THE CYTOGENETIC LOCALIZATION OF THE ALCOHOL DEHYDROGENASE-1 LOCUS IN MAIZE

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ABSTRACT

The alcohol dehydrogenase-1 (*Adh*) locus in maize has been positioned relative to thirteen reciprocal translocations that have breakpoints in the long arm of chromosome *1* (*1L*). The methods of GOPINATH and BURNHAM (1956) to produce interstitial segmental trisomy with overlapping translocations and of RAKHA and ROBERTSON (1970) to produce compound *B-A* translocations were coupled with the co-dominant nature of the ADH isozymes to allow the cytological placement. The results of several crosses are consistent with *Adh* being in the region of 0.80–0.90 of *1L*.——The duplication that results from the overlap of translocations *1–3*(5267) and *1–3*(5242) and that includes *Adh* was studied with respect to meiotic segregation and pollen transmission. When heterozygous with normal chromosomes, a low level of recombination within the duplicated regions is detectable and the duplication and normals are recovered with equal frequencies through the female. In the pollen, the hyperploid grains cannot compete equally with the euploids in achieving fertilization.——The use of co-dominant heteromultimeric isozymes as genetic markers for the development of a series of interstitial segmental trisomics in maize is discussed.

THE alcohol dehydrogenase-1 (*Adh*) locus in maize has been extensively studied as a model system of enzyme expression (*e.g.*, SCHWARTZ 1971; FREELING 1975; LAI and SCANDALIOS 1977). It has been shown to be uncovered by *TB-1La* and to map approximately 1.5 units from *lw*(*1*–128) (SCHWARTZ 1971); however, a more precise cytogenetic localization would advance this work, primarily by allowing the dosage of the structural locus to be more easily manipulated. In this paper, experiments are described that localize this gene relative to thirteen reciprocal translocations having a breakpoint in *1L*. The approach used to further define its position was to combine two genetic techniques with starch gel electrophoresis of ADH isozymes. The overlapping translocation procedure of GOPINATH and BURNHAM (1956) was employed to generate interstitial trisomic regions along *1L*, which were then tested for inclusion of *Adh*. To confirm these results, compound B-A translocations (RAKHA and ROBERTSON 1970) involving various lengths of *1L* were synthesized and then crossed to a line with a unique *Adh* allele in order to determine whether the locus was uncovered.

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The use of overlapping translocations to produce segmental trisomy has been extensively applied to a number of problems in Drosophila genetics, including the cytogenetic localization of several enzyme structural genes (O'BRIEN and GETHMAN 1973; STEWART and MERRIAM 1974, 1975; HODGETTS 1975; OLIVER, HUBER and WILLIAMSON 1978; HALL and KANKEL 1976; MOORE and SULLIVAN 1978; PATTERSON, STONE and SUCHE 1938; PATTERSON, BROWN and STONE 1940; PIPKIN 1940; LINDSLEY *et al.* 1972). Their use in maize, however, has been limited by a lack of the appropriate genetic markers for the various classes of aneuploids in segregating progenies. Isozyme variants, encoded by co-dominant alleles, can serve in this capacity, allowing one to distinguish a dosage series of the region being investigated. Although the *Adh* locus was used as the specific example in the study reported here, the methodology is generally applicable. Other isozymes for which variants have been described (SCANDALIOS 1974) can be localized in a similar manner and then used as markers for aneuploids involving other chromosome arms. Since a wide spectrum of reciprocal translocations is available in maize (LONGLEY 1961), aneuploids involving most of the genome can eventually be produced.

MATERIALS AND METHODS

Experimental rationale and protocol: GOPINATH and BURNHAM (1956) outlined procedures for producing interstitial deficiencies and duplications by combining two translocations involving different breaks in the same two chromosomes. Depending upon the relative positions of the breakpoints of the chosen translocations and the types of segregation, the regions of the two chromosomes between the breaks will be deficient-deficient, duplicate-deficient or duplicate-duplicate. Since deficient gametophytes generally abort, only the last type (double duplicates) were generated in this study. Basically, the experimental design was to cross together two different translocations that have breakpoints in the same two chromosome arms. In one of these translocations, the break in chromosome *1L* was proximal, while the break in the other chromosome was distal. For the other translocation, the relative positions of the breakpoints were reversed, as diagrammed in Figure 1a.

When a plant heterozygous for such interchanges undergoes meiosis, four types of gametes are formed, assuming segregation of homologous centromeres: (1) balanced translocation A; (2) balanced translocation B; (3) deficiencies for the regions between the breakpoints; and (4) duplications for the regions between the breakpoints. If the deficiencies produced by type three are large, the resulting gametophytes will abort. If adjacent-2 segregations occur, severely deficient gametophytes are formed and would also abort. The gametic types produced are shown in Figure 1b.

If the heterozygote of the two interchanges is used as a female and pollinated by a normal male, the resulting ear is approximately 25% sterile due to the abortion of deficient gametophytes and has 33% of the kernels heterozygous for interchange A, 33% of the kernels heterozygous for interchange B and 33% segmentally trisomic for all the regions between the translocation breakpoints. If a crossover occurs between the breakpoints on one of the two chromosomes, the complementary products are 1) a normal chromosome, and (2) a chromosome duplicated for the regions delineated by the two translocation breakpoints on one chromosome, whose two components are separated by an insertion of the segment between the breakpoints of the second chromosome. Only when the two crossover strands segregate together would a viable gametophyte be produced. This gamete type would be duplicate for the regions between the breakpoints of both translocations. It is technically difficult to recognize such crossovers, but it is important to note that comparable situations in Drosophila permit little or no recombination (BEADLE 1933; PATTERSON, STONE and BEDICHEK 1937).

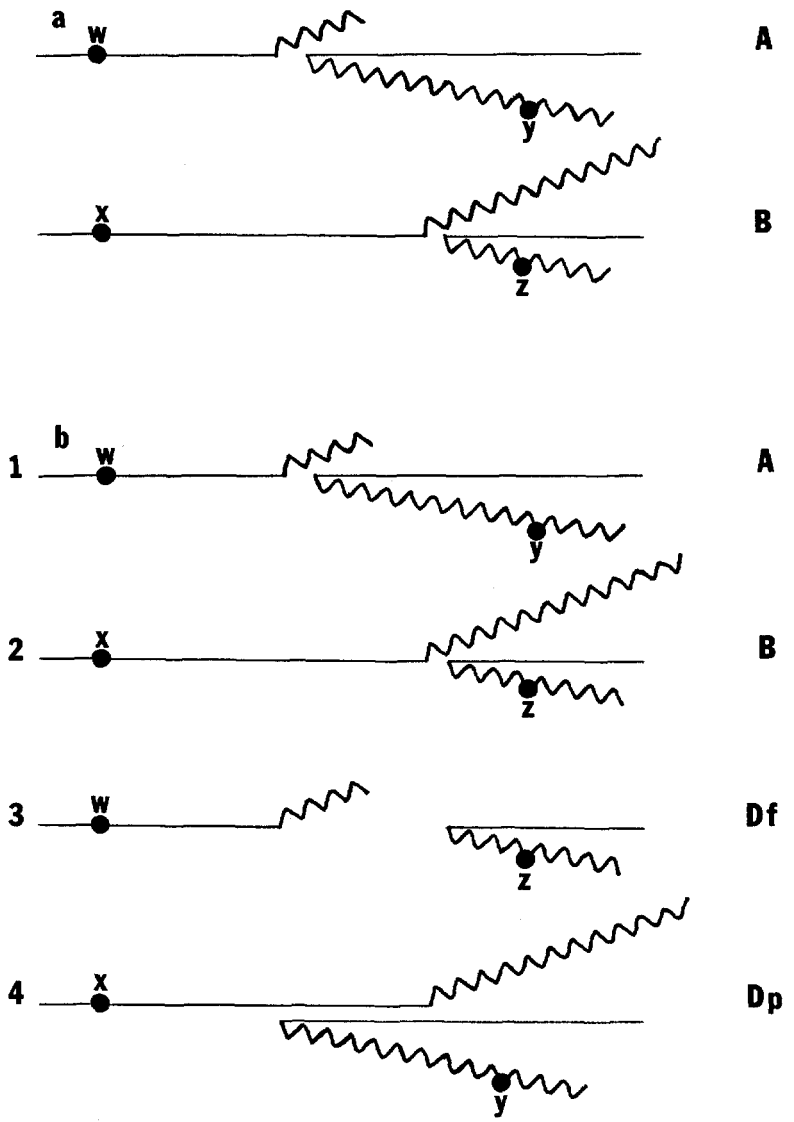

FIGURE 1.—(a) Chromosomal constitution of a heterozygote of two translocations with displaced breakpoints. Straight lines represent chromosome *1* and the wavy lines represent the other chromosome involved in any particular combination. w and x represent centromeres of chromosome *1* and y and z those of the other chromosome. (b) Chromosomal constitutions of gametes expected from segregation of homologous centromeres: 1. Balanced translocation A; 2. Balanced translocation B; 3. Deficiency for the regions between the breakpoints; 4. Duplication for the regions between the breakpoints. The possible crossover classes and adjacent-2 segregations have been omitted.

In order to locate the *Adh* gene cytologically, heterozygotes of two appropriate interchanges were crossed as females by normal pollen from plants that were homozygous for *Adh-C-70–86*, an ethyl methanesulfonate-induced electrophoretic variant discovered by D. SCHWARTZ. Since this variant is not found in natural populations of maize, there will be a detectable heterozygosity of ADH mobility in the resulting progeny. A majority of maize varieties have an *Adh-F* allele. If the *Adh* gene is located between the breakpoints of the two translocations, a third of the kernels would show an approximate 4:4:1 zymogram ratio for the F·F homodimer: C·C heterodimer:C·C homodimer isozyme bands. This would be the case because twice the number of F to C subunits would be produced and would randomly dimerize in these proportions. Such scutella are segmentally trisomic for the regions of the two chromosome arms between the breakpoints. The remaining two-thirds (euploids) of the kernels will have a zymogram ratio of approximately 1FF : 2FC : 1CC isozyme bands, since there will be nearly equal numbers of F and C subunits produced.

If, however, one of the translocation chromosomes carries an *Adh-S* allele while the other carries an *Adh-F*, the segmentally trisomic scutella from crosses with *Adh-C* would be *Adh-F/S/C*. The zymogram pattern of the segmental trisomic would show all three types of homodimers and their respective heterodimers. The remaining scutella will have S/C or F/C genotypes and produce zymograms with only the two variants and the intermediate heterodimer.

If the *Adh* locus lies outside the regions spanned by the breakpoints, none of the scutella would give zymograms that indicate trisomy for the *Adh* locus. All kernels will show a 1F·F : 2F·C : 1C·C isozyme band ratio if both translocation stocks have the *Adh-F* allele. If the heterozygote of the two interchanges is also heterozygous for *Adh-F* and *S*, there will be only F/C and S/C scutella. The frequency of the two classes will depend upon the genetic distance separating the *Adh* locus from the translocations. The ratio of the two classes will deviate from 1:1 because one variant will be more often linked to the lethal deficiency produced in the cross.

The combinations of translocations tested are listed in Table 1. The choice of those used was based on the published breakpoints (LONGLEY 1961) and the availability of homozygous stocks. All were obtained from the Maize Stock Center, University of Illinois, Urbana.

Compound TBA's produced: In order to verify the correct order of the breakpoints in certain combinations that duplicated the *Adh* locus, compound *B-A* translocations (RAKHA and ROBERTSON 1970) were constructed. The following *1L-3L* compounds were synthesized: *TB-1La-3L4759–3*(*1L* 0.20–0.39; *3L* 0.20 to tip); *TB-1La-3Le*(*1L* 0.20–0.58; *3L* 0.45 to tip); *TB-1La-3L5267*(*1L* 0.20–0.72; *3L* 0.73 to tip); and *TB-1La-3L5242*(*1L* 0.20–0.90; *3L* 0.65 to tip).

This series was produced as follows, using *TB-1La-3L4759–3* as an example: Plants hyperploid for *TB-1La*($11^{B}B^{1}B^{1}$) and homozygous for the standard *Adh-S* allele were used as females in crosses by a stock of translocation *1–3*(4759–3). The F_1 plants included heterozygotes for both *TB-1La* and *T1–3*(4759–3). These F_1 plants were crossed as males onto an *a-m-1 A2 C C2 R-scm2* tester. This tester is homozygous for the *R-scm2 allele* (WEYERS 1961) of the *R* locus on chromosome ten. The presence of this allele allows expression of anthocyanin in scutellar tissue if the other complementary color factors are present (see ROBERTSON 1967). The *A2*, *C* and *C2* loci are on chromosomes 5, *9* and *4*, respectively and are required for anthocyanin production. Anthocyanin expression in the tester line is blocked due to the recessive *a-m-1* allele on *3L*. If in the *TB-1La/T1–3*(4759–3) translocation heterozygote, a crossover occurs between the *B-A* translocation breakpoint(at 0.20 in *1L*) and the *T1–3*(4759–3) break(at 0.39 in *1L*), a *TB-1La-3L4759–3* compound translocation is produced. The chromosome with the *B* centromere has the segment of *1L* between 0.20 and 0.39 combined with the translocated portion of *3L*. If the proper segregation occurs to bring the 1^{B}, the B^{1-3L} and 3^{1} chromosomes into the same microspore, and if the *B* centromere undergoes nondisjunction (80% for *TB-1La*, BIRCHLER, unpublished) and fertilizes the egg instead of the polar nuclei, the resulting kernels can easily be recognized as a potential new compound *TB-A* because the scutellum will be colored, although the aleurone will be colorless. This will be the case because the *A* locus will be carried in the distal portion of such a chromosome and, when added to the genome of the tester, will allow anthocyanin production. Each such isolate was crossed again to the *a-m-1 A2 C C2*

R-scm2 tester to confirm the presence of a new compound *TB-1La-3L*, as well as to establish a stock. The remaining three compounds were synthesized in a similar manner.

Tests for the inclusion of Adh *in compound* TB-A's: Plants homozygous for the *Adh-C* allele were used as females and crossed by hyperploid *A* A^{B} B^{AA} B^{AA} males of each compound *TB-A*. If the *Adh* locus is contained in the portion of *1L* present in the compound translocation, large numbers of C/- and C/F/F (or C/S/S) progeny will result. If *Adh* is not included in the *1L* region translocated to the *B* centromere, all of the scutella will exhibit zymograms typical of *Adh-C/F* (or *C/S*) heterozygotes. In addition to the four compound *TB-1La-3L*'s synthesized, a test for the inclusion of *Adh* in *TB-1La-5S8041* (*1L* 0.20–0.80; *5S* 0.20 to tip) (ROBERTSON 1975) was conducted.

Electrophoresis and staining: Starch gel electrophoresis and staining for ADH isozymes was by the method of SCHWARTZ and ENDO (1966). Electrophoresis was conducted at 5°.

RESULTS

The types of progeny found when each of the heterozygotes of two translocations was crossed as females by *Adh-C* are given in Table 1. Combinations 3, 4, 6 and 9 produced overlaps that are segmentally trisomic for *Adh*. One of the breakpoints of each of these combinations must lie distal to and the other proximal to the *Adh* locus.

In the two cases (#'s 6, 9) in which segmental trisomics were detected on the basis of skewed isozyme ratios rather than the introduction of three variants, each of the four individual translocation stocks involved was crossed by *Adh-C* and the isozyme ratio examined to determine whether any anomalous variants were present in these lines. This test was necessary to eliminate the possibility that *Adh* lies outside the duplication, but one of the two translocations was linked to an allele whose expression is much greater than normal. Such a situation could produce the observed results, if the usual allele were linked to the more distally broken translocation, but none of these lines showed particularly unusual zymograms—an observation that makes this alternative unlikely.

It is possible to gain additional information from these data by examining the frequency of ADH types from combinations that were not only heterozygous for two translocations, but also heterozygous for *Adh-F* and *S*. Combinations #15 and #16 were *Adh-F/S* heterozygotes. When these F_1 plants were crossed as females by *Adh-C* pollen, the allele linked to the breakpoint nearer the *Adh* locus was more frequently included in the deficient gametic types. Since these gametophytes aborted, the FC:SC segregation deviated from a 1:1 ratio. Table 2 gives the chi-square test of a one to one segregation and shows that both deviate significantly from the expected. Since it was also known which *Adh* allele is linked to each translocation, the breakpoints could be ordered with respect to the centromere and *Adh*. The translocation *1–10*(8375) is used in both combinations and is linked to an *Adh-S*. In combination #15, ADH-S is recovered less frequently than F, but in #16 it is recovered more often. Using the above reasoning, the order is centromere — *T1–10(d)*—*T1–10*(8375)—*T1–10*(001–3)—*Adh*. This order is consistent with the cytological observations of LONGLEY (1961).

With combination #8, each translocation is linked to a different allele, but the two variants are recovered with equal frequency with a low percentage of

TABLE 1

Combinations of translocations tested and types of progeny

Combination no.	Translocations	*Adh* alleles	*1L* Breakpoints	Other breakpoints	*Adh* genotypes of progeny from crosses by *Adh-C* F/C	S/C	F/S/C	F/F/C	S/S/C	Comments
1	*1–3e/1–3*(8405)	F;F	0.58/0.60	*3L* 0.45/*3L* 0.31	33					
2	*1–3*(5476)/*1–3*(5267)	F;F	0.66/0.72	*3L* 0.87/*3L* 0.73	36					
3	*1–3*(5476)/*1–3*(5242)	F;S	0.66/0.90	*3L* 0.87/*3L* 0.65	137	144	126	1		Duplicates *Adh*
4	*1–3*(5267)/*1–3*(5242)	F;S	0.72/0.90	*3L* 0.73/*3L* 0.65	296	288	264			Duplicates *Adh*
5	*1–6*(5225–4)/*1–6*(4456)	F;F	0.61/0.71	*6L* 0.72/*6L* 0.30	76					
6	*1–7*(4891)/*1–7*(5693)	F;F	0.12/0.92	*7L* 0.69/*7L* 0.18	83			40		Duplicates *Adh*
7	*1–8*(026–2)/*1–8*(6766)	S;S	0.49/0.54	*8L* 0.80/*8L* 0.77		60				
8	*1–8*(6766)/*1–8*(5821)	S;F	0.54/0.65	*8L* 0.77/*8L* 0.31	100	109	12			Breakpoints in error
9	*1–8b/1–8*(6697)	S;S	0.59/0.89	*8L* 0.82/*8L* 0.52		89			47	Duplicates *Adh*
10	*1–9*(4997–6)/*1–9*(4398)	F;F	0.37/0.51	*9S* 0.28/*9S* 0.19	54					
11	*1–10*(068–14)/*1–10*(001–3)	F;F	0.16/0.86	*10L* 0.79/*10L* 0.48	45					
12	*1–10*(5273)/*1–10*(001–3)	F;F	0.17/0.86	*10L* 0.69/*10L* 0.48	59					
13	*1–10*(8491)/*1–10*(001–3)	F;F	0.45/0.86	*10L* 0.76/*10L* 0.48	46					
14	*1–10d/1–10*(001–3)	F;F	0.50/0.86	*10L* 0.68/*10L* 0.48	44					
15	*1–10d/1–10*(8375)	F;S	0.50/0.69	*10L* 0.68/*10L* 0.64	131	69				Deviant segregation
16	*1–10*(8375)/*1–10*(001–3)	S;F	0.69/0.86	*10L* 0.64/*10L* 0.48	95	166				Deviant segregation

Breakpoints are those listed by LONGLEY 1961. They are expressed as the fraction of the chromosome arm involved.

TABLE 2

Chi-square analysis of segregation ratios from combinations heterozygous for Adh-F/S

Combination	Translocations	Observed frequency F/C	Observed frequency S/C	Expected frequency F/C	Expected frequency S/C	χ^2	P
15	*1-10d/1-10*(8375)	131	69	100	100	19.22	< 0.001
16	*1-10*(8375)*/1-10*(001-3)	95	166	131	131	19.24	< 0.001

gametes duplicated for *Adh*. To account for these results, one must conclude that the relative position of one or both of the *1L* or *8L* breakpoints is in error. This conclusion is necessary to explain the equal frequencies of F and S gametes. Such results could occur if a proximal break in *1L* is associated with a proximal *8L* breakpoint in one translocation, with the other interchange having both breakpoints distal *or* if one of the breakpoints lies on the other side of centromere *1* or *8* from its reported location. With these conditions, two possibilities could explain the *AdhF/S* gametes: (1) the true breakpoints in *1L* surround the *Adh* locus and are associated with a deficiency of *8L* that allows only a low frequency of such gametophytes to survive or (2) the *Adh* locus lies outside of the breakpoints with occasional 3:1 segregation to produce gametes with one of the two balanced translocations plus the element of the other that carries the *Adh* gene. The data reported here cannot discriminate among the possibilities and the analysis was not carried beyond this point.

By comparing those combinations that include the *Adh* locus with those that exclude it, one finds that, *prima facie*, *Adh* lies between 0.86 and 0.89 on *1L*. This is based on its inclusion in combination #9 and its exclusion from #16. This finding must be tempered with the knowledge that the cytological determinations are, at best, approximations and that the pachytene cross configurations observed in translocation heterozygotes — from which the determinations were made, do not necessarily coincide with the actual physical breakpoints (BURNHAM 1934). A negative result from a translocation combination should not be construed as providing information on the location of *Adh* or the translocation breakpoint. Only those combinations that duplicate *Adh* or that give deviant segregation ratios can be considered to have provided information.

Test for inclusion of Adh *in compound* B-A *translocations:* The B-A translocations of maize have proved quite useful for locating genes to chromosome arms (ROMAN and ULLSTRUP 1951; BECKETT 1978). This is derived from the fact that the *B* chromosome centromere undergoes nondisjunction in the majority of second microspore divisions, resulting in the production of large numbers of deficient sperm. However, because there are few chromosome arms for which more than one *TB-A* exists, their utility for further localizations is limited. The compound *TB-A*'s, first synthesized by RAKHA and ROBERTSON (1970) provide the necessary means to subdivide a particular chromosome. These translocations combine the proximal portion of a simple *TB-A* with the distal portion of another chromosome arm.

TABLE 3

Phenotypes of kernels from crosses of a-m-1, A2, C, C2, R-scm2 *females by compound* TB-1La-3L's†

Translocation	Concordant *A* aleurone *A* scutellum	Concordant *a* aleurone *a* scutellum	Nonconcordant *a* aleurone‡ *A* scutellum	Nonconcordant *A* aleurone *a* scutellum
TB-1La-3L4759–3	51	40	98	27
TB-1La-3Le	23	31	40	9
TB-1La-3L5267	50	4	231	92
TB-1La-3L5242	27	31	78	40

† A low frequency of mosaic and defective kernels was encountered, but were not included in these data.

‡ The *a* aleurone; *A* scutellum class of translocations *TB-1La-3L4759–3* and *TB-1La-3Le* exhibit a slight but phenotypically recognizable reduction in endosperm size; this class produced by *TB-1La-3L5267* and *TB-1La-3L5242* have a more pronounced reduction.

Four compound *TBA*'s were synthesized: *TB-1La-3L4759–3*, *TB-1La-3Le*, *TB-1La-3L5267* and *TB-1La-3L5242*. All of these involve *TB-1La* and have the distal region replaced by the respective portion of *3L*. When hyperploid $AA^{B}\,B^{AA}\,B^{AA}$ plants were crossed as males onto the *a-m-1 A2 C C2 R-scm 2* tester, the phenotypes listed in Table 3 were found. The high frequency of nonconcordant (*i.e.*, different aleurone and scutellum phenotypes) kernels for each cross confirms the production of the compound translocations and indicates that the *3L* breakpoints in each of these is proximal to the *A* locus. When these translocations were, in turn, crossed as males onto the *Adh-C* line, the classes of progeny with respect to ADH are as given in Table 4. In addition, the *TB-1La-5S8041* translocation, synthesized by ROBERTSON (1975), was crossed to *Adh-C* females.

Of these translocations, only *TB-1La-3L5242* uncovers *Adh*. In three of the other four, a low frequency of cases was observed in which C/— or C/F/F types were present. These exceptional scutella are believed to have arisen from self-pollination (for C/— only) or from an occasional crossover event in the translocation parent reconstituting *TB-1La*, which would then uncover *Adh*. The frequency of C/— and C/F/F scutella was not sufficiently great to warrant the conclusion that this gene is present in the compound. All of these compounds

TABLE 4

Tests for inclusion of Adh *in compound* TBA's

Translocation	*1L* Region	*Adh* genotypes Hypoploid C/–	Euploid C/F	Euploid C/S	Hyperploid C/F/F	Hyperploid C/S/S
TB-1La-3L4759–3	0.20–0.39	1	103		4	
TB-1La-3Le	0.20–0.58		78		3	
TB-1La-3L5267	0.20–0.72		141			
TB-1La-5S8041	0.20–0.80	2	103	22		
TB-1La-3L5242	0.20–0.90	38		31		74

The maternal line in all crosses was homozygous for *Adh-C*, whereas the *TBA* lines were marked by *Adh-F* or *Adh-S*.

have a small endosperm phenotype (of varying severity depending upon the translocation) associated with the hyperploid embryos. Such kernels were present on the tested ears and did not have ADH zymogram ratios different from those of the normal-sized classes. Their presence indicated that nondisjunction in the male parent was unimpaired and justifies the conclusion that *Adh* is distal to the *A-A* breakpoints involved in these compounds.

The summation of the data from compound *TB-A*'s locates *Adh* between about 0.80 and 0.90 on *1L*. The fact that *TB-1La-3L5242* uncovers *Adh*, but *TB-1La-3L5267* does not, confirms the relative order of the breakpoints in the overlapping segmental trisomic produced from these two *1L-3L* translocations.

The genetics of Dp 1–3(5267):1–3(5242): The smallest segmentally trisomic region that includes the *Adh* locus is produced from the *1–3*(5267)/*1–3*(5242) *Adh-F/Adh-S* heterozygotes. These segmental trisomics were examined in detail with respect to the male and female transmission properties, as well as the amount of recombination between the breakpoints. Table 1 gives the data for the frequency of production of the segmental trisomics when the heterozygote for these two translocations (combination #4) is used as a female. Zymograms of the F/C, S/C and F/S/C classes are shown in Figure 2. When the reciprocal cross [*Adh-C* ♀ × *1–3*(5267)/*1–3*(5242)] was made, 79 C/S, 99 C/F and 4 C/F/S genotypes were observed from a total of 182 kernels analyzed. This result indicates that the pollen grains containing the duplication cannot equally compete against the euploid balanced translocation gametes marked by *Adh-F* and *S*.

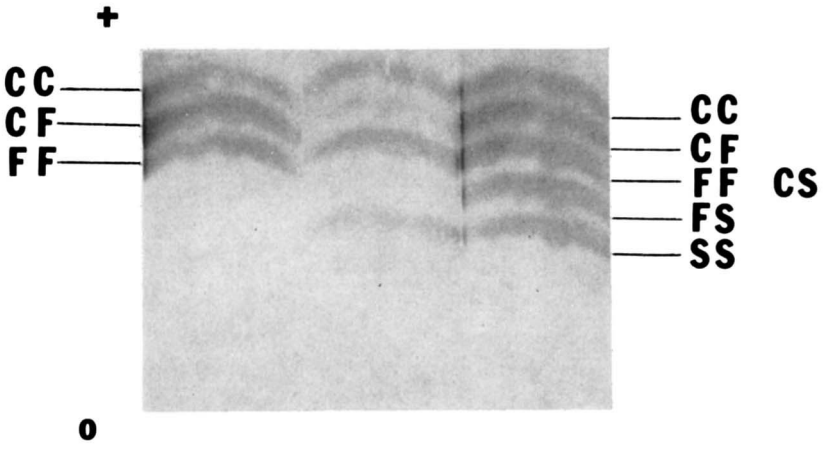

FIGURE 2.—Zymograms of progeny from the cross of Δ*1–3*(5267)/Δ*1–3*(5242) *Adh-F/S* by *Adh-C*. Left to right: *Adh-F/C*; *Adh-S/C*; *Adh-F/S/C* (segmental trisomic). The individual types of ADH dimers are labelled along the margin. The F/S/C kernels have only five bands because C·S and F·F dimers co-migrate. 0 = origin; + = anode.

The ears of the heterozygous plants exhibit irregular spacing of the kernels, which is due to the abortion of the deficient gametophytes, allowing the remaining progeny to fill out the ear. The range of pollen abortion of these plants is 20–30%.

The meiotic segregation behavior in the segmentally trisomic plants was also studied. Kernels from an ear segregating for the segmental trisomic were classified by subjecting an extract of a sliver of the scutellum to starch gel electrophoresis. Kernels with *Adh-F/S/C* embryos were selected and transplanted to the field. At anthesis, they were crossed as males onto an *Adh-C* line and used as females for *Adh-W* [which produces an enzyme that migrates slower than *S* (SCHWARTZ 1969)]. These plants have one normal chromosome *1* and one normal chromosome *3*, but also contain the duplicated regions of *1L* and *3L* that are between the two translocation breakpoints. The chromosomal constitution is diagrammed in Figure 3a. The genetic constitutions of the resulting gametophytes is given in Figure 3b. If, as illustrated, the homologous centromeres proceed to the opposite poles at random, the only viable combinations would be: (1) the normal chromosome *1* with the normal chromosome *3*, and (2) the double-duplication combinations. The other two possible segregations would result in severely deficient gametophytes that would be haplo-inviable. If adjacent-2 segregations are absent or rare, the parental plants should show about 50% ovule and pollen abortion and produce progeny in roughly a 1:1 ratio for the duplication and the normal chromosomes.

In the example studied, the duplication had the *Adh-F* and *Adh-S* alleles present in the overlapping sections. The normal chromosome *1* carried *Adh-C*. The surviving progeny would be present in a ratio of 1 F/S : 1 C. Recombination in the regions of the overlap would result in a chromosome duplicated for the regions delineated by the two translocation breakpoints on one chromosome, whose two components are separated by an insertion of the segment between the breakpoints of the second, as well as the complementary normal chromosome. It is also possible that crossing over could occur between the normal chromosomes and one of the regions of the duplication to produce newly marked gametes. CS, CF, F and S gametic genotypes might result from the various regions of crossing over and segregation. Certain F/S gametes might also be recombinants in which a normal chromosome *1* and the insertion are the complementary products of the crossover event, with subsequent migration of both products to the same meiotic pole. The recovery of the exceptional types would indicate that recombination can indeed occur within the complex chromosomal configuration, but the particular chromosomes involved can not be ascertained.

The progeny types from the cross *1/Dp 1–3*(5267):*1–3*(5242) (*Adh-C/F/S*) females by *Adh-W* are given in Table 5. When the segmentally trisomic plants were used as males onto *Adh-C*, 207 C/C, 7 C/F/S, 2 C/F and 2 C/S kernels were found from a total of 218 tested. These results indicate that the duplication and normal chromosomes are recovered in a 1:1 ratio through the female and that, although recombination can occur within the limits of the breakpoints, the detectable frequency is very low. Moreover, these data reaffirm the observation that the duplication pollen cannot compete equally with normals.

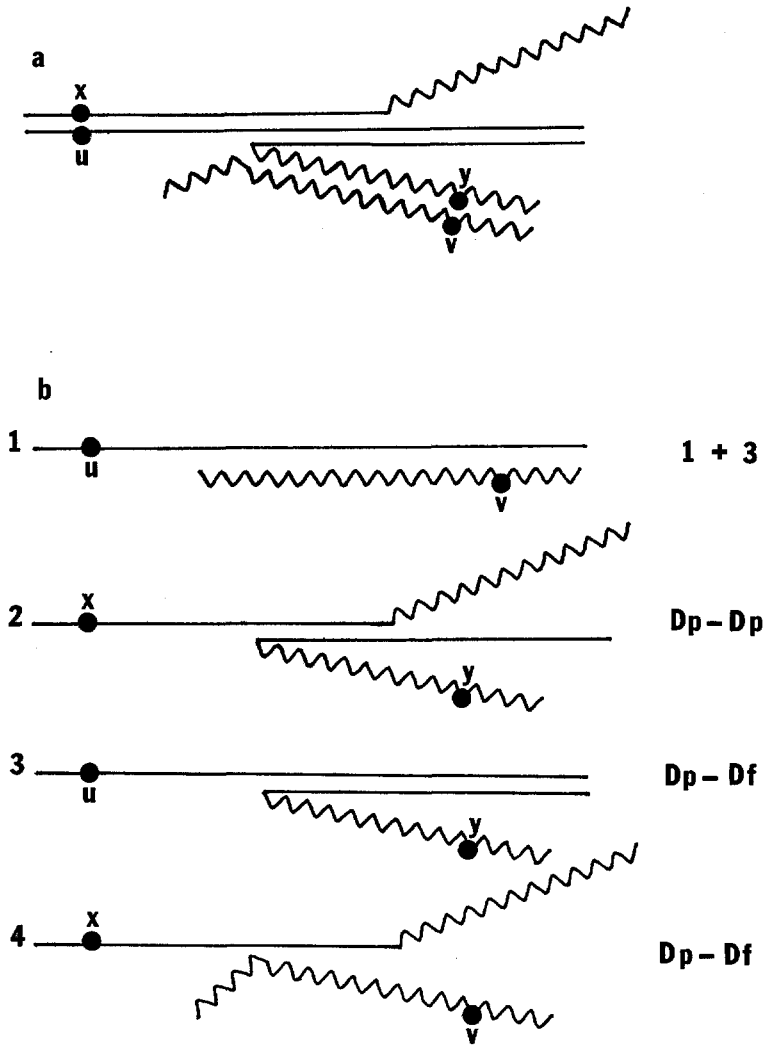

FIGURE 3.—(a) Chromosomal constitution of *1/Dp1–3*(5267):*1–3*(5242). Straight lines represent chromosome *1*; wavy lines chromosome *3*. This schematic should not be construed as representing meiotic pairing. (b) Chromosomal constitutions of gametes expected from segregation of homologous centromeres: 1. Normal chromosomes *1* and *3*. Duplication for the regions between the breakpoints; 3. Duplicate *1L*-deficient *3L*; 4. Duplicate *3L*-deficient *1L*. The possible crossover classes have been omitted.

TABLE 5

Progeny of cross 1/Dp 1–3(*5267*):1–3(*5242*)Adh C/F/S *by* Adh-W

Parental types†		Recombinant types			
F/S/W	C/W	C/S/W	C/F/W	F/W	S/W
209	209	1			

† As discussed in the text, certain FS/ gametes could be recombinants.

DISCUSSION

From the placement of *Adh* relative to the translocation breakpoints studied here, it is reasonable to conclude that this gene lies within the penultimate tenth of *1L*. As stated above, this locus is genetically within 1.5 map units of *lw*, which would place it roughly at two-thirds of the *1L* genetic map. Not unexpectedly, the genetic and cytological positions are quite divergent. The distal third of the genetic map is confined to little more than the last tenth of the arm. Higher frequencies of recombination per cytological unit in distal regions than in proximal is commonly observed in maize (see CARLSON 1977).

Due to the lack of stocks with suitable breakpoints, no segmental monosomics for *Adh* were observed during the course of this study. GOPINATH and BURNHAM (1956) reported two cases of transmissible deficiencies. They were not, however, able to demonstrate genetically the presence of duplications, due to the absence of markers. The co-dominant ADH isozyme alleles used here confirm the production of duplicate gametes and allow a determination of their frequency.

The genetic behavior of one of these duplications was studied. The results indicate that the duplication and normal chromosomes are recovered in equal frequencies through the female, but the duplicated pollen cannot equally compete with normal in achieving fertilization. It is important to note, however, that other duplications might not necessarily behave in the same manner as *Dp 1–3(5267): 1–3(5242)*. Other duplications with different breakpoints could allow more or less recombination, both within or surrounding the regions involved, which might affect the segregation properties (see BURNHAM 1949, for a study of these effects in reciprocal translocation heterozygotes).

The use of co-dominant allozymes as genetic markers for interstitial segmental trisomics: The use of *B-A* translocations is unparalleled as a means of locating genes to a chromosome arm (BECKETT 1978). However, because they are few in number, their utility for interstitial placement is limited. The method described here, using *Adh* as an example, will allow the positioning of genes encoding multimeric enzymes to a more precise cytogenetic location. The wealth of reciprocal translocations available in maize spans most of the genome, including much of the proximal regions not covered by the available set of *TB-A*'s. The procedure requires only two generations and few progeny need to be scored—unlike conventional placement of morphological mutants relative to translocations.

Once an isozyme gene is located, it can be used to classify the various classes of progeny in studies requiring segmental aneuploidy. If the marker gene is located within the duplicated region, its use is straightforward. Skewed ratios of isozyme heteromultimers or the introduction of three variants into a single individual would indicate the segmentally trisomic progeny. Such individuals could then be compared with euploid siblings. Once produced, duplication stocks could be maintained by self pollination with selection for ears exhibiting approximately 50% ovule abortion. Since recombination can produce new types of gametes, albeit at low frequency, it would be necessary to confirm the presence of the duplication by electrophoretic analysis. Certain cases of extremely close

linkage of isozyme loci to translocations (*e.g.*, KLEESE and PHILLIPS 1972) might prove useful in eliminating this need.

Interstitial duplications not involving the isozyme locus itself, but present on the same chromosome arm, could possibly be marked by introducing an inverted chromosome carrying an allele specifying an electrophoretically distinguishable enzyme from that linked to the duplication or perhaps carrying a recessive endosperm mutant. The inversion must span the region including the marker locus, as well as the length of the chromosome overlap. Segregation from a heterozygote would produce a 1:1 ratio of duplication to inversion, each marked by a distinct allele. Since such a situation would greatly expand the utility of any particular marker gene, and because each duplication involves portions of two chromosome arms, relatively few isozyme loci would be required to mark a substantial portion of the total genome.

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