Dosage Effects on Gene Expression in a Maize Ploidy Series

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Manuscript received August 24, 1995
Accepted for publication December 22, 1995

ABSTRACT

Previous studies on gene expression in aneuploids revealed numerous trans-acting dosage effects. Segmental aneuploidy of each varied chromosomal region exhibited predominantly inverse effects on several target genes. Here, dosage regulation was examined in a maize (Zea mays L.) ploidy series where the complete genomic complement is varied. Total RNA from leaf tissue of monoploid, diploid, triploid, and tetraploid plants (1X, 2X, 3X, and 4X, respectively) was analyzed for the expression of 18 genes. For most tested genes, the transcript level per cell is directly proportional to structural gene dosage; that is, on a per genome basis, there is approximately equal expression among the four ploidies. Exceptional cases show a negative correlation of expression with ploidy or a positive correlation greater than expected from the structural gene dosage. These studies suggest that, in general, as structural gene dosage increases in multiples of the monoploid complement, the absolute level of gene expression per cell decreases. In contrast, addition or subtraction of only a single chromosome arm tends to alter gene expression patterns extensively. The combined results of the euploid and aneuploid studies suggest that aneuploid effects result from an altered stoichiometry of the factors contributing to the mechanisms of gene expression.

The gene dosage changes affecting structural gene expression have been studied in several species including human, mouse, Drosophila and plants (BIRCHLER 1979, 1981; BIRCHLER and NEWTON 1981; WHATLEY et al. 1984; EPSTEIN 1986; REICHERT 1986; DEVLIN et al. 1988; BIRCHLER et al. 1990; GUO and BIRCHLER 1994). Changes in dosage include an aneuploid series in which a chromosome or chromosomal segment is added or subtracted relative to the genomic complement or a ploidy series where a complete genomic increment is increased or decreased. When the dosage of a structural gene is varied, typically two types of dosage regulation on its expression are observed. One is a gene dosage effect where there are increases or decreases in expression in proportion to the gene copy number. Gene dosage effects are often observed (e.g., GRELL 1962; EPSTEIN 1986). This is particularly the case when the aneuploid segments surrounding the structural gene are small (BIRCHLER 1981). The other type of dosage regulation is called dosage compensation in which there is an equal expression regardless of the copy number of the respective gene in aneuploids involving significant cytological length. Studies of such dosage series from various organisms, especially Drosophila and plants, reveal that gene expression is often dosage compensated (BIRCHLER 1979; BIRCHLER and NEWTON 1981). This phenomenon is found with X chromosome linked genes, as well as genes encoded on the autosomes of Drosophila (MULLER 1932; DEVLIN et al. 1982; BIRCHLER et al. 1990).

In addition, when the dosage of a chromosomal segment is varied, trans-acting effects, either positive (direct) or negative (inverse), on genes elsewhere in the genome have been observed. We previously studied gene dosage effects of maize aneuploids using B-A translocations to add or subtract single chromosome arms relative to normal (GUO and BIRCHLER 1994). Expression patterns of six genes were examined in a dosage series of 14 chromosomal segments. Transcript levels of each tested gene were affected by multiple chromosomal regions. Each varied chromosomal region showed trans-effects on several genes. Such modulations are predominantly inverse (negative) effects on gene expression. When the dosage of a trans-acting inverse regulator of a target gene is varied simultaneously with the structural gene, the dosage effect of the latter is cancelled by the former, resulting in the compensation described above (BIRCHLER 1981; GUO and BIRCHLER 1994).

Because of the predominant lethality of ploidy changes in animal systems, most of the studies on dosage regulation of ploidy series have been conducted on plants, although a few studies on yeast, Drosophila and animal cell lines have been reported (CIFFERI et al. 1969; PRIEST and PRIEST 1969; LUCCHESE and RAWLS 1973). Among isozymes and proteins examined in various plant species, some are positively or negatively affected by ploidy changes, while most of them exhibit an increased level as the ploidy level rises, that is, a gene dosage effect (DEMACCIO and LAMBRUKOS 1974; LEVIN et al. 1979; TIMKO et al. 1980; BIRCHLER and NEWTON 1981). Morphologically documented effects with increased ploidy in plants include increased cell size and organ size while the vigor decreases beyond the tetraploid level (RHODES 1982).
and McClintock 1935; Blakeslee 1941; Rhodes and Dempsey 1966). No previous studies have examined specific RNA levels in a ploidy series.

In the present study, we investigated gene dosage effects further by varying the ploidy, where genomic balance is maintained as compared with aneuploids. We sought to test whether the aneuploid effects were cumulative, in principle, or whether a “balance” was operating as suggested by classical phenotypic observations. The transcript levels of 18 genes expressed in leaf tissue were analyzed in monoploid, diploid, triploid and tetraploid maize plants. Most genes exhibited a gene dosage effect relative to the ploidy level, although interesting exceptions occurred in which a strong positive correlation or a negative correlation occurred between gene expression and ploidy.

MATERIALS AND METHODS

Genetic stocks and production of a ploidy series: The diploid (Oh 40B) and tetraploid (Alexander synthetic B) stocks were obtained from the Maize Genetics Cooperation. The 1X–4X ploidy series was generated as follows: The monoploid was produced by using the indeterminate gametophyte (ig) mutation, which causes the production of androgenetic haploids when used as a female parent (Kermicle 1969). The ig stock is homozygous for R-nj, which conditions anthocyanin development in the anther, endosperm and embryo. This line was crossed to the Oh 40B/elongate (el) diploid male (see below). The androgenetic haploid was selected based on the lack of pigmentation in the embryo. Triploids were produced by crossing the tetraploid with the diploid. Each ploidy was confirmed by cytological examination of the chromosome number in root tips.

The homozygous elongate female produces normal haploid as well as diploid gametes (Rhoades and Dempsey 1966). The el stock was crossed as a female parent with the Oh 40B diploid. Normal (diploid embryos) as well as defective kernels (triploid embryos) are produced. The normal kernels in the cross were grown and selfed. Females grown from the progeny were crossed with the Oh 40B/elongate (el) diploid male (see below). The androgenetic haploid was selected based on the lack of pigmentation in the embryo. Triploids were produced by crossing the tetraploid with the diploid. Each ploidy was confirmed by cytological examination of the chromosome number in root tips.

For each gene, triplicate loadings of each dosage series were made on each gel. After probing with each specific gene, the blots were washed at 50°C for 15 min and then hybridized with 32P labeled antisense RNA of the respective genes. The hybridization conditions were 60°C in 5X SSC (1X SSC consists of 0.15 M NaCl, 0.015 M sodium citrate), 50% formamide, 10× Denhardt’s, 0.5% SDS, 10× dextran sulfate and 0.2 mg/ml salmon sperm DNA, for 16 h. The blots were washed at 75°C with 0.2X SSC and 0.05% SDS (Bircher and Hiebert 1989).

Data analysis: The radioactivity was quantitated using a Fuji Phosphorimagger. The specific RNA quantity of each lane was normalized by calculating the ratio of mRNA/rRNA of the phosphorimagger readings. The means and standard errors of the respective normalized values from each ploidy were calculated from three replicates. The mRNA levels of each ploidy relative to that of the diploid were determined by taking the ratios of the respective mean values of three replicates: monoploid/diploid, triploid/diploid and tetraploid/diploid, respectively. Such ratios represent mRNA levels relative to that of the diploid on a per genome basis because each genome level. Therefore it appears that in this background both pentaploids and hexaploids fail to germinate or survive beyond the early seedling stage. Plants of the ploidy series were transferred from the greenhouse to the field after cytological confirmation by root tip chromosome counts. At the six-leaf stage the younger four leaves of each plant were harvested. The tissue was frozen in liquid nitrogen and stored at −80°C until use.

RNA isolation: Leaf tissue from single plants of each ploidy was used for RNA preparations. Total RNA was extracted using a modified method of Cone et al. (1986). Tissue was ground to a fine powder in liquid nitrogen with a mortar and pestle. Cells were lysed in 2.5–3.0 volumes of buffer containing 0.1 m NaCl, 50 mM Tris-HCl (pH 7.4), 50 mM disodium ethylenediaminetetraacetic acid (EDTA), 2% sodium dodecyl sulfate (SDS) and protease K (200 μg/ml). The supernatant was extracted twice with an equal volume of phenol/chloroform/isoamyl alcohol (100:98:1) and twice with chloroform/isoamyl alcohol (99:1). Total RNA was precipitated in a final concentration of 2 m LiCl solution and reprecipitated with 2.5 volumes of absolute ethanol and one-tenth volume of 3.0 m sodium acetate (pH 5.0).

Probe sources and Northern analysis: Except for the three well-characterized genes, Alcohol dehydrogenase 1 (Adh1), Alcohol dehydrogenase 2 (Adh2) and Sucrose synthase (Sus1), those examined were mapped isolates derived from a maize leaf cDNA library and were provided by the University of Missouri, Columbia (UMC) Maize RFLP laboratory. All clones for probe synthesis were in or subcloned into the Bluescript vector (Stratagene), which contains T3 and T7 promoters. Antisense RNA probes were prepared by in vitro transcription of the respective genes in such constructs using either the T3 or T7 promoter. Five micrograms of total RNA from each sample was loaded in each lane and subjected to electrophoresis in 1.5% formaldehyde–agarose gels. RNA was then capillary transferred to Biotrans nylon membrane (ICN) and cross linked by UV irradiation. Blots were prehydrized for 6 hr and then hybridized with 32P labeled antisense RNA of the respective genes. The hybridization conditions were 60°C in 5X SSC (1X SSC consists of 0.15 M NaCl, 0.015 M sodium citrate), 50% formamide, 10× Denhardt’s, 0.5% SDS, 10× dextran sulfate and 0.2 mg/ml salmon sperm DNA, for 16 h. The blots were washed at 75°C with 0.2X SSC and 0.05% SDS (Bircher and Hiebert 1989).

For each gene, triplicate loadings of each dosage series were made on each gel. After probing with each specific gene, the blots were reprobed with antisense ribosomal RNA (rRNA) as a measure of the loading of total RNA. To test that rRNA is a valid loading control, i.e., that constant rRNA level is produced per genome among the ploidy series, total nucleic acid isolated from leaf tissue was separated on an agarose gel and stained with ethidium bromide. Negatives of film (type 55, Polaroid) were prepared, and the DNA and RNA bands were scanned with a laser densitometer. DNA/rRNA ratios were then calculated according to the densitometric readings. No evidence of variation was found among the ploidy series.

Data analysis: The radioactivity was quantitated using a Fuji Phosphorimagger. The specific RNA quantity of each lane was normalized by calculating the ratio of mRNA/rRNA of the phosphorimagger readings. The means and standard errors of the respective normalized values from each ploidy were calculated from three replicates. The mRNA levels of each ploidy relative to that of the diploid were determined by taking the ratios of the respective mean values of three replicates: monoploid/diploid, triploid/diploid and tetraploid/diploid, respectively. Such ratios represent mRNA levels relative to that of the diploid on a per genome basis because RNA/DNA ratios are constant among the ploidy series. The values on a per cell basis were calculated by multiplying the per genome values by the respective genomic ratios of each ploidy.
to diploid: i.e., 0.5 for monoploid, 1.0 for diploid, 1.5 for triplold and 2.0 for tetraploid. Using csu16 as an example, the per genome values from monoploid to tetraploid are 0.97, 1.00, 1.01 and 1.00, respectively; their per cell values are 0.49 (0.97 × 0.5), 1.00 (1.00 × 1.00), 1.52 (1.01 × 1.5) and 2.00 (1.00 × 2.00), respectively.

RESULTS

Numerous dosage effects were found in the previous study of aneuploid dosage series of 14 chromosomal segments (Guo and Birchler 1994). This work led us to investigate dosage regulation of gene expression in a euploid series, consisting of monoploid, diploid, triploid and tetraploid maize plants to determine whether such effects are additive or nonadditive in general when the collective regions of the genome are varied together. Northern analyses were used to determine the transcript levels of 18 different genes including 15 random cDNA clones from maize leaf tissue, as well as Adhl, Adh2 and Sus1. To confirm that ribosomal RNA could be used to standardize equal loading of total RNA on Northern blots, total nucleic acid from the ploidy series was quantified and compared. The ratios of rRNA/DNA were calculated and the following values were obtained for each ploidy: 0.97 (monoploid), 1.04 (diploid), 0.95 (triploid) and 1.08 (tetraploid). These results indicate that approximately equal RNA levels are present per genome among the ploidy series. Therefore, rRNA could be used as a valid internal loading control.

Values from the Northern analysis (see Table 1) are expressed as relative transcript levels of each gene from each ploidy to that of the diploid and are normalized to a per genome basis as well as a per cell basis. If a gene is expressed in proportion to its dosage, an equal expression value will be measured per genome regardless of the ploidy level. To obtain the per cell level, the expression in each ploidy was multiplied by the number of genomes present in a cell, that is, 1X, 2X, 3X and 4X in monoploid, diploid, triploid and tetraploid, respectively (because the value of the diploid is standardized as 1.00, the per cell values of each ploidy were calculated in proportion to the diploid; see MATERIALS AND METHODS). Results from the study indicate that the transcript level per genome of most genes is approximately equal among different ploidies or, on a per cell basis, the transcript level of these genes increases proportionally with increasing ploidy. Thus, most genes display gene dosage effects, although variations are observed with some of the clones. A Northern blot probed with csu 16, a clone encoding a protein with homology to NADP malic enzyme, is presented in Figure 1 as an example of the gene dosage effect of ploidy. Because approximately equal amounts of total RNA were loaded and rRNA/DNA ratios are constant among ploidies, the mRNA levels on Northern blots represent a per genome measure. The predominant gene dosage effect observed with the ploidy series is in contrast to what was found in the aneuploid dosage series, where the expression of most genes located on the varied chromosome arm was dosage compensated. Moreover, the multitude of trans-acting aneuploid effects does not appear to be cumulative.

In contrast to the standard dosage effects, there were several interesting exceptions among the 18 genes analyzed. The first one is csu 3, a gene with homology to thiol protease, on chromosome 7. The transcript level of csu 5 decreases as the ploidy increases (Table 1 and Figure 1). If the reduction is inversely proportional to the number of genomes, on a per cell level, taking the diploid as 100%, the predicted value of the monoploid will be a twofold increase (200%, inverse of 1/2), whereas in the triploid and tetraploid, it would approach 67% (inverse of 3/2) and 50% (inverse of 4/2), respectively. However, in the higher ploidies, the actual decrease is more extreme with the relative levels at 48 and 28% of the diploid, respectively. In other words, the reduction is more than that expected from an inverse effect due to the increasing ploidy level.

Another exceptional pattern was exhibited by csu 31, which is on chromosome 8. There is a striking increase in the amount of mRNA from 1X to 4X. The increased messenger RNA level per genome with increasing ploidy was 68% (1X), 100% (2X), 251% (3X) and 214% (4X), or per cell values of 54% (1X), 100% (2X), 377% (3X) and 428% (4X). These levels exceed those predicted from a direct gene dosage effect (Figure 1 and Table 1). A direct gene dosage effect will give an equal transcript level per genome among ploidies, or per cell values of 50% for monoploid, 100% for diploid, 150% for triploid and 200% for tetraploid.

A third exceptional expression pattern was exhibited by Sus1, which is encoded on chromosome 9. The messenger RNA level of Sus1 exhibits a curious odd/even effect as illustrated in Table 1 and Figure 1. That is, in the monoploid and triploid the transcript levels increase dramatically relative to the diploid but the mRNA levels of the diploid and tetraploid are not significantly different from each other. The magnitude of the increase is so extreme that there is a nearly three fold higher mRNA level per genome in the monoploid and 6.7-fold higher in the triploid as compared with the diploid and tetraploid. Although less dramatic, two other genes, csu 28 (unmapped) and csu 116 (chromosome 6), also display similar patterns of the odd/even effect (Table 1). The transcript level of csu 28 has a 1.36-fold increase in the monoploid and a 2.44-fold increase in the triploid as compared with that of the diploid; csu 116 expresses 1.41-fold higher mRNA level in the monoploid and twofold higher in the triploid as compared with the diploid.

DISCUSSION

For gene dosage studies it is desirable to have a uniform genetic background through the dosage series. As
TABLE 1
The mRNA levels of 18 genes expressed in leaf tissue of four ploidies relative to the diploid

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ploidy</th>
<th>Mean</th>
<th>SE</th>
<th>Ploidy</th>
<th>Mean</th>
<th>SE</th>
<th>Ploidy</th>
<th>Mean</th>
<th>SE</th>
</tr>
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<tr>
<td>Thiol protease</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>csu5 (7)</td>
<td></td>
<td>2.00</td>
<td>0.05 (1.00)</td>
<td>1 0.97</td>
<td>0.17 (0.49)</td>
<td>1 0.84</td>
<td>0.13 (0.42)</td>
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<td></td>
</tr>
<tr>
<td>NADP malic enzyme</td>
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<td>1.00</td>
<td>0.04 (1.00)</td>
<td>2 1.00</td>
<td>0.16 (1.00)</td>
<td>2 1.00</td>
<td>0.12 (1.00)</td>
<td></td>
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<tr>
<td>31k ribonucleoprotein</td>
<td></td>
<td>0.32</td>
<td>0.00 (0.48)</td>
<td>3 1.01</td>
<td>0.12 (1.52)</td>
<td>3 1.29</td>
<td>0.14 (1.94)</td>
<td>4 0.13</td>
<td>0.09 (2.06)</td>
</tr>
<tr>
<td>ATP/ADP translocator</td>
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<td>0.14</td>
<td>0.02 (0.28)</td>
<td>4 1.00</td>
<td>0.10 (2.00)</td>
<td>4 1.03</td>
<td>0.09 (2.06)</td>
<td>4 1.03</td>
<td>0.09 (2.06)</td>
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<tr>
<td>csu26 (5)</td>
<td></td>
<td>1.10</td>
<td>0.02 (0.55)</td>
<td>1 1.36</td>
<td>0.02 (0.68)</td>
<td>1 1.44</td>
<td>0.03 (0.72)</td>
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<td></td>
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<tr>
<td>Ribosomal protein S22</td>
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<td>1.56</td>
<td>0.03 (3.24)</td>
<td>2 1.00</td>
<td>0.01 (1.00)</td>
<td>2 1.00</td>
<td>0.01 (1.00)</td>
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<tr>
<td>Vacuolar ATPase proteolipid subunit</td>
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<td>1.25</td>
<td>0.01 (2.50)</td>
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<td></td>
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<td>csu31 (8)</td>
<td></td>
<td>0.68</td>
<td>0.01 (0.34)</td>
<td>1 1.47</td>
<td>0.02 (0.74)</td>
<td>1 1.19</td>
<td>0.02 (0.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>2.00</td>
<td>0.03 (1.00)</td>
<td>2 1.00</td>
<td>0.03 (1.00)</td>
<td>2 1.00</td>
<td>0.01 (1.00)</td>
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<td></td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td></td>
<td>2.51</td>
<td>0.08 (3.77)</td>
<td>3 1.24</td>
<td>0.06 (1.86)</td>
<td>3 1.23</td>
<td>0.02 (1.85)</td>
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<tr>
<td>csu91-1 (4)</td>
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<td>2.14</td>
<td>0.06 (4.28)</td>
<td>4 1.52</td>
<td>0.04 (3.04)</td>
<td>4 1.02</td>
<td>0.01 (2.04)</td>
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</tr>
<tr>
<td>Unknown</td>
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<td>1.99</td>
<td>0.05 (0.50)</td>
<td>1 1.65</td>
<td>0.35 (0.83)</td>
<td>1 1.23</td>
<td>0.05 (0.62)</td>
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<tr>
<td>csu92 (5)</td>
<td></td>
<td>1.00</td>
<td>0.01 (1.00)</td>
<td>2 1.00</td>
<td>0.22 (1.00)</td>
<td>2 1.00</td>
<td>0.03 (1.00)</td>
<td></td>
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<tr>
<td>Elongation factor la</td>
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<td>1.34</td>
<td>0.01 (2.01)</td>
<td>3 1.26</td>
<td>0.29 (1.89)</td>
<td>3 0.96</td>
<td>0.03 (1.44)</td>
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<td>1.11</td>
<td>0.03 (2.22)</td>
<td>4 0.81</td>
<td>0.20 (1.62)</td>
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<td>0.03 (2.26)</td>
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<tr>
<td>csu116 (6)</td>
<td></td>
<td>1.41</td>
<td>0.04 (0.71)</td>
<td>1 0.92</td>
<td>0.04 (0.46)</td>
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<td>0.05 (0.62)</td>
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<tr>
<td>Alcohol dehydrogenase 1</td>
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<td>1.00</td>
<td>0.01 (1.00)</td>
<td>2 1.00</td>
<td>0.06 (1.00)</td>
<td>2 1.00</td>
<td>0.01 (1.00)</td>
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<tr>
<td>MADS box gene</td>
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<td>2.01</td>
<td>0.03 (3.02)</td>
<td>3 1.45</td>
<td>0.07 (2.18)</td>
<td>3 1.43</td>
<td>0.01 (2.15)</td>
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<tr>
<td>csu137 (5)</td>
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<td>1.46</td>
<td>0.02 (2.92)</td>
<td>4 0.69</td>
<td>0.02 (1.38)</td>
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<td>Alcohol dehydrogenase 2</td>
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<td>1.40</td>
<td>0.16 (0.70)</td>
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<td>0.14 (0.47)</td>
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<td>0.64 (1.47)</td>
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<tr>
<td>Sucrose synthase 1</td>
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<td>0.10 (1.00)</td>
<td>2 1.00</td>
<td>0.15 (1.00)</td>
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<td>0.05 (1.00)</td>
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<tr>
<td></td>
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<td>0.75</td>
<td>0.06 (1.13)</td>
<td>3 0.93</td>
<td>0.15 (1.40)</td>
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<td>0.15 (2.30)</td>
<td>4 0.87</td>
<td>0.03 (1.74)</td>
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</table>

Mean values and SE are presented on a per genome basis with the corresponding per cell levels in parentheses.
*Indicates the chromosome where each gene is mapped.
**Ploidy 1, 2, 3, and 4 refers to monoploid, diploid, triploid, and tetraploid, respectively.
**Mean is an average value of the relative mRNA levels from three replicates. Mean values that are in bold face are significantly (P < 0.01 in t tests) different from that of diploids.
#SE, Standard error of three replicates.

we were unsuccessful in generating diploid plants with the Alexander synthetic B stock crossed onto zg females, a modified approach was taken (see MATERIALS AND METHODS). The monoploid and diploid are derived from the Oh 40B/el stock. The tetraploid is derived from a selfed progeny from crosses of Oh 40B/el × Alexander synthetic B tetraploid. Therefore, the monoploid and diploid are derived from the same genetic stock, which is partially related to the tetraploid. If this potential heterogeneity affected expression patterns, it would be found as pronounced differences between the monoploid/diploid lines and the triploid/tetraploid lines. No such pattern is obvious in the data. Therefore, we do not believe that the minor differences in background have a profound effect on the major results, but one should consider this situation in interpretation of differences.
It should also be noted that there is a certain degree of aneuploidy in triploid and tetraploid maize (RANDOLPH 1935). That is, a tetraploid plant often consists of more or less than exactly 40 chromosomes. The plants used in this study were cytologically confirmed as exact multiples of the monoploid number. However, a tetraploid with 40 chromosomes may not have four sets of each chromosome; instead, it may have, for example, four sets of chromosomes 1–8 plus three chromosomes 10 and five chromosomes 9 (8 IV+III+V). Such aneuploidy of triploid and tetraploid plants might also contribute to minor deviations from the expected gene dosage effect.

Results from this study demonstrate that increasing gene dosage from one to four via a ploidy series increases the expression of most genes proportionally. Polyploids in numerous plant species have shown higher levels of enzyme activity or proteins than diploids (DE-MAGGIO and LAMBRUKOS 1974; NAKAI 1977; LEVIN et al. 1979; TIMKO et al. 1980). Similar observations were also made with yeast (CIFERRI et al. 1969) and Drosophila (LUCCHESI and RAWLS 1975). In the fibroblast-like cell lines of rat, the rate of collagen synthesis was correlated with the ploidy (PRIEST and PRIEST 1969). Although exceptions exist, such direct correlations of gene expression with the copy number of the genome in a ploidy series are the predominant situation.

In contrast, results from our earlier studies on the transcript levels of six genes examined in an aneuploid dosage series of 14 chromosomal segments of maize revealed a high level of modulation (GUO and BIRCHLER 1994). For instance, in the case of Adhl and Adh2, within the ploidy dosage series, no significant variation of the transcript amount per genome was found. However, in the aneuploid dosage series, the expression of both genes was dosage compensated and modulated by several chromosomal regions in trans. Our hypothesis is that the effect of dosage regulation on gene expression is the result of the stoichiometric interactions of multiple dosage sensitive trans regulatory factors among themselves and with their target genes. In aneuploids, chromosomal imbalance would change the stoichiometry of these factors, resulting in altered gene expression patterns. In the euploid series, the dosage of the genomic complement is changed proportionally and the stoichiometric relationship are presumably maintained. Therefore, the level of gene expression will be increased or decreased according to the ploidy variation.

Nevertheless, in the present study of the 18 genes analyzed, several do not follow this trend and show remarkable expression patterns. The transcript level of the thiol protease-like gene, csu 5, decreases dramatically with increasing genomic equivalents. This result suggests that the absolute level of a regulatory product responsible for this effect negatively influences the level of csu 5 mRNA. In Brassica oleracea, NAKAI (1977) also noted decreased activity for some esterase isozymes in the tetraploid in comparison with its diploid. There were even more extreme cases in which some isozyme bands found in diploids were absent in the tetraploids.

A consistent decrease in esterase isozyme activity with increased ploidy was also observed in monoploid, diploid and tetraploid individuals of Ricinus (TIMKO et al. 1980) and peroxidase isozyme activity in a ploidy series of the gametophyte and sporophyte of ferns (DE-MAGGIO and LAMBRUKOS 1974). It was suggested that an increased dosage of a regulatory gene(s) may have played a role in determining the activity of the affected isozyme bands (NAKAI 1977). On the other hand, for certain genes like csu 31, the positive correlation of transcript level to ploidy can go beyond the level predicted from a strict gene dosage effect. MITRA and BHATIA (1971) also observed such “super” positive correlation of several isozyme activities (ADH, malate dehydrogenase and glutamate dehydrogenase) in a ploidy series including diploid (AA), tetraploid (AABB), and hexaploid (AABBDD) wheat and an octaploid of Triticeae (AABBDDRR) derived from wheat and rye. These observations further indicate an interaction of structural gene dosage and their regulatory genes in producing the net effects on gene expression.
The interesting expression pattern exhibited by Sus1 is that the transcript level is elevated nearly threefold in the monoploid on a per genome level and more than sixfold in the triploid compared with the diploid and tetraploid. A similar expression pattern is also represented by two other genes, csu 28 and csu 116. This type of result is reminiscent of the odd/even effect found with B chromosomes in rye and maize (Jones and Rees 1982). In these two cases, the levels of total nuclear protein and RNA (Kirk and Jones 1970; Ayonoaudi and Rees 1971) and also the straw weight and the number of tillers per plant (Muntzing 1963) were elevated when an even number of B chromosomes were present in the cell and reduced to a lower level when there was an odd number of B chromosomes present. The direction of the effects between the B chromosomes and the ploidy series is opposite, but the two have in common an unusual response due to chromosome number. It is not known what factors are involved in this phenomenon or whether one or more A chromosomes might produce such an effect as suggested by the Sus1 result. It is of interest to note that Sus1 is the only one of six genes examined in our previous aneuploidy study that exhibited effects that fell outside the limits of an inverse or direct correlation with dosage (Guo and Birchler 1994). For example, a transacting direct effect will reduce the mRNA level of a two dose endosperm to 67% (2/3) and enhance that of a four dose endosperm to 133% (4/3) of the normal three dose endosperm. However, the transcript level of Sus1 (on chromosome 9) was 58 and 246% in endosperm tissue containing two and four doses of the short arm of chromosome 4, respectively. An interesting future study would be to examine the exceptional ploidy cases in the aneuploidy series to determine whether they all exhibit unique aneuploid effects. If present, the odd/even effect could then be experimentally approached using chromosomal manipulation techniques available in diploid maize.

In conclusion, gene regulation in response to segmental dosage variation results from a combined effect of the structural genes and the interactive relationship among dosage sensitive modifiers. When this relationship is maintained in euploidy, gene dosage effects are often observed. When this system is altered, as in the aneuploid condition, modifications of expression patterns result. In most multicellular eukaryotes, aneuploidy has a more severe effect upon phenotype than altering ploidy level. Our molecular results are in parallel with these observations. We have previously proposed that the reductions in gene expression found in both monosomics and trisomics are rate limiting on growth and could be the molecular basis of aneuploidy syndromes (Birchler and Newton 1981; Guo and Birchler 1994). In a ploidy series, gene expression patterns are more constant as are the phenotypes, although each ploidy has characteristic properties. However, further increases in autopolyploidy result in plants with decreased vigor. Based upon our results, these effects could be brought about by the exceptional cases in which gene expression decreases with increasing ploidy, although the relative changes of gene products or the effects of different cell size on developmental processes might also contribute.

We thank L. C. Hannah, M. M. Sachs, R. Phillips and the UMC maize RFLP lab for providing clones. Research was supported by a grant from the Department of Energy Biosciences Division.

LITERATURE CITED


Communicating editor: W. F. Sheridan