MODULATION OF PROTEIN LEVELS IN CHROMOSOMAL DOSAGE SERIES OF MAIZE: THE BIOCHEMICAL BASIS OF ANEUPLOID SYNDROMES

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ABSTRACT

Genetically defined dosage series of chromosome arms *1L*, *3L*, *4S*, *5L*, *7L*, *9S*, *10L* and combinations of *1L–3L*, collectively spanning approximately one-third of the maize genome, were examined for alterations in the expression of total protein profiles in scutellar tissue. The major effects found were negative correlations of specific proteins with the dosage of particular regions in a manner similar to that previously described for enzyme activity levels (BIRCHLER 1979). Chromosome arms *1L*, *4S* and *5L* produced the most severe negative effects, with *3L* and *7L* exhibiting this phenomenon to a lesser degree. Positive correlations of certain proteins were observed with the dosage of the *1L*, *3L*, *5L* and *7L* regions. The structural locus of one of the major scutellar proteins (PRO) is present in the long arm of chromosome *1* (SCHWARTZ 1979), but exhibits compensation in a dosage series involving whole-arm comparisons. Multiple factors in *1L* affect the level of the protein. The compound *TB-1La-3L4759-3* (*1L* 0.20–0.39) has a slight negative effect on PRO, while *TB-1La-3Le* (*1L* 0.20–0.58) and *TB-1La-3L5267* (*1L* 0.20–0.72) have a more pronounced negative influence. The level of this protein is not altered by the dosage of *3L*. These observations suggest that compensation is brought about by the cancellation of a positive structural gene dosage effect by the negative inverse effect. Other regions of the genome that contribute to the control of PRO levels are *4S* and *5L*. Total protein profiles were also compared in haploid, diploid and tetraploid maize as a comparison to the aneuploid series. Most proteins exhibit structural-gene-dosage effects through the ploidy series, but others show a positive effect greater than expected from varying the structural genes. Still others are negatively affected by ploidy changes. In general, the ploidy alterations are not as great as predicted from the cumulative action of the aneuploid effects. The bearing of these observations on the biochemical basis of aneuploid syndromes is discussed.

IN a study of enzyme expression in a dosage series of the long arm of chromosome one (*1L*), BIRCHLER (1979) observed that the levels of specific enzyme activities were negatively correlated with segmental chromosomal dosage. The range of the elevations in the monosomic rose to as much as 200% of the diploid

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level, and the reductions in the trisomic approached a lower limit of two-thirds. Since the enzyme levels fell within the inverse of the dosage relative to normal, the phenomenon has been termed the inverse effect.

The present study was designed to test for other dosage-sensitive regions that specifically alter the expression of proteins. Approximately one-third of the maize genome was examined. Total protein extracts of scutellar tissue were subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis to determine the number of specific proteins affected by any one region. To compare the effects of varying portions of the genome with varying the total chromosomal complement, a ploidy series involving haploid, diploid and tetraploid maize was also analyzed. Two-dimensional native-SDS electrophoretic analyses were performed to test for the unique expression of any particular polypeptide in the aneuploid and euploid series. Examination of the protein profile represents a more comprehensive approach to the understanding of the total response from varying a particular chromosomal region than is possible by testing only one or a few selected enzymes. This provides a minimum estimate of the number of effects—positive and negative—produced by a particular aneuploid series.

The present experiments involve all proteins that are in sufficient quantity to be recognized as discrete Coomassie Blue-staining bands in SDS polyacrylamide gel electrophoresis. The location of the structural loci are unknown, with the exception of Protein-1 (*Pro*), which was defined and mapped by SCHWARTZ (1979) to a region between *Adh* and bronze-2 on *1L*. It produces one of the major proteins expressed in the scutellum and serves as an example for determining the number and type of responses that are observed for any one polypeptide.

The results found in this study bear on the problem of the biochemical basis of aneuploid syndromes. It has long been recognized that both additions and subtractions of substantial chromosomal segments to or from the normal diploid complement result in less vigorous individuals (BLAKESLEE 1934; SATINA, BLAKESLEE and AVERY 1937; GOODSPEED and AVERY 1939; PATTERSON, STONE and BEDICHEK 1935, 1937). Classically, it was assumed that such effects were due to an imbalance of protein products that results from structural-gene-dosage effects (PATTERSON, BROWN, STONE 1940; MULLER 1950). However, the data presented here indicate that specific reductions, which might slow metabolism, are found in both monosomics and trisomics. On the other hand, an increase in vigor occurs in the ploidy series where the protein levels per cell are positively correlated with the number of genomic equivalents. By comparison of both the aneuploid and euploid series, there is a greater correlation of vigor with the absolute amounts of proteins than with their balance. The levels of particular proteins might be a major contributing factor to the phenotypic effects associated with each aneuploid and euploid level.

MATERIALS AND METHODS

In order to define genetically the various dosage series, it was necessary to develop a set of specific tester stocks that would allow the detection of one, two and three doses of various regions of the genome. The following is a list of the regions examined and a description of the particular

lines used. *1L*: The *Adh* allozyme markers were utilized to distinguish the dosage series. *Adh-F* and *S* female lines were crossed by males hyperploid for *TB-1La*, *1* 1^{B} B^{1} B^{1}, and homozygous for *Adh-C*. The details of these stocks and the detection of the various doses are given by BIRCHLER (1979). *3L*: By crossing *a-m-1 A2 C C2 R-scm-2* females by males hyperploid for *TB-3La*, a dosage series of the terminal 80% of *3L* included in the translocation can be distinguished. Kernels having a colorless scutellum with colored aleurone have one-dose embryos, kernels with colored aleurone and scutellum are diploid and kernels with colored scutellum and colorless aleurone have trisomic embryos. *4S*: Females homozygous for *su* were crossed by *TB-4Sa* hyperploid males. The sugary kernels have three-dose embryos, and the normal kernels have one or two. Backcross ears segregating for *Su* and *su* served as a control on any effects of the sugary endosperm on embryo protein profiles. No differences were observed. *5L*: The dosage series for *5L* was genetically distinguished by the use of an *A A2 C C2 R-sc-122 pr* tester. The determination of the dosage series was analogous to the *a* system described above except that *pr* was used as the marker locus. *7L*: The Dotted-3 locus was transferred by recombination to *TB-7Lb*. To accomplish this, hyperploid females *7* 7^{B} B^{7} B^{7} were crossed by *Dt3* males. The F_1 plants showing higher than normal pollen abortion were crossed as males onto an *a-m-1 A2 C C2 R-scm-2* tester, and kernels with *Dt3* embryos and dotless endosperm were selected as putative hyperploids marked by *Dt3*. Successive crosses to the *a-m-1* tester established the stock. *9S*: An *A A2 C C2 R-scm-2* stock was crossed as females by a stock homozygous for *TB-9Sb* marked by C^{I}. In this case, colored scutellum, colorless aleurone kernels have one-dose embryos, completely colorless kernels are diploid and colorless scutellum, colored aleurone kernels have three-dose embryos. *10L*: Females of *A A2 C C2* r^{g} constitution were crossed by hyperploid heterozygotes of *TB-10L-4* that were genetically marked by *R-scm*. Compounds *TB-1La-3L*4759-3 (*1L* 0.20–0.39; *3L* 0.20–1.00); *TB-1La-3Le* (*1L* 0.20–0.58; *3L* 0.45–1.00); *TB-1La-3L*5267 (*1L* 0.20–0.72; *3L* 0.73–1.00) and *TB-1La-3L*5242 (*1L* 0.20–0.90; *3L* 0.65–1.00), all containing the *A* locus in the distal region, were crossed as males to the *a-m-1 A2 C C2 R-scm-2* tester for production of each dosage series. Further details of the construction of these translocations can be found in BIRCHLER (1980).

The high haploid line of COE and SARKAR (1964), termed stock 6, was used to select haploids by crossing stock 6 carrying the necessary constellation of aleurone and scutellum color factors as females by males of genotype *A A2* C^{I} *C2 R*, stock 6. Since the C^{I} allele inhibits anthocyanin expression, those kernels with a purple scutellum but colorless aleurone were considered to be *1N*. The diploid comparison utilized the colorless scutellum and aleurone kernels from the same crosses. Diploid and tetraploid stocks of inbred W23 and N6 were obtained from the Maize Genetics Cooperation, Urbana, Illinois.

Kernels were genetically classified into the various dosage classes described above. Twenty to 30 dry scutella of each class were excised and ground to a meal in a Wiley Mill (mesh 20). One hundred mg of meal were extracted in 500 μl of sample buffer (0.0625 M Tris-HCl, pH 6.8, 10% glycerol, 2.3% SDS and 5% β-mercaptoethanol) at room temperature and centrifuged in an Eppendorf centrifuge, each for 15 min. The supernatant was removed with a Pasteur pipet, boiled 2 min and 10 μl were applied to the sample wells in the gel. One-dimensional polyacrylamide gel electrophoresis was by the method of LAEMMLI (1970). Extracts were first examined on 7.5% and/or 10% gels, 10 cm in length and subsequently analyzed on 8.75–15% gradient gels of 20 cm length. Gels were scanned in a Helena integrating densitometer. Two-dimensional native/ SDS gel analysis was by the method of FERL, DLOUHY and SCHWARTZ (1979). Replica extractions of each aneuploid series from 3 to 10 ears were examined in one-dimensional gels. Two-dimensional analysis were performed for each aneuploid and euploid series.

The linearity of the densitometric quantitation was tested as follows. A dilution series of bovine serum albumin from 0.01 mg/ml to 0.50 mg/ml was subjected to the preparation procedure and 20 μl (0.20 to 10 μg per channel) applied to gels for electrophoresis. After staining, the gel channels were scanned by a Helena integrating densitometer and the areas under each curve calculated. The amount of Coomassie Blue staining was linearly related to the amount of protein applied to the gel.

Protein estimates (LOWRY *et al.* 1951) per unit dry weight in each dosage of the different series did not vary by more than 8% in the monosomic or trisomic compared to the corresponding

disomic, with the exception of the compounds *1L-3L*(5267) and *1L-3L*(5242). The monosomic of *TB-1La-3L5267*(*1L* 0.72) has a mean protein level 12% greater than the diploid; the monosomic of *TB-1La-3L5242*(*1L* 0.90) has a mean protein value 20% greater than the diploid. Moreover for *1L*, *3L*, *4S*, *5L*, *7L*, *9S*, *10L* and *1L-3L*(5267), various combinations of enzyme activities were determined per mg dry weight. For each chromosomal region, at least some enzyme levels are constant through the series. These observations indicate that, by extracting identical dry weights of the three classes in each case, an equivalent number of cells has been sampled for individual protein concentration after electrophoretic separation.

The various marker loci for the dosage series are of necessity from different backgrounds. There is considerable variation in relative protein concentrations from different inbred lines, a fact that is reflected in the present study. This, however, does not affect the ability to determine whether a certain protein responds to a particular chromosomal region since such is revealed by the relative changes in the monosomic and trisomic of the segment in question. The critical observation is a change in the quantitative relationships correlated with the dosage level. The *1L-3L* compounds were constructed from the same *TB-1La* stock and then repeatedly crossed to the *a* tester. In this case, cross comparisons are possible.

RESULTS

Analyses of the protein profiles of the one-to-three series for *9S* and *10L-4* did not reveal major differences in the various dosage levels. A few of the less intensely staining polypeptides were altered in the *10L* series, exhibiting a slight negative correlation with dosage. When the long arm of chromosome 5 is varied (Figure 1), one observes elevations of particular proteins in the monosomic relative to the disomic and, in most cases, corresponding relative decreases of the same proteins in the trisomic. This is illustrated with the densitometric scans shown in Figure 2. Certain proteins of intermediate concentration and molecular weight are positively correlated with the dosage of this region. For *7L*, also shown in Figure 1, most of the proteins show constant expression with ascending dosage, but at least one major protein (marked by an arrowhead) and several less predominant ones are negatively correlated with *7L* dosage. A few of the minor polypeptides are positively correlated with *7L* dosage. A densitometric tracing is presented in Figure 3. With *4S*, the phenotypically indistinguishable one- and two-dose embryos were compared to those with three doses of this chromosome arm. The former group is weighted toward the one-dose kernels because of the high rate of nondisjunction of the *B* centromere at the second microspore division. Thus, the magnitude of any differences will be greater than when only a single dosage differential is examined. The gel shown in Figure 1 demonstrates that many of the major proteins are strongly negatively correlated with *4S* dosage. These effects, however, are clearly specific since many proteins do not vary. The densitometric measurement is presented in Figure 4. Examples of the pattern observed with *1L* are shown in Figures 5 and 8. In addition to the numerous negative effects, at least one major and some minor proteins are positively correlated with the dosage of *1L*. These effects are evident from the densitometric analysis presented in Figure 6. The gels of the *3L* series are shown in Figure 11. One of the major proteins (marked by an arrowhead) is specifically reduced in the monosomic but not elevated in the trisomic. In addition, several intermediate level proteins respond negatively to the dosage level. The densitometric tracings are shown in Figure 7.

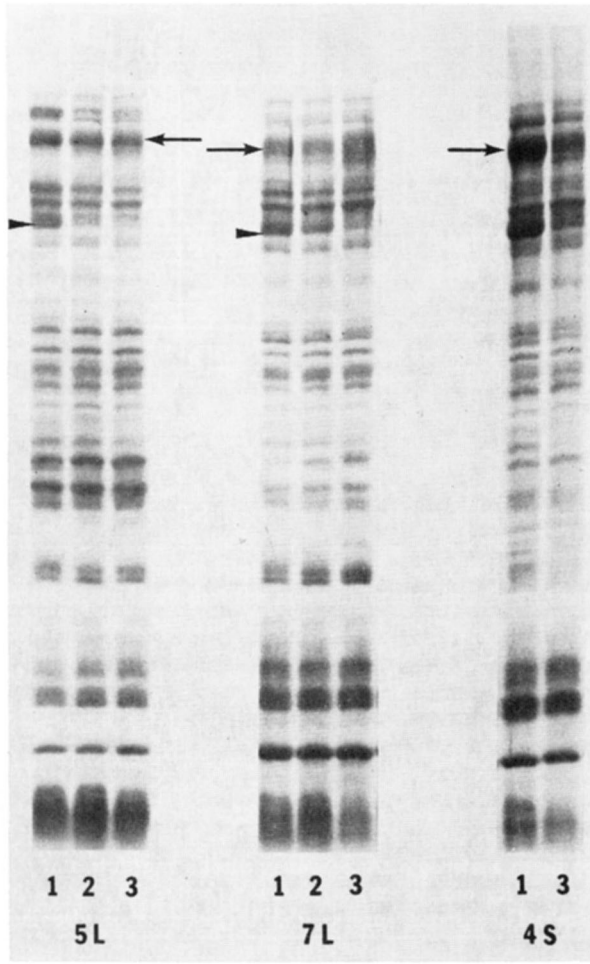

FIGURE 1.—Profiles from dosage series of *5L*, *7L* and *4S*. The chromosomal dose is indicated at the bottom of each channel. The origin is at the top. The arrows designate the PRO protein.

The genetic location of the gene encoding a major slowly migrating protein (PRO) was delineated by SCHWARTZ (1979). The locus maps between *Adh1* and *bz2* on *1L*. In most lines, two electrophoretic forms appear to be the result of a modification of a single gene product. The expression of this protein was examined in the *1L* dosage series. Figure 8 presents profiles from a cross in which the electrophoretic mobility of the products of the *Pro* alleles differs in the two parents. The maternal form has a faster migration than the one encoded by the *TB-1La* line. Therefore, the expression of each allele can be followed in all three chromosomal doses. Although there is only one copy of the maternal allele present in each case, its expression is increased in the segmental monosomic and decreased in the segmental trisomic relative to the diploid. The paternal form is present only in the diploid and the trisomic, but its expression is increased in the

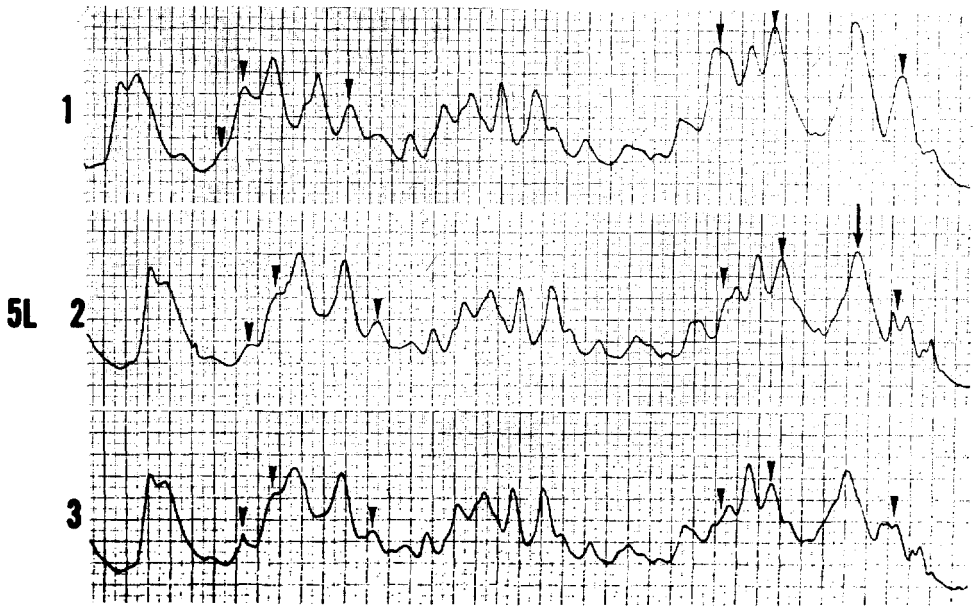

FIGURE 2.—Densitometer scan of *5L* series. The dosage is labeled along the left margin. The origin of the gel (20 cm) is to the right. The arrow designates the PRO peak. The triangular arrowheads identify examples of negative effects. The indented arrowheads identify positive responses.

latter where two doses of this allele are present (Figure 9). The densitometric integration units for the total amount of protein remain fairly constant: one dose = 2100; two doses = 1974; three doses = 1833.The slight overall negative effect observed might be explained by the apparent lower expression of the paternal allele. When material was examined in which all electrophoretic variants for PRO were identical, the total values are quite similar (see Figures 9 and 6): Thus, the amount of protein-1 present is relatively constant with 1, 2 and 3 doses of *1L*, although the numbers of structural genes present have been progressively increased. The alcohol dehydrogenase-1 (*Adh*) locus is also present in *1L* and exhibits compensation in the dosage series (BIRCHLER 1981).

The basis of this dosage compensation was studied further by examining the protein responses in a group of compound *B-A* translocations involving various lengths of *1L* and *3L*. These chromosomes have been described in detail elsewhere (BIRCHLER 1980). The compounds *TB-1La-3L4759-3* (*1L* 0.39), *TB-1La-3Le* (*1L* 0.58) and *TB-1La-3L5267*(*1L* 0.72) include increasing lengths of *1L* and decreasing lengths of *3L*. The number of proteins affected and the severity of the response increases with the length of the *1L* segment included in the dosage series. Some difficulty was encountered in assigning the chromosome arm responsible for the effects, due to the fact that *3L* as a whole produces aneuploid responses. Nevertheless, because fewer effects are found with *3L* than with *1L* and because decreasing lengths of *3L* are associated with increasing lengths of *1L*, it appears that there are multiple factors in the latter that negatively alter

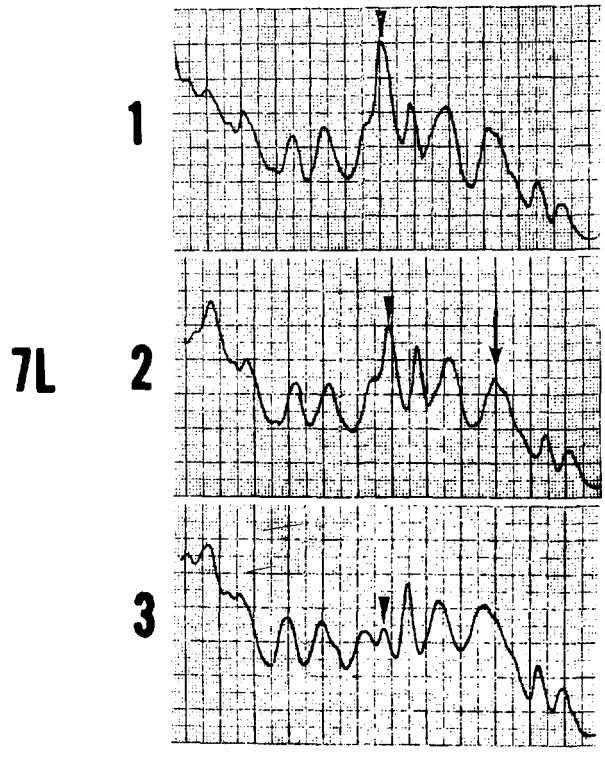

FIGURE 3.—Densitometer scan of *7L* series (10 cm gel). The arrow designates the PRO peak. The arrowheads identify the negative responses of the protein labeled in the *7L* series in Figure 1.

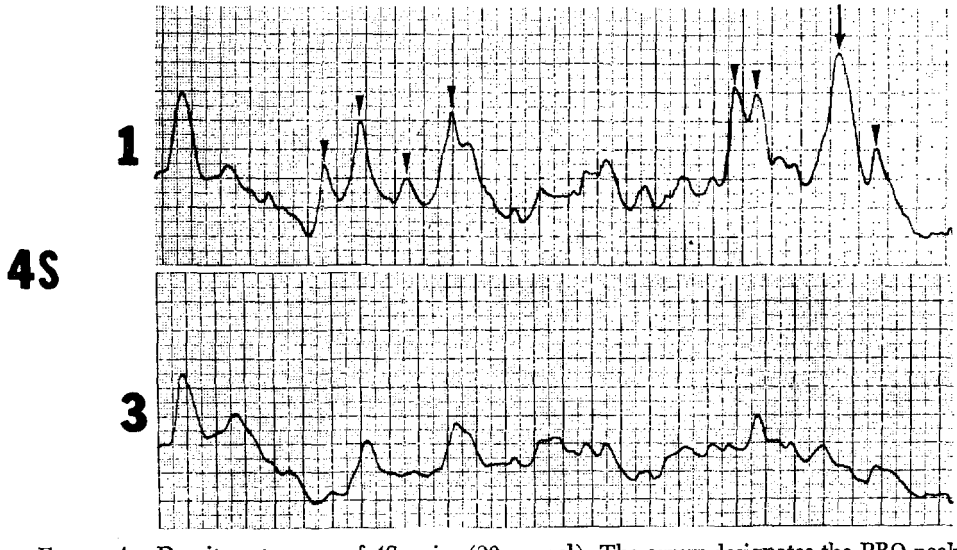

FIGURE 4.—Densitometer scan of *4S* series (20 cm gel). The arrow designates the PRO peak. The arrowheads identify examples of negative responses.

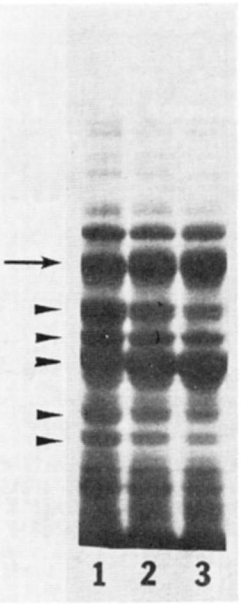

FIGURE 5.—Profiles from a *1L* dosage series (10 cm gel). The chromosomal dose is indicated at the bottom of each channel. The arrow designates the PRO protein. The arrowheads identify examples of negative responses. The indented arrowhead identifies a positive effect.

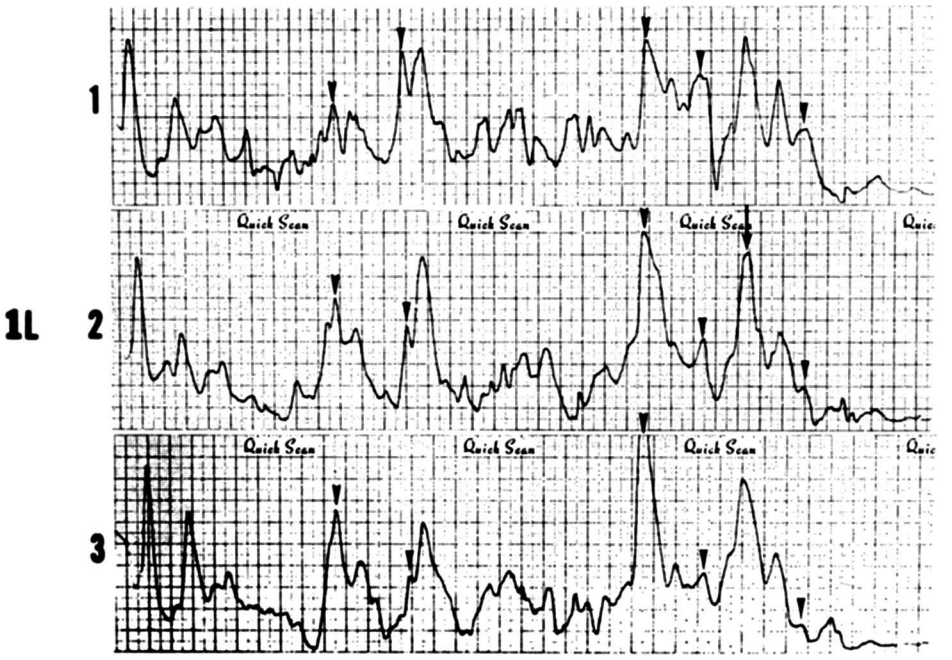

FIGURE 6.—Densitometer scan of *1L*. The dosage is labeled along the left margin. The origin of the gel (20 cm) is to the right. The arrow designates the PRO peak. The triangular arrowheads identify examples of negative responses, and the indented arrowheads identify positive effects.

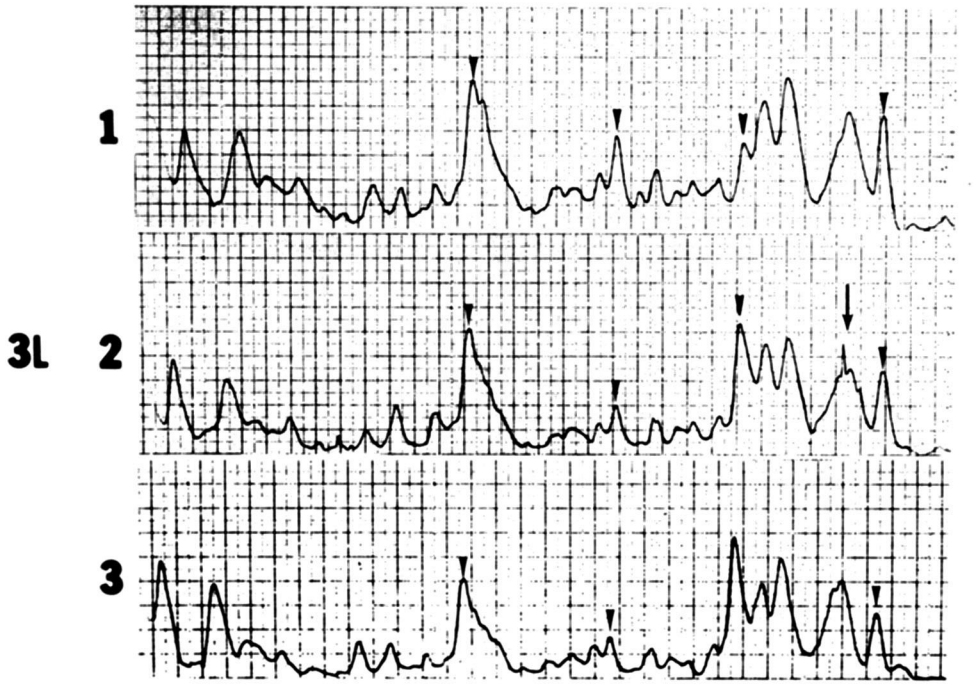

FIGURE 7.—Densitometer scan of *3L* dosage series. Chromosomal dosage is noted along the left margin. Arrow designations are as in Figure 6.

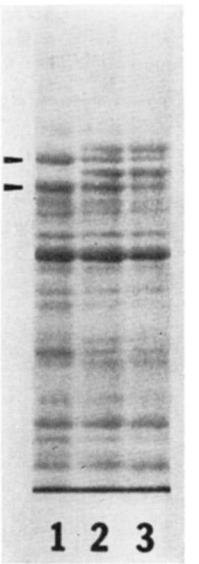

FIGURE 8.—Dosage series of *1L* in which the electrophoretic mobility of the maternal and paternal alleles of *Pro* differ. The chromosomal dosage is noted at the bottom. The two forms produced by the maternal allele are marked by arrows on the left. The paternal forms are slower in migration and are present only in the two- and three-dose embryos. The origin is at the top.

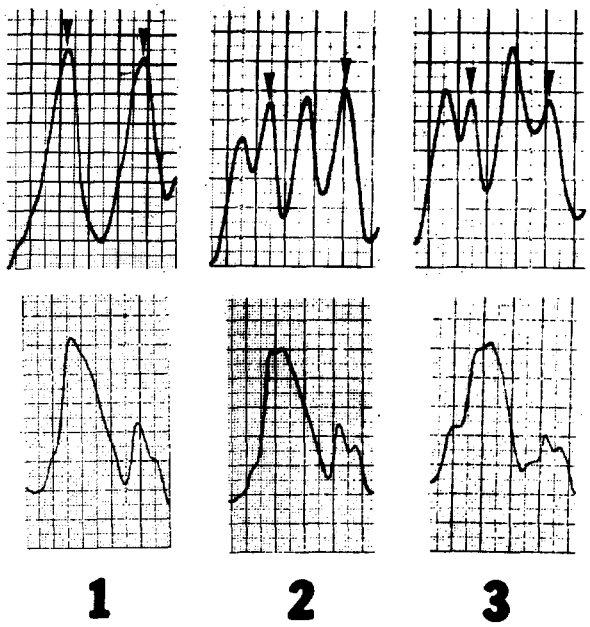

FIGURE 9.—Densitometer scan of PRO in *1L* series. Top, tracing of the PRO region from the profiles shown in Figure 8. The dosage of *1L* is noted at the bottom. The segmental monosomic has only the two maternal forms (labeled with arrowheads), while the diploid and trisomic have the two maternal and two paternal forms. Bottom, tracings from a *1L* dosage series in which the maternal and paternal alleles are of identical electrophoretic mobility. The major peak represents PRO.

the level of certain polypeptides, including PRO. The levels of PRO are negatively correlated with the dosage of *TB-1La-3L*4759-3 (*1L* 0.39), *TB-1La-3Le* (*1L* 0.58) and *TB-1La-3L*5267 (*1L* 0.72), but are not sensitive to the dosage of *3L*. When *TB-1La-3L*5242 (*1L* 0.90) was examined, the response of PRO returns to a more nearly equal expression relative to the other proteins. This translocation extends beyond the others in *1L* to include the genetic region surrounding the *Pro* structural locus (see SCHWARTZ 1979; BIRCHLER 1980; NEWTON and BIRCHLER 1980). The simultaneous inclusion of the structural gene and a modifying locus that inversely affects each copy relative to the diploid would result in the dosage compensation observed in *1L* as a whole. Densitometric scans of the *1L–3L* series are given in Figure 10 and the gel patterns in Figure 11. In addition to the factors in *1L*, the levels of PRO are also sensitive to the dosage of *5L* and *4S*.

Two-dimensional analyses were performed on each segmental dosage series and the euploid comparisons. These were conducted to test whether the protein alterations were the result of modulation or unique expression in any particular dosage. None of the proteins was observed to be lacking in any of the aneuploids. In the *5L* series, one of the major proteins appears to be unusually high in its expression in the monosomic. The two-dimensional analysis confirmed that this

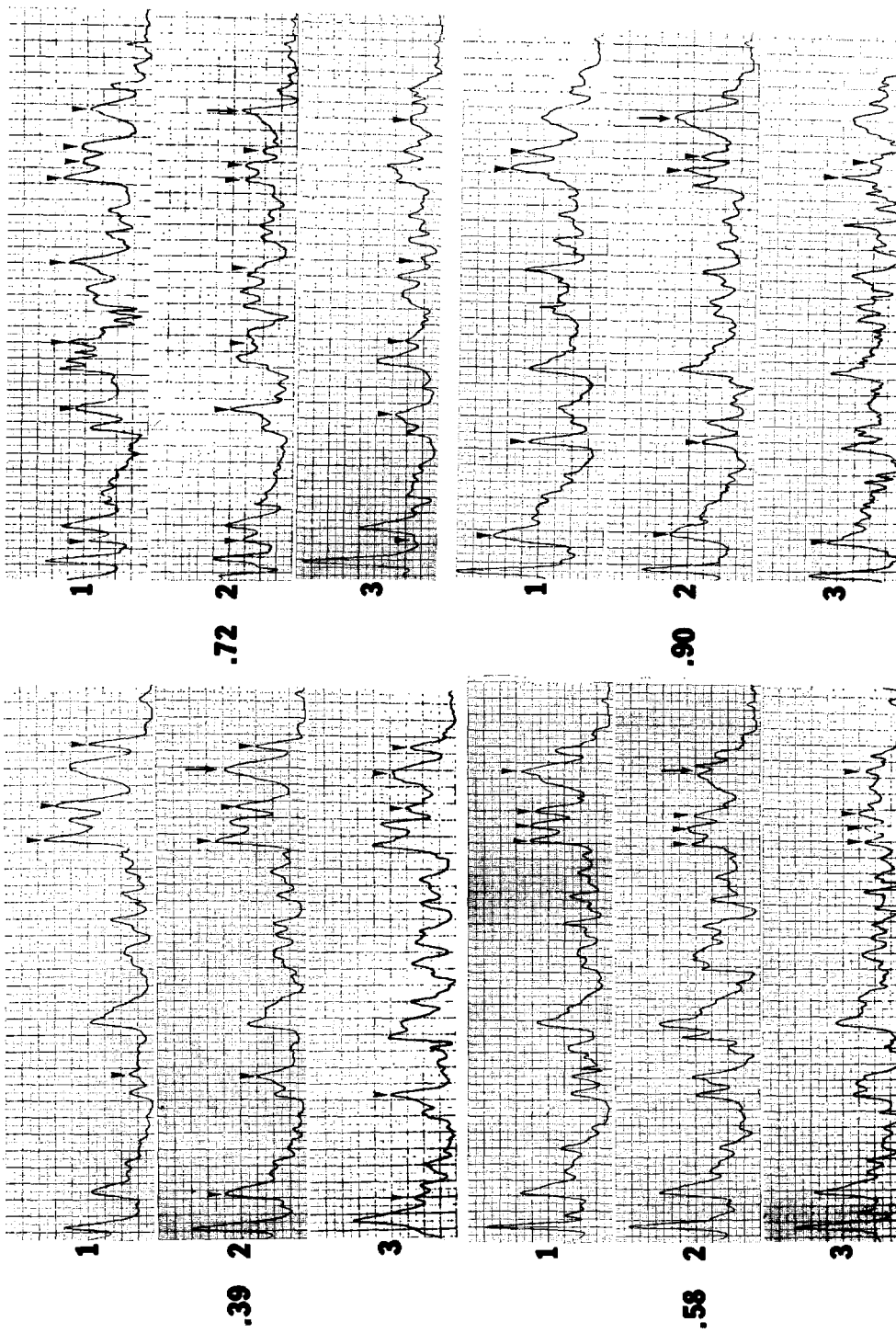

FIGURE 10.—Densitometer scans of the dosage series of the four compound *1L-3L* translocations. The *1L* breakpoint of each is noted along the left margin. The cytological composition of the four *1L-3L* chromosomes is described in MATERIALS AND METHODS. The arrow designates the PRO peak. The triangular arrowheads identify examples of negative effects and the indented arrowheads identify positive responses.

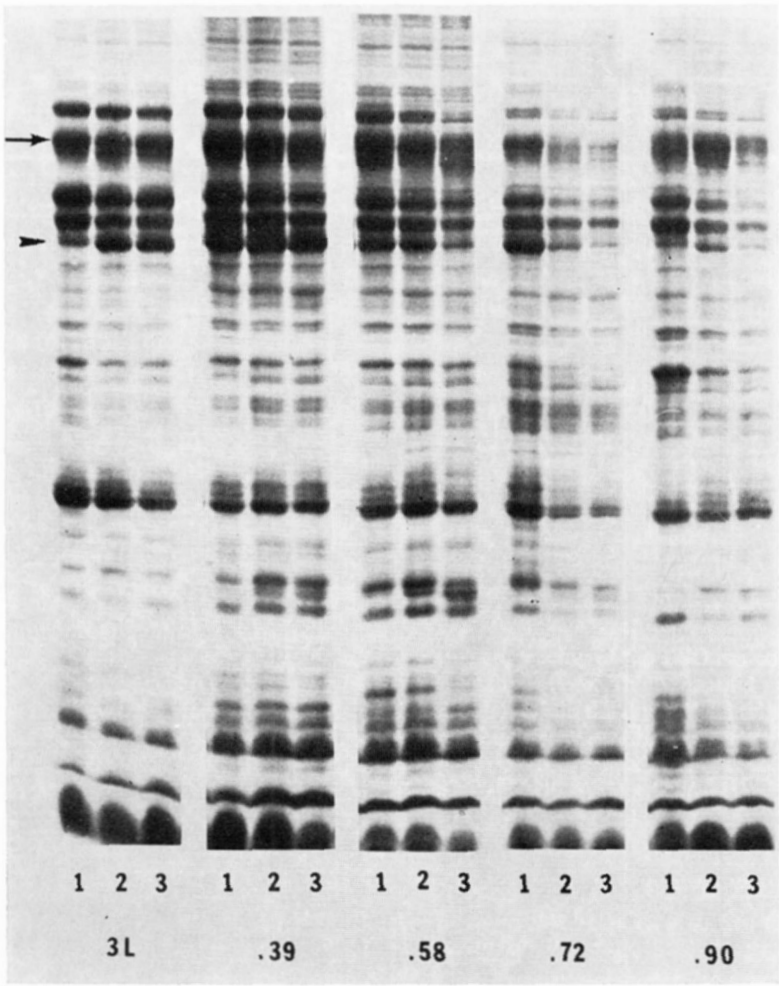

FIGURE 11.—Protein profiles of the *3L* and *1L-3L* compound dosage series. The dosage of each series is noted at the bottom of each channel. The arrow designates the PRO protein.

involved a modulation rather than a novel expression, because this protein was present in all three doses. In the *3L* series, the monosomic shows a considerable reduction for one of the major proteins (marked by an arrowhead); the two-dimensional study demonstrated this effect to also involve a modulation (Figure 12).

Haploid, diploid and tetraploid maize were examined as a comparison to the aneuploidy results. In maize, cell size is proportional to the level of ploidy (RHOADES and MCCLINTOCK 1935; RHOADES and DEMPSEY 1966). Thus, as the number of genomic complements increases, there are fewer but larger cells per mg dry mass. When similar dry weights of the haploid, diploid and tetraploid are extracted, fewer cells are sampled, but each is greater in size. If each gene were equally active and not altered in expression by the ploidy changes, then the pro-

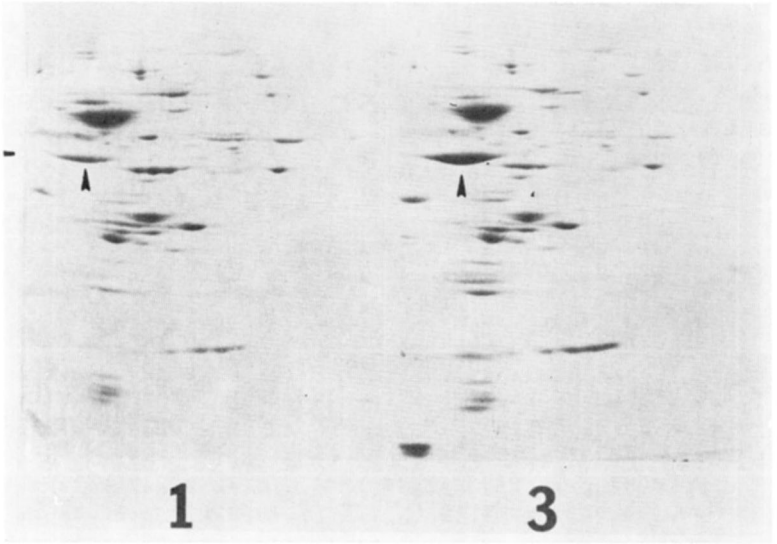

FIGURE 12.—Two-dimensional native-SDS analysis of the dosage series of *3L*. The chromosomal dosage is noted along the bottom. The native first dimension extends from left to right, the SDS dimension from top to bottom. The arrow designates the major protein that is marked in the *3L* series in Figure 11.

tein profiles from the different ploidies should be similar when the same dry weights are compared. To confirm that the cell size and number considerations were reasonably correct, the total hydrolyzable DNA levels were determined in the same lines as used in the present study (BIRCHLER 1981). The results indicate that the amounts of DNA per mg dry weight were similar in the haploid-diploid and diploid-tetraploid comparisons. Since the DNA per cell doubles in each case, there must be a corresponding decrease in the number of cells per dry weight to obtain this result. Estimates of total protein were performed on aliquots of extracts of fifty mg of meal per ml of buffer from each ploidy level. The stock 6 haploid extract had 1.54 mg/ml (mean of five extracts) and the corresponding diploid 1.53 (five extracts). The inbred W23 diploid and tetraploid were examined from four extracts each: 2*N*, 1.36 mg/ml—4*N*, 2.07 mg/ml; for inbred N6, 2*N*, 1.23 mg/ml —4*N*, 1.55 mg/ml.

As noted above, if in the ploidy series each gene is expressed in proportion to gene dosage, then the profiles would be the same. The haploid-diploid comparison revealed that many proteins exhibit a relatively similar expression in the two ploidy levels (Figures 13 and 14). This in fact indicates that these polypeptides are present at roughly half the level in the haploid cells as in the diploid. Some proteins, however, show indications of altered expression. The levels of PRO and a few others are slightly elevated in the haploid, whereas still other major proteins show reduced levels. In these cases the expression is greater or less than the gene dosage would predict. In the diploid-tetraploid study (Figures 13 and 15), most protein levels are similar, which indicates a near doubling per cell in the 4*N*.

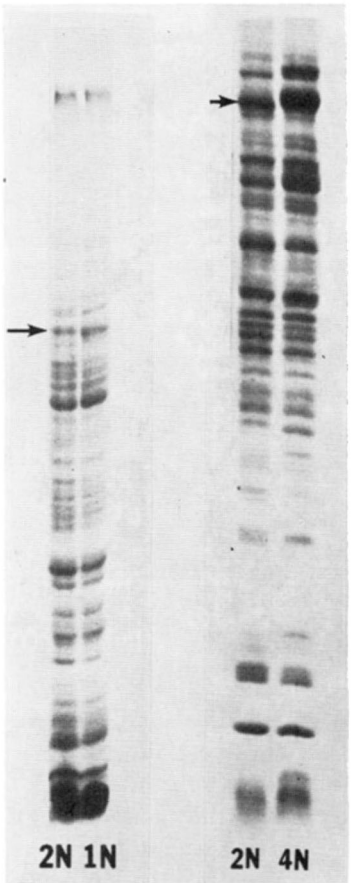

FIGURE 13.—Protein profiles from the haploid(1*N*)-diploid(2*N*) and diploid (2*N*)-tetraploid(4*N*) comparisons. The arrow designates the PRO protein.

However, several of the major proteins show a greater expression in the tetraploid, while a few are reduced. The former effect provides the basis for the greater total protein per mg dry mass described above.

It is unlikely that the haploid-diploid and diploid-tetraploid differences are the result of different electrophoretic mobilities of alternative alleles in each type. This is the case because both stocks used for haploid detection were converged to stock 6, and both 2*N*–4*N* comparisons involved tetraploid derivatives of the respective diploid inbred. While W23 and N6 showed differences, the overall effects were comparable.

DISCUSSION

One dimensional gels obviously do not resolve all of the polypeptides into discrete bands. The precise quantitation of the protein levels is not possible, but it is clear that quantitative differences are discernible and allow qualitative state-

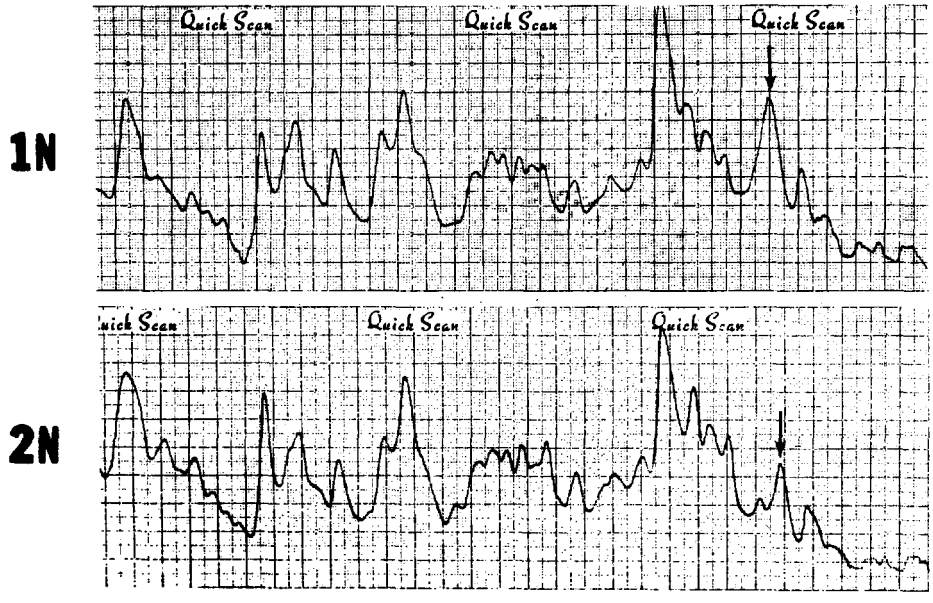

FIGURE 14.—Densitometer tracing of haploid and diploid profiles from the stock 6 inbred. The arrow designates the PRO peak.

ments concerning the positive or negative alteration in the expression of specific proteins in the various aneuploids. The positive changes might represent gene dosage effects or positive modifiers that affect the expression of unlinked genes. The negative responses appear to be similar to the inverse effect described by BIRCHLER (1979) for enzyme activities in the *1L* dosage series. The fact that there are increases in the one-dose classes and decreases in three doses indicates that the same basic phenomenon is operative. Studies designed to locate structural genes

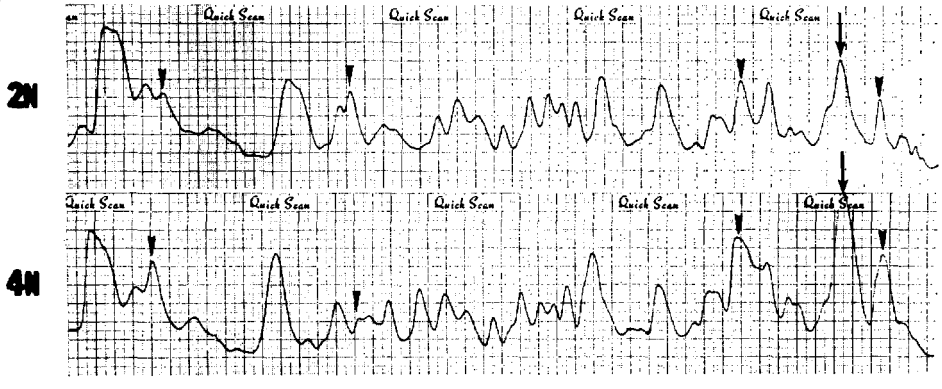

FIGURE 15.—Densitometer tracing of diploid and tetraploid profiles from the W23 inbred. The arrow designates the PRO peak. The triangular arrowheads identify examples of reductions with increasing ploidy and the idented arrowheads identify examples of positive correlations with ploidy.

via segmental trisomic effects on enzyme levels show that this response is common in aneuploids of other species as well (CARLSON 1972; DETWILER and MACINTYRE 1978; HALL and KANKEL 1976; HODGETTS 1975; MOORE and SULLIVAN 1978; O'BRIEN and GETHMAN 1973; OLIVER, HUBER and WILLIAMSON 1978; PIPKIN, CHAKRABARTTY and BREMNER 1977; RAWLS and LUCCHESI 1974; SMITH and CONKLIN 1975).

The various polypeptides are expressed to widely varying degrees in the diploid. Yet for individual proteins, the negative modifying effects of chromosomal dosage appear to fall within the limits of the inverse level relative to normal. One explanation of these observations might be that the results reflect degradative events mediated by gene products encoded in the varied region. This, however, would require that the degradative enzymes be limiting and occur at precisely the correct concentration in the diploid to yield an inverse effect in the monosomic and trisomic. Since the degradation reaction acts on the level of synthesized product, the kinetic parameters involved would not result in an inverse effect. None of the reactions could have gone to completion at any level of aneuploidy since the 2-D analyses did not reveal differences in the number of proteins present. Cellular processes that go to completion or for which the reactants are in excess in the diploid would not be detected by segmental aneuploidy. The improbability that such conditions would be found in each cell for each protein in each dosage series leads to the genetic argument that most of the effects result from action in an inverse manner on some synthetic step in the total mechanism of gene expression. Eukaryotic genes exhibit rather strict gene dosage effects (*e.g.*, GRELL 1962; SCHIMKE *et al.* 1978); thus, the primary rate limiting step in expression is at the gene level. Because of this fact, other loci could potentially be identified, *via* segmental aneuploidy, that act in *trans* to regulate this process. Since aneuploid changes might alter the efficiency of some later step or make it rate limiting, the genetic data do not allow a determination of the step involved. This, however, is inconsequential to the interpretations on dosage compensation and aneuploid syndromes, since they rest on phenomenological observations.

The two-dimensional native-SDS polyacrylamide gel analysis did not reveal any recognizable changes in the number of proteins present in any dosage series. This result indicates that the alterations in intensities are not a matter of presence or absence in any particular dosage, but rather a modulation of the individual levels. All-or-none events such as turning on or off of expression were not detected in any genetic constitution examined. It should be noted, however, that positive or negative modifiers that do not exhibit a dosage effect would not be observed in this study. The distinction between all or none and modulation, as well as between positive or negative, might be arbitrary and could potentially be manifestations of the same set of genes (see PTASHNE *et al.* 1980).

Haploid, diploid and tetraploid maize were examined to compare the effects of varying the total genomic constitution with the effects observed in the aneuploid series. Many of the protein levels are roughly correlated with the increase in ploidy, while a few are elevated or depressed. This result suggests that, in general,

the expression of these proteins is directly proportional to gene dosage. This situation has been previously observed in ploidy comparisons from several other organisms including Rattus (PRIEST and PRIEST 1969), Odontophrynus (BECAK and PUEYO 1970); Triticinae (MITRA and BHATIA 1971), Drosophila (LUCCHESI and RAWLS 1973), Todea (DEMAGGIO and LAMBRUKOS 1974), Datura (SMITH and CONKLIN 1975), Pleurodeles (AUDIT *et al.* 1976) and Ricinus (TIMKO, VASCONCELOS and FAIRBROTHERS 1980). While the protein balance, on the whole, is maintained, it should be noted that haploids and autopolyploids often differ from the respective diploid in characteristics that can not readily be attributed to mere increases or decreases of all the gene products (for examples, see PATTERSON, BROWN and STONE 1940; CHASE, 1969).

With certain proteins, the expression is not only positively correlated with the genomic level but goes beyond the response expected from a strict gene dosage effect. That is, the haploid appears to have less than half as much *per cell* as the diploid and the tetraploid appears to express more than twice the level compared to the diploid. Still other proteins are negatively correlated with the number of genomic equivalents. The diploid-tetraploid comparison should be interpreted with the knowledge that tetraploid maize is often subject to a certain degree of aneuploidy (RANDOLPH 1935).

On the biochemical basis of aneuploid syndromes

Since less than a third of the total cytological length of the genome was analyzed in the aneuploids, only a fraction of the potential number of modifying factors is evident. The preponderance of negative, as opposed to positive, responses would have predicted a completely different result from that observed in the ploidy series on the assumption of simple cumulative effects. The positive correlation of most proteins with the ploidy level is in contrast to the negative modulations found in the aneuploids.

The aneuploidy-euploidy difference parallels a similar phenomenon long known phenotypically throughout eukaryotic organisms, *i.e.*, that more extreme deviations from the diploid are observed with aneuploidy than with varied ploidy. Classically, the detrimental effects of aneuploidy were attributed to an imbalance of gene products (BLAKESLEE, 1934; PATTERSON, BROWN and STONE 1940). This idea evolved into the concept that loci present in the varied segment produced gene dosage effects for the levels of the enzymes encoded therein. Relative to the expression of genes in the unvaried regions, it was postulated that the cellular proteins were not present at the appropriate belanced levels for the normal functioning of metabolic processes.

The concept of imbalance of gene products as the basis for aneuploid depression was formulated before the extent of natural variation in enzyme levels was realized. It was believed that there was a selection for a very precise relationship of one gene product to another (MULLER 1950). It is now known, however, that considerable quantitative variation exists both at the structural loci for enzymes and at regulatory or modifying loci (*e.g.*, HUBBY and LEWONTIN 1966; SINGH, LEWONTIN and FELTON 1976; JOHNSON 1977; WARD 1975; MCDONALD and

AYALA 1978; POWELL and LICHTENFELS 1979; DICKINSON 1980a,b; LAURIE-AHLBERG *et al.* 1980). The strict balance of gene products, once thought to exist, in fact does not.

The positive and negative effects observed in aneuploids provide a molecular approach to an understanding of the phenotypic observation that aneuploidy is accompanied by a general lack of vigor. It is reported here that aneuploidy produces numerous reductions of specific proteins in both partial monosomy and trisomy and allows the interpretation that the reductions *per se* are a major factor contributing to the aneuploid phenotypes. In monosomics, the reductions are due to structural gene responses, to unlinked positive modifiers or to a combination of both. If a positive effector is simultaneously present with a structural locus for an affected enzyme, even greater reductions would result. The potential exists for the monosomic to produce much more extreme reductions than the corresponding trisomic. Phenotypically, monosomy is considerably more detrimental than trisomy—providing a further correlation of biological response to the molecular expression.

In trisomics, the reductions are the result of the numerous inverse effects present in the genome. The greater detrimental phenotypes of larger trisomics might result from more proteins coming under the influence of the increasing number of negative modifiers.

The data indicate major reductions in monosomics, trisomics and in the haploid. While the normal balance is considerably upset in the former two, the relative expression of the various proteins is reasonably maintained in the haploid, but the absolute levels per cell are decreased. Since the haploid is considerably reduced in stature and other characteristics (CHASE 1969), it appears that the correlation of vigor is more with protein level than with protein balance. Apparently the lowered levels of certain enzymes and structural components in the aneuploid and haploid become limiting on the rate of metabolism.

While the altered relative levels of enzymes or structural proteins might play a role in the detrimental effects observed with chromosomal variation, it is proposed that the major imbalance aspect of aneuploidy involves modifying or regulatory loci *per se*, which by the nature of their interaction produce the protein reductions. Since the aneuploid responses are not manifested to the same extent in the euploid changes, the differential relationship of the modifying genes is perhaps the cause of the positive and negative effects and would therefore be the indirect, but ultimate, cause of the aneuploid syndromes.

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LITERATURE CITED

AUDIT, I., P. DEPANIS, M. FLAVIN and R. ROSE, 1976 Erythrocyte enzyme activities in diploid and triploid salamanders (*Pleurodeles waltlii*) of both sexes. Biochem. Genetics **14**: 759–769.

BECAK, W. and M. T. PUEYO, 1970 Gene regulation in the polyploid amphibian *Odontophyrnus americanus*. Exp. Cell Res. **53**: 448–451.

BIRCHLER, J. A., 1979 A study of enzyme activities in a dosage series of the long arm of chromosome one in maize. Genetics **92**: 1211–1229. ——, 1980 The cytogenetic localization of the alcohol dehydrogenase-1 locus in maize. Genetics **94**: 687–700. ——, 1981 The genetic basis of dosage compensation of alcohol dehydrogenase-1 in maize. Genetics **97**: 625–637.

BLAKESLEE, A. F., 1934 New jimson weeds from old chromosomes. J. Heredity **25**: 81–108.

CARLSON, P. S., 1972 Locating genetic loci with aneuploids. Molec. Gen. Genet. **114**: 273–280.

CHASE, S. S., 1969 Monoploids and monoploid-derivatives of maize (*Zea mays* L.). Bot. Review **35**: 117–167.

COE, E. H. and K. R. SARKAR, 1964 The detection of haploids in maize. J. Heredity **55**: 231–233.

DEMAGGIO, A. E. and J. LAMBRUKOS, 1974 Polyploidy and gene dosage effects on peroxidase activity in ferns. Biochem. Genetics **12**: 429–440.

DETWILER, C. and R. MACINTYRE, 1978 A genetic and developmental analysis of an acid deoxyribonuclease in *Drosophila melanogaster*. Biochem. Genetics **16**: 1113–1134.

DICKINSON, W. J., 1980a Complex cis-acting regulatory genes demonstrated in Drosophila hybrids. Develop. Genetics **1**: 229–240. ——, 1980b Evolution of patterns of gene expression in Hawaiian picture winged Drosophila. J. of Molec. Evol **16**: 73–94.

FERL, R. J., S. R. DLOUHY and D. SCHWARTZ, 1979 Analysis of maize alcohol dehydrogenase by native-SDS two-dimensional electrophoresis and autoradiography. Molec. Gen. Genet. **169**: 7–12.

GOODSPEED, T. H. and P. AVERY, 1939 Trisomic and other types of *Nicotiana sylvestris*. J. Genetics **38**: 381–459.

GRELL, E. H., 1962 The dose effect of *ma-l*+ and *ry*+ on xanthine dehydrogenase activity in *Drosophila melanogaster*. Z. Vererbungsl. **93**: 371–377.

HALL, J. and D. R. KANKEL, 1976 Genetics of acetylcholinesterase in *D. melanogaster*. Genetics **83**: 517–533.

HODGETTS, R. B., 1975 Response of DOPA decarboxylase activity variations in gene dosage in Drosophila: A possible location of the structural gene. Genetics **79**: 45–54.

HUBBY, J. L. and R. C. LEWONTIN, 1966 A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. Genetics **54**: 577–594.

JOHNSON, G. B., 1977 Evaluation of the stepwise mutation model of electrophoretic mobility: comparison of the gel sieving behavior of alleles at the esterase-5 locus of *Drosophila pseudoobscura*. Genetics **87**: 139–157.

LAEMMLI, U. K., 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**: 680–685.

LAURIE-AHLBERG, C. C., G. MARONI, G. C. BEWLEY, J. C. LUCCHESI and B. S. WEIR, 1980 Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **77**: 1073–1077.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265–275.

LUCCHESI, J. C. and J. M. RAWLS, JR., 1973 Regulation of gene function: A comparison of enzyme activity levels in relation to gene dosage in diploids and triploids of *Drosophila melanogaster*. Biochem. Genetics **9**: 41–51.

MCDONALD, J. F. and F. J. AYALA, 1978 Genetic and biochemical basis of enzyme activity variation in natural populations. I. Alcohol dehydrogenase in *Drosophila melanogaster*. Genetics **89**: 371–388.

MITRA, R. and C. R. BHATIA, 1971 Isoenzymes and polyploidy. I. Qualitative and quantitative isoenzymes in the triticinae. Genet. Research **18**: 57–69.

MOORE, G. P. and D. T. SULLIVAN, 1978 Biochemical and genetic characterization of kynurenine formamidase from *Drosophila melanogaster*. Biochem. Genetics **16:** 619–634.

MULLER, H. J., 1950 Evidence for the precision of genetic adaptation. The Harvey Lectures, Charles C Thomas, Publ. 1947–48 Series **43:** 165–229.

NEWTON, K. J. and J. A. BIRCHLER, 1980 A further cytogenetic localization of *bz-2*. Maize Genet. Coop. Newsl. **54:** 24.

O'BRIEN, S. J. and R. C. GETHMAN, 1973 Segmental aneuploidy as a probe for structural genes in Drosophila: Mitochondrial membrane enzymes. Genetics **75:** 155–167.

OLIVER, M. J., R. E. HUBER and J. W. WILLIAMSON, 1978 Genetic and biochemical aspects of trehalase from *Drosophila melanogaster*. Biochem. Genetics **16:** 927–940.

PATTERSON, J. T., M. S. BROWN and W. S. STONE, 1940 Experimentally produced aneuploidy involving the autosomes of *Drosophila melanogaster*. Univ. Texas Publ. **4032:** 167–189.

PATTERSON, J. T., W. STONE and S. BEDICHEK, 1935 The genetics of X-hyperploid females. Genetics **20:** 259–279. ——, 1937 Further studies on X-chromosome balance in Drosophila. Genetics **22:** 407–426.

PIPKIN, S. B., P. K. CHAKRABARTTY and T. A. BREMNER, 1977 Location and regulation of *Drosophila* fumarase. J. Heredity **68:** 245–252.

POWELL, J. R. and J. M. LICHTENFELS, 1979 Population genetics of Drosophila amylase. I. Genetic control of tissue-specific expression in *D. pseudoobscura*. Genetics **92:** 603–612.

PRIEST, R. E. and J. H. PRIEST, 1969 Diploid and tetraploid colonal cells in culture: Gene ploidy and synthesis of collagen. Biochem. Genetics **3:** 371–382.

PTASHNE, M., A. JEFFREY, A. D. JOHNSON, R. MAURER, B. J. MEYER, C. O. PABO, T. M. ROBERTS and R. T .SAVER, 1980 How the λ repressor and cro work. Cell **19:** 1–11.

RANDOLPH, L. F., 1935 Cytogenetics of tetraploid maize. J. Agric. Res. **50:** 591–605.

RAWLS, J. M. and J. C. LUCCHESI, 1974 Regulation of enzyme activities in *Drosophila*. I. The detection of regulatory loci by gene dosage responses. Genet. Research **24:** 59–72.

RHOADES, M. M. and E. DEMPSEY, 1966 Induction of chromosome doubling at meiosis by the elongate gene in maize. Genetics **54:** 505–522.

RHOADES, M. M. and B. MCCLINTOCK, 1935 The cytogenetics of maize. Bot. Review **1:** 292–325.

SATINA, S., A. F. BLAKESLEE and A. G. AVERY, 1937 Balanced and unbalanced haploids in *Datura*. J. Heredity **28:** 193–202.

SCHIMKE, R. T., R. J. KAUFMAN, F. W. ALT and R. F. KELLEMS, 1978 Gene amplification and drug resistance in cultured murine cells. Science **202:** 1051–1055.

SCHWARTZ, D., 1979 Analysis of the size alleles of the *Pro* genes in maize—evidence for a mutant protein processor. Molec. Gen. Genetics **174:** 233–240.

SINGH, R. S., R. C. LEWONTIN and A. A. FELTON, 1976 Genetic heterogeneity within electrophoretic "alleles" of xanthine dehydrogenase in *Drosophila pseudoobscura*. Genetics **84:** 609–629.

SMITH, H. H. and M. E. CONKLIN, 1975 Effects of gene dosage on peroxidase isozymes in *Datura stramonium* trisomics. pp. 603–618. In: *Isozymes*, Vol. 3: *Developmental Biology*. Edited by C. L. MARKET. Academic Press, New York.

TIMKO, M. P., A. C. VASCONCELOS and D. E. FAIRBROTHERS, 1980 Euploidy in *Ricinus*. I. Euploidy and gene dosage effects on cellular proteins. Biochem. Genetics **18:** 171–183.

WARD, R. D., 1975 Alcohol dehydrogenase activity in *Drosophila melanogaster*: a quantitative character. Genet. Research **26:** 81–93.

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