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Genetic variation for the response to ploidy change in *Zea mays* L.

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Abstract Polyploidization is an important process in the evolutionary history of most eukaryotic species. It oftentimes causes large-scale genomic reorganizations and is accompanied by a wide variety of phenotypic alterations in morphology, niche preference and fitness characteristics. Despite their importance, the morphological effects of alterations in ploidy are not well understood. We investigated these changes in four diverse maize inbred lines, using monoploid, diploid, triploid and tetraploid derivatives, measuring 13 characters in a randomized field study. Employing several analysis of variance approaches, we find that all characters investigated strongly respond to alterations in ploidy. This response appears to have two sources: one source is shared by all inbred lines and constitutes a common response to ploidy change. The other source is genotype specific and results in a response to ploidy change that varies among inbred lines. This finding demonstrates the existence of genetic variation for the morphological response to ploidy change in *Zea mays*.

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Introduction

Polyploidization has been an important process in the evolutionary history of many diverse lineages, including animal groups such as fish and amphibians in addition to a large number of plant species (Levin 2002; Ptacek et al. 1994; Sidow 1996; Uyeno and Smith 1972). Recent estimates show that a high percentage of angiosperms have undergone some form of polyploidization during their history (Masterson 1994). Studies also suggest that these polyploidization events are important gateways to phenotypic novelty, not only in the case of allopolyploids which bring together the full genomes of two parent species, but also in autopolyploids that are formed by uniting two identical genomes. Redundancy generated by the presence of multiple copies of the same genetic material allows for mutation, genetic drift and natural selection to alter some of the copies, while keeping others constant, potentially leading to neo- or sub-functionalization of genes and ultimately phenotypic diversification (Ohno 1970).

In addition to these long-term modes of evolutionary change in polyploids, studies of newly synthesized allo- and autopolyploids have shown that the unification of multiple genomes in one nucleus has a surprisingly large number of immediate effects as well. On the molecular level, such a restructuring takes place in many newly synthesized polyploids. Genetic changes are common, including genome rearrangements and

the loss or expansion of repeated elements (Feldman et al. 1997; Liu et al. 1998; Madlung et al. 2002, 2005; Pires et al. 2004; Pontes et al. 2004; Song et al. 1995; Weiss and Maluszynska 2000). Other consequences of polyploidization include alterations in gene expression patterns such as gene silencing or novel tissue specificity as well as changes in epigenetic modifications (Adams et al. 2003; Blanc and Wolfe 2004; Mittelsten Scheid et al. 2003).

Most evident are the morphological changes often observed in polyploids, which have been reported in the literature since the early 1900s. Many of the phenotypes commonly observed in polyploid derivatives of flowering plants were grouped together under the ''gigas'' description, which includes increased size of the floral organs and fruits, larger leaves, and often fleshier appearance of the plants (Blakeslee 1941; Bretagnolle and Lumaret 1995; Ramsey and Schemske 2002), making polyploids also of great interest to both agriculture and horticulture. Increased pollen size and enlarged stomatal guard cells are also common occurrences with increasing ploidy (Blakeslee 1941; Laane et al. 1983; Quadt 1955). In addition to these more typical changes in morphology, polyploids can exhibit a large number of novel characters, some of which are potentially responsible for their large success as a group (Levin 2002; Ramsey and Schemske 2002). Various polyploids have been reported to show developmental delays (Blakeslee 1941; Keller and Gerhardt 2001; Lowcock 1994) leading for example to shifts in flowering time (Schranz and Osborn 2000; Werlemark and Nybom 2001), altered niche preference (Laane et al. 1983; Levin 2002) and changes in mating systems (Keller and Gerhardt 2001; Pandey 1968; Stout and Chandler 1941). Sometimes, polyploid individuals are capable of out-competing the parent species for instance in the newly formed Tragopogon allopolyploids of the New World (Soltis et al. 1995), but this trend is not universal (Stebbins 1985).

Despite the fact that common phenotypic alterations in response to changes in ploidy level occur, the extent to which a specific polyploid exhibits them varies widely between species and genotypes (Doyle 1986; Ramsey and Schemske 2002; Werlemark and Nybom 2001). This finding indicates that there are multiple factors that determine the morphological response to ploidy change. Using autopolyploids that vary only in genome dosage and not genetic content, it is possible to investigate these factors in detail. For *Zea mays*, a ploidy series has been generated from monoploid to octoploid (Rhoades and Dempsey 1966). In recent years, methods have been improved that allow the derivation of monoploid, triploid and tetraploid individuals from diploid maize inbred lines under controlled conditions (Kato 1997, 1999; Kato and Birchler 2006). Studying individuals of varying ploidy and genotypes allows us to determine the portion of morphological variance that is due to alterations in ploidy, genetic background, or a genotype-specific response to ploidy change.

In this article, we examine the morphological changes associated with alteration of ploidy level in *Z. mays*. Using a ploidy series developed in our lab, we conducted a field study measuring 13 morphological characters in four different inbred lines and three ploidy levels. Thus, we were able to determine which phenotypic characters respond to ploidy change, and determine if there is an overall (multi-character) response to ploidy change. Since *Z. mays* is an autopolyploid, one can investigate the effects of ploidy change per se on plant morphology and study genotype-specific responses with this experimental design. We were able to determine if different lines of maize respond similarly to ploidy change, or if the phenotypic changes associated with ploidy are mainly genotype-specific. We find that in addition to the influence of genetic background and ploidy level, there is a highly significant interaction effect that demonstrates the existence of genetic variation among maize inbred lines in their response to alterations in ploidy.

Materials and methods

Plant material

Starting materials were the diploid inbred lines A188, B73, Oh43 and W22, which were selected to represent a wide range of the genetic variability within *Z. mays* (Liu et al. 2003; Senior et al. 1998).

Monoploid individuals were derived from the four diploid inbred lines using the monoploid inducing line Stock 6. When used as a pollen parent, Stock 6 results in an unusually high monoploid production rate (Coe 1959). In order to be able to easily identify monoploid kernels, we used the following color selection scheme, which was described previously (Auger et al. 2004). The four inbred lines used carry recessive color markers for the aleurone layer and the embryo of the kernel. The Stock 6 line employed in our study carries the *R1-scm2* allele, which specifies purple coloration for both the endosperm aleurone layer and embryo. Thus, if the colorless inbred lines are pollinated by this version of Stock 6, most kernels will be diploid showing purple coloration of embryo and aleurone layer. Monoploid kernels, however, will be distinguished by the fact that they exhibit discordant coloration of the

embryo and aleurone layer. Monoploid embryos are colorless—they do not receive any genetic material from the purple pollen parent—and are surrounded by an endosperm with purple aleurone layer—as the central cell receives the dominant *R1-scm2* allele from Stock 6. The success of this color-based selection of monoploids is, to some degree, dependent on the inbred line due to differences in the penetrance of the color marker. However, monoploid individuals are readily distinguished from their diploid counterparts in the field due to their much shorter stature and sterility.

Trifluralin treatment was used to generate triploids of B73 as described (Kato 1997, 1999). Approximately 1 week to 10 days before flowering, the immature tassel of selected individuals was exposed by making a vertical cut through the surrounding leaves. The immature tassel was sprayed with a solution of 0.2% Treflan containing 43% trifluralin (DowElanco, Indianapolis, IN, USA). Trifluralin disturbs microtubule polymerization and can result in the formation of diploid sperm cells by preventing the second pollen mitosis. Pollen from treated plants thus contains a mixture of normal pollen with two monoploid sperm cells and pollen that carries only one diploid sperm cell. To generate triploid embryos, silks have pollen applied twice: the first time with trifluralin-treated pollen, during which a diploid sperm cell might fertilize the egg cell; the second time with untreated pollen, so the central cell can be fertilized by a haploid sperm cell. Again, a color-based selection scheme was employed to identify triploid embryos. While the inbred lines do not carry embryo or aleurone color markers, the pollen used for the second pollination carried the *R1-scm2* allele mentioned above. Only if a diploid sperm from the first pollination fertilizes the egg cell and a monoploid sperm from the second pollination fertilizes the central cell will a kernel with purple aleurone layer and colorless embryo be generated, which uniquely identifies the desired triploid embryos.

Tetraploids were previously derived from inbred diploid stocks of A188, B73, Oh43 and W22 using nitrous oxide treatment (Kato and Birchler 2006). These materials have been propagated by self-pollination for several generations and are the source of the material used in this study.

Phenotypic measurements

Phenotypic measurements were collected during the summer of 2004 when all maize lines were grown at the University of Missouri Genetics Farm near Columbia, Missouri, USA. Monoploid, diploid and tetraploid derivatives of the maize inbred lines A188, B73, Oh43 and W22 as well as triploids of B73 were planted in a randomized complete block design. Each of the 13 maize lines was represented once in each of the three blocks by a row of ten individuals. No monoploid plants of A188 were recovered in the field due to the poor expression of *R1-scm2* allele in this background. All diploid and tetraploid plants were self-fertilized, while monoploid and triploid individuals were open pollinated. Proper ploidy of putative monoploid and triploid individuals was confirmed by the almost complete lack of extruded anthers and sterile ears (monoploids) and by aberrant kernel development on the resulting ears (triploids). Measurements were taken on all ten plants if possible.

Plant height was measured (from the soil to the top of the plant) at three different stages of development: 4 weeks after planting, 6 weeks after planting and at maturity after flowering. The number of days from planting until the emergence of both silks and anthers was recorded for each individual. The number of leaves at maturity and tassel branches as well as the node number for the topmost ear were determined after flowering of the plants. In addition, we measured the position/height of the topmost ear in relation to the soil. After flowering, the width and length of the third leaf from the top of the plant were recorded as was the stem circumference in the second internode above the ear. Finally, after harvesting the ears from all individuals, ear length was measured.

Statistical analysis

All statistical analyses were performed using the SAS System for Windows Version 9.

B73 1×, 2×, 3×, 4×

For the inbred line B73, a two-way analysis of variance (ANOVA) based on a randomized complete block design with replication was conducted including individuals of all four ploidies. The model used included effects for 'block' and 'ploidy' and is shown below:

$$y = \mu + \text{Block} + \text{Ploidy} + \varepsilon$$

It was assumed that no interaction effect between 'block' and 'ploidy' exists, and 'block' was treated as a random factor. The mean square associated with the interaction term was used as error mean squared and denominator in the *F* test to test for significance of the main effects in the ANOVA. For all character measurements the analysis was conducted in two ways: (a) using the raw data with the measures from individual plants within a row representing subsamples and (b)

using row means as input for the ANOVA. In the results below, we report the analysis using means, as both analyses yielded very similar results (while the same factors were called significant in all analyses carried out, the *P* values varied slightly).

All inbred lines 1×, 2×, 4×

To investigate the relationship among all 13 character measurements included in our study, we calculated pair-wise Pearson correlation coefficients between all possible character pairs. They were evaluated for statistical significance, and a Bonferroni correction was applied to result in an overall significance level of $P < 0.05$ for this analysis.

A three-way ANOVA was carried out based on a randomized complete block design. As in the analysis of B73 described above, two analyses were carried out, one on the row means, the other on the raw data for all characters with measurements within a row specified as subsamples. The model used was identical for all characters and included the main effects of block, ploidy and line, i.e., the effect of the genetic background on the phenotype. Also included was the interaction between the factors ploidy and line, and the simplest model is shown below:

$$y = \mu + \text{Block} + \text{Ploidy} + \text{Line} + \text{Ploidy} \times \text{Line} + \varepsilon$$

For the analysis of means, the various interaction effects involving the factor block were considered part of the experimental error and thus not included in the model. For the analysis of the raw data, block as well as all interactions involving block were treated as random factors. Overall, the two analyses gave very similar results, and we will focus our discussion on the analysis of means.

Factor analysis was used to identify a small number of factors that could contain the variance structure present in the 13 characters measured in this study (Spearman 1904). Factor analysis was carried out using PROC FACTOR with prior communality estimates being calculated using the MAX option. This option sets the prior communality estimate for each variable to its maximum absolute correlation with any other variable, which was necessary due to the singular nature of the correlation matrix. The factors were rotated using the VARIMAX option. Due to low values for Kaiser's measure for sampling adequacy (MSA) (Cerny and Kaiser 1977; Kaiser 1970; Kaiser and Rice 1974), the variables 'tassel branch number' and 'days to silk emergence' were excluded from the analysis (tassel branch number MSA = 0.45; days to silk emergence MSA = 0.48). Based on their eigenvalues, four factors were retained for later analysis.

Analysis of covariance (ANCOVA) was carried out using PROC MIXED. The four factors identified in the factor analysis were included in the ANCOVA to represent the 11 original variables from which they were derived. This analysis was carried out using only the row means. The model used was identical to the ANOVA model from above with the addition of the various factors as covariables (x):

$$y = \mu + x + \text{Block} + \text{Ploidy} + \text{Line} + \text{Ploidy} \times \text{Line} + \varepsilon$$

For each factor, models with no, one, two or three covariables were compared using the Akaike Information Criteria (AIC) (Akaike 1973) and a Chi-square test for the difference in –2 log likelihoods of the two models.

Multivariate analysis of variance (MANOVA) was conducted using the MANOVA option in PROC GLM. Instead of the original variables, the four factors identified in the previously described factor analysis were used. The model is shown below:

$$y_1, \ldots, y_n = \mu + \text{Block} + \text{Ploidy} + \text{Line} + \text{Ploidy} \times \text{Line} + \varepsilon$$

The analysis was carried out once with the row means of factors and once with all the individual measures included as subsamples. Because the results were very similar, only the analysis of factor means will be discussed.

Results

B73 1×, 2×, 3×, 4×

Using the maize inbred line B73, we first examined the effect of ploidy change on morphological traits by comparing monoploid, diploid, triploid and tetraploid individuals. As these individuals were all originally derived from the same diploid stock, they do not differ in their genetic make-up other than in genome dosage. Comparisons between individuals of varied ploidy illustrate the strong effect genome dosage has on plant morphology (Fig. 1). The same phenotypic differences demonstrated in Fig. 1 are also evident in the morphological measurements collected under common environmental field conditions in the summer of 2004. For example, for plant height, there is a clear effect of ploidy on the character at all stages of development that were examined (Fig. 2). Interestingly, even though there are large differences in adult height between

Fig. 1 B73 ploidy series. From left to right are fully grown monoploid, diploid, triploid and tetraploid individuals of the maize inbred line B73. A meter stick is included in the picture for scale

diploid and tetraploid individuals as seen in Fig. 1 and the bottom panel of Fig. 2, it appears that at four and 6 weeks after planting, both tetraploids and diploids grow at similar rates (Fig. 2, top and middle panel), and that the growth rate of the tetraploid decreases sometime later in development. The dependency of all other characters on ploidy change is illustrated in Table 1, which contains mean measurements for each ploidy level. They clearly reflect the smaller stature of the monoploid individuals, and also show evidence of a stockier appearance of the tetraploids.

To evaluate quantitatively the effect of ploidy on the various characters, we used an ANOVA, which included main effects for 'block' and 'ploidy'. As shown in Table 2 for the character 'adult height', we find that the factor 'ploidy' in our model is highly significant with a P value of 0.001. For other characters, similar levels of significance are obtained in both analyses carried out, with the exception of the characters ''days to anther emergence'' and ear node number, which were marginally not significant in the analysis of means but significant at the $P = 0.05$ level in the subsampling analysis (see Supplemental Table 1). Overall, these results indicate that alterations in genome dosage and ploidy level in the maize inbred line B73 lead to global changes in plant morphology.

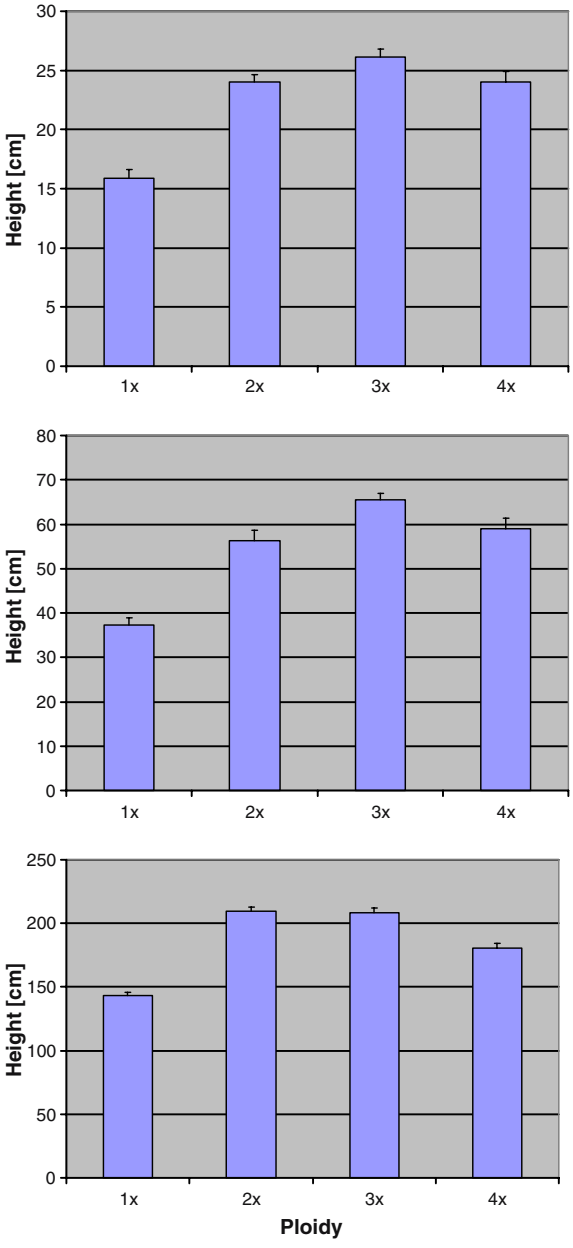

Fig. 2 Growth pattern of the B73 ploidy series Histograms are presented comparing the plant height of monoploid (1×), diploid (2×), triploid (3×) and tetraploid (4×) B73 lines at three stages of development. The *top panel* illustrates plant height at 4 weeks after planting, the *middle panel* plant height at 6 weeks after planting, and the *bottom panel four bars* adult height measures. The *four bars* represent the mean plant height in centimeters (*Y*-axis) for each of the four ploidy groups (*X*-axis). *Error bars* correspond to standard errors

All inbred lines 1×, 2×, 4×

Correlation analysis

A second analysis was carried out using monoploid, diploid and tetraploid derivatives of the maize inbred lines A188, B73, Oh43 and W22. To gain an understanding of the relationships among the 13 characters measured, we investigated all possible pair-wise correlation coefficients using the monoploid, diploid and tetraploid lines. The results of this analysis are shown in Table 3. Many of the characters are highly corre-

Table 1 Summary of character means for the B73 ploidy series

	1×	2×	3×	4×
Days to anther emergence	71.18 ± 1.73	67.33 ± 0.4	68.69 ± 0.24	70.2 ± 0.72
Days to silk emergence	66.78 ± 0.4	66.54 ± 0.59	66.47 ± 0.31	71.65 ± 0.49
Stem circumference (cm)	4.88 ± 0.08	6.23 ± 0.13	7.97 ± 0.13	7.78 ± 0.21
Ear node number	3.67 ± 0.16	5.27 ± 0.22	4.65 ± 0.19	4.81 ± 0.25
Ear height (cm)	41.81 ± 1.7	76 ± 2.38	72.47 ± 2.98	73.27 ± 2.21
Ear length (cm)	7.85 ± 0.23	12.5 ± 0.28	12.53 ± 0.27	8.38 ± 0.27
Height at 4 weeks (cm)	15.85 ± 0.75	24 ± 0.64	26.15 ± 0.64	24.05 ± 0.89
Height at 6 weeks (cm)	37.3 ± 1.58	56.35 ± 2.32	65.59 ± 1.32	59.01 ± 2.29
Adult height (cm)	143.23 ± 2.45	209.38 ± 3.51	208.35 ± 3.72	180.25 ± 3.99
Leaf number	10.7 ± 0.25	11.8 ± 0.26	12.24 ± 0.26	10.87 ± 0.2
Leaf length (cm)	44.29 ± 0.75	62.67 ± 1.07	63.88 ± 1.16	62.71 ± 1.13
Tassel branch number	6.81 ± 0.37	6.74 ± 0.26	5.12 ± 0.22	4.03 ± 0.19
Leaf width (cm)	5.5 ± 0.11	8.19 ± 0.22	8.82 ± 0.15	7.75 ± 0.22

For each measurement, mean and standard error are reported

Table 2 ANOVA results estimating the effect of ploidy change on ''dult height'' in the B73 ploidy series

Source	*DF*	Type III SS	Mean square	*F* value	Pr > *F*
Block	2	35.84	17.92	0.15	0.87
Ploidy	3	8,630.10	2,876.70	23.67	0.001
Error	6	729.32	121.55		

lated with each other, demonstrated by the fact that 18 of the 78 correlations are 0.5 or higher. For example, days to anther and silk emergence show a strong positive correlation with each other while exhibiting a negative correlation with almost all other characters. The reason for this behavior is the fact that early flowering is found in the taller diploid lines, while late flowering occurs in the shorter tetraploid and monoploid plants.

The strength of these various correlation coefficients was evaluated by testing them against the null hypothesis of no correlation. Of the 78 tests conducted, 53 were significantly different from zero, when using an analysis-wide P value of 0.05 (Bonferroni correction). This result shows that most characters are highly interrelated with each other. One exception is tassel branch number, which is significantly correlated only with the two flowering time measurements. As shown below, this finding is partially explained by the ANOVA results, which demonstrate that most of the variation in tassel branch number among the monoploid, diploid and tetraploid lines is explained by genetic background alone, evident in the strong effect of 'line' that was detected ($P < 0.001$). In B73 as well as in Oh43, tassel branch numbers are consistently much lower than in A188 and W22 (see Table 4).

ANOVA

To investigate the effect of ploidy change and genetic background on the various character measurements, we conducted a second ANOVA. The model included the main effects of ''ploidy,'' ''line'' or genetic background (in our case A188, B73, Oh43 and W22), and ''block.'' In addition, the interaction effect between ploidy and line was included. Our main goal was to determine if there was a line-specific aspect to the phenotypic response to ploidy change, which is measured in the ''ploidy × line'' interaction effect.

An example of the ANOVA carried out is illustrated in Table 5 for the character height at 4 weeks after planting. We found that for all 13 characters, ploidy had a significant effect on the phenotype (see Supplemental Table 2). The same was true for genetic background, with one exception: height at 4 weeks after planting did not exhibit a significant genetic background effect. With regards to the main effects, our second ANOVA which used the raw data instead of row means showed a few differences in which effects are significant. For leaf number, the factor ploidy was no longer considered significant, while for ear node number and height at 6 weeks after planting, ''line'' was no longer significant. Overall, the results were very consistent between the two analyses and demonstrate that both ploidy level and the inbred line strongly influence morphological traits.

In addition, we discovered that the ''ploidy × line'' interaction effect has a strong influence on plant phenotypes as well. The ''ploidy × line'' interaction effect measuring the line-specific aspect of the phenotypic response to ploidy change is statistically significant for 6 of the 13 traits examined (Table 5 and Supplemental Table 2). These characters include ear

Table 3 Correlation between characters: pair-wise Pearson correlation coefficients for each possible character pair are shown

	Days to anther emerg.	Days to silk emerg.	Stem circ.	Ear node #	Ear height	Ear length	Height at 4 weeks	Height at 6 weeks	Adult height	Leaf #	Leaf length	Tassl br #	Leaf width
Days to anther emerg.	1.00												
Days to silk emerg.	0.74**	1.00											
Stem circ.	–0.08	0.25**	1.00										
Ear node #	–0.07	0.05	0.29**	1.00									
Ear height	–0.09	0.09	0.50**	0.58**	1.00								
Ear length	–0.31**	–0.31**	0.14	0.16	0.23*	1.00							
Height at 4 weeks	–0.55**	–0.37**	0.48**	0.29**	0.59**	0.30**	1.00						
Height at 6 weeks	–0.51**	–0.42**	0.50**	0.36**	0.65**	0.38**	0.85**	1.00					
Adult height	–0.27**	–0.09	0.43**	0.58**	0.80**	0.39**	0.62**	0.71**	1.00				
Leaf #	–0.02	0.06	0.35**	0.31**	0.59**	–0.09	0.43**	0.37**	0.49**	1.00			
Leaf length	–0.27**	0.06	0.51**	0.38**	0.69**	0.29**	0.47**	0.52**	0.59**	0.18	1.00		
Tassel branch #	–0.46**	–0.41**	0.00	–0.04	0.00	–0.02	0.10	0.04	–0.07	0.08	0.10	1.00	
Leaf width	–0.44**	–0.23*	0.41**	0.17	0.22*	0.63**	0.38**	0.45**	0.28**	–0.13	0.40**	0.22*	1.00

*The coefficients that are significant at the experiment-wide significance level of $P < 0.05$

**The coefficients that are significant at the experiment-wide significance level of $P < 0.01$

Table 4 Character means

Variable	A188 1×	B73 1×	Oh43 1×	W22 1×	A188 2×	B73 2×	Oh43 2×	W22 2×	A188 4×	B73 4×	Oh43 4×	W22 4×
Days to anther emerg.	–	71.18 ± 1.73	69.39 ± 1.06	69.29 ± 0.87	60.38 ± 0.32	67.33 ± 0.40	64.81 ± 0.95	66.41 ± 0.51	66.54 ± 0.84	70.20 ± 0.72	69.47 ± 1.24	72.63 ± 0.71
Days to silk emerg.	–	66.78 ± 0.4	65.21 ± 1.02	66.70 ± 0.43	60.71 ± 0.34	66.54 ± 0.59	65.00 ± 1.01	68.69 ± 0.66	67.75 ± 0.90	71.65 ± 0.49	72.24 ± 1.17	74.72 ± 0.83
Stem circ.	–	4.88 ± 0.08	5.00 ± 0.14	5.98 ± 0.15	5.81 ± 0.14	6.23 ± 0.13	6.53 ± 0.14	7.13 ± 0.12	5.98 ± 0.15	7.78 ± 0.21	6.55 ± 0.22	7.39± 0.18
Ear node #	–	3.67 ± 0.16	2.89 ± 0.19	2.87 ± 0.16	3.92 ± 0.16	5.27 ± 0.22	4.88 ± 0.20	3.97 ± 0.19	4.58± 0.18	4.81 ± 0.25	4.22 ± 0.19	4.50 ± 0.20
Ear height	–	41.81 ± 1.70	28.00 ± 3.01	44.22 ± 2.38	52.11 ± 1.89	76.00 ± 2.38	60.50 ± 2.28	69.72 ± 2.27	41.96 ± 2.12	73.27 ± 2.21	46.34 ± 2.02	59.00 ± 2.97
Ear length	–	7.85 ± 0.23	12.8 ± 0.42	7.91 ± 0.38	11.54 ± 0.29	12.50 ± 0.28	17.88 ± 0.42	12.86 ± 0.27	8.26 ± 0.53	8.38± 0.27	12.81± 0.71	7.65 ± 0.37
Height at 4 weeks	–	15.85 ± 0.75	12.88 ± 1.60	18.04 ± 1.12	22.75 ± 0.83	24.00 ± 0.64	22.19 ± 1.51	21.59 ± 0.81	16.09± 0.77	24.05 ± 0.89	21.06 ± 1.40	16.71 ± 0.97
Height at 6 weeks	–	37.30 ± 1.58	40.84 ± 4.12	43.90 ± 1.32	54.82 ± 1.96	56.35 ± 2.32	59.32 ± 3.61	52.20 ± 1.82	32.67 ± 1.68	59.01 ± 2.29	43.38 ± 2.45	39.90 ± 1.36
Adult height	–	143.23 ± 2.45	121.63 ± 7.81	115.91 ± 4.26	167.25± 3.68	209.38 ± 3.51	187.87 ± 4.42	176.25 ± 2.99	123.63 ± 4.28	180.25 ± 3.99	148.10 ± 6.94	154.78 ± 5.54
Leaf #	–	10.70 ± 0.25	7.74 ± 0.26	11.00 ± 0.32	10.00 ± 0.27	11.8 ± 0.26	10.00 ± 0.35	11.27 ± 0.20	9.43 ± 0.35	10.87 ± 0.20	9.11 ± 0.17	11.68 ± 0.38
Leaf length	–	44.29 ± 0.75	43.67 ± 1.00	50.43 ± 0.99	56.13 ± 0.81	62.67 ± 1.07	59.27 ± 1.31	60.82 ± 0.81	53.78 ± 1.15	62.71 ± 1.13	52.79 ± 1.59	50.47 ± 2.02
Tassel branch #	–	6.81 ± 0.37	5.00 ± 0.56	11.91 ± 0.60	15.17 ± 0.58	6.74 ± 0.26	7.94 ± 0.44	12.83 ± 0.53	14.48 ± 0.97	4.03 ± 0.19	4.00 ± 0.34	7.47 ± 0.76
Leaf width	–	5.50 ± 0.11	8.56 ± 0.20	8.26 ± 0.22	9.88 ± 0.14	8.19 ± 0.22	12.20 ± 0.28	9.83 ± 0.20	7.83 ± 0.35	7.75 ± 0.22	10.05 ± 0.39	8.26 ± 0.30

Mean and standard error for all measurements are given for monoploid, diploid and tetraploid lines of the four inbred lines. Unless otherwise noted, measurements are in centimeters

Table 5 ANOVA results for the interaction effect ploidy × line for the character ''height at 6 weeks after planting''

Source	*dF*	Type III SS	Mean square	*F* value	Pr > *F*
Block	2	773.65	386.83	9.30	0.0015
Ploidy	2	1,456.53	728.26	17.50	<0.0001
Line	3	481.32	160.44	3.86	0.0260
Ploidy × line	5	737.11	147.42	3.54	0.0197
Error	19	790.53	41.61		

node number, tassel branch number, leaf width, leaf length, stem circumference and height at 6 weeks after planting. Two other characters, leaf number, and ear height are marginally non-significant in the analysis of means, but significant in the subsampling analysis (data not shown). This finding is illustrated in Fig. 3 for the three plant height measures. At 4 weeks after planting, when the ploidy × line interaction effect is marginally non-significant, the growth pattern for the four genetic backgrounds is similar, with the exception of W22, where the tetraploid plants already are markedly reduced in height. This genotype-specific response to ploidy change becomes more accentuated in the measurements from 6 weeks after planting. Here, diploids and tetraploids of the inbred line B73 are very similar at this stage in development, which is in stark contrast with the other three genetic backgrounds where tetraploids are markedly shorter than diploids. An overall summary of the differences among the four genetic backgrounds as well as the effects of ploidy change are provided in Table 4, which shows character means for all lines included in this study.

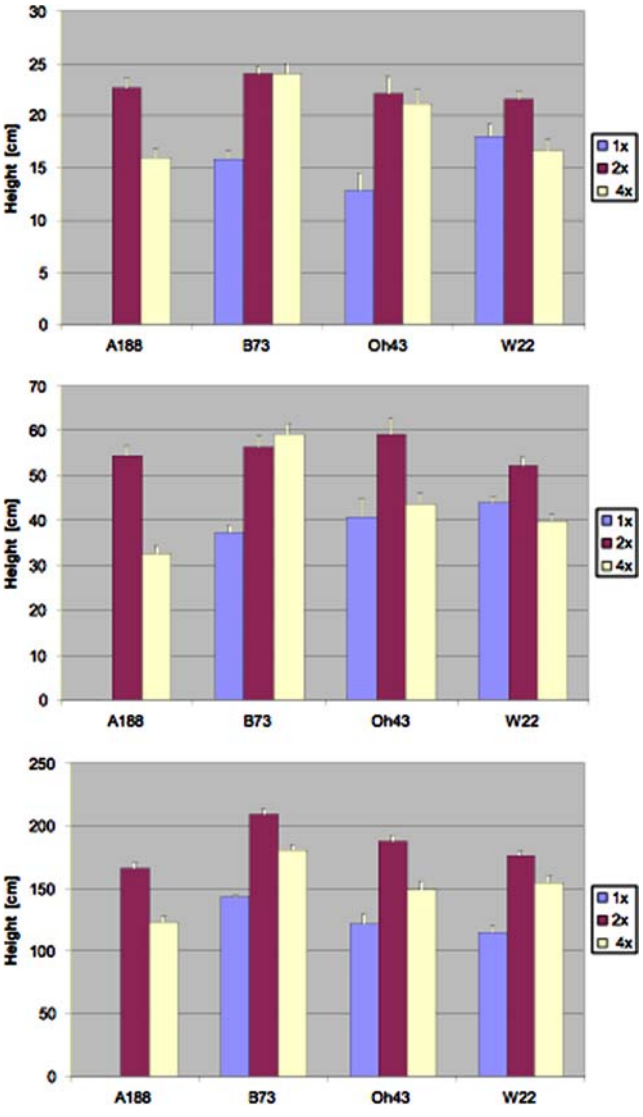

Fig. 3 Line-specific response to ploidy change. Response of the three height characters, ''height at 4 weeks after planting'' (*top panel*), ''height at 6 weeks after planting'' (*middle panel*) and ''adult height'' (*bottom panel*), to alterations in ploidy change is dependent on the genetic background of the individuals. Mean height in centimeters for each group is given on the *Y*-axis, while the four groupings of *bars* represent the monoploid, diploid and tetraploid lines of each inbred line. Monoploid, diploid and tetraploid lines are shown in *blue*, *red* and *yellow*, respectively. *Error bars* represent standard errors

Factor analysis

To investigate further the nature of phenotypic change associated with alterations in ploidy, we conducted a factor analysis to identify a small number of factors that represents the original variables measured in this experiment. As the characters ''tassel branch number'' and ''days to silk emergence'' did not meet the criteria for factor analysis, the factors identified represent only 11 variables. The analysis recognized four factors that contribute significantly to the model based on their eigenvalues. Table 6 shows the contribution of each of the identified factors to the original variables. Factor 1 shows a strong positive loading for all height-related measures, while factor 2 most strongly contributes to the flowering time measures in a negative manner. Together, factors 1 and 2 explain more than 80% of the original variance. Factors 3 and 4 contribute 10% and 6%, respectively, and their loading on the original variables is less easily interpreted. Factor 3 has its strongest effect negatively on the measurement of height at 4 weeks, while factor 4 influences stem circumference most strongly.

A similar pattern is observed for the four factors after rotation (see Table 7). Factor 1 again demonstrates strong loading for height-related measures in the adult stage, while factor 2 most strongly influences the growth measures taken during the juvenile stage of

Table 6 Factor analysis: loading of the four factors identified onto the original variables

	Factor 1	Factor 2	Factor 3	Factor 4
Days to anther emerg.	–0.39	0.41	0.24	0.19
Stem circumference	0.55	0.10	–0.15	0.43
Ear node #	0.50	0.23	0.36	–0.18
Ear height	0.85	0.32	0.17	0.06
Ear length	0.39	–0.63	0.25	–0.11
Height at 4 weeks	0.79	–0.05	–0.44	–0.15
Height at 6 weeks	0.85	–0.09	–0.25	–0.09
Adult height	0.84	0.12	0.28	–0.15
Leaf #	0.55	0.53	–0.17	0.00
Leaf length	0.71	–0.04	0.21	0.22
Leaf width	0.41	–0.67	0.06	0.23

the plants. Factor three most strongly impacts ear length and leaf width, and factor 4 wields most influence over the leaf size measurements.

ANCOVA

To investigate the covariance relationships among the variables measured in our field study, we conducted an ANCOVA. In particular, we used the factors identified in the previous analysis to investigate all possible variable/covariable combinations. When comparing models with zero, one, two or three covariants, we find that no model including covariables is superior to the simple ANOVA model presented above.

MANOVA

A multivariate analysis was conducted to evaluate the overall trend in the behavior of variables. The four factors identified by factor analysis were used as a substitute for the original variables. These four factors represent most of the variance present among the 11 variables included in the factor analysis. The multivariate model included all four factors. We find that in addition to significant main effects, there is also a highly significant ploidy × line interaction effect ($P = 0.01$, Wilk's lambda, data not shown).

Table 7 Rotated factor pattern

	Factor 1	Factor 2	Factor 3	Factor 4
Days to anther emerg.	–0.02	–0.53	–0.38	0.00
Stem circumference	0.19	0.20	0.06	0.66
Ear node #	0.68	0.05	0.03	0.03
Ear height	0.76	0.26	0.01	0.46
Ear length	0.20	0.17	0.75	–0.07
Height at 4 weeks	0.29	0.80	0.10	0.33
Height at 6 weeks	0.41	0.68	0.22	0.35
Adult height	0.81	0.30	0.19	0.22
Leaf #	0.46	0.33	–0.38	0.38
Leaf length	0.51	0.14	0.32	0.46
Leaf width	–0.01	0.17	0.76	0.26

Discussion

In the field study reported here, we investigated the morphological response of four maize inbred lines to alterations in ploidy level. Thirteen phenotypic measures were observed in a ploidy series of B73 that included monoploid, diploid, triploid and tetraploid individuals. ANOVA demonstrated that all characters strongly respond to changes in ploidy (Tables 1, 2). In particular, the smaller stature of the monoploid individuals is reflected in most measurements.

A more extensive study included monoploids, diploids and tetraploids from four diverse inbred lines, for which the same 13 characters were measured. We find that all characters are highly correlated with each other with the possible exception of tassel branch number, which seems to be more strongly dependent on the genetic background and less influenced by genome dosage (Table 3). ANOVA results demonstrate that for all the variables there is a strong effect of ploidy level on the phenotype observed (data not shown). These differences in phenotypes induced by ploidy and genetic background are illustrated by the data in Table 4. We also find that for many characters there is a significant interaction between line and ploidy level (Table 5). These findings indicate that the phenotypic differences observed between the 12 lines included in this study are mainly due to 3 factors: a common response to ploidy change, an effect of genetic background (i.e., the inbred line they were derived from) and a response to ploidy change that is dependent on the genetic background.

We used factor analysis to discover a smaller number of factors to be used in multivariate analyses. A total of four significant factors were identified (see Tables 6, 7 for details) that subsequently were used in an ANCOVA and the MANOVA. The MANOVA with the four factors representing our original morphological measures was carried out to determine if the trends seen in the univariate analyses could be confirmed. Again our data show that ploidy in general has a large effect on plant morphology. In addition to an overall ploidy effect, there is a genotype-specific response to changes in genome dosage that demonstrated the presence of genetic variation for the response to ploidy change among maize inbred lines.

Overall, our results demonstrate the existence of two sources of phenotypic change in response to ploidy

change, one common to all genotypes, and one genotype-specific. Similar observations have been made in other plant systems. Kermani et al. (2003) produced tetraploid and hexaploid derivatives from diploid and triploid rose varieties and found that most of the characters such as pollen fertility and floral structures showed phenotypic responses dependent on the variety used. Comparable results were obtained in a study of diploid, triploid and tetraploid *Dactylis glomerata* individuals; broader and thicker leaves were observed in all polyploids while other characters such as mean seed weight were strongly influenced by genotype (Bretagnolle and Lumaret 1995).

A strong genetic influence on the response to triploidy has recently been discovered by examining interploidy crosses in *Arabidopsis thaliana* (Henry et al. 2006). Similar to our results, the authors find that certain changes such as increasing size of floral structures with increased ploidy are common among genotypes. However, they also find that the rate of successful seed set of triploids strongly depends on the genetic nature of the triploids. In addition, they show that in recombinant inbred lines generated from a diploid/tetraploid cross, certain genomic regions are overrepresented in the diploid or tetraploid classes, respectively (Henry et al. 2006). These findings support our conclusion of the existence of genetic variation for the response to ploidy change.

Many morphological responses that are common between genotypes and occur in autopolyploid as well as allopolyploid species such as larger leaves increased pollen size and the almost ubiquitous increase in stomatal guard cell size raise questions regarding their basis. This communality indicates that a portion of the morphological response to ploidy change is independent of a potential hybrid state or genotype, while a second portion is clearly genotype-dependent. Rather it points to the possibility that many of these changes are due to the increased nuclear volume and cell size observed in polyploids. Alterations in the size of the nucleus can lead to changes in the ratio between nuclear and cytoplasmic volume. In addition, increased cellular size alters the cell's surface-to-volume ratio. These alterations potentially disturb regulatory and developmental processes, accounting for the common responses to ploidy change observed, while the genotype-dependent response represents a modulation thereof.

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References

Adams KL, Cronn R, Percifield R, Wendel JF (2003) Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. Proc Natl Acad Sci USA 100:4649–4654

Akaike H (1973) Information theory as an extension, Akademiai Kiado, Budapest, 267–281

Auger DL, Ream TS, Birchler JA (2004) A test for a metastable epigenetic component of heterosis using haploid induction in maize. Theor Appl Genet 108:1017–1023

Blakeslee AF (1941) Effect of induced polyploidy in plants. Am Nat 75:117–135

Blanc G, Wolfe KH (2004) Functional divergence of duplicated genes formed by polyploidy during *Arabidopsis* evolution. Plant Cell 16:1679–1691

Bretagnolle F, Lumaret R (1995) Bilateral polyploidization in *Dactylis glomerata L.* subsp. lusitanica: occurence, morphological and genetic characteristics of first polyploids. Euphytica 84:197–207

Cerny BA, Kaiser HF (1977) A study of a measure of sampling adequacy for factor-analytic correlation matrices. Multivariate Behav Res 12:43–47

Coe EJ (1959) A line of maize with high haploid frequency. Am Nat 93:381–382

Doyle G (1986) The allotetraploidization of maize. 4. Cytological and genetic evidence indicative of substantial progress. Theor Appl Genet 71:585–594

Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM (1997) Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. Genetics 147:1381–1387

Henry IM, Dilkes BP, Young K, Watson B, Wu H, Comai L (2005) Aneuploidy and genetic variation in the *Arabidopsis thaliana* triploid response. Genetics 170:1979–1989

Kaiser HF (1970) A second generation little jiffy. Psychometrika 35:401–415

Kaiser HF, Rice J (1974) Little jiffy mark IV. Educ Psychol Meas 34:111–117

Kato A (1997) Induced single fertilization in maize. Sex Plant Reprod 10:96–100

Kato A (1999) Induction of bicellular pollen by trifluralin treatment and occurrence of triploids and aneuploids after fertilization in maize. Genome 42:154–157

Kato A, Birchler JA (2006) Induction of tetraploid derivatives of maize inbred lines by nitrous oxide gas treatment. J Hered 97:39–44

Keller MJ, Gerhardt HC (2001) Polyploidy alters advertisement call structure in gray treefrogs. Proc Biol Sci 268:341–345

Kermani MJ, Sarasan V, Roberts AV, Yokoya K, Wentworth J, Sieber VK (2003) Oryzalin-induced chromosome doubling in Rosa and its effect on plant morphology and pollen viability. Theor Appl Genet 107:1195–200

Laane MM, Croff BE, Wahlstrom R (1983) Cytotype distribution in the *Campanula rotundifolia* complex in Norway, and cyto-morphological characteristics of diploid and tetraploid groups. Hereditas 99:21–48

Levin DA (2002) The role of chromosomal change in plant evolution, Oxford University Press, New York

Liu B, Vega JM, Feldman M (1998) Rapid genomic changes in newly synthesized amphiploids of Triticum and Aegilops. II. Changes in low-copy coding DNA sequences. Genome 41:535–542

Liu K, Goodman M, Muse S, Smith JS, Buckler E, Doebley J (2003) Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics 165:2117–2128

Lowcock LA (1994) Biotype, genomotype, and genotype: variable effects of polyploidy and hybridity on ecological partitioning in a bisexual-unisexual community of salamanders. Can J Zool 72:104–117

Madlung A, Masuelli RW, Watson B, Reynolds SH, Davison J, Comai L (2002) Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic *Arabidopsis* allotetraploids. Plant Physiol 129:733–746

Madlung A, Tyagi AP, Watson B, Jiang H, Kagochi T, Doerge RW, Martienssen R, Comai L (2005) Genomic changes in synthetic *Arabidopsis* polyploids. Plant J 41:221–230

Masterson J (1994) Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science 264:421–423

Mittelsten Scheid O, Afsar K, Paszkowski J (2003) Formation of stable epialleles and their paramutation-like interaction in tetraploid *Arabidopsis thaliana*. Nat Genet 34:450–454

Ohno S (1970) Evolution by gene duplication, Springer, Berlin Heidelberg New York

Pandey KK (1968) Colchicine-induced changes in the self-incompatibility behaviour of *Nicotiana*. Genetica 39:257–271

Pires JC, Zhao Y, Schranz ME, Leon EJ, Quijada PA, Lukens LN, Osborn TC (2004) Flowering time divergence and genomic rearrangements in resynthesized *Brassica* polyploids (Brassicaceae). Biol J Linn Soc 82:675–688

Pontes O, Neves N, Silva M, Lewis MS, Madlung A, Comai L, Viegas W, Pikaard CS (2004) Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid *Arabidopsis suecica* genome. Proc Natl Acad Sci USA 101:18240–18245

Ptacek MB, Gerhardt HC, Sage RD (1994) Speciation by polyploidy in treefrogs: multiple origins of the tetraploid *Hyla versicolor*. Evolution 48:898–908

Quadt F (1955) Beobachtungen an den Nachkommen tetraploider Tomatenbastarde. Der Züchter 25:241–245

Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. Annu Rev Ecol Syst 33:589–639

Rhoades MM, Dempsey E (1966) Induction of chromosome doubling at meiosis by the *elongate* gene in maize. Genetics 54:505–522

Schranz ME, Osborn TC (2000) Novel flowering time variation in the resynthesized polyploid *Brassica napus*. J Hered 91:242–246

Senior ML, Murphy JP, Goodman MM, Stuber CW (1998) Utility of SSRs for determining genetic similarities and realtionships in maize using an agarose gel system. Crop Sci 38:1088–1098

Sidow A (1996) Gen(om)e duplications in the evolution of early vertebrates. Curr Opin Genet Dev 6:715–722

Soltis PS, Plunkett GM, Novak SJ, Soltis DE (1995) Genetic variation in Tragopogon species: additional origins of the allotetraploids *T. mirus* and *T. miscellus* (Compositae). Am J Bot 82:1329–1341

Song K, Lu P, Tang K, Osborn TC (1995) Rapid genome change in synthetic polyploids of Brassica and its implications for polyploid evolution. Proc Natl Acad Sci USA 92:7719–7723

Spearman C (1904) General Intelligence objectively determined and measured. Am J Psychol 15:201–293

Stebbins GL (1985) Polyploidy, hybridization and the invasion of new habitats. Ann MO Bot Gard 72:824–832

Stout AB, Chandler C (1941) Change from self-incompatibility to self-compatibility accompanying change from diploidy to tetraploidy. Science 94:118

Uyeno T, Smith GR (1972) Tetraploid origin of the karyotype of catostomid fishes. Science 175:644–646

Weiss H, Maluszynska J (2000) Chromosomal rearrangement in autotetraploid plants of *Arabidopsis thaliana*. Hereditas 133:255–261

Werlemark G, Nybom H (2001) Skewed distribution of morphological character scores and molecular markers in three interspecific crosses in Rosa section Caninae. Hereditas 134:1–13