A STUDY OF ENZYME ACTIVITIES IN A DOSAGE SERIES OF THE LONG ARM OF CHROMOSOME ONE IN MAIZE

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ABSTRACT

The enzyme activity levels of alcohol, malate, isocitrate, glucose-6-phosphate and 6-phosphogluconate dehydrogenases were determined in mature maize scutella in a series of one to four doses of the long arm of chromosome *1*, produced by the *B-A* translocation *1La*. Although the *Adh* structural locus was varied, ADH levels did not exhibit a gene-dosage effect. The levels of G6PDH, 6PGDH and IDH were negatively correlated with the dosage of *1L*. MDH was unresponsive. The esterase-8 enzyme, whose structural locus was demonstrated to be elsewhere in the genome, was also negatively correlated with *1L* dosage. The portion of the *B* chromosome involved in the translocation was shown to have no effect on the enzyme levels. Measurements of cell size and hydrolysable DNA per mg dry weight revealed no change in the number of cells through the one, two and three dose series. The topic of enzyme alterations in aneuploids is reviewed.

AS part of a characterization of the alcohol dehydrogenase (ADH) isozyme system in maize, SCHWARTZ (1971) compared the activity of ADH in mature scutella monosomic for the terminal 80% of the long arm of chromosome *1* to that found in the normal diploid. Since the *Adh* gene is located in this chromosome arm, one would expect the expression in the monosomic to be approximately 50% of the euploid value. He found, however, that the specific activity in the partial monosomic was 76% of the euploid. Because the corresponding partial trisomic of this region was associated with a defective endosperm phenotype in his experiment, he did not examine the level of ADH in the partial trisomic scutella. The work reported here extends SCHWARTZ's (1971) study to determine the relative ADH expression in scutella trisomic and tetrasomic for the distal 80% of chromosome arm *1L*.

When such a large portion of chromatin is varied, problems with specific activity measurements might arise due to dosage effects for at least some of the proteins encoded by the genes in the varied segment. Thus, correcting the enzyme level by total protein estimates may actually produce a quotient that appears to reflect a change in the level of enzyme, when in actuality only the total protein has been altered. If some of the major proteins are encoded in the

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varied section and exhibit gene-dosage effects, the degree of deviation may become significant. Perhaps a more important consideration is that certain loci on the varied chromosome arm may affect the level of proteins encoded elsewhere in the genome. Studies on trisomic *21* of humans (BENSON 1967) and on various chromosome arms in maize (BIRCHLER 1978) indicate that aneuploidy can indeed influence the level of specific proteins or protein synthesis in general. If the dosage and aneuploid effects do not cancel, the use of total protein would not be the best standard. For this reason, a number of other enzyme levels, namely MDH, IDH, G6PDH, 6PGDH, PGM and esterase were examined as possible controls. Since the levels of some of these enzymes appeared to be negatively correlated with *1L* dosage, hydrolyzable DNA per mg dry weight of scutellum estimates were also made.

Although considerable variation at numerous isozyme loci has been detected in corn (SCANDALIOS 1974), few of the structural genes for enzymes that are expressed at reasonable levels in the scutellum have been mapped. Consequently, the genetic locations of the structural genes for the enzymes studied are not known, with the exception of *Adh*. It was therefore necessary to seek a variant of one of the enzymes that was negatively affected and test for its inclusion in *1L*.

The dosage series was produced by using a translocation between a *B* chromosome and the long arm of chromosome *1*. The inheritance of the supernumerary *B* chromosomes and *B-A* translocations has been reviewed recently by CARLSON (1977) and BECKETT (1978), where references to the pertinent literature may be found. The *B* chromosomes of maize are found in certain strains and are almost completely genetically inert. The centromere of the *B* chromosome undergoes nondisjunction at the second microspore division in 50 to 98% of the pollen grains containing them. If a translocation is produced between the *B* chromosome and one of the *A* (normal) chromosomes, the distal portion of the *A* chromosome that is attached to the *B* centromere will also nondisjoin at this particular mitotic division. Since this division gives rise to the maize sperm, the two male gametes will differ in their genetic constitution. One sperm has no B^A chromosomes and the other has two. In most backgrounds, the sperm with the two *B-A* chromosomes preferentially fertilizes the egg nucleus at an approximate frequency of 67%. Since nondisjunction does not always occur, gametes with zero, one or two doses of the translocated arm are produced. When a normal female is pollinated by a male carrying such a translocation, zygotes will be formed having one, two or three doses of the critical chromosome arm. The frequency of these three classes of progeny will depend upon the rate of nondisjunction in the male, the extent of preferential fertilization of the egg nucleus by the sperm carrying the two *B* centromeres and whether the translocation is in the homozygous, euploid heterozygous or hyperploid heterozygous condition. The pairing and recombination frequencies in these three chromosomal constitutions have been delineated by ROMAN (1947) and ROBERTSON (1967). When *B-A* translocations are appropriately marked, a dosage series of the *A* region can

be produced and genetically distinguished. In the present study, *Adh* isozyme markers have been used.

The ADH isozyme system in maize has been the subject of many studies on the processes involved in the expression of enzyme levels (SCHWARTZ 1971; FREELING 1975; LAI and SCANDALIOS 1977). The discovery by SCHWARTZ (1971) that scutellar ADH levels were not proportional to gene dosage in the partial monosomics relative to the euploid provides an interesting exception to the general rule of gene dosage effects. The purpose of this article is to report the compensation of ADH in the partial trisomic and tetrasomic and the phenomenon of a negative correlation of certain enzyme activities with *1L* chromosome dosage.

MATERIALS AND METHODS

Maize lines and Adh *alleles*: Four *Adh* alleles were used in these experiments. The standard *Adh-F* and *-S* alleles were described by SCHWARTZ and ENDO (1966). In addition, *Adh-C-70-86*, a mutant which has a greater electrophoretic mobility than *F*, was needed. This allele was derived from the standard *F* in an ethyl methanesulfonate (EMS) mutagenesis study conducted by SCHWARTZ. The *TB-1La* line used in this study originally carried an *Adh-F* allele and was in a W23/L317 hybrid background.

The specific activity (units per mg total protein) of the standard *S* allele used in these experiments was 5% greater than the standard *F* when comparisons were made on homozygous kernels from F_2 ears (LAUGHNER 1970). The data in Table 1 also compare the mutant *C* with the standard *F* and with the *F* originally in TB-1La. The mutant *C* has a specific activity similar to both the standard *F* and standard *S*. The *F* originally in *TB-1La* has only 82% of the standard alleles specificity. This fact should be considered in interpreting the aneuploid comparisons presented later. However, because of the way in which the comparisons were made, this difference can only account for a 5 to 6% modulation of the aneuploid/euploid ratio, all other factors being equal.

Construction and classification of 1L *dosage series*: The dosage series of *1L* was produced by using the *B-A* translocation, *1La*. This translocation has the terminal 80% of chromosome

TABLE 1

Specific activities of Adh *homozygotes from* F_2 *ears*

Adh alleles	*n*	ADH units/mg protein ± S.E.
C-70–86/C-70–86	3	321 ± 3
standard *S*/standard *S*	3	318 ± 18
C-70–86/C-70–86	3	390 ± 9
standard *F*/standard *F*	3	369 ± 18
standard *S*/standard *S*	3	631 ± 12
standard *F*/standard *F*	3	603 ± 26
C-70–86/C-70–86	3	616 ± 1
F/*F* (from *TB-1La*)	3	503 ± 20

n = number of extracts for which ADH units/mg protein was determined; specific activity is the number of ADH units (1 unit = 0.001ΔO.D. 340/min) per mg. total protein. The *F* originally from *TB-1La* was removed from the translocation by recombination and was present in a structurally normal chromosome for these assays.

arm *1L* linked to a *B* centromere. It was X-ray-induced by ROMAN (1947) and obtained for this study from the Maize Genetics Coop., Urbana, Illinois.

In order to determine genetically the various doses of *1L*, it was necessary to transfer the *Adh-C* and *S* alleles on to the *TB-1La* translocation. The original stock carried an *F* allele. In order to produce a line that carries a *C* or *S* on the translocated arm, the following protocol was performed. Hyperploid *1 1^{B}B^{1}B^{1}* (*Adh-F/F/F*) plants were used as females and crossed by males homozygous for *C* or *S*. Approximately half of the progeny are balanced euploids (*1 1^{B}B^{1}*) that are heterozygous for two *Adh* alleles and are distinguishable from the other classes of progeny because they exhibit higher than normal pollen abortion. When recombination occurs in the euploid heterozygote between the normal chromosome and the *B^{1}* chromosome at a point between the *Adh* locus (*1*–128±1.5) (SCHWARTZ 1971) and the translocation breakpoint (proximal to *hm*, *1*–64) (ROMAN and ULLSTRUP 1951), a different *Adh* allele is transferred to the *B^{1}* chromosome and can be recovered if the *B^{1}* chromosome segregates with *1^{B}*, undergoes nondisjunction and fertilizes the egg nucleus. Such kernels have hyperploid embryos (*1 1^{B}B^{1}B^{1}*) and, in the case of *TB-1La*, are associated with a distinctive small endosperm phenotype. By analyzing only the small kernel class, one has selected the embryos in which all of these criteria have been met except recombination between *Adh* and the breakpoint. The frequency of ADH types present in the small kernel class is a direct measure of the percent recombination. In a cross of a normal *Adh-C/C* female by a euploid heterozygote (*1 1^{B}B^{1}*) (*Adh-F/C*), kernels with the small endosperm phenotype were genotyped by electrophoretic analysis of an extract of a sliver of the scutellum. Fourteen out of 36 hyperploids analyzed were homozygous for *Adh-C*. Crosses to other electrophoretic variants confirmed the presence of the *TB-1La* translocation in all tested cases. Backcrosses to *Adh-C* served to establish a *TB-1La* line marked with an electrophoretically distinguishable allele. The standard *Adh-S* allele was transferred to the translocation in a similar manner.

Partial monosomic vs. *disomic*: A *1L* dosage series having partial monosomic, disomic and trisomic scutella can be produced and distinguished in the following way. Plants homozygous for a particular *Adh* allele were pollinated by hyperploid *1 1^{B}B^{1}B^{1}* plants whose *Adh* allele produces an electrophoretically distinguishable enzyme. Such hyperploid plants produce *1B^{1}* and *B^{1}1^{B}* microspores in approximately equal frequencies (ROMAN 1947; ROBERTSON 1967). The *1B^{1}* pollen grains are duplicated for *1L* and do not compete in fertilization with the euploid pollen. Only two successful fertilizations by hyperploid pollen were observed in over 10,000 kernels from this type of cross electrophoretically analyzed in the course of this and related studies. In the *B^{1}1^{B}* microspore, the *B* centromere undergoes nondisjunction in the second microspore division in approximately 80% (BIRCHLER, unpublished) of the pollen grains so as to yield gametes with zero or two regions of *1L*.

If the egg is fertilized by a sperm lacking the *B^{1}* chromosome, a zygote monosomic for the distal portion of *1L* is formed. If the egg is fertilized by a sperm from a microspore in which disjunction was normal or by the normal chromosome, which is occasionally transmitted, there is an equal contribution of *A* chromatin from the male and female such that a disomic zygote is produced. If the egg is fertilized by a sperm with two *B^{1}* chromosomes, a partial trisomic zygote is formed. Because the chromosomes are marked by different *Adh* alleles, the monosomics will have only the maternal electrophoretic variant, whereas the disomics will have both the maternal and paternal variants. The trisomic class is distinguishable on the basis of isozyme ratios. Also, the hypotriploid endosperm associated with the partial trisomic is reduced in size relative to the endosperm of kernels with monosomic and disomic embryos.

For example, if an *Adh-F/F* plant is crossed by a hyperploid (*1 1^{B}B^{1}B^{1}*) plant that is homozygous for *Adh-C/C/C*, the resulting zygotes and approximate percentage of the total will be: *F/—*, partial monosomic, 31%; *F/C*, disomic, 27% and *F/C/C*, partial trisomic, 42%. With random association of enzyme subunits, the *F/C* embryos have a 1 *FF*:2 *FC*:1 *CC* isozyme band ratio, which is discernible from the 1 *FF*:4 *FC*:4 *CC* ratio produced by *FCC* embryos. Representative zymograms are given in Figure 1.

Partial trisomic vs. *disomic*: Ears segregating for scutella trisomic and disomic for the distal region of chromosome arm *1L* were produced as follows. Hyperploid (*1 1^{B}B^{1}B^{1}*) females with

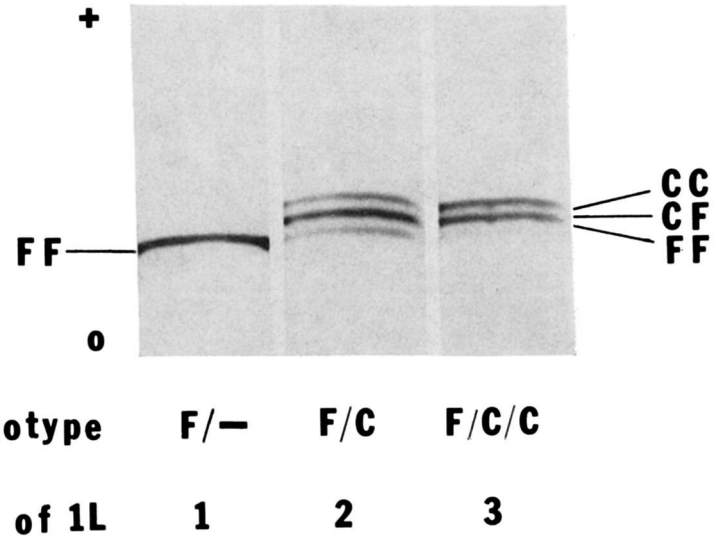

FIGURE 1.—Zymograms of ADH in a partial monosomic, disomic, and partial trisomic dosage series produced from the cross *Adh-F/F* by *Adh-C/C/C* (*1 1^{B}B^{1}B^{1}*). The partial monosomic with the hemizygous *Adh-F* is shown in the left gel. In the center is the disomic, *Adh-F/C*. On the right is the partial trisomic, *Adh-F/C/C*. The origin is denoted as 0; the anode by +. The isozyme bands corresponding to *C·C*, *C·F* and *F·F* ADH dimeric molecules are labeled along the margin.

Adh-S on the normal chromosome one and the original *Adh-F* on the two *B^{1}* chromosomes were pollinated by males homozygous for *Adh-C*. The resulting classes of progeny are *Adh-S/F/C*, partial trisomic, 39%; *Adh-F/C*, disomic, 52% and *Adh-S/C*, disomic, 9%. Recombination between the normal one and one of the *B^{1}* chromosomes might change the linkage relationship of the *Adh* alleles and the translocation, but does not alter the ability to distinguish the chromosome dosage. Rarely are *FFC* or *SSC* kernels found, the classes expected from recombination between the breakpoint and *Adh* with subsequent double reduction. Zymograms of *SFC* and *FC* scutellar extracts are given in Figure 2.

Partial tetrasomic vs. *disomic*: Ears bearing kernels tetrasomic for *1L* were produced as follows. Hyperploid females (*1 1^{B}B^{1}B^{1}*) that carried the standard *Adh-F* allele in the normal chromosome and the standard *Adh-S* in the two *B^{1}* chromosomes were crossed by hyperploid males (*1 1^{B}B^{1}B^{1}*) homozygous for *Adh-C*. The classes of zygotes from such a cross will then be as illustrated below:

		Male gametes (*Adh* genotype)		
		..	*C*	*C/C*
Female gametes	*F*	*F/—*	*F/C*	*F/C/C*
(*Adh* genotype)	*S*	*S/—*	*S/C*	*S/C/C*
	F/S	*F/S/—*	*F/S/C*	*F/S/C/C*

All classes of scutella are distinguishable on the basis of the ADH isozymes present and their band ratios. An additional aid in classification is the fact that all of the classes in the right-most column are associated with the distinctive small endosperm phenotype. All of these classes lack a *1L* paternal contribution to the endosperm and therefore exhibit the small kernel phenotype

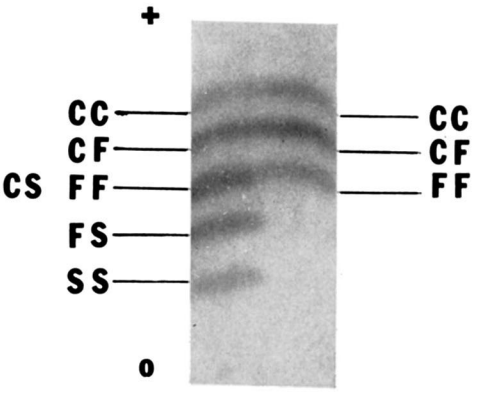

FIGURE 2.—Zymogram of ADH in partial trisomics and disomic scutella produced from the cross *Adh-S/F/F* (*1 1^{B}B^{1}B^{1}*) by *Adh-C/C*. On the left is the *Adh-S/F/C*; on the right, *Adh-F/C*. Each type of ADH dimer is labeled along the margin. The trisomic has only five isozyme bands because *F·F* homodimers and *C·S* heterodimers co-migrate under the conditions of electrophoresis employed.

regardless of *1L* dosage in a similar, but not identical, fashion to the case of *TB-10L19* described by LIN (1975). The partial tetrasomic *F/S/C/C* scutella were compared to the *F/S* disomic. The *FSCC* scutella are discernible from *FSC* embryos on the basis of their isozyme ratios. The former has an approximate 4*CC* : 4 *CF* : 5 *FF*+*CS* : 2 *FS* : 1 *SS* ratio, while the latter has an approximate 1 *CC* : 2 *CF* : 3 *FF*+*CS* : 2 *CS* : 1 *SS* ratio.

Since recombination might occasionally occur between the normal chromosome *1* and one of the *B^{1}* chromosomes, it is conceivable that double reduction of the *Adh* locus would produce exceptional kernels with only *F* or *S* alleles present that were actually *F/F/—* or *S/S/—*. In addition, certain "*F/C* or *S/C*" kernels may actually by *FFCC* or *SSCC*. The use of only the *F/S/—* kernels as disomics and *F/S/C/C* kernels as partial tetrasomics does not involved this complication. Zymograms of *F/S/—*; *F/S/C* and *F/S/C/C* scutellar extracts are shown in Figure 3.

Root-tip chromosome counts are not useful in verifying the series because, in each cross, certain genotypes of differing dosage have the same number of chromsomes. It was therefore necessary to progeny test a sample. To this end, scutella of a *1L* dosage series were genotyped by electrophoretic analysis and the kernels grown into plants under field conditions. The plant height, from greatest to least, in the background studied was disomic, trisomic, tetrasomic and monosomic. The partial monosomics are shorter and have thinner leaves than the euploid, but flower at about the same time. The partial trisomics have shorter and broader leaves, a more compact tassel, smaller ears and later flowering time than their diploid sibs. The tetrasomics are much shorter than the trisomics, and although they produce ear shoots, no silks emerge and thus they are completely female sterile. Pollen examination of partial tetrasomics revealed variable, but high, levels (90% ±) of abortion.

Determination of enzyme activities: The enzymes assayed in this study and the assay procedures are as follows: Alcohol dehydrogenase (ADH) (E.C. 1.1.1.1) activity was monitored as

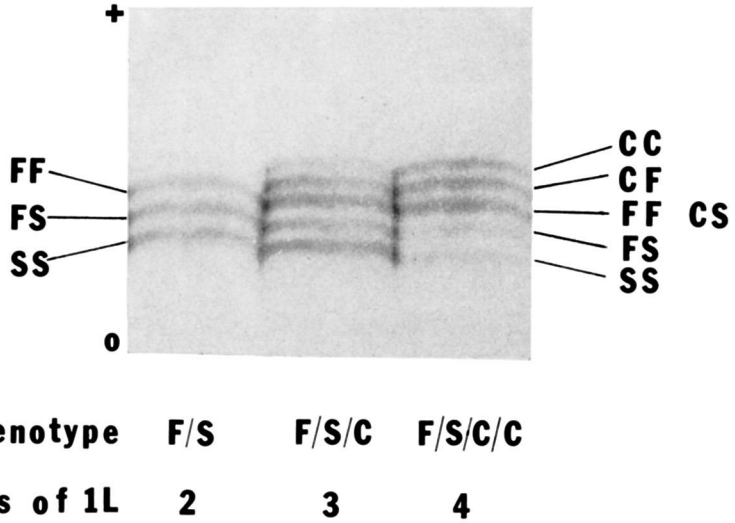

FIGURE 3.—Zymograms of ADH from disomic, partial trisomic and partial tetrasomic scutella produced from the cross of *Adh-S/F/F* (*1 1^{B}B^{1}B^{1}*) by *Adh-C/C/C* (*1 1^{B}B^{1}B^{1}*). The order from left to right is disomic (*F/S*), partial trisomic (*F/S/C*) and partial tetrasomic (*F/S/C/C*). The partial trisomic and partial tetrasomic are discernible on the basis of their different isozyme band intensities.

described by EFRON and SCHWARTZ (1968) with the expection that only 0.02 ml of extract was used per assay. The linear change in absorbance was measured with a Perkin-Elmer Coleman 54-B spectrophotometer with digital read out.

Glucose-6-phosphate dehydrogenase (G6PDH) (E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH) (E.C. 1.1.1.44) were assayed as modified from the procedure of SCHNARRENBERGER, OESER and TOLBERT (1973). The assay solution was 50 mM tris-HCl (Sigma) pH 8.0, 7.5 mM $MgCl_2$ (Nutritional Biochemicals), 250 μM nicotinamide adenine dinucleotide phosphate (Sigma) and 2 mM glucose-6-phosphate, monosodium salt (Sigma), or 2mM 6-P-gluconate trisodium salt (Sigma), respectively. Final volume of the assay was 3.0 ml. One-tenth ml of extract was used per assay.

Malate dehydrogenase (MDH) (E.C. 1.1.1.37) was assayed in the following manner. To 4.88 ml of activity solution [150 mM tris-HCl, pH 8.0; 1 mM oxalacetic acid (Sigma)], 0.02 ml of scutellar extract (diluted one part original plus three parts extraction buffer) was added. The spectrophotometer was zeroed and 0.1 ml of reduced nicotinamide adenine dinucleotide (Sigma) sufficient to bring the assay solution to a concentration of 0.012 mM was added. The linear loss of absorbance at 340 nm was followed at 15 sec intervals for one min.

Isocitrate dehydrogenase (IDH) (E.C. 1.1.1.42) was assayed by a modification of KORNBERG (1955). The assay solution was 50 mM tris-HCl, pH 8.0; 0.054 mM ethylenediamine tetracetic acid, disodium salt (Sigma); 7.9 mM magnesium chloride; 250 μM nicotinamide adenine dinucleotide phosphate; 2.2 mM isocitric acid, trisodium salt (Sigma). Total volume was 3.0 ml. One-tenth ml of extract was added to 2.9 ml of activity solution to begin the reaction.

Phosphoglucomutase (PGM) (E.C. 2.7.5.1) was assayed by a modification of TSAI, SALAMINI and NELSON (1970). The assay solution was 5.6 mM tris-HCl, pH 8.0, 7.9 mM $MgCl_2$, 250 μM nicotinamide adenine dinucleotide phosphate, 0.15 mg per ml glucose-1-phosphate (Nutritional Biochemicals), one I.U. sulfate-free yeast glucose-6-phosphate dehydrogenase per ml (Sigma). Total volume of the assay was 3.0 ml. One-tenth ml of extract was added to 2.9 ml of solution. Sufficient glucose-1,6 diphosphate was present in the G-1-P preparation for the re-

action to proceed. Change in absorbance in the absence of G-1-P was less than 1% of standard assay conditions.

Total esterase activity was measured according to the method of ENDO and SCHWARTZ (1966).

An enzyme unit of ADH, G6PDH, 6PGDH, IDH, MDH and PGM is defined as that amount of enzyme which causes a change of 0.001 absorbance units per min at 340 nanometers under the standard assay conditions. All assays were monitored at 15-sec intervals for one min and were linear over this time period even when an extract five times as concentrated at the experimental level was used.

Sample preparation: Kernels were removed from an ear segregating for the particular aneuploid and euploid being studied. A portion of each scutellum was excised, soaked in distilled water for 12 to 18 hr and then genotyped for ADH in starch gel electrophoresis as described by SCHWARTZ and ENDO (1966). On the basis of the ADH isozymes present, the kernels were classified into aneuploid and euploid classes as described in "construction of *1L* dosage series." Kernels of like types were pooled (20 to 30 kernels per pool) and scutella removed with a scalpel and ground to a meal in a Wiley Mill (mesh 20). Two hundred milligrams of meal were extracted in two ml of 5 mM Na phosphate, pH 7.5, 6 mM β-mercaptoethanol buffer for 15 min at room temperature. The slurry was centrifuged at 48,000 $\times$ g for 15 min in a refrigerated Sorvall centrifuge and the supernatant was filtered through Miracloth. Thereafter the extract was kept at 0 to 1°. All extracts were subjected to electrophoresis and stained for ADH to verify correct classification of the kernels.

Absolute values of enzyme activity per mg dry weight vary greatly from ear to ear due possibly to different moisture contents and/or segregation of genetic modifiers. Since the ultimate goal of the experiments is to compare aneuploid to euploid levels, the data for each ear are expressed as the aneuploid-euploid ratio. The data for each ear represent the determination of enzyme units per mg dry weight of mealed aneuploid scutella divided by the enzyme units per mg dry weight of the sibling euploids.

Estimation of total protein: Proteins were estimated by the method of LOWRY *et al.* (1951), after precipitation with ice-cold 10% trichloroacetic acid (Sigma) and redissolved in 0.1 N NaOH. Bovine serum albumin (Sigma) was used as a standard. For each determination, 0.05 ml of extract was used.

Electrophoresis of ADH and esterase: Electrophoresis of ADH in starch gels and development of ADH zymograms were performed as described by SCHWARTZ and ENDO (1966). Electrophoresis and staining of esterase were by the method of SCHWARTZ (1960). All were conducted at 5°.

Cell size examination: In order to investigate whether aneuploidy affects the cell size in scutellar tissue, embryos were classified as partial trisomic, disomic and monosomic and imbibed at 30° overnight. Then the scutella were excised and mounted on a Histo freeze microtome. Sections were cut and stained with 1% aqueous safranin. Fifty cells in each of two sections of each type of aneuploid and euploid were measured in the longest dimension. The sections were taken between the embryo and the edge of the scutellum near the face of the germ.

Estimation of total cell hydrolyzable DNA: As an additional control on the use of dry weight as a standard and in order to determine if the number of cells per unit mass was altered in the aneuploid types, total cellular hydrolysable DNA per unit mass of tissue was estimated by a modification of the procedure of WEBB and LEVY (1955). For comparisons in the dosage series produced by *TB-1La*, the partial monosomic *Adh-S/—* and *Adh-S/F* (disomic) kernels from a segregating ear were analyzed. For the partial trisomic comparison to disomics, *Adh-S/C/F* and *Adh-C/F* kernels from segregating ears were used. To complete the dosage series partial tetrasomic, *Adh-F/S/C/C* kernels were compared to the disomic, *Adh-F/S*.

Approximately twenty kernels of each type of material to be tested had their scutella excised and mealed with a Wiley Mill (mesh 20). One-tenth gram of scutellar meal was hydrolyzed in 6 ml of 5% trichloroacetic acid (TCA) in glass Sorvall centrifuge tubes in a boiling water bath for 40 min. The remaining steps were as described by WEBB and LEVY (1955). A linear response in absorbance was found in the range 0.025 to 0.200 grams of scutellar meal used in the assay. Comparisons of maize lines with zero or approximately eight *B* chromosomes

established that the assay could discriminate differences in DNA per unit dry mass (BIRCHLER 1977).

When comparing the relative hydrolyzable DNA contents between the aneuploids and diploid tissue, the amount of DNA per cell that is altered in each aneuploid should be considered. The calculation of the approximate percentage of DNA involved in *TB-1La* was based on the cytological breakpoints and the DNA content of the *B* chromosome. The *B* chromosome has 3.8% of the DNA content of the diploid complement (RAMIREZ 1974). The fraction of the total length of the *A* chromosomes translocated to the *B* in *TB-1La* is 0.067. Since the break in the *B* in *TB-1La* is very near the centromere (E. WARD, personal communication), the portion of the *B* chromosome present in the partial monosomic compensates the loss of *A* chromatin, and therefore the partial monosomic/disomic ratio was 0.97. The partial trisomic/disomic ratio was found to be 0.996 because the disomic has most of the *B* chromosome, which is similar to the extra amount of DNA in *1L* present in the partial trisomic. The partial *FSCC* tetrasomic has two *1L* regions more than the *FS* disomic and therefore the ratio is 1.067 (BIRCHLER 1977). These values can be considered only as rough estimates, but they illustrate that none of the aneuploids deviates greatly from the euploid.

RESULTS

When enzyme activity levels in the *1L* partial monosomics were compared to the diploid, the results found in Table 2 were observed. Three types of crosses are shown. The comparisons made were standard *F* hemizygotes to *F*/standard *S* heterozygotes, standard *F* hemizygotes to *F/C* heterozygotes and standard *S* hemizygotes to *S/F*, where the latter was originally present in *TB-1La*. Although differences exist, all three types of crosses exhibit similar phenomena. ADH levels show a partial compensation in all three types of crosses, indicating that compensation is not allele specific. G6PDH and 6PGDH are significantly increased in the partial monosomic compared to the disomic. IDH is significantly

TABLE 2

*Partial monosomic/disomic ratios of enzyme activities**

Adh alleles	*n*	ADH	G6PDH	6PGDH	IDH	MDH	Protein
F:F/S	5	0.81	1.49	1.44	1.39	1.01	1.19
		±0.07	±0.08	±0.09	±0.09	±0.02	±0.07
F:F/C	4	0.70	1.58	1.37	1.19	0.98	1.00
		±0.05	±0.12	±0.13	±0.11	±0.06	±0.08
S:S/F	3	0.85	1.62	1.50	1.30	1.02	1.07
		±0.08	±0.10	±0.06	±0.10	±0.02	±0.05

* Ratios represent the mean ± standard error of *n* number of ears for which the enzyme units per mg dry weight of mealed scutella for the partial monosomic extract was divided by the enzyme units per mg dry weight of mealed scutella of sibling disomics; 1 enzyme unit = 0.001 O.D. $_{340}$/min.; protein ratio is the mg total protein per mg dry weight of the partial monosomic divided by the total protein per mg dry weight of disomic scutella. The comparisons were made from standard *F/—* partial monosomics *vs.* sibling standard *F*/standard *S* disomics, standard *F/—* partial monosomics *vs.* sibling standard *F/C* disomics, and standard *S/—* partial monosomics *vs.* standard *S/F* (originally in *TB-1La*) from the crosses; standard *F* females by $11^{B}B^{1}B^{1}$ (*Adh-S/S/S*) males, standard *F* females by $11^{B}B^{1}B^{1}$ (*Adh-C/C/C*) males and standard *S* females by $11^{B}B^{1}B^{1}$ (*Adh-F/F/F*) males, respectively.

TABLE 3

*Partial trisomic/disomic ratios of enzyme activities**

Adh alleles	*n*	ADH	G6PDH	6PGDH	IDH	MDH	Protein
S/F/C:F/C	8	1.17 ±0.05	0.79 ±0.04	0.84 ±0.03	0.89 ±0.02	1.02 ±0.03	0.94 ±0.04

* Ratios represent the mean ± standard error of *n* number of ears for which the enzyme units per mg dry weight of mealed scutella for the partial trisomic extract was divided by the enzyme units per mg dry weight of mealed scutella of sibling disomics. The partial trisomic, *Adh S/F/C*, and disomic, *Adh F/C*, result from the cross $11^{B}B^{1}B^{1}$ (*Adh-S/F/F*) females by normal males (*Adh-C/C*) as described in MATERIALS AND METHODS.

increased in two (*F:FS* and *S:SF*) of the three types of crosses. MDH partial monosomic/disomic ratios in all cases do not significantly deviate from unity.

When the same set of enzymes were measured in partial trisomics and compared to their sibling disomics, the results in Table 3 were found. The mean ADH trisomic/disomic ratio was 1.17 ± 0.05, which is significantly less than the 1.50 value expected from a gene dosage effect. G6PDH, 6PGDH and IDH are all significantly reduced below the euploid level. MDH values, however, were unchanged.

When ADH levels in tetrasomics were compared to sibling disomics, the ratio was 1.18 ± 0.02. The data in Table 4 show that for G6PDH, 6PGDH and IDH, there are consistent further reductions below the trisomic level; MDH levels were unaffected. It is important to note that these values must be interpreted with the knowledge of the difficulties, discussed below, involved with standardizing the tetrasomic data.

In addition to MDH, PGM levels were found to be unresponsive to *1L* dosage. The mean (± s.e.) ratio of six monosomic/disomic comparisons of phosphoglucomutase was 1.05 ± 0.03. The mean (± s.e.) of 12 trisomic/disomic comparisons was 0.96 ± 0.03. The observation that MDH and PGM levels are insensitive to *1L* dosage does not necessarily indicate that the structural genes are located elsewhere in the genome, since their levels could be compensated. However, the total MDH levels monitor the products of five or possibly more unlinked loci (GOODMAN *et al.* 1978), so that it is reasonable to conclude that at least some of the MDH genes are not located in *1L*.

TABLE 4

*Partial tetrasomic/disomic ratios of enzyme activities**

Adh alleles	*n*	ADH	G6PDH	6PGDH	IDH	MDH	Protein
F/S/C/C:F/S	6	1.18 ±0.02	0.64 ±0.05	0.66 ±0.05	0.72 ±0.05	1.06 ±0.04	0.84 ±0.06

* Ratios represent the mean ± standard error of *n* number of ears for which the enzyme units per mg dry weight of mealed scutella for the partial tetrasomic extract was divided by the enzyme units per mg dry weight of mealed scutella of sibling disomics. The partial tetrasomic, *Adh-F/S/C/C*, and the disomic, *Adh-F/S*, result from the cross $11^{B}B^{1}B^{1}$ (*Adh-F/S/S*) females by $11^{B}B^{1}B^{1}$ (*Adh-C/C/C*) males as described in MATERIALS AND METHODS.

The partial trisomics reported in Table 3 were produced by using hyperploid *1 1^{B}B^{1}B^{1}(Adh-S/F/F)* females that were crossed by normal males. The partial trisomics consist of one paternal and two maternal *1L* segments. To test if the parental source of the varied region affects the results (LIN 1975), trisomics consisting of two paternally derived *B^{1}* chromosomes and one maternal chromosome were compared to their sibling disomics. This type of partial trisomic is produced when a normal female is crossed by a hyperploid *1 1^{B}B^{1}B^{1}* male, as described in MATERIALS AND METHODS. The mean ± s.e. for 11 ears analyzed for ADH ratios and normalized to MDH levels in the (2♂:1♀) trisomic/disomic comparisons was 1.17 ± 0.03, which is comparable to the ADH (1♂:2♀) trisomic/disomic comparisons reported above. Likewise, the mean ± s.e. for ten ears analyzed in the same manner for G6PDH/MDH was 0.76 ± 0.04. These results indicate that the enzyme relationships (ADH, G6PDH and MDH) are essentially the same in trisomics produced from reciprocal crosses.

The genetic locations of the loci encoding the enzymes studied are not known, with the exception of *Adh*. It was believed unlikely that the structural genes for G6PDH, 6PGDH and IDH were located in *1L* in view of their expression in the dosage series. However, in the absence of electrophoretic mobility variants, this point could not be tested. Since some of the loci contributing to the total esterase have known electrophoretic mobility variants, esterase zymograms of scutellar extracts were examined for the presence of the negative correlation of enzyme level with chromosome dosage. One of the major scutellar esterase isozymes is the product of the Esterase-8 locus (MACDONALD and BREWBAKER 1974). The intensity of this isozyme band is negatively correlated with the dosage of *1L* (Figure 4). An estimation of total esterase, performed in triplicate, from a cross of an *Adh S/S* female by a *TB-1La Adh F/F/F* male gave an esterase/MDH partial monosomic/disomic ratio of 1.94 ± 0.02. The trisomic/disomic ratio was 0.60 ± 0.04.

In the course of an ethyl methanesulfonate mutagenesis project, an electrophoretic mobility variant of *E8* had been recovered which migrated more slowly than the normal (BIRCHLER, unpublished). Heterozygotes between the slow

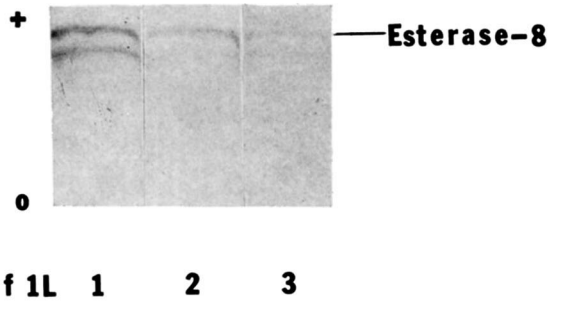

FIGURE 4.—Zymogram of esterase from a dosage series of *1L*. To minimize the number of esterase bands present, seedling extracts were used. Twenty μg of total protein were applied to each gel. The esterase-8 band is designated with an arrow.

form, *E8-S*, and the normal, *E8-N*, produce three *E8* isozymes corresponding to *N·N*, *N·S* and *S·S* dimers. Self pollination of a heterozygote gave the following progeny: 31 *N/N*, 44 *N/S* and 21 *S/S*. A χ^2 test of a 1:2:1 segregation ratio gave $\chi^2 = 2.75$, $P > 0.25$.

To test for the inclusion of *E8* in the region of *1L* translocated to the *B* centromere, an *Adh-S/S*; *E8-S/S* plant was pollinated by a hyperploid male from the *TB-1La* line homozygous for *Adh-C* and *E8-N*. Scutella from this cross were scored for both ADH and Esterase-8 by staining the top and bottom slices of the gel for the separate enzymes. The following classes of progeny were found: *Adh-S/—*, *E8-N/S* (15); *Adh-S/C*, *E8-N/S* (34) and *Adh-S/C/C*, *E8-N/S* (34). Since the *E8-S* allele is not uncovered in the *1L* partial monosomics, it is believed that the structural locus is elsewhere in the genome. This example suggests that the effect produced by *TB-1La* operates on unlinked loci or on their products.

The total protein values (per mg dry weight) in some aneuploids deviate from the euploid levels in the same direction as the responsive enzymes. Such a result might be expected if the negative aneuploid effect operates on the protein level. It was believed that dry weight was the appropriate standard by which to determine the level of deviation of the aneuploid effect because two different enzymes, namely MDH and PGM, were unresponsive to *1L* dosage. Nevertheless, an additional control independent of protein or enzyme levels was sought. Accordingly, the amounts of hydrolyzable DNA were compared per unit dry mass of partial monosomic, disomic, trisomic and tetrasomic scutella. Table 5 presents the data. There are no significant differences between the partial monosomic and disomic, nor between the partial trisomic and disomic, but the difference between the means of the partial tetrasomic and disomic is significant. This is of note since the MDH values are not reduced on a per mg dry weight basis. This discordance between these two controls could be due to (1) fewer cells per mg dry weight in the partial tetrasomic with a concomitant slight dosage effect for

TABLE 5

Comparisons of hydrolyzable DNA in aneuploid and euploid scutella

Aneuploid/euploid comparison	*Adh* genotype	Absorbance Δ 560*	Ratio DNA
Partial monosomic	*S/—*	0.277 ± 0.011	1.05
Disomic	*S/F*	0.264 ± 0.019	
Partial trisomic	*S/F/C*	0.465 ± 0.052	0.96
Disomic	*F/C*	0.483 ± 0.013	
Partial trisomic	*S/F/C*	0.446 ± 0.008	1.00
Disomic	*F/C*	0.446 ± 0.009	
Partial tetrasomic	*F/S/C/C*	0.356 ± 0.006	0.88
Disomic	*F/S*	0.406 ± 0.012	

* Values represent the mean ± S.E. of three determinations of absorbance at 560 nanometers per 100 mg of TCA hydrolysed meal minus the absorbance produced by 100 mg of meal of like type, carried through the procedure with water substituted for TCA.

MDH, (2) the same number of cells per mg dry weight but the number of plastids and/or mitochondria is reduced and hence the total DNA measurement, or (3) a combination of the above, or other possibilities may account for it. The data in the table have not been corrected for the variations in DNA present. In the case of the tetrasomic, the value would be lowered even more by this correction. The reduction of enzyme levels may become so great in the tetrasomic that secondary effects also become evident. The nearly equal amounts of DNA through the one, two, three dosage series is consistent with the fact that MDH and PGM levels are constant and corroborates the use of dry mass as a standard in those cases.

Since cell size and number have been found to vary in some tissues of certain aneuploids of Datura (SINNOTT and BLAKESLEE 1922), the possibility that the observed changes in enzyme activities could be attributed to this phenomenon was examined. Scutellar sections were made from ears segregating for partial trisomics and disomics as well as for partial monosomics and disomics. The cells varied in size within each section, but the magnitude of any differences between the euploids and aneuploids is small and would not appear to account for the alterations in enzyme levels due to a change in the number of cells per dry mass. The data are in Table 6.

Since the aneuploids produced by *TB-1La* not only vary in the amount of *A* chromatin present, but also in the amount of *B* chromatin, tests were conducted to determine if the portion of the *B* chromosome present in each aneuploid was responsible for the observed effects. Two tests are reported. The first involves producing trisomics for *1L* in two ways such that different amounts of *B* chromatin are present. When partial trisomics are made by using a hyperploid *TB-1La* as a female, there will be a *B* centromere segment in the partial trisomic and a *B* centromere plus a distal *B* segment in the euploid. If one generates partial trisomics by using the *TB-1La* as a male, the trisomic has two *B* centromeres and a distal *B* segment, whereas the euploid will usually have a balanced *1L·B* translocation containing the whole *B* chromosome. Both trisomic types are iden-

TABLE 6

Cell size observations in the dosage series

Dosage of *1L*	Adh genotype	Section no.	Mean length* (μm) ± S.E.
3	*SFC*	1	56.74 ± 1.37
3	*SFC*	2	59.09 ± 1.25
2	*FC*	1	61.50 ± 1.57
2	*FC*	2	63.11 ± 0.44
2	*FC*	1	57.03 ± 1.23
2	*FC*	2	58.90 ± 1.52
1	*F/—*	1	57.42 ± 1.47
1	*F/—*	2	59.19 ± 1.76

* Measurement of the approximate longest dimension is expressed in micrometers. Values are the means of 50 cells.

TABLE 7

Comparison of ADH, G6PDH and MDH in maize lines with and without B *chromosomes**

No. B chromosomes	ADH	G6PDH	MDH
None	28.6 ± 5.1 (5)	2.24 ± 0.40 (5)	146.5 ± 7.4 (3)
Six	24.8 ± 1.7 (5)	2.48 ± 0.32 (5)	185.0 ± 18.7 (3)

* Values represent the mean ± S.E. of the determination of the enzyme units (1 unit = O.D. $_{340}$/min) per mg dry weight of scutellar meal for the number of ears listed in parentheses.

tical in their *A* chromosome constitution, but differ in the amounts of the *B* present. Both classes of trisomics as noted above show similar ADH/MDH and G6PDH/MDH levels relative to the euploids. This indicates that the *A* chromatin is responsible for the effects.

Secondly, ADH, MDH and G6PDH were examined in related lines with no *B* chromosomes and with approximately six. The data are given in Table 7. The variability of enzyme levels in ear to ear comparisons is great, but there were no significant differences between the 6*B* line and the zero *B* line for the activity levels of ADH, MDH or G6PDH.

Considered together, the above data indicate that the long arm of chromosome *1* produces a negative aneuploid effect on the levels of G6PDH, 6PGDH, IDH and esterase. The MDH and PGM enzyme levels are unaffected. Although the *Adh* locus is varied, a gene dosage effect was not found.

DISCUSSION

Despite considerable attention, the mechanisms regulating enzyme levels during development and differentiation are largely unknown. The genetic approach to this problem has mainly been concerned with enzyme structural genes and possible contiguous regulatory sites. One of the general rules that has emerged from such studies is that structural genes produce a directly proportional gene dosage effect on the level of product found when the locus is varied (for examples see GRELL 1962; CARLSON 1972; MACINTYRE and O'BRIEN 1976). In the study reported here, the *Adh* locus was varied in a one, two, three, four dosage series, but the level of activity was not proportional to the number of genes present. It is difficult to explain this observation by suggesting that the *Adh* locus is not in *1L*. SCHWARTZ (1971) mapped electrophoretic variants to within 1.5 map units from *lw* and demonstrated that they were uncovered by *TB-1La*. Mutants that produce ADH molecules with little enzymatic activity (the C^m allele) (SCHWARTZ and ENDO 1966), as well as those that lack ADH cross-reacting material (CRM) (see FREELING 1976), are allelic to the electrophoretic mobility locus. Thus, different alleles of *Adh* affect the electrophoretic mobility, the activity of the enzyme or the presence or absence of ADH protein. It is therefore reasonable to conclude that this locus encodes the ADH enzyme. A second locus, *Adh 2*, (SCHWARTZ 1966, 1969; FREELING and SCHWARTZ 1973) is on chromo-

some *4* (see FREELING and CHENG 1978), but is negligibly expressed in the dry scutellum and is therefore assumed to have no effect on the results.

The observation that structural loci produce gene-dosage effects has been interpreted in the literature (*e.g.*, CARLSON 1972) to mean that eukaryotic loci are constitutive and that the number of genes is the rate-limiting aspect of the total process of gene expression. In the tissue and chromosomal constitutions studied here, this does not appear to be true for ADH. Studies of the relative expression of various *Adh* alleles in endosperm, seedling and pollen tissues had led several investigators to postulate the existence of a factor limiting ADH expression (SCHWARTZ 1971; FREELING 1975; PRYOR and MARSHALL 1977). Such hypotheses predict that the ADH level would not depend solely on the number of structural genes present. In this study, scutellar tissue was examined. This organ may be fundamentally different from the others with respect to ADH regulation because the different alleles are more nearly equal in expression in the scutellum than in the other tissues (SCHWARTZ 1971). Nevertheless, a limiting factor hypothesis could explain the results obtained with the *1L* dosage series. Alternatively, the fact that several other enzymes show a negative correlation with *1L* dosage makes it necessary to consider the possibility that ADH compensation is brought about by a cancellation of a positive gene-dosage effect by the *1L* negative aneuploid effect.

The experiments reported here demonstrate that G6PDH, 6PGDH, IDH and esterase activities in the mature scutellum are negatively correlated with the dosage of the region of *1L* involved in *TB-1La*. The levels of MDH and PGM activities are constant through the dosage series. This indicates that the response observed for the former group is specific for certain enzymes and not due to a general effect on all protein synthesis. The negative correlation of enzyme activity levels with *1L* dosage suggests that the enzyme activities or their encoding loci are modulated by the product(s) of a locus or loci in *1L*, which exhibit gene-dosage effects. Because the portion of the total genome that is varied is rather substantial, one must consider that the observed alterations may be due to a single gene, the additive or cooperative effects of several or even to chromosomal sites such as heterochromatin. Further subdivision of *1L* and a search for mutants producing similar consequences may serve as a first approximation at discerning among the possibilities. Although two enzymes of the same biochemical pathway, namely G6PDH and 6PGDH of the pentose phosphate cycle, are affected in a similar fashion, it is not yet possible to ascertain whether this is brought about by a single locus coordinately influencing both enzymes.

The use of chromosomal dosage studies have the potential to reveal several aspects of gene expression in addition to structural gene effects. The genetic location of at least some of the factors involved in the expression of an enzyme at various levels can be determined. The use of segmental aneuploidy to identify modifying loci does not rely on the availability of natural or induced variation and furthermore provides information on whether the modifier augments or depresses the level or activity of an enzyme.

There exist in the literature considerable data that indicate specific enzyme

or protein deviations in trisomics. Much of these data come from studies designed to locate enzyme loci cytogenetically or to identify trisomics that could not be distinguished morphologically. McDANIEL and RAMAGE (1970) used electrophoresis of germ proteins to identify the various trisomics of barley. They observed three types of effects: (1) increases of specific protein bands to 140 to 150% of the diploid intensity, (2) appearance of a new protein band accompanied by a loss of an adjacent protein band, and (3) suppression of specific protein bands. CARLSON (1972) used the trisomics of Datura to locate chromosomally the genes for nine of 15 enzymes tested on the basis of increased enzyme levels. In addition, the specific activities of most of these enzymes were reduced below the diploid level in one or more trisomics. The levels of lactate, isocitrate, 6-phosphogluconate, malate and glutamate dehydrogenases, as well as hexokinase, were reduced to between 70 to 80% of the diploid for at least one trisomic. Five different trisomics each reduced one of the enzymes and a sixth chromosome reduced two. The other enzymes studied (alcohol, glucose-6-P and glyceraldehyde-3-P dehydrogenases) were reduced in some trisomics, but none below 80%.

The data of O'BRIEN and GETHMAN (1973) in a study to locate structural loci by gene-dosage effects show that 12 regions of 31 tested reduced Drosophila α-glycerophosphate oxidase below 80%. In each of these comparisons, the other enzyme tested, succinic dehydrogenase (SDH), was near the euploid level. SDH was reduced in flies trisomic for one region in which α-GPO levels were normal. RAWLS and LUCCHESI (1974) examined autosomal segmental trisomic regions of Drosophila for activity levels of glucose-6-phosphate dehydrogenase (chromosome *1*), α-glycerophosphate dehydrogenase (chromosome *2*) and isocitrate dehydrogenase (chromosome *3*) in an effort to identify regulatory loci. One segmental trisomic region on chromosome *2* reduced G6PDH to 79% of the euploid. Five regions on chromosome *2* decreased α-glycerophosphate dehydrogenase to between 66 and 79% of the disomic control. Isocitrate dehydrogenase was increased to 126–127% by one region on chromosome *2* and one on chromosome *3*.

SMITH and CONKLIN (1975) examined eight of the 12 trisomics of Datura for four peroxidase isozyme bands in a study of the biochemical consequences of aneuploidy. One band was decreased to approximately two-thirds of the diploid level by three trisomics; two bands were decreased by four and one band decreased by five different trisomics. In addition, two different trisomics each increased one and the same band to 150% of the diploid, which they construed as representing structural loci. PIPKIN, CHAKRABARTTY and BREMNER (1977) examined fumarase activity in a set of autosomal Drosophila hyperploids similar to those of O'BRIEN and GETHMAN (1973). Their data indicate that five regions of 27 tested have hyperploid/euploid activity ratios less than 0.80. One region on the *X* chromosome, which was only temporally expressed, reduced the amount of activity to 70%.

In the absence of monosomic data, it is formally impossible to generalize that the above-mentioned cases are similar to those reported for the maize *1L*

dosage series. It would be instructive in each of the above to know the enzyme response in segmental monosomics corresponding to each trisomic. The experimental systems were not, however, readily amenable to such analysis. It is known that aneuploids often lag behind euploids in developmental time and that enzyme levels fluctuate greatly during ontogeny. One might attribute the results obtained from the trisomic studies to these types of aneuploid effects. However, in the maize case, the fact that the enzyme activities reduced in the partial trisomic are increased in the partial monosomic indicates that the deviations from the euploid level are not due to reduced vigor or slight developmental asynchrony of the aneuploids. Since both the monosomics and trisomics are less vigorous than the disomics, there is no correlation of enzyme activity with vigor—but rather a negative correlation with chromosome dosage. The studies with other organisms must be extended to determine if further parallels to the maize case are observed.

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