome *8*, the most poorly mapped of all maize chromosomes (NEWTON and SCHWARTZ 1980). These parallel linkages of IDH and MDH loci might also be explained by chromosome segment duplication as hypothesized for two pairs of MDH and PGM loci.

Thus, among the 19 isozyme loci in this study we have two sets of pairs of linked loci showing analogous linkages and analogous enzyme activities: (A) *Mdh4-Pgm1* on chromosome *1* with 23% recombination, and *Mdh5-Pgm2* on chromosome 5 with 17% recombination; (B) *Idh1-Mdh1* on chromosome *8* with 22% recombination, and *Idh2-Mdh2* on chromosome *6* with 1% recombination. This evidence suggests the possibility that chromosome segment duplication may be fairly common for enzyme loci. Previously, WEBER and ALEXANDER (1972)

provided cytogenetic evidence for nonadjacent duplicated chromosomal regions in the maize genome.

NEWTON and SCHWARTZ (1980) reported linkage between *sh2* and *Mdh3* on chromosome *3* (2.6 recombination units). Here, we report that *Mdh3* is proximal to *A* and somewhat farther from *A* (9.4 recombination units) than would be expected from the *sh2 data.*

In Table 4, we also report linkage (2.3% ± 1.6%) between *Got1* and *Me.* We had suspected that *Got1* was on chromosome *5* along with *Got2* and *Got3* (STUBER and GOODMAN 1979a), but essentially free recombination has been observed between *Got1* and *Got2*, between *Got1* and *Got3*, between *Got1* and *v2*, and between *Got1* and *Pgm2*, which would appear to eliminate that hypothesis.

Both *Mdh4* and *Pgm1* map to the portion of the long arm of chromosome *1* included in *TB-1La* used in dosage studies of enzyme activities by BIRCHLER (1979). BIRCHLER (1980) then mapped *Adh1* cytologically to the 0.80 to 0.90 region of chromosome *1*. NEWTON and SCHWARTZ (1980) further demonstrated that *Mdh4* maps cytologically to the *1L* region proximal to the breakpoint of *TB1La-5S8041* at 0.80. Preliminary results suggest that both *Mdh4* and *Pgm1* map cytologically proximal to the breakpoint of *TB1La-3L5267* at 0.72. Reciprocal crosses, using the same procedure as outlined by BIRCHLER (1980), uncovered *Mdh4* and *Pgm1* on chromosome *1* and *Mdh3* on chromosome *3* (but not *Adh1* on chromosome *1*) in several plants when the *TB1La-3L5267* stock was used as the pollen parent.

Our work, when combined with BIRCHLER's (1979) results, suggests either that dosage compensation occurs specifically for the products of *Pgm1* and *Mdh4* (the most active of the five major, independently segregating MDH structural loci) or that overall enzyme activity decreases as the dosage level of the chromosome *1* segment increases *at almost precisely the same rate* that specific *Mdh4* and *Pgm1* activities increase due to gene dosage. The former seems more plausible. Since both IDH loci and at least one of the two 6-PGD loci map to chromosomes other than *1*, it appears that BIRCHLER's results of lower enzyme activities for these enzymes with increased doses of *1L* are most likely explained by the presence of a negative modifying factor (or factors) within the segment of *1L* included in *TB-1La*, as BIRCHLER (1979) suggested without knowledge of the gene localizations.

In the most recent summary of maize genetics, COE and NEUFFER (1977) list only seven isozyme loci localizations. Since then, an additional 23 such localizations have been made, eight of these formally reported here for the first time (Table 6). The selective effects, if any, of the commonly segregating alleles at polymorphic loci must be small, since both alleles at most such loci can be replaced by nulls or deficiencies with relatively minor effects on the development of the organism (VOELKER *et al.* 1980). In maize, the only commonly polymorphic enzyme that has so far been shown to be necessary for normal kernel development is mitochondrial MDH. Even in that case, a single active allele (of the total of six at three loci) is sufficient for normal development and

TABLE 6

Summary of the known chromosomal locations of isozyme loci in maize

Symbol	Name	Location	Reference
Acph	acid phosphatase	*9*	STUBER *et al.* (1980)
Adh1	alcohol dehydrogenase-1	*1L*	SCHWARTZ (1971)
Adh2	alcohol dehydrogenase-2	*4S*	FREELING and CHENG (1978); DLOUHY (1979)
Amp1	aminopeptidase-1	*1L*	OTT and SCANDALIOS (1978)
Amp2	aminopeptidase-2	*1L*	OTT and SCANDALIOS (1978)
Amp3	aminopeptidase-3	*5S*	McMILLIN and SCANDALIOS (1980a); OTT and SCANDALIOS (1978)
Cat1	catalase-1	*5S*	ROUPAKIAS, McMILLIN and SCANDALIOS (1979)
Cat2	catalase-2	*1S*	TSAFTARIS, SCANDALIOS and McMILLIN (1980)
Cx	catechol oxidase	*10*	PRYOR and SCHWARTZ (1973)
E1	esterase-1	*7*	SCHWARTZ in BROWN and ALLARD (1969)
E3	esterase-3	*3*	BROWN and ALLARD (1969)
E4	esterase-4	*3S*	HARRIS (1968); KLEESE and PHILLIPS (1972)
E16	esterase-16	*7*	BROWN and ALLARD (1969)
Ep	endopeptidase	*6*	NIELSEN and SCANDALIOS (1974)
β-Glu	β-glucosidase	*10*	PRYOR (1978); STUBER *et al.* (1980)
Gdh1	glutamic dehydrogenase	*1L*	PRYOR (1979)
Got2	glutamate-oxaloacetate transaminase-2	*5L*	This paper
Got3	glutamate-oxaloacetate transaminase-3	*5S*	This paper
Idh1	isocitrate dehydrogenase-1	*8*	STUBER and GOODMAN (1980a)
Idh2	isocitrate dehydrogenase-2	*6L*	GOODMAN *et al.* (1980)
Mdh1	malate dehydrogenase-1	*8*	NEWTON and SCHWARTZ (1980)
Mdh2	malate dehydrogenase-2	*6L*	GOODMAN *et al.* (1978, 1980); NEWTON (1979b); McMILLIN, ROUPAKIAS and SCANDALIOS (1979); NEWTON and SCHWARTZ (1980)
Mdh3	malate dehydrogenase-3	*3L*	NEWTON (1979b); GOODMAN, NEWTON and STUBER (1979); NEWTON and SCHWARTZ (1980)
Mdh4	malate dehydrogenase-4	*1L*	NEWTON (1979b); NEWTON and SCHWARTZ (1980)
Mdh5	malate dehydrogenase-5	*5S*	NEWTON and SCHWARTZ (1980)
Mmm	modifier of mitochondrial MDH's	*1L*	NEWTON (1979a,b); NEWTON and SCHWARTZ (1980)
Pgd1	6-phosphogluconate dehydrogenase-1	*6L*	WEISSINGER, STUBER and GOODMAN (1979)
Pgm1	phosphoglucomutase-1	*1L*	WEISSINGER, STUBER and GOODMAN (1979)
Pgm2	phosphoglucomutase-2	*5S*	GOODMAN *et al.* (1980)
Phi	phosphohexose isomerase	*1L*	WEISSINGER, STUBER and GOODMAN (1979); GOODMAN *et al.* (1980)

reproduction (GOODMAN, NEWTON and STUBER 1980). Most of the other enzymes have yet to be tested, although null alleles are known to exist at many of the loci.

The suggestions of C. R. BURNHAM are greatly appreciated.

Note added in proof: Further studies have suggested that *Got1*, *Me*, *Mdh3* (on *3L*), *Pgd2*, and an esterase locus (probably *E8*) belong to the same linkage group. A 5-point testcross (144 plants) has established the order of the loci with the following recombination percentages:

(Est)	*Pgd2*	*Got1*	*Me*	*Mdh3*
	38.9 ± 4.1	23.6 ± 3.5	5.6 ± 1.9	20.8 ± 3.4

(The esterase location needs confirmation.)

In addition, *Cat3* has recently been localized to *1L* (ROUPAKIAS, MCMILLIN and SCANDALIOS 1980).

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