Theor Appl Genet (2006) 113:831–845
DOI 10.1007/s00122-006-0335-x

ORIGINAL PAPER

Genome-wide transcript analysis of maize hybrids: allelic additive gene expression and yield heterosis

Mei Guo · Mary A. Rupe · Xiaofeng Yang · Oswald Crasta · Christopher Zinselmeier · Oscar S. Smith · Ben Bowen

Received: 16 February 2006 / Accepted: 3 June 2006 / Published online: 26 July 2006

Abstract Heterosis, or hybrid vigor, has been widely exploited in plant breeding for many decades, but the molecular mechanisms underlying the phenomenon remain unknown. In this study, we applied genome-wide transcript profiling to gain a global picture of the ways in which a large proportion of genes are expressed in the immature ear tissues of a series of 16 maize hybrids that vary in their degree of heterosis. Key observations include: (1) the proportion of allelic additively expressed genes is positively associated with hybrid yield and heterosis; (2) the proportion of genes that exhibit a bias towards the expression level of the paternal parent is negatively correlated with hybrid yield and heterosis; and (3) there is no correlation between the over- or under-expression of specific genes in maize hybrids with either yield or heterosis. The relationship of the expression patterns with hybrid performance is substantiated by analysis of a genetically improved modern hybrid (Pioneer® hybrid 3394) versus a less improved older hybrid (Pioneer® hybrid 3306) grown at different levels of plant density stress. The proportion of allelic additively expressed genes is positively associated with the modern high yielding hybrid, heterosis and high yielding environments, whereas the converse is true for the paternally biased gene expression. The dynamic changes of gene expression in hybrids responding to genotype and environment may result from differential regulation of the two parental alleles. Our findings suggest that differential allele regulation may play an important role in hybrid yield or heterosis, and provide a new insight to the molecular understanding of the underlying mechanisms of heterosis.

Communicated by F. van Eeuwijk

Electronic Supplementary Material Supplementary material is available to authorised users in the online version of this article at http://dx.doi.org/10.1007/s00122-006-0335-x.

M. Guo (✉) · M. A. Rupe · X. Yang · C. Zinselmeier · O. S. Smith · B. Bowen
Pioneer Hi-Bred International, Inc., 7250 NW 62nd Avenue, Johnston, IA 50131, USA
e-mail: mei.guo@pioneer.com

O. Crasta
CuraGen Corp., New Haven, CT, USA

Present Address:
O. Crasta
Virginia Bioinformatics Institute, Virginia, VA 24061, USA

Present Address:
B. Bowen
Gene-fx, Inc., Berkeley, CA 94705, USA

Introduction

Heterosis is a term first introduced by Shull (1908) to describe the superior performance of hybrid progeny compared to their inbred parents. Heterosis in plants is associated with increases in grain yield, vegetative growth rate, tolerance to pests and environmental stress, accelerated maturity, and many other changes in desirable agronomic characteristics. In maize breeding, heterosis has been widely exploited for many decades, but there is still a very limited understanding of the underlying genetic or molecular mechanisms.

Two fundamental questions regarding the molecular mechanism of heterosis are the relationship between

yield heterosis and gene expression in the hybrids, and how the two different alleles brought together in the hybrids are expressed. The superior performance of the hybrid over the mean of the inbred parents may result from the altered regulation of gene expression in the hybrids, either at the global level or for specific classes of genes. One possible scenario is that the two different alleles brought together in the hybrid create a combined allelic expression pattern in hybrids. Complementation of the allelic expression differences may result in different overall developmental expression patterns in the hybrid compared to the inbred parents. For example, the timing of gene expression may differ in the parents such that the expression pattern in the hybrid results in an extended time of gene expression. Alternatively, at some loci, allelic interaction or a change in the spectrum of trans-acting factors causes gene expression in the hybrid to deviate from simple additive allelic expression patterns of the parents (Birchler et al. 2003; Gibson and Weir 2005).

Early studies reported examples of increased mRNA quantity or protein amount expressed in hybrids as compared to their inbred parents (Leonardi et al. 1991; Romagnoli et al. 1990; Tsaftaris et al. 1995, 1999) and suggested that increased gene expression level in the hybrids may contribute to heterosis. More recent studies of gene expression in hybrid maize have shown both allelic additive types of expression regulation and deviation from it in the triploid endosperm tissue (Guo et al. 2003; Son and Messing 2003) and in leaf tissue (Auger et al. 2005). However, there is still a paucity of data demonstrating any relationship between any expression patterns in maize or those documented in other plants (Bao et al. 2005; Vuylsteke et al. 2005) and yield heterosis.

We have previously shown with a subset of randomly selected genes that the two parental alleles in maize hybrids can be regulated differentially, at the cumulated transcript level, in different tissues and in different environments (Guo et al. 2004). The study shows that transcript regulation can be allele-specific and that specific expression patterns of the two parental alleles in the hybrid manifest during development and in different environments is possibly associated with hybrid performance.

In the present study, we used GeneCalling technology (Shimkets et al. 1999) to profile mRNA expressed in the immature ear tissue (before pollination) of a series of 16 maize hybrids that varied in yield heterosis. GeneCalling technology is an open-ended, gel-based method that permits comprehensive profiling of mRNA abundance for both known and novel genes in an unbiased way (Crasta and Folkerts 2003), although identification of corresponding genes of profiled cDNA fragments requires additional process (such as isolation, cloning and sequencing). This technology detects 80–90% of expressed genes in a given tissue (Shimkets et al. 1999). GeneCalling mRNA profiling has been successfully used in various maize gene expression studies, identifying genes involved in flavonoid biosynthesis (Bruce et al. 2000), root-lodging resistance (Bruce et al. 2001), stress response during maize seed maturation and germination (Kollipara et al. 2002), and demonstrating allelic expression in the maize endosperm of reciprocal hybrids and inbred parents (Guo et al. 2003).

In the present study, we used GeneCalling mRNA profiling technology to analyze a series of 16 maize hybrids that shared a common female parent and vary in the degree of yield heterosis. The objectives of this study are (1) to gain a global view of the level of expression of genes during one stage of development (immature ear tissue at stage V19) in a series of maize hybrids and their inbred parents; (2) to determine whether specific gene expression patterns in the hybrid are associated with differences in heterosis and/or hybrid yield both among genotypes and in different environments with varying levels of stress; (3) whether up- or down-regulated gene expression in the hybrid correlates with heterosis; and (4) what are the effects of differential regulation of the two parental alleles on gene expression in the hybrid background.

Materials and methods

Experimental design and tissue sampling

A series of 16 maize (*Zea mays* L.) hybrids and their respective inbred parents were selected from the collection at Pioneer Hi-Bred International, Inc. (Table 1). The parental inbreds are either Stiff Stalk Iowa Synthetic (S) or Non-Stiff Stalk (NS) lines, which comprise two heterotic pools used widely in maize breeding (Labate et al. 1997). Hybrids were produced by crossing a common female inbred (S1) with a series of male inbreds that share different percentages of pedigree relationship with the female parent. The crosses were either between different heterotic pools (S/NS), or within a heterotic pool (S/S). The resulting hybrids therefore range from highly heterotic to hybrids exhibiting little heterosis. Yield trials were conducted for all 16 hybrids and 17 inbreds in 1997 and 1999. There were four locations and two replicates per location in each year. At one of these four locations where yield trials

Table 1 Description of inbred parents, their pedigree relationships with inbred S1, and the grain yield of their hybrids when crossed as a male to inbred S1

	Characteristics	Pedigree relationship to S1 inbred (%)	Hybrid yield (bu/acr)	Heterosis (bu/acr)
Male parent				
S3	Mid maturity SS (YD) type Central/eastern US CB adaptation	85.5	67.7	24.9
S4	Mid maturity SS (YD) type Central/western US CB adaptation	45.9	75.3	15.6
S5	Mid/early maturity SS (YD) type	47.3	82.4	36.1
S6	Public line from Iowa SS (100%), YD type	63.9	93.8	52.4
S7	Public line from Iowa SS (100%), YD type	60.5	98.6	60.6
S8	Mid maturity SS (YD) type Eastern/central US CB adaptation	48.8	105.9	56.0
S9	Mid maturity SS (YD) type B37 derivative	62.4	112.4	44.3
S10	Mid maturity SS (YD) type B73 derivative	48.1	113.5	55.2
S11	Mid maturity SS (YD) type Western US CB adaptation	71.0	118.0	66.0
B73	Public US Iowa SS (100%), YD type	20.7	131.9	68.1
NS3	Mid maturity NS (YD) type Central US adaptation	3.1	136.7	53.6
NS4	Mid maturity NS (YD) type Central US adaptation	1.1	142.2	87.1
NS1	Mid maturity NS (YD) type Central US adaptation	0.8	142.6	87.4
NS5	Mid maturity NS (YD) type Central US adaptation	4.5	148.0	88.5
NS6	Mid maturity NS (YD) type Central US adaptation	1.5	156.0	90.8
NS7	Mid maturity NS (YD) type Central US adaptation	2.4	157.8	98.2
Female parent				
S1	Mid maturity Stiff Stalk (YD) type Central/eastern US CB adaptation			

S stiff stalk synthetic, *NS* non-stiff stalk, *YD* reid yellow dent, *CB* corn belt, *S1* the common female parent for the 16 hybrids. Pedigree was used to estimate percentage relationship to the common female parent, S1. Yield heterosis (F1 yield−mid-parent yield) is expressed as bushels/acre (bu/acr)

were conducted, tissue samples were collected for RNA analysis (see below).

For tissue sampling and RNA profiling in 1997, hybrids and inbreds were grown in the field at Johnston, IA and planted at three different times, in the months of April, May and June, approximately one month apart. Such an experimental design was intended to create growing environment variations; thus, we refer to these different plantings as three environmental replicates. Three primary, immature ears of pre-pollination (approximately 6–8 cm in length) at the stage of V19 were collected from three individual plants and pooled as one biological sample from each environmental replicate. One biological sample from each environmental replicate was used for RNA profiling in 1997 except for the common female S1 inbred, in which three biological replicates were submitted for GeneCalling. In 1999, five of the 16 hybrids and their respective inbred parents: S1/S3, S1/S4, S1/S11, S1/NS1, and S1/NS4 were grown in the field at Johnston, IA. Reciprocal hybrids (made by crossing the S1 inbred as a common male parent) of these five were also grown in the same location. Three primary immature ears of pre-pollination at the stage of V19 were collected from three individual plants and pooled as one biological replicate, and three biological replicates were sampled for GeneCalling RNA profiling.

In the density stress treatment experiment, we selected two commercial maize hybrids, S1/NS1 (Pioneer® hybrid 3394) and S2/NS2 (Pioneer® hybrid 3306) that were developed in the 1990s and 1960s, respectively. The parental inbreds S1 and NS1 are described in Table 1. S2 is in the parentage of S1, and both are derived from the same public line plus other Stiff Stalk public and proprietary lines. The NS2 inbred is a Non-Stiff Stalk line (Labate et al. 1997) and is derived from

a cross of two first-cycle lines out of Midwestern dent open-pollinated populations. The NS1 inbred is a line of complex parentage involving NS2. The inbred parents of both hybrids are adapted to the central US Corn Belt and have very similar maturity. The hybrid 3306 is one of the earliest single cross hybrids released commercially by Pioneer Hi-Bred International and is typical of the germplasm that farmers used in the mid-late 1960s, whereas hybrid 3394 was grown most widely in the early 1990s. In contrast to 3306, the improved yield characteristics of 3394 were selected using very different agronomic management practices, including higher plant density and increased levels of nitrogen fertilizer application. The two hybrids, 3394 and 3306 were grown at Johnston, IA in 1999 at three plant densities: 4,000, 18,000, and 35,000 plants per acre, respectively. The same tissue sampling protocol as above was used for RNA expression profiling. Three primary, immature ears of pre-pollination at the stage of V19 were collected from three individual plants and pooled as one biological sample and three biological replicates were used for GeneCalling analysis.

RNA isolation and transcript profiling

Protocols for RNA isolation and GeneCalling profiling have previously been described (Guo et al. 2003, 2004) The tissue was ground to a fine powder in liquid N_2. Total RNA was extracted using TriPure reagent (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to the manufacturer's protocol. Poly-A^+ RNA was purified from total RNA using oligo (dT) magnetic beads (PerSeptive, Cambridge, MA, USA) and quantified by fluorometry. Poly-A^+ RNA was then subjected to GeneCalling analysis as described in Shimkets et al. (1999). Briefly, the following steps were involved in the GeneCalling process. Double-stranded cDNA was synthesized from the mRNA and digested with 48 different pairs of restriction enzymes (6-bp recognition sites). Adapters were ligated to the cDNA fragments, which was then PCR amplified for 20-cycles using adapter-specific primers. After size fractionation on an electrophoresis gel, the fluorescamine (FAM)-labeled PCR products were quantified by a laser scanner into digital tracefiles. The fluorescent intensity from FAM-labeled cDNA fragments is proportional to the abundance of the corresponding mRNA expressed in the given tissue.

The same 48 pairs of PCR primers were used for all the samples in this study, and these cover 80–90% of the expressed genes represented in the mRNA pool from the tissue analyzed (Shimkets et al. 1999). For each primer pair, three independent PCRs were made from an individual mRNA sample. A composite trace is calculated based on the average peak height and variance of the three PCR reactions from each sample (Shimkets et al. 1999). One mRNA sample from each genotype per environmental replicate was analyzed (three PCR reactions), except for one genotype (the S1 inbred) in which three experimental replicates consisting of nine PCR reactions were profiled. We analyzed the three biological replicates of the S1 genotype and did not find a significant difference between the replicates (see Fig. 3d for representative traces).

Data analysis

Using the original GeneCalling data, we selected inter-parental differentially expressed (IPDE) cDNA fragments based on the criteria that the average intensities of the cDNA fragments differed by at least two fold and a *P*-value of 0.01. The IPDE cDNA fragments could be due to expression level differences as well as allelic sequence polymorphism. We chose two fold as an empirical cut-off based on CuraGen's prior analysis on the reproducibility, sensitivity and false-positive rate of the GeneCalling technology (Shimkets et al. 1999). The two fold cut-off is somewhat arbitrary, but is used here, as it has also been used in other studies from maize (Bruce et al. 2000, 2001; Guo et al. 2003). Depending on the expression level of each gene, however, a lower fold change cut-off would favor false positives and a higher fold change cut-off would increase the chance of missing differentially expressed genes.

In order to obtain a quantitative measurement of the F_1 hybrid expression level relative to the mid-parental level for each IPDE cDNA fragment, we adapted the d/a ratio from quantitative genetics as a metric. In this measurement, d (dominant gene action) = F_1 (hybrid) − μ (average of the parents); a (additive gene action) = P_1 (parent 1) − μ. In the case of a complete dominant gene action of the P_1 allele, $F_1 = P_1$ then, $d/a = 1$; similarly, $d/a = -1$ if the other parental allele (P_2) is dominant. In the case of additive gene action, $F_1 = \mu$, then, $d/a = 0$. Using this concept, we considered the RNA expression level as a phenotype of each gene and measured the F1 hybrid expression level relative to the allelic additive expression (Guo et al. 2003). Transcript expression may be affected by multiple loci, the estimates for "a" and "d" for each transcript of interest would represent composite additive and dominance genetic effects, respectively. In the hybrid, the two parents contribute one dose each to the genetic constitution. Additive allelic expression in the hybrid would give a mid-parent level (μ) of $(P_{female} + P_{male})/2$. For each IPDE cDNA fragment, we

first calculated the deviation of the actual hybrid expression level from the average of the parents, as $d = F_1 - \mu$, and then calculated the deviation of the male parent from the average as $a = P_{male} - \mu$. The d/a ratio was then used to measure the hybrid expression level relative to the average of the parental level. If the hybrid expression level is equal to the average expression level, then $d = F_1 - \mu = 0$, which results in $d/a = 0$. Therefore, a zero value of the d/a ratio indicates that the level of expression in the hybrid is the same as the mid-parent value and fits the prediction of allelic additive expression. If the hybrid expression deviates from the average expression level and is biased towards the male parent's level, then the values $d = F_1 - \mu$ and $a = P_{male} - \mu$, would be both negative or both positive, resulting in $d/a > 0$. Likewise, $d/a < 0$ will be obtained if the hybrid expression is biased towards the female parent's level, where the values $d = F_1 - \mu$ and $a = P_{male} - \mu$, would be opposite in sign, one is negative and the other is positive. While the absolute value of the d/a ratio indicates the degree of the deviation from allelic additive expression, the sign of the d/a ratio indicates the direction of the deviation, maternal or paternal.

Results

Hybrid yields and heterosis

In order to examine gene expression patterns in hybrids in relation to heterosis, we crossed a Stiff Stalk (S1) line as a common female parent with 16 different inbred lines as males, that include Stiff Stalk and non-stiff stalk (NS) lines sharing different percentages of pedigree relationship with the female parent (Table 1). This experimental design created a series of hybrids that share a common parent with a wide range in yield and heterosis. Grain yield of hybrids and their inbred parents was tested in 2 years, 1997 and 1999 in four locations with two replicates per location in each year. The grain yield of all hybrids was lower in 1997 than in 1999. In general, 1997 was a low yielding season due to various environmental stresses, including a combination of low temperature and frost early in the season, prolonged local flooding, and water-deficient conditions later in the season. The growing season of 1999 in contrast, was a mild season and resulted in a higher grain yield (Fig. 1). The average yield and heterosis data from the 2 years are shown in Table 1. Yield heterosis was calculated by the yield advantage of the hybrid over the mid-parent values.

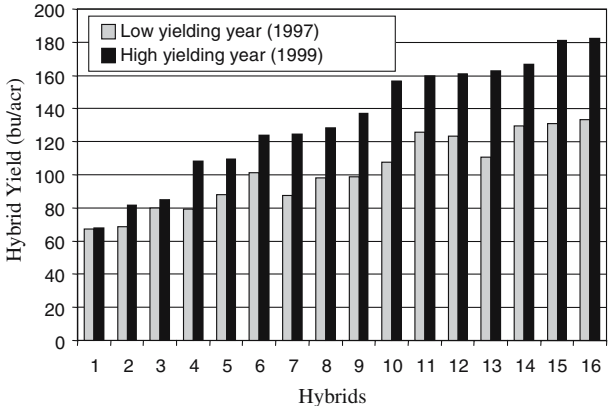

Fig. 1 Hybrid yields from a high yielding year (1997) and a low yielding year (1999). The hybrids are listed in Table 1. Yield was measured in bushels/acre (*bu/acr*) at four locations per year and in two replicates per location. Hybrids are ranked by yield data from 1999. All hybrids yielded higher in 1999 than in 1997

Gene expression differences between the inbred parents

Poly $(A)^+$ RNA from non-pollinated immature ear tissues (V19 stage) of 16 hybrids and 17 inbred parents harvested in 1997 was subjected to GeneCalling analysis as described in Shimkets et al. (1999, Materials and methods). In this study, we used the original GeneCalling data, the profile of each sample consisted of approximately 70,000 cDNA fragments. As described by Shimkets et al. (1999), each transcript can frequently be represented by more than one cDNA fragment using the GeneCalling approach since several restriction fragments from each cDNA can be amplified by this method. In comparing cDNA fragments from different genotypes using GeneCalling, cDNA sequence polymorphism can further complicate the expression analysis. Such polymorphisms can exist at the restriction site used prior to amplification or between sites to generate a restriction fragment length polymorphism. When comparing two inbreds of different genotypes with their F_1 hybrid, a cDNA sequence with no polymorphism will appear as one fragment size in all three genotypes. In contrast, a cDNA fragment that exhibits a sequence polymorphism detected by GeneCalling will yield two fragments of different lengths in the F1 hybrid, but only one or the other of these two fragments in each parental inbred. In either case, it is still valid to determine if the expression level of the hybrid bands deviates from the mid-parent. Indeed, sequence polymorphisms provide an opportunity to determine allele-specific levels of expression in the F_1 hybrid rather than the total expression level produced by the two combined alleles at a given locus.

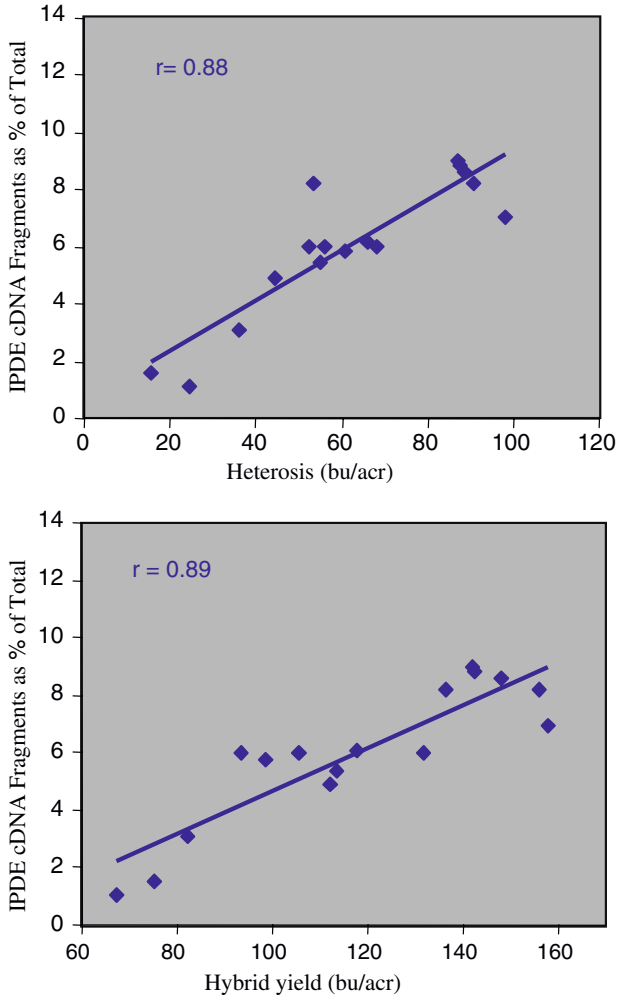

Fig. 2 Correlation between the proportion of Inter-Parental Differentially Expressed (IPDE) cDNA fragments with yield heterosis (*upper panel*) and hybrid yield (*lower panel*). Heterosis was calculated as yield of single cross minus mid-parent yield as shown in Table 1. Yield was measured in bushels/acre (*bu/acr*) in two years, 1997 and 1999, at four locations per year, and two replicates per location. Yield and heterosis data shown are the mean values of the two years

mRNA profiles of the hybrids and inbreds grown in 1997 were obtained from three environmental replicates, and each environmental replicate was sampled as three RT-PCR replicates in GeneCalling analysis. The cDNA fragments that are differentially expressed between the inbred parents by at least two fold were selected for expression analysis in the hybrids, and are hereafter referred to as IPDE (Inter-Parental Differentially Expressed) cDNA (Materials and methods). We found that the proportion of IPDE cDNA fragments varied between 1 and 10% among the different pairs of inbreds chosen as hybrid parents. The percentage of IPDE cDNA fragments correlated positively with hybrid yield and yield heterosis (Fig. 2).

Gene expression in F_1 hybrids relative to the mid-parent prediction

In order to obtain a quantitative measurement of the F_1 hybrid expression level relative to the average of the parental levels for each IPDE cDNA fragment, the *d*/*a* ratio, a metric often employed in quantitative genetics, was used, where "*d*" and "*a*" stand for a dominant and an additive gene action, respectively (Comstock and Robinson 1952; Gardner et al. 1953). This metric has been used for quantitative analysis of transcript level relative to allelic dosage regulation in the maize endosperm (Guo et al. 2003). The *d*/*a* ratio is a measure of the level of gene expression in the hybrid in relation to the allelic dosage, i.e. the mid-parent value. The farther the deviation of the F_1 expression from the mid-parent, the greater the absolute *d*/*a* value. For example, when the F_1 hybrid expresses at the same level as the maternal parent, the *d*/*a* ratio is -1. When the F_1 hybrid expression level is the same as the paternal parent, the *d*/*a* ratio is $+1$. If the hybrid expression level is outside the range of the parents, the *d*/*a* ratio is < -1, if the deviation is towards the maternal parent, and $> +1$ if towards the paternal parent. The *d*/*a* ratio is 0 when the F_1 expression level is the same as the mean of the expression level of the two parents.

If most of the IPDE cDNA fragments exhibit allelic additive expression in the F_1 hybrid, the *d*/*a* ratios should exhibit a normal distribution with the peak at zero and a narrow variance (mostly due to inherent variation of the GeneCalling method). We calculated the *d*/*a* ratio for every IPDE cDNA fragment in each individual hybrid and the distributions of the *d*/*a* ratios from all 16 hybrids (approximately 125,000 data points total) are shown in Fig. 3a. The majority ($\sim 80\%$) of the IPDE cDNA fragments have a *d*/*a* ratio between -1 and $+1$, indicating that, for most genes, the expression level in the hybrid was within the range of the parents. To estimate the proportion of genes that expressed at the approximate mid-parent level, we set an arbitrary cutoff of a *d*/*a* ratio between -0.5 and $+0.5$ and found that among the different hybrids 35–55% of IPDE cDNAs fell within this range of approximate mid-parent expression. The remaining IPDE cDNAs ($\sim 20\%$) were expressed at a level in the F_1 hybrid beyond the range of the two parents, either higher than the higher parent, or lower than the lower parent (*d*/*a* ratio $> +1$ or < -1), but none of these fragments was uniformly under- or over-expressed in all or even a majority of the hybrids analyzed.

We also looked for IPDE cDNA fragments that exhibited similar *d*/*a* ratios between -1 and $+1$ among all hybrids analyzed, but none were conclusively identi-

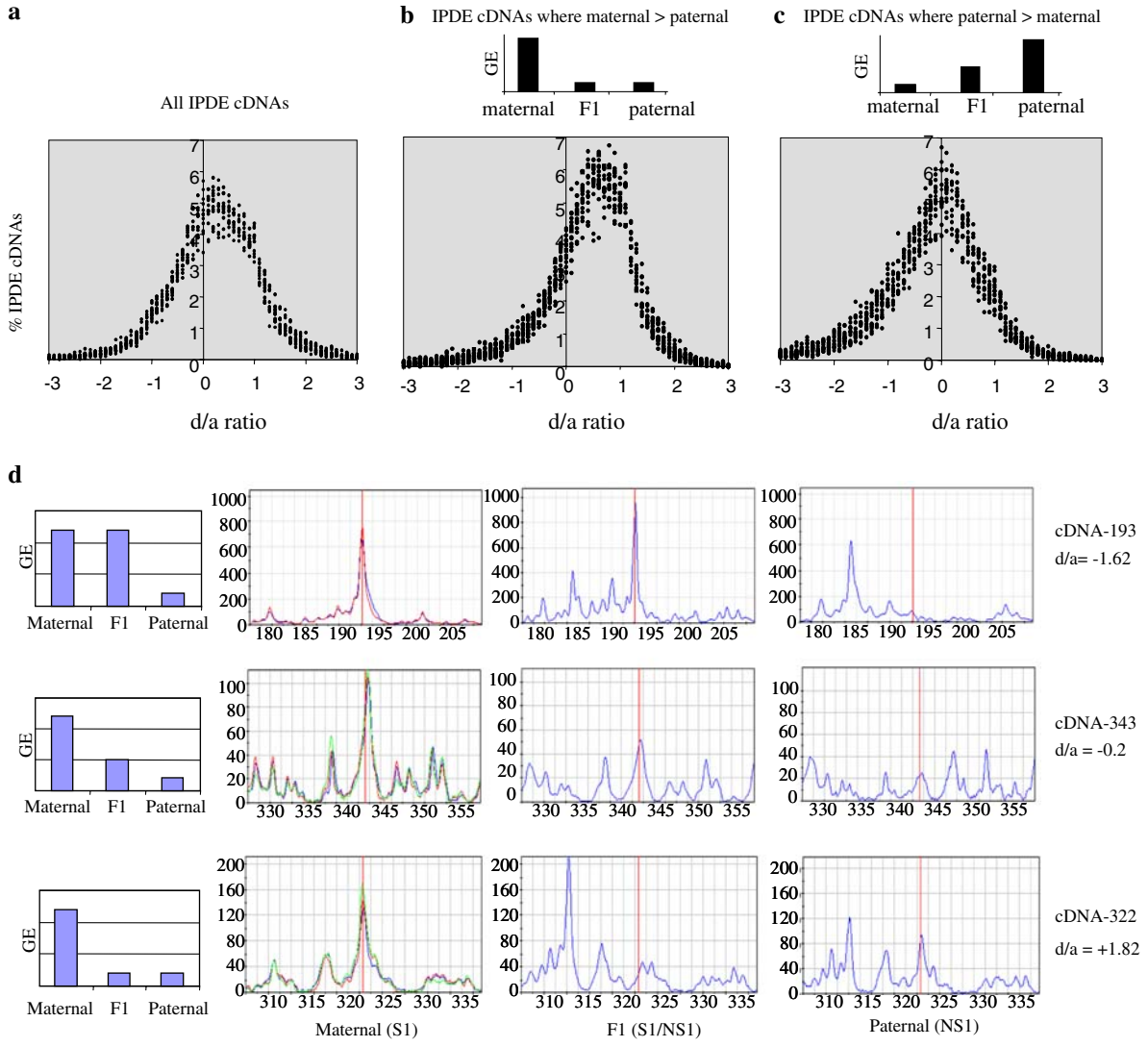

Fig. 3 Distributions of *d*/*a* ratio for IPDE cDNA fragments from 16 hybrids grown in 1997. **a** Hybrid *d*/*a* ratio distribution of all IPDE cDNAs. For each hybrid, IPDE cDNAs were binned by *d*/*a* ratio interval of 0.10. The percentages of IPDE cDNAs in each bin were calculated from the total IPDE cDNA fragments of each individual hybrid. Each data point in **a–c** represents the percentage of IPDE cDNAs in each bin per hybrid, but the total number of IPDE *d*/*a* ratios computed in **(a)** was near 125,000, the summation of all 16 hybrids. **b** Hybrid *d*/*a* ratio distribution of IPDE cDNAs where the level of maternal parent expression was higher than the paternal parent. The diagram above the distribution chart illustrates the predominant pattern where the S1-derived maternal alleles were expressed below mid-parent prediction in the F_1 hybrid. GE on the *y*-axis of the chart represents Gene Expression level. The predominance of this type of gene expression contributed to the bias in the *d*/*a* ratio distribution towards the paternal parent in **(a)**. See also Supplemental Fig. 1. **c** Hybrid *d*/*a* ratio distribution of IPDE cDNAs where the level of paternal parent expression was higher than the maternal parent. The diagram above the distribution chart illustrates expression of these paternal alleles at the mid-parent level in the F_1 hybrid. The predominance of this type of expression results in the symmetric *d*/*a* ratio distribution for cDNAs in which the paternal parent expression was higher than the maternal parent. **d** GeneCalling trace presentation of examples of three expression scenarios in F1 hybrid (S1/NS1) where the expression of an IPDE cDNA fragment is higher in the S1 parent (the common maternal parent) than in the other parent. The top panel represents an IPDE cDNA fragment where the maternal (S1) allele in the hybrid is expressed at a level higher than the mid-parent and biased to the maternal parent's level, resulting in a *d*/*a* ratio of −1.62. The middle panel depicts an instance of additive or mid-parent expression for an IPDE cDNA fragment with a *d*/*a* ratio of −0.2 (near 0). The bottom panel shows the F1 hybrid expression level of an IPDE cDNA fragment below the mid-parent prediction and biased toward the paternal level, resulting in a *d*/*a* ratio of + 1.82. . The higher frequency of genes with under-expression of the maternal S1 allele in the F1 hybrid (as depicted in the bottom panel) contributed to the paternally biased distribution in the *d*/*a* ratio seen in **(a)** and **(b).** The *y*-axis of the GeneCalling traces is RNA expression level in arbitrary units. GeneCalling traces were composite of three PCR replications. For the S1 parent, three biological replicate traces, each a composite of three PCR replications, are shown to indicate reproducibility of the GeneCalling procedure. The traces highlighted with a line correspond to the IPDE cDNA fragments described. The schematic diagrams on the left of each panel depict simplified versions of the expression patterns shown. GE on the *y*-axis of the diagram represents Gene Expression level

fied. Unfortunately, both the high rate of sequence polymorphism in maize and the nature and resolution of the GeneCalling methodology made it difficult to confirm if IPDE cDNA fragments of a given size (± 0.5 base pair; Shimkets et al. 1999) that exhibited similar *d*/*a* ratios among several hybrids were identical in sequence in every electrophoresis lane. Considerable further work (i.e., fragment isolation, cloning and sequencing) would be required to investigate this in each instance. Thus, the focus for the remainder of this study was on global trends in expression rather than on the correlation between the expression pattern of any specific gene and heterosis.

Deviation of hybrid gene expression from the mid-parent prediction and allele-specific regulation

The data in Fig. 3a indicate that hybrid gene expression can approximate the mid-parental level, be similar to either the maternal or paternal level or fall outside the parental range. However, the distribution of the *d*/*a* ratios in Fig. 3a is noticeably biased towards the paternal parent. To investigate this further, we analyzed *d*/*a* ratios of IPDE cDNA fragments separated into two groups: those where the expression level in the maternal parent is higher than the paternal parent (Fig. 3b) and vice versa (Fig. 3c). This analysis showed that IPDE cDNAs that had a higher expression level in the maternal parent (inbred S1) are mainly responsible for the biased *d*/*a* distribution seen in Fig. 3a (Fig. 3b), whereas those IPDE cDNAs expressed at a higher level in the paternal parents generally exhibit mid-parent expression and the *d*/*a* ratio distribution for this class of IPDE cDNAs is symmetric (Fig. 3c). The difference is exemplified by the proportion of IPDE cDNAs with a *d*/*a* ratio between − 0.5 and + 0.5, which was 32–46% among hybrids for the data in Fig. 3b and 41–54% among hybrids for the data in Fig. 3c. Thus, in this series of F1 hybrids, maternal S1-derived alleles that are more highly expressed than their paternal counterparts are generally expressed at a level lower than that predicted by the null hypothesis of allelic additivity. This results in hybrid expression closer to the paternal level and, as a consequence, the distribution of the *d*/*a* ratio is paternally biased, as seen in Fig. 3a, b.

Figure 3d shows examples of GeneCalling traces representing three scenarios of S1-derived maternal allele expression in a representative F_1 hybrid (S1/NS1) from this series: hybrid expression at the same level as maternal parent S1 (top panel), at the mid-parent level (middle panel), and below the mid-parent level (bottom panel), respectively. The predominance of genes that exhibited under-expression of the more active maternal S1-derived allele (as shown in the bottom panel in Fig. 3d) in this series of F_1 hybrids contributes to the biased *d*/*a* ratio distribution seen in Fig. 3a, b.

Next, we analyzed the IPDE cDNA *d*/*a* ratio distributions for each of the 16 hybrids individually and found that, in every case, the *d*/*a* ratio distribution is biased towards the non-common, paternal parent, but varies in the degree, with lower yielding hybrids showing more bias, i.e., having more genes expressed closer to the paternal level. The trend becomes more obvious when the *d*/*a* ratio distributions of fewer hybrids with bigger differences in heterosis [(S1/S3, S1/S11 and S1/NS1) out of the 16 hybrids shown in Fig. 3b] are visualized (Supplemental Fig. 1). We calculated the proportion of the IPDE cDNA fragments that fell into the range of approximate mid-parent expression (*d*/*a* ratio of − 0.5 to + 0.5) and those that were biased toward the paternal parent (*d*/*a* ratio > + 1), respectively in each hybrid. The proportion of IPDE cDNA fragments in the mid-parent expression category is highest in high yielding hybrids exhibiting the most heterosis. As shown in Fig. 4a, there is a significant positive correlation between hybrid yield/heterosis and the percentage of genes with mid-parent expression. In contrast, a significant negative correlation is observed between hybrid yield/heterosis and the percentage of IPDE cDNA fragments in the paternally biased expression category (Fig. 4b, Supplemental Fig. 1).

Hybrid gene expression in relation to stress

To test whether different growing environments affect the gene expression pattern in F_1 hybrids, we repeated RNA profiling of five of the initially selected hybrid/inbred combinations grown in a different year (1999). Two of the hybrids chosen (S1/S3, S1/S4) were lower yielding, two (S1/NS1, S1/NS4) were high yielding and one (S1/S11) had intermediate yield.

IPDE cDNA fragments were selected as before and the distribution of their *d*/*a* ratios is shown in Fig. 5a. We also included hybrids of the reciprocal crosses (i.e. hybrids in which S1 was a common female parent, as well as those in which S1 was a common male parent) in this second experiment (Fig. 5b). No difference in the *d*/*a* distributions was found between hybrids of reciprocal crosses in the growing season of 1999. Among reciprocal hybrids, we found no differences in the *d*/*a* distributions for IPDE cDNA fragments where expression in the female parent was higher than the male or vice versa (Supplemental Fig. 2). Similarly, there was very little difference in the proportion of IPDE cDNA fragments that showed mid-parent or

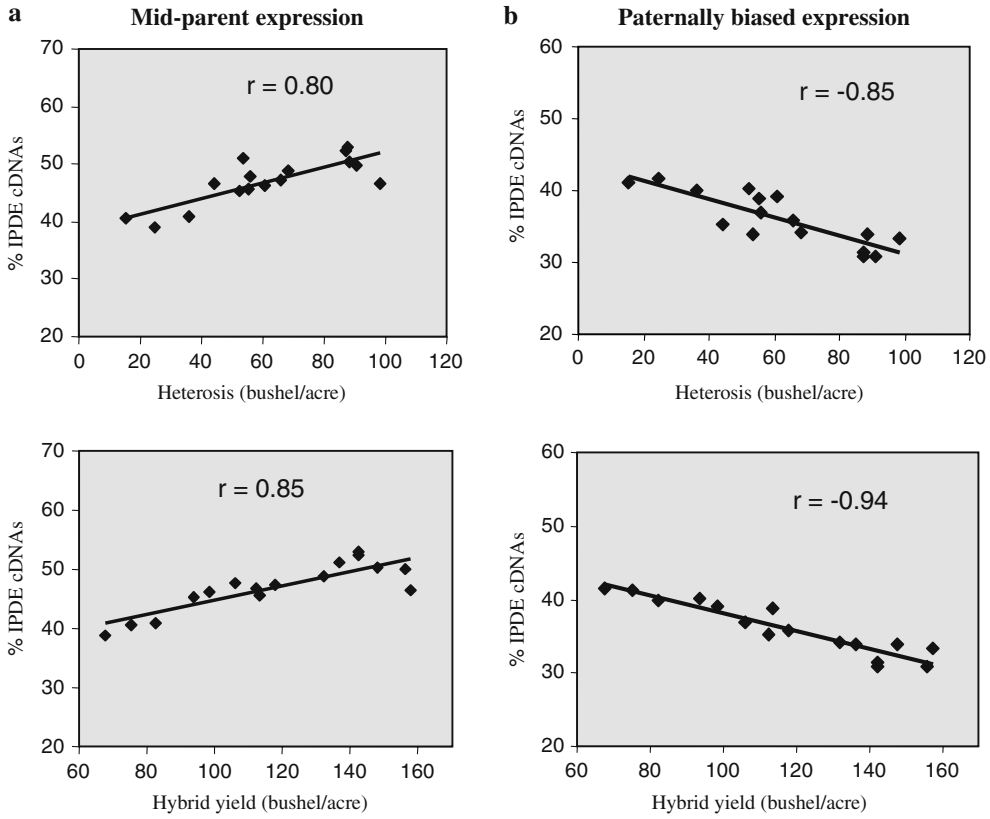

Fig. 4 Correlation between patterns of gene expression with yield heterosis and hybrid yield. **a** Significant positive correlations ($P < 0.001$) between the percentage of IPDE cDNAs with mid-parent expression and yield heterosis (*upper panel*) and hybrid yield (*lower panel*). **b** Significant negative correlations ($P < 0.001$) between the percentage of IPDE cDNAs with paternally biased expression and yield heterosis (*upper panel*) and hybrid yield (*lower panel*). Expression profiling data from three environmental replicates were combined. The heterosis of grain yield was calculated in bushel per acre as F_1 yield–mid-parent yield

paternally biased expression between reciprocal hybrids (Supplemental Fig. 3).

As compared to the first experiment performed in 1997, the *d*/*a* ratio distributions for IPDE cDNA fragments in 1999 peak around zero and are not biased toward the paternal parent (Fig. 5a, b). When we compared the two seasons for their mid-parent versus paternally biased gene expression, we found that hybrids grown in 1999 had a much higher proportion of genes with the mid-parent expression and a concomitantly lower proportion of paternally biased expression, as compared to those in 1997 (Fig. 6a). 1999 was a mild and high yielding season, whereas 1997 was a stressed and low yielding season. The difference between the two years' growing environment was reflected in the higher grain yields seen in 1999 (Fig. 1). The results suggest that environmental stress reduced the proportion of genes that were expressed in an allelic additive manner in the hybrid, and that at many loci, each parental allele can respond differentially to environmental stress in hybrids.

To further test whether adverse or stressed environments can contribute to hybrid gene expression, we conducted a density study using two contrasting hybrids, a modern hybrid, Pioneer ® hybrid 3394 (S1/NS1) and a less improved hybrid, Pioneer ® hybrid 3306 (S2/NS2). The hybrid 3306 is one of the earliest single cross hybrids released commercially by Pioneer Hi-Bred International and is typical of the germplasm that farmers used in the mid-late 1960s, whereas hybrid 3394 was grown most widely in the early 1990s. In contrast to 3306, the improved yield characteristics of 3394 were selected using very different agronomic management practices, including higher plant density and increased levels of nitrogen fertilizer application. As has been noted previously, the yield gains of corn hybrids over time have primarily resulted from genetic improvement in tolerance to both biotic and abiotic stresses (Duvick 2001; Duvick et al. 2004). We sought to compare the stress-tolerant modern hybrid, 3394, with the stress-susceptible less improved hybrid, 3306, by growing them under increasing levels of plant density. If the deviation of mid-parent expression in the

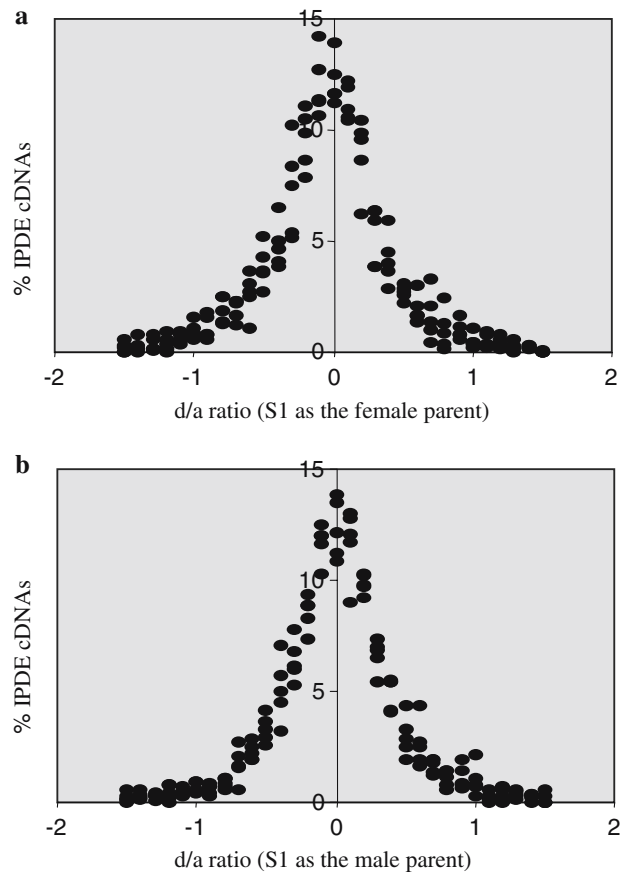

Fig. 5 Distributions of the IPDE cDNA *d*/*a* ratio for hybrids grown in 1999. Five of the sixteen hybrids were grown in 1999, as described in the text. Data for the original hybrids (where S1 was used as the female parent: *upper panel*, **a**) and hybrids of reciprocal crosses (where S1 was used as the male parent: *lower panel*, **b**) are shown. All IPDE cDNA fragments (approximately 7,500) are included in the analysis, and the data were binned for graphical representation, as described in the legend to Fig. 3

hybrid is related to a stressed growing condition, the deviation should be greater in both hybrids grown under higher plant density than in low density, and be more pronounced in the stress-susceptible hybrid, 3306, than in the more stress-tolerant hybrid, 3394.

We profiled three biological replicates of the two hybrids and their inbred parents grown at three population densities. The relationship between the proportions of these IPDE cDNAs exhibiting mid-parent or paternally biased gene expression and plant density is shown in Fig. 6b. In both hybrids, the percentage of genes with paternally biased expression increased as the level of plant density stress increased from 4,000 to 35,000 plants per acre. The higher yielding, stress tolerant hybrid 3394 had a higher percentage of genes with mid-parent expression and a lower percentage of paternally biased expression as compared to the lower yielding and stress susceptible hybrid, 3306. Thus, the results were consistent with our prediction about the relationship between these two patterns of hybrid gene expression, hybrid yield and plant density stress (i.e., environmental stress).

Discussion

In this study, we used GeneCalling, a genome-wide transcript profiling method that is widely used in several plant species (Crasta and Folkerts 2003; Shimkets et al. 1999), to gain a global view of the level of expression of a large proportion of genes during one stage of development (immature ear tissue at stage V19) in a carefully chosen series of maize hybrids and their inbred parents. We also attempted to address whether specific patterns of expression are associated with differences in heterosis and/or hybrid yield both among genotypes and in different environments with varying levels of stress.

We focused our subsequent analysis on the subset of cDNA fragments that were differentially expressed between the parents of each hybrid analyzed (IPDE cDNAs, as defined in Results). The majority ($\sim 80\%$) of IPDE cDNAs were expressed in each hybrid at levels between that of the two inbred parents (i.e., where $-1 < d/a < +1$), and 35–55% were expressed more or less additively at or near the mid-parental value (i.e., where $-0.5 < d/a < +0.5$). About 20% of IPDE cDNAs were expressed in hybrids at higher or lower levels than either inbred parent (i.e., where $d/a < -1$ or $d/a > +1$). However, we were unable to identify any specific set of IPDE cDNA fragments that was consistently up- or down-regulated in all hybrids, indicating that neither pattern of gene expression in immature ear tissue is generally associated with either heterosis or hybrid yield in maize. It should be pointed out that our analysis was restricted to IPDE cDNAs: thus, cDNAs that show no difference in expression between inbred parents but that are consistently up- or down-regulated in hybrids would not have been identified. In other gene-expression profiling studies of inbreds and hybrids, genes that are up- or down-regulated in hybrids have been seen previously, and genes that are consistently up- or down-regulated between reciprocal crosses have been identified (Auger et al. 2005; Bao et al. 2005; Gibson et al. 2004; Gibson and Weir 2005; Guo et al. 2003; Kollipara et al. 2002; Swanson-Wagner et al. 2006; Vuylsteke et al. 2005). However, no previous study has involved such an extensive series of hybrids as that presented here, so it will be interesting to see if our observations hold up in other species.

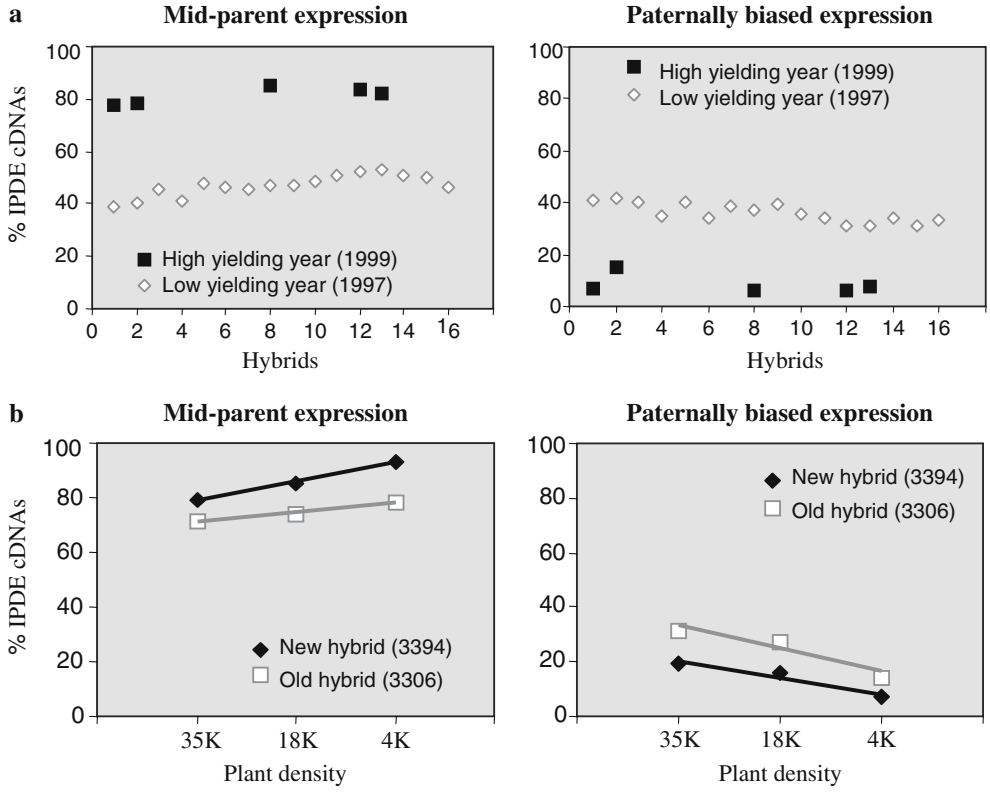

Fig. 6 Patterns of hybrid gene expression in response to environmental stresses (**a**) and comparison between a modern stress-tolerant hybrid and an older stress-susceptible hybrid (**b**). **a** Comparison between hybrids grown in two seasons (sixteen hybrids in 1997 and five of the same set in 1999). The percentages of IPDE cDNAs with mid-parent expression and paternally biased expression increased and decreased, respectively, in the high yielding season of 1999, as compared to the low yielding season of 1997. The hybrids were ranked by their yield data from low to high based on data from 1999 (see Fig. 1). **b** Gene expression patterns of a modern stress-tolerant hybrid (3394) and an older stress-susceptible hybrid (3306) grown under three different plant densities: 35,000 (35 K), 18,000 (18 K), and 4,000 (4 K) plants/acre

In contrast to the absence of association between under- or over-expressed genes and heterosis, we found that the proportion of additively expressed IPDE cDNAs correlates well with both yield and heterosis amongst hybrid genotypes and with yield across environments that vary in the overall level of stress. We also found that a fraction of IPDE cDNAs exhibit a paternal expression bias in hybrids. This subset of IPDE cDNAs is not necessarily identical or even extensively overlapping between different hybrids. But, in general, paternally biased IPDE cDNAs are expressed at higher levels in the female parent than in the male, suggesting an under expression of the maternal allele in the hybrid. The proportion of paternally biased IPDE cDNAs correlates negatively with yield and heterosis amongst hybrid genotypes and between environments. In contrast, the proportions of genes with expression biased toward the maternal parent showed little correlation with yield or heterosis (data not shown).

Could the associations between different classes of IPDE cDNA observed in this work be due to systematic bias or some other artifact of the GeneCalling methodology? Systematic bias could arise if the assumptions of mRNA sampling and normalization are incorrect or if there were differences in mRNA extraction or quantitation among genotypes, neither of which we find convincing as arguments for the trends observed. With GeneCalling, we used an equal amount of mRNA from each genotype, extracted and quantified by uniform methods. The normalization is therefore based on the assumption that each hybrid and its inbred parents express the same amount (i.e., mass) of total mRNA. This assumption has been the basis of global normalization in most of the genome-wide RNA profiling studies involving hybrid-inbred comparisons (Bao et al. 2005; Guo et al. 2003; Kollipara et al. 2002; Vuylsteke et al. 2005). An alternative normalization strategy is based not on mRNA, but on the level of total RNA (Auger et al. 2005) which is comprised mostly of ribosomal RNA. Unfortunately, there are no data to validate which approach is more appropriate than the other, or if the interpretation of the results obtained here would be any different following the alternative normalization strategy.

By profiling a series of 16 hybrids made between a common female inbred and 16 different male inbreds that varied in genetic relatedness to the female parent, we found that the percentage of IPDE cDNAs was positively correlated with hybrid yield and yield heterosis (Fig. 2). This correlation is highly reminiscent of the correlation between restriction fragment length polymorphisms and hybrid yields observed previously (Smith et al. 1990; Smith and Smith 1992), and, as explained in Results, sequence polymorphism may be a contributing factor to the trends seen in Fig. 2. Because there may also be an association between genomic polymorphism and gene expression variation, the interpretation of Fig. 2 may be further confounded. However, even if some fraction of the IPDE cDNA fragments scored by GeneCalling represents allelic transcripts as a result of sequence polymorphism, this does not invalidate the subsequent analysis we employed using the *d*/*a* ratio as a metric. Two other observations further suggest that sequence polymorphism may be only a minor contributor to many of the conclusions we have made in this study. First, if all the IPDE cDNA fragments were due to sequence polymorphism and there was no allele-specific variation in gene expression, the distribution of *d*/*a* ratios should peak at zero and have a near-zero variance, which we never observed (Figs. 3a–c and 5; Supplemental Fig. 2). Second, the fact that the proportions of mid-parent and paternally biased IPDE cDNA fragments vary not only between genotypes, but also between different environments when the genotype is constant (Fig. 6), indicates that a substantial proportion of IPDE cDNA fragments cannot be due to sequence polymorphism per se, but must be due to differential regulation of parental alleles in each hybrid.

To further test the relationship of the expression patterns with hybrid performance and environmental stress, we analyzed gene expression of two contrasting hybrids, a genetically improved modern hybrid (3394) versus a less improved older hybrid (3306), grown under different levels of stress imposed by increasing plant density. The older hybrid 3306 is one of the earliest commercially available single-cross hybrids and was developed in the early 1960s, whereas hybrid 3394 was developed in the 1990s and is known for its tolerance to environmental stress. Consistent with our a priori predictions, the older lower yielding hybrid (3306) had more paternally biased IPDE cDNAs and fewer additively expressed IPDE cDNAs than the more modern higher yielding hybrid (3394) developed some 30 years later. When both hybrids were deliberately subjected to environments of elevated stress by increasing plant density, the proportion of paternally biased IPDE cDNAs increased concomitantly with a decrease in both the proportion of additively expressed IPDE cDNAs and the yield per plant.

Commercial maize breeding involves selection of hybrid genotypes that exhibit high yield in both stressful and high yield environments (Janick 1999). The yield gains of improved hybrids are primarily due to the genetic improvement in tolerance to both biotic and abiotic stresses (Duvick 2001; Duvick et al. 2004). The available data suggest that genetic improvement through breeding selection might involve selection for regulatory allelic variants that respond to stress. The role of selection in shaping gene expression patterns has been observed previously in various other organisms including *Escherichia coli* (Gall et al. 2005), *Drosophila* (Ranz et al. 2003) and primates (Enard et al. 2002; Ogura et al. 2004). Because heterotic combinations of maize inbreds are selected on the basis of testcross yield, the method most commonly used in breeding of new maize hybrids may be ideally suited for selecting patterns of coordinated allelic regulation in the hybrid state. Previous observations of the contrasting allele-specific expression patterns and stress responses between the older maize hybrid 3306 and the modern hybrid 3394 are consistent with this idea (Guo et al. 2004). Bi-allelic expression is more prevalent in the high yielding modern hybrid and unstressed environments, whereas mono-allelic expression is more prevalent in the low yielding hybrid and stressed environments.

Results from this study and previous work (Guo et al. 2003, 2004) suggest that deviation from mid-parent expression is due to differential allele regulation in the hybrids. Our previous GeneCalling study with triploid hybrid endosperm demonstrated that allelic additive expression is the norm and deviation from allelic additive gene expression is indicative of down-regulation of one allele in the hybrid state due to either genomic imprinting or heterochronic allelic regulation (Guo et al. 2003). Similarly, in diploid immature ear and seedling tissues of maize hybrid S1/NS1 (3394), differential allelic expression has been documented at the cumulative transcript level both during development and in response to stress (Guo et al. 2004).

Several additional findings from our previous studies (Guo et al. 2003, 2004) are also consistent with the results of this work: (1) alleles are often differentially regulated in different tissues of maize hybrids; (2) the S1 inbred alleles appear to be down-regulated more often than the allele of other parent [among eleven examples of allele-specific regulation documented by Guo et al. (2004), nine exhibit down-regulation of the S1 allele]; (3) mono-allelic expression is more frequent

in 3306 than in 3394 and in stressed versus unstressed environments; and (4) analysis of reciprocal cross hybrids showed that a parental effect was not involved in the differential allele regulation. In summary, our data suggest that deviations from mid-parent gene expression observed in the current study reflect the differential regulation and environmental responses of the parental alleles at multiple loci in each hybrid. The paternally biased expression is likely attributed to the under expression of the allele of the common maternal parent.

Two key questions that remain to be answered are: (1) what are the regulatory mechanisms responsible for the deviation from allelic additive expression in maize hybrids; and (2) are the patterns of additive and paternally biased gene expression observed here in maize hybrids the cause or consequence of heterosis and/or higher yield.

If differences in allelic regulation are due to *cis*-regulatory divergence, allelic expression is expected to be additive in the hybrids. Numerous studies (Cowles et al. 2002; Doss et al. 2005; Gibson and Weir 2005; Guo et al. 2004; Schadt et al. 2003; Wittkopp et al. 2004; Yan et al. 2002) have shown that such *cis*-regulatory differences play a major role in the regulation of allelic expression regulation in hybrids of yeast, plants, insects and mammals. Recent findings have shown that genomic DNA sequences among maize inbred lines not only differ by single nucleotide polymorphisms and other minor insertions or deletions (Bhattramakki et al. 2002; Ching et al. 2002), but also exhibit rearrangements, are non-collinear, and can show differences in gene copy number, all of which may be important genetic factors affecting allele-specific expression (Brunner et al. 2005; Fu and Dooner 2002; Song and Messing 2003). However, when alleles are brought together in hybrid combination, they may also be exposed to a different spectrum of trans-acting factors as compared to those in the parental inbreds. As a result, allelic expression in inbred parents could be different from that in the hybrid and the combined allelic expression at any given locus may deviate significantly from additivity (Auger et al. 2005; Birchler et al. 2005; Bao et al. 2005; Gibson et al. 2004; Gibson and Weir 2005; Vuylsteke et al. 2005). Trans-regulation has been shown to affect 55% of the genes with divergent expression between *Drosophila* species (Wittkopp et al. 2004) and up to 80% of the genes segregating in an *Arabidopsis* recombinant inbred population (B. Bowen, C. Haudenschild, S. DeCola, J–Z. Lin, B. Rauh and E. Buckler IV, unpublished data). Trans-acting factors are also known to play an important role in allelic regulation in maize (Birchler et al. 2005; Doebley 2004; Guo and Birchler 1994; Szalma et al. 2005), yeast (Papp et al. 2003; Yvert et al. 2003) and mammals (Schadt et al. 2003). Finally, the interaction between different sets of trans-acting factors and allele-specific *cis*-regulatory sequences may contribute further to the dynamics of the allelic additive expression and its deviation. However, whether the regulatory mechanisms occur at the level of transcription initiation, rate of transcription, post-transcription (RNA stability), or involve epigenetic regulation, remains to be investigated.

The key observations made in this study are: (1) the proportion of additively expressed genes appears to be positively associated with maize yield and heterosis; (2) there exists a fraction of genes that exhibit a bias towards the expression level of the paternal parent and an increase in proportion of these genes is negatively correlated with maize yield and heterosis; and (3) there does not appear to be any relationship between the over- or under-expression of specific genes in maize hybrids and either yield or heterosis. In addition, our observations have added to the growing body of evidence that differential allelic expression is not only commonly seen in diploid plant and animal tissues, but also is an important contributor to the natural genetic variation of quantitative traits and human diseases (Cong et al. 2002; Doebley 2004; Knight 2004; Yan et al. 2002). Whether these associations are responsible for heterosis and/or increased yield in maize or are merely an additional consequence of these phenotypes remains to be determined. At the very least, however, any model attempting to explain heterosis in maize at the molecular level must account for these associations in addition to other parameters associated with this phenomenon that have been documented in the literature (Birchler et al. 2003; Duvick et al. 2004). Given that a 50% yield increase in grain production is estimated to be required by the year 2030 to meet the demand from worldwide population growth (Horton 2000), and that breeders have apparently improved maize yields in the past without increasing heterosis except when hybrids are grown under severe abiotic stress (Duvick et al. 2004; Duvick 2005), future research in this area may lead to novel methods that will accelerate genetic gain.

Novel materials described in this publication may be available for non-commercial research purposes upon acceptance and signing of a material transfer agreement. In some cases, such materials may contain or be derived from materials obtained from a third party. In such cases, distribution of material will be subject to the requisite permission from any third-party owners, licensors or controllers of all or parts of the material.

Obtaining any permission will be the sole responsibility of the requestor. Plant germplasm and transgenic material will not be made available except at the discretion of the owner, and then only in accordance with all applicable governmental regulations.

Acknowledgments We thank S. Zhao, W. Bruce, and B. Zeka for tissue sampling and RNA preparation; R. Luedtke, D. Ritland, and L. Heetland for field experiment support; O. Folkerts, T. Jarvie, P. Pochart, G. Vijayadamodar, and D. Marks for their support in the GeneCalling technology and database development from the CuraGen Corporation. We specially thank D. Duvick and J. Birchler for critical reading of the manuscript.

References

Auger DL, Gray AD, Ream TS, Kato A, Coe EH Jr, Birchler JA (2005) Nonadditive gene expression in diploid and triploid hybrids of maize. Genetics 169:389–9397

Bao J, Le S, Chen C, Zhang X, Zhang Y, Liu S, Clark T, Wang J, Cao M, Yang H, Wang S, Yu J (2005) Serial analysis of gene expression study of a hybrid rice strain (LYP9) and its parental cultivars. Plant Physiol 138:1216–1231

Bhattramakki D, Dolan M, Hanafey M, Wineland R, Vaske D, Register JC III, Tingey S, Rafalski A (2002) Insertion-deletion polymorphisms in 3′ regions of maize genes occur frequently and can be used as highly informative genetic markers. Plant Mol Biol 48:539–547

Birchler JA, Auger DL, Riddle NC (2003) In search of the molecular basis of heterosis. The Plant Cell 15:2236–2239

Birchler JA, Riddle NC, Auger DL, Veitia RA (2005) Dosage balance in gene regulation: biological implications. Trends Genet 21:219–226

Brunner S, Fengler K, Morgante M, Tingey S, Rafalski A (2005) Evolution of DNA sequence nonhomologies among maize inbreds. The Plant Cell 17:343–360

Bruce W, Desbons P, Crasta O, Folkerts O (2001) Gene expression profiling of two related maize inbred lines with contrasting root-lodging traits. J Exp Bot 52:459–468

Bruce W, Folkerts O, Garnaat C, Crasta O, Roth B, Bowen B (2000) Expression profiling of the maize flavonoid pathway genes controlled by estradiol-inducible transcription factors CRC and P. The Plant Cell 12:65–80

Ching A, Caldwell KS, Jung M, Dolan M, Smith OS, Tingey S, Morgante M, Raflaski AJ (2002) SNP frequency, haplotype structure and linkage disequilibrium in elite maize lines. BMC Genet 3:19

Comstock RE, Robinson HF (1952) Estimation of average dominance of genes. In: Gowen JW (ed) Heterosis. Iowa State College Press, Ames, Iowa, pp. 494–516

Cong B, Liu J, Tanksley SD (2002) Natural alleles at a tomato fruit size quantitative trait locus differ by heterochronic regulatory mutations. Proc Natl Acad Sci 99:13606–13611

Cowles CR, Hirschhorn JN, Altshuler D, Lander ES (2002) Detection of regulatory variation in mouse genes. Nat Genet 32:432–437

Crasta OR, Folkerts O (2003) Open architecture expression profiling of plant transcriptomes and gene discovery using GeneCalling® technology. In: Grotewold E (ed) Plant functional genomics: methods and protocols. Humana, Totawa, NJ, pp 381–394

Doebley J (2004) The genetics of maize evolution. Ann Rev Genet 38:37–59

Doss S, Schadt EE, Drake TA, Lusis AJ (2005) *cis*-acting expression quantitative trait loci in mice. Genome Res 15:681–691

Duvick DN (2001) Biotechnology in the 1930s: the development of hybrid maize. Nat Genet Rev 2:69–74

Duvick DN (2005) The contribution of breeding to yield advances in maize (*Zea mays L.*). In: Sparks DN (ed) Adv Agron, Vol. 86. Academic, San Diego, pp 83–145

Duvick DN, Smith JSC, Cooper M (2004) Long-term selection in a commercial hybrid maize breeding program. In: Janick J (ed) Plant breeding reviews, Vol. 24, part 2. Long term selection: crops, animals, and bacteria. Wiley, New York, pp 109–151

Enard W, Khaitovich P, Klose J, Zollner S, Heissig F, Giavalisco P, Nieselt-Struwe K, Muchmore E, Varki A, Ravid R, Doxiadis G, Bontrop R, Pääbo S (2002) Intra- and interspecific variation in primate gene expression patterns. Science 296:340–343

Fu H, Dooner HK (2002) Intraspecific violation of genetic colinearity and its implications in maize. Proc Natl Acad Sci 99:9573–9578

Gall T, Darlu LP, Escobar-Páramo P, Picard B, Denamur E (2005) Selection-driven transcriptome polymorphism in *Escherichia coli/Shigella* species. Genome Res 15:260–268

Gardner CO, Harvey PH, Comstock RE, Robinson HF (1953) Dominance of genes controlling quantitative characters in maize. Agron J 45:186–191

Gibson G, Weir B (2005) The quantitative genetics of transcription. Trends Genet 21:616–623

Gibson G, Riley-Berger R, Harshman L, Kopp A, Vacha S, Nuzhdin S, Wayne M (2004) Extensive sex-specific non-additivity of gene expression in *Drosophila melanogaster*. Genetics 167:1791–1799

Guo M, Birchler JA (1994) Trans-acting dosage effects on the expression of model gene systems in maize aneuploids. Science 266:1999–2002

Guo M, Rupe MA, Danilevskaya ON, Yang X, Hu Z (2003) Genome-wide mRNA profiling reveals heterochronic allelic variation and a new imprinted gene in hybrid maize endosperm. The Plant J 36:30–44

Guo M, Rupe MA, Zinselmeier C, Habben J, Bowen BA, Smith OS (2004) Allelic variation of gene expression in maize hybrids. The Plant Cell 16:1707–1716

Horton P (2000) Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. J Exp Bot 51:475–485

Janick J (1999) Exploitation of heterosis: uniformity and stability. In: Coors JG, Pandey S (eds) The genetics and exploitation of heterosis in crops (American Society of Agronomy, Inc. Crop Science Society of America, Inc.) Madison, pp 319–333

Knight JC (2004) Allele-specific gene expression uncovered. Trends Genet 20:113–116

Kollipara KP, Saab IN, Wych RD, Lauer MJ, Singletary GW (2002) Expression profiling of reciprocal maize hybrids divergent for cold germination and desiccation tolerance. Plant Physiol 129:974–992

Labate JA, Lamkey KR, Lee M, Woodman WW (1997) Molecular genetic diversity after reciprocal recurrent selection in BSSS and BCCB1 maize populations. Crop Sci 37:416–423

Leonardi A, Damerval C, Herbert Y, Gallais A, de Vienne D (1991) Association of protein amount polymorphism (PAP) among maize lines with performances of their hybrids. Theor Appl Genet 82:552–560

Ogura A, Ikeo K, Gojobori T (2004) Comparative analysis of gene expression for convergent evolution of camera eye between octopus and human. Genome Res 14:1555–1561

Papp B, Pal C, Hurst LD (2003) Dosage sensitivity and the evolution of gene families in yeast. Nature 424:194–197

Ranz JM, Castillo-Davis CI, Meiklejohn CD, Hartl DL (2003) Sex-dependent gene expression and evolution of the Drosophila transcriptome. Science 300:1742–1745

Romagnoli S, Maddaloni M, Livini C, Motto M (1990) Relationship between gene expression and hybrid vigor in primary root tips of young maize (*Zea mays L*) plantlets. Theor Appl Genet 80:767–775

Schadt EE, Monks SA, Drake TA, Lusis AJ, Che N, Colinayo V, Ruff TG, Milligan SB, Cavet G, Linsley PS, Mao M, Stoughton RB, Friend SH (2003) Genetics of gene expression surveyed in maize, mouse and man. Nature 422:297–302

Shimkets RA, Lowe DG, Tai JT, Sehl P, Jin H, Yang R, Predki PF, Rothberg BEG, Murtha MT, Roth ME, Shenoy SG, Windemuth A, Simpson JW, Simons JF, Daley MP, Gold SA, McKenna MP, Hillan K, Went GT, Rothberg JM (1999) Gene expression analysis by transcript profiling coupled to a gene database query. Nat Biotech 17:798–803

Shull GH (1908) The composition of a field of maize. Am Breed Assoc Rep 4:296–301

Smith OS, Smith JSC (1992) Measurement of genetic diversity among maize hybrids; a comparison of isozymic, RFLP, pedigree, and heterosis data. Maydica 37:53–60

Smith OS, Smith JSC, Bowen SL, Tenborg RA, Wall SJ (1990) Similarities among a group of elite maize inbreds as measured by pedigree, F1 grain yield, heterosis, and RFLPs. Theor Appl Genet 80:833–840

Song RT, Messing J (2003) Gene expression of a gene family in maize based on non-collinear haplotypes. Proc Natl Acad Sci 100:9055–9060

Swanson-Wagner RA, Jia Y, DeCook R, Borsuk LA, Nettleton D, Schnable PS (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F1 hybrid and its inbred parents. PNAS 103:6805–6810

Szalma SJ, Buckler ES IV, Snook ME, McMullen MD (2005) Association analysis of candidate genes for maysin and chlorogenic acid accumulation in maize silks. Theor Appl Genet 110:1324–1333

Tsaftaris AS (1995) Molecular aspects of heterosis in plants. Physiol Plant 94:362–370

Tsaftaris AS, Kafka M, Polidoros A, Tani E (1999) Epigenetic changes in Maize DNA and Heterosis. In: Coors JG, Pandey S (eds) The genetics and exploitation of heterosis in crops. (American Society of Agronomy, Inc. Crop Science Society of America, Inc.) Madison, pp 195–203

Vuylsteke M, van Eeuwijk F, van Hummelen P, Kuiper M, Zabeau M (2005) Genetic analysis of variation in gene expression in *Arabidopsis thaliana.* Genetics 171:1267–1275

Wittkopp P, Belinda J, Haerum K, Andrew GC (2004) Evolutionary changes in *cis* and *trans* gene regulation. Nature 430:85–88

Yan H, Yuan W, Velculescu VE, Vogelstein B, Kinzler KW (2002) Allelic variation in human gene expression. Science 297:1143

Yvert G, Brem RB, Whittle J, Akey JM, Foss E, Smith FN, Mackelprang R, Kruglyak L (2003) Trans-acting regulatory variation in *Saccharomyces cerevisiae* and the role of transcription factors. Nat Genet 35:57–64