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Duplicated chromosome segments in maize (*Zea mays* L.): further evidence from hexokinase isozymes*

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Summary. The genetic control of hexokinase isozymes (ATP: d-hexose-6-phosphotransferase, E.C. 2.7.7.1, HEX) in maize (*Zea mays* L.) was studied by starch gel electrophoresis. Genetic analysis of a large number of inbred lines and crosses indicates that the major isozymes observed are encoded by two nuclear loci, designated *Hex1* and *Hex2*. Five active allozymes and one null variant are associated with *Hex1*, while *Hex2* has nine active alleles in addition to a null variant. Alleles at both loci govern the presence of single bands, with no intragenic or intergenic heteromers visible, suggesting that maize HEX's are active as monomers. Organelle preparations demonstrate that the products of both loci are cytosolic. All alleles, including the nulls, segregate normally in crosses. Vigorous and fertile plants were synthesized that were homozygous for null alleles at both loci, suggesting that other hexose-phosphorylating enzymes exist in maize that are undetected with our assay conditions. Linkage analyses and crosses with B-A translocation stocks place *Hex1* on the short arm of chromosome 3, 27 centimorgans from *Pgd2* (phosphogluconate dehydrogenase) and *Hex2* on the long arm of chromosome 6, approximately 45 centimorgans from *Pgd1*. It is suggested that the parallel linkages among these two pairs of duplicated genes reflects an evolutionary history involving chromosome segment duplication or polyploidy.

Key words: Hexokinase (HEX) – *Zea mays* L. – Isozymes – Gene duplication – Genetic linkage

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Introduction

Approximately 380 genes have been mapped in *Zea mays* L. (at least to chromosome arm; see Hoisington and Coe 1985), making maize the best mapped higher plant. Included among the mapped loci are a number of apparent gene duplications, usually involving unlinked factors which have, by convention, been assigned similar gene symbols differing only in their subscripts. Based on an idea which originally traces to Marcus Rhoades (Rhoades 1951), these gene duplications suggest the possibility that the genome of maize contains duplicated chromosome segments, or may in fact have evolved from stock which was phylogenetically tetraploid.

With respect to morphological markers, it is often difficult to establish with certainty the evolutionary homology (or homoeology) of factors which confer similar phenotypes. Phenotypic similarity may be due to causes other than actual gene duplication, such as mutations affecting different parts of a common pathway, or mutations in different pathways leading to phenotypic convergence. This problem is to some extent circumvented with biochemical markers such as isozyme loci, where the in vivo function of the genetic products is often clearer, resulting in an increased probability of correctly identifying homologous genes. Because of this, isozyme gene number has proven to be a valuable source of evidence regarding the ploidy level of various plant taxa (Gottlieb 1981, 1982).

Zea mays contains a number of isozyme systems that show gene multiplicity above the diploid level (Goodman and Stuber 1983; Stuber and Goodman 1983b, 1984; Wendel et al. 1985). The suggestion that these duplications arose through chromosome segment duplication or tetraploidy rather than some other mechanism (such as tandem duplication) can be supported if it can be shown that members of different putative duplications show similar or parallel linkage relationships. Two cases of parallel linkages among pairs of duplicated isozyme loci are reported in maize (Goodman et al. 1980a, b; Goodman and Stuber 1983; Stuber and Goodman

1983 b), suggesting the presence of at least two duplicated chromosome segments in the maize genome.

In this paper we present evidence for a third duplicated chromosome segment in maize, based on two new hexokinase isozyme loci, *Hex1* and *Hex2,* and their linkage relationships with previously mapped genes. Hexokinase (ATP: d-hexose-6-phosphotransferase, E.C. 2.7.7.1; HEX) and fructokinase (ATP: d-fructose-6-phosphotransferase, E.C. 2.7.1.4; FK) catalyze the initial step in the metabolism of plant hexoses, which is phosphorylation by ATP. HEX is thought to be responsible for the phosphorylation of glucose and mannose, while FK primarily phosphorylates fructose (Feingold and Avigad 1980; Turner et al. 1977 a, b; Turner and Copeland 1981).

Materials and methods

Plant materials

Plant materials studied included publicly available inbred lines, over 100 racial accessions of maize from the United States and Central and South America, and numerous progenies (F_1, BC, F_2, and F_3) involving plants generated from crosses made in field nurseries. Many of these progenies have complex pedigrees resulting from many generations of intercrossing individuals from racial collections and/or inbred lines. The B-A translocation stocks TB-3Sb and TB-6Lc were generously supplied by J. Beckett.

Enzyme extraction and electrophoresis

Maize hexokinase is found in most plant parts, but analyses were usually performed on etiolated coleoptiles from 5-day old seedlings, following previously described techniques (see Cardy et al. 1983; Goodman et al. 1980 b; Stuber and Goodman 1983 b). The primary advantage of using this tissue is that the coleoptile can be removed without sacrificing the plant, which can then be transplanted to the nursery for crosses and subsequent genetic analyses. Hexokinase was occasionally extracted from mature leaves according to the procedures of Wendel and Parks (1982). Coleoptile extracts were frozen at −80 °C until electrophoresis without noticable loss of activity, but it was necessary to run leaf extracts fresh. Samples prepared either way were absorbed onto filter paper wicks and inserted into 12.8% starch gels as described earlier (Cardy et al. 1983; Goodman et al. 1980 b; Stuber and Goodman 1983 b). Although many different gel and electrode buffer systems adequately resolved the products of *Hex2,* optimal separation and visualization of the products of *Hex1* could only be achieved with pH 7.0 tris-citrate gel and electrode buffers (electrode buffer: 0.135 M tris, 0.043 M citric acid; gel buffer: 1 part electrode buffer to 14 parts water). Gels were run at 15 watts constant power for 6 h. Subsequent to electrophoresis, gels were sliced into 1.2 mm thick slices and stained for hexokinase, and where necessary, fructokinase and various other enzymes (see Wendel et al. 1985, for a list of currently studied enzyme systems). The stain for hexokinase was modified from Shaw and Prasad (1970): 250 mg adenosine triphosphate (ATP), 250 mg d-glucose, 50 mg $MgCl_2$, 10 mg nicotinamide adenine dinucleotide phosphate (NADP), 40 units of glucose-6-phosphate-dehydrogenase (Sigma No. G-6378), 10 mg tetrazolium thiazolyl blue (MTT), and 5 mg phenazine methosulfate (PMS) were added to 30 ml of 0.05 M tris-HCl pH 8.0. This solution was added to a solution of pre-boiled and cooled (to 60 °C) agar (30 ml of 0.05 M tris-HCl, pH 8.0, plus 1.0% (w/v) agar), and the resulting mixture was poured onto the gel slice, allowed to solidify, and incubated at 37 °C until the isozymes appeared (usually 1–2 h). The staining mixture for fructokinase was identical to that of hexokinase, except that d-fructose was substituted for d-glucose, and 100 units of phosphoglucose isomerase (Sigma No. P-5381) were added.

Organelle isolation

Chloroplast preparations were obtained according to the procedures presented in Jenkins and Russ (1984) with little modification. Isolation of intact mitochondria was accomplished using differential centrifugation: 75 etiolated 5-day old coleoptiles were gently ground in a chilled mortar and pestle with 30 ml of extraction buffer (0.4 M sucrose, 0.05 M tris, 0.005 M EDTA, 0.1% bovine serum albumin (w/v), titrated to pH 7.5 with concentrated HCl). The homogenate was filtered through four layers of cheesecloth and two layers of miracloth and centrifuged for 10 min at 1,000 × g. The supernatant was centrifuged at 16,000 × g for 5 min, and the resulting pellet was resuspended in 10 ml of resuspension buffer (same as extraction buffer except with 0.01 M $MgCl_2$ added). Mitochondria were pelleted by centrifugation at 17,000 × g for 15 min, washed again in 10 ml of resuspension buffer, and finally pelleted with an additional centrifugation at 17,000 × g for 15 min.

The preparations were microscopically inspected for intact organelles (under oil immersion) and their purity verified by electrophoresis followed by staining for the mitochondrial markers *Mdh1, Mdh2, Mdh3* (Goodman et al. 1980 a), and the plastid markers *Got2, Tpi1* and *Tpi2* (Stuber and Goodman 1983 a; Wendel et al., unpublished). For electrophoresis, organelles were burst using a combination of osmotic shock (with deionized water) and grinding with a power driven acetal pestle in a 400 µl microcentrifuge tube.

Linkage estimation

Recombination fractions between linked loci were estimated by the method of maximum likelihood (Allard 1956), using the computer program Linkage-1 (Suiter et al. 1983).

Results and discussion

Subcellular localization of hexokinase isozymes

Studies of the intracellular localization of hexokinase and fructokinase in plant cells indicate that activity is distributed between the mitochondrial and soluble fractions (Baldus et al. 1981; Tanner et al. 1983). Hexokinase and fructokinase are reported to be associated with the mitochondria and cytosol, respectively, in pea (*Pisum sativum*) seeds (Tanner et al. 1983), but both fractions have been reported to contain hexokinase activity in pea leaves (Dry et al. 1983).

Our results indicate that the primary isozymes which stain under our assay conditions are soluble. Neither hexokinase nor fructokinase bands appeared in the mitochondrial and chloroplast preparations, although these same preparations displayed darkly staining isozymes for the plastid markers *Got2, Tpi1,* and *Tpi2,* and the mitochondrial markers *Mdh1, Mdh2,* and

Mdh3. No contamination was evident in either preparation.

Electrophoretic variation and genetic analyses

Inbred lines stained for hexokinase usually display two bands (zones 1 and 2), with the more anodal of these (zone 1) often lightly staining and more poorly resolved. Only the more slowly migrating region (zone 2) appears with the fructokinase stain, suggesting that the two isozymes are the products of different loci. Crosses among plants with different phenotypes generate hybrids having all parental isozymes, but no additional hybrid bands (heteromers). Segregation studies demonstrated that the two zones vary independently of each other, and that their behavior follows the pattern expected for codominant alleles specifying functionally monomeric enzymes; i.e., in both zones 1 and 2, one and two-banded phenotypes are observed in material expected to be homozygous and heterozygous, respectively.

A large number of crosses confirmed the above digenic, monomeric enzyme model, and the loci encoding the isozymes in zones 1 and 2 have accordingly been designated *Hex1* and *Hex2*. Screening of over 5,000 plants representing a diverse assemblage of exotic and domestic maize germplasm has resulted in the identification of 5 and 9 allelic variants of *Hex1* and *Hex2*, respectively. Alleles and their designations are schematically illustrated in Fig. 1, and photographs of some of the variants appear in Fig. 2. Locus assignment of new variants was facilitated by taking advantage of the fact that *Hex1* products fail to appear with the fructokinase stain, and was nearly always verified with allelism tests. These analyses are too lengthy to fully present here, but segregation data for some alleles are presented in Table 1. Most progenies segregate *Hex1* and *Hex2* alleles in accordance with Mendelian expectations, but distorted segregation is occasionally observed. A dramatic example is provided by the F_2 progeny of the inbred lines CM37 and T232, where *Hex1* ($\chi^2_2 = 5.42$; $P < 0.05$) and *Hex2* ($\chi^2_2 = 12.50$; $P < 0.01$), as well as 9 of the 14 other loci scored, segregated in a skewed fashion (unpublished data).

In addition to the active alleles shown in Fig. 1, individual plants and some lines appear to lack activity for one or both of the zones of staining. These "null" phenotypes breed true, segregate normally in crosses, and as a consequence are considered null allelic variants. An F_1 was constructed between a line which is null at *Hex1* (*Hex1-n/nHex2-2/2*) and a line homozygous null at *Hex2* (*Hex1-4/4Hex2-n/n*). The F_1 displayed intermediate staining intensity at both loci, and upon selfing, segregated null alleles according to Mendelian expectations (χ^2_2 for 3 : 1 = 2.69 and 3.02 for

Table 1. Segregation patterns in several progenies demonstrating genetic control of some hexokinase variants in maize

Locus	Alleles	Expected[a] ratio	No. observed	Chi-square[b]
Hex1	4, n	3:1	1,488:437	5.42*
	4, n	3:1	93:42	2.69
	3, 4	1:2:1	50:122:22	1.82
	4, 5	1:2:1	36:82:32	0.75
	2, 4	1:2:1	45:97:40	1.07
	2, 6	1:2:1	27:47:21	0.77
	2, 5	1:2:1	26:49:20	0.85
Hex2	1, 2	1:1	54:58	0.14
	2, n	3:1	110:25	3.02
	1, 2	1:2:1	419:845:413	0.15
	2, 4	1:2:1	44:73:42	1.11
	1, 2[c]	1:2:1	320:664:357	2.11
	1, 2	1:2:1	416:1,021:488	12.50**

[a] Degrees of freedom are 1 for 1:1 and 3:1 expected ratios and 2 for 1:2:1 segregations
[b] Significance levels are 0.05 and 0.01 for single and double asterisks, respectively
[c] Pooled F_2 data from 14 families

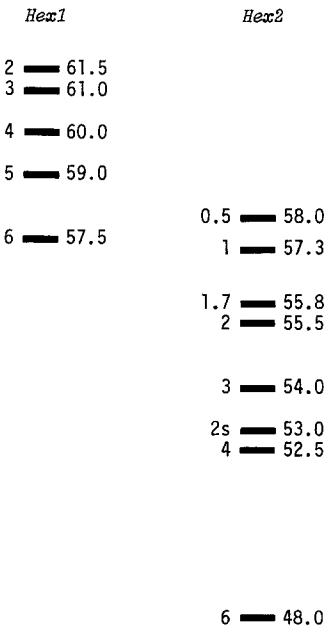

Fig. 1. Schematic illustration of allelic variation of hexokinase isozymes in maize. Protein migration was towards the anode (*top* of the figure). Monomers encoded by *Hex1* and *Hex2* are shown in the *left and right halves* of the figure, respectively. Allele designations are shown to the *left* of each band, and the approximate migration distance from the origin (in mm) is shown to the *right* of each band. The allele *Hex2-2* actually represents two alleles: the standard *Hex2-2* and lightly staining allele *Hex2-2l*. *Hex2-2s* is a "streaky" allele

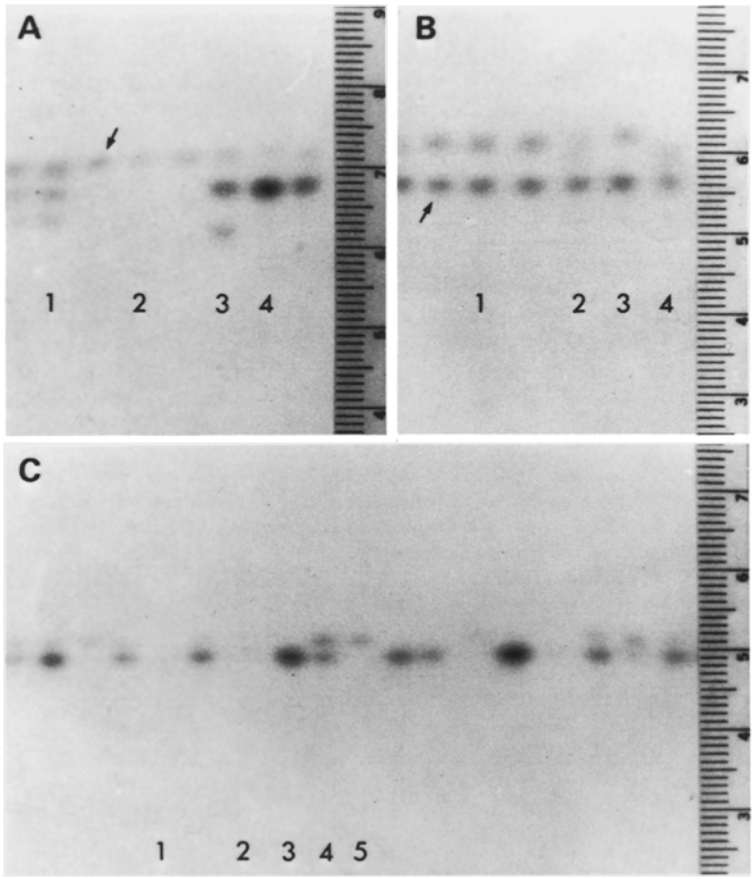

Fig. 2A–C. Photographs of gels stained for hexokinase. In all figures the origin is at the bottom and the anode is at the top. **A** Allelic variation at *Hex2* with *Hex1* fixed for the most common allele (*arrow*). Homozygotes and heterozygotes are one and two-banded,, respectively. *Channels 1, 2, 3* and *4* illustrate the genotypes *Hex2-2/4, Hex2-n/n* (null), *Hex2-2/6* and *Hex2-2/2,* respectively. **B** Allelic variation at *Hex1,* with *Hex2* fixed for the most common allele (*arrow*). *Channels 1, 2, 3,* and *4* have genotypes *Hex1-4/4, Hex1-2/6, Hex1-2/2* and *Hex1-6/6,* respectively. **C** Segregation of null alleles in an F_2 of *Hex1-n/nHex2-2/2×Hex1-4/4Hex2-n/n.* Individuals homozygous null at both loci are evident in *channels 1* and *2. Channels 3, 4,* and *5* have genotypes *Hex1-n/nHex2-2/2, Hex1-4/4Hex2-2/2* (or *2/n*) and *Hex1-4/4* (or *4/n*) *Hex2-n/n,* respectively

Hex1 and *Hex2,* respectively, $P > 0.05$). Of the 135 F_2 plants examined, eight were homozygous null at both loci (Fig. 2C), which is the number expected under the hypotheses of no differential selection or viability among the null and active variants. Some of the double homozygous-null individuals were transplanted to the field nursery, where they appeard fully vigorous. Upon selfing, these plants produced a true breeding HEX-null stock.

The occurrence of vigorous plants that apparently lack cytosolic hexokinase activity, and the inability to detect mitochondrial hexokinase suggests that other hexose-phosphorylating enzymes exist in maize which are not visualized under our electrophoretic and staining conditions. In this regard, it is noteworthy that a few slowly migrating and faint bands are occasionally observed in some of our gels. A reasonable suggestion is that cytosolic hexokinase is not necessary for normal growth and development and that the faint, slowly migrating bands result from the activity of mitochondrial hexokinases. The ability of plants to grow and reproduce without cytosolic hexokinase is similar to the situation for maize malate dehydrogenase (MDH). Goodman et al. (1981) showed that lines lacking cytosolic MDH's are viable, but that at least one active allele specifying mitochondrial MDH is required for normal growth.

Most widely used inbred lines have the genotype *Hex1-4/4Hex2-2/2,* but some variation does occur in publicly availables lines. Examples of lines which contain variant *Hex1* alleles are as follows: *Hex1-null* – CM37, K6; *Hex1-5* – OS420, A632; *Hex1-6* – A641, Ky201. Some lines with variant *Hex2* alleles are *Hex2-1* – CM37, CO159; *Hex2-1.7* – Ky201; *Hex2-4* – OS420, CI64, NC7, C123, CH701-30.

Mapping of Hex1 and Hex2

Hex1 and *Hex2* were mapped by studying joint segregations with previously mapped allozyme loci (Goodman et al. 1980b; Goodman and Stuber 1983; Wendel et al., unpublished) and by crosses with B-A translocation stocks (Beckett 1978). The placement of *Hex1* was revealed through the analysis of a large F_2 between the inbred lines CM37 and T232, which segregated for 5 allozyme markers on chromosome 3. Parental genotypes, joint segregation data, and maximum likelihood estimates of the recombination percentage between pairs of loci are presented in Table 2. These data are consistent with the previously reported relationships

Table 2. Two locus-segregation data from an F_2 of the inbred lines CM37 and T232, and maximum linkelihood estimates of the recombination fractions (r) between 5 loci on chromosome 3

Locus pair	N	Genotypes[a]									χ^2 (df)	r (se)[b]
		X_1Y_1	X_1Y_2	X_1Y_3	X_2Y_1	X_2Y_2	X_2Y_3	X_3Y_1	X_3Y_2	X_3Y_3		
E8-Hex1	1,925	259	234	–	160	811	–	18	443	–	364.3 (2)	0.243 (0.011)
E8-Tpi4	1,928	–	409	85	–	724	247	–	239	224	124.2 (2)	0.358 (0.013)
E8-Pgd2	1,928	168	227	99	212	483	276	66	198	199	88.6 (4)	0.399 (0.011)
E8-Mdh3	1,928	125	241	128	228	488	255	89	242	132	5.4 (4)	–
Hex1-Tpi4	1,925	–	422	15	–	948	540	–	–	–	177.7 (1)	0.169 (0.022)
Hex1-Pgd2	1,925	279	136	22	166	771	551	–	–	–	552.5 (2)	0.199 (0.010)
Hex1-Mdh3	1,925	112	225	100	330	744	414	–	–	–	5.0 (2)	–
Tpi4-Pgd2	1,928	–	–	–	442	819	111	4	89	463	1,078.6 (2)	0.104 (0.007)
Tpi4-Mdh3	1,928	–	–	–	345	680	347	97	291	168	14.4 (2)	0.451 (0.013)
Pgd2-Mdh3	1,928	145	211	90	215	474	219	82	286	206	65.8 (4)	0.411 (0.011)

[a] Genotypes of the parents are as follows: CM37 – *E8-4.5, Hex1-n, Tpi4-4, Pgd2-5, Mdh3-16;* T232 – *E8-4, Hex1-4, Tpi4-n, Pgd2-2.8, Mdh3-18.* X and Y refer to the genotypes at the first and second locus listed for each locus pair. The subscripts 1, 2, and 3, indicate homozygosity for the CM37 allele, heterozygosity for the CM37 and T232 alleles, and homozygosity for the T232 allele, respectively. Two detectable classes of progeny exist for *Hex1* and *Tpi4,* and the subscript 2 indicates the null/active heterozygotes pooled with the active variant homozygotes. The 1 or 3 indicates the null variant class

[b] Recombination fraction not estimated if χ^2 for independence has an associated probability of greater than 0.05

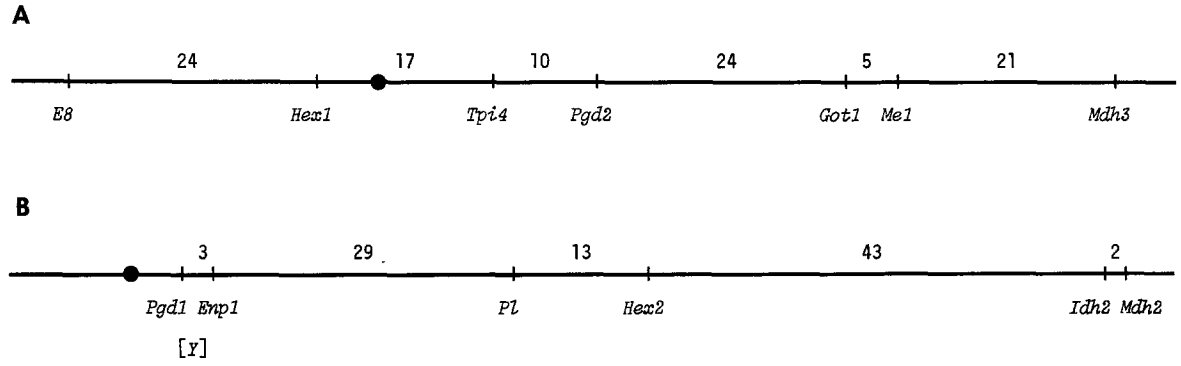

Fig. 3A, B. Linkage relationships among allozyme markers on maize chromosomes 3 and 6. Centromeres (*circles*) divide the maps into short (*to the left*) and long (*to the right*) arms. Recombination fractions are given *above* the maps, and locus symbols *below*. Locus symbols are as follows: *E8:* esterase; *Tpi4:* triose phosphate isomerase; *Pgd1, Pgd2:* phosphogluconate dehydrogenase; *Got3:* glutamate oxaloacetate transaminase; *Me:* malic enzyme; *Mdh2, Mdh3:* malate dehydrogenase; *Idh2:* isocitrate dehydrogenase; *Enp1:* endopeptidase; *Hex1, Hex2:* hexokinase. **A** Map for 7 allozyme markers on chromosome 3. **B** Map for 5 allozyme markers and two color factors on chromosome 6. *Y* and *Pl* are the pigment factors yellow endosperm and purple plant, respectively

among the loci *E8, Pgd2,* and *Mdh3,* and further indicate that *Hex1* and *Tpi4* (one of the three genes encoding cytosolic triose phosphate isomerase; Wendel, unpublished) lie between *E8* and *Pgd2.* The localization of *Hex1* to the short arm of chromosome 3 was confirmed with a cross of the B-A translocation stock TB-3Sb (*Hex1-4/4)* onto the *Hex1-null* tester Ky201. Postmeiotic, mitotic nondisjunction in the male (TB-3Sb) results in some gametes which fail to transmit the portion of the chromosome arm distal to the translocation breakpoint, which results in zygotes that are hypoploid for this part of the genome (Beckett 1978). Null phenotypes (i.e. hypoploids) were recovered in 5 of 13 plants examined. These data collectively place *Hex1* on 3S approximately 24 centimorgans proximal to *E8,* and lead to the 7-point isozyme map for chromosome 3 illustrated in Fig. 3A. The discovery that two additional allozyme markers, *Hex1* and *Tpi4,* lie between *E8* and *Pgd2* forces the genetic map for this chromosome segment to enlarge by approximately 12 centimorgans over previous estimates (Goodman et al. 1980b).

Hex2 was placed on the long arm of chromosome 6 through the analysis of two large F_2 populations generated for other reasons (Edwards et al., in preparation). One of these (the F_2 of the inbreds Tx303 and CO159)

Table 3. Two locus-segregation data from F_2's of inbred lines, and maximum likelihood estimates of the recombination between loci on the long arm of chromosome 6

Locus pair	N	Genotypes[a]									χ^2 (df)	r (se)[b]
		X_1Y_1	X_1Y_2	X_1Y_3	X_2Y_1	X_2Y_2	X_2Y_3	X_3Y_1	X_3Y_2	X_3Y_3		
A. F_2 of Tx303 and CO159:												
Pgd1-Enp1	1,842	446	17	1	31	880	22	0	28	417	3,117.2 (4)	0.028 (0.003)
Pgd1-P1	1,835	408	–	54	738	–	190	231	–	214	180.5 (2)	0.316 (0.013)
Pgd1-Hex2	1,677	157	206	68	204	455	186	52	184	165	110.3 (4)	0.377 (0.011)
Pgd1-Idh2	1,840	166	226	122	228	465	239	100	228	116	1.0 (4)	–
Pgd1-Mdh2	1,841	115	230	119	231	463	238	99	235	111	1.6 (4)	–
Enp1-P1	1,835	427	–	49	739	–	181	211	–	228	239.0 (2)	0.291 (0.012)
Enp1-Hex2	1,677	173	204	61	195	461	184	45	180	174	155.7 (4)	0.354 (0.011)
Enp1-Idh2	1,842	129	231	117	228	472	220	120	222	103	1.6 (4)	–
Enp1-Mdh2	1,842	117	226	103	232	471	225	128	228	112	1.1 (4)	–
P1-Hex2	1,674	404	754	93	–	–	–	10	90	323	815.6 (2)	0.125 (0.009)
P1-Idh2	1,835	361	673	343	–	–	–	83	244	131	12.4 (2)	0.457 (0.014)
P1-Mdh2	1,835	355	685	337	–	–	–	88	243	127	8.3 (2)	0.463 (0.014)
Hex2-Idh2	1,677	125	190	98	211	442	192	65	210	144	37.6 (4)	0.434 (0.012)
Hex2-Mdh2	1,677	118	197	98	214	439	192	72	209	138	25.4 (4)	0.447 (0.012)
Idh2-Mdh2	1,842	427	18	0	19	888	13	0	22	455	3,265.4 (4)	0.020 (0.002)
B. F_2 of CM37 and T232:												
Hex2-Idh2	1,924	129	210	76	233	512	276	88	229	171	40.6 (4)	0.426 (0.011)
Hex2-Mdh2	1,925	119	215	82	233	508	280	95	231	162	24.1 (4)	0.444 (0.011)
Idh2-Mdh2	1,927	427	19	5	15	906	32	5	30	488	3,051.4 (4)	0.031 (0.003)

[a] Genotypes of the parents are as follows: Tx303 – *Pgd1-2, Enp1-6, P1, Hex2-2, Idh2-6, Mdh2-3;* CO159 – *Pgd1-3.8, Enp1-10, p1, Hex2-1, Idh2-4, Mdh2-6;* CM37 – *Hex2-1, Idh2-6, Mdh2-3.5,* T232 – *Hex2-2, Idh2-4, Mdh2-6.* X and Y refer to the genotypes at the first and second locus listed for each locus pair. The subscripts 1, 2, and 3, indicate homozygosity for the Tx303 (or CM37) allele, heterozygosity for the Tx303 and CO159 (or CM37 and T232) alleles, and homozygosity for the CO159 (or T232) allele, respectively. In the case of *P1,* 1 indicates pooled *P1/P1* and *P1/p1* genotypes and 3 indicates *p1/p1* genotypes

[b] Recombination fraction not estimated if χ^2 for independence has an associated probability of greater than 0.05

segregated for 6 loci on 6L (allozyme loci *Pgd1, Enp1, Hex2, Idh2,* and *Mdh2,* and the plant color factor *Pl*[1]), and the other (from inbreds CM37 and T232) was segregating alleles at *Hex2, Idh2,* and *Mdh2.* Parental genotypes, joint segregation data, and maximum likelihood estimates of the recombination fractions between loci are presented in Table 3. These data allow the map shown in Fig. 3 B to be constructed. *Hex2* is located approximately 12.5 map units distal to *Pl* near the center of the chromosome arm. As illustrated, the map of Fig. 3 B has two particularly noteworthy features:

1) *Idh2* and *Mdh2,* which were previously shown to be on 6L (Goodman et al. 1980 a, b) perhaps 12 units from *py* (pygmy; footnote in McMillan et al. 1979) at approximately position 77, must be moved a minimum of 23 units distal to their previously assigned locations. As a consequence, the genetic map for 6L must be expanded by a third over its prior size.

2) The B-A translocation stock TB-6Lc, which uncovers *y* (the recessive white endosperm allele at the yellow locus) also uncovers all allozyme markers on 6L except *Pgd1* (Table 4). Consequently, *Pgd1* must be proximal, and *Enp1* distal, to the TB-6Lc breakpoint.

The proper gene order of the two closely linked loci *Idh2* and *Mdh2* cannot unequivocally be determined from the F_2 data at hand, but they suggest that *Mdh2* is the more distal locus. The order illustrated must be considered tentative until testcross progenies are evaluated.

Duplicated chromosome segments in maize

Previous linkage work among maize allozyme loci has demonstrated parallel linkages among two sets of duplicated genes: 1) *Mdh4-Pgm1* on chromosome 1 with

[1] *Pl* scoring was as follows: those individuals without any visible anthocyanin pigmentation were scored as recessive homozygotes (*plpl*), while individuals that carried the dominant allele (*Pl*) variably expressed maroon pigmentation in the silks, leaf margins, and internerval spaces of the glumes. While there are no other plant color factors mapped in the region of *Pl,* this expression is apparently not conventional (Coe, personal communication). Two possibilities are suggested: 1) A hitherto unrecognized variant at the *Pl* locus was scored; 2) There is an additional pigmentation factor near *Pl* which has gone unmapped to date. Studies are currently underway to test these possibilities

Table 4. The B–A translocation stock TB-6Lc uncovers all isozyme markers on 6L with the exception of *Pgd1*

Tester	Locus tested	No. hypoploid for the tester allele	No. heterozygous for the TB stock and tester alleles
Ky201	*Enp1*	4	8
	Hex2	4	8
	Idh2	4	8
	Mdh2	4	8
T226	*Pgd1*	0	18
	Enp1	11	7

23% recombination, and *Mdh5-Pgm2* on chromosome 5 with 17% recombination; 2) *Idh1-Mdh1* on chromosome 8 with 22% recombination and *Idh2-Mdh2* on chromosome 6 with about 2–3% recombination (Goodman et al. 1980b and present study). Data presented here suggest a third duplicated chromosome segment based on an additional parallel linkage: *Hex1-Pgd2* on chromosome 3 (27 map units apart – Fig. 3A) and *Hex2-Pgd1* on chromosome 6 (45 map units apart – Fig. 3B). If these interpretations are correct, it suggests a complex evolutionary history for the long arm of chromosome 6, in that it appears to possess regions homologous to both the central part of chromosome 3 (*Pgd-Hex* segments), and the long arm of chromosome 8 (*Idh-Mdh* segments). Unpublished data for triose phosphate isomerase (TPI) loci lends support to this hypothesis. Of the three loci encoding cytosolic TPI's, two have been mapped, to chromosomes 3 and 8 (Wendel et al., unpublished). The remaining locus has not yet been mapped, but it is intriguing to speculate that it will ultimately be found to reside on chromosome 6. Stocks are currently being synthesized to test this prediction.

Concluding remarks

Despite the ubiquity of hexokinase and fructokinase in plant tissues, they have rarely been studied genetically, and little comparative data are available on their phenotypes and variation patterns in electrophoretic gels. The few but distantly related diploid taxa which have been examined to date display the products of only a single hexokinase locus: *Citrus* and *Poncirus* (Torres et al. 1985); *Salicornia europaea* and *S. ramosissima* (Jefferies and Gottlieb 1982); *Pinus halepensis* (Loukas et al. 1983); and notably the more closely related (to maize) diploids within the wheat genera *Triticum* and *Aegilops* (Brody and Mendlinger 1980). In these studies, the hexokinase locus was either monomorphic or was not investigated genetically. In contrast, three loci were responsible for only five of the thirteen hexokinase isozymes resolved by isoelectric focusing in hexaploid wheat (Ainsworth 1983). The genetic control of the remaining bands has not yet been reported, but additional loci are clearly active.

The data presented here indicate that maize contains two hexokinase loci, an observation that is consistent with an interpretation of gene duplication by some mechanism. Other maize isozyme systems that display a similar pattern of gene multiplicity above the diploid level (sensu Gottlieb 1982) include aminopeptidase (Ott and Scandalios 1978), superoxide dismutase (Baum and Scandalios 1981), aconitase, isocitrate dehydrogenase, phosphoglucomutase, 6-phosphogluconate dehydrogenase, malate dehydrogenase, glutamate dehydrogenase, and triose phosphate isomerase (Goodman et al. 1980a, 1980b; Goodman and Stuber 1983; Stuber and Goodman 1983b, 1984; Wendel et al. 1985). The parallel linkages found among pairs of these loci clearly suggest that chromosome segment duplication has occurred during the evolution of the maize genome, a suggestion which finds cytogenetic support in the work of Weber and Alexander (1972) and Bennett (1984). The chromosome number of maize ($2n=20$) is the most common number reported in its tribe (Andropogoneae). There are, however, a few genera in the Andropogoneae that contain members with a haploid complement of five chromosomes; i.e., *Coix* (Mangelsdorf and Reeves 1939; Nirodi 1955), *Elyonurus* (Celarier 1957), and *Sorghum* (Carnahan and Hill 1961). Because of this, it is tempting to speculate that chromosome segment duplication in maize reflects an evolutionary history for the genome which includes tetraploidy. Indeed, Bennett (1984) presents evidence for an allotetraploid origin of the ancestral stock of maize based on the spatial arrangement of mitotic metaphase chromosomes in the cell. It should be noted, however, that certain features of the data presented here suggest additional evolutionary complexities unaccounted for by a simple model of gene and chromosome segment duplication through polyploidy; i.e., the apparent homology of separate parts of the long arm of chromosome 6 with regions of chromosomes 3 and 8, and the fact that the Hex-Pgd segment on chromosome 3 spans the centromere whereas on chromosome 6 it does not. More evidence is required before the role of polyploidy in maize evolution can be fully evaluated, and one of the critical sources of additional data is likely to be comparative linkage relationships among unique or nearly unique fragments of the maize genome.

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