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Detection of QTL for flowering time in multiple families of elite maize

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Abstract Flowering time is a fundamental quantitative trait in maize that has played a key role in the postdomestication process and the adaptation to a wide range of climatic conditions. Flowering time has been intensively studied and recent QTL mapping results based on diverse founders suggest that the genetic architecture underlying this trait is mainly based on numerous small-effect QTL. Here, we used a population of 684 progenies from five connected families to investigate the genetic architecture of flowering time in elite maize. We used a joint analysis and identified nine main effect QTL explaining approximately 50 % of the genotypic variation of the trait. The QTL effects were small compared with the observed phenotypic variation and showed strong differences between families. We detected no epistasis with the genetic background but four digenic epistatic interactions in a full 2-dimensional genome scan. Our results suggest that flowering time in elite maize is mainly controlled by main effect QTL with rather small effects but that epistasis may also contribute to the genetic architecture of the trait.

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Introduction

Maize (*Zea mays* L.) is grown worldwide in both, tropical and temperate regions which illustrates its amazing adaptability to a wide range of environments. Maize was domesticated in Central America and during the postdomestication spread from tropical to temperate regions of the Americas (Goodman 1988). This expansion of maize was only possible because of the adaptation of maize flowering time to different climatic conditions which was facilitated by the tremendous natural variation for flowering time that evolved due to the adaptation of its wild relatives to distinct ecological zones (Camus-Kulandaivelu et al. 2006). Flowering time is still a major trait in maize breeding required not only for the adaptation of germplasm to different maturity zones, but also as a component affecting grain yield, grain moisture, or drought stress (Veldboom and Lee 1996; Mechin et al. 2001).

Flowering time is controlled by different signaling pathways: the vernalization, photoperiod, autonomous flowering, and the gibberellic acid response pathways. It has been best studied in Arabidopsis and many genes from the different pathways have been identified (Bäurle and Dean 2006). In grasses including maize, rice, and wheat some of the same genes are present, but their functions are not always conserved (Hayama et al. 2003), and in addition genes that were not previously known from Arabidopsis

have been identified as key regulators (Turner et al. 2005; Xue et al. 2008). In maize only a few genes that have been cloned have been associated with flowering time, including *DWARF8* (*D8*), *INDETERMINATE GROWTH1* (*ID1*), and *VEGETATIVE TO GENERATIVE TRANSITION1* (*VGT1*) (Thornsberry et al. 2001; Colasanti et al. 2006; Salvi et al. 2007).

Genetic studies of flowering time have been conducted intensively in biparental populations of maize (e.g., Austin and Lee 1996; Beavis et al. 1994). Based on a meta-analysis comprising 22 flowering time QTL mapping studies Chardon et al. (2004) identified more than 60 flowering time QTL and six major QTL affecting flowering time across populations. The focus of plant geneticists has recently shifted to the combined analysis of multiple biparental families (Rebai and Goffinet 1993; Blanc et al. 2006; Verhoeven et al. 2006; Myles et al. 2009; Steinhoff et al. 2011; Würschum et al. 2012a). This multiple-family analysis offers the advantage of an improved QTL detection power, a higher precision in QTL position estimates, and a better understanding of the allelic variation at QTL positions (Würschum 2012). Blanc et al. (2006) suggested two models for multiple-line cross QTL (MC-QTL) mapping: the disconnected model which assumes variable QTL effects in the families and the connected model which assumes uniform QTL effects. Recent studies based on connected families have investigated the genetic architecture of flowering time in maize. The studies of Buckler et al. (2009) and Coles et al. (2010) were based on diverse founder lines with temperate and tropical origin. The results suggest that flowering time in maize is mainly controlled by numerous QTL each having only a small impact on the trait (Buckler et al. 2009). Coles et al. (2010) detected four of the major regions identified in the meta-analysis. One of them is located on chromosome 10 and fine mapping revealed a gene encoding for a protein homologous the rice *Ghd7* heading regulator though its causative role has not been demonstrated yet (Ducrocq et al. 2009).

Epistasis refers to interactions between two or more loci in the genome (Carlborg and Haley 2004). The interactions within and among pathways controlling flowering time suggest that these should be reflected in a similar amount of epistasis at the genetic level. Epistatic interactions have indeed been detected in the self-pollinating plants Arabidopsis and rice (El-Lithy et al. 2006; Uwatoko et al. 2008), whereas in the outbreeding species maize epistasis appears to be less prominent (Buckler et al. 2009; Coles et al. 2010).

The main objective of this study was to dissect the genetic architecture underlying flowering time in elite maize based on a large population of 684 genotypes. In particular, the objectives were to (1) perform a QTL analysis for main effects, (2) investigate the allele substitution effects of the QTL in different families, and (3) assess the contribution of epistasis to the expression of flowering time in elite maize.

Materials and methods

Plant materials and field experiments

Five F_3 families, with a total of 684 unselected individuals (Table 1) were obtained from an incomplete diallel cross between four dent inbreds (A, B, C, and D) (Fig. 1). From each cross between the parental lines an F_2 family was established and the individuals were selfed to obtain F_3 plants. F_3 plants were then selfed to obtain $F_{3:4}$ families. Testcross (TC) progenies were produced by mating each of the 684 $F_{3:4}$ families to the same inbred tester. The tester was an elite inbred from the opposite heterotic pool and unrelated by pedigree. The plant material is identical to that described by Steinhoff et al. (2011) with the exception that five families have been used in this study as one of the six families described by Steinhoff et al. (2011) has not been phenotyped for flowering time. All plant materials used in this study are from Syngenta Seeds, Bad Salzuflen, Germany.

The testcross progenies were evaluated in 2007 in Italy at three climatically similar locations with unreplicated trials. Each of the segregating families was evaluated in separate but adjacent field trials connected with common checks. Two-row plots (8.4 m^2) were machine planted (8 plants m^{-2}), and female flowering time (days to silking)

Table 1 First- and second degree statistics for maize testcross (TC) progenies evaluated for female flowering time in three environments

Family	*n*	TC mean (range)	σ^2_G	σ^2_e	h^2
A × B	131	82.62 (79.93–84.93)	0.55***	1.29	0.56
A × C	143	83.00 (79.84–85.84)	1.03***	1.53	0.67
A × D	140	83.28 (80.75–85.41)	0.27**	1.82	0.31
B × C	129	83.20 (80.58–85.58)	0.32***	1.56	0.38
B × D	141	83.37 (80.78–85.45)	0.11*	1.53	0.18
Population	684		0.49***	1.99	0.42

Genotypic variance (σ^2_G), error variance (σ^2_e), and heritability (h^2) are shown

***,**,*significant at the 0.01, 0.05, and 0.1 probability level, respectively

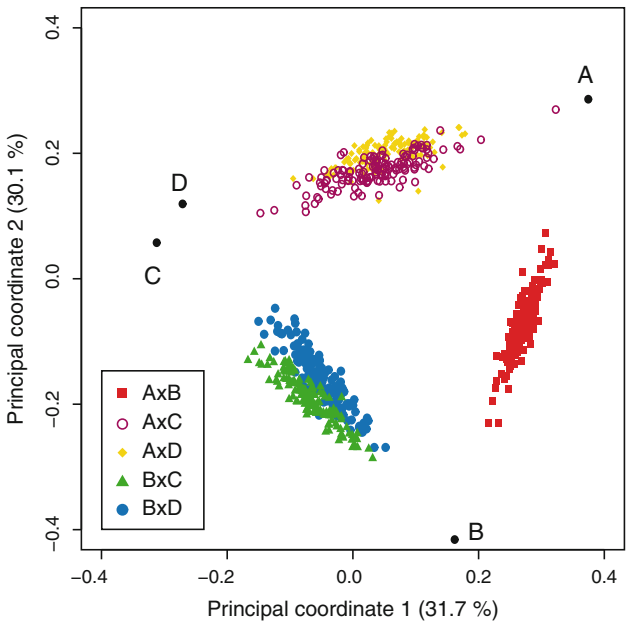

Fig. 1 Principal coordinate analysis of the four parents and the 684 progenies based on modified Rogers' distance estimates. Percentages in parentheses refer to the proportion of variance explained by the principal coordinate

was defined as the number of days until 50 % of plants in a plot were exerting silks.

Genotypic analysis

Each F_3 plant was represented by 15 bulked $F_{3:4}$ plants. DNA extraction was conducted following a modified SDS-potassium-acetate protocol (Dellaporta et al. 1983). Single nucleotide polymorphism (SNP) detection was performed using Taqman technology (Applied Biosystems 2002). Observed genotype frequencies at each marker locus were checked for deviations from the expected Mendelian segregation ratio and allele frequencies of 0.5 using a χ^2 test. High-quality molecular data were produced with 857 SNP markers, which were used for further analysis. The genetic linkage map is described in Steinhoff et al. (2011). Associations among the 4 parents and the 684 progenies were analyzed by applying principal coordinate analysis (PCoA) (Gower 1966) based on the modified Rogers' distances of the individuals (Wright 1978). PCoA analysis was performed using software Plabsoft (Maurer et al. 2008). Segregation distortion was assessed in each population by a Chi-square test for each marker segregating in that population.

Agronomic data analysis

In each environment, phenotypic data values were adjusted for block effects with four checks. Estimates of variance components σ^2_e (genotype by environment interaction variance confounded with experimental error variance) and σ^2_G (genotypic variance) of testcross progenies were calculated by assuming random genotypic effects. As the experiment was based on unreplicated trials, the genotype by environment interaction variance cannot be separated from the experimental error variance. Heritability (h^2) on a testcross progeny-mean basis was calculated as the ratio of genotypic to phenotypic variance according to the method described by Melchinger et al. (1998). Moreover, Best Linear Unbiased Estimates (BLUEs) of testcross progenies were determined by assuming fixed genotypic effects. Analyses were performed using the statistical software SAS (SAS Institute 2008).

Multiple-line cross QTL mapping

For QTL mapping, an additive genetic model was chosen for the testcross progenies as described by Melchinger et al. (1998). A joint analysis was performed with a model assuming (1) specific QTL effects for every family (disconnected model) and (2) uniform QTL effects of parental inbred lines across families (connected model) (Blanc et al. 2006). Both models were described in detail by Steinhoff et al. (2011). In brief, the disconnected model was

$$Y = JM + X_q B_q + \sum_{c \neq q} X_c B_c + \varepsilon$$

where Y was a $N \times 1$ column vector of those BLUE values of phenotypic data of N testcross progenies coming from P families. J was a $N \times P$ matrix whose elements were 0 or 1 according to whether individual i belonged to family p or not and M was a $P \times 1$ vector of family specific means. X_q (X_c) an $N \times P$ matrix containing the expected number (ranging from 0 to 2) of allele k for each individual in family p at QTL q (cofactor c), and B_q (B_c) was a $P \times 1$ vector of the expected allele substitution effects of QTL q (cofactor c) in family p. ε was the vector of the residuals.

The connected model which accounts for the relationships between the parents was

$$Y = JM + X^*_q B^*_q + \sum_{c \neq q} X^*_c B^*_c + \varepsilon$$

where Y, J, M, and ε were as described in the disconnected model, X^*_q (X^*_c) was an $N \times K$ matrix containing the expected number of alleles of parent k at QTL q (cofactor c) given the marker data for each progeny i, and B^*_q (B^*_c) was a $K \times 1$ vector of the expected allele substitution effects of QTL q (cofactor c).

Cofactor selection was performed for both models using PROC GLMSELECT implemented in the statistical software SAS (SAS Institute 2008). Testing for presence of a putative QTL in an interval was performed using a likelihood-ratio test using statistical software R (R Development Core Team 2010). LOD-thresholds of 4.98 for the

disconnected model and of 5.59 for the connected model were used corresponding to an experiment-wise type I error of $P < 0.10$, based on 2,000 permutations (Doerge and Churchill 1996).

The proportion of the genotypic variance explained by all detected QTL was estimated as R^2_{adj}/h^2 (Utz et al. 2000). The support interval of a QTL was defined as a LOD fall-off of 1.0 expressed as position on the chromosome in centimorgans (cM) (Lander and Botstein 1989), and cofactors were excluded within a distance to the marker interval under consideration smaller than 10 cM. To compare our results with other QTL mapping experiments, we took the relative position of the QTL on the chromosomes in our study (e.g., QTL in the middle of a chromosome) and compared it with the relative position of published QTL on the published chromosomes (e.g., QTL also in the middle of the same chromosome).

The scan for QTL × genetic background epistasis was performed by model comparison as suggested by Blanc et al. (2006), which is a modified version of the test suggested by Jannink and Jansen (2001). The test for background epistasis is based on a model with QTL as defined in the disconnected analysis and cofactors as defined in the connected analysis and a model with QTL as defined in the connected analysis and cofactors as defined in the connected analysis. An F-threshold of 7.7 was used corresponding to an experiment-wise type I error of $P < 0.10$, based on 2,000 permutations.

The epistasis scan for pairwise interactions was done with the model described above which was extended by the term $X_{q'}B_{q'}$ for the second locus and the interaction term between the two loci q and q' $X_{qq'}B_{qq'}$. We used an α-level of 0.05 and followed the suggestion of Holland et al. (2002) dividing the α-level by the number of possible independent pairwise interactions between chromosome regions, assuming two separate regions per chromosome ($P < 2.6e\text{-}4$).

Results

The genotypic variances (σ^2_G) estimated in the five families with family sizes ranging between 129 and 143 were all significantly larger than zero (Table 1). Heritability estimates in single families ranged between 0.18 and 0.67 and the total heritability amounted 0.42. The average and the range of the adjusted entry means of days to silking were comparable for all five families. The difference between the earliest and the latest flowering genotypes was between 4.7 and 6 days in the five families. The first two principal coordinates together explained 61.8 % of the total variation. Principal coordinate analysis (PCoA) revealed that the progenies within segregating families cluster together and that the five families show different degrees of relatedness with progenies from families A × C and A × D, and B × C and B × D, respectively, being more closely clustered due to the close relationship between parents C and D (Fig. 1). The analysis of the haplotype structure of the four parental lines confirmed the similarity between parents C and D and revealed a different haplotype pattern for parent B compared with the other parents (Figure S1).

Our test for segregation distortion revealed that in the different families certain chromosomal regions showed deviations from the expected Mendelian segregation ratio (Fig. 2). This was most obvious for a region on chromosome 1 which was affected in most families. Regarding the

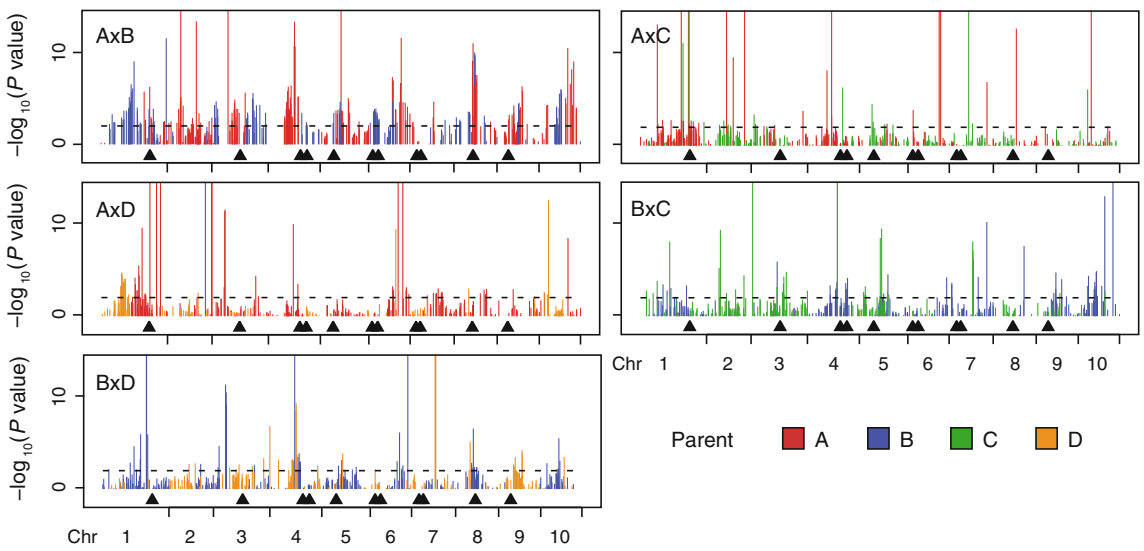

Fig. 2 Segregation distortion in the five families underlying this study. The positions of the detected QTL are indicated by *arrowheads*

Table 2 QTL detected with the disconnected and the connected model for female flowering time, position of the QTL and the support interval in brackets, their proportion of explained genotypic variance (p_G), potential candidate genes underlying the QTL, and the ratio of the variance explained by the QTL (σ^2_{QTL}) and by the QTLxLocation interaction ($\sigma^2_{QTLxLoc}$)

QTL	Chr.	Disconnected model Position (cM)	Connected model p_G	Position (cM)	p_G	Candidate gene (*s*)	$\sigma^2_{QTL}/\sigma^2_{QTLxLoc}$
1	1	157.9 (157.6–158.7)	12.9	157.9 (157.6–158.7)	9.3	*ID1*	0.99
2	3	84.8 (81.6–89.1)	5.7				0.01
3	4	87.8 (86.4–88.0)	8.1				0.01
4	4	113.8 (112.6–114.3)	6.2				14.41
5	5	42.3 (41.1–44.1)	7.2			*ZmPHYA*, *OsGAI*, *OsPHYC*	7.12
6	6			13.1 (12.8–13.3)	9.1	*OsELF3*, *OsZTL_L1*	0.31
7	6	26.8 (25.8–34.3)	12.0				1.52
8	7	15.4 (13.3–21.6)	11.2	16.4 (12.3–21.6)	9.1		1.52
9	8	59.2 (58.9–59.5)	6.7			*VGT1*	10.27
10	9	45.6 (44.6–46.4)	7.2			*OsCO_Hd1*	2.17
Total			77.2		27.4		

entire genome, family A × B showed the strongest segregation distortion. In general, the regions exhibiting segregation distortion did not appear to co-localize with the positions at which flowering time QTL were detected (Fig. 2). The exception may be the region surrounding the QTL on chromosome 1 which appeared to show a distorted segregation ratio in all three families involving parent A.

QTL analysis with the disconnected model identified nine main-effect QTL, whereas only three QTL were detected with the connected model (Table 2; Fig. 3). Two of these three QTL were also detected with the disconnected model and only one was specific for the connected model. No QTL was detected at this position in the analysis of the five single families (Figure S2) which might indicate that this QTL is a false-positive QTL, but could also be due to the reduced QTL detection power in single families as opposed to the QTL detection across all families. It must be noted that for testcross progenies only half of the additive variance is exploited compared with evaluation of the *per se* performance. This inevitably leads to a reduced power to detect QTL of interest. In addition, if we assume a biparental population and fixation of a completely dominant allele in the tester, then we will not be able to detect any difference between testcross progenies even though the population is segregating for the QTL. The problem related to the use of strong testers was outlined in detail by Hallauer et al. (1988).

The genotypic variance explained by all detected QTL simultaneously amounted 77.2 and 27.4 % for the disconnected and the connected model, respectively. The estimates for the proportion of explained genotypic variance of single QTL ranged between 6.2 and 12.9 %. The size of the support intervals averaged 3.8 cM for the disconnected model and 3.6 cM for the connected model. We estimated the variances of the detected QTL (σ^2_{QTL}) as well as of their interaction with locations ($\sigma^2_{QTLxLoc}$). We observed that for six of the ten detected QTL, the variance σ^2_{QTL} was larger than $\sigma^2_{QTLxLoc}$. The strong differences in the ratio of the two variances illustrate the variability in the importance of the interaction of the QTL with the environment. As expected for an adaptive trait like flowering time, some QTL show a strong interaction with the environment and consequently these QTL should be treated carefully in marker-assisted selection programs for different environments.

The allele substitution effects estimated in each family for the QTL detected with the disconnected model showed strong variation between families and even changed in sign (Fig. 4a). The allele substitution effects estimated for each parental line for the QTL detected with the connected model also revealed differences between the parents, even for the closely related parents C and D (Fig. 4b). These parental allele substitution effects were small compared with the range of flowering time observed in the families.

One of the potential causes for the observed variation in QTL effects between families are multiple alleles at QTL. The effect of multiple alleles at one QTL locus on the allele substitution (α) effect is exemplified in Fig. 4c. Assuming a population in Hardy–Weinberg equilibrium and absence of epistasis the α-effect is $\alpha = a + (q–p)$d, which with equal allele frequencies of $p = q = 0.5$ becomes $\alpha = a$ (Fisher 1918). If we assume three alleles carried by three parents, the α-effect will vary substantially in the three resulting populations, depending on the difference of lines homozygous for these alleles. In this study, we evaluated the testcross performance of $F_{3:4}$ families and not the *per se* performance. Following Schnell (1965) we expect the following allele substitution effect for the alleles originating from our $F_{3:4}$ families assuming absence of epistasis: $\alpha = a + (q'–p')$d, where p' and q' are the allele frequencies of the tester. With the expected allele frequencies at the

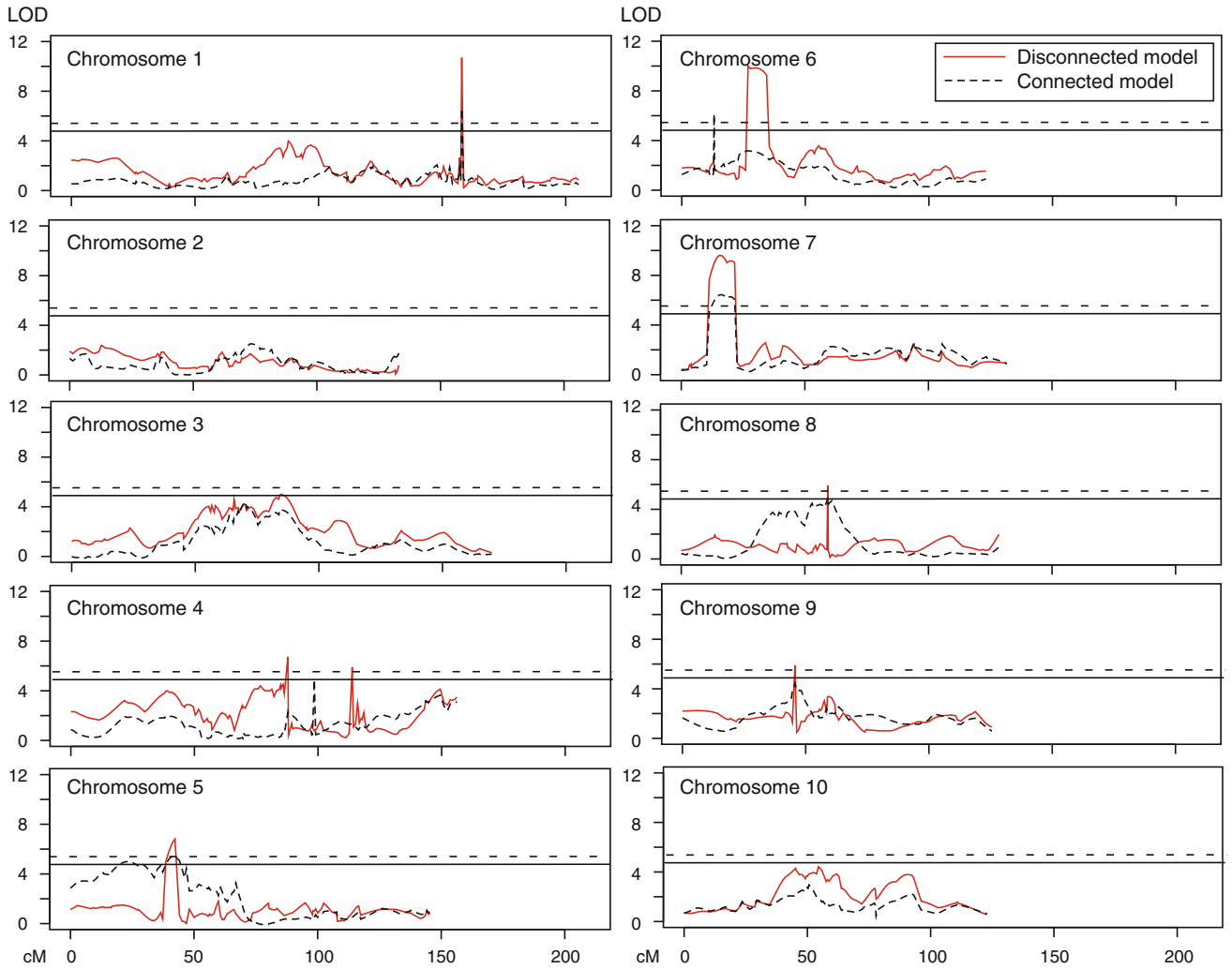

Fig. 3 QTL for female flowering time. The LOD curves from the joint QTL analyses based on the disconnected and connected model. The significance thresholds are shown as *horizontal lines*

tester the α-effect becomes $\alpha = a–d$ ($p' = 1$ and $q' = 0$) or $\alpha = a + d$ ($p' = 0$ and $q' = 1$) (Fig. 4d). Consequently, as QTL effect we can map the α-effect which is sometimes referred to as additive effect, but does not correspond to the '*a*' effect. The α-effect is actually determined by the '*a*' and '*d*' effect and also by the allele frequencies (in our case simplified by only one single tester). Multiple alleles will have the same effect for testcross progenies as described above. In addition, the α-effect is strongly influenced by the degree of dominance which can vary between alleles and thus between families (Fig. 4e).

We first tested for epistatic interactions with the approach suggested by Blanc et al. (2006). This test, however, revealed no significant interactions of markers with the genetic background (Fig. 5a). Two regions on chromosome 1 and on chromosome 10 showed the strongest interaction with the genetic background. The peak on chromosome 1 also co-localized with a main effect QTL that was detected by both models. Next, we performed a full 2-dimensional genome scan for interactions. This scan revealed that the region on chromosome 1 which showed a strong, albeit non-significant interaction with the genetic background was not involved in any significant digenic epistasis (Fig. 5b). Four regions were identified which showed significant epistatic interactions (Fig. 6). Three of these involved chromosome 9 which interacted with regions on chromosomes 2, 7, and 8. The interactions with chromosomes 7 and 8 appear to involve the same region on chromosome 9 between 40 and 60 cM, a region in which also a main effect QTL was identified. The interaction with chromosome 2 likely includes a different region on chromosome 9 between 60 and 80 cM. In addition to the interaction with chromosome 9, we also identified an interaction of chromosome 8 with chromosome 4. The two

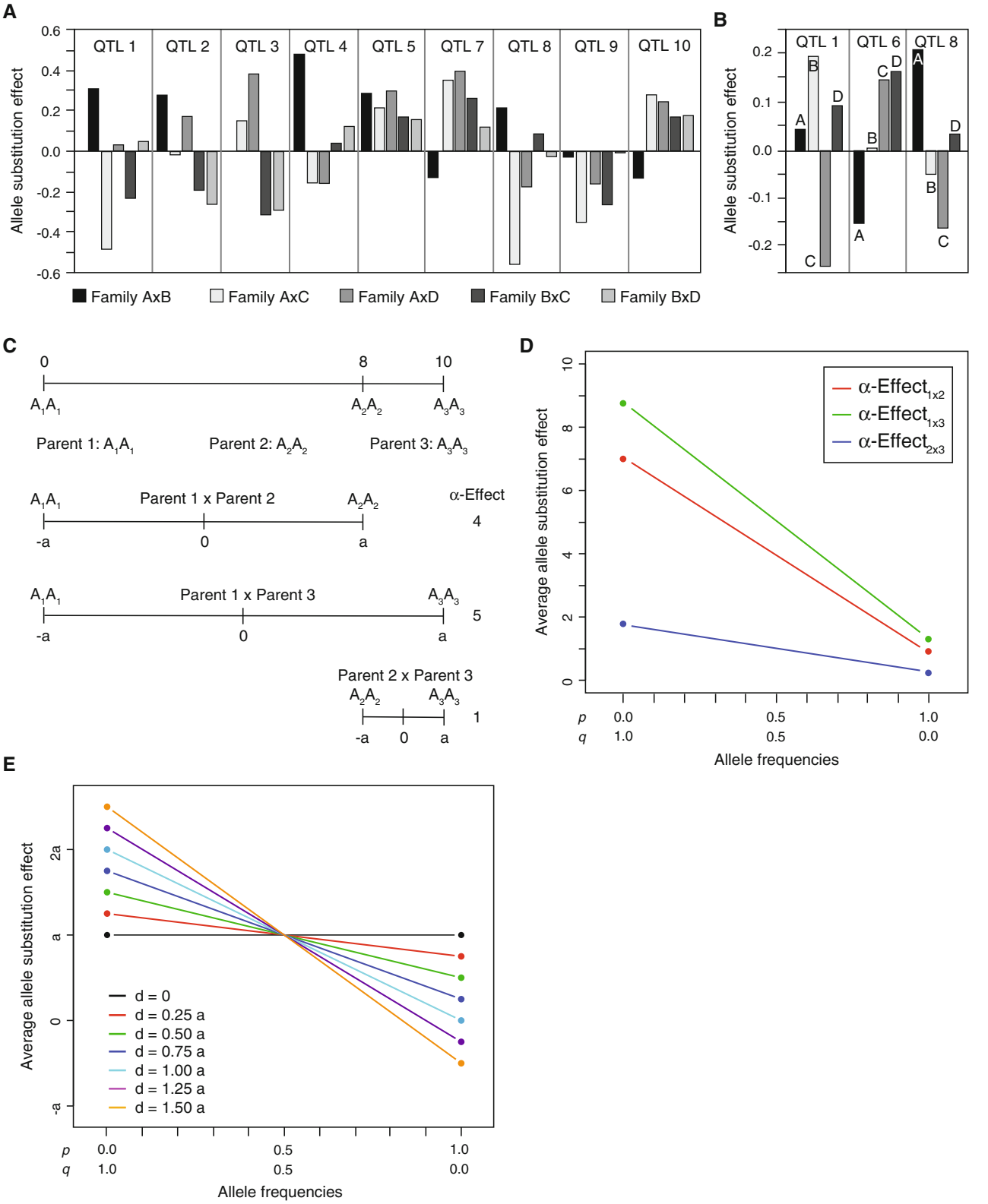

Fig. 4 **a** Allele substitution (α) effect in each of the families for the QTL detected with the disconnected model (the QTL are as described in Table 2). **b** Allele substitution effect of each parental line for the QTL detected with the connected model. **c** Schematic representation of the effect of multiple alleles on the α-effect, assuming a population in Hardy–Weinberg equilibrium and equal allele frequencies of $p = q = 0.5$. **d** Effect of the allele frequency on the α-effect of multiple allele loci, assuming $d = ¾\ a$. For testcrosses the allele frequencies are those of the tester population: $p = p'$ and $q = q'$. For a single tester this becomes $p' = 0$ and $q' = 1$, or $p' = 1$ and $q' = 0$. **e** Dependency of the α-effect on d

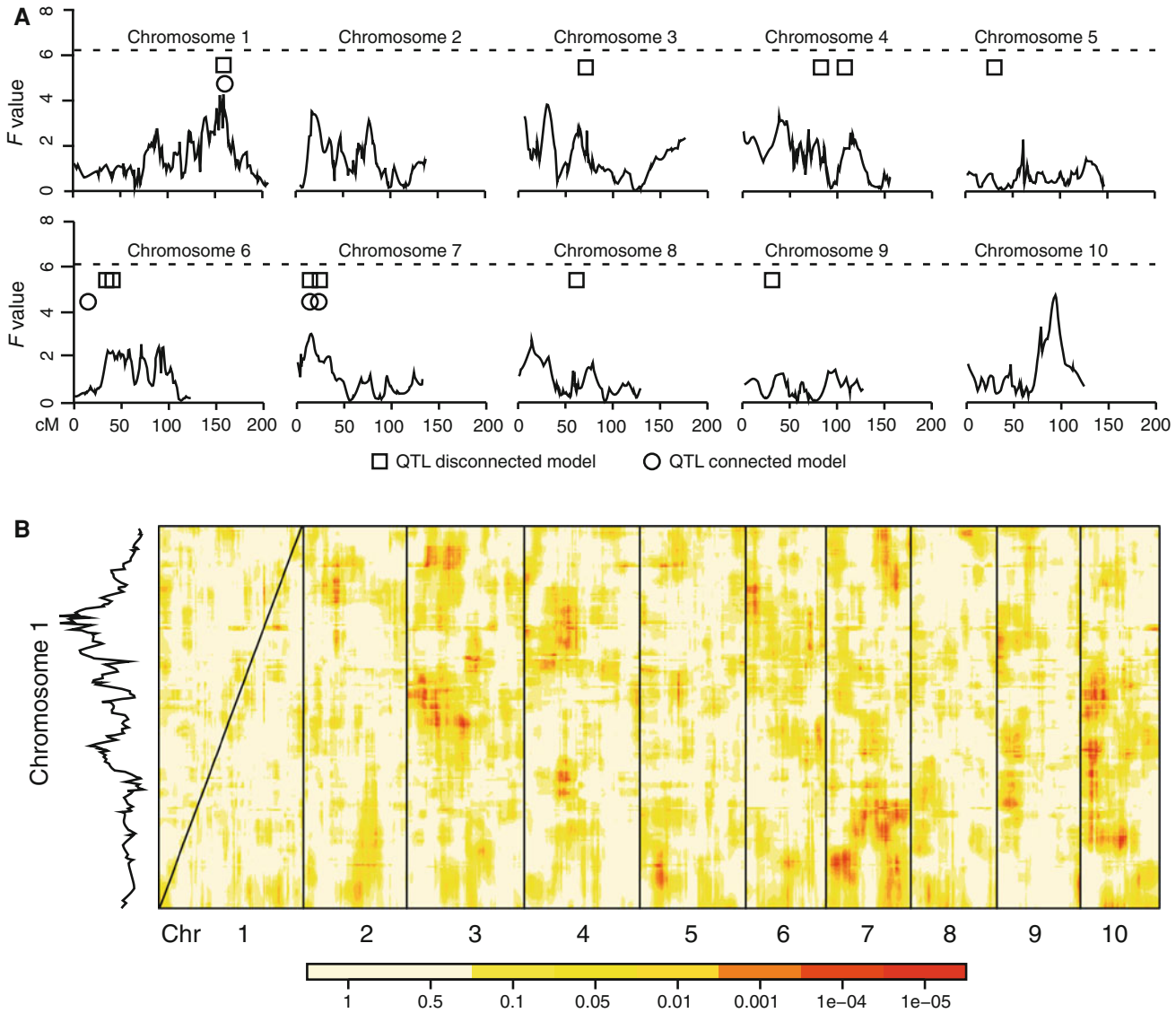

Fig. 5 **a** *F*-value curves of the background epistasis scan. The main effect QTL detected with the disconnected and the connected model are indicated. **b** Results from the full 2-dimensional epistasis scan shown for chromosome 1

interactions appear to involve different regions on chromosome 8. The proportion of genotypic variance explained by these epistatic interactions ranged between 5.3 and 6.4 %.

Discussion

Flowering time is an important trait in maize breeding not only for the adaptation of germplasm to a maturity zone, but also for the optimization of grain yield and grain moisture. Whereas the genetic architecture underlying this trait has been investigated in populations specifically designed for QTL detection, the genetic control of flowering time in elite material remains less well understood.

Properties of the maize population

Our study was based on 684 progenies derived from five families with family sizes ranging between 129 and 143 which constitutes a fairly large population size for QTL mapping studies. The heritability was lower than that observed by Buckler et al. (2009) and also compared with the study from Blanc et al. (2006) which also investigated elite material. The difference may be attributed to the higher number of environments or a higher genotypic variance in these studies (Lynch and Walsh 1998). The four parents were all elite lines but the PCoA (Fig. 1) revealed that parents C and D are closely related and consequently the parents likely do not fully represent the alleles and the genotypic variation present in elite material. Nevertheless,

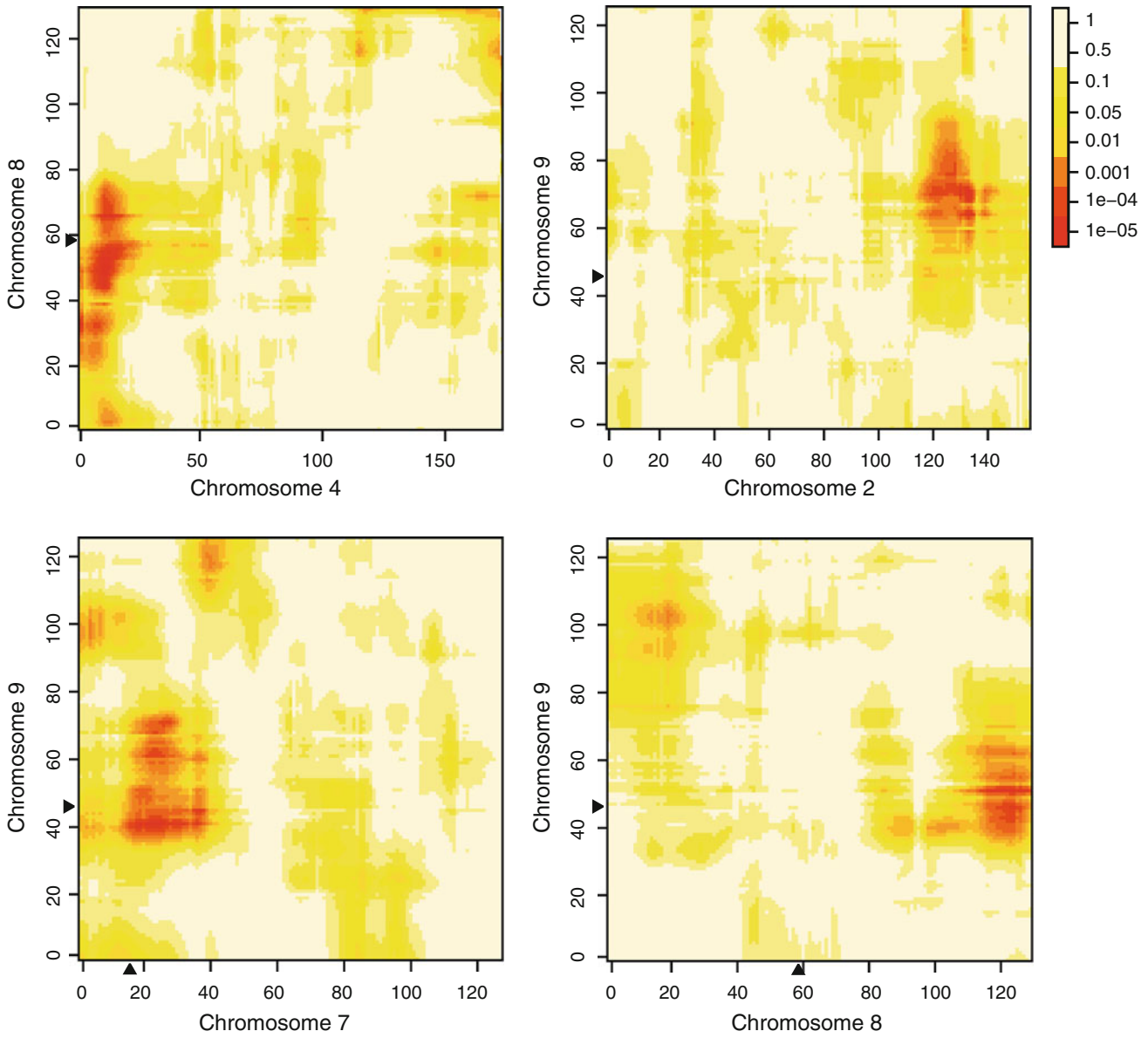

Fig. 6 Heat plots showing significant epistatic interactions detected by a full 2-dimensional genome scan. The positions of the detected main effect QTL are indicated by *arrowheads*

we observed a significant genotypic variance in all families. The large population size and the obtained heritability constitute a good basis to dissect the genetic architecture underlying the genetic variation for flowering time in elite maize material.

Detection of main effect QTL

Buckler et al. (2009) based their study on the NAM population including tropical and temperate genotypes (McMullen et al. 2009) and identified 39 QTL for days to silking which together explained 95 % of the genotypic variance. Their results showed that the differences in flowering time between maize lines were not caused by few QTL with large effects, but rather by numerous small-effect QTL. By comparison, Coles et al. (2010) examining four populations also derived from crosses between temperate and tropical parents detected eight QTL for days to silking under long day conditions and Blanc et al. (2006) observed eight QTL using the disconnected model.

In our study we identified nine QTL using the disconnected model and three QTL with the connected model (Table 2). This is in contrast to the results from Coles et al. (2010) who detected a higher number of QTL with the connected model. This illustrates that the appropriate MC-QTL mapping model must be determined specifically

for each data set. The total proportion of genotypic variance explained by the QTL detected with the disconnected model was 77.2 % and the highest variance was explained by the QTL on chromosome 1 with 12.9 %. The meta-analysis of 22 maize flowering time QTL mapping studies (including temperate and tropical germplasm) by Chardon et al. (2004) identified six regions affecting flowering time across experiments and four of them were also identified as major photoperiod response QTL by Coles et al. (2010) (*ZmPR1-4*). Five of the QTL detected in our study were also identified in the meta-analysis, but only one of them may correspond to one of the major regions described by Coles et al. (2010), namely the QTL on chromosome 8 named *ZmPR2*.

Even though the different genetic maps underlying the studies make comparisons difficult, we attempted to compare our results with those of Chardon et al. (2004) and Coles et al. (2010) who have placed candidate genes for flowering time on their genetic maps. For five of the detected QTL we found candidate genes mapped to the regions of these QTL (Table 2). The QTL on chromosome 1 maps to the same region as *INDETERMINATE1* (*ID1*) which encodes a zinc finger transcription factor and the mutant of which fails to undergo the transition of the apical meristem from the vegetative to the generative state (Colasanti et al. 1998). *ID1* also falls within a QTL interval in the NAM population study (Buckler et al. 2009). The QTL on chromosome 8, which may be identical to *ZmPR2*, may be caused by *VEGETATIVE TO GENERATIVE TRANSITION1* (*VGT1*). The *VGT1* locus contains a cis-acting regulatory region of *ZmRap2.7*, an *AP2*-like gene (Salvi et al. 2007) whose effect on flowering time was confirmed by Buckler et al. (2009). Alternatively, the effects of this QTL may be due to *VGT2* which is tightly linked to *VGT1* and which may be the maize gene *CENTRORADIALIS8* (*ZCN8*) (Danilevskaya et al. 2008), a homolog of the Arabidopsis *FT* gene. Candidates for the QTL on chromosome 9 are homologs of three major QTL cloned in rice: *OsCO* (*Hd1*), *OsELF3*, and *OsHY1* (*se5*). However, using a candidate gene approach *CO* was mapped to a plant height photoperiod QTL interval but not to a photoperiod response QTL (Coles et al. 2010). Verification of the candidate genes potentially underlying the QTL in elite maize will require markers based on these genes.

No QTL were detected in the regions of the maize *LEAFY*-like genes, *ZFL1* and *ZFL2* (Bomblies et al. 2003), which were both found to associate with flowering time in the meta-analysis (Chardon et al. 2004) and *ZFL2* also by Buckler et al. (2009). Also the *DWARF8* (*D8*) region which was initially identified as an important regulator of flowering time and latitudinal adaptation in maize (Thornsberry et al. 2001) was not identified as QTL in our study which confirms similar findings (Myles et al. 2009). We also did not identify the major QTL on chromosome 10 (*ZmPR4*) that was detected by Blanc et al. (2006) and by Coles et al. (2010). The reason may either be a fixation of this QTL in elite material or within the founder lines used for this study. Taken together, no single major QTL were identified in all five families of elite maize and the contribution of the candidate genes to the genetic variation in elite material requires validation.

Variation in allele substitution effects

The number of days to silking varied by approximately 4.7 to 6 days within the five families (Table 1) whereas the largest effect of a days to silking QTL allele was only 0.6 days (Fig. 4a). We, however, observed a strong variation of the estimated allele substitution effects of all QTL among families (Fig. 4a). This is in accordance with other studies on multiple families which also observed strong differences in allele substitution effects (e.g., Liu et al. 2011). The analysis of the NAM population in maize also revealed an allelic series for many of the detected QTL including changes in sign, i.e., positive and negative effects at one locus (Buckler et al. 2009).

Explanations that have been brought forward for this variation in allele substitution effects include epistasis, i.e., a dependency of the QTL effect on the genetic background (Liu et al. 2011) and multiple variants of a QTL, i.e., functionally distinct alleles of a locus (Buckler et al. 2009). Multiple alleles of a QTL can be caused by different alleles of the causal gene underlying a QTL. With the current marker densities, however, a QTL will often not identify a single gene, but rather a chromosomal region including several genes affecting the trait. In that case each haplotype, i.e., combination of alleles at genes within the QTL region, will mimic a different QTL allele. Another factor that strongly influences the estimated allele substitution effect is the allele frequency (Fig. 4d). Our test for segregation distortion has revealed several regions which show allele frequencies deviating from the expected Mendelian ratio and more importantly differing among families (Fig. 2). A similar picture was observed for the individual NAM families which showed segregation distortion at loci throughout the genome resulting in different, family-specific allele frequencies (McMullen et al. 2009). Thus, for experiments based on *per se* performance the different allele frequencies in individual families are likely to contribute to the observed variation in allele substitution effects. By contrast, for testcrosses the allele frequencies in the population of the testers (p' and q') determine the allele substitution effect of the QTL alleles from the population under study.

Given the presence of multiple alleles, the degree of dominance among combinations of them may also vary. Except for equal allele frequencies, the dominance effect

'*d*' will affect the allele substitution effect and is therefore also of relevance for QTL mapping experiments based on testcrosses (Fig. 4e). In summary, the strong differences in allele substitution effects between the families observed here can be caused by multiple alleles at the QTL loci, different degrees of dominance among alleles, and may in addition be influenced by epistasis. This variation in allele substitution effects is of paramount importance for applied plant breeding as it affects the implementation of marker-assisted selection programs as well as the estimation of effects in genomic selection.

Genetic background epistasis scan

The test for interactions of a locus with the genetic background compares the connected model which assumes consistent allele effects across backgrounds with the disconnected model which allows the allele effects to vary depending on the genetic background (Blanc et al. 2006). It is thus based on a comparison of the allele substitution effects of that locus in different families. Blanc et al. (2006) observed that one of the eleven loci detected as main effect QTL for silking date showed a significant interaction with the genetic background. By contrast, Coles et al. (2010) observed no epistatic interactions of QTL with the genetic background. In our study we tested each locus for its interaction with the genetic background and detected no significant interaction (Fig. 5a). We next focused on the region on chromosome 1 which was comparably close to the significance threshold and for which a main effect QTL was detected. Considering the results from the full 2-dimensional genome scan for epistatic interactions no two-way epistasis appeared to be present in that region (Fig. 5b). Given the low number of digenic epistatic interactions (Fig. 6), it appears unlikely that this peak is caused by higher-order epistasis. Possible reasons for the failure of this test to detect epistasis are the absence of epistasis for this trait in the studied population or that with this test, positive and negative effects can cancel each other out. In addition, as the test relies on differences in allele substitution effects, which as shown above can have causes different from epistasis, epistatic interactions identified with this test should in any case be verified by different approaches such as pairwise interaction scans.

Genetic architecture of flowering time

Epistasis has recently been shown to contribute substantially to the expression of complex traits in breeding populations (Reif et al. 2011; Würschum et al. 2011, 2012b). Flowering time pathways are controlled by extensive molecular interactions (Bäurle and Dean 2006) but surprisingly Buckler et al. (2009) detected only a low contribution of epistasis in the joint analysis. Based on their result they speculated that in the outbreeding species maize, selection may have favored a genetic architecture with numerous small-effect QTL underlying flowering time to ensure synchronous flowering of plants within a population. In addition, the dispersion of the genetic control of the expression of this trait to approximately 50–100 QTL may permit the adaptation of the species to a wide range of environments by different combinations of QTL which either decrease or increase flowering time. In accordance with this Coles et al. (2010) also found no evidence for pairwise epistasis.

Our full 2-dimensional genome scan for pairwise interactions revealed four potential epistatic interactions despite the fact that only one quarter of the additive variance is exploited in testcross populations compared with experiments based on *per se* performance. Some of the regions involved in epistatic interactions have also been identified as main effect QTL (Fig. 6). The total proportion of genotypic variance explained by these epistatic QTL was much lower than that of the main effect QTL and the proportion explained by individual epistatic QTL was slightly less than the average of the main effect QTL. This suggests that the contribution of epistasis to the genetic architecture of flowering time may be higher in elite maize than in the diverse panel investigated by Buckler et al. (2009). As the constraints of natural selection on the outbreeding species maize as described above are not effective in breeding populations a number of epistatic interactions may be maintained in elite maize, thus contributing to the trait.

Conclusions

Our study based on five connected families revealed that flowering time in elite maize is largely controlled by main effect QTL but to a smaller extent also by epistasis. The identified QTL may facilitate a faster adaptation of material to different environments and can assist in tailoring flowering time to local climatic conditions to optimize the important agronomic traits grain yield and grain moisture.

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