Theor Appl Genet (2013) 126:2717–2736
DOI 10.1007/s00122-013-2167-9

ORIGINAL PAPER

Combined linkage and linkage disequilibrium QTL mapping in multiple families of maize (*Zea mays* L.) line crosses highlights complementarities between models based on parental haplotype and single locus polymorphism

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Received: 19 December 2012 / Accepted: 12 July 2013 / Published online: 23 August 2013

Abstract Advancements in genotyping are rapidly decreasing marker costs and increasing marker density. This opens new possibilities for mapping quantitative trait loci (QTL), in particular by combining linkage disequilibrium information and linkage analysis (LDLA). In this study, we compared different approaches to detect QTL for four traits of agronomical importance in two large multi-parental datasets of maize (*Zea mays* L.) of 895 and 928 testcross progenies composed of 7 and 21 biparental families, respectively, and genotyped with 491 markers. We compared to traditional linkage-based methods two LDLA models relying on the dense genotyping of parental lines with 17,728 SNP: one based on a clustering approach of parental line segments into ancestral alleles and one based on single marker information. The two LDLA models generally identified more QTL (60 and 52 QTL in total) than classical linkage models (49 and 44 QTL in total). However, they performed inconsistently over datasets and traits suggesting that a compromise must be found between the reduction of allele number for increasing statistical power and the adequacy of the model to potentially complex allelic variation. For some QTL, the model exclusively based on linkage analysis, which assumed that each parental line carried a different QTL allele, was able to capture remaining variation not explained by LDLA models. These complementarities between models clearly suggest that the different QTL mapping approaches must be considered to capture the different levels of allelic variation at QTL involved in complex traits.

Communicated by T. Würschum.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-013-2167-9) contains supplementary material, which is available to authorized users.

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Introduction

Most agronomical traits of interest show continuous variation resulting from the combined effect of many quantitative trait loci (QTL) (Fisher 1930). A major issue in quantitative genetics is to map QTL and get insight into their number, mode of action and effect with the aim of improving the efficiency of animal or crop selection. In plants, the relative ease of producing progenies from crosses between two inbred lines leads to a wide use of biparental populations to map QTL (Bernardo 2008; Lander and Bolstein 1989; Jansen and Nap 2001). However, most QTL analyses in biparental populations lead to limited accuracy of QTL positions in large chromosomal regions because of the low number of recombination events occurring in the mapping populations. Thereby, costly complementary works, involving specific development of recombinant plants within targeted region, is required to assign QTL to the shortest possible genetic intervals for eventual positional cloning. Besides the poor resolution of QTL location, the use of biparental cross for QTL mapping is also

largely questioned in terms of relevance for breeding and marker-assisted selection (MAS) due to the small fraction of the possible alleles sampled in the population. To reflect the allelic diversity of elite germplasm used for breeding, it is necessary to explore a larger genetic base. The use of populations derived from multiple founders allows evaluation of more alleles over multiple genetic backgrounds than what is possible in a single-cross family and thus increases the probability to find alleles of interest. Therefore, QTL mapping in complex population designs has been proposed as a more efficient and relevant approach for plant breeding and MAS (Beavis 1994; Muranty 1996; Xu 1998). Additional to the possibility of exploring a larger genetic base, simulation studies (Rebai and Goffinet 2000; Jannink and Jansen 2001; Jansen et al. 2003) have shown that these complex populations may increase the statistical power of QTL detection and the accuracy of their location and allelic effect estimates, especially when some inbred lines are used as parents for different segregating populations. In this situation, it creates connections between the segregating populations that may lead to reduction in the number of allelic effects to be estimated, under the hypothesis of additivity. According to these results, a few studies were carried out on real data sets (Rebai et al. 1997; Blanc et al. 2006; Verhoeven et al. 2006; Coles et al. 2010; Steinhoff et al. 2011) and generally confirmed the advantages of using multiple lines crosses for QTL mapping compared to single-cross analysis.

However, statistical models used in the plant community assume that there is a different QTL allele for each parental allele segregating in the multi-allelic population. Such assumption clearly does not reflect the reality of most breeding programs where parental lines are often related. Indeed, at a given tested position parental lines may share the same allele inherited from common ancestor (in this case the lines are said to be locally Identical By Descent, IBD). Meuwissen and Goddard (2001) proposed to combine linkage and linkage disequilibrium analyses (LDLA analyses) for QTL mapping in animal populations with variance component methods where correlation between random effects of QTL alleles are estimated with IBD probabilities computed locally around each test position from dense-marker information. Such approach takes into consideration historical recombination events between markers through LD analysis in addition to the recent recombination events occurring within families at the stage of the parental gametes formation, resulting in a better resolution of QTL locations besides a gain of power. The potential of such strategy has been demonstrated through simulation studies (Lund et al. 2003; Uleberg and Meuwissen 2007) and for fine mapping of some candidate genes in previously mapped QTL regions in cattle (Meuwissen et al. 2002; Blott et al. 2003).

In plants, the new availability of high genotyping density offers the possibility to perform such LDLA analyses for QTL mapping. Inspired from methods developed in human and animal genetics, Jansen et al. (2003) proposed an advanced haplotype-based method for QTL mapping of multi-allelic plant populations where parental alleles are grouped based on local marker similarity of parents. They showed by simulation that such a strategy led to a significant gain of power, precision and accuracy in allele effects estimates in QTL analyses, due to a reduction of parameters to estimate in the model. Recently, Bink et al. (2012) confirmed through a simulation study the potential of LDLA analyses in plants using a bayesian framework for QTL mapping, where parental alleles were clustered using marker-based local IBD probabilities. However, while the advantages of LDLA models for QTL mapping have been demonstrated, marker-based methods used for parental alleles clustering are still questioned for their relevance to identify the real number of ancestral alleles. Because of these limitations, it has been proposed in plants to analyze multipopulation designs without trying to infer ancestral haplotypes but just by considering that individuals that carry the same genotype at a given marker are IBD which can be considered as a reasonable assumption if QTL are mostly bi-allelic and if the marker density used to genotype parental lines is high enough to ensure that there is at least one marker in perfect LD with the causal polymorphism. This approach has been applied to multi-parental advanced generation inter-cross (MAGIC) populations, obtained by intermating inbred lines for several generations and proved to be efficient in several species to increase QTL mapping resolution compared to conventional populations (Kover et al. 2009; Bandillo et al. 2010). In maize (*Zea mays* L.), a nested association mapping (NAM) design which consists of several biparental populations connected by one common parental line was developed to exploit simultaneously advantages of linkage and linkage disequilibrium information. In this design, only parental lines need to be densely genotyped. The small number of recombination events expected per chromosome in the progenies makes it possible to easily infer dense genotyping information from the parents by using conventional marker density genotyping of the progenies. Analysis of such design was performed either using a joint linkage approach in which each parental line is expected to carry a different allele (Buckler et al. 2009) or considering an association mapping approach including in the model allelic effects observed at individual SNP (Yu et al. 2008; Tian et al. 2011; Kump et al. 2011). This latter model is expected to enhance statistical power of QTL detection and resolve QTL position at the level of individual genes (Yu et al. 2008; McMullen et al. 2009; Guo et al. 2010), which was validated for several traits (Tian et al. 2011; Kump et al. 2011). The same type of model was also

successfully applied to jointly analyze several biparental populations densely genotyped (Lu et al. 2010, Liu et al. 2011, 2012; Würschum et al. 2012). However, besides these encouraging results such approach assumes bi-allelic QTL, which might not be realistic in some cases compared to other methods assuming multi-allelic QTL. Thus, questions still remain about the best method to use for QTL mapping in multi-parental plant populations.

The objective of this study was to compare empirically different LDLA models and different linkage analysis methods to map QTL, in two multi-parental designs for four different traits of agronomical interest in maize. The two multi-parental mapping populations come from an applied breeding program where parental lines are highly related and families partially connected. Dense genotyping information was used first to group parental alleles into ancestral alleles using an approach recently developed by Leroux et al. (2013), and in a second step to simply consider single-marker model after imputation of high-density marker information from lines to progenies. Each approach was combined with linkage information within segregating progenies to map QTL with the MCQTL (Jourjon et al. 2005) software using a composite interval mapping method based on linear multiple regression model (Haley and Knott 1992; Charcosset et al. 2000). Results of analyses on the multiple population designs, based only on linkage information, were used as reference to assess the potential gain of the two different LDLA mapping methods. Based on these results, we discussed the potential benefits of the different QTL mapping models for crop improvement.

Materials and methods

Plant material and experimental designs

This study relies on two different multi-parental QTL mapping populations, belonging to the French seed company, Euralis Semences. A total of 21 maize (*Zea mays* L.) inbred lines of the dent heterotic group were used to create the two different experimental designs. These inbred lines belong to the same breeding pool and some are related.

The first multi-parental population, further referred to as dataset 1, is composed of 895 F_2 individuals split into seven families, obtained from crosses between ten inbred lines, among the 21 mentioned above. On average, 128 plants per cross were produced. The smallest and the largest F_2 families comprised, respectively, 60 and 180 individuals. The mating design between parental lines created connections between some families, leading to two sub-populations of connected families, involving, respectively, 2 and 4 crosses, and one "non-connected" family (see Fig. 1). The second mapping population further referred to as dataset 2, is composed of a total 928 F_2 or F_3 individuals. Plants were obtained from 21 crosses between 16 maize inbred lines, of which five were common with the parental lines of dataset 1. On average 44 individuals per cross were produced, with a minimum and maximum family size of 25 and 87 plants. All families were connected to at least another one, yielding a fully connected mapping population.

Field trials

Each F_2 or F_3 plant was selfed to obtain, respectively, $F_{2:3}$ and $F_{3:4}$ families. Testcross progenies were obtained in isolation plots in Chile by crossing plants from the $F_{2:3}$ or $F_{3:4}$ families, as well as the parental inbred lines, with a complementary inbred line (used as a tester) from the flint heterotic group. A different tester was used for each dataset. The testcross progenies were then evaluated in field trials located in Northern France and Germany. Hybrids from datasets 1 and 2 were evaluated in two different years: in 2007 for dataset 1 and in 2005 for dataset 2. Seven different locations were used for the 2007 field trials, and four different locations in 2005. In a given location, testcross progenies were not replicated within the field trial. Each testcross was grown in two-row plots of 9 or 9.6 m^2 with population densities ranging from 75,000 and 95,000 plants ha^{-1} depending on the location. To assess the precision of the trials, 15 % of the plots within each trial were devoted to check varieties replicated twice within trials. All hybrids were randomized within a given trial.

Three agronomic traits were measured: silking date (defined as the number of days after January 1st when half of the plants in a plot exhibited silks), grain moisture at harvest (in % of fresh weight) and grain yield adjusted to 15 % of grain moisture (quintal per hectare, q ha^{-1}). As the interest of breeders is to increase grain yield while keeping low drying costs at harvest, an economic index was also included in the analyses. This index was calculated as grain yield + 2.5 × (32-grain moisture). Trial management and measurement of all traits were conducted by the company Euralis Semences.

Agronomic data analysis

Data from plots with <70 % of the expected stand or neighbor with plots having 60 % of missing plant were excluded from the analysis. Plots with phenotype clearly out of the trait distributions were excluded. This led to exclude 3 and 5 % of plot data for dataset 1 and dataset 2, respectively. For each biparental family, adjusted means for environmental effects were calculated for each genotype, according to the following model:

$$y_{ij} = \mu + \delta_j + g_i + e_{ij},$$

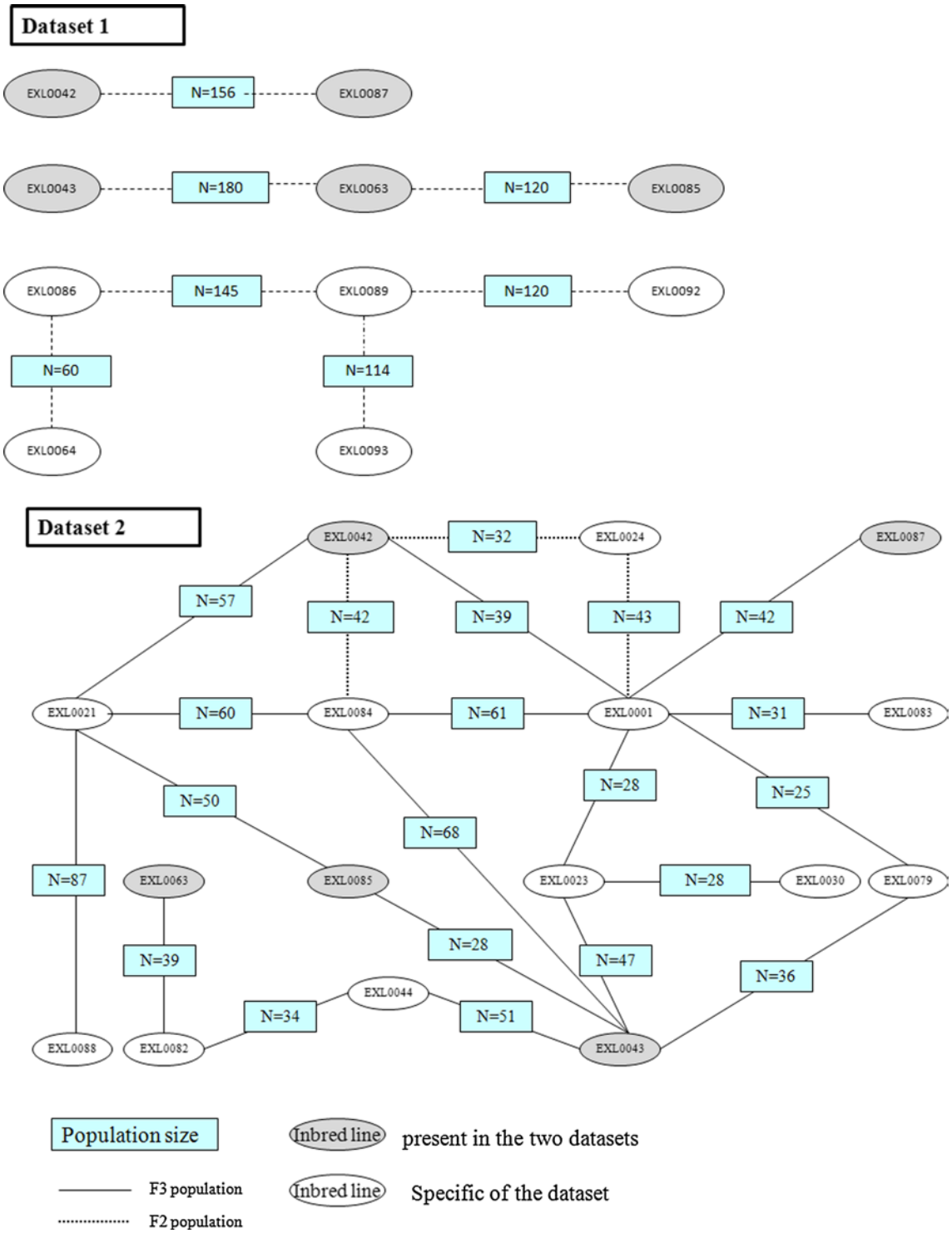

Fig. 1 Mating design of dataset 1 and dataset 2

where y_{ij} indicates performance of testcross progeny of genotype i within the environment j, μ is the fixed overall mean, δ_j is the fixed effect of environment j, g_i the genotypic fixed effects of individual i and e_{ij} random residuals of the model. Genetic and residual variances were also estimated by this model in the global multi-parental design by considering genotype effects as random. They were used to calculate the broad sense trait heritabilities (h^2) for each dataset, as $h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_\varepsilon^2}{L}}$, with σ_g^2 being the genetic variance,

σ_e^2 the residual variance (as only one replication was present in each environment, the genotype × environment interaction and plot error variance were confounded in σ_e^2) and L was the average number of environments. Phenotypic correlations were calculated on these adjusted means over all populations. All analyses were performed using the statistical software SAS (SAS Institute 2008) with the Proc MIXED procedure.

Genotyping and linkage maps

A total of 491 molecular markers (SSR and SNP) were used for genotyping the F_2 and F_3 individuals of the 2 datasets and the 21 parental inbred lines. The average number of polymorphic markers within each family in dataset 1 and 2 was, respectively, 142 and 73. All markers with unexpected segregation within family, tested with a Chi-squared test at $\alpha = 5$ % were not used. A consensus linkage map between all families of the two datasets was built with the software CarthaGène (de Givry et al. 2005) by considering non-segregating markers in a given family as missing data. All genetic positions were projected on a robust reference map based on 126 common markers. These positions were used as consensus linkage map for both datasets.

An additional dense genotyping was done on the 21 parental inbred lines with SNPs coming from two different DNA chip array, a 12K SNPs chip described in Mezmouk et al. (2011) and a 50K SNPs chip described in Ganal et al. (2011). Physical positions of these SNPs were projected through linear interpolation onto a genetic reference map developed by Euralis, thanks to 153 SNPs having both genetic and physical positions. The projected genetic positions of the SNPs coming from the 50K SNP chip were then compared with their position on the LHRE (F2 × F252) and IBM (B73 × Mo17) linkage genetic maps (Ganal et al. 2011). Only SNPs having consistent order between the reference map and these two genetic maps were retained. Then, only SNPs being polymorphic in the set of inbred lines were kept for analyses.

Those SNPs, as well as the 491 markers genotyped on the mapping populations, were used as dense genetic map for parental inbred lines.

Clustering analysis of parental inbred lines

All the marker data available on the 21 inbred lines were used for clustering analyses, yielding a total of 17,728 markers.

Clustering of inbred lines was carried out with the R package clusthaplo (Leroux et al. 2013). This tool performs clustering of parental inbred lines into shared ancestors, through a similarity score measured between each pair of individuals along the genome. The similarity score was measured at each 2 cM, taking into account information from neighbor markers located in a window of 10 cM around the considered test positions. The similarity between individuals i and j for the test position t was measured according to the Li and Jiang (2005) formulae:

$$S_{i,j}^t = \sum_{1}^{K} W_1(x_k^t) \times I(h_{ik}^t, h_{jk}^t) + \sum_{k=l'}^{r'} W_2(x_k^t), \quad (1)$$

where h_i^t and h_j^t denote the marker haplotypes of lines i and j around the test position t, h_{ik}^t denotes the allele of individual i at marker k (with $k \in [1, K]$, K being the total number of markers in the window) and x_k^t is the distance between the marker k and the test position t. $I(a, b)$ is a function where $I(a, b) = 1$ if alleles a and b are alike-in-state and $I(a, b) = 0$ otherwise. $W_1(x_k)$ and $W_2(x_k)$ are two weight functions. A Laplace function was used for the first weight function W_1, giving more weight to similarity for markers close to the test position and a constant function was used for the second weight function W_2. The indices l' and r' are the two boundary markers such that the two individuals i and j have alike-in-state alleles between these two markers and different alleles at both marker $l' - 1$ and $r' + 1$. The first term of the sum shown in Eq. (1) can be seen as a weighted measure of the number of alleles in common, and the second term, as a weighted measure which is proportional to the length of the common region around the test position t when markers are equally spaced and W_2 is a constant function. As the similarity computed with Eq. (1) increases with the number of markers in the sliding window, it was normalized to the interval [0, 1] by dividing it by the maximum possible similarity value around position t, i.e., the similarity of an haplotype h_i^t with itself assuming no missing data.

Clustering of inbred lines was based on a similarity threshold th for a risk level α of 1 % and transitivity closure rules. If $S_{i,j}^t >$ th, then individuals i and j were assumed to share the same ancestral allele at the test position t, and if individuals i and k shared the same ancestral allele, then by transitivity individuals j and k shared also the same ancestral allele. Note that this procedure may lead to assign all lines to a same ancestral allele class even if some pairs had an initial similarity score below threshold (see Leroux et al. 2013).

The similarity threshold was estimated by the "mosaic" procedure which simulates a set of nearly independent parental inbred lines while considering the observed allelic marker frequencies. Following recommendations made by Leroux et al. (2013), independency between lines was simulated by crossing lines along 500 generations to mix their genome and break linkage disequilibrium between loci. The empirical distribution of similarity scores between the simulated independent lines, yielded the direct estimation

of the threshold by reading the quantile value at the level of the chosen risk of 1 %. Threshold estimations were performed separately for each dataset, according to this process.

The first prototype of clusthaplo that was run for the analyses was used to compute the dissimilarity score equal to $1 - S^t_{i,j}$. Thus, all the results are reported with the dissimilarity score.

After the clustering process, each parental line was locally attributed to an ancestral allele at different positions of the genome. We used these results to estimate similarities between lines based on the proportion of positions for which the two lines were attributed to the same ancestral allele. This similarity was compared with a similarity based on marker allele sharing.

QTL detection

QTL analyses were conducted on the adjusted means for environmental effects because of limited precision of performances within a single trial. Analyses were performed separately for the two datasets due to the differences in evaluation of performances in terms of the year and the tester.

QTL detection were conducted using MCQTL_LD software, a new version of MCQTL software (Jourjon et al. 2005), which performs composite interval mapping (CIM) in bi- or multi-parental populations, using a linear regression model. Several genetic models, described below were considered. The use of testcross progenies for phenotypic evaluation does not allow estimating dominance genetic effects. Thus estimates of QTL allelic effects were reported as additive effects.

First, analyses were performed in each biparental population. Family sizes in the dataset 2 (44 plants on average) were not large enough to detect QTL within population, hence single-population analyses were only carried out in dataset 1. The following model (further referred to as single-population model) was applied:

$$\boldsymbol{y_p} = \mathbf{1}_{N_p}\mu_p + \boldsymbol{X_{q_p}}.\boldsymbol{a_{q_p}} + \sum_{c \neq q}(\boldsymbol{X_{c_p}}.\boldsymbol{a_{c_p}}) + \boldsymbol{e_p}, \quad \text{model (1)}$$

where $\boldsymbol{y_p}$ was the vector of adjusted mean performance of the N_p individual of population p; $\mathbf{1}_{N_p}$ was a $N_p \times 1$ column vector of 1s; μ_p was the mean of population p; $\boldsymbol{X_{q_p}}$ and $\boldsymbol{X_{c_p}}$ were N_p × 2 matrices containing the expected number (ranging from 0 to 2) of the 2 parental alleles of population p at QTL q (cofactor $\boldsymbol{c}$) given the marker data for each individual i; $\boldsymbol{a_{q_p}}$ and $\boldsymbol{a_{c_p}}$ were column vectors of length 2 corresponding to the additive effects associated with each parental allele at QTL q and cofactor c, respectively, in population p; and $\boldsymbol{e_p}$ was a $N_p \times 1$ column vector of the residuals of the model. The additive effects were estimated so that their sum equaled zero for each QTL or cofactor.

Second, analyses were conducted by taking into account all the populations of each experimental dataset, according to four genetic models: two based on linkage analysis and two others combining linkage and linkage disequilibrium information.

In each dataset, families were first considered as independent (further referred to as multipopulation disconnected analyses), according to the following intra-population model:

$$\boldsymbol{y} = \boldsymbol{J\mu} + \boldsymbol{X_q}.\boldsymbol{a_q} + \sum_{c \neq q}(\boldsymbol{X_c}.\boldsymbol{a_c}) + \boldsymbol{e}, \quad \text{model (2)}$$

where $\boldsymbol{y}$ was the vector of adjusted mean performances of the N individuals of the dataset; $\boldsymbol{J}$ was a $N \times P$ matrix whose elements were 0 or 1 according to whether or not individual i belonged to population p; $\boldsymbol{\mu}$ was a $P \times 1$ column vector of population-specific means; $\boldsymbol{X_q}$ and $\boldsymbol{X_c}$ were $N \times K$ matrices containing the expected number (ranging from 0 to 2) of allele k at QTL q or cofactor c given the marker data for each individual i. At a given QTL or cofactor, the number of parameters to estimate was $2P$ corresponding to the two parental QTL allele effects within each population p. As only P parameters were estimable, the sum of the two parental alleles of each population was constrained to equal zero; $\boldsymbol{a_q}$ and $\boldsymbol{a_c}$ were $K \times 1$ column vectors of the within population allelic additive effects at QTL q and cofactor c, respectively, and $\boldsymbol{e_p}$ was a $N \times 1$ column vector of the residuals of the model. This model is close to the "joint inclusive composite interval mapping" model used to detect QTL in the NAM design (Buckler et al. 2009).

Then, connections between populations were considered through shared parental inbred lines with the assumption that each parental line carried a different QTL allele and that each allelic effect did not depend on the population where it segregated (i.e., assuming no epistasis, i.e., no QTL × genetic background interaction). The third model below further referred to as multipopulation connected, was applied:

$$\boldsymbol{y} = \mathbf{J\mu} + \boldsymbol{X^*_q}.\boldsymbol{a^*_q} + \sum_{c \neq q}(\boldsymbol{X^*_c}.\boldsymbol{a^*_c}) + \boldsymbol{e}, \quad \text{model (3)}$$

where $\boldsymbol{y}$, $\mathbf{J}$, $\boldsymbol{\mu}$ and $\boldsymbol{e}$ were the same as described in model (2). $\boldsymbol{X^*_q}$ and $\boldsymbol{X^*_c}$ were $N \times K^*$ matrices containing the expected number of parental allele k^* at the QTL q and cofactor c, respectively, given the marker data for each individual i, K^* being the total number of parental inbred lines, different for each dataset ($K^* = 10$ and 16, respectively, for dataset 1 and 2). The number of parameters to estimate at a given QTL (cofactor) was K^*. A constraint was added so that the sum of parental alleles within a connected design

equaled zero. It is important to notice that in dataset 1, as all families were not connected to each other, a constraint was applied for each subgroup of connected families. $\boldsymbol{a}_{\boldsymbol{q}}^{*}$ and $\boldsymbol{a}_{\boldsymbol{c}}^{*}$ were $K^{*} \times 1$ column vectors of the additive effects associated with each parental allele k^{*} at QTL q and cofactor c, respectively.

Afterwards, connections between families were considered taking into account similarity between parental alleles thanks to the clustering approach. The following model, further referred to as multipopulation LDLA analysis with clustering of parental alleles, was considered:

$$\boldsymbol{y} = \mathbf{J}\boldsymbol{\mu} + \boldsymbol{X}_{\boldsymbol{q}}^{*}\boldsymbol{Q}_{\boldsymbol{q}}.\boldsymbol{h}_{\boldsymbol{q}} + \sum_{\boldsymbol{c}\neq\boldsymbol{q}}(\boldsymbol{X}_{\boldsymbol{c}}^{*}.\boldsymbol{Q}_{\boldsymbol{c}}.\boldsymbol{h}_{\boldsymbol{c}}) + \boldsymbol{e}, \quad \text{model (4)}$$

where $\boldsymbol{y}$, $\mathbf{J}$, $\boldsymbol{\mu}$,$\boldsymbol{X}_{\boldsymbol{q}}^{*}$,$\boldsymbol{X}_{\boldsymbol{c}}^{*}$ and $\boldsymbol{e}$ were the same as described in model (3). $\boldsymbol{Q}_{\boldsymbol{q}}$ and $\boldsymbol{Q}_{\boldsymbol{c}}$ were $K^{*} \times A$ matrices whose elements were 0 or 1 according to whether or not parental allele k^{*} belong to ancestral allele a at the QTL q and cofactor c, respectively, A being the number of ancestral alleles identified by the clustering analysis at the QTL q or cofactor c position. $\boldsymbol{h}_{\boldsymbol{q}}$ and $\boldsymbol{h}_{\boldsymbol{c}}$ were $A \times 1$ column vectors of additive effects associated with ancestral allele a at QTL q and cofactor c, respectively. At a given QTL (or cofactor), the number of parameters to estimate was A. A constraint was added so that the sum of ancestral allele effects equaled zero.

The same type of model was also directly applied to the data by considering marker alleles at 17,237 SNPs and 491 markers as an ancestral origin, that is to say by considering that two parental lines carrying the same marker allele were IBD. This approach can also be viewed as an association mapping approach in which the 17,237 SNPs genotyped on parental lines were inferred for the segregating individuals and in which the structure is controlled by the population effect. This model (model 5) is referred to as multipopulation single-marker LDLA. This model is equivalent to the genomewide association mapping model used to analyze the NAM design (Yu et al. 2008; Tian et al. 2011; Kump et al. 2011) and is closed to the "model B" used by Liu et al. (2011, 2012, 2013) or Würschum et al. (2012) to analyze jointly designs composed of several biparental populations.

For inference and QTL mapping, probabilities of the different possible genotypes were computed at each marker position and every 2 cM, taking into account genotypes from informative neighbor markers. Presence of a putative QTL (cofactor) in an interval was tested based on the –log 10 (p value) of F tests. Significance thresholds were determined by 1,000 permutation tests to reach a global type I risk of 10 % (across all families and total genome). In dataset 1, to compare intra-family analyses performed with model 1 to global analyses performed with the other models, using the same genomewide significance level of 10 % over the seven families, a significance level per family was determined by applying the Bonferroni correction. All significance thresholds applied are reported in Table 1 for each dataset, traits and models. Thresholds used to select cofactors were fixed at 95 % of QTL significance threshold values. The QTL detection was performed using an iterative composite interval mapping approach (iQTLm) (Charcosset et al. 2000). In a first step, markers associated with the studied trait were selected as cofactors by forward regression considering a minimal distance of 20 cM between two selected cofactors. The identified cofactors were next used to detect QTL by composite interval mapping (CIM). Then, chromosome by chromosome, the identified QTL positions were used as cofactors in a new CIM mapping to refine QTL positions. The model stopped after convergence of the QTL positions. At the end of the detection process, confidence regions (or LODdrop regions) were estimated on the basis of a 1.5 LOD unit fall. The additive allelic effects at each QTL, as well as the phenotypic variance explained, either by each QTL or by all the detected QTL were estimated.

After performing QTL detection, the different analyses were compared in terms of number of QTL detected, size of confidence regions and proportion of phenotypic variance explained by the detected QTL (R^2, estimated as the ratio between the variation explained by detected QTL and the residual variation of phenotypes adjusted by family means). For the sake of simplicity, QTL with overlapping confidence regions were considered as corresponding to the same QTL.

For QTL detected by models 3–5, a hierarchical analysis of variance was performed to assess if less parsimonious models (3 and 4) captured an additional part of the phenotypic variation compared to more parsimonious LDLA models (4 and 5). For each QTL the genetic variability was dissected according to the following procedure: first, a F test was performed to know if ancestral alleles at the given QTL may capture remaining variation not explained by the significant single-marker model (5); and then a second F test was performed to assess if model based on parental alleles may capture the remaining variation not explained by both ancestral alleles model (4) and single-marker model (5). For each QTL, the incidence matrices used for this hierarchical analysis were built by considering genotypic probabilities at position with the highest p value for each model, since the genome scan for QTL detection differed from one model to the other (with model 5 genome scan was performed at each SNP position of the dense genotyping whereas with model 3 and 4, it was conducted every 2 cM and at each marker position being typed within populations).

Table 1 Significance thresholds in −log10 (*p* values) applied for testing presence of putative QTL in intervals

	Silking date	Grain moisture	Grain yield	Index
Dataset 1				
Single-population analyses				
EXL0042-EXL0047	4.05	3.94	3.82	3.86
EXL0043-EXL0063	4.07	3.87	4.08	4.07
EXL0085-EXL0063	4.13	3.95	3.91	4.13
EXL0086-EXL0064	3.98	4.03	4.20	4.00
EXL0086-EXL0089	4.22	4.08	3.99	3.92
EXL0093-EXL0089	3.81	4.00	3.90	4.13
EXL0092-EXL0089	4.01	3.90	4.05	4.15
Multipopulation analyses				
Disconnected (model 2)	3.51	3.32	3.23	3.22
Connected (model 3)	3.47	3.16	3.32	3.22
LDLA with ancestral alleles (model 4)	3.76	3.41	3.51	3.37
LDLA with single marker (model 5)	4.40	4.24	4.30	4.29
Dataset 2				
Multipopulation analyses				
Disconnected (model 2)	4.01	3.00	3.19	3.03
Connected (model 3)	4.39	3.37	3.71	3.42
LDLA with ancestral alleles (model 4)	4.85	3.86	4.29	3.89
LDLA with single marker (model 5)	5.16	4.76	5.17	4.77

Values correspond to a global type I risk of 10 % genomewide for multipopulation analyses and of 1.5 % for single-population analyses

Results

Genetic maps

The consensus linkage map was constructed on the basis of the 28 populations of the 2 datasets and comprised a total of 491 polymorphic markers. It had a total length of 2,297 cM and an average interval length between two markers of 4.66 cM.

The dense genetic map (supplemental Figure S5) used for the clustering analysis comprised 14,089 SNPs coming from the 50K SNP chip and 3,148 SNPs coming from the 12K SNPs chip, leading to a total of 17,237 polymorphic SNPs between parental lines. The total length of the dense map was 2,413 cM with a mean distance between two markers of 0.13 cM.

Agronomic results

Grain yield and index were higher on average in dataset 1 than in dataset 2, whereas silking date and grain moisture were lower (Table 2). The four traits followed normal distributions (supplemental Figure S2) for both datasets. There was more phenotypic variability in the global population than within families in the two datasets, for the four traits. Trait heritability ranged from 0.58 to 0.88 in dataset 1 and from 0.37 and 0.88 in dataset 2 (Table 2). As expected, trait heritabilities were higher for silking date and grain moisture than for the other traits. Except for the silking date, trait heritabilities at mean level were higher in dataset 1 than in dataset 2 (Table 2), consistent with the evaluation of traits in almost twice as many environments in dataset 1 than dataset 2. Silking date, grain yield and grain moisture were all positively correlated in both datasets (supplemental Fig. S3).

Clustering analysis of parental alleles

The distribution of the dissimilarity scores obtained with the "mosaic" procedure that simulates nearly independent parental inbred lines showed a high proportion of values close to one (Fig. 2). This led to a threshold value at the 1th percentile of 0.36 for dataset 1 (Fig. 2a). The same threshold of 0.36 was obtained for dataset 2. On the observed parental line dataset, local dissimilarity scores were mainly distributed around 0 or 1 (Fig. 2b), with a very low proportion of intermediate dissimilarity scores. There were several large chromosomal regions where values of dissimilarity scores were null or close to zero between pairs of inbred lines (Fig. 3), clearly suggesting that the two lines were IBD. Outside these regions, values of dissimilarity showed more erratic pattern but were clearly higher than the threshold value of 0.36 (Fig. 3). Based on these local dissimilarity scores, parental alleles were clustered on average along

Table 2 Trait means, genetic variances and heritability for the four traits in each dataset

	Silking date (days)	Grain moisture (%)	Grain yield (q ha^{-1})	Index
Dataset 1				
Mean[a] (STD_G^b/STD_F^c)	202.14 (1.90/1.21)	32.92 (1.7/0.8)	114.51 (6.9/4.6)	112.39 (7.4/4.6)
Genetic variance[d] (SE[e])	1.91 (0.13)	1.71 (0.09)	24.22 (1.60)	14.68 (1.22)
Heritability[f] (h^2)	0.70	0.88	0.74	0.58
Dataset 2				
Mean[a] (STD_G^b/STD_F^c)	207.36 (2.3/1.2)	33.17 (1.8/0.9)	102.04 (6.2/4.5)	80.96 (6.8/4.4)
Genetic variance[d] (SE[e])	4.66 (0.27)	2.31 (0.12)	19.68 (1.72)	9.75 (1.41)
Heritability[f] (h^2)	0.84	0.88	0.58	0.37

[a] Overall location adjusted mean

[b] Standard deviation of adjusted means in the global population

[c] Mean of standard deviations of adjusted means within families

[d] Genetic variance in the global population (accounting for inter and intra-family variation)

[e] Standard error

[f] Broad sense heritability

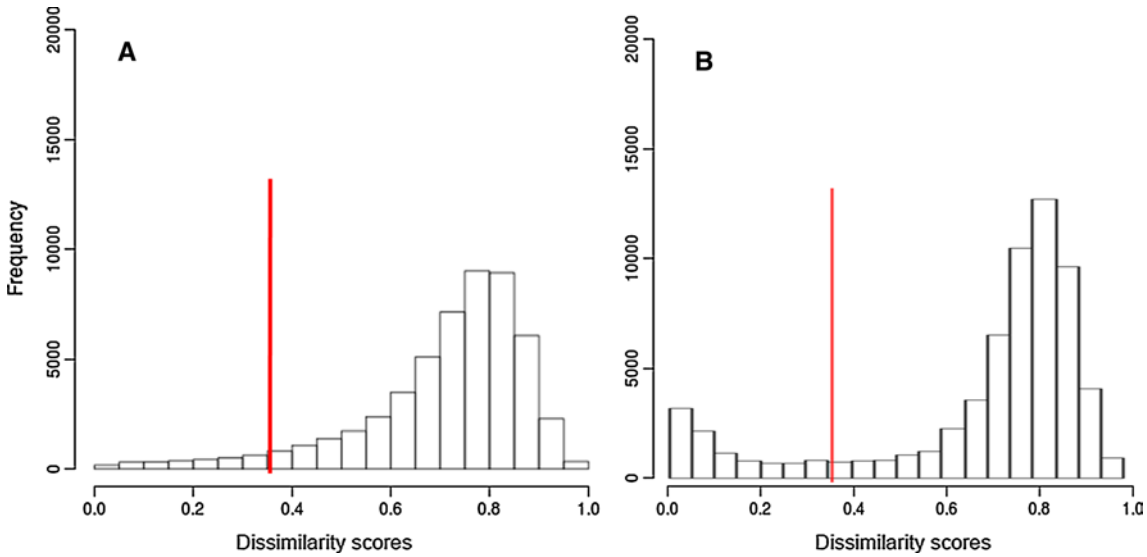

Fig. 2 Distribution of local dissimilarity scores estimated at each test position along the genome between pairs of parental lines of dataset 1. Distribution A was obtained by simulating independency between lines, distribution B was obtained with the real dataset. The *vertical red line* corresponds to the dissimilarity threshold value of 0.36

the genome in half as many ancestral groups than the actual number of parents, i.e., 5.86 ancestral alleles on average along the genome over 10 parental lines in dataset 1, and 7.86 ancestral alleles over 16 parental alleles in dataset 2 (Fig. 4).

The similarity between pairs of parental lines based on marker allele sharing ranged from 0.50 to 0.89, whereas similarity based on ancestral allele sharing (resulting from the clustering analysis) ranged from 0 to 0.78. These two measures of similarity were correlated to 0.96 (supplemental Fig. S4) illustrating that the clustering approach tended to group alleles carried by related parental lines.

Linkage-based QTL detection within families and in global design

Single-population analysis

A total of 35 QTL were detected for the four traits in the single-population analysis (Table 3) performed in dataset 1, with on average one or two QTL detected within population for a given trait. The detected QTL explained individually between 10 and 29 % of phenotypic variation. On average over population, all the detected QTL explained 17.2, 18.9, 14.5 and 13.8 % of the phenotypic variance for silking

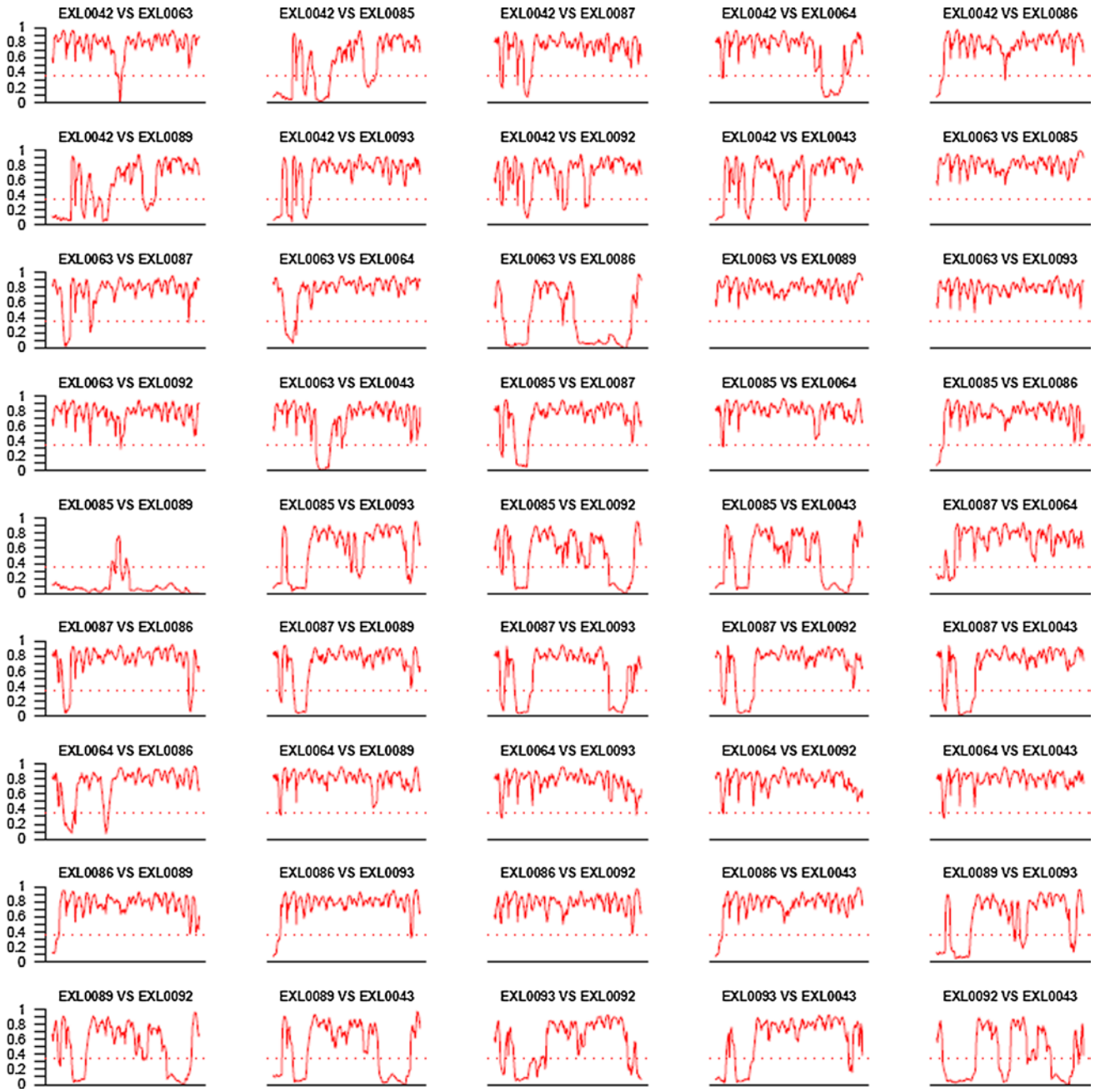

Fig. 3 Dissimilarity between pairs of the 10 parental inbred lines of dataset 1, for chromosome 1. The *vertical red dotted lines* represent the threshold value of 0.36

date, grain moisture, grain yield and index (Table 3). The number of QTL detected for each trait was positively related to trait heritability. The confidence regions varied between 35 and 42.6 cM for the different traits. Considering globally the results found in the seven populations, 24 different regions were identified with three, nine, six and six regions for silking date, grain moisture, grain yield and index, respectively.

Global design, linkage-based analysis

Model 2 (disconnected model) and model 3 (connected model) in dataset 1 gave the same results. For both models threshold values were very close (Table 1) and profiles of −log10 (*p* values) along the genome were identical for the four traits (see Fig. 5 for grain moisture and Supplemental Figure S5 for other traits). Both models identified a total of

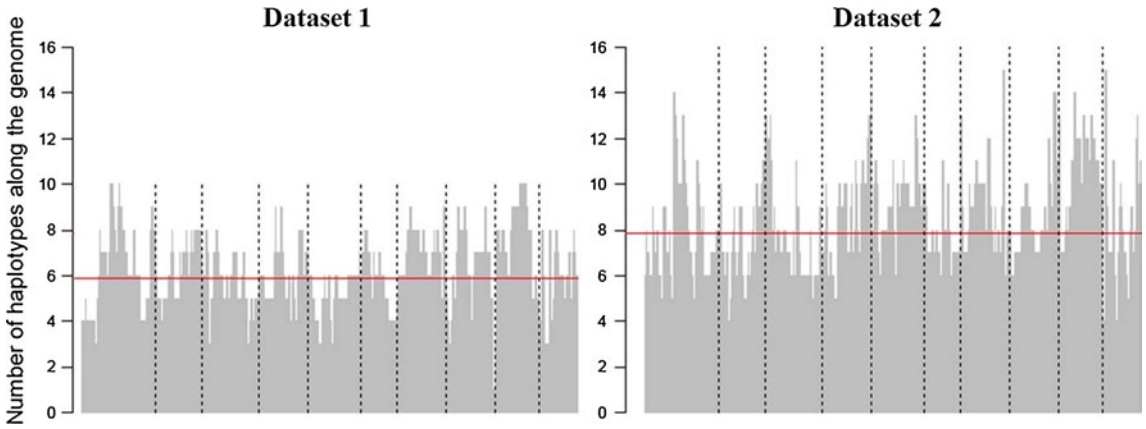

Fig. 4 Number of ancestral alleles along the genome for the two datasets. *Horizontal red lines* correspond to the average of number of alleles along the whole genome. *The vertical black dotted lines* correspond to the limits of each chromosome

31 QTL for all traits with same percentages of explained phenotypic variation ranging from 24.5 % for silking date to 53.7 % for grain moisture (Table 3). In comparison with single-population analysis in dataset 1, QTL analyses carried out in the global design detected 19 QTL over the 24 QTL found with single-cross analyses and identified 15 new QTL. For silking date, two additional genomic regions were identified with the multipopulation analyses and no QTL found in single-cross analyses was lost. For grain moisture, three new QTL were identified and only one QTL (on chromosome 7) among the nine different regions detected with single-analyses was lost. For grain yield, four new QTL were detected with the multipopulation analyses, three regions were re-identified and three QTL were lost (located on chromosomes 1, 2 and 7). Concerning these three lost regions "almost significant" peaks of −log10 (*p* values), obtained with the global design analysis, were distinguishable on chromosome 1, 2 and 7 at the corresponding positions estimated in single-cross analyses (Supplemental Fig. S5). For index, two new QTL were identified in the global design and one QTL was not re-detected but a small peak of −log10 (*p* values) (around 2.80) was present at this position, around 33 cM on chromosome 1. Considering the 19 common QTL detected in single cross and in the global design, confidence regions averaged over these common QTL were significantly reduced for all traits, with a reduction from 42.6 to 15.3 cM for silking date, from 31.3 to 27.7 cM for grain moisture, from 55.5 to 29.2 cM for grain yield and from 37.4 to 31.2 cM for index (Table 3). The total percentage of phenotypic variance explained by all QTL detected in the global design was higher for all traits than with single-cross analyses, with 24.5, 53.7, 26.3 and 30.8 % for silking date, grain moisture, index and grain yield, respectively.

Less QTL were detected in dataset 2 and results were different between the models 2 and 3. Disconnected analyses (model 2) identified a total of 18 QTL for all traits and connected analyses (model 3) a total of 13 QTL (Table 3). Confidence regions were quite large, varying on average between 22 and 60 cM for the four traits with model 2 and between 12 and 67 cM with model 3. With model 2, QTL were mostly detected for grain moisture and grain yield, with, respectively, nine and six QTL out of the 18 QTL. With model 3 (connected model), most of QTL were detected for grain moisture with eight out of 13 QTL and for silking date, grain yield and index only one, two and two QTL were detected, respectively. Consequently, percentage of explained phenotypic variance was high for grain moisture with both models, with 49.7 and 38.7 % for models 2 and 3, respectively. For grain yield, percentage of explained phenotypic variance was high with model 2 (30.9 %) and decreased with model 3 (9.7 %). Silking date and index percentages of explained phenotypic variance were low for both models and slightly higher with model 2 (Table 3). For silking date, models 2 and 3 identified the same QTL with a confidence regions reduction with model 3. One QTL was lost with model 3 compared to model 2 for grain moisture. Two QTL were detected with model 3 for grain yield and six were identified with model 2 including the two QTL detected with model 3 (Table 3). Finally for index, the two QTL detected were the same for both models and had large confidence regions on average (60.0 for model 2 and 67.2 cM for model 3).

QTL detection by combining linkage and linkage disequilibrium analysis

Multipopulation analyses taking into account the LD information (models 4 and 5) allowed to identify more or equal number of QTL than with the previous models (models 1, 2 and 3) in both datasets and for all traits, except for grain yield and grain moisture in dataset 2 (Table 3).

Table 3 Comparison of number of detected QTL, average confidence regions (CR) (in cM), and percentage of variance explained (global R^2) in the two datasets for each model

Models	Silking date (days)			Grain moisture (%)		
	Number of QTL	CR	R^2	Number of QTL	CR	R^2
Dataset 1						
Single-population analyses (model 1)	1[a]/3[b]	42.6	15.7	2.1[a]/8[b]	35	29.5
Disconnected (model 2)	5	16.5 (15.3[c])	24.5	12	25.8 (24.7[c])	53.7
Connected (model 3)	5	16.5	24.5	12	25.8	53.7
LDLA with ancestral alleles (model 4)	5	24.5	21.7	13	20.2 (19.3[d])	53.2
LDLA with single marker (model 5)	7	27.9 (31.8[d])	38.8	15	28.0 (24.8[d])	46.1
Dataset 2						
Disconnected (model 2)	1	21.7	7.3	9	42.7	49.7
Connected (model 3)	1	11.8	6.2	8	38.7	38.7
LDLA with ancestral alleles (model 4)	2	55.6 (12.6[d])	8.3	9	44.7 (47.2[d])	36.0
LDLA with single marker (model 5)	2	62.8	5.1	7	11.1	24.3
Models	Grain yield (q ha^{-1})			Index		
	Number of QTL	CR	R^2	Number of QTL	CR	R^2
Dataset 1						
Single-population analyses (model 1)	1[a]/7[b]	42.5	13.0	0.8[a]/6[b]	37.4	10.0
Disconnected (model 2)	7	22.3 (29.2[c])	30.8	7	36.8 (31.2[c])	26.3
Connected (model 3)	7	22.3	30.8	7	36.8	26.3
LDLA with ancestral alleles (model 4)	12	17.0 (16.1[d])	37.5	11	25.5 (28.1[d])	30.5
LDLA with single marker (model 5)	9	17.3 (21.8[d])	35.8	7	24.3 (28.0[d])	31.7
Dataset 2						
Disconnected (model 2)	6	45.5	30.9	2	60.0	12.7
Connected (model 3)	2	55.2	9.7	2	67.2	9.1
LDLA with ancestral alleles (model 4)	2	53.5	7.9	6	65.8 (48.0[d])	18.4
LDLA with single marker (model 5)	1	14.7	3.7	4	42.4	11.2

[a] Average number of QTL detected per population

[b] Number of different regions detected by single-population analyses (model 1)

[c] Average of CR of QTL also detected by single-population analyses (model 1)

[d] Average of CR of QTL also detected by multipopulation connected analyses (model 3)

Remark[c] and [d] not specified when same QTL are detected by the different methods

In dataset 1, model 4 with the ancestral alleles detected a total of 41 QTL for all traits, with one, five and four additional QTL for grain moisture, grain yield and index compared to linkage analyses. Confidence regions were on average smaller for common QTL. Compared to models 2 and 3 (linkage-based multipopulation analyses), no QTL were lost for any trait. Percentages of explained phenotypic variation were more important for grain yield and index, and almost the same than linkage analysis for grain moisture and silking date. For silking date, no additional QTL were detected and no gain in confidence regions was observed. For grain moisture, one new QTL was identified and confidence region was reduced from 25.8 to 19.3 cM. For grain yield and index, five and four additional QTL were, respectively, identified. For both traits, confidence regions for the QTL jointly detected with models 3 and 4

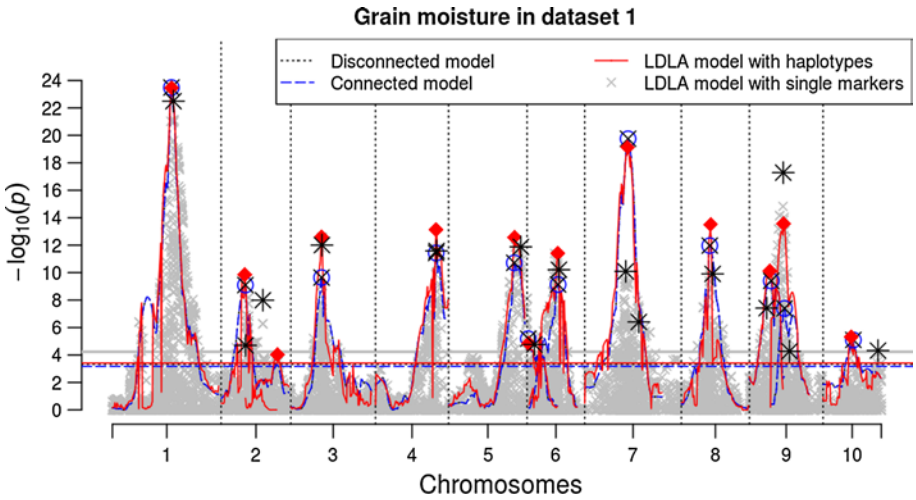

Fig. 5 Comparison of −log10 (*p* values) profiles obtained with the four multipopulation analyses (models 2, 3, 4 and 5) for grain moisture in dataset 1. *Horizontal lines* correspond to significance thresholds for each model and *dotted vertical lines* separate chromosomes. *Black crosses* correspond to QTL detected with the disconnected model (model 2), *blue empty circles* correspond to QTL detected with the connected model (model 3), *red diamonds* correspond to QTL detected with LDLA model through clustering (model 4) and *black asterisk* corresponds to QTL detected with LDLA model with single markers (model 5). It might be noticed that profiles of −log10 (*p* values) for model disconnected and connected are confounded in this dataset

were clearly smaller with model 4. In dataset 2, model 4 detected a total of 19 QTL detected for all traits. Compared to model 3 (connected model), the same number of QTL were detected for grain yield, one more QTL was identified for silking date and grain moisture and four new QTL for index (Table 3).

In dataset 1, model 5 (LDLA based on single-marker alleles) detected a total of 38 QTL for all traits. For both silking date and grain moisture, two additional QTL were detected with model 5 than with model 4. Conversely, for grain yield and index, model 5 detected fewer QTL than model 4 (four and five QTL were, respectively, lost) but still more QTL than simple linkage analyses (models 1, 2 and 3). Confidence regions and percentages of explained variance were close with models 5 and 4 for all traits except silking date for which we observed a higher percentage of variance with model 5. In dataset 2, model 5 identified a total of 14 QTL for all traits (Table 3). Compared to model 4, no additional QTL were detected for silking date. Confidence regions were larger with model 5 only for silking date. For grain moisture, grain yield and index two, one and two QTL were, respectively, lost compared to model 4, leading to a total of seven, one and four QTL, respectively, for the three traits. Compared to linkage analyses in the global design (models 2 and 3), more QTL were identified for silking date and index. For grain yield, fewer QTL were detected than with models 2 and 3. The QTL detected with model 5 was also detected with models 2 and 3 with a reduction of the confidence region to 14.7 cM (Table 3). For grain moisture, seven QTL already identified with models 2 and 3 were also detected with model 5, with smaller confidence regions.

Hierarchical analysis to compare different parental allele modeling

A total of 5, 11, 6 and 6 QTL were jointly detected in dataset 1 by the three considered models, for silking date, grain moisture, index and grain yield, respectively (Table 4). In dataset 2, one, five, two and one QTL were jointly detected by models 3, 4 and 5 for silking date, grain moisture, index and grain yield, respectively (Table 4). For dataset 1, hierarchical analyses from model 5 to 4 showed that a significant part of remaining phenotypic variation not explained by model 5 (based on single-marker alleles) was captured by model 4 (based on ancestral alleles) for one QTL of silking date, four QTL of grain moisture, one QTL of index and two QTL of grain yield. Model 3 explained a significant part of phenotypic variation not explained by LDLA models 5 and 4 for one QTL of silking date and grain moisture and two QTL of grain yield. So in total, model 3 and/or model 4 still capture phenotypic variation remaining from model 5 for more than 65 % of the detected QTL for grain yield. These proportions were 45, 33 and 17 % for grain moisture, silking date and index, respectively. In dataset 2, part of the unexplained variation by model 5 was captured by model 4 for one QTL of silking date and two QTL of grain moisture. Model 3 was also able to capture remaining variation from the combination of models 5 and 4, for one QTL of silking date and two QTL of grain moisture.

Table 4 List of common QTL detected with the multipopulation analyses (models 3, 4 and 5). Comparison of positions, confidence regions (CR) and R^2

Trait	QTL no.	Chr. no.	Model 5 (LDLA with single marker)			Model 4 (LDLA with ancestral allele)			Model 3 (connected model)			Hierarchical model significance	
			Pos. (cM)	CR (cM)	R^2 (%)	Pos. (cM)	CR (cM)	R^2 (%)	Pos. (cM)	CR (cM)	R^2 (%)	Model 4/model 5[a]	Model 3/ model 4–5[b]
Dataset 1													
Silking date	1	1	184	184–191	8.6	178	174–185	8.7	178	174–185	8.7	NS	NS
	2	3	110	54–143	1.8	98	76–139	2.8	98	94–100	3.6	NS	NS
	3	6	126	104–131	3.0	116	106–117	3.9	114	106–126	6.2	NS	**
	4	8	88	87–89	6.2	91	85–94	6.8	91	86–100	6	**	NS
	5	10	95	94–130	2.5	94	90–122	2.8	94	90–120	3.2	NS	NS
Grain moisture	1	1	191	191–192	10.7	185	181–189	13.8	185	181–190	14.5	NS	NS
	2	2	76	75–94	1.6	74	65–79	6.7	76	60–80	6.7	***	NS
	3	3	97	95–97	5.6	96	91–99	7.5	96	90–105	7.0	NS	NS
	4	4	191	191–205	5.4	190	188–202	8.5	190	187–202	8.0	NS	NS
	5	5	226	208–233	5.6	206	202–225	7.5	206	201–227	7.6	NS	NS
	6	6	23	4–51	2.1	4	0–53	3.5	4	0–52	4.3	NS	NS
	7	6	99	86–100	4.8	97	88–100	6.5	97	89–110	6.7	NS	NS
	8	7	128	128–137	4.7	136	125–145	11.3	134	129–145	12.6	***	NS
	9	8	98	87–98	4.6	91	85–95	8.0	89	85–100	8.3	**	NS
	10	9	54	41–82	3.4	64	55–89	6.5	68	49–94	6.9	NS	**
	11	9	106	105–106	9.5	107	95–113	9.0	110	99–147	5.7	*	NS
Index	1	1	165	136–168	2.1	157	145–167	4.0	149	131–163	4.1	NS	NS
	2	1	252	234–253	2.7	247	241–332	4.6	246	241–271	4.5	NS	NS
	3	3	110	102–112	5.6	112	107–119	8.1	110	107–118	9.1	NS	NS
	4	6	95	27–95	2.0	41	37–59	4.0	79	24–96	3.3	*	NS
	5	7	149	130–149	3.0	126	104–131	3.1	118	104–129	4.0	NS	NS
	6	9	85	65–85	4.0	87	78–97	5.2	87	79–98	5.3	NS	NS
Grain yield	1	1	165	147–179	2.2	159	153–171	4.6	168	148–174	3.8	**	NS
	2	3	103	102–111	6.6	107	103–113	9.1	110	107–116	10.4	NS	*
	3	4	188	162–206	3.4	198	193–210	5.8	196	179–203	6.2	NS	NS
	4	6	45	30–52	3.1	32	23–42	5.1	28	24–32	5.5	NS	NS
	5	8	88	88–97	3.4	91	84–95	6.2	91	85–122	6.5	*	NS
	6	9	73	60–76	6.3	62	55–74	4.9	87	63–96	7.7	NS	*

Table 4 continued

Trait	QTL no.	Chr. no.	Model 5 (LDLA with single marker)			Model 4 (LDLA with ancestral allele)			Model 3 (connected model)			Hierarchical model significance	
			Pos. (cM)	CR (cM)	R^2 (%)	Pos. (cM)	CR (cM)	R^2 (%)	Pos. (cM)	CR (cM)	R^2 (%)	Model 4/model 5[a]	Model 3/ model 4–5[b]
Dataset 2													
Silking date	1	8	91	70–156	2.0	85.4	78–91	6.9	87	81–93	6.3	***	*
Grain moisture	1	1	127	121–139	3.7	121	112–137	6.0	177	109–178	5.6	NS	***
	2	2	109	96–111	5.4	104	78–112	6.2	102	93–131	7.8	*	NS
	3	4	191	190–192	3.3	186	180–191	5.4	186	168–197	6.0	NS	NS
	4	6	81	81–88	5.2	78	71–88	5.9	82	53–97	6.3	NS	*
	5	8	83	83–98	4.4	78	74–83	8.8	78	54–88	7.0	***	NS
Index	1	1	43	16–57	3.2	11	7–48	4.4	28	0–50	5.8	NS	NS
	2	3	176	166–176	3.2	179	141–195	4.2	173	138–222	5.1	NS	NS
Grain yield	1	3	169	161–176	3.75	179	141–196	4.0	179	152–212	5.07	NS	NS

NS not significant

[a] Test if model 4 explained remaining variation from model 5

[b] Test if model 3 explained remaining variation from the combination of model 5 and 4

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Discussion

The objective of this study was to compare different statistical models regarding their ability to detect QTL and evaluate their biological relevance in multi-parental populations. Motivations for this work resulted from the large use of such multi-parental population designs in applied breeding programs and the fact that the density of marker information available now on the parental inbred lines gives new possibilities in terms of QTL detection. It allows one to take into account that different parental lines might have inherited the same IBD segments and it also permits to take advantage of historical recombination events between markers and causal molecular polymorphisms to potentially increase the resolution of QTL mapping.

Numerous studies on mating design with multiple line crosses have shown an improved power and resolution for QTL mapping compared to the use of single populations (Rebai and Goffinet 1993; Xu 1998; Rebai and Goffinet 2000; Yi and Xu 2002; Jansen et al. 2003; Li et al. 2005; Verhoeven et al. 2006; Blanc et al. 2006). Our results in dataset 1 confirmed the gain of QTL detection and resolution in the multi-parental population than in single crosses. Such results, being well discussed in the literature we will focus here on the comparison of multipopulation models (models 2–5).

Comparison of connected vs. disconnected models

Under the hypothesis of absence of epistatic interactions one would expect that models taking into account connection between populations either through the use of same parental lines (model 3) or possible identity of parental alleles (models 4, 5) would detect more QTL than model 2 (disconnected), thanks to a reduction of the number of parameters to be estimated in the model. Contrary to what was observed in Blanc et al. (2006), model 3 (connected) did not detect more QTL than model 2 (disconnected). In dataset 1, the two models were strictly equivalent. This was expected since in this design the seven families were not all connected through common parental lines and thus, the same number of independent parameters had to be estimated with model 2 (disconnected) and model 3 (connected). In dataset 2, the families were fully connected but we surprisingly observed that disconnected analyses outperformed connected analyses for grain moisture and grain yield. This might be due to potential epistatic effects occurring within families (Jannink and Jansen 2001; Blanc et al. 2006; Steinhoff et al. 2011). Another explanation might be related to the fact that in this dataset the thresholds for declaring QTL as significant with model connected (model 3) were higher than with model disconnected (model 2) for all traits. In addition to the additivity of allelic effects,

models 2 and 3 assume homoscedasticity of within-family variances. This hypothesis is false in both datasets, but more particularly in dataset 2. This might be due to epistasis but also to the fact that in this dataset, families derived from crosses between pairs of lines with variable level of relatedness, whereas in dataset 1 parental lines were more homogeneously related. Heteroscedasticity which is variable according to the trait considered might explain differences of threshold between traits. It is also expected to impact more the threshold found by permutations for model 3 (connected) which takes into account connections between families (Mangin, personal communication).

Benefits of LDLA models compared to parental alleles-based models

Numbers of QTL detected clearly show that LDLA-based models (models 4 and 5) lead to identify more QTL than models only based on linkage analysis (models 2 and 3), whatever the dataset considered (except for grain yield in dataset 2). According to previous studies (Lund et al. 2003; Jansen et al. 2003; Uleberg and Meuwissen 2007; Bink et al. 2012; Leroux et al. 2013), this notable gain might be explained by the potential of LDLA models for reducing the number of parameters to estimate. Concerning model 4 (based on parental allele clustering), the reduction of allele number resulted from the clustering analysis and its ability to group parental alleles into ancestral alleles. The clustering approach identifies large IBD segments between inbred lines. On average, the number of ancestral alleles was close to six for dataset 1 (10 parents) and eight for dataset 2 (16 parents). The parental lines used in the two datasets were all derived from the same breeding company and were related to each other. In conformity with our expectation, a higher number of shared regions were found between closely related inbred pairs (Supplemental Figure S4). This consistency with pedigree information and the fact that the clustering approach increased the number of detected QTL clearly shows the interest of this approach in our example. However, its interest still needs to be studied in other situations.

In agreement with Grapes et al. (2004), the single-marker LDLA model (model 5) was also an effective method to detect QTL compared to linkage-based methods (disconnected model 2 and connected model 3) and LDLA with ancestral alleles-based method (model 4). Other studies also reported the ability of high-density SNP for capturing both LD and linkage information to map QTL in the maize NAM design (Yu et al. 2008; Tian et al. 2011) or in other multi-parental designs composed of several biparental populations (Liu et al. 2011, 2012; Würschum et al. 2012). These studies showed that a model similar to our model 5 was more powerful than a linkage model considering QTL effect nested within population as our model 2 (disconnected model). One expects model 5 to be more efficient than others in cases when a single SNP is in perfect LD with an actual bi-allelic QTL or with a multi-allelic QTL with alleles grouped into two distinct classes of effects. Compared to model 4 (based on clustering of parental alleles), model 5 (based on single-marker alleles) is expected to be able to capture the effect of recent mutations occurring within a given IBD segment. On the contrary, the ability of single SNPs for capturing variation explained by actual multi-allelic QTL is expected to be low, especially when allelic effects do not differ substantially from each other (Jannink and Wu 2003). We observed several cases where significance of model 4 (based on clustering of parental alleles) was higher than that of model 5 (based on single-marker alleles). Thus a compromise must be found between the reduction of the number of parameters in the detection model, offering possibly higher statistical power for QTL detection, and the adequacy of the model to the actual number of QTL alleles segregating in the mapping population.

In order to address this issue, hierarchical analyses were performed at the significant QTL positions to assess if the remaining variations not explained by the simplest bi-allelic model (model 5) could be captured by models assuming more complex allelic diversity (model 4, then connected model 3). For all traits, in some cases, model 4 based on clustering of parental alleles explained a significant part of the variation beyond model 5 (single-marker LDLA), suggesting the presence of a multi-allelic QTL. We even found cases where the connected model (model 3), assuming that each parental line carries a different allele, was able to capture remaining variation from combination of models 4 and 5. This suggests that even if two inbred lines appear to be similar based on dense-marker genotyping, they may carry different alleles at QTL. This may be due to incomplete LD between markers and QTL that might be improved by using higher marker density. It may also be due to recent causal mutation not captured by the SNP genotyping or even the presence of epigenetic effects occurring at the level of the parental lines. These results suggest that models assuming specific allele effects for each parent across populations are clearly complementary to LDLA models. This is consistent with experimental results that showed that considering haplotypes (Lu et al. 2010, 2012) or parental alleles (Kump et al. 2011) in addition to single SNP effects can increase the percentage of variance explained by the models. In a recent study, Würschum et al. (2012) compared different statistical models and concluded that, even if the single SNP model (model 5 in our study) was the more powerful in terms of detection, the model considering QTL effects nested within population (disconnected model 2 in our study) was better

adapted to estimate QTL effect in case of multi-allelic series, corroborating experimental results of Liu et al. (2011). These findings suggest that these different models are complementary to reflect different genetic architectures at QTLs (see "Conclusion").

Besides the gain in terms of power of detection, LDLA models are also expected to increase the resolution of QTL position in multi-parental designs (Bink et al. 2012; Liu et al. 2012) by the joint use of recombinations within segregating populations and historical recombinations at the level of the parental lines. To our knowledge, there is no well-established method for estimating relevant confidence intervals with LDLA analyses. So it is difficult to conclude from our results and further investigations need to be done in this direction. We generally observed lower confidence regions with LDLA models (models 4 and 5) than with linkage-based models (models 2 and 3). This is consistent with simulation results of Leroux et al. (2013). However, confidence regions observed in our study were very large compared to what was found in other studies where the approach was able to resolve QTL at the level of individual gene (see Tian et al. 2011 for instance in the NAM design). First, the size of our datasets (about 900 progenies) was not large enough to achieve the same resolution as in the NAM design. Second, in our design, parental lines were closely related to each other and they often share large chromosome segments. Thus even if our parental lines were densely genotyped, compared to multi-parental designs issued from more diverse parental lines such as the NAM design, we do not expect to gain much resolution from historical recombinations.

Prospects for IBD-based allele clustering

Previous results show that IBD-based clustering can be a valuable approach (Bink et al. 2012) and therefore deserves further investigations. The dissimilarity computation we used for the clustering approach was based on a formulae proposed by Li et al. (2006). Around each position test, for each pair of parental lines, this computation includes two types of information: (1) the dissimilarity between the parental alleles at markers weighted by the distance between the marker and the position test and (2) the length of the intact segment they share. The use of weighting functions based on distance between markers and the test position opens the possibility of using a relatively large window capturing more marker information to estimate dissimilarity. Moreover, the combined use of a dissimilarity score with a threshold for accepting or not dissimilarity between lines might avoid creation of false ancestral allele, due to possible recent marker mutations, missing values or genotyping errors and might thus give more accurate grouping of parental alleles compared to a simple identity by state (IBS) approach in a fixed size window, as proposed by Jansen et al. (2003). In our case, we used a relatively large window around each position test (20 cM), which is in accordance with the high level of relatedness between the different parental lines and the linkage disequilibrium extent observed (results not shown). Indeed, we observed large segments with dissimilarities values close to zero which clearly corresponded to large IBD segments. This maybe specific from our design where such pattern was expected due to the history of the parental lines. In the case where inbred lines are of more diverse origin, the ancestral genomes might be more recombined leading certainly to more erratic patterns than in our case and making the choice of threshold, window size and weighting functions more difficult as they need certainly to be adapted depending on the LD extent among the parental lines.

There are several ways of estimating IBD probabilities at specific positions of the genome. In ter Braak et al. (2010) the authors used an approach based both on markers and recent pedigree. In our study, the pedigree of the parental lines was not taken into account in the dissimilarity score computation, even if it was possible to include this information in "clusthaplo". Indeed, in "clusthaplo", the pedigree information is only used to compute an expected genomewide IBD value that is useful only in cases when the marker density is not high enough to estimate accurately local IBD. Beyond the inclusion or not of pedigree information, ter Braak et al. (2010) suggested the use of a bayesian approach for identifying ancestral alleles (called Latent Ancestral Model), which avoids issues of threshold value settings. In the last version of the R tool "clusthaplo", a new approach based on a Hidden Markov Model (HMM) has been proposed to overcome this threshold setting issue. Bink et al. (2012) included clustering approaches of ter Braak et al. (2010) in a bayesian framework for QTL detection and observed an increase of power compared to a more simple linkage-based approach in a design close to the one analyzed in this study. It would certainly be interesting to test this and other approaches on our data set.

Whatever the approach considered, IBD computations rely on the ability of marker genotyping to reflect local IBD status of inbred lines. It could be questioned regarding the set of 17, 237 SNP used to estimate dissimilarity scores. Indeed, this set of markers was selected from a 50K SNP chip by considering markers that were included in the genetic maps build from LHRE and IBM populations used as references in the maize community (Ganal et al. 2011). This leads to only select markers being polymorphic between the parental lines of LHRE and IBM populations, namely F2 and F252 and, B73 and Mo17, respectively. This might create an ascertainment bias in the estimation of dissimilarity. In our case, the clustering seems to be consistent with what was expected from pedigree, but this maybe

a risk one needs to be aware of when performing such an analysis for other populations.

Variation among populations and traits

Comparison of QTL results in the two multi-allelic designs highlighted a general tendency of detecting more QTL in dataset 1 than in dataset 2 for all models and all traits. The two datasets having approximately the same global size (with 895 plants in dataset 1 and 928 in dataset 2) and being representative of similar genetic material (five parents were common to the two experimental designs and strong similarities between parents of the two designs were observed, testers were from the same genetic group), we would expect to observe similar results in the two designs. For grain yield and index, differences might be due to the notable lower trait heritabilities in dataset 2, power of QTL detection being strongly affected by the proportion of genetic variance involved in the observed phenotypic variation (van Ooijen 1992). This lower heritability is the direct consequence of the lower number of trials carried out for this dataset than for dataset 1. Besides trait heritability, the lower statistical power for detecting QTL in dataset 2 for all traits might also be explained by differences in terms of number of families and family sizes, since dataset 2 included more families of small sizes. Previous studies (Verhoeven et al. 2006; Wu and Jannink 2004) reported similar results. It was shown that for a fixed experiment size and number of parental lines, QTL detection is more powerful in mating design with few families of large size (like dataset 1) than with a higher number of families with few individuals within family (like dataset 2). Recently, Liu et al. (2013) considered a fixed experimental capacity in terms of total number of experimental units and optimized the number of parents, the number of individuals per family and the number of field trials considering two different parental crossing schemes. They concluded that the most powerful designs were composed of families composed of at least 100 progenies and derived from 7 to 12 inbred lines depending on the crossing scheme and that it was important to have a balanced contribution of the different parental lines. This is closer to our dataset 1 than to our dataset 2.

Comparing results across the four traits, this work also revealed different gains of LDLA mapping models according to the trait under study (Table 3). For grain yield and index, model 4 using ancestral alleles was more efficient than model 5 based on single-marker alleles and for silking date and grain moisture opposite results were observed. Huang et al. (2010) results suggested that QTL of grain yield detected in conventional mapping populations correspond mainly to clusters of linked QTL with small individual effects. Such linked QTL mimic a single locus with a multi-allelic determinism whose contribution to the phenotypic variance can be better captured by the clustering-based approach of model 4 than by model 5. If these QTL are considered separately, as in model 5, their individual effect may be too small to be detected.

Finally, it should be noted that these investigations highlighted the presence of a highly significant QTL of silking date on chromosome 8, detected by all models in the two datasets. SNPs associated with this QTL in model 5 (based on single-marker alleles) were physically located at 126.7 Mb in dataset 1 and at 135.7 Mb in dataset 2, which correspond to the genomic region of *Vgt1*, a flowering time QTL in maize (Salvi et al. 2007; Ducrocq et al. 2008). Moreover, allelic clustering of the parental inbred lines estimated for this QTL was consistent with the one obtained considering other molecular polymorphisms of the *Vgt1* region found to be associated with silking date by Ducrocq et al. (2008) (results not shown). This result is also consistent with a recent genomewide association study (Bouchet et al. 2013) on a diversity panel of inbred lines of maize for flowering time which reported for this region a high level of allelic diversity in the dent heterotic group considered in this study.

Conclusion

Dealing with multi-allelic populations for QTL mapping appears to be an interesting way of valorizing the numerous line crosses produced each year in applied breeding programs. The detected QTL can then be directly used in marker-assisted selection to increase the genetic value of the population by cumulating favorable alleles. In this context, LDLA models take advantage of dense-marker genotyping that is often available on parental lines to provide a classification of parental alleles into ancestral groups that can be compared in terms of effect on trait variation. Thus, they might yield a better management of the allelic diversity at the level of applied breeding programs than the implementation of conventional linkage analysis in separate biparental populations. Moreover, adding linkage disequilibrium information in QTL models may improve the resolution of QTL mapping, allowing the identification of marker–QTL associations that are more likely to remain stable in different genetic backgrounds and along generations. Even if our results showed that on average LDLA methods led to the detection of a higher number of QTL than linkage mapping, it is important to note that models performed inconsistently over datasets and traits. Potential reasons for this relate to differences in (1) the complexity of allelic variation, (2) magnitude of epistatic effects or (3) experimental designs. It is thus important to test different QTL models to take advantage of their complementarities. As proposed for instance by Kump et al. (2011), it

might be interesting to then include in a single model all the detected QTL positions, considering for each the most likely allelic model. However, this clearly requires additional development.

Acknowledgments Part of this work was financed by Euralis Semences; we thank them as well for the phenotypic and genetic material. We also thank the National Agency of French Research (ANR) which financed the MCQTL-LD project. We are grateful to the platform of bioinformatics Toulouse Midi-Pyrénées which partially supported this project. We also thank Frank Gauthier for helpful scripts to deal with the large amount of data. We are grateful to the editor and three anonymous reviewers for insightful comments that improved the manuscript.

References

Bandillo N, Muyco PA, Caspillo C, Laza M, Sajise AG, Singh RK et al (2010) Development of multiparent advanced generation intercross (magic) populations for gene discovery in rice (*Oryza sativa* L.). Philipp. J Crop Sci 35(suppl 1):96

Beavis WD (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies. In: The forty-ninth annual corn and sorghum industry research conference, vol 49, pp 250–266

Bernardo R (2008) Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Sci 48: 1649–1664

Bink MCAM, Totir LR, ter Braak CJF, Winkler CR, Boer MP, Smith OS (2012) QTL linkage analysis of connected populations using ancestral marker and pedigree information. Theor Appl Genet 124:1097–1113

Blanc G, Charcosset A, Mangin B, Gallais A, Moreau L (2006) Connected populations for detecting quantitative trait loci and testing for epistasis: an application in maize. Theor Appl Genet 113:206–224

Blott S, Kim JJ, Moisio S, Schmidt-Kuntzel A, Cornet A et al (2003) Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. Genetics 163:253–266

Bouchet S, Servin B, Bertin P, Madur D, Combes V, Dumas F, Brunel D, Laborde J, Charcosset A, Nicolas S (2013) Adaptation of maize to temperate climate: mid-density genome-wide association genetics and diversity patterns reveal key genomic regions. PLoS ONE (in press)

Buckler ES et al (2009) The genetic architecture of maize flowering time. Sciences 325:714–718

Charcosset A, Mangin B, Moreau L, Combes L, Jourjon MF et al (2000) Heterosis in maize investigated using connected RIL populations. In: Quantitative genetics and breeding methods: the way ahead. INRA, Paris, France

Coles ND, McMullen MD, Balint-Kurti PJ, Pratt RC, Holland JB (2010) Genetic control of photoperiod sensitivity in maize revealed by joint multiple population analysis. Genetics 184:799–812

de Givry S, Bouchez M, Chabrier P, Milan D, Schiex T (2005) CarthaGene: multipopulation integrated genetic and radiation hybrid mapping. Bioinformatics 21:1703–1704

Ducrocq S, Madur D, Veyrieras JB, Camus-Kulandaivelu L, Kloiber-Maitz M, Presterl T, Ouzunova M, Manicacci D, Charcosset A (2008) Key impact of Vgt1 on flowering time adaptation in Maize: evidence from association mapping and ecogeographical information. Genetics 178(4):2433–2437

Fisher RA (1930) The genetical theory of natural selection. Clarendon Press, Oxford

Ganal MW, Durstewitz G, Polley A, Bérard A, Buckler ES, Charcosset A, Clarke JD, Graner EM, Hansen M, Joets J, Le Paslier MC, McMullen MD, Montalent P, Rose M, Schön CC, Sun Q, Walter H, Martin O, Falque M (2011) A large maize (*Zea mays* L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. PLoS ONE 334:6–28

Grapes L, Dekkers JCM, Rothschild MF, Fernando RL (2004) Comparing linkage disequilibrium-based methods for fine mapping quantitative trait loci. Genetics 166:1561–1570

Guo B, Sleper DA, Beavis WD (2010) Nested association mapping for identification of functional markers. Genetics 186(373):383

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324

Huang YF, Madur D, Combes V, Ky CK, Coubriche D, Jamin P, Jouanne S, Dumas F, Bouty E, Bertin P, Charcosset A, Moreau L (2010) The genetic architecture of grain yield and related traits in Zea maize L. revealed by comparing intermated and conventional populations. Genetics 186:395–404

Jannink JL, Jansen R (2001) Mapping epistatic quantitative trait loci with one-dimensional genome searches. Genetics 157: 445–454

Jannink JL, Wu XL (2003) Estimating allelic number and identity in state of QTLs in interconnected families. Genet Res 81:133–144

Jansen RC, Nap JP (2001) Genetical genomics: the added value from segregation. Trends Genet 388:388–391

Jansen RC, Jannink JL, Beavis WD (2003) Mapping quantitative trait loci in plant breeding populations: Use of parental haplotype sharing. Crop Sci 43:829–834

Jourjon MF, Jasson S, Marcel J, Ngom B, Mangin B (2005) MCQTL: multi-allelic QTL mapping in multi-cross design. Bioinformatics 21(128):130

Kover PX, Valdar W, Trakalo J, Scarcelli N, Ehrenreich IM, Purugganan MD, Durrant C, Mott R (2009) A multiparent advanced generation inter-cross to fine-map quantitative traits in Arabidopsis thaliana. PLoS Genet 5:e1000551

Kump K, Bradbury PJ, Wisser RJ, Buckler E, Belcher A, Oropeza-Rosas MA, Zwonitzer JC, Kresovich S, McMullen MD, Ware D, Balint-Kurti PJ, Holland JB (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43:163–167

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

Leroux D, Rahmani A, Jasson S, Ventelon M, Louis F, Moreau L, Mangin B (2013) Clusthaplo: a plugin for MCQTL to enhance QTL detection using ancestral alleles in multi-cross design. Bioinformatics (review)

Li J, Jiang T (2005) Haplotype-based linkage disequilibrium mapping via direct data mining. Bioinformatics 21:4384–4393

Li R, Lyons MA, Wittenburg H, Paigen B, Churchill GA (2005) Combining data from multiple inbred line crosses improves the power and resolution of quantitative trait loci mapping. Genetics 169:1699–1709

Li J, Zhou Y, Elston RC (2006) Haplotype-based quantitative trait mapping using a clustering algorithm. BMC Bioinf 7:258

Liu W, Gowda M, Steinhoff J, Maurer HP, Wurschum T, Longin CF, Cossic F, Reif JC (2011) Association mapping in an elite maize breeding population. Theor Appl Genet 123:847–858

Liu W, Reif JC, Ranc N, Della Porta G, Wurschum T (2012) Comparison of biometrical approaches for QTL detection in multiple segregating families. Theor Appl Genet 125:987–998

Liu W, Maurer HP, Reif JC, Melchinger AE, Utz HF, Tucker MR, Ranc N, Della Porta G, Wurschum T (2013) Optimum design of family structure and allocation of resources in association mapping with lines from multiple crosses. Heredity 110:71–79

Lu Y, Zhang S, Shah T, Xie C, Hao Z, Li X, Farkhari M, Ribaut JM, Cao M, Rong T, Xu Y (2010) Joint linkage–linkage disequilibrium mapping is a powerful approach to detecting quantitative trait loci underlying drought tolerance in maize. PNAS 107(45):19585–19590

Lu Y, Xu J, Yuan Z, Hao Z, Xie C, Li X, Shah T, Lan H, Zhang S, Rong T, Xu Y (2012) Comparative LD mapping using single SNPs and haplotypes identifies QTL for plant height and biomass as secondary traits of drought tolerance in maize. Mol Breed 30:407–418

Lund MS, Sorensen P, Guldbrandtsen B, Sorensen DA (2003) Multitrait fine mapping of quantitative trait loci using combined linkage disequilibria and linkage analysis. Genetics 163:405–410

McMullen MD et al (2009) Genetic properties of the maize nested association mapping population. Science 325:737–740

Meuwissen THE, Goddard ME (2001) Prediction of identity by descent probabilities from marker-haplotypes. Genet Sel Evol 33:605–634

Meuwissen THE, Karlsen A, Lien S, Olsaker I, Goddard ME (2002) Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping. Genetics 161:373–379

Mezmouk S, Dubreuil P, Bosio M, Decousset L, Charcosset A, Praud S, Mangin B (2011) Effect of population structure corrections on the results of association mapping tests in complex maize diversity panels. Theor Appl Genet 122:1149–1160

Muranty H (1996) Power of tests for quantitative trait loci detection using full-sib families in different schemes. Heredity 76:156–165

Rebai A, Goffinet B (1993) Power of tests for QTL detection using replicated progenies derived from a diallel cross. Theor Appl Genet 86:1014–1022

Rebai A, Goffinet B (2000) More about quantitative trait locus mapping with diallel designs. Genet Res 75:243–247

Rebai A, Blanchard P, Perret D, Vincourt P (1997) Mapping quantitative trait loci controlling silking date in a diallel cross among four lines of maize. Theor Appl Genet 95:451–459

Salvi S, Sponza G, Morgante M, Tomes D, Niu X, Fengler KA, Meeley R, Ananiev EV, Svitashev S, Bruggemann E, Li B, Hainey CF, Radovic S, Zaina G, Rafalski JA, Tingey SV, Miao GH, Phillips RL, Tuberosa R (2007) Conserved noncoding genomic sequences associated with a flowering-time quantitative trait locus in maize. PNAS 104(27):11376–11381

SAS Institute (2008) SAS/STATÒ 9.2 User's Guide. SAS, Cary, NC

Steinhoff J, Liu W, Maurer HP, Würschum T, Longin H, Friedrich C, Ranc N, Reif JC (2011) Multiple-line cross quantitative trait locus mapping in European elite maize. Crop Sci 51:2505–2516

ter Braak CJF, Boer MP, Totir LR, Winkler CR, Smith OS, Bink MCAM (2010) Identity-by-descent matrix decomposition using latent ancestral allele models. Genetics 185:1045–1057

Tian F, Bradbury PJ, Brown PJ, Hung H, Sun Q, Flint-Garcia S, Rocheford TR, McMullen MD, Holland JB, Buckler ES (2011) Genome-wide association study of leaf architecture in the maize nested association mapping population. Nat Genet 43:159–162

Uleberg E, Meuwissen THE (2007) Fine mapping of multiple QTL using combined linkage and linkage disequilibrium mapping: a comparison of single QTL and multi QTL methods. Genet Sel Evol 39:285–299

van Ooijen (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84:803–811

Verhoeven KJF, Jannink JL, McIntyre LM (2006) Using mating designs to uncover QTL and the genetic architecture of complex traits. Heredity 96:139–149

Wu XL, Jannink JL (2004) Optimal sampling of a population to determine QTL location, variance, and allelic number. Theor Appl Genet 108:1434–1442

Würschum T, Liu W, Gowda M, Maurer HP, Fischer S, Schechert A, Reif JC (2012) Comparison of biometrical models for joint linkage association mapping. Heredity 108:332–340

Xu SZ (1998) Mapping quantitative trait loci using multiple families of line crosses. Genetics 148:517–524

Yi NJ, Xu SZ (2002) Linkage analysis of quantitative trait loci in multiple line crosses. Genetica 114:217–230

Yu J, Holland JB, McMullen MD, Buckler SE (2008) Genetic design and statistical power of nested association mapping in maize. Genetics 178:539–551