Tissue-specific effects of aneuploidy on gene expression

Corresponding Author:
Irina Makarevitch
Biology Department
Hamline University
1536 Hewitt Ave
Saint Paul, MN 55104
Phone: 651-523-2341
e-mail: imakarevitch01@hamline.edu
Aneuploidy causes tissue-specific qualitative changes in global gene expression patterns in maize

Irina Makarevitch, Carolyn Harris
Hamline University, 1536 Hewitt Ave, Saint Paul, MN 55104
Financial support: Hamline Biology Lund Fund and Fairchild Sherman foundation.
Corresponding Author: Irina Makarevitch, e-mail: imakarevitch01@hamline.edu
ABSTRACT

Segmental aneuploidy refers to the relative excess or deficiency of specific chromosome regions. This condition results in gene dosage imbalance and often causes severe phenotypic alterations in plants and animals. The mechanisms by which gene dosage imbalance effects gene expression and phenotype are not completely clear. The effects of aneuploidy on the transcriptome may depend on the types of cells analyzed and on the developmental stage. We performed global gene expression profiling to determine the effects of segmental aneuploidy on gene expression levels in two different maize tissues and a detailed analysis of expression of 30 genes affected by aneuploidy in multiple maize tissues. Different maize tissues varied in frequency at which genes located outside of the aneuploid regions are positively or negatively regulated, as well as in the degree of gene dosage compensation. Multiple genes demonstrated qualitative changes in gene expression due to aneuploidy, when the gene became ectopically expressed or completely silenced in aneuploids relative to wild type plants. Our data strongly suggested that quantitative changes in gene expression at developmental transition points caused by variation in gene copy number progressed through tissue development and resulted in stable qualitative changes in gene expression patterns. Thus, aneuploidy in maize results in alterations of gene expression patterns that differ between tissues and developmental stages of maize seedlings.
For most eukaryotic genomes, the balance in gene dosage is essential for normal function. Aneuploidy is a deviation from the normal chromosome number that involves the loss (monosomy) or gain (trisomy) of one or more individual chromosome(s) or large chromosomal segments (segmental aneuploidy) and results in a dosage imbalance of genes on the affected chromosome(s). Such imbalance can cause severe phenotypic syndromes in both plants and animals (reviewed in (Birchler and Veitia, 2007; Dierssen et al., 2009)). In humans, all autosomal aneuploid conditions are either lethal or result in severe phenotypic syndromes that include mental retardation and multiple anomalies in development of internal organs (Epstein, 2001). Although plants are generally more tolerant to aneuploidy than animals (Matzke et al., 2003), aneuploid plants exhibit a variety of phenotypic syndromes including developmental delays, partial sterility, and alterations in plant architecture (Birchler et al., 2001; Birchler and Veitia, 2007; Makarevitch et al., 2008). Interestingly, there exist substantial variation in ability of plants to tolerate gene dosage imbalance caused by aneuploidy, both between different plants species and between varieties of the same species (reviewed in (Henry et al., 2005; Henry et al., 2007)). However, despite the widespread interest in aneuploidy, there is a limited understanding of the molecular mechanisms that lead to phenotypic alterations in aneuploid organisms as well as gene interactions involved in coping with gene dosage imbalance caused by aneuploidy on the global genomic level.

Several studies assayed RNA levels or activity of enzymes encoded by genes that were located within segmental aneuploid regions, or were exposed to segmental aneuploidy of other chromosomal regions in maize (Birchler, 1979, 1981; Guo and Birchler, 1994; Guo et al., 1996) and Drosophila (Devlin et al., 1988; Birchler et al., 1990). These studies suggested that a specific chromosome arm dosage series can affect the expression of multiple genes located throughout the genome resulting in both positive and negative correlations of gene expression with the dosage of the varied chromosome arm. In addition, genes located in the affected region frequently do not exhibit alterations in their expression level, suggesting the occurrence of some level of dosage compensation. Interestingly, the expression level for most of the studied genes showed tissue-specific differences in response to aneuploidy. Only recently global gene expression profiling studies have been performed in aneuploid tissues from human and mice (Mao et al., 2003; Kahlem et al., 2004; Lyle et al., 2004; FitzPatrick, 2005; Mao et al., 2005; Ait Yahya-Graison et al., 2007; Laffaire et al., 2009), Drosophila flies (Stenberg et al., 2009),
Arabidopsis (Huettel et al., 2008), and maize seedlings (Makarevitch et al., 2008). Several models explaining the effects of aneuploidy on global gene expression have been proposed (Birchler et al., 2001; FitzPatrick, 2005; Birchler and Veitia, 2007; Birchler et al., 2007). According to the simplest models, genes primarily follow gene dosage in their expression, so that the genes located on the duplicated chromosomal regions in partial trisomics would show 1.5-fold increase in expression. More complicated models suggest that slight alterations in the relative expression level of transcription factors, or other regulatory proteins, located in the affected chromosomal region might affect expression of multiple genes located throughout the genome, thus causing massive alterations in gene network functioning. Such response in trans can lead to large quantitative differences in gene expression (up-regulation as well as down-regulation) or even qualitative changes in gene expression patterns and affect many genes due to complex gene interactions. All of the studies assaying global gene expression in aneuploids reported trans effects as well as some level of functional gene dosage compensation, or a “buffering” effect, when the level of RNA transcript read from genes present in three copies due to segmental aneuploidy were found to be similar to wild type levels (Mao et al., 2003; Kahlem et al., 2004; Lyle et al., 2004; FitzPatrick, 2005; Mao et al., 2005; Potier et al., 2006; Ait Yahya-Graison et al., 2007; Huettel et al., 2008; Makarevitch et al., 2008; Moldrich et al., 2009; Stenberg et al., 2009). The degree of reported dosage compensation varied substantially between studies; from 3 -15% in Arabidopsis (Huettel et al., 2008) and developing human brain cells (Mao et al., 2003; Mao et al., 2005) to over 65% in human lymphoblastoid cells (Ait Yahya-Graison et al., 2007). A portion of this reported variation is likely due to the different treatment of weakly expressed genes in the analysis and due to difficulties in accurately monitoring small changes (~1.5-fold) in gene expression. The reported differences between maize, mice, Drosophila, Arabidopsis, and human aneuploid studies may also reflect species-specific differences in response to aneuploidy or tissue-specific effects on gene regulation.

Indirect comparisons of different cell types in the mouse model of Down syndrome and human patients suggests variation in response to aneuploidy between different tissues (Mao et al., 2003; Kahlem et al., 2004; Lyle et al., 2004; FitzPatrick, 2005; Mao et al., 2005; Potier et al., 2006; Ait Yahya-Graison et al., 2007; Moldrich et al., 2009) and between different developmental stages (Laffaire et al., 2009). Global expression profiling performed in cerebellum cells of trisomic mice at multiple time points showed that only a small number of
genes were consistently affected during development, while the majority of differentially expressed genes were affected only at some developmental stages (Laffaire et al., 2009). In plants, tissue-specific aneuploidy effects have been investigated only for a limited number of genes and tissues (Guo and Birchler, 1994; Cooper and Birchler, 2001). Although these studies demonstrated tissue-specific aneuploidy effects on gene expression levels and the progression of aneuploidy effects through plant development, both studies focused on very small number of genes (Guo and Birchler, 1994; Cooper and Birchler, 2001). Moreover, comparisons between diploid (embryo) and triploid (endosperm) tissues (Guo and Birchler, 1994) might not completely reflect the differences in aneuploidy effects between different diploid tissues. Genome-wide effects of aneuploidy on gene expression have not been investigated in multiple tissues or at multiple developmental stages in plants.

Segmental aneuploid maize plants analyzed in this study carry three copies of a short arm of chromosome 5 (duplication) and only one copy of a small region of chromosome 6 (deficiency) and are referred to as duplicate-deficient (DpDf) plants. DpDf plants exhibit phenotypic abnormalities when compared to their siblings, including leaf knotting, developmental delay, partial tassel sterility, and growth reduction (Makarevitch et al., 2008). Since this phenotypic syndrome clearly affects multiple tissues, we would expect that expression of different genes would be affected by aneuploidy in different tissues. In our previous study, we reported evidence suggesting that aneuploidy resulted in ectopic expression of a meristem-specific gene, knox10, in mature leaves, possibly causing leaf knotting displayed by DpDf plants (Makarevitch et al., 2008). This finding made us believe that qualitative changes in gene expression patterns as opposed to quantitative changes in the levels of gene expression could cause some of the phenotypic alterations displayed by aneuploids. We hypothesize that stable qualitative alterations of gene expression patterns caused by misregulation of gene expression during developmental transition stages (in meristem tissues, for example) are more common in aneuploids than previously thought.

Here we report the results of two experiments: (i) an investigation of effects of aneuploidy on global gene expression in meristem-enriched and leaf tissues using microarray analysis of over 15,000 maize genes, and (ii) a study of gene expression changes in response to aneuploidy for 30 genes affected by aneuploidy in six different maize tissues and at three early developmental stages after germination. At least 23 out of 30 genes analyzed were either
ectopically expressed or erroneously silenced in mature aneuploid tissues. Our data strongly suggest that quantitative changes in gene expression at developmental transition points caused by variation in gene copy number progress through tissue development and result in stable qualitative changes in gene expression patterns.

RESULTS

Aneuploidy affects global gene expression in tissue-specific manner. We were interested in comparing genome-wide expression profiles of DpDf plants that are trisomic for a short arm of chromosome 5 and monosomic for a small region of chromosome 6 and their diploid siblings in multiple tissues. Specifically, we wanted to address the tissue-specific differences in the frequency of primary or cis effects (variation in the expression levels of genes located within the affected portions of the genome), and secondary or trans effects (variation in the expression levels of diploid genes located elsewhere in the genome). We hypothesized that meristem tissues would show a larger degree of trans effects, as a developmentally active tissue with an elaborate gene regulation network. In this study, we performed global gene expression profiling of aneuploid maize meristem-enriched tissues and compared this data to whole seedling expression data for these same genotypes from a previous study (Makarevitch et al., 2008). These two tissues were selected because there are relatively few phenotypic differences between wild-type and DpDf plants at the seedling stage and, therefore, the expression analyses should not be complicated by morphological differences. Pooled RNA samples were prepared from three biological replicates from DpDf plants and their wild-type siblings and used for microarray hybridization. The microarray data from both experiments were analyzed using the same statistical criteria and cut-off values (discussed in detail in Materials and Methods).

Aneuploidy causes limited changes at transcription level in meristem-enriched and seedling tissues. A series of statistical tests, ratio cut-offs and expression level criteria (see Materials and Methods for detailed description of handling cut-offs and several normalization methods used in the analysis) were applied to identify a set of 1,447 genes that are differentially expressed in DpDf plants relative to wild-type siblings (Table S1; Table 1). These 1,447 differentially expressed genes include 971 genes (6.5% of all genes present on the microarrays) that displayed higher transcript levels either in DpDf meristem-enriched tissue or in DpDf total seedlings and 474 genes (3.1% of genes) that displayed lower transcript levels in either one or
both DpDf tissues (Table 1). One gene was transcribed at higher level in DpDf meristem-enriched tissues and at lower level in DpDf total seedling tissues. In total, less than 10% of all the genes assessed by this platform demonstrated altered gene expression levels. In conformity with the expected dosage-dependent change in expression (1.5 fold increase in trisomic region and 2 fold decrease in monosomic region), the vast majority (1411/1447) of the differentially expressed genes exhibited less than a two-fold increase in transcript levels in DpDf plants relative to wild type siblings (Table 1). Analysis of the GO annotations for the differentially expressed genes did not reveal evidence for over-representation of any functional categories relative to their abundance on the microarray (data not shown). We also did not notice a correlation between the expression level of a gene and its likelihood to be sensitive to dosage changes in one or both tissues. Applying expression ratio cut-offs could potentially affect the number and distribution of genes identified as differentially expressed. To test for this possibility, we performed additional analyses for all genes displaying statistically significant differences in expression levels between DpDf and wild type tissues (for details see Materials and Methods, Tables S2 and S3, and Text S1). This analysis indeed revealed larger number of differentially affected genes (1,846 genes compared to 1,447). However, the proportion and distribution of trans- and cis- effects between different tissues remained very similar regardless of whether cut-offs were used or not.

**Aneuploidy affects different sets of genes in meristem-enriched and seedling tissues.** Interestingly, the effects of aneuploidy on gene expression varied substantially in the two tissues that were assessed. Only 37% of the genes with higher transcript levels in DpDf plants (358/972) were differentially expressed in both meristem and seedling tissues, whereas 40% (385/972) and 24% (229/972) of the genes with higher transcript levels in DpDf plants were affected only in meristem-enriched tissue or whole seedlings, respectively. Even more strikingly, only 56 (12% of all genes with lower transcript levels in DpDf plants) genes were differentially expressed in both tissues, whereas 207 and 211 genes were affected only in meristem or whole seedling tissue, respectively (Table 1). One possible explanation for these tissue-specific differences in aneuploidy effects could be that genes affected in only one tissue were not expressed at all in another tissue and therefore did not exhibit dosage-dependent differences in expression levels in the other tissue. However, the vast majority (over 85%) of genes differentially expressed in either meristem-enriched or whole seedling tissue were
expressed in the other tissue (genes differentially expressed in one of the tissues and not expressed in another tissue are shown in parentheses in Table 1). Another possible explanation for the tissue-specific differences could be the failure of some genes to reach a statistical threshold level, such that a particular gene was affected in both tissues, but was only statistically significant in one of the tissues. Genes affected only in meristems or seedlings (shown in white squares or gray triangles on Figure 1) could be clearly divided into two groups: likely affected in both tissues (located in the upper-right or lower-left quadrant) and affected only in one of two tissues (located along the axes). We performed t-tests to identify genes that demonstrated statistically significant differences between ratios of gene expression in DpDf and wild type seedlings in meristem-enriched tissues versus seedling tissues. Over 57% of genes expressed in both tissues and identified as differentially expressed only in one of the two tissues by microarrays demonstrated statistically significant differences (t-test, p-value <0.1) between meristem-enriched and seedling tissues (Table S1, Figure 1). Taken together, our microarray data suggested that the effects of aneuploidy on gene expression differ in meristem-enriched and predominantly leaf tissues.

Tissue-specific effects of aneuploidy on gene expression are predominantly due to trans effects. The maize genome sequence and genetic and physical map resources were used to determine location of 10,143 genes probed with Affymetrix microarrays (67% of all genes present on microarrays, Table 1, Figure 2). In the subsequent analysis of aneuploidy effects on gene expression, we primarily focused on trisomic genes located on the short arm of chromosome 5. Although expression changes for the monosomic genes could cause some of the trans-effects and phenotypic alterations of DpDf plants, we believe that effects of the monosomic genes are likely less significant, since the monosomic region of chromosome 6 in DpDf plants is very short, with only 14 genes present on the arrays used in this study (less than 2% compared to trisomic genes located on short arm of chromosome 5). The majority (418/752) of the genes with higher transcript levels in either tissue of DpDf plants with available map positions are located on the trisomic portion of chromosome 5 (contigs 204 - 238). However, the genomic localization of genes displaying higher transcript levels in DpDf plants varied depending on whether the genes exhibit common response in both tissues or tissue-specific expression response to aneuploidy. Over 91% (262/287) of the genes with higher transcript levels in both DpDf tissues are located in the segmental aneuploid portion of the genome (Table...
1). However, only 65% (107/164) or 16% (49/301) of the genes displaying higher transcript levels only in seedling or meristem, respectively, are located within the segmental aneuploid region (Table 1). This suggests that aneuploidy induced trans effects are much more common in meristem tissue than in whole seedling tissues. Trans effects were equally distributed across all chromosomes (Figure 2, Fisher’s exact test, p=46%), indicating that segmental aneuploidy of a short arm of chromosome 5 can affect the expression levels of genes throughout the maize genome in a tissue-specific manner. The data also suggested that dosage-dependent alterations in expression levels of genes located in trisomic regions of chromosome 5 are often maintained in multiple tissues, whereas trans effects involving genes from other chromosomes and diploid regions of chromosome 5 are more dependent on a particular tissue. Similar analyses for the monosomic region of chromosome 6 were inconclusive because this region is very short and contains only 14 genes present on the microarrays. We did note that 10 out of 14 genes in this monosomic region exhibited lower expression in DpDf plants than in wild-type plants in one or both tissues, potentially causing some of the trans-effects reported in this study. Similar trends were noticed when only genes with higher than 2-fold changes in gene expression were analyzed (Table 2).

**Approximately 50% of trisomic genes exhibit dosage compensation in each of two tissues.** Our data provided evidence for dosage compensation effects for some genes within the trisomic region of chromosome 5. Out of 694 trisomic genes expressed in either tissue and assayed by 17K maize microarrays, 38% (265/694) genes did not exhibit altered expression in any of two tissues, while another 38% (262/694) showed higher transcript levels in both DpDf tissues and 22% (156/694) demonstrated higher transcript levels in one of the two DpDf tissues studied (Table 1, Figures 2 and 3). To verify this general trend for trisomic genes, we used quantitative RT-PCR to quantify transcript levels of five genes with higher transcript levels in DpDf plants and five dosage-compensated genes showing moderate and low expression. The qRT-PCR data supported the microarray data and suggested that some genes exhibit dosage-dependent up-regulation while other genes exhibit dosage compensation (Table S4). Comparing gene expression ratios for genes located in contigs 204 through 225 and contigs 230 through 238 (Figure 2) revealed a higher proportion of genes with higher transcript levels in either or both DpDf tissues in contigs 204 through 225. These data suggest that the actual breakpoint in the DpDf plants occurred somewhere between contigs 225 and 230, leading to overestimation of the
number of dosage-compensated trisomic genes. Genes located in the contigs 230 through 238 and considered trisomic in this study may actually be diploid, since most of the genes in these region, as opposed to contigs 204 through 225, did not exhibit changes in gene expression. Cytological and FISH studies are necessary to confirm the actual location of the breakpoint. However, even when these genes were taken into account, more than 45% of trisomic genes were dosage compensated in at least one of DpDf tissues analyzed. Interestingly, despite the increased gene dosage, 11 genes located on the short arm of chromosome 5 had significantly lower expression levels in DpDf plants compared to the wild type seedlings. Four of these 11 genes showed lower expression values in meristem-enriched DpDf tissues, while 7 showed lower transcript levels in only whole seedling DpDf tissue. Whether the observed lower transcript levels in DpDf tissues is due to epigenetic silencing, altered transcription factor availability, or other mechanism remains to be investigated.

Large changes in gene expression are frequently due to qualitative changes in gene expression patterns. Only 36 genes (0.2% of all genes present on the microarrays) showed higher than 2-fold change in gene expression between DpDf and wild type seedlings in either meristem-enriched or whole seedling tissue (Table 2). A large proportion of the differentially expressed genes showing large fold changes demonstrated qualitative changes in the expression state (on/off) as opposed to quantitative changes in gene transcript levels (Table 2). To more fully investigate the pattern of aneuploidy effects on gene expression, we performed quantitative RT-PCR analysis for genes showing high fold changes in gene expression in at least one of the tissues.

Many large-fold changes in gene expression are tissue-specific and result from qualitative changes in gene expression patterns. We were intrigued by the tissue-specific effects of aneuploidy on gene expression levels and decided to further characterize this phenomena in additional tissues. We selected 30 genes, including genes displaying both higher and lower transcript levels in either one or both DpDf tissues (Table 3) and analyzed their expression using quantitative RT-PCR in 6 tissues: meristem, young leaf, developing leaf, mature leaf, root, and immature ear (see Materials and Methods for details of tissue collection). The majority of these 30 genes showed clear tissue-dependent patterns of gene expression changes in response to aneuploidy (Table 3, Figure 4). For example, gene CF629635 was not affected in meristem-enriched tissues, demonstrated quantitatively higher transcript levels in all
DpDf leaf tissues, and was turned on in immature ear tissue of DpDf plants (Table 3). A gene was termed “turned on” when its transcript was absent in a wild type tissue and present in an aneuploid tissue. Similarly, a gene was defined as “turned off” when its transcript was present in a wild type tissue and absent in an aneuploid tissue. We defined the “absence” of the transcript as no detectable PCR product after 40 cycles of RT-PCR or noise level amplification signal in quantitative RT-PCR. Most of the genes (25/30) with large-fold change differences in meristem or total seedlings exhibit qualitative (presence/absence) variation for gene expression between DpDf and wild-type plants in at least one tissue analyzed.

Our experiments revealed multiple examples of altered gene expression patterns in aneuploid tissues that progressed throughout the leaf development. Although these meristem-specific genes were progressively turned off during leaf development in wild-type seedlings, they failed to turn off and remained expressed in mature leaf tissues of DpDf seedlings, thus resulting in qualitative changes in gene expression patterns. Five of 14 genes with higher transcript levels in DpDf tissues and 11 of 16 genes with lower transcript levels in DpDf plants analyzed by qRT-PCR showed relatively mild quantitative changes in expression levels in DpDf meristems that progressed throughout the leaf development and resulted in qualitative (absence/presence) variation and ectopic gene expression or erroneous transcriptional silencing of these genes in mature leaf tissues (Figure 4). For example, in wild type plants gene BM379473 was expressed in meristem tissues and displayed progressively lower transcript levels during leaf development, so that it was not expressed at all in mature leaves. This gene was expressed at two-fold higher level in DpDf meristems and remained ectopically expressed at meristem tissue level in DpDf mature leaf tissues.

Similar examples of progressive alteration of gene expression patterns were discovered during early seedling development right after germination (Table 4). We found that at least 6 out of 9 genes with higher transcript levels in DpDf tissues and 4 out of 6 genes with lower transcript levels in DpDf tissues showed a pattern of progressive alteration of gene expression patterns during early development, when gene expression levels were tested in seedlings at three time points after germination. In these examples, 5 mm DpDf seedlings did not show significant changes in expression relative to wild type seedlings, while DpDf seedlings at later time points during development (15 mm or 30 mm seedlings) demonstrated significant quantitative or qualitative changes in gene expression levels (Table 4). Although we could not directly link
changes in expression of a particular gene to a particular phenotypic alteration of DpDf plants, taken together our data strongly suggested that tissue-specific qualitative changes in gene expression patterns are a common response to aneuploidy in plants and are a likely cause of phenotypic alterations seen in DpDf plants.

**DISCUSSION**

The concept of “gene dosage balance” has always been viewed as an important mechanism for regulation of gene function. It has been long known that aneuploidy causes different phenotypic abnormalities both in plants and animals. Interestingly, despite existence of similar phenotypic defects in different aneuploids (Torres et al., 2007), many of such phenotypic abnormalities are frequently specific depending on the particular chromosome that is being duplicated. For example, in humans, trisomy 13 (Patau Syndrome), 18 (Edwards Syndrome), 21 (Down Syndrome), or trisomy of sex chromosomes (Kleinfelter Syndrome) have different phenotypic characteristics and different degree of mental retardation and lethality (reviewed in (Altug-Teber et al., 2007)). Trisomic Datura lines differ in the structure of the leaves and flowers (Blakeslee et al., 1920). Trisomic maize plants possess characteristic features in leaf and tassel structure, plant architecture, developmental stages, and other characteristics (maizegdb.org). Such differences suggest that there might be specific “key” genes on each of the chromosomes that cause these phenotypic effects when their copy number is out of balance with other genes. However, even with a small duplicated regions (chromosome 21 in Down Syndrome contains only 350 genes), it is still impossible to create genetic models for each of duplicated genes to identify such “key” genes responsible for specific phenotypes (Laffaire et al., 2009). Therefore, most of the recent investigations of effects of aneuploidy have been focused on global gene expression profiling. However, plants studies of tissue-specific effects of aneuploidy on gene expression have been limited to several genes and a limited number of tissues (Guo and Birchler, 1994; Cooper and Birchler, 2001). Tissue-specific aneuploidy effects on global gene expression have not been studied in plants.

We performed global gene expression profiling in meristem-enriched and predominantly leaf maize seedling tissues and found that these two tissues differed substantially in the prevalence of *trans* and *cis* effects and dosage compensation. Interestingly, meristem-enriched tissues showed a much higher rate of *trans* effects and a much stronger level of dosage
compensation for the trisomic genes. The results of more detailed study of 30 genes in six tissues of DpDf and wild type maize seedlings and at three early developmental points further suggest that effects of aneuploidy on global gene expression depend on the tissue analyzed. In a study of mouse cerebellar regions enriched in granule cell precursors, similar differences in primary (cis) and secondary (trans) effects of trisomy have been observed between cells at different developmental stages, and likely even between different cells of the same stage (Laffaire et al., 2009). Varying degrees of tissue-specificity of gene expression response to aneuploidy have also been reported for human heart and brain tissues (Mao et al., 2005), and for multiple mice tissues (Kahlem et al., 2004; Lyle et al., 2004). In all of these studies, similar to our results in maize, primary (cis) effects were more common between different tissues, while secondary (trans) effects exhibited higher tissue-specificity.

Stress-response genes, transcription factors, and other potentially regulatory genes have been frequently reported to be overrepresented among the genes affected by aneuploidy both in plants and animals (Huettel et al., 2008; Makarevitch et al., 2008; Laffaire et al., 2009). We believe that various sets of transcription factors could be initially affected in different tissues due to the fact that some of these regulatory genes are completely silenced in particular tissues. Such initial variation could lead to variation in trans effects in different tissues observed in aneuploids. The larger variety of transcription factors and other regulatory genes potentially expressed in meristems as in developmentally immature and potentially pluripotent tissues could explain the prevalence of trans effects in meristems, when compared to leaf tissues. Some of these mild cis and trans effects in meristem tissues could be resolved during development, while others could lead to fixed changes in gene expression patterns, when a particular gene becomes ectopically expressed or erroneously silenced in developed tissues, as we showed for leaf development and early developmental stages after germination. More detailed investigation following development of other plant organs through multiple stages could provide further support for this hypothesis.

It is commonly assumed that phenotypic alterations caused by aneuploids occur due to quantitative changes in expression of genes with altered dosage (cis-effects) or genes located throughout the genome (trans-effects). It has been previously reported that aneuploidy causes greater quantitative changes in gene expression of two maize genes (sus1 and shl) in two-week old plants compared to embryo and endosperm tissues (Cooper and Birchler, 2001). In our
previous study (Makarevitch et al., 2008), we demonstrated that ectopic expression of a meristem-specific gene, *knox10*, could cause leaf knotting, displayed by aneuploid plants. This finding made us look for more evidence suggesting that qualitative changes in gene expression levels are common in aneuploid plants. Results of the study presented in this manuscript strongly suggest that during development of mature tissues, relatively mild quantitative initial effects of gene dosage imbalance lead to fixed qualitative changes in gene expression patterns. In this case, many of the phenotypic abnormalities are likely caused by ectopic expression of certain “key” genes, not necessarily located on the affected chromosomal region, and influenced by many steps in the gene network chains. We previously showed a clear connection established between an ectopic expression of particular gene and a phenotype, when ectopic expression of *knox10* in developed leaves of maize DpDf seedlings correlated to a formation of knots on the leaves of DpDf plants (Makarevitch et al., 2008). In the present study, we identified at least 23 new examples of qualitative gene expression changes resulting in ectopic expression or erroneous silencing of particular genes in different tissues in aneuploid plants (Tables 3 and 4). It remains unresolved, whether qualitative changes of expression patterns of these 23 genes cause specific phenotypic alterations displayed by DpDf plants. Our ability to better understand their potential involvement in causing phenotypic abnormalities in maize aneuploid seedlings would depend on detailed investigation of the function and expression patterns of these genes in transgenic plants carrying a dosage series for one “candidate” gene at a time.

Based on the results of global gene expression profiling of aneuploids, it could be concluded that regardless of the system analyzed aneuploidy causes (i) *cis* effects, correlated to gene dosage and predominantly common to multiple tissues; (ii) varying degree of gene dosage compensation for trisomic genes; (iii) tissue-specific *trans* effects (likely as a result of misregulation due to the slight variation in the presence of a regulatory protein), and (iv) tissue-specific fixed qualitative variation in gene expression patterns that is more frequent in mature tissues. However, all of these changes have been reported for gene mRNA levels and it is still unclear which of these effects are translated to the protein level and are indeed important for phenotypic abnormalities, given the existence of posttranscriptional and posttranslational regulation mechanisms.

MATERIALS AND METHODS
Plant materials and tissue collection: Maize stocks carrying a T5-6b translocation, backcrossed into the B73 genetic background for over 10 generations, were obtained from the University of Minnesota collection. The interchange T5-6b carries a break at 5S.1 (ca. 350 cM on IBM2 2008 Neighbors genetic map; in contig 238, according to maizesequence.org) and between the middle and distal chromomere satellites of 6S (break occurs prior to 70 cM on IBM2 2008 Neighbors genetic map; in contig 257, according to maizesequence.org) (Phillips, 1969; Phillips and Suresh, 1997). Duplicate-Deficient (DpDf) heterozygous plants were identified among progeny derived from crossing a female B73/T5-6b translocation heterozygote by a male B73 plant. The DpDf plants contain one normal chromosome 6 and one 65 chromosome that is lacking the terminal chromomere of the chromosome 6 satellite and contains ~90% of the short arm of chromosome 5 (Makarevitch et al., 2008). Thus, the DpDf plants are trisomic for the majority of the short arm of chromosome 5 and monosomic for a small region of chromosome 6.

For microarray experiments, three biological replicates were grown using standard greenhouse conditions (1:1 mix of autoclaved field soil and MetroMix; 16 hours light and 8 hours dark; daytime temperature of 30°C and night temperature of 22°C) and sampled for gene expression on the 14th day after planting between 9:00 and 10:00 am. For each biological replicate, sibling seeds produced by self pollination of a DpDf plant that segregate for wild-type and DpDf plants were planted individually and thirty plants were collected and genotyped using an SSR marker (bnlg161) that is tightly linked to the translocation breakpoint on chromosome 5, as described in (Makarevitch et al., 2008). A pool of eight wild-type plants and a separate pool of eight DpDf plants were generated for each of the biological replicates. For each plant, a shoot apical meristem-enriched tissue (approximately 1 cm of tissue from the shoot apical meristems containing leaf primordium) was collected for RNA isolation. The sampled tissues were flash frozen in liquid nitrogen and stored at -80°C prior to RNA isolation. For quantitative RT-PCR expression studies, the plants were grown as described above and five tissue types were collected from individual plants. The samples collected included the following tissues: (i) shoot apical meristems-enriched tissue, (ii) un-expanded leaf, (iii) developing leaf (the third leaf of the seedling), (iv) mature leaf (the first true leaf of the seedling), and (v) root apical meristems (approximately 1 cm of tissue from the root tips). In addition, (vi) immature ear (approximately 7 cm in length) tissues were collected from DpDf and wild type maize siblings grown in the field.
of University of Minnesota Saint Paul Agricultural Experimental Station (Saint Paul, MN) during the summer of 2008. For analysis of gene expression during early developmental stages, maize seeds were germinated on sterile filter paper and total green tissues were collected for three sets of seedlings, 5 mm, 15 mm, and 30 mm of length. Following genotyping with the bnlg161 SSR marker, five DpDf and five wild type siblings were selected for each of the tissue samples and were assayed separately.

**RNA isolation and microarray hybridization:** RNA isolation and Affymetrix (Santa Clara, CA, USA) microarray hybridizations were performed as described (Makarevitch et al., 2008) with some modifications for three biological replicates of wild-type B73 and DpDf plants. Briefly, tissues from 8 seedlings per genotype per biological replicate were pooled and ground in liquid nitrogen. RNAs were extracted using Trizol reagent according to the manufacturer’s instructions (Invitrogen Corp., Carlsbad CA) and purified using the RNeasy kit, according to the manufactures instructions (Qiagen Corp., Valencia, CA). The quality and quantity of all purified RNA samples were assessed using agarose gel electrophoresis and the Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE). RNA samples were sent to the University of Minnesota Microarray Facility for RNA labeling and hybridization to the Affymetrix Maize GeneChip where eight µg of RNA from each sample were used for labeling.

**Microarray data analysis:** We combined the microarray data from meristem-enriched tissue (described in this experiment; NCBI GEO Series Submission Number GSE19212) and the data from total seedlings (NCBI GEO Series Submission Number GSE10243 (Makarevitch et al., 2008)) and analyzed them using the same criteria. Affymetrix microarray data analysis was performed as described (Makarevitch et al., 2008) with some modifications. Briefly, the GCOS software package v1.2 (Affymetrix) was used for signal acquisition and initial analysis. GeneSpring (Agilent Technologies, Palo Alto CA) software was used for GC-RMA (GC-content robust multi-array) processing of the .cel files that involved normalization between the arrays and a subsequent per gene normalization of the resulting values. Genes differentially expressed in DpDf plants relative to wild-type siblings were identified by performing a one-way ANOVA on the GC-RMA values using a parametric test with no assumption of equal variance. A Benjamin and Hochberg multiple testing correction was applied using a false-discovery rate significance threshold of 0.1. Genes identified using this statistical test were further filtered based on criteria of expression level (at least 50 units for GC-RMA values in at least one of the
genotypes) and expression change fold (at least >1.24 or <0.8 fold change in wild-type versus DpDf comparisons either in meristems-enriched or total seedling tissues). The GeneSpring software was used to perform hierarchical clustering analyses using a Pearson correlation method to create gene or condition trees based on specified gene lists, conditions and genotypes.

To address the possibility that average gene expression could differ between wild type and aneuploid tissues, thus skewing results of the analysis in case of normalization between the arrays, alternative analyses were performed. In the first approach, normalization between arrays was performed, while leaving out the genes located on either all chromosome 5 and 6 or in aneuploid regions of chromosomes 5 and 6 and expected to be differentially expressed in aneuploid tissues. In the second approach, microarray data were normalized using “per-gene” normalization and genes differentially expressed in DpDf plants relative to wild type siblings were identified by a one-way ANOVA on the GC-RMA values using nonparametric test. Despite some differences in the number of genes identified as differentially expressed, all of these analyses yielded similar results concerning the frequency and distribution of trans- and cis-effects between two tissues. To confirm that applying the cut-off to filter genes with very small changes in gene expression does not alter the results of our analysis, the cut-off requirement was dropped and genes with significant changes in expression levels in DpDf versus wild type tissues were identified using a false-discovery rate significance threshold of 0.05 and an expression level threshold of at least 50 units for GC-RMA values in at least one of the genotypes. In this analysis, the number of differentially expressed genes increased to 1,846. However, the proportion and distribution of trans- and cis- effects between different tissues remained very similar (for details of this additional analysis see Tables S2 and S3 and Text S1).

**cDNA synthesis and quantitative RT-PCR (qRT-PCR):** One µg of total RNA was treated with DNAsel I (Qiagen, CA) and used for cDNA synthesis using Qiagen Omniscript reverse transcriptase (Qiagen, CA) according to manufacturer’s instructions and was diluted 1:5 for use in qRT-PCR experiments. Thirty genes that exhibited variation in expression patterns between DpDf and wild type genotypes in meristems-enriched and/or whole seedling tissues were selected for qRT-PCR analysis. Primers for these thirty genes (Table S5) and three control genes (actin1, Gene ID 100282267, GAPC, Gene ID 542367, and mez1, Gene ID 541954) were designed using Primer 3.0 software (Rozen and Skaletsky, 2000). qPCR reactions were performed using SYBR Green I (Bio-Rad, Hercules, CA) incorporation, according to iCyclerIQ
manufacturer’s recommendations. Each primer pair was tested for PCR efficiency using serial dilutions of pooled cDNA samples. PCR conditions were optimized to at least 90 – 95% efficiency and amplification efficiency for each primer pair was calculated (see Table S5 for primer sequences). For each tissue sample and each genotype, we analyzed five individual plants that were considered biological replicates. Three technical replicate qPCR reactions were performed for each of the samples. This approach provided the opportunity to assess variation between individual aneuploid plants as well as between wild-type and aneuploid plants. The relative expression levels in each sample were determined based on the threshold cycle (Ct) value for each PCR reaction. A Ct mean value and a standard error were obtained for three technical replicates, normalized to the expression of control genes, actin, GAPC, and mez1, and compared between individual samples of the same genotype. During normalization, the primer efficiency was included in calculations. The control genes did not show significant differences in transcript levels between DpDf and the wild type seedlings as evaluated by microarrays and PCR semi-quantitative analysis (data not shown). In all cases, Ct mean values for individual biological replicates were similar and they were combined to calculate mean Ct values for each genotype-tissue combination. A ΔCt value (difference in number of cycles to reach a threshold) was calculated by subtracting the Ct mean value for the samples to be compared (DpDf versus the wild type). Fold differences (FDs) for a given primer combination were calculated as (primer efficiency)ΔCt. Additional t-tests were performed using five values, each representing the average of the technical replicates for each gene/tissue combination to assess the statistical significance of fold changes between DpDf and wild type seedlings.

Bioinformatics analysis: Annotations for differentially expressed genes were based on information available at the TIGR Maize Gene Index (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=maize). The gene ontology (GO) annotations were obtained based on the assignment of the best Arabidopsis hits from the TAIR website (http://www.arabidopsis.org/tools/bulk/go/index.jsp) and based on microarray annotations provided by Affymetrix. The genetic map positions for Affymetrix array probe sets were predicted based on identity with genetically mapped sequences or inferred based upon identity with BAC contig sequences that contained genetically mapped markers (www.maizesequence.org, www.maizegdb.org, http://www.genome.arizona.edu).
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FIGURE LEGENDS

**Figure 1.** Different sets of genes are affected by aneuploidy in two different tissues. The expression ratios of the DpDf versus wild-type tissues were compared for meristem-enriched and green seedling tissues in order to assess whether genes were differentially expressed in both genotypes or just one of the genotypes. Only genes differentially expressed in at least one of the aneuploid tissues and expressed at both tissues are shown. Genes equally affected in both tissues are expected to fall on the diagonal line, while genes affected only in one tissue are expected to be located along the axes. Shades and shapes indicate gene behavior in two tissues: black circles designate genes with significant changes in expression levels in both tissues; grey triangles and white squares indicate genes with significant changes in total seedling tissue or meristem-enriched tissue, respectively.

**Figure 2.** Aneuploidy causes both *cis* and *trans* variation of gene expression levels. Distribution of genes with significant expression changes across maize chromosomes. Chromosome 1 is shown as a representative for chromosomes other than 5, since no clear preferences in gene distribution along non-affected chromosomes were detected. Each transcript is represented by a mark. The x-axes correspond to the gene location on a maize contig along the chromosomes, the y-axes show expression ratios with positive values indicating increased expression in DpDf plants. Shades report on relative significance (white is highest, black is lowest). Genes on chromosome 5 that are dosage compensated are at the zero line; any gene significantly above is not dosage compensated.

**Figure 3.** Trisomic genes demonstrate different patterns of expression changes. Trisomic genes from a representative 15 Mb region of the chromosome 5 are shown. The x-axis corresponds to the chromosomal location of a gene along the chromosome 5 (in Mb), the y-axis shows expression ratios with positive values indicating increased expression in DpDf plants. Each trisomic transcript is represented by two marks: a square shows expression change in meristem-enriched tissues, while a circle shows expression change in seedlings. Color indicates gene behavior in two tissues: black refers to genes with significant changes in expression levels in both tissues; blue and red indicate genes with significant changes in meristem-enriched or seedling tissue, respectively, while white shows genes with no significant changes in either tissues. Genes on chromosome 5 that are dosage compensated are at the zero line; any gene
significantly above is not dosage compensated. Horizontal line ($y = 0.32$) indicates the $\log_2$ value of the cutoff ratio used in this study ($\log_2 1.25 = 0.32$).

**Figure 4.** Aneuploidy causes progressive qualitative changes in gene expression patterns. (A) Average ratios of gene expression detected by qRT-PCR and normalized to expression in meristem of wild type plants are shown. Error bars denote standard error values for each experiment. (B) Semi-quantitative RT-PCR was performed for 40 cycles. $Mez1$ gene served as a normalization control for cDNA concentration. All RNAs were tested without RT enzyme (-RT) and showed negative results (data not shown). Lanes are as follows: 1 - wild type meristem tissue, 2 – wild type young leaf, 3 – wild type developing leaf, 4 – wild type mature leaf, 5 – DpDf meristem, 6 – DpDf young leaf, 7 – DpDf developing leaf, 8 – DpDf mature leaf, 9 – negative control (water)
TABLES

Table 1. Analysis of genes differentially expressed in DpDf plants compared to wild-type based on microarray analysis.

| Gene Categorya | Total number of genesb | Genes with predicted map positionsb | Trisomic genes (mapped to 5S)b | Monosomic genes (mapped to deleted portion of 6)b |
|----------------|------------------------|-----------------------------------|-------------------------------|-----------------------------------------------|
| Genes with higher transcript levels in DpDf plants | 972 | 752 | 418 | 0 |
| Higher transcript levels in DpDf meristem only | 385 (77) | 301 (51) | 49 (12) | 0 |
| Turned on in DpDf meristem | 0 | 0 | 0 | 0 |
| Higher transcript levels in DpDf seedlings only | 229 | 164 | 107 | 0 |
| Turned on in DpDf seedlings | 10 | 8 | 4 | 0 |
| Higher transcript levels in both DpDf tissues | 358 | 287 | 262 | 0 |
| Turned on in DpDf seedlings/meristems | 12/0 | 7/0 | 4/0 | 0/0 |
| Genes with lower transcript levels in DpDf | 474 | 352 | 11 | 10 |
| Lower transcript levels in DpDf meristem only | 207 (67) | 146 (43) | 4 (1) | 1 (0) |
| Turned off in DpDf meristem | 2 (2) | 2 (2) | 0 (0) | 0 (0) |
| Lower transcript levels in DpDf seedlings only | 211 | 162 | 7 | 3 |
| Turned off in DpDf seedlings | 18 | 16 | 1 | 0 |
| Lower transcript levels in both DpDf tissues | 56 | 44 | 0 | 6 |
| Turned off in DpDf seedlings / meristem | 10/0 | 8/0 | 0/0 | 4/0 |
| Genes displaying mixed effects in two tissues | 1 | 1 | 0 | 0 |
| Genes on the microarray | 15052 | 10143 | 694 | 14 |

a A gene was defined as “turned on” when its transcript is absent in a wild type tissue and present in an aneuploid tissue. Similarly, a gene was defined as “turned off” when its transcript is present in a wild type tissue and absent in an aneuploid tissue.

b Shown in parentheses is the number of genes in each category that are not expressed in seedlings at all. All of the differentially expressed genes are expressed in meristem tissues.
Table 2. List of genes with the largest changes in gene expression in aneuploid plants.
Normalized microarray signals are shown for DpDf and wild type plants for meristem and total seedling tissues.

| Accession | DpDf meristem | Wild type meristem | Fold change (DpDf/Wild type) meristem | DpDf seedlings | Wild type seedlings | Fold change (DpDf/Wild type) seedlings | Chromosome | Annotation |
|-----------|---------------|--------------------|--------------------------------------|----------------|---------------------|---------------------------------------|------------|------------|
| Genes with higher transcript levels in both DpDf tissues |
| AW289130  | 537           | 60                 | 8.94*                                | 414            | 18                  | 23.09*                                | 5          | cyclophilin |
| BM380426  | 1033          | 146                | 7.09                                 | 339            | 31                  | 11.10*                                | 6          | unknown    |
| BM379473  | 1790          | 764                | 2.34                                 | 262            | 140                 | 1.88                                  | 5          | unknown    |
| BM269210  | 804           | 351                | 2.29                                 | 198            | 81                  | 0.41                                  | 5          | splicing factor Prp18 |
| AF783234  | 1424          | 656                | 2.17                                 | 429            | 169                 | 2.53                                  | 5          | aminoacoholphosphotransferase |
| BF792948  | 846           | 422                | 2.00                                 | 544            | 327                 | 1.66                                  | 1          | unknown    |
| AF105653  | 2273          | 1199               | 1.90                                 | 842            | 358                 | 2.35                                  | 5          | malate oxidoreductase |
| CK369759  | 298           | 172                | 1.74                                 | 145            | 63                  | 2.32*                                 | 5          | unknown    |
| AF107589  | 2918          | 1809               | 1.61                                 | 633            | 317                 | 2.00                                  | 5          | unknown    |
| Genes with higher transcript levels in DpDf meristem |
| CF626580  | 927           | 451                | 2.06                                 | 123            | 110                 | 1.12                                  | 4          | unknown    |
| Genes with higher transcript levels in DpDf seedlings |
| L16798    | 105           | 105                | 1.00                                 | 140            | 12                  | 0.09*                                 | 5          | class I acidic chitinase |
| AF659019  | 384           | 293                | 1.31                                 | 119            | 49                  | 2.41*                                 | 5          | phosphate transport protein |
| U17897    | 1242          | 845                | 1.47                                 | 415            | 200                 | 2.07                                  | 5          | starch branching enzyme I (sbe1) |
| CF629635  | 542           | 489                | 1.11                                 | 375            | 187                 | 2.01                                  | 5          | sec14 like protein |
| Genes with lower transcript levels in both DpDf tissues |
| AY109249  | 1122          | 2814               | 0.40                                 | 242            | 571                 | 0.42                                  | 2          | unknown    |
| AF677337  | 615           | 1217               | 0.51                                 | 427            | 874                 | 0.49                                  | 6          | unknown    |
| AF622092  | 399           | 714                | 0.56                                 | 92             | 200                 | 0.46                                  | 6          | expressed protein |
| AF737202  | 407           | 711                | 0.57                                 | 76             | 192                 | 0.40                                  | 6          | unknown    |
| BM073880  | 456           | 764                | 0.60                                 | 48             | 109                 | 0.44*                                 | ND         | GCN5L1 family protein |
| BM379784  | 375           | 608                | 0.62                                 | 119            | 237                 | 0.50                                  | 6          | unknown    |
| AY107007  | 280           | 419                | 0.67                                 | 37             | 78                  | 0.47*                                 | 6          | unknown    |
| BM079913  | 120           | 179                | 0.67                                 | 62             | 135                 | 0.46*                                 | 1          | protease inhibitor/lipid transfer |
| Genes with lower transcript levels in DpDf meristem |
| 11990232-113 | 3351         | 12121              | 0.28                                 | 72             | 162                 | 0.45                                  | 5          | unknown    |
| 11990232-89 | 96            | 231                | 0.41                                 | 49             | 55                  | 0.89                                  | 7          | unknown    |
| 11990232-114 | 5528         | 12872              | 0.43                                 | 6071           | 9528                | 0.64                                  | ND         | unknown    |
| 11990232-42 | 171           | 389                | 0.44                                 | 229            | 316                 | 0.72                                  | 5          | unknown    |
| 11990232-86 | 129           | 289                | 0.45                                 | 41             | 71                  | 0.58                                  | 7          | unknown    |
| 11990232-82 | 130           | 290                | 0.45                                 | 125            | 205                 | 0.61                                  | 10         | unknown    |
| 11990232-33 | 158           | 353                | 0.45                                 | 31             | 39                  | 0.81                                  | 5          | unknown    |
| 11990232-49 | 527           | 1141               | 0.46                                 | 358            | 519                 | 0.69                                  | 5          | unknown    |
| 11990232-44 | 92            | 196                | 0.47                                 | 60             | 85                  | 0.71                                  | 1          | unknown    |
| 11990232-90 | 80            | 170                | 0.47                                 | 17             | 17                  | 1.01                                  | 9          | unknown    |
| 11990232-43 | 163           | 341                | 0.48                                 | 248            | 280                 | 0.88                                  | 1          | unknown    |
| 11990232-8  | 415           | 855                | 0.49                                 | 119            | 256                 | 0.47                                  | ND         | unknown    |
| Genes with lower transcript levels in DpDf seedlings |
| BG873775  | 468           | 621                | 0.75                                 | 448            | 938                 | 0.48                                  | 1          | unknown    |
| CD991129  | 898           | 1097               | 0.82                                 | 311            | 626                 | 0.50                                  | 6          | harpin-induced protein 1 family |

*a* Fold ratios that are not statistically significant (t-test, p-value > 0.5) are shown in italic

*b* A star indicates a gene with a likely qualitative pattern of gene expression changes

*c* Chromosome positions predicted by BLASTing maize genome sequence and validated using oat x maize chromosome addition lines (Kynast et al., 2001). ND - not determined by either method.

All of the genes on chromosome 5 in this table are trisomic.
Table 3. Quantitative RT-PCR analysis of 30 genes differentially expressed in DpDf and wild type plants in six tissues.

| Accession | Ploidy* | Fold ratio (DpDf/wild type) | Fold ratio (DpDf/wild type) | Fold ratio (DpDf/wild type) | Fold ratio (DpDf/wild type) | Fold ratio (DpDf/wild type) | Fold ratio (DpDf/wild type) |
|-----------|---------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|           |         | meristem<sup>b</sup>       | unexpanded leaves<sup>b</sup> | developing leaves<sup>b</sup> | mature leaves<sup>b</sup> | roots<sup>b</sup>         | immature ears<sup>b</sup>   |
| AW289130  | trisomic | Turned on                   | Turned on                   | Turned on                   | Turned on                   | Turned on                   | Turned on                   |
| BM380426  | monosomic | 7.31                        | 8.62                       | Turned on                   | Turned on                   | 2.12                       | 4.94                       |
| AI783234  | trisomic | 2.38                        | 2.53                       | 1.97                       | 2.03                       | Turned on                   | Turned on                   |
| BM331974  | trisomic | 2.24                        | 2.12                       | 1.97                       | 1.99                       | Turned on                   | 1.12                       |
| AY105653  | trisomic | 2.14                        | 2.32                       | 2.05                       | 1.89                       | 1.19                       | Turned on                   |
| BM379473  | trisomic | 2.12                        | 1.97                       | Turned on                   | Turned on                   | NE                         | Turned on                   |
| BM332751  | disomic  | 2.08                        | NE                         | NE                         | NE                         | NE                         | 3.12                       |
| CF626580  | trisomic | 2.08                        | 1.91                       | 1.63                       | 1.12                       | 1.35                       | Turned on                   |
| BM335301  | trisomic | 2.05                        | NE                         | NE                         | NE                         | 5.16                       | Turned on                   |
| CK367959  | trisomic | 1.71                        | 4.31                       | Turned on                   | Turned on                   | Turned on                   | 4.41                       |
| U17897    | trisomic | 1.36                        | 1.65                       | 1.97                       | 2.35                       | NE                         | NE                         |
| AY639019  | trisomic | 1.28                        | 3.15                       | Turned on                   | Turned on                   | Turned on                   | Turned on                   |
| CF629635  | trisomic | 1.07                        | 1.53                       | 1.82                       | 2.53                       | NE                         | Turned on                   |
| L16798    | trisomic | 0.95                        | 1.14                       | Turned on                   | Turned on                   | Turned on                   | Turned on                   |

| Genes with lower transcript levels in DpDf |
|------------------------------------------|
| AY109249 | disomic | 0.37 | 0.44 | Turned off | Turned off | Turned off | NE           |
| 11990232-86 | disomic | 0.38 | Turned off | Turned off | 0.83 | Turned off | NE           |
| 11990232-44 | disomic | 0.45 | 0.38 | Turned off | Turned off | Turned off | NE           |
| 11990232-33 | disomic | 0.47 | NE | NE | NE | NE | 0.34 |
| 11990232-89 | disomic | 0.48 | 0.18 | Turned off | Turned off | 0.98 | 0.13 |
| AI622092 | monosomic | 0.48 | 0.45 | 0.48 | Turned off | Turned off | 0.91 |
| 11990232-90 | disomic | 0.50 | NE | NE | NE | 0.91 | Turned off |
| 11990232-8 | ND | 0.52 | 0.42 | 0.39 | Turned off | NE | Turned off |
| 40794996-111 | disomic | 0.55 | NE | NE | NE | 0.52 | Turned off |
| BM073880 | ND | 0.56 | 0.54 | Turned off | Turned off | 0.36 | 0.66 |
| BM379784 | monosomic | 0.57 | 0.62 | 0.42 | Turned off | 0.95 | Turned off |
| AI737202 | monosomic | 0.59 | 0.52 | Turned off | Turned off | Turned off | 0.91 |
| BM331837 | ND | 0.64 | 0.47 | 0.56 | 0.39 | 0.89 | 0.54 |
| AY107007 | monosomic | 0.72 | 0.22 | Turned off | Turned off | 0.56 | 0.25 |
| CD991129 | monosomic | 0.79 | 0.27 | 0.18 | Turned off | 0.47 | NE |
| BM079913 | disomic | 0.79 | 0.61 | 0.62 | 0.49 | 0.85 | 0.40 |

*Fold ratios are indicated for genes showing quantitative changes in expression. For genes with detected expression only in DpDf or wild type plants, the direction of the change is indicated. A gene was defined as “turned on” when its transcript is present in a wild type tissue and absent in an aneuploid tissue. Similarly, a gene was defined as “turned off” when its transcript is present in a wild type tissue and absent in an aneuploid tissue.

Statistically insignificant changes in gene expression (t-test, p-value>0.5) are indicated in italic.

NE (Not expressed) - Expression of the gene is not detected in both DpDf and wild type plants in a particular tissue.
Table 4. Effect of aneuploidy on expression levels of 15 genes assayed by qRT-PCR at several time points early after germination

| Accession   | Ploidy | 5 mm seedlings<sup>bde</sup> | 15 mm seedlings<sup>bde</sup> | 30 mm seedlings<sup>bde</sup> |
|-------------|--------|-------------------------------|-------------------------------|-------------------------------|
|             |        | Turned on                     | Turned on                     | Turned on                     |
| Genes with higher transcript levels in DpDf |        |                               |                               |                               |
| AW289130    | trisomic | 1.94                          | 1.87                          | 1.95                          |
| AI783234    | trisomic | 2.13                          | 2.35                          | 2.26                          |
| AY105653    | trisomic | 1.47                          | 1.32                          | 2.56                          |
| BM379473    | trisomic | 1.18                          | 3.41                          | Turned on                     |
| CK369759    | trisomic | 1.04                          | 1.01                          | 2.83                          |
| U17897      | trisomic | 1.47                          | 4.63                          | Turned on                     |
| AY639019    | trisomic | 1.43                          | 1.24                          | 2.56                          |
| CF629635    | trisomic | 1.07                          | 0.89                          | 8.84                          |
| L16798      | trisomic | 0.42                          | 0.38                          | Turned off                    |
| Genes with lower transcript levels in DpDf |        |                               |                               |                               |
| AY109249    | disomic  | 0.86                          | 0.92                          | 0.63                          |
| AI737202    | monosomic | 0.64                          | 0.47                          | 0.56                          |
| BM331837    | ND       | 1.14                          | 0.91                          | 1.02                          |
| AY107007    | monosomic | 0.92                          | 0.64                          | Turned off                    |
| CD991129    | monosomic | 1.03                          | 0.98                          | 0.57                          |

<sup>a</sup>ND-Not determined

<sup>b</sup>Seedlings were germinated on filter paper and total green tissues were collected of seedlings of appropriate length

<sup>c</sup>Fold ratios are indicated for genes showing quantitative changes in expression. For genes with detected expression only in DpDf or wild type plants, the direction of the change is indicated. A gene was defined as “turned on” when its transcript is absent in a wild type tissue and present in an aneuploid tissue. Similarly, a gene was defined as “turned off” when its transcript is present in a wild type tissue and absent in an aneuploid tissue.

<sup>d</sup>Statistically insignificant changes in gene expression (t-test, p-value>0.5) are indicated in italic.

<sup>e</sup>NE (Not expressed) - Expression of the gene is not detected in both DpDf and wild type plants in a particular tissue.
SUPPLEMENTAL MATERIALS

Table S1. Annotation and identification of 1446 genes that are differentially expressed in either total seedlings or meristems of DpDf plants compared to their wild type siblings

Table S2. Characterization of genes differentially expressed in DpDf plants compared to their wild type siblings identified without the use of expression ratio cut-offs.

Table S3. Analysis of genes differentially expressed in DpDf plants compared to their wild-type siblings identified without the use of expression ratio cut-offs.

Table S4. qRT-PCR validation of microarray data on expression of selected trisomic genes

Table S5. The list of primers used in genotyping, RT-PCR, and quantitative RT-PCR studies of gene expression

Text S1. Analysis of genes identified as differentially expressed between DpDf and wild type tissues without using any ratio cut-offs.
Figure 1. Different sets of genes are affected by aneuploidy in two different tissues. The expression ratios of the DpDf versus wild-type tissues were compared for meristem-enriched and green seedling tissues in order to assess whether genes were differentially expressed in both genotypes or just one of the genotypes. Only genes differentially expressed in at least one of the aneuploid tissues and expressed at both tissues are shown. Genes equally affected in both tissues are expected to fall on the diagonal line, while genes affected only in one tissue are expected to be located along the axes. Shades and shapes indicate gene behavior in two tissues: black circles designate genes with significant changes in expression levels in both tissues; grey triangles and white squares indicate genes with significant changes in total seedling tissue or meristem-enriched tissue, respectively.
**Figure 2.** Aneuploidy causes both *cis* and *trans* variation of gene expression levels. Distribution of genes with significant expression changes across maize chromosomes. Chromosome 1 is shown as a representative for chromosomes other than 5, since no clear preferences in gene distribution along non-affected chromosomes were detected. Each transcript is represented by a mark. The *x*-axes correspond to the gene location on a maize contig along the chromosomes, the *y*-axes show expression ratios with positive values indicating increased expression in DpDf plants. Shades report on relative significance (white is highest, black is lowest). Genes on chromosome 5 that are dosage compensated are at the zero line; any gene significantly above is not dosage compensated.
Figure 3. Trisomic genes demonstrate different patterns of expression changes. Trisomic genes from a representative 15 Mb region of the chromosome 5 are shown. The x-axis corresponds to the chromosomal location of a gene along the chromosome 5 (in Mb), the y-axis shows expression ratios with positive values indicating increased expression in DpDf plants. Each trisomic transcript is represented by two marks: a square shows expression change in meristem-enriched tissues, while a circle shows expression change in seedlings. Color indicates gene behavior in two tissues: black refers to genes with significant changes in expression levels in both tissues; blue and red indicate genes with significant changes in meristem-enriched or seedling tissue, respectively, while white shows genes with no significant changes in either tissues. Genes on chromosome 5 that are dosage compensated are at the zero line; any gene significantly above is not dosage compensated. Horizontal line (y = 0.32) indicates the log₂ value of the cutoff ratio used in this study (log₂ 1.25 = 0.32).
Figure 4. Aneuploidy causes progressive qualitative changes in gene expression patterns. (A) Average ratios of gene expression detected by qRT-PCR and normalized to expression in meristem of wild type plants are shown. Error bars denote standard error values for each experiment. (B) Semi-quantitative RT-PCR was performed for 40 cycles. *Mez1* gene served as a normalization control for cDNA concentration. All RNAs were tested without RT enzyme (-RT) and showed negative results (data not shown). Lanes are as follows: 1 - wild type meristem tissue, 2 – wild type young leaf, 3 – wild type developing leaf, 4 – wild type mature leaf, 5 – DpDf meristem, 6 – DpDf young leaf, 7 – DpDf developing leaf, 8 – DpDf mature leaf, 9 – negative control (water)