THE GENETIC BASIS OF DOSAGE COMPENSATION OF ALCOHOL DEHYDROGENASE-1 IN MAIZE

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ABSTRACT

The levels of alcohol dehydrogenase (ADH) do not exhibit a structural gene-dosage effect in a one to four dosage series of the long arm of chromosome one (*1L*) (BIRCHLER 1979). This phenomenon, termed dosage compensation, has been studied in more detail. Experiments are described in which individuals aneuploid for shorter segments were examined for the level of ADH in order to characterize the genetic nature of the compensation. The relative ADH expression in segmental trisomics and tetrasomics of region *1L* 0.72–0.90, which includes the *Adh* locus, approaches the level expected from a strict gene dosage effect. Region *1L* 0.20–0.72 produces a negative effect upon ADH in a similar manner to that observed with other enzyme levels when *1L* as a whole is varied (BIRCHLER 1979). These and other comparisons have led to the concept that the compensation of ADH results from the cancellation of the structural gene effect by the negative aneuploid effect. The example of ADH is discussed as a model for certain other cases of dosage compensation in higher eukaryotes.

IN a study of the expression of various enzyme levels in a one-to-four dosage series of the long arm of chromosome one (*1L*), it was observed that the level of alcohol dehydrogenase (ADH), whose structural locus is in *1L*, exhibited compensation rather than the customary gene-dosage effect (BIRCHLER 1979). Among the possibilities to explain this observation, BIRCHLER discussed two. In the first case, the level of ADH expression would be compensated because a factor encoded elsewhere in the genome limits the expression of ADH (SCHWARTZ 1971; FREELING 1975). This factor would be specific for the *Adh* locus; thus, an equal expression of enzyme level regardless of the dosage of the gene would result.

An alternative explanation became necessary upon the discovery of the inverse effect on certain other enzyme levels in the dosage series: the levels of glucose-6-phosphate, 6-phosphogluconate and isocitrate dehydrogenases, as well as esterase, were negatively correlated with the dosage of *1L* with the extreme limits being the inverse of the dosage relative to the diploid (BIRCHLER 1979). The compensatory phenomenon involving ADH levels could be brought about by a cancellation of a structural gene-dosage effect by the negative effect simultaneously produced by chromosome arm *1L*. That is, as the number of *Adh* al-

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leles increases through the series, the level of expression of each is correspondingly decreased such that the total amount of enzyme remains fairly constant.

It was reasoned that these alternatives could be distinguished by subdividing the chromosome arm in such a way as to vary smaller regions that include the *Adh* structural locus and to test for a region in *1L* that exerts the negative effect upon ADH. The limiting-factor hypothesis predicts that the level of ADH would be exactly equal regardless of the gene dosage and would not depend upon the genetic size of the chromosomal segment that includes the *Adh* gene. If the alternative hypothesis were true, it should be possible to find a genetically smaller region surrounding the *Adh* locus that exhibits a dosage effect and, as a concomitant, to vary another portion of *1L* and to realize a negative effect upon ADH. The contingency of this approach is the availability of chromosomal aberrations that would separate the structural locus from at least some of the presumptive modifiers.

The purpose of this article is to present data indicating that the latter explanation is indeed the case, and to introduce the concept that certain types of dosage compensation result from the opposing forces of simultaneously varying both the structural gene and a negative modifier in a dosage series.

MATERIALS AND METHODS

Adh *alleles*: The basic electrophoretic mobility classes of *Adh* alleles were described by SCHWARTZ and ENDO (1966). The migration to the anode at pH 8.3 is C, F and S. The translocations *1-3* (5242) and *1-3* (5267) used in these experiments are linked to an *Adh-S* and *-F*, respectively. The *Adh-C-70-86* allele was induced with ethyl methanesulfonate by D. SCHWARTZ.

There are two genes for alcohol dehydrogenase in maize. *Adh* is expressed in several tissues, including the scutellum, developing endosperm, pollen and seedling. It is genetically located on chromosome *1* near lemon-white (*lw*) (SCHWARTZ 1971) and cytologically in region 0.80–0.90 of the long arm (BIRCHLER 1980a). *Adh2* resides in the short arm of chromosome *4* (FREELING and CHENG 1978; DLOUHY 1979). The latter is weakly expressed in the developing endosperm and in seedling tissues, but is induced by anaerobiosis to levels comparable to that of *Adh1* (FREELING and SCHWARTZ 1973; FREELING 1975). The present study is concerned only with the *Adh* locus in scutellar tissue, where its expression is high. The *Adh2* gene is negligibly expressed in this tissue and is assumed, therefore, to have no effect upon the results.

To compare the expression of the *F*, *S* and *C* alleles used in these experiments, the respective translocation stocks were crossed to *Adh-C* and backcrossed to the same. Ears segregating for *C/C* and *C/F*, as well as *C/C* and *C/S*, were classified by subjecting an extract of a sliver of the scutellum to electrophoresis. Extracts of pooled sibling scutella were compared. The results are presented in Table 1.

Experimental procedures: Electrophoresis of ADH, ADH enzyme assay, sample preparation and estimation of hydrolyzable DNA and total protein were as described by BIRCHLER (1979). Electrophoresis was conducted at 3°, and enzyme assays were performed at 25°. Aspects of these procedures that should be emphasized or that were modified are as follows: All assays were performed on extracts of mealed scutella that were excised away from the endosperm. Fifty mg of meal were extracted in 1 ml of buffer. The enzyme assay was altered to a volume of 3.0 ml, with the enzyme extract constituting 50 μl of the total. To classify the ADH allozymes, a portion of each scutellum was excised, soaked in distilled H_2O for 12–18 hr, extracted in a drop of 0.005 M Tris buffer, pH 7.5, and subjected to electrophoresis for identification of the *Adh* alleles present. Scutella from phenotypic classifications utilizing the *A* locus described below were directly excised. Enzyme activity units are expressed on a per mg total protein basis. Previous determinations of total protein and hydrolyzable DNA per mg dry mass in the *1L* dosage series

TABLE 1

Comparison of Adh *alleles linked to* T1-3 *(5267) and* T1-3 *(5242)*

Adh genotypes	*n*	Mean ratio ± s.e.
CF: *CC*	5	1.11 ± 0.08
CS: *CC*	5	1.15 ± 0.06

The mean ratio is the average of the *CF* to *CC* or *CS* to *CC* ratio for *n* ears analyzed from backcrosses of *T1–3* (5267) *Adh-F/Adh-C* and *T1–3* (5242) *Adh-S/Adh-C* heterozygotes to the *Adh-C* line. Each ratio for individual ears is the number of units of ADH activity per mg total protein of the heterozygote divided by the units of activity per mg protein of the *CC* homozygous sibs. One enzyme unit = $\Delta 0.001$ OD_{340}/min. at 25°. From the above values, the calculated ratio for *FF* to *CC* is 1.22 and for *SS* to *CC* is 1.30. Therefore, the relative expression of *S* to *F* is 1.07.

indicated that the protein values do not fluctuate so greatly as to obscure the results when expressed in this way (BIRCHLER 1979).

Construction of segmental trisomics including the Adh *locus*: To produce ears segregating for kernels with 3 *vs.* 2 doses of *Adh*, the heterozygote of translocations *1–3* (5242), *Adh-S*, and *1–3* (5267) *Adh-F*, was crossed by a chromosomally normal stock homozygous for *Adh-C*. This cross was described more fully by BIRCHLER (1980a), but the salient features pertinent to this study are as follows. The heterozygote of the two translocations produces three types of viable gametes: (1) *T1-3* (5242) marked by *Adh-S*, (2) *T1-3* (5267) marked by *Adh-F*, and (3) the overlap of the two translocations, which is duplicated for all regions of *1L* and *3L* that are delineated by the translocation breakpoints. In crosses to the *Adh-C* stock, zygotes are formed that are euploid and contain two doses of *Adh* with *Adh-S/C* or *F/C* genotypes, as well as segmentally trisomic *Adh-F/S/C* zygotes that contain three doses of the *Adh* structural gene.

It is unlikely that recombination within the region between the breakpoints produces a significant number of duplicate gametes marked by the same *Adh* allele. Single recombinational events within the limits of the *1L* or *3L* breakpoints cannot result in products that will allow double reduction at the *Adh* locus. The only recombinants that are recovered are those in which both strands of the single crossover event segregate together. These gametes consist of a normal chromosome and an insertion of the region between the breakpoints of one chromosome into a duplication of the region of the other chromosome delineated by the translocations. These exceptional types are duplicated for the regions between the *1L* and *3L* breakpoints and, with respect to *Adh* genotype, are *F/S*. For double reduction to occur, two recombinational events between the *1L* breakpoint would be required, with the two exchanges surrounding the *Adh* locus, followed by the appropriate segregation. Control crosses capable of detecting *FF* or *SS* gametes failed to recognize any such kernels from a total of 838 tested. It is reasonable to conclude that those gametes with only *F* or *S* present have but one *Adh* gene and those with *F/S* have two.

Single recombinational events simultaneously occurring between the breakpoints of *1L* and *3L*, followed by the appropriate segregation, could conceivably give rise to segmentally trisomic gametes that are *SSF* or *SFF* in *Adh* genotype, depending on whether the event occurs proximally or distally to *Adh*. Such kernels would be recognizable on the basis of their altered isozymes ratios, but due to the multiplicity of requirements to produce such gametes, their frequency would be expected to be extremely low, if they occur at all.

The total genetic distance in a normal chromosome *1* between the two breakpoints is less than 26 map units. This is based on the fact that *Adh* lies proximal to the breakpoint of *T1-3* (5242) (*1L*.90) (BIRCHLER 1980a) and the bronze-2 locus is proximal to *T1-3* (5267) (*1L*.72) (NEWTON and BIRCHLER 1980). The genetic distance separating these two loci is 26% recombination (SCHWARTZ 1979).

Construction of segmental tetrasomics including the Adh *locus*: Tetrasomic individuals were recovered by self-pollinating segmentally trisomic *Adh-FS/C* plants. The trisomics produce as their viable gametes the Dp *1–3* (5242): *1–3* (5267) and the normal chromosome *1* in a 1:1

ratio (BIRCHLER 1980a). The competition of the duplication pollen is greatly reduced, but is sufficiently high to recover homozygotes upon sparse pollination. Such kernels are *Adh-FS/FS* in genotype and are readily distinguished from *C/C* or *FS/C* siblings. These kernels were selected from selfed ears by subjecting an extract of a sliver of the scutellum to starch gel electrophoresis.

Construction of a dosage series of 1L0.20–0.72 proximal to Adh: To examine the effect of the proximal region of *1L* on ADH levels, the compound *B-A* translocation *1 La-3L* (5267) (BIRCHLER 1980a) was used. This translocation has the region of 0.20–0.72 of *1L* and of 0.73 to tip of *3L* attached to the *B* centromere. The *1L* break is proximal to *Adh* and the *3L* portion carries the dominant *A* allele. When crossed as a male onto an *a-m-1 A2 C C2 R-scm-2* tester (described by BIRCHLER 1980), the dosage of the included regions can be distinguished as follows: kernels with *a* scutella, *A* endosperm have one-dose embryos; kernels with *A* endosperm and scutellum have two doses and kernels with *A* scutella and *a* endosperm have three doses of the respective regions.

In all three doses of the *TB-1La-3L* dosage series, the *Adh* alleles are identical. In order to form a functional gametophyte, the *B-1L-3L* chromosome must segregate with the 1^B and 3^1 chromosomes, the latter carrying *Adh*. Since nondisjunction occurs at the second microspore division, all doses of *1L* 0.20–0.72, *3L* 0.73–tip are linked to only one copy of *Adh*. Thus, upon fertilization, all zygotes will be heterozygous for the *Adh* allele in the tester (electrophoretically F) and the allele linked to the translocation (also F).

The hyperploid heterozygotes have the following chromosomes with respect to *1* and *3*: a normal *1* and *3*, 1^B, 3^1 and two B^{1L-3L} chromosomes. The recessive *a* marker is carried on the normal chromosome *3* and the B^{1L-3L} chromosomes are marked with *A*. Crosses of hyperploids as females by a recessive *a* line result in ears that are almost totally *A* in phenotype. When *1L* compound *TBA* hyperploids with one *Adh* allele on the normal *1* (*C*) and a different one on 3^1 (*F*) are crossed as females by yet a third variant (*S*), three classes of zygotes are found. These are *Adh C/S, F/S* and *CF/S* zygotes in roughly equal proportions. These two observations suggest that as a general rule the *1*, *B* and *3* centromeres assort independently, which gives rise to four types of gametes: (1) balanced euploid: 1^B, B^{1L-3L}, 3^1; (2) duplicated for *1L* 0.20–1.00: *1*, B^{1L-3L}, 3^1; (3) duplicated for *1L* 0.20–0.72, *3L* 0.73–1.00: *1*, B^{1L-3L}, *3*; (4) duplicated for *3L* 0.73–1.00, deficient for *1L* 0.72–1.00: 1^B, B^{1L-3L}, *3*. The latter type is expected to abort due to the large size of the deficiency.

Despite the generality of the above, recombination must occur to some degree between the normal *1* and one of the B^{1L-3L} chromosomes. This is evident from the fact that *TB-1La* is occasionally regenerated in crosses of compounds involving portions of *1L* (BIRCHLER 1980a). In the case of *TB-1La-3L*5267, recombinational events between the normal *1* and one of the B^{1L-3L} chromosomes can result in two types of viable gametes: (1) *TB-1La* regenerated, which must segregate with the *a*-marked chromosome *3*, and will therefore be found in the colorless class of kernels and not affect the classification scheme, and (2) the reciprocal product generates a 1^s chromosome that can form a viable gametophyte only upon segregation with the *A*-marked 3^1. This class will produce zygotes with *A* aleurones and scutella and have two doses of all chromosomal regions, as do the remainder in this phenotypic class.

A second class of recombinants of note would occur between the B^{1L-3L} chromosome and the normal *3* that carries *a*. This would transfer *a* to the compound translocation and result in *a* kernels, regardless of the disjunctional events at the second microspore division. The reciprocal product transfers *A* to the normal *3*, which can form a viable competitive pollen grain only if it segregates with a normal *1*, again producing *A* kernels with two doses of all regions.

Double-crossover events involving proximal *1L* of B^{1L-3L} and chromosome *1* as one site and distal *3L* of the same B^{1L-3L} with normal *3* as the second could introduce ambiguity of dosage into the *A* kernel class, but these would be expected at a multiplicatively lower frequency than the two single events. The testcrosses described above indicate that single events do occur, but at low frequency. The level of double events, if they occur at all, is considered inconsequential to the interpretation of the results.

It is believed that duplicated gametes do not contribute to the *A* scutellum, *A* aleurone class. Since pollen grains duplicated for *1L* (BIRCHLER 1979) or *3L* (WARD 1971) cannot compete and the duplications produced by this translocation are comparable in size, their transmission is

considered to be nil. It is known that the class of duplicated gametes that carry an extra dose of *1L* 0.20–1.00 are not present, since this has been directly tested by crossing the translocation to *Adh-C* (BIRCHLER 1980a). The presence of *FF* gametes would be readily detected, but none were found. The occurrence of transmission of the duplicated *1L* 0.20–0.72, *3L* 0.73–1.00 gametes, if indeed it occurs, cannot be genetically determined. Since the balanced euploid and both types of duplicated gametes will give rise to zygotes with 21 chromosomes, root tip counts are not useful in determining the frequency.

Combination of Adh *structural gene and proximal* 1L *dosage series*: To test the effect of the combination of the *Adh* locus and the above *1L–3L* region, the compound *B-A* translocation, *TB-1La-3L*5242 was crossed to the *a-m-1 R-scm-2* tester. This translocation contains the *1L* 0.20–0.90 and *3L* 0.65–1.00 regions. It therefore includes all of the chromosomal material of *TB-1La-3L*5267, plus the region of *1L* 0.72–0.90 that includes the *Adh* locus. Since *TB-1La-3L*-5242 and *TB-1La-3L*5267 were constructed from the two *1L–3L* translocations used to produce the *Adh* duplication and segmental tetrasomic, the above mentioned test of a gene dosage effect is directly comparable to the results obtained with the two compound *B-A* translocations, regardless of the accuracy of the cytologically determined breakpoints.

The *TB-1La-3L*5242 chromosome carries an *Adh-S* allele. Crosses to *Adh-C* could detect the successful competition of both types of duplicate gametes, but none were found (BIRCHLER 1980a).

Dosage series of 3L: As a control on the effect of the *3L* region involved in the above compound translocations, the *a-m-1 R-scm-2* tester was crossed with hyperploid heterozygotes of *TB-3La* (0.20–1.00). The phenotypic distinction of the dosage was as described above. Duplicated pollen grains of *3L* cannot compete with normals (WARD 1972).

The occasional cases of recombination between the normal *3* marked with *a* and one of the B^{3L} chromosomes carrying *A* will not affect the ability to distinguish chromosomal dosage. A crossover event will transfer *a* to the B^{3L} chromosome and thus, will be found among the colorless kernels, which were not used. The reciprocal product transfers *A* to the normal chromosome *3*. This gamete can form a competitive gametophyte only if the B^{3L} is lost. The resulting zygote would have two doses of all regions, as do the remainder of the *A* scutellum, *A* endosperm class.

Dosage series of Adh *superimposed on a* 1L *dosage series*: To vary the *1L* dosage of *Dp 1–3* (5242)*/1–3* (5267) independently of the *3L* region and to compare a 0.72–0.90 dosage series with a whole-arm dosage series from the same ear, the following cross was made. The heterozygote of *T1–3* (5242)*/T1–3* (5267), *Adh-S/F* was pollinated by a *TB-1La* stock homozygous for *Adh-C*. The construction of this latter chromosome has been described elsewhere (BIRCHLER 1979).

The female plants of this cross produce haploid gametes marked by *Adh-F* or *-S* and segmentally disomic ones that are *Adh-F/S*, as described above. The *TB-1La* stock produces sperm with 0, 1 or 2 doses of the region of *1L* translocated to the *B* centromere in *TB-1La*. Since these gametes are marked by *Adh-C*, the array of zygotes formed is as illustrated below.

		Male gametes		
		-	C	CC
		1L Monosomics	*1L* Disomics	*1L* Trisomics
Female gametes	F	F/-	F/C	F/CC
	S	S/-	S/C	S/CC
	FS	FS/-	FS/C	FS/CC

Those zygotes in the left column are monosomic for the long arm of *1L*; those in the center are disomics and those in the rightmost column are trisomics for *1L*. The first two rows vary only in *1L*, but are marked by the different *Adh* alleles, *F* and *S*. The bottom row represents those cases that contribute two doses of the region *1L* 0.72–0.90; *3L* 0.65–0.73. This results in two, three and four doses of *Adh* in *1L* monosomics, disomics and trisomics, respectively. The dosage of *3L* 0.65–0.73 is three in all cases, but the *1L* region 0.72–0.90 is independently varied. Thus, in contrast to the simple segmental trisomics and tetrasomics, the *1L* and *3L* regions are separately manipulated.

Haploid vs. *diploid*: The system of detection of haploids developed by COE and SARKAR (1964) was used to recognize 1*N* embryos. Female plants of stock 6 that were *A A2 C C2 R* in genotype and carried an appropriate constellation of genetic factors for scutellum color were crossed by males of stock 6 that were *A A2 C^I C2 R* with respect to anthocyanin loci. Progeny were screened for cases of *C* scutellum and *C^I* endosperm. The germ was transsected to insure proper identification. These kernels were considered to be haploid; the cytological studies of COE and SARKAR (1964) indicate a 98% accuracy of classification. Sibling kernels of *C^I* scutellum and endosperm were used as a diploid comparison.

Tetraploid vs. *diploid*: Tetraploid derivatives of standard inbred lines W23 and N6 were compared to the diploid stocks. It is recognized that tetraploid maize is subject to frequent aneuploid variation (RANDOLPH 1935). This fact requires that the interpretation of the results be considered as only a general measure of the effects of tetraploidy. Aneuploidy for chromosome *1* would not affect the results due to compensation, but other chromosome variation might.

RESULTS

The concept that the compensation of ADH levels in a one-to-four dosage series of *1L* is due to a cancellation of a positive structural gene-dosage effect by the negative effect of modifying loci in *1L* enables one to make two predictions: (1) a gene-dosage effect should be demonstrable in a smaller dosage series surrounding *Adh* and (2) a negative modifying effect should be found when another region of *1L*, excluding *Adh*, is varied.

A test of the first prediction was performed by producing ears segregating for segmental trisomics for the 0.72–0.90 region of *1L*, which includes the *Adh* gene. The average value for six ears analyzed gave a 3:2 dose ratio of 1.48 ± 0.03 (see Table 2). The predicted value, based on the relative expressions of the *C*, *F* and *S* alleles described above is 1.59. This region then exerts a gene-dosage effect reasonably close to the expected value and significantly greater at the 1% level in statistical tests than the whole-arm trisomic value of 1.17 (BIRCHLER 1979).

A further test was performed by determining the ADH expression in segmental tetrasomics for the *1L* 0.72–0.90 region. These measurements were conducted by MARY ALLEMAN. If the expression of each allele present in the disomic and tetrasomic were identical, one would expect, from a gene-dosage effect, an increase in the tetrasomic to 200%. However, as noted above, the F and S alleles linked to the translocations used to construct the tetrasomic are greater in expression than that of the *C* allele homozygous in the disomic. This results in a corrected ratio of 2.52. The observed value was found to be 2.63, as shown in Table 3. These data demonstrate that the *Adh* gene is capable of exhibiting a structural

TABLE 2

Trisomic/disomic ratios of region 1L *0.72–0.90 including the* Adh *structural locus*

Adh genotype	*n*	Mean ratio ± s.e.
C/FS: *C/F*	6	1.48 ± 0.03

The mean ratio (± standard error) is the average of *n* ears for which the ratio of units of ADH activity per mg protein in the segmental trisomic *Adh-C/F/S* was divided by the units of ADH activity per mg protein in the disomic *Adh-C/F*. Based on the relative expressions of *C*, *F* and *S*, a complete dosage effect would give a ratio of 1.59.

TABLE 3

ADH expression in the segmental tetrasomic 1L *0.72–0.90*

Adh genotype	Dosage of *Adh*	*n*	Units activity/mg dry weight
C/C	2	4	56.7 ± 5.6
C/SF	3	9	120.3 ± 5.8
SF/SF	4	8	149.7 ± 2.4

Ratio		Expected	Observed
SF/SF: *C/C*	=	2.52	2.63
C/SF: *C/C*	=	1.76	2.11

The activity measurements represent determinations of units of ADH activity per mg dry weight of scutellar sections taken from ears segregating for disomics, trisomics and tetrasomics for region *1L* 0.72–0.90. The number of extracts examined is designated by *n*. The ratios expected based on the relative expressions of *C*, *F* and *S* for a complete dosage effect are listed, as are the observed values. The activity measurements were performed by MARY ALLEMAN. The means of the disomic and tetrasomic are significantly different at the 0.1% level.

gene-dosage effect in a manner similar to that found with other eukaryotic genes (*e.g.*, GRELL 1962; SCHIMKE *et al.* 1978).

To test for a negative effect upon ADH levels by a region of *1L* excluding the structural locus, a one to three dosage series for *1L* 0.20–0.72, produced by *TB-1La-3L*5267 was examined. The data are in Table 4. There is a significant (at the 5% level) increase in the monosomic to the 1.25 level relative to the diploid. In the trisomic, there is a significant (at the 1% level) decrease to 77% of the diploid value. The level of magnitude of the effect exerted by this region approaches a value sufficiently great to account for the degree of compensation in *1L* as a whole, but it is important to note that this region may not be the only segment of *1L* affecting ADH.

The 0.20–0.72 *1L* region was combined with a dosage series for the section around *Adh*, including *1L* 0.72–0.90, by examining ADH levels in a one to three dosage series of *TB-1La-3L*5242. This compound *B-A* translocation includes all of the *1L* and *3L* regions of *TB-1La-3L*5267, plus the regions of *1L* and *3L* in *Dp 1–3* (5242): (5267) used to test for a gene-dosage effect. The results are presented in Table 5. The two effects cancel each other to give a very slight compensation in the monosomic and complete compensation in the trisomic. A control test for any effects of *3L* was conducted by examining one to three doses pro-

TABLE 4

ADH expression in a dosage series of 1L 0.20–0.72

Adh genotype	Dosage comparison	*n*	Mean ratio ± s.e.
F/F: *F/F*	1/2	5	1.25 ± 0.08
F/F: *F/F*	3/2	5	0.77 ± 0.04

The mean ratio ± standard error is the average of *n* ears for which the ratio of ADH units per mg protein in the aneuploid was divided by the ADH units per mg protein in the diploid. In all genotypes, *Adh-F* from the tester stock is heterozygous with the *Adh-F* allele present in the compound *B-A* translocation.

TABLE 5

ADH expression in a dosage series of 1L *0.20–0.90*

Adh genotype	Dosage of *1L* 0.20–0.90; *3L* 0.65–1.00	*n*	ADH units / mg protein
F	1	3	585 ± 11
F/S	2	4	1011 ± 35
F/SS	3	4	1019 ± 30
Ratios			
Monosomic: disomic = 0.58			
Trisomic: disomic = 1.01			

ADH units are the mean ± s.e. of the ADH activity units per mg total protein for *n* extracts examined from a single ear of the cross *a-m-1 R-scm-2* females by hyperploid *TB-1La-3L*5242. The disomic and trisomic values are not significantly different.

duced by *TB-3La*. The means (±s.e.) for the three ears are as follows: monosomic/disomic, 1.10 ± 0.06 and trisomic/disomic, 1.01 ± 0.07. In neither case is there a significant effect of *3L* dosage on ADH.

It perhaps could be argued that the level of an *Adh*-specific positive effector is elevated in the segmental trisomic and tetrasomic lines to such a level that it is no longer rate limiting, thus allowing the dosage effect of the gene to become evident. This possibility was tested by producing ears that bear a dosage series for both the *Adh* region and the whole of *1L*. This was accomplished by crossing females heterozygous for translocations *1–3* (5267)/*1–3* (5242) *Adh-F/S* by males of the *TB-1La, Adh-CCC* stock. This cross produces *1L* monosomics with one or two doses of the *Adh* region, disomics with two or three and trisomics with three or four doses of *Adh*. If the *1L* 0.72–0.90 and whole-arm comparisons of ADH levels are identical, either both showing dosage effects or compensation, the above possibility must be considered. The results are shown in Table 6. For whole-arm comparisons, the monosomics show the partial compensation normally observed, and the trisomic values exhibit a nearly complete compensation. Two doses of *Adh* in the monosomic are significantly greater (at the 1% level) than either the single dose of *F* or *S*. Among disomics, the three-dose value is significantly greater (1% level) than the diploid values. Finally, four doses in the trisomic exhibit a dosage effect (significant at the 1% level) relative to the normal trisomics. This experiment reaffirms the conclusion that ADH levels are affected by the structural gene dosage *and* by the whole of *1L*, in opposing ways.

Since ADH levels are compensated (partially in monosomics; nearly completely in trisomics and tetrasomics) in a dosage series of *1L*, the expression in a total genomic dosage series was examined. This analysis was previously conducted by LEVITES and NOVOZHILOVA (1978). They measured ADH specific activity (nmole NAD/min/mg protein) in haploid, diploid and tetraploid maize. There was no significant difference between the haploid and diploid. For the tetraploid-diploid comparisons, a number of different lines were examined. Depending upon the genotype, there was either an increase or decrease in the

TABLE 6

Comparison of gene dosage and whole-arm effects

Adh genotype	Dosage of *Adh*	Dosage of *1L*	*n*	ADH units/mg protein
F	1	1	4	1741 ± 26
S	1	1	4	2310 ± 20
FS	2	1	4	3176 ± 99
F/C	2	2	4	2724 ± 51
S/C	2	2	4	2976 ± 54
FS/C	3	2	4	3948 ± 62
F/CC	3	3	4	2805 ± 106
S/CC	3	3	4	2974 ± 90
FS/CC	4	3	4	3726 ± 119

Ratios

F: *F/C* = 0.64	*FS/C*: *F/C* = 1.45
S: *S/C* = 0.78	*FS/C*: *S/C* = 1.33
FS: *F/C* = 1.17	*F/CC*: *F/C* = 1.03
FS: *S/C* = 1.07	*S/CC*: *S/C* = 1.00
FS: *F* = 1.82	*FS/CC*: *F/CC* = 1.33
FS: *S* = 1.37	*FS/CC*: *S/CC* = 1.25

ADH units are the mean ± s.e. of the ADH activity per mg total protein for *n* extracts examined from a single ear of the cross *T1-3* (5242)/*T1-3* (5267), *Adh-S/F* females by a *TB-1La* hyperploid heterozygote, *Adh-C/C/C*.

tetraploid, but most stocks showed a relatively similar expression in the two ploidy levels.

These comparisons were also investigated in this study. The data are presented in Table 7. In the haploid, there is a significant reduction relative to the diploid when the data are expressed as enzyme units per mg protein or per mg dry weight. For the tetraploid-diploid analysis, two inbred lines were used, W23 and N6. When the data are expressed as units per mg protein, there is little difference

TABLE 7

Ploidy comparisons of ADH expression

	ADH units / mg protein	ADH units / mg dry weight	ΔA_{560} / 50 mg
Stock 6. Haploid, *Adh-S*	984 ± 40 (5)	29.92 ± 1.16 (5)	0.608 ± 0.025 (5)
Stock 6. Diploid, *Adh-S*	1241 ± 32 (5)	37.76 ± 1.95 (5)	0.541 ± 0.020 (5)
W23. Diploid, *Adh-F*	980 ± 60 (4)	26.6 ± 1.85 (4)	0.642 ± 0.026 (3)
W23. Tetraploid, *Adh-F*	1171 ± 24 (4)	48.5 ± 1.30 (4)	0.605 ± 0.016 (4)
N6. Diploid, *Adh-S*	736 ± 13 (4)	18.10 ± 0.44 (4)	0.817 ± 0.012 (4)
N6. Tetraploid, *Adh-S*	1252 ± 34 (4)	38.90 ± 1.32 (4)	0.762 ± 0.021 (4)

ADH units are as described in previous tables. The haploid-diploid and both diploid-tetraploid comparisons (per mg dry weight) are significantly different at the 1% level. The number of extracts examined is given in parentheses. The hydrolyzable DNA values are expressed as the change in the absorbance at 560 nanometers per 50 mg of TCA hydrolyzed meal. None of the DNA ploidy comparisons are significantly different.

between the 2*N* and 4*N* derivatives of W23, but for N6 there is an increase in the tetraploid. However, when both are expressed as ADH units per mg dry weight, there is nearly a doubling in expression in both cases.

In maize, cell size is proportional to the level of ploidy (RHOADES and MCCLINTOCK 1935). If a strict gene-dosage relationship were present, similar activities per mg dry mass would result because each cell would have more ADH with ascending ploidy, but there would be a correspondingly smaller number of cells per unit mass. However, in the ploidy study, there appears to be a positive correlation of ADH expression above and beyond that expected from a mere increase of gene dosage. To verify dry mass as an appropriate standard, total hydrolyzable DNA levels were compared in the ploidy series. In all comparisons, the hydrolyzable DNA per mg dry mass is more nearly equal than the ADH levels. This result strengthens the above interpretation. A similar phenomenon of positive effect occurs for some of the major scutellar proteins when a ploidy series is examined on SDS polyacrylamide gels (BIRCHLER and NEWTON, in preparation). This may be due to a positive effect on ADH from the total genomic increase, or to an overall greater metabolic rate through the ploidy series.

DISCUSSION

The results presented in this paper demonstrate that the compensation of ADH levels in scutellar tissue in the dosage series of *1L* is due to a cancellation of a positive increase with structural gene dosage by the negative effect of a modifying locus or loci in *1L*. In the monosomic, the number of *Adh* alleles is reduced, but their expression is elevated. In the trisomic, *Adh* gene dosage increases, but the expression of each is lowered. The two opposing forces result in compensation.

It is now possible to evaluate some of the unresolved parameters in the study of BIRCHLER (1979), due to the genetic mapping of an extensive array of allozyme loci by M. M. GOODMAN *et al.* (1980). The levels of malate dehydrogenase and phosphoglucomutase were determined in a dosage series of *1L* and found to be constant relative to both total protein and hydrolyzable DNA levels. It was recognized that this result did not exclude the presence of their structural genes being in *1L*, since their levels could be compensated. The mapping of the major soluble *Mdh4* and *Pgm1* loci to the long arm of *1* (NEWTON and SCHWARTZ 1980; GOODMAN *et al.* 1980) suggests, in fact, that these enzyme levels might also be dosage compensated. The multiplicity of loci involved in contributing to the total enzyme level in these two cases complicates an accurate understanding of the situation. However, the enzymes glutamate dehydrogenase (PRYOR, A. J. 1979) and phosphohexoisomerase (GOODMAN, M. M. *et al.* 1980), as well as a major protein of unknown function (SCHWARTZ 1979), are encoded by genes in *1L*, and each of these is compensated to individual degrees in the dosage series (BIRCHLER, unpublished; BIRCHLER and NEWTON, in preparation). Although the underlying mechanisms may be different in each case, the phenomenon of compensation is not unique to ADH.

The finding that six of the known enzymes and proteins mapped to *1L* are compensated to one degree or another raises the possibility that all enzymes, indeed, protein levels in general, are affected by the dosage of *1L*. That this is not true is indicated by the following observations: (1) total protein per mg dry weight estimates deviate only slightly from the total hydrolyzable DNA levels per mg dry weight (BIRCHLER 1979) and (2) protein profiles of the *1L* dosage series separated on SDS polyacrylamide gels show that, while many negative effects are evident, the majority of proteins are constant in 1, 2 and 3 doses (BIRCHLER and NEWTON, in preparation).

The observation that the enzyme level is directly proportional to structural gene dosage regardless of the dosage of *1L*, but in all cases being negatively modulated by the dosage of *1L* as a whole, suggests that each allele is more-or-less equally affected by the linked modifiers. That is, compensation is not due to a differential allelic response; instead, all *Adh* alleles introduced into monosomics, disomics, trisomics and tetrasomics are expressed in all instances, as detected by electrophoresis. Although none were evident from these experiments, mutant alleles with altered response would serve as a useful approach to the further study of the phenomenon.

It might be proposed that the *Adh* locus and a second gene (or genes) located in the *1L* 0.20–0.72 segment compete for the same limiting factor. In order for this hypothesis to be consistent with the gene-dosage effect observed in the segmental trisomic and tetrasomic, the competing gene or genes must do so only weakly. On the other hand, for the competing gene concept to be accommodated with the data from the compound translocation *TB-1La-3L5267*, it would be necessary to postulate that their competitive ability is much stronger than that of *Adh*. Thus, the two observations lead to conflicting modifications of the hypothesis.

It is not our intention to imply that the region of *1L* between 0.72 and 0.90 that includes the *Adh* gene contains no modifiers of gene expression, for, indeed, protein profiles of this region suggest that some level of effects do occur. If a modifier of *Adh* is present within the limits of the duplication, its influence in these backgrounds and tissue are minimal. The results of the study of region *1L* 0.20–0.72 indicate that at least some of the modifiers of ADH present in *1L* are readily separable from the structural locus. The region distal to the *Adh* gene (*1L* 0.90–1.00) has not been examined in this study.

Since ADH levels are compensated in the dosage series of *1L*, one might expect ADH expression not to be increased with increasing ploidy. This is not the case, however. Through the *1L* series, the dosage of *Adh* and the modifying region *1L* 0.20–0.72 remains constant, and the level of ADH is compensated. In the ploidy series, the dosage of *Adh* and this region are maintained in the same relationship, but the enzyme levels exhibit a near dosage effect (or greater) per cell. This comparison leads to the concept that the genic imbalance in the aneuploids produces the inverse effect, directly or indirectly.

The example of ADH compensation described here might serve as a model for other cases of compensation found in higher eukaryotes. It has previously been

noted that evidence exists for the inverse effect in such diverse organisms as maize, Datura and Drosophila (see BIRCHLER 1979). Their presence in the latter can account for dosage compensation of *X*-linked genes (BIRCHLER 1980b). Recently, DEVLIN, GRIGLIATTI and HOLM (1980) have reported the phenomenon of autosomal dosage compensation of α-glycerophosphate dehydrogenase (αGPDH), phosphoglycerokinase and cytoplasmic malate dehydrogenase in trisomic *2L* larvae. When this observation on αGPDH is coupled with the segmental trisomy studies on this enzyme by RAWLS and LUCCHESI (1974), a situation analogous to that of ADH in maize emerges. There are three regions in the left arm of chromosome *2* that reduce αGPDH to 0.66, 0.75 and 0.73, respectively, relative to the diploid controls. The structural locus produces a gene-dosage effect. The simultaneous inclusion of the two responses would result in dosage compensation.

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