Theor Appl Genet (2007) 115:1147–1162
DOI 10.1007/s00122-007-0640-z

ORIGINAL PAPER

Identification of quantitative trait loci that affect endoreduplication in maize endosperm

Cintia M. Coelho · Song Wu · Youchun Li · Brenda Hunter · Ricardo A. Dante · Yuehuai Cui · Rongling Wu · Brian A. Larkins

Received: 29 January 2006 / Accepted: 28 August 2007 / Published online: 2 October 2007

Abstract Endoreduplication in maize endosperm precedes the onset of starch and storage protein synthesis, and it is generally thought to influence grain filling. We created four backcross populations by reciprocally crossing the F_1 progeny of a cross between Sg18 and Mo17 to the parental inbreds, which differ in endoreduplication by two parameters—mean ploidy and percentage of endoreduplicated nuclei. This four-backcross design allowed us to estimate and test the additive and dominant genetic effects of quantitative trait loci (QTLs) affecting endoreduplication. An analysis of endosperm from the four backcross populations at 16 days after pollination using a modified triploid mapping approach identified three endosperm QTLs influencing mean ploidy and two endosperm QTLs affecting the percentage of endoreduplicated nuclei. Some of these QTLs may manifest their effects on endoreduplication via expression in the embryo. The QTLs detected display strong dominance or over-dominance and interacted epistatically with an embryo-expressed QTL. This helps to explain the genetic basis for transgressive segregation in the backcross progeny. Although the favorable alleles that increase mean ploidy and percentage of endoreduplicated nuclei can be contributed by both parents, the Mo17-derived alleles for endoreduplication were often dominant or over-dominant to the Sg18-derived allele. One QTL on chromosome 7 that may be expressed in both the embryo and endosperm exerted a pleiotropic effect on two different parameters of endoreduplication. The results from this study shed light on the regulation of endoreduplication in maize endosperm and provide a marker-assisted selection strategy for potentially improving grain yield.

Communicated by J.-L. Jannink.

C. M. Coelho and S. Wu contributed equally to this work and should be considered as first authors.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-007-0640-z) contains supplementary material, which is available to authorized users.

C. M. Coelho · Y. Li · B. Hunter · R. A. Dante · B. A. Larkins (✉)
Department of Plant Sciences, Forbes Hall 303, University of Arizona, Tucson, AZ 85721, USA
e-mail: Larkins@Ag.Arizona.edu

S. Wu · Y. Cui · R. Wu
Department of Statistics, University of Florida, Gainesville, FL 32611, USA

Introduction

Endoreduplication describes a cell cycle in plants and animals that leads to genome replication without an ensuing reduction in chromosome number. This process is found widely in Angiosperms, having been observed in many cell types, except gametes, meristematic parenchyma and guard cells (Nagl 1978). Endoreduplication has been well characterized in maize endosperm, where cells can attain ploidy levels of 96C and higher (Kowles and Phillips 1985; Dilkes et al. 2002). Maize endosperm is a triploid tissue (3C) formed by the fusion of a sperm nucleus from the pollen tube and two polar nuclei in the female gametophyte. During the first few days of endosperm development, cells undergo a period of rapid mitotic activity. Around 8–9 days after pollination (DAP), cells in the center of the endosperm begin to undergo endoreduplication, and it progresses centripetally toward peripheral

cell layers. Though not synchronous, endoreduplication persists throughout the starchy endosperm until mid-kernel development (20 DAP), at which time programmed cell death begins in the central cells of the starchy endosperm (Young and Gallie 2000).

Although endoreduplication is common in plants and animals, its function is not well understood. It is thought that this process could provide a mechanism to create high levels of gene expression by increasing the availability of DNA templates. Transcriptional and translational activity are thought to increase proportionately with each doubling of the genome, so the metabolic activity of a highly polyploid cell can be functionally equivalent to that of many diploid cells (D'Amato 1984). Cells that undergo endoreduplication are typically larger than other cells. It was suggested that large cells have the capacity to increase their volume faster than smaller cells, and in the case of rapidly growing fruits and seeds, this could be advantageous (Grime and Mowforth 1982). Because endoreduplication occurs frequently in seed storage tissues, it has also been suggested that this process could also provide a mechanism for storing nucleotides or nitrogen for the embryo (reviewed in Larkins et al. 2001).

Several genetic factors have been reported to influence the endoreduplication process in plants. For example, mutations have been described that limit endoreduplication in *Arabidopsis* trichomes (kaktus, rastafari, polychrome, and siamese), and leaves and hypocotyls (hypocotyls 6 and root hairless) (Perazza et al. 1999; Walker et al. 2000; Sugimoto-Shirasu et al. 2002). Altered expression of cell cycle genes has also been reported to affect endoreduplication. In *Arabidopsis*, ectopic-expression of genes involved in the G1/S transition and S-phase, such as E2Fa (AtE2F3) and its dimerization partner DPa, AtCDC6a, and AtCDT1a leads to increased ploidy in leaves and trichomes (DeVeylder et al. 2002; Castellano et al. 2001, 2004). On the other hand, ectopic expression of genes involved in M-phase, such as the mitotic cyclin CYCB1;2, were shown to induce mitotic cycles in endoreduplicating *Arabidopsis* trichomes (Schnittger et al. 2002). In maize, Leiva-Neto et al. (2004) demonstrated that expression of a dominant negative mutant of CDKA, which appears to function in the S phase of the cell cycle, decreased endoreduplication in starchy endosperm cells.

Because of the high frequency with which endoreduplication occurs in plants and its presumed importance, there is interest in understanding the regulation of this process. Several studies suggested maternal control of endoreduplication in maize endosperm. Poneleit and Egli (1983) and Seka and Cross (1995) observed that genes from both the male and female parents influenced grain-filling rates, but maternal effects controlled the effective filling period. The effective grain-filling period in maize endosperm is concomitant with endoreduplication, which could indicate maternal control of this process. Cavallini et al. (1995) showed a maternal effect influencing the extent of DNA endoreduplication in the endosperm of Illinois high protein and Illinois low protein maize lines, and Kowles et al. (1997) reported that the endoreduplication pattern of F_1 hybrid endosperm is more similar to the maternal parent than the paternal parent. Further evidence for maternal control of endoreduplication in maize endosperm was reported by Dilkes et al. (2002).

It has been shown that maintenance of a 2:1 maternal to paternal genome ratio is important for endoreduplication in maize endosperm. Leblanc et al. (2002) reported that maternal genomic excess forces cells to enter early into endoreduplication, while paternal genomic excess prevented its establishment, indicating that endoreduplication in maize endosperm depends on parental gene dosage events. Although several genetic factors influencing endoreduplication have been described, the mechanisms controlling this process and their regulation are not well understood. Consequently, the objective of this study was to investigate the genetic basis of endoreduplication in maize endosperm. Elucidation of the regulation of this cell cycle would allow a better understanding of endosperm development and ultimately could provide insight regarding the manipulation of the genetic components affecting grain yield.

The control of endoreduplication in maize endosperm could be influenced by several different genetic sources, including the maternal genome and the genomes of the embryo and endosperm. The embryo and endosperm have the same genetic composition, but because of the difference in their ploidy, a quantitative trait locus (QTL) could perform differently between the two. To consider this potential difference, we used the terminology "embryo genome" and "endosperm genome". In order to address this question, it is necessary to determine how QTLs in different locations affect the phenotype of endoreduplication in the endosperm. In particular, it was interesting to test whether these QTLs interact in a coordinated fashion to affect endoreduplication.

In an effort to map genes influencing the endoreduplication process in maize endosperm, four backcross populations were created by reciprocally crossing the F_1 progeny of a cross between Sg18 (a popcorn inbred with a high endoreduplication level) and Mo17 (a Midwestern dent inbred with a lower level of endoreduplication) to the parental inbreds. Molecular markers and traits that describe endoreduplication were measured for backcross ears on plants of the F_1 genotype. This design can be used to identify QTLs expressed from the genomes of the embryo and endosperm. We performed a joint analysis with the four backcross populations to estimate the additive and dominance effects of a segregating QTL affecting endoreduplication. The Lander and Botstein technique was

used to map QTLs in the diploid embryo, but we used a modified mapping approach for the triploid endosperm based on the method proposed by Wu et al. (2002) to identify endosperm QTLs. The combination of the four backcross populations allowed us to detect and test how different QTLs associated with the embryo and endosperm interact and influence the process of endoreduplication. These tissue-specific QTLs for endoreduplication were detected by using Cui and Wu's (2005) genome–genome epistasis model.

Materials and methods

Plant materials

Sg18, a popcorn inbred line, was crossed as the maternal parent with Mo17, a Midwestern dent inbred line, to produce F_1 progeny. Subsequently, four backcross (BC) populations were created by using the following strategy. One F_1 plant was crossed as the maternal parent to one Sg18 plant ($F_1 \times$ Sg18). The Sg18 plant that was the pollen source was also used as a maternal parent and received pollen from the F_1 plant (Sg18 $\times$ F_1). A second F_1 plant was crossed as the maternal parent to a Mo17 plant ($F_1 \times$ Mo17), and this same Mo17 plant was used as a maternal parent and received pollen from the second F_1 plant (Mo17 $\times$ F_1). In addition to the plants used to produce the four backcross populations, one plant for each of the two inbred lines, Mo17 and Sg18, was selfed. All plants used to produce the backcross populations, plus the two parental inbred lines, were grown at the same time and in the same location in the green house facility at the University of Arizona. The ears were broken from the stalk and placed in crushed ice for transport to the laboratory. Approximately 100 kernels were removed with a razor blade from the central region of single, well-filled 16 DAP ears of Sg18, Mo17, Sg18 $\times$ F_1, $F_1 \times$ Sg18, Mo17 $\times$ F_1 and $F_1 \times$ Mo17, respectively. Endosperms from the parental inbred lines and the four backcross mapping populations were dissected and analyzed by flow cytometry, and their corresponding embryos were rescued by tissue culture and grown to seedlings, as described by Dilkes et al. (2002). Because some embryos did not germinate due to fungal contamination, the final mapping populations were composed of 92, 89, 85 and 82 endosperms of Sg18 $\times$ F_1, $F_1 \times$ Sg18, Mo17 $\times$ F_1 and $F_1 \times$ Mo17, respectively.

DNA extraction, PCR analysis and map construction

Seedlings of the backcross progeny were lyophilized at –40°C with a Labconco vacuum drier (VWR, Tempe, AZ). DNA was prepared by the hexadecyltrimethyl-ammonium bromide method (Shen et al. 1994) and diluted to a final concentration of approximately 10 ng/μl for PCR reactions. Simple sequence repeat (SSR) primers were purchased from Research Genetics (Huntsville, AL) or Invitrogen (Carlsbad, CA). The primer sequences are available in the Maize Genomic Database (http://www.maizegdb.org/ssr.php). DNA from the parental inbreds, Sg18 and Mo17, was analyzed to identify polymorphic SSR markers, and these markers were then used to screen the backcross populations. PCR reactions were initiated by denaturing the DNA at 95°C for 5 min, followed by 45 cycles of PCR, which consisted of the following: 94°C for 1 min, 65–53°C for 45 s (depending on the SSR primer), and 72°C for 1 min. The final cycle was extended at 72°C for 6–8 min. Reactions were conducted in 96 well plates in an Eppendorf Mastercycles PCR machine (Eppendorf, Hamburg, Germany). Following DNA amplification, the PCR products were separated by electrophoresis in 4% (w/v) agarose gels (Amresco, Solon, Ohio), and visualized by staining with 0.5 μg of ethidium bromide per milliliter of gel.

Of approximately 500 SSR primer pairs screened, only 65 amplified clear and unambiguous polymorphic DNA fragments in Sg18 and Mo17. Given the genetic differences between popcorn and dent corn (Liu et al. 2003), this result was surprising, and we are unsure of the explanation. The informative SSR markers were grouped into ten different maize chromosomes (Fig. 4). The markers in each group were ordered according to the published maize genome database (http://www.maizegdb.org/). The map distances between each pair of adjacent markers were calculated with the four backcross populations. The average interval between markers for the entire linkage map was very close to 16 cM.

Flow cytometric analysis of endosperm nuclei

Dissected 16 DAP endosperms were chopped with a single-edged razor blade in the presence of 0.8 ml of filtered, ice cold PARTEC buffer (200 mM Tris–HCl, pH 7.5, 4 mM $MgCl_2$ and 0.1% Triton X-100) to release the nuclei (Dilkes et al. 2002). The homogenate was aspirated through two layers of cheesecloth, passed through 100 μm nylon mesh and combined with an additional 0.8 ml of PARTEC buffer. Nuclei were stained with 40 μl of a 100 mg/ml solution of 4′,6-diamidino-2-phenylindole (DAPI, Sigma, St Louis) and analyzed with a PARTEC CCAII flow analyzer (PARTEC, Munster, Germany). For each sample, at least 10,000 nuclei were collected and analyzed using a logarithmic scale display. Each flow cytometric histogram was saved with PARTEC CA3 software and analyzed with WinMDI 2.8 software

(available at http://facs.scripps.edu/software.html). The accuracy and reproducibility of flow cytometric measurements was evaluated by analyzing at least 50 kernels from the same ear of each parental inbred line. The data were consistent between kernels from each parental ear (data not shown). Furthermore, we have grown multiple generations of these genotypes over several years, and the ploidy measurements are highly reproducible for each inbred in multiple generations. A single endosperm sample cannot be analyzed twice, because of starch grain deposition in the tube during the assay. The portion of the sample remaining in the tube after the first run clogs the tubes in the PARTEC machine, resulting in inaccurate data.

Estimation of endoreduplication levels

Two parameters were used to estimate endoreduplication in the backcross progeny and parental inbred lines, mean ploidy and percentage of endoreduplicated nuclei (Dilkes et al. 2002). Mean ploidy was calculated from the DNA content by multiplying the nuclear ploidy level by the number of nuclei in each ploidy class and dividing by the total number of nuclei. The percentage of endoreduplicated nuclei was calculated as the number of nuclei with 6C and greater DNA content, divided by the total number of nuclei, and multiplied by 100.

Statistical models

We used the diploid (Lander and Botstein 1989) and triploid models (Wu et al. 2002) to map embryo and endosperm QTLs, respectively, and a genome–genome interaction model (Cui and Wu 2005) to detect interactions between the QTLs. The models that were modified to suit our four-backcross design are described below.

Diploid model. For this analysis, endoreduplication levels were measured in the endosperm and molecular markers were used to genotype the corresponding embryo. Suppose there are two inbred lines, P_1 and P_2, between which a QTL is segregating with two alleles Q and q. When the F_1 as an egg or pollen donor is backcrossed to each inbred line, different QTL genotypes will be generated. In Table 1, we list the segregation of genotypes in each of these backcrosses for the QTL expressed in the (diploid) embryo. These backcrosses together generate all three possible embryo QTL genotypes, QQ, Qq, and qq, with the genotypic values described in Table 1, where μ_m's are the overall mean with the subscripts denoting the type of backcross, a_m is the additive effect, d_m is the dominance effect of an embryo QTL. Thus, by estimating the set of parameters (a_m, d_m), the mode of genetic control in the diploid embryo can be characterized.

We combined four backcrosses, $F_1 \times$ Mo17, $F_1 \times$ Sg18, Mo17 $\times F_1$ and Sg18 $\times F_1$, for a joint likelihood analysis to estimate the parameters (μ_{m1}, μ_{m2}, μ_{m3}, μ_{m4}, a_m, d_m) for the inheritance of a QTL affecting mean ploidy and percentage of endoreduplicated nuclei in the endosperm based on the EM algorithm. After these parameters were estimated, the significance of the embryo QTL was tested by formulating the following hypotheses:

$$H_0 : a_m = d_m = 0 \text{ versus } H_1 : \text{ Not all equalities in } H_0 \text{ hold.} \quad (1)$$

The log-likelihood ratio test statistic under the H_0 and H_1 of hypotheses (1) is calculated and compared with the critical threshold determined from permutation tests (Churchill and Doerge 1994). The proportion of the total phenotypic variance in an endoreduplication variable explained by a detected QTL was estimated. Additive and dominance genetic effects of the embryo QTL can be tested according to the hypotheses as follows:

$$H_0 : a_m = 0 \text{ versus } H_1 : a_m \neq 0, \quad (2)$$

$$H_0 : d_m = 0 \text{ versus } H_1 : d_m \neq 0. \quad (3)$$

Test statistics for each of the above hypotheses (2) and (3) can be thought to be asymptotically χ^2-distributed with one degree of freedom.

Triploid model. This design allows us to map endosperm-specific QTLs that follow the mode of triploid inheritance (Wu et al. 2002). Table 1 also tabulates the segregation of genotypes in each backcross for the QTL expressed in the triploid endosperm. There are four possible endosperm QTL genotypes, QQQ, QQq, Qqq and qqq, with the genotypic values described in Table 1 (Gale 1976; Mo 1987), where μ_n's are the overall mean with the subscripts denoting the type of backcross, a_n is the additive effect, d_{n1} is the dominance effect of QQ to q and d_{n2} is the dominance effect of qq to Q. As for the diploid model, by estimating the set of parameters (a_n, d_{n1}, d_{n2}), the mode of genetic control in the triploid endosperm can be characterized.

We derived the EM algorithm to estimate the parameters (μ_{n1}, μ_{n2}, μ_{n3}, μ_{n4}, a_n, d_{n1}, d_{n2}) for the inheritance of a QTL affecting mean ploidy and percentage of endoreduplicated nuclei in the endosperm by combining four backcrosses, $F_1 \times$ Mo17, $F_1 \times$ Sg18, Mo17 $\times F_1$ and Sg18 $\times F_1$, for a joint likelihood analysis (Supplement 1). After these parameters were estimated, the significance of the endosperm QTL was tested by formulating the following hypotheses:

Table 1 Genotypic segregation and values for the QTLs expressed in the embryo (diploid) and endosperm (triploid) for four backcrosses

Backcross		QTL genotype			Genotypic value
No	Type	Female	Male	Offspring embryo/endosperm	
The diploid model					
1	$F_1 \times P_1$	*Qq*	*QQ*	*QQ*	$\mu_{m1} + a_m$
				Qq	$\mu_{m1} + d_m$
2	$F_1 \times P_2$	*Qq*	*qq*	*Qq*	$\mu_{m2} + d_m$
				qq	$\mu_{m2} - a_m$
3	$P_1 \times F_1$	*QQ*	*Qq*	*QQ*	$\mu_{m3} + a_m$
				Qq	$\mu_{m3} + d_m$
4	$P_2 \times F_1$	*qq*	*Qq*	*Qq*	$\mu_{m4} + d_m$
				qq	$\mu_{m4} - a_m$
The triploid model					
1	$F_1 \times P_1$	*Qq*	*QQ*	*QQQ*	$\mu_{n1} + 3/2a_n$
				Qqq	$\mu_{n1} - 1/2a_n + d_{n2}$
2	$F_1 \times P_2$	*Qq*	*qq*	*QQq*	$\mu_{n2} + 1/2a_n + d_{n1}$
				qqq	$\mu_{n2} - 3/2a_n$
3	$P_1 \times F_1$	*QQ*	*Qq*	*QQQ*	$\mu_{n3} + 3/2a_n$
				QQq	$\mu_{n3} + 1/2a_n + d_{n1}$
4	$P_2 \times F_1$	*qq*	*Qq*	*Qqq*	$\mu_{n4} - 1/2a_n + d_{n2}$
				qqq	$\mu_{n4} - 3/2a_n$

$$H_0 : a_n = d_{n1} = d_{n2} = 0 \text{ versus}$$
$$H_1 : \text{ Not all equalities in } H_0 \text{ hold.} \quad (4)$$

The log-likelihood ratio test statistic under the H_0 and H_1 of hypotheses (1) is calculated and compared with the critical threshold determined from permutation tests (Churchill and Doerge 1994). The proportion of the total phenotypic variance in an endoreduplication variable explained by a detected QTL was estimated. Additive and dominance genetic effects of the endosperm QTL are further tested according to the hypotheses as follows:

$$H_0 : a_n = 0 \text{ versus } H_1 : a_n \neq 0, \quad (5)$$

$$H_0 : d_{n1} = 0 \text{ versus } H_1 : d_{n1} \neq 0, \quad (6)$$

$$H_0 : d_{n2} = 0 \text{ versus } H_1 : d_{n2} \neq 0. \quad (7)$$

Test statistics for hypotheses (5)–(7) can be thought to be asymptotically χ^2-distributed with one degree of freedom.

The triploid model will be reduced to the diploid model when the two dominance effects specified by the triploid model are the same, i.e., $d_{n1} = d_{n2}$. This can be tested by formulating this equality as a null hypothesis and then calculating the log-likelihood ratio under the null and alternative hypotheses.

Genome-genome epistasis model. Cui and Wu (2005) and Cui et al. (2006) have recently developed a statistical model for mapping and estimating two interacting QTLs from different genomes during seed development. This method allows estimation of the QTL locations in the embryo and endosperm genomes, and QTL additive and genome–genome epistatic effects. Suppose that there are two epistatically interacting QTLs, denoted as **P** (with alleles *P* and *p*) and **Q** (with alleles *Q* and *q*), responsible for an endosperm-specific trait. In our four-backcross design derived from the F_1 and each inbred parents (P_1 and P_2), these two QTLs generate a total of 16 joint endosperm–embryo genotypes (Table 2), assuming that QTL **P** is expressed in the endosperm and **Q** is expressed in the embryo. Together, QTLs **P** and **Q** form a total of 11 genetic effects which are

1. Additive (a_n), *PP* to *p* dominance (d_{n1}) and *pp* to *P* dominance genetic effects (d_{n2}) of QTL **P**,
2. Additive (a_m) and dominance genetic effects (d_m) of QTL **Q**,
3. Additive × additive genetic effect (i_{aa}) between QTLs **P** and **Q**,
4. Additive × dominance genetic effect (i_{ad}) between QTLs **P** and **Q**,
5. *PP* to *p* dominance × additive (i_{da1}) and *pp* to *P* dominance × additive genetic effects (i_{da2}) between QTLs **P** and **Q**,
6. *PP* to *p* dominance × dominance (i_{dd1}) and *pp* to *P* dominance × dominance genetic effects (i_{dd2}) between QTLs **P** and **Q**.

Each of the joint endosperm–embryo genotypes contains a backcross-specific overall mean (μ_1, μ_2, μ_3, or μ_4) and genetic effects whose number and type depend on the

structure of the joint genotype. Note that, of the 16 joint genotypes, 6 are present twice (Table 2), which leads to ten unique genotypes. By considering a different overall mean for each backcross, we will finally have 12 independent equations, which do not adequately provide estimates of the four overall means and all the 11 genetic effects defined above. To the largest extent, 12 independent equations can be used to estimate four backcross-specific means and 8 of the 11 possible genetic effects. The numbers and types of those estimable genetic effects are given in various options as follows:

Option	Endosperm QTL effect	Embryo QTL effect	Endosperm–embryo interaction effect	Number of parameters
1	a_n, d_{n1}, d_{n2}	a_m	i_{aa}, i_{da1}, i_{da2}	8
2	a_n, d_{n1}, d_{n2}	d_m	i_{ad}, i_{dd1}, i_{dd2}	8
3	a_n, d_{n1}	a_m, d_m	i_{aa}, i_{ad}, i_{da1}, i_{dd1}	9
4	a_n, d_{n2}	a_m, d_m	i_{aa}, i_{ad}, i_{da2}, i_{dd2}	9
5	d_{n1}, d_{n2}	a_m, d_m	i_{da1}, i_{dd1}, i_{da2}, i_{dd2}	9
6	a_n, d_{n1}, d_{n2}	a_m, d_m	i_{aa}, i_{ad}, i_{da1}, i_{dd1}	9
…	…	…	…	…
20	a_n, d_{n1}, d_{n2}	a_m, d_m	i_{da2}, i_{ad}, i_{dd1}, i_{dd2}	9

Options 1 and 2 contain a full set of genetic effects for the endosperm-expressed QTL, one genetic effect for the embryo-expressed QTL and all possible genome–genome epistatic effects given the effect specification of the endosperm and embryo QTLs. Options 3–5 each involve both additive and dominance effects for the embryo-expressed QTL, two of the genetic effects for the endosperm-expressed QTL, as well as all possible epistatic effects for these specified main effects. Options 6–20 specify a full set of genetic effects for both the endosperm- and embryo-expressed QTLs, as well as four arbitrarily chosen endosperm–embryo interaction effects. An optimal option that gives the best fit to the data can be determined with a model selection criterion, such as AIC or BIC. In fact, Options 1 and 2 cannot be distinguished from one another because they contain the same number of parameters (eight). This is true for Options 3–20 where each contains nine parameters. For this reason, we can only make a selection between Options 1 or 2 and Options 3–20. In this study, our interest is to detect all possible genetic effects triggered by the endosperm QTL and their possible interaction effects with the embryo QTL. Thus, our selection is Option 1 or 2 in which only one genetic effect is estimated for the embryo QTL. Because the additive effect can be better estimated than the dominance effect, especially for a small to moderate sample size like the one used in this study, we used Option 1 to estimate endosperm–embryo interaction effects on endoreduplication.

The EM algorithm has been developed to estimate the four backcross-specific means and seven effect parameters (a_n, d_{n1}, d_{n2}, a_m, i_{aa}, i_{da1}, i_{da2}) for Option 1 by maximizing a joint likelihood that combines the four backcrosses (Supplement 2). The existence of any significant QTLs for the endosperm trait can be tested by formulating the following hypotheses:

$$H_0 : a_m = a_n = d_{n1} = d_{n2} = i_{aa} = i_{da1} = i_{da2} = 0 \text{ versus}$$
$$H_1 : \text{ Not all equalities in } H_0 \text{ hold.} \quad (8)$$

The critical value for the declaration of significant QTLs can be determined from permutation tests (Churchill and Doerge 1994). The significance of the endosperm- (**P**) and embryo-expressed QTLs (**Q**) can be tested, respectively, based on

$$H_0 : a_n = d_{n1} = d_{n2} = 0 \text{ versus}$$
$$H_1 : \text{ Not all equalities in } H_0 \text{ hold.} \quad (9)$$

$$H_0 : a_m = 0 \text{ versus } H_1 : a_m \neq 0. \quad (10)$$

Whether genome–genome epistatic interactions between QTL **P** and **Q** are significant, is tested by formulating the hypotheses:

$$H_0 : i_{aa} = i_{da1} = i_{da2} = 0 \text{ versus}$$
$$H_1 : \text{ Not all equalities in } H_0 \text{ hold.} \quad (11)$$

As needed, each specific component of the genotypic values can be tested in a similar way. The test statistics for all these hypotheses follow a χ^2 distribution with the degree of freedom equal to the difference in the number of unknown parameters between the null and alternative hypotheses.

Results

Phenotypic characterization of endoreduplication in Mo17 and Sg18 endosperm and their backcross populations

To investigate the genetic basis, for the phenotypic variation in endoreduplication observed in developing maize

Table 2 Genotypic segregations for two QTLs, **P** (with two alleles *P* and *p*), expressed in the endosperm, and **Q** (with two alleles *Q* and *q*), expressed in the embryo, in the four backcrosses as well as genotypic values of these two QTLs under Option 1 for the triploid × diploid model

Backcross		QTL genotype			Genotypic value
No	Type	Female	Male	Offspring (endosperm) (embryo)	
1	$F_1 \times P_1$	(*Pp*) (*Qq*)	(*PP*) (*QQ*)	(*PPP*) (*QQ*)	$\mu_1 + \frac{3}{2}a_n + a_m + \frac{3}{2}i_{aa}$
				(*PPP*) (*Qq*)	$\mu_1 + \frac{3}{2}a_n$
				(*Ppp*) (*QQ*)	$\mu_1 - \frac{1}{2}a_n + d_{n2} + a_m - \frac{1}{2}i_{aa} + i_{ad2}$
				(*Ppp*) (*Qq*)	$\mu_1 - \frac{1}{2}a_n + d_{n2}$
2	$F_1 \times P_2$	(*Pp*) (*Qq*)	(*pp*) (*qq*)	(*PPp*) (*Qq*)	$\mu_2 + \frac{1}{2}a_n + d_{n1}$
				(*PPp*) (*qq*)	$\mu_2 + \frac{1}{2}a_n + d_{n1} - a_m - \frac{1}{2}i_{aa} + i_{da1}$
				(*ppp*) (*Qq*)	$\mu_2 - \frac{3}{2}a_n$
				(*ppp*) (*qq*)	$\mu_2 - \frac{3}{2}a_n - a_m + \frac{3}{2}i_{aa}$
3	$P_1 \times F_1$	(*PP*) (*QQ*)	(*Pp*) (*Qq*)	(*PPP*) (*QQ*)	$\mu_3 + \frac{3}{2}a_n + a_m + \frac{3}{2}i_{aa}$
				(*PPP*) (*Qq*)	$\mu_3 + \frac{3}{2}a_n$
				(*Ppp*) (*QQ*)	$\mu_3 + \frac{1}{2}a_n + d_{n1} + a_m + \frac{1}{2}i_{aa} + i_{da1}$
				(*Ppp*) (*Qq*)	$\mu_3 + \frac{1}{2}a_n + d_{n1}$
4	$P_2 \times F_1$	(*pp*) (*qq*)	(*Pp*) (*Qq*)	(*Ppp*) (*Qq*)	$\mu_4 - \frac{1}{2}a_n + d_{n2}$
				(*Ppp*) (*qq*)	$\mu_4 - \frac{1}{2}a_n + d_{n2} - a_m + \frac{1}{2}i_{aa} - i_{da2}$
				(*ppp*) (*Qq*)	$\mu_4 - \frac{3}{2}a_n$
				(*ppp*) (*qq*)	$\mu_4 - \frac{3}{2}a_n - a_m + \frac{3}{2}i_{aa}$

endosperm, four backcross populations were created by crossing Sg18 and Mo17, two inbred lines that differ in their degree of endoreduplication (Dilkes et al. 2002). Developing kernels were harvested at 16 DAP; the endosperm was phenotyped for endoreduplication by flow cytometry, and DNA was subsequently obtained from the developing embryo. Figure 1 shows representative flow cytometric profiles of nuclei from 16 DAP endosperms obtained from Sg18 and Mo17. Two parameters were used to describe the level of endoreduplication in the parental inbred lines, mean ploidy and the percentage of endoreduplicated nuclei. Mean ploidy was calculated as a weighted average, by multiplying the nuclear ploidy level by the number of nuclei in each ploidy class, and dividing this value by the total number of nuclei. Percentage of endoreduplicated nuclei was estimated as the number of nuclei with 6C or greater DNA content, divided by the total number of nuclei and multiplied by 100. It was observed that Sg18 possess a higher mean ploidy (15.4C) and percentage of endoreduplicated nuclei (72.3%) at 16 DAP than Mo17, for which the mean ploidy was 9.8C and the percentage of endoreduplicated nuclei is 54.8% (Fig. 1).

By crossing the F_1 progeny with the parental inbreds, $Mo17 \times F_1$ and $Sg18 \times F_1$ were produced, and by reciprocal crosses, $F_1 \times Mo17$ and $F_1 \times Sg18$, were developed. Mean ploidy and the percentage of endoreduplicated nuclei were calculated for 92, 89, 85 and 82 endosperms obtained from the central region of single, well-filled 16 DAP ears of $Sg18 \times F_1$, $F_1 \times Sg18$, $Mo17 \times F_1$ and $F_1 \times Mo17$, respectively. Figures 2 and 3 show the distribution of values for mean ploidy and percentage of endoreduplicated nuclei calculated for two of the backcross populations, $Mo17 \times F_1$ and $Sg18 \times F_1$; similar ranges of phenotypic variation were measured for the two other crosses (data not shown). Tests performed for the two parameters used to

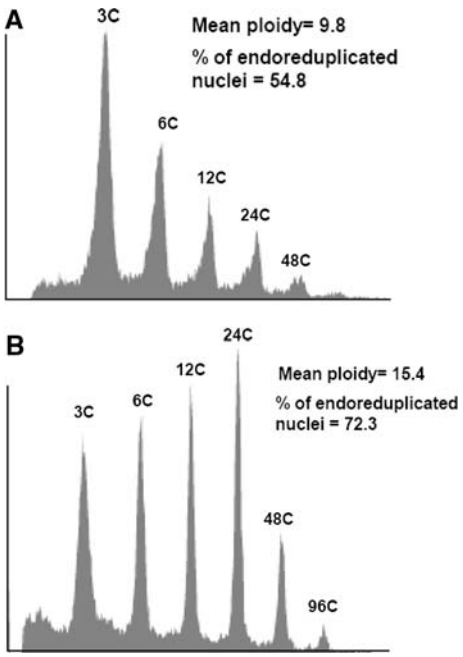

Fig. 1 Flow cytometric analysis of nuclei from 16 DAP endosperms of Mo17 (**a**) and Sg18 (**b**). Nuclei were isolated, analyzed by flow cytometry and the mean ploidy and percentage of endoreduplicated nuclei were calculated as described in "Materials and methods"

phenotype endoreduplication showed continuous variation and a normal distribution in the four backcross populations. For Mo17 × F_1 (Fig. 2a) and F_1 × Mo17 (data not shown), mean ploidy ranged from 6.8C to 14.9C and 8.4C to 14.9C, respectively. The percentage of endoreduplicated nuclei for these two populations ranged from 37.9 to 63.7% for Mo17 × F_1 (Fig. 2b) and 50.4 to 73.3% for F_1 × Mo17 (data not shown). For Sg18 × F_1 (Fig. 3a) and F_1 × Sg18 (data not shown) the estimated mean ploidy values ranged from 9.3C to 19.8C and 8.8C to 19.1C, respectively. The percentage of endoreduplicated nuclei ranged from 56.9 to 94.2% for Sg18 × F_1 (Fig. 3a) and 42.2 to 77.9% for F_1 × Sg18 (data not shown). It was observed that transgressive segregants, for which the phenotypic values were greater or lesser than the parents, were present in the four backcross populations. Analysis of variance indicated that the backcross populations and the parental inbred lines are statistically different at 16 DAP at the 1% significance level (data not shown).

Identification of embryo and endosperm QTLs influencing endoreduplication

An integrated linkage map was constructed with 65 SSR markers for the four backcrosses (Fig. 4). This map was used to genome-wide map and test for the existence of QTLs that affect endoreduplication using the triploid mapping model. A profile of the log-likelihood ratios (LR) used to test the significance of an embryo-expressed QTL calculated under hypothesis (1) for mean ploidy (Fig. 5) and the percentage of endoreduplicated nuclei (Fig. 6) was drawn over the linkage map. The LR peaks that surpass the chromosome- and genome-wide critical thresholds determined from permutation tests indicate the locations of suggestive and significant embryo-expressed QTLs. Table 3 shows estimates of the locations of the QTLs detected by flanking markers and the genetic effects of the QTLs on the two endoreduplication variables. One suggestive QTL on chromosome 4 and one significant QTL on chromosome 7 that affect mean ploidy were detected, with each explaining about 17% of the phenotypic variance. At the QTL on chromosome 4, the Mo17 parent contributes a favorable allele to increased mean ploidy, although the allele from the Sg18 parent is dominant to the Mo17 allele. Compared with this QTL, the one on chromosme 7 manifests an inverse mode of inheritance (Table 3). A second QTL on chromosome 7 at the same location and with a similar mode affects the percentage of endoreduplicated nuclei (Fig. 6; Table 3).

The triploid model detects more QTLs for the two endoreduplication variables—mean ploidy (Fig. 7) and the percentage of endoreduplicated nuclei (Fig. 8). The QTLs detected by the triploid model cannot be detected by the diploid model, because two dominance effects characteristic of an endosperm QTL are significantly different from one another, i.e., $d_{n1} \neq d_{n2}$ ($P < 0.05$). Table 4 shows estimates of the locations of the QTLs detected by flanking markers and the genetic effects of the QTLs on the two endoreduplication variables. Three suggestive QTLs were observed for mean ploidy on chromosomes 4, 6 and 7, respectively, with each explaining 11–18% of the total phenotypic variance (Table 4). It appears that the three triploid endosperm QTLs detected behave in a dominant

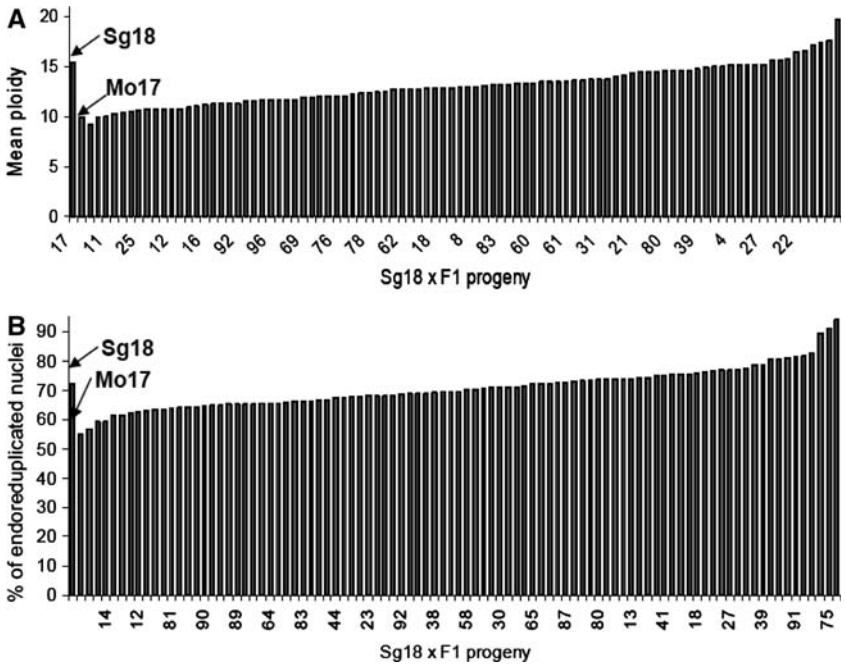

Fig. 2 Distribution of mean ploidy values (**a**) and percentage of endoreduplicated nuclei (**b**) of 16 DAP endosperms from the backcross Sg18 × F_1. Nuclei were analyzed as described in Fig. 1. The data for the reciprocal backcross F_1 × Sg18 were not shown

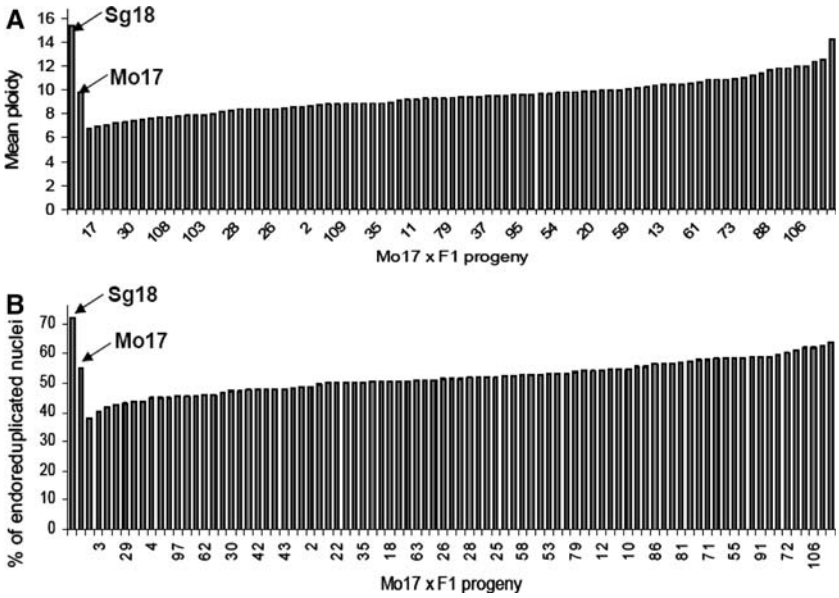

Fig. 3 Distribution of mean ploidy values (**a**) and percentage of endoreduplicated nuclei (**b**) of 16 DAP endosperms from the backcross Mo17 × F_1. Nuclei were analyzed as described in Fig. 1. The data for the reciprocal backcross F_1 × Mo17 were not shown

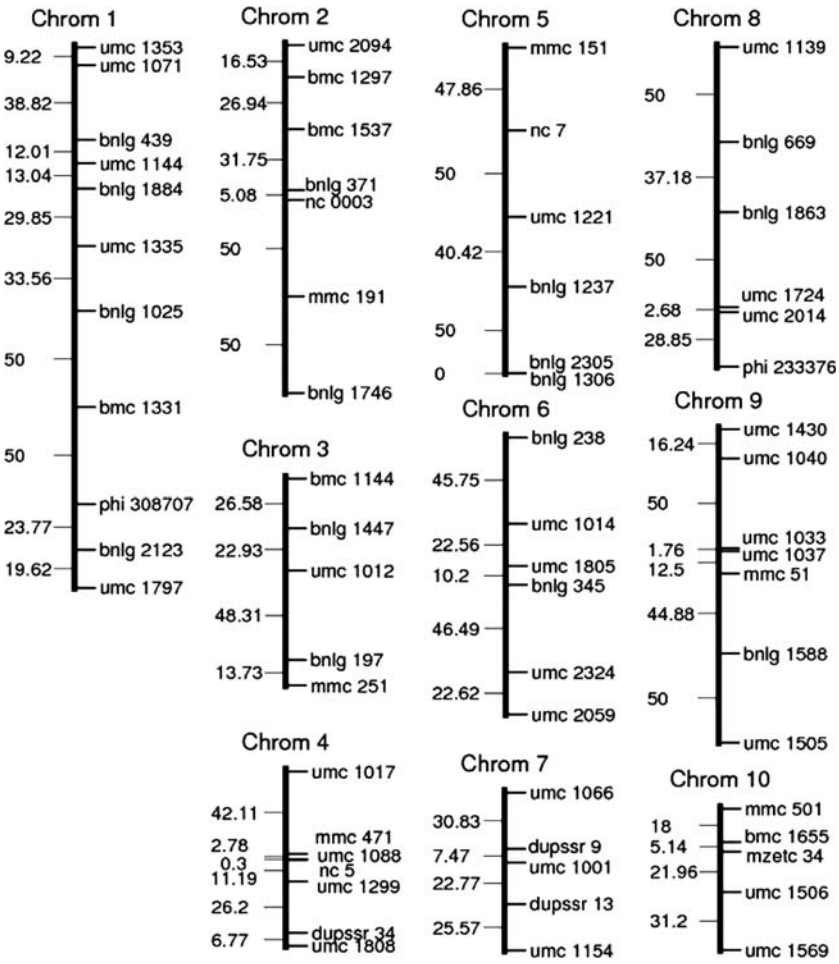

Fig. 4 An integrative genetic linkage map constructed by polymorphic SSR markers for the four backcross populations F_1 × Sg18, F_1 × Mo17, Sg18 × F_1 and Mo17 × F_1. These SSR markers were grouped and ordered on ten different maize chromosomes according to the published maize genome database (http://www.maizegdb.org/)

over-dominance manner, because the two types of dominance effects (i.e., dominance of two Sg18-derived alleles over one Mo17-derived allele, d_{n1}, and dominance of two Mo17-derived alleles over one Sg18-derived allele, d_{n2}) are similar to, or strikingly larger than, the additive effect (a_n). For the QTLs on chromosomes 4 and 6, the Mo17 parent contributes the favorable allele to an increase in mean ploidy ($a_n < 0$), and the Mo17-derived allele is always

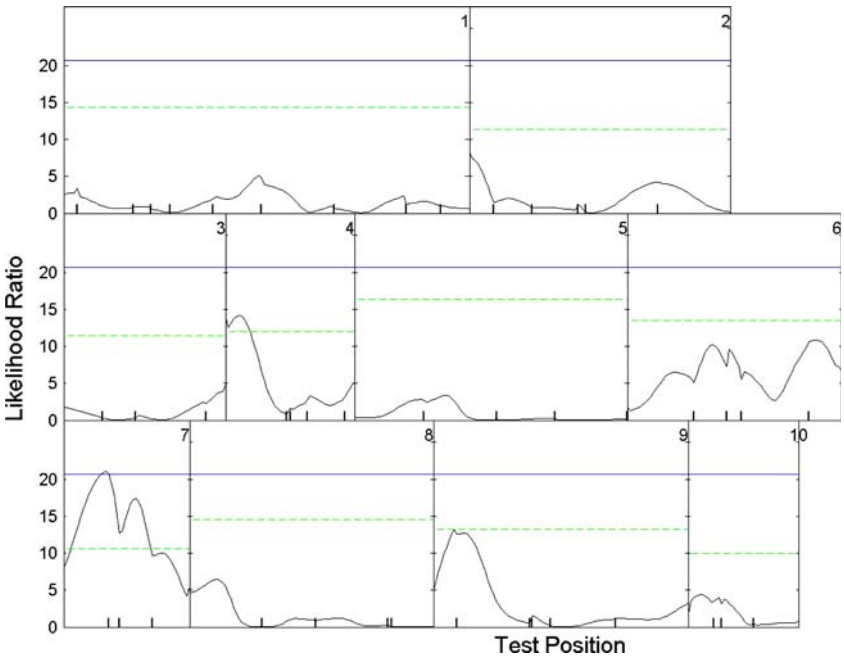

Fig. 5 Profiles of the log-likelihood ratio (LR) test statistics for testing embryo-expressed QTLs affecting mean ploidy based on a joint analysis of the four backcross populations, calculated as a function of genome position across the linkage map. Chromosome- and genome-wide critical thresholds determined from permutation tests are indicated by the *dashed* and *solid horizontal lines*, respectively

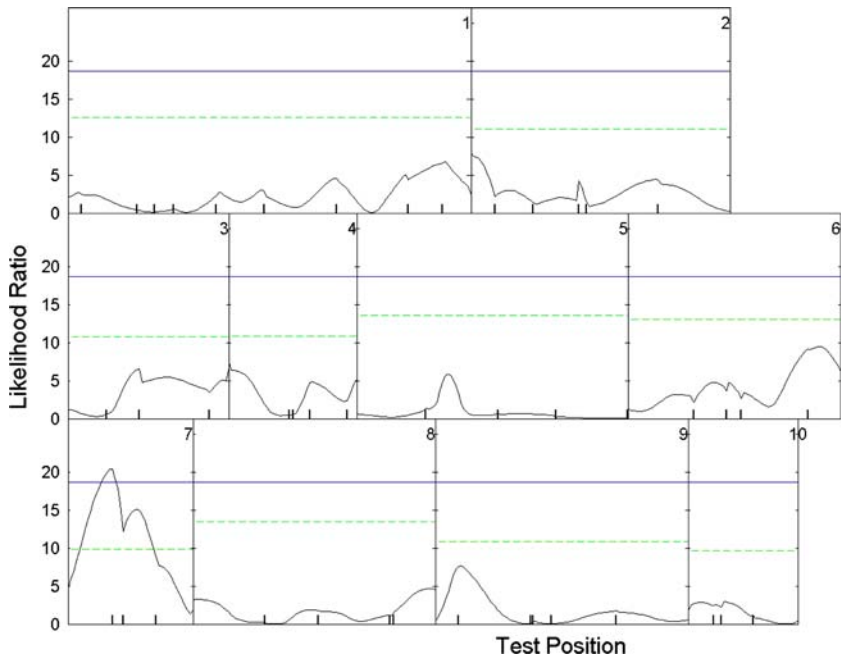

Fig. 6 Profiles of the log-likelihood ratio (LR) test statistics for testing embryo-expressed QTLs affecting percentage of endoreduplicated nuclei based on a joint analysis of the four backcross populations, calculated as a function of genome position across the linkage map. Chromosome- and genome-wide critical thresholds determined from permutation tests are indicated by the *dashed* and *solid horizontal lines*, respectively

Table 3 Additive (a_m) and dominance effects (d_m) of embryo-expressed QTLs for two endoreduplication variables estimated from the diploid mapping model and the proportion of the total phenotypic variance explained by the QTLs in the four backcrosses

Trait	Chromosome	QTL location	Genetic effects		R^2
			a_m	d_m	
Mean ploidy	4	umc1017–mmc471	–0.65	0.76	0.17
	7	umc1066–dupssr9	0.70	–0.59	0.16
% Endoreduplicated nuclei	7	umc1066–dupssr9	1.94	–2.49	0.13

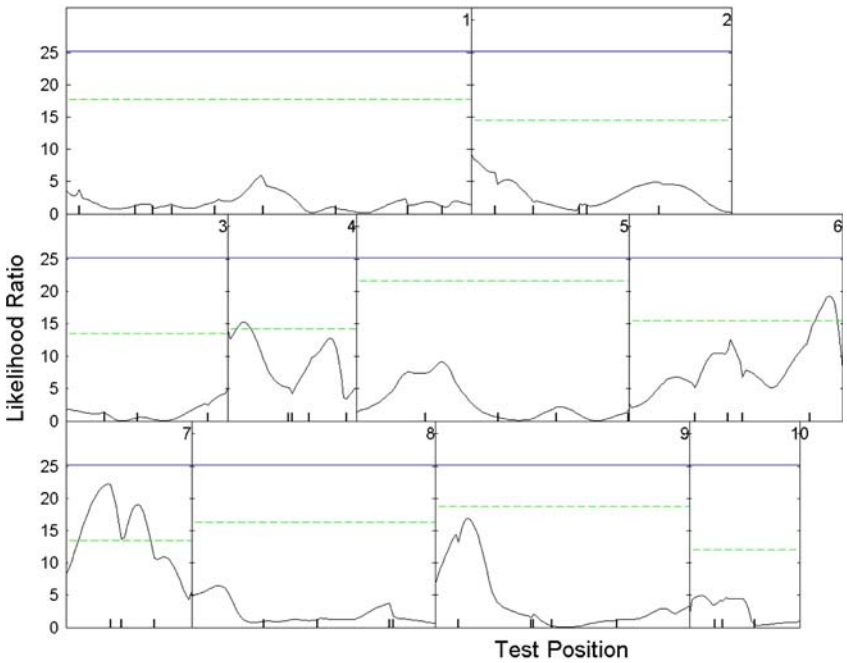

Fig. 7 Profiles of the log-likelihood ratio (LR) test statistics for testing endosperm QTLs affecting mean ploidy based on a joint analysis of the four backcross populations, calculated as a function of genome position across the linkage map. Chromosome- and genome-wide critical thresholds determined from permutation tests are indicated by the *dashed* and *solid horizontal lines*, respectively

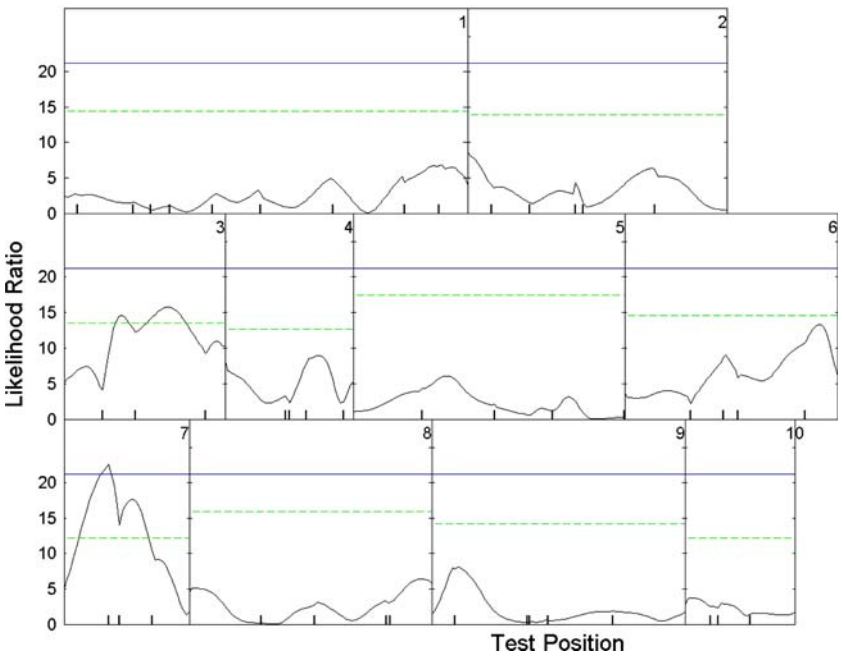

Fig. 8 Profiles of the log-likelihood ratio (LR) test statistics for testing endosperm-expressed QTLs affecting percentage of endoreduplicated nuclei based on a joint analysis of the four backcross populations, calculated as a function of genome position across the linkage map. Chromosome- and genome-wide critical thresholds determined from permutation tests are indicated by the *dashed* and *solid horizontal lines*, respectively

dominant to the Sg18-derived allele, regardless of whether there is one dose of the Mo17 allele compared with two doses of the Sg18 allele ($d_{n1} > 0$), or two doses of the Mo17 allele compared with one dose of the Sg18 allele ($d_{n2} > 0$). The QTL on chromosome 7 has a favorable allele contributed by the Sg18 parent ($a_n > 0$), which is recessive to the Mo17-derived allele (d_{n1}, $d_{n2} < 0$) (Table 4).

It was observed that chromosome 7 harbors a significant QTL, whereas chromosome 3 harbors a suggestive QTL responsible for the percentage of endoreduplicated nuclei (Fig. 8). These QTLs are over-dominant and explain approximately 8 to 18% of the total phenotypic variance (Table 4). The significant QTL on chromosome 7 is located at the same position as the QTL affecting mean ploidy, suggesting that this QTL is pleiotropic, exerting its effects on both endoreduplication traits. This pleiotropic QTL affects mean ploidy and the percentage of endoreduplicated nuclei with the same direction of additive and dominant

Table 4 Additive effect (a_n) and two dominance effects (d_{n1} and d_{n2}) of the endosperm-expressed QTLs for two endoreduplication variables estimated from the triploid mapping model and the proportion of the total phenotypic variance explained by the QTLs in the four backcrosses

Trait	Chromosome	QTL location	Genetic effects			R^2
			a_n	d_{n1}	d_{n2}	
Mean ploidy	4	umc1017–mmc471	–0.46	0.77	0.82	0.13
	6	umc2324–umc2059	–0.16	0.37	1.68	0.18
	7	umc1066–dupssr9	0.47	–0.61	–0.56	0.11
% Endoreduplicated nuclei	3	umc1012–bnlg197	–1.17	–1.07	3.78	0.15
	7	dupssr9	1.26	–2.03	–2.78	0.08

effects. At the QTL in chromosome 3, the Mo17 parent contributes a favorable allele to an increase in the percentage of endoreduplicated nuclei, but one dose of the Sg18-derived allele is highly over-dominant to two doses of the Mo17-derived allele (Table 4).

It should be pointed out that, although there is a distinction between expression of QTLs in the embryo and endosperm, the two types of QTLs are not totally independent, because of the similarity in their genotypic values (see Table 1). This implies that the triploid model can possibly detect an embryo-expressed QTL, whereas the diploid model can also possibly find an endosperm QTL. An issue thus naturally arises about whether the origin of a QTL—embryo or endosperm—can be claimed to affect endoreduplication if both diploid and triploid models obtain significant results. For the QTLs detected on chromosomes 3 and 6, it is safe to suggest that they are endosperm-derived because the diploid model failed to detect them (Table 3 vs. 4). To determine a more likely source for the QTLs on chromosomes 4 and 7, we calculated AIC values under the diploid and triploid mapping models. For the QTL on chromosome 4 for mean ploidy, AICs are 1322.6 for the diploid model and 1323.6 for the triploid model, and therefore this QTL most likely has an embryo origin. It appears that the QTL on chromosome 7 for both mean ploidy and the percentage of endoreduplicated nuclei is an embryo-derived QTL, because the AICs for these two endoreduplication traits are smaller under the diploid model (1315.273 and 2141.998) than the triploid model (1316.128 and 2142.240). However, it could also happen that these two QTLs originate in both the embryo and endosperm, because they were found to be significant by the diploid and triploid models.

Epistatic effects between the QTLs expressed in the endosperm and embryo on endoreduplication

Different QTLs from the embryo and endosperm may interact to affect endoreduplication. Cui and Wu's (2005) genome–genome epistasis model, in conjunction with the four-backcross design used in this study, is able to estimate the main genetic effects of the endosperm- and embryo-expressed QTLs, respectively, and their interaction effects. For our four-backcross design (see Table 2), only a subset of genetic parameters can be estimated because the number of independent equations is less than that of parameters. Considering the purpose of this study for mapping endosperm traits, we attempted to estimate as many genetic effects as possible that are related to endosperm QTLs. The genetic effects of interest to be estimated are the additive (a_n) and dominance effects (d_{n1} and d_{n2}) of the endosperm-expressed QTL, the additive effect (a_m) of the embryo-expressed QTL and the additive × additive (i_{aa}), dominance × additive (i_{da1} and i_{da2}) epistatic effects between the endosperm and embryo QTLs. Hypothesis (11) was used to test whether the endosperm–embryo interaction is important for endoreduplication through pair-wise scanning of the entire genome (Fig. 4). The critical threshold for testing the significance of genome–genome epistasis is directly determined from the χ^2 table, subject to the Bonferroni correction by assuming independence among marker intervals in a sparse linkage map (Lander and Botstein 1989). The corrected threshold at the 5% significance level is 32.93. Although no significant epistasis was detected for the percentage of endoreduplicated nuclei, two pairs of QTL interactions between the endosperm and embryo display an evident impact on mean ploidy (Fig. 9). These two significant genome–genome epistatic pairs occur between two endosperm-expressed QTLs on chromosomes 4 and 10 and the embryo-expressed QTL on chromosome 9, respectively (Table 5), although the main effects due to the two endosperm-expressed QTLs were found to be non-significant. Yet a small peak was detected at each of the two endosperm-expressed QTL positions with the triploid QTL mapping model (see Fig. 7). For both pairs, there is a large value for the dominance × additive epistatic effect (i_{da2}) due to the interaction between the dominance of two doses of the Mo17-derived allele over one dose of the Sg18-derived allele for the endosperm-

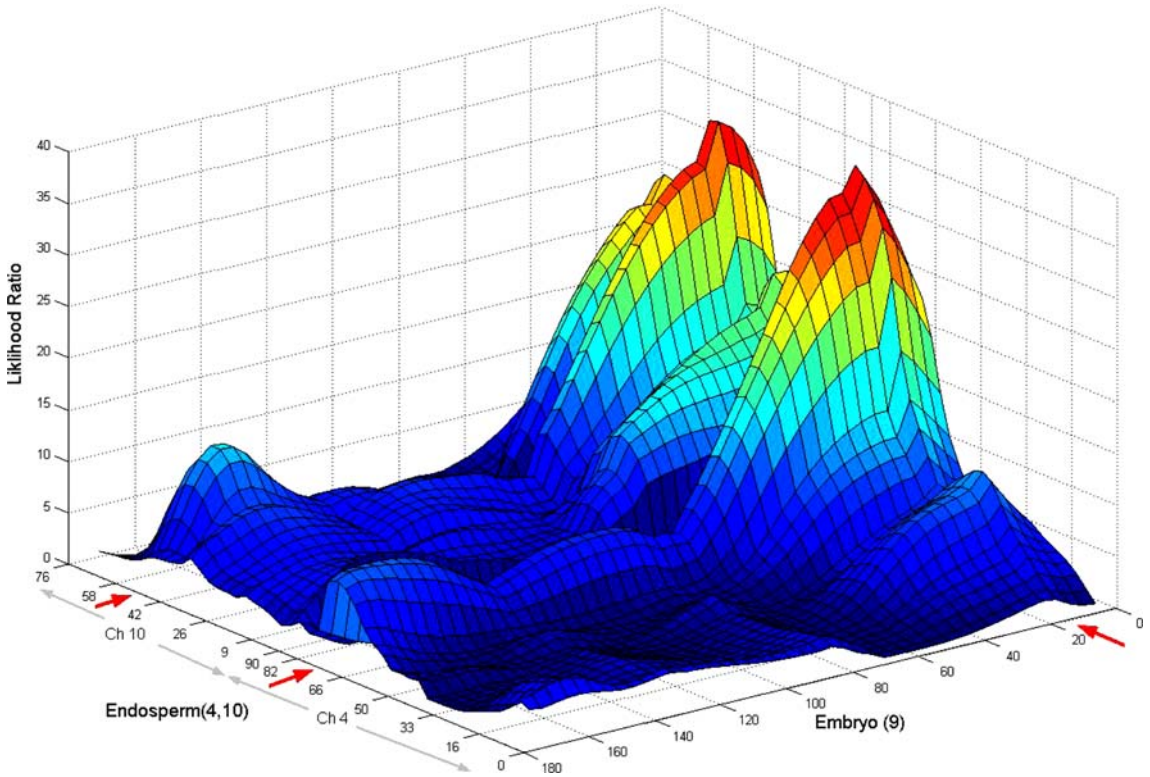

Fig. 9 Profiles of the log-likelihood ratio (LR) test statistics for testing the genome–genome epistatic QTL affecting mean ploidy based on a joint analysis of the four backcross populations, calculated as a function of the endosperm and embryo genome positions across the linkage map. The *arrows* indicate the two peaks of the LR profiles at the locations of the endosperm (in chromosomes 4 and 10) and embryo QTLs (in chromosome 9) that display significant epistatic effect

Table 5 Additive × additive (i_{aa}) and dominance × additive (i_{da1} and i_{da2}) epistatic effects between the endosperm- and embryo-expressed QTLs on mean ploidy estimated by a triploid × triploid model

Pair	Endosperm QTL		Embryo QTL		Main effect				Epistatic effect			LR[a]
	Chrom	Location	Chrom	Location	a_n	d_{n1}	d_{n2}	a_m	i_{aa}	i_{da1}	i_{da2}	
1	4	umc1299–dupssr34	9	umc1040–umc1430	0.10	–0.54	1.06	–0.47	–0.56	0.06	1.88	35.96
2	10	mzetc34–umc1506	9	umc1040–umc1430	–0.37	0.06	1.18	–0.10	–0.37	–0.81	1.49	33.00

[a] LR = log-likelihood ratio for testing the significance of epistasis based on hypothesis (8)

expressed QTL and an additive effect for the embryo-expressed QTL.

As stated by Cui and Wu (2005), significant endosperm (triploid) × embryo (diploid) interactions may be due to the interactions between two QTLs expressed in the endosperm or between two QTLs expressed in the embryo, because the three models, triploid × diploid, triploid × triploid and diploid × diploid, are not totally independent from each other. The full triploid × triploid model contains 15 genetic effect parameters (two additive effects, four dominance effects, and one additive × additive, two additive × dominance, two dominance × additive and four dominance × dominance epistasis), plus four backcross-specific means. To estimate these parameters from 12 independent equations in our four-backcross design (Table 2), we need to make similar assumptions as shown in different options for the triploid × diploid model in Table 3. One option is to consider all three genetic effects for one triploid QTL and only one genetic effect for the second triploid QTL, but this will not make an adequate distinction between the triploid × triploid model from the triploid × diploid model. However, these two models are distinguishable from the diploid × diploid model in which ten independent equations are generated (Table 2). By assuming the additive effect for each embryo QTL, the diploid × diploid model can provide the estimates of four backcross means, two additive genetic effects and one additive × additive epistatic effect. The diploid × diploid

model found a significant embryo × embryo interaction between two QTLs on chromosomes 6 and 7 (Table 6), with the log-likelihood ratio (25.1) for the epistasis test being larger than the 5% significance threshold determined with a Bonferroni correction. But the diploid × diploid model did not find significant endosperm × embryo interactions at the QTL locations detected by the triploid × diploid model (see Table 5), suggesting that the results regarding QTL interactions given in Table 5 can be better explained by the epistasis between the endosperm and embryo.

Discussion

Maize endosperm is formed by the fusion of two nuclei in the mega-gametophyte with a sperm nucleus from the micro-gametophyte, and as a consequence there is a 2:1 balance of maternal to paternal genomes (reviewed in Birchler 1993). Following formation of this triploid nucleus, three distinct cell cycles characterize the phases of endosperm development: syncitial, mitotic and endoreduplication. The latter is an unusual cell cycle that consists of one or more rounds of DNA replication without nuclear and cell division. Many studies have investigated the genetic control of endoreduplication in plants, although the molecular mechanisms by which it is controlled remain poorly understood. Poneleit and Egli (1983) and Seka and Cross (1995) observed that genes from both parents influence the rate of grain-filling, but only the maternal parent controlled the effective grain-filling period. This is consistent with maternal control of endoreduplication, since this process is concomitant with the effective grain-filling period. Indeed, several studies have demonstrated maternal genetic control of endoreduplication during endosperm development (Cavallini et al. 1995; Kowles et al. 1997). Dilkes et al. (2002) obtained robust data showing a maternal influence on endoreduplication in crosses of two popcorn inbred lines, A1-6 and Sg18, with the Midwestern dent inbred, B73, and also for the cross between two Midwestern dent inbreds, Mo17 and B73. Additionally, Dilkes et al. (2002) showed that maternal zygotic components, specifically, were significant for two of the three parameters used to measure endoreduplication in crosses involving A1-6, Sg18, and B73. On the other hand, research has also shown the importance of the 2:1 maternal to paternal genome ratio for endosperm development (Leblanc et al. 2002).

In this study, four backcross populations were developed from crosses between Sg18 and Mo17 to investigate the genetic components influencing endoreduplication. Because this design utilized the backcrosses between the F_1 and each of the parental inbreds, the progeny genotypes included all possible allele combinations, allowing the estimation of additive and dominance effects for a QTL expressed in the embryo or endosperm. In this four-backcross design, two traits, mean ploidy and percentage of endoreduplicated nuclei, previously described by Dilkes et al. (2002), were used as measurements of endoreduplication. Mean ploidy considers both mitotic and endoreduplicated nuclei; therefore, with this parameter we expected to identify loci affecting the transition from a mitotic to an endoreduplication cell cycle. Percentage of endoreduplicated nuclei considers nuclei equal to and above 6C. Kowles and Phillips (1985) showed that the majority of 6C nuclei in 19 DAP endosperm are not poised to divide. So, by using the percentage of endoreduplicated nuclei to assess endoreduplication, we hypothesized we could identify QTLs related to unique aspects of endoreduplication, such as its extent and rate.

Although Fig. 1 shows only one representative flow cytometric image for the two parents, data obtained with each kernel from these ears produced a phenotype consistent with this pattern of endoreduplication. The flow cytometric profiles of endoreduplication in the Sg18 popcorn inbred were similar to those described several years earlier by Dilkes et al. (2002). These authors also analyzed the Mo17 inbred line, and while those results were not reported, the data were consistent with those presented here. As with B73, in Mo17 there is a larger number of nuclei in the 6C class and a decreasing number of nuclei in the higher ploidy classes. It is important to note that the flow cytometric profiles reported for Sg18 and B73 by Dilkes et al. (2002) were obtained from endosperms at a more advanced stage of kernel development than that reported here, and this explains the higher mean ploidy values they reported. Dilkes et al. (2002) proposed that the phenotypic differences in endoreduplication between popcorn and dent corn might occur because this cell cycle begins earlier, is more rapid, or involves a greater proportion of cells than in dent inbreds, such as the Mo17 and B73.

Table 6 Additive × additive (i_{aa}) epistatic effects between two embryo-expressed QTLs on mean ploidy estimated by a diploid × diploid model

Pair	QTL1		QTL2		Main effect		Epistatic effect	LR	Threshold
	Chrom	Location	Chrom	Location	a_1	a_2	i_{aa}		
1	6	bnlg238–umc1014	7	umc1001–dupssr13	0.65	0.83	1.72	25.08	24.71

Based on the phenotypic variation in the backcross populations, the lowest value for mean ploidy was related to the maternal parent. For example, in the backcross population in which Sg18 was the maternal parent, the lowest mean ploidy value was approximately equal to the Mo17 parent (9.8C). And, in the backcross population for which Mo17 was the maternal parent, the lowest mean ploidy value observed was even smaller (6.8C) than the value calculated for Mo17. $F_1 \times$ Mo17 and $F_1 \times$ Sg18, which have the same maternal parent, also had approximately the same lowest mean ploidy values, 8.4C and 8.8C, respectively. On the other hand, the highest mean ploidy value for all of the backcross populations was dependent on the inbred lines used in these crosses, and was independent of the direction in which these crosses were made. The highest mean ploidy values for Mo17 $\times F_1$ and $F_1 \times$ Mo17 were the same, 14.9C, and these values are close to the mean ploidy value calculated for the Sg18 parent (15.4C). The highest mean ploidy values measured for Sg18 $\times F_1$ and $F_1 \times$ Sg18 were approximately the same, 19.8C and 19.1C, and are much higher than the value calculated for the highest mean ploidy inbred parent, Sg18. These results may indicate that maternal control genes, plus genes with a different mode of inheritance, would determine mean ploidy value.

For percentage of endoreduplicated nuclei, it seems that the lowest and the highest values in the backcross populations were dependent on the maternal inbred parent of each mapping population. The Sg18 $\times F_1$ progeny consisted of individuals for which the lowest value of percentage of endoreduplicated nuclei is approximately the same as that calculated for Mo17 (54.8%), and the highest individual value for this trait (94.2%) is much greater than the value measured for Sg18 (72.3%). For the Mo17 $\times F_1$ population, the lowest individual value (37.9%) is much smaller than the value calculated for Mo17, and the highest value (63.7%) is between the parental inbred lines. For $F_1 \times$ Mo17 and $F_1 \times$ Sg18, the lowest percentage of endoreduplicated nuclei, 50.4 and 42.2%, were greater than the value calculated for Mo17, and the highest values, 73.3 and 77.9%, respectively, were greater than that measured for Sg18. These results may indicate that genes with maternal effect control influence the percentage of endoreduplicated nuclei.

By implementing appropriate statistical models (see Wu et al. 2002; Cui and Wu 2005), the joint analysis of these four backcross populations provided the power to dissect endoreduplication into individual QTLs arising from the embryo and endosperm and estimate the additive, dominance and epistatic genetic effects between the QTLs. We detected three endosperm-expressed QTLs on chromosomes 4, 6 and 7 for mean ploidy and two triploid QTLs on chromosomes 3 and 7 for the percentage of endoreduplicated nuclei (Figs. 7, 8; Table 4). Some of these QTLs may also trigger an effect on endoreduplication by the embryo (Figs. 5, 6). The results describing the genetic effects of the QTLs detected can be summarized as follows: First, each of the QTLs accounts for a moderate proportion (8–18%) of the total phenotypic variance for the two endoreduplication variables, and they display strong dominance or overdominance effects. The dominance due to the interactions between the QTL alleles derived from the parents, Sg18 and Mo17, was found to be larger than, or equal to, the additive effect. Second, the triploid endosperm QTLs interact strongly with the QTL expressed in the embryo to influence the degree of endoreduplication. There is abundant evidence for physiological and genetic interactions between the embryo and endosperm in maize kernels, e.g. both organs compete for sucrose transported into the kernel. In the endosperm, sucrose ends up as starch, while in the embryo it is converted to oil, and there is a reciprocal, negative relationship between starch and oil accumulation in the seed (Moose et al. 2004). By crossing maize ears that produce abnormal numbers of polar nuclei with diploid and polyploid pollen, Rhoades and Dempsey (1966) and Lin (1982, 1984) observed that any deviation from a maternal to a paternal nuclear dosage ratio of 2:1 resulted in abnormal or failed endosperm development that generally did not support embryo germination. Remarkable dominance/over-dominance effects may have contributed to transgressive segregants for endoreduplication that were detected in the backcross progeny populations (see also deVicente and Tanksley 1993). The existence of genetic episatsis may also provide an explanation for the formation of transgressive segregants (see also Yu et al. 1997), although our experimental design by phenotyping and genotyping four backcrosses is limited to precisely distinguish among the sources of QTL epistasis due to endosperm–embryo (triploid × diploid), endosperm–endosperm (triploid × triploid) and embryo–embryo (diploid × diploid) interactions.

The QTL detected on chromosome 7 jointly affects mean ploidy and the percentage of endoreduplicated nuclei, suggesting the importance of pleiotropy in the regulation of endoreduplication. The phenotypic coefficient of determination (r^2) between mean ploidy and percentage of endoreduplicated nuclei was estimated for each of the four backcross populations. This coefficient, reported as an average of the four backcross populations, was high, positive, and equal to 0.662, suggesting that approximately 66% of the variance for mean ploidy in the mapping populations can be explained by the variance in percentage of endoreduplicated nuclei. The detection of this pleiotropic QTL explains the strong relationship between the two measures of the degree of endoreduplication.

In conclusion, having as a primary goal the fine mapping and ultimate cloning of QTLs involved in endoreduplication in maize endosperm, the QTLs identified in this study will be confirmed in nearly isogenic lines, which contain small introgressed genome fragments in an isogenic background or in heterogeneous inbred families that are derived from a single recombinant inbred line that segregates a single QTL region in an inbred background that is a mixture of the two parents.

Acknowledgments We thank Dr. Jean-Luc Jannink and the two anonymous reviewers for their comments on the earlier versions of this manuscript that led to a much better presentation of our work. The research described in this manuscript was supported by a grant from the Department of Energy (DE-96ER20242) to BAL and NSF grant (No. 0540745) to RLW. C.M.C. and R.A.D. were supported by scholarships from Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Brazil.

References

Birchler JA (1993) Dosage analysis of maize endosperm development. Ann Rev Genet 27:181–204

Castellano MM, del Pozo JC, Ramirez-Parra E, Brown S, Gutierrez C (2001) Expression and stability of *Arabidopsis* CDC6 are associated with endoreduplication. Plant Cell 13:2671–2686

Castellano MM, Boniotti MB, Caro E, Schnittger A, Gutierrez C (2004) DNA replication licensing affects cell proliferation or endoreduplication in a cell type-specific manner. Plant Cell 16:2380–2393

Cavallini A, Natali L, Balconi C, Rizzi E, Motto, et al (1995) Chromosome endoreduplication in endosperm cells of two maize genotype and their progenies. Protoplasma 189:156–162

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Cui YH, Wu RL (2005) Mapping genome-genome epistasis: a multidimensional model. Bioinformatics 21:2447–2455

Cui YH, Wu JG, Shi CH, Zhu J, Littell RC, Wu RL (2006) Modeling epistatic effects of embryo and endosperm QTL on seed development. Genet Res 87:61–71

D'Amato F (1984) Role of polyploidy in reproductive organs and tissues In: Johri BM (ed) Embryology of angiosperms. Springer, New York, pp 523–566

De Veylder L, Beeckman T, Beemster GTS, de A. Engels J, Ormenese S et al (2002) Control of proliferation, endoreduplication and differentiation by *Arabidopsis* E2Fa-Dpa transcription factor. EMBO J 21:1360–1368

deVicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134:585–596

Dilkes BP, Dante RA, Coelho C, Larkins BA (2002) Genetic analysis of endoreduplication in *Zea mays* endosperm: evidence of sporophytic and zygotic maternal control. Genetics 160:1163–1177

Fulker DW, Cardon LR (1994) A sib-pair approach to interval mapping of quantitative trait loci. Am J Hum Genet 54:1092–1103

Gale MD (1976) High α-amylase breeding and genetical aspects of the problem. Cereal Res Commun 4:231–243

Grime JP, Mowforth MA (1982) Variation in genome size—an ecological interpretation. Nature 299:151–153

Kowles RV, Phillips RL (1985) DNA amplification patterns in maize endosperm nuclei during kernel development. Proc Natl Acad Sci USA 82:7010–7014

Kowles RV, Yerk GL, Haas KM, Phillips RL (1997) Maternal effects influencing DNA endoreduplication in developing endosperm of *Zea mays*. Genome 40:798–805

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Larkins BA, Dilkes BP, Dante RA, Coelho CM, Woo Y-M, Liu Y (2001) Investigating the hows and whys of DNA endoreduplication. J Exp Bot 52:183–192

Leblanc O, Pointe C, Hernandez M (2002) Cell cycle progression during endosperm development in *Zea mays* depends on parental dosage effects. Plant J 32:1057–1066

Leiva-Neto JT, Grafi G, Sabelli PA, Dante RA, Woo Y et al (2004) A dominant negative mutant of cyclin-dependent kinase A reduces endoreduplication but not cell size or gene expression in maize endosperm. Plant Cell 16:1854–1869

Lin BY (1982) Association of endosperm reduction with parental imprinting in maize. Genetics 100:475–486

Lin BY (1984) Ploidy barrier to endosperm development in maize. Genetics 107:103–115

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

Mo HD (1987) Genetic expression for endosperm traits. In: Proceedings of the 2nd international conference on quantitative genetics. Sinauer Associates, Sunderland, pp 478–487

Moose SJ, Dudley JW, Rocheford TR (2004) Maize selection passes the century mark: a unique resource for 21st century genomics. Trends Plant Sci 9:358–364

Nagl W (1978) Endopolyploidy and polyteney in differentiation and evolution. Elsevier, Amsterdam

Perazza D, Herzog M, Hülskamp M, Brown S, Dorne A-M, Bonneville J-M (1999) Trichome cell growth in *Arabidopsis thaliana* can be derepressed by mutation in at least five genes. Genetics 152:461–476

Poneleit CG, Egli DB (1983) Differences between reciprocal crosses of maize for kernel growth characteristics. Crop Sci 23:871–875

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

Schnittger A, Schöbinger U, Stierhorf Y-D, Hülskamp M (2002) Ectopic B-type cyclin expression induces mitotic cycles in endoreduplicating *Arabidopsis* trichomes. Curr Biol 12:415–420

Seka D, Cross HZ (1995) Xenia and maternal effects on maize kernel development. Crop Sci 35:80–85

Shen B, Carneiro N, Torres-Jerez I, Sterverson B, McCreery T et al (1994) Partial sequencing and mapping of clones from two maize cDNA libraries. Plant Mol Biol 26:1085–1101

Sugimoto-Shirasu K, Stacey NJ, Corsar J, Roberts K, McCann MC (2002) DNA topoisomerase VI is essential for endoreduplication in *Arabidopsis*. Curr Biol 12:1782–1786

Walker JD, Oppenheimer DG, Concienne J, Larkin JC (2000) SIAMESE, a gene controlling endoreduplication cell cycle in *Arabidopsis thaliana* trichomes. Development 127:3931–3940

Wu R, Lou X-Y, Ma C-X, Wang X, Larkins BA, Casella G (2002) An improved genetic model generates high-resolution mapping of QTL for protein quality in maize endosperm. Proc Natl Acad Sci 99:11281–11286

Young TE, Gallie DR (2000) Programmed cell death during endosperm development. Plant Mol Biol 44:283–301

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231