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Multivariate whole genome average interval mapping: QTL analysis for multiple traits and/or environments

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Abstract A major aim in some plant-based studies is the determination of quantitative trait loci (QTL) for multiple traits or across multiple environments. Understanding these QTL by trait or QTL by environment interactions can be of great value to the plant breeder. A whole genome approach for the analysis of QTL is presented for such multivariate applications. The approach is an extension of whole genome average interval mapping in which all intervals on a linkage map are included in the analysis simultaneously. A random effects working model is proposed for the multivariate (trait or environment) QTL effects for each interval, with a variance–covariance matrix linking the variates in a particular interval. The significance of the variance–covariance matrix for the QTL effects is tested and if significant, an outlier detection technique is used to select a putative QTL. This QTL by variate interaction is transferred to the fixed effects. The process is repeated until the variance–covariance matrix for QTL random effects is not significant; at this point all putative QTL have been selected. Unlinked markers can also be included in the analysis. A simulation study was conducted to examine the performance of the approach and demonstrated the multivariate approach results in increased power for detecting QTL in comparison to univariate methods. The approach is illustrated for data arising from experiments involving two doubled haploid populations. The first involves analysis of two wheat traits, α-amylase activity and height, while the second is concerned with a multi-environment trial for extensibility of flour dough. The method provides an approach for multi-trait and multi-environment QTL analysis in the presence of non-genetic sources of variation.

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Introduction

In plant-based studies, several traits or variates may be of interest, or a single trait may be observed under different conditions or treatments. Often genetic information, in the form of molecular markers, may be available on the plant lines being studied, and determination of quantitative trait loci (QTL) may be a primary aim of the study. If several traits are measured or observed in an experiment, the QTL effects across traits will be examined; these are essentially QTL by trait interactions. Multi-environment trials are common in plant-based studies and understanding QTL by environment interactions in such trials is of importance to the plant breeder.

There are various forms for such QTL by trait, environment or treatment interactions. The variates, whether they relate to traits, environments or treatments, may be pleiotropic or have a common QTL at a locus, and in the multi-environment or treatment situations may have equal QTL size or unequal QTL size. Some variates may not be associated with the QTL so that the level of pleiotropy or commonality may vary. It may be that QTL for individual or groups of variates are closely linked rather than being pleiotropic. Understanding the nature of these effects is important in making genetic progress. This is particularly true for marker assisted selection where co-location or closely linked QTL for several traits may inhibit the ability to pyramid such QTL.

Past researchers have investigated multivariate methods for QTL analysis. For example, Tinker and Mather (1995) consider an approach to multi-environment analysis. Jiang and Zeng (1995) present an approach for QTL analysis for multiple traits using composite interval mapping (CIM) (Zeng 1994; Jansen 1994). These authors demonstrate the efficacy of their approach using a simulation study. Wang et al. (1999) consider QTL by environment interactions using a mixed model containing additive and epistatic effects for possible pairs of QTL. They include random effects for markers and marker by marker interactions to control for background variation. A mixed model approach using CIM is discussed by Piepho (2000). Multi-trait analysis has been considered by Korol et al. (1995), (1998), Zeng et al. (1999), Knott and Haley (2000), Gilbert and Le Roy (2003) and Lund et al. (2003). Hackett et al. (2001) present a review and an interval mapping method based on multivariate regression. In a simulation study, Sorensen et al. (2003) showed the improved power in using a bivariate as opposed to a univariate analysis. Verbyla et al. (2003) use a factor analytic model in an interval mapping setting for multi-environment analysis and Vargas et al. (2006) consider factorial regression and partial least squares methods, also for multi-environment analysis. Gonzalo et al. (2006) develop a treatment by QTL approach using random regression techniques and multi-trait locus by plant density analysis. Boer et al. (2007) consider QTL analysis for multi-environment trials, using a genome scan approach within a mixed models setting, and move to using environmental variable by QTL interaction terms in an attempt to explain the QTL by environment interactions found. Malosetti et al. (2008) investigate multi-trait multi-environment analysis. These authors use mixed models, a preliminary genome scan and a backward elimination approach for putative QTL selected using the preliminary genome scan. A nice review of methods for multi-environment QTL analysis is presented by van Eeuwijk et al. (2010).

Most of the methods proposed both in the univariate and multivariate setting, involve some type of genome scan, often at various levels. Thus, usually a large number of analyses is required. For example, CIM might be utilized in an attempt to allow for background genetic variation. A preliminary scan is required. Subsequently, it may not be clear how many co-factors to use. In addition, these methods also suffer from multiple testing issues and hence the need to use LOD scores.

Verbyla et al. (2007) presented a whole genome average interval mapping approach for QTL analysis of a single trait in a single trial. This method uses all the intervals on a linkage map simultaneously and avoids the difficult issues regarding repeated genome scans. The approach involves a working model in which every interval is allowed a QTL size that is initially assumed a random effect. The working model provides a mechanism for determining if QTL are present and a stopping rule for the selection process. An outlier detection technique is used in a forward selection process to select the QTL. The method was shown to be much more powerful than CIM, although there is a small increase in selecting false positives.

In this paper, a method is presented for multivariate QTL analysis using a whole genome interval mapping approach. Multivariate QTL effects are included for all intervals on the linkage map in the model simultaneously. The multivariate genetic QTL sizes are modelled as random effects with an associated variance–covariance matrix allowing correlation between the variates, be they traits, environments or treatments. A likelihood ratio test is presented for testing the significance of the QTL variance–covariance matrix. If the test is significant, a multivariate outlier detection method based on a Cholesky decomposition (Golub and van Loan 1996) of the QTL variance–covariance matrix is used to select the most likely interval for a QTL. Multivariate QTL are chosen in a forward selection process and are progressively moved to the fixed effects model. The approach allows both genetic and non-genetic effects to be included in the model simultaneously. A simulation study provides an indication of the performance of the method in comparison to univariate analyses. Both a multi-trait and a multi-environment analysis are presented to illustrate the approach.

Methods

Overview

The multivariate whole genome average interval mapping (MVWGAIM) approach presented in this paper mirrors the univariate method (WGAIM) presented by Verbyla et al. (2007). A working model is proposed for random QTL

effects for intervals and incorporates differing QTL variances and correlations for the multivariate response. An approximate likelihood ratio test is used to determine if the QTL random effects provide sufficient variance–covariance structure to warrant selection of a putative QTL, and an outlier model based on a Cholesky decomposition allows selection of a QTL to be carried out. As for WGAIM, the forward selection process firstly involves determining the chromosome or linkage group most likely to contain a QTL and then selecting the interval on that chromosome or linkage group as most likely to contain the putative QTL. One difference is that the multivariate outlier detection leads to fixed QTL effects for each of the variates. Some of the individual variate QTL effects may not be significant as a multivariate outlier can exist in a dimension lower than the full multivariate dimension. This reflects the possible varying level of common (possibly pleiotropic) effects in the multi-trait situation, and QTL by environment or treatment interactions in the multi-environment or multi-treatment situation.

Linear mixed model

Mixed models form the basis for analysis. These models are of the form

$$\mathbf{y} = \mathbf{X}\boldsymbol{\tau} + \mathbf{Z}_g\mathbf{u}_g + \mathbf{Z}_0\mathbf{u}_0 + \mathbf{e} \tag{1}$$

where the vector $\mathbf{y}$ consists of data that has multivariate structure. This might simply be multiple traits, responses on multiple treatments or multiple environments, or combinations of these; the term variates is used for the generic multivariate vector. We denote by t the dimension of the underlying multivariate response, that is the number of variates, for a single experimental unit.

The components of (1) reflect the nature of the study. Thus $\mathbf{X}$ and $\mathbf{Z}_0$ are known design matrices for the fixed terms and random terms respectively, $\boldsymbol{\tau}$ is the vector of fixed term parameters, and $\mathbf{u}_0$ is a vector of random terms. These terms will incorporate effects due to the experimental design and the conduct of the experiment; see for example Smith et al. (2005, 2006). The vector of residual effects is denoted by $\mathbf{e}$ and this term and $\mathbf{u}_0$ are assumed independent, mean zero with variance–covariance matrices $\mathbf{R}$ and $\mathbf{G}_0$ respectively. The form that $\mathbf{R}$ takes will reflect the nature of the multivariate analysis. For example, for a multi-trait analysis, it will be appropriate to provide for different variances for different traits, and for correlation between traits.

If there are n_g lines of interest, the genetic effects $\mathbf{u}_g$ is an $n_g t \times 1$ vector formed by stacking the columns of an $n_g \times t$ matrix of genetic effects $\mathbf{U}_g$. The elements of this matrix will be denoted by u_{gij} and this is the genetic effect for line i for variate j. The associated design matrix $\mathbf{Z}_g$ in (1) assigns the appropriate genetic effect to each observation.

Multivariate whole genome average interval mapping

As in Verbyla et al. (2007), QTL effects are allowed for each interval in the model formulation. Thus, the whole genome model for the genetic effects $\mathbf{u}_g$ in the multivariate case is given by

$$u_{gij} = \sum_{k=1}^{c}\sum_{l=1}^{r_k-1} q_{i;kl}a_{j;kl} + u_{pij} \tag{2}$$

where there are c chromosomes (or linkage groups), and r_k markers on chromosome k and hence $r_k - 1$ intervals. The total number of markers is $r_\cdot = \sum_k r_k$ and hence the number of intervals is $r_\cdot - c$. The terms $q_{i;kl}$ are the unknown QTL indicators for line i, either -1 or 1 for doubled haploid, recombinant inbred lines or backcross lines, depending on the parental origin of the QTL allele, for the lth interval on the kth chromosome. The QTL size for variate j in interval l is denoted by $a_{j;kl}$. The term u_{pij} is a polygenic effect that provides a genetic residual and reflects the possible small contribution of a large number of genes that may impact on the genetic expression of variate j in line i.

We use the regression approach (Haley and Knott 1992; Martinez and Curnow 1992) for QTL mapping and hence for each interval, $q_{i;kl}$ is replaced by its expected value given the two markers defining the interval. If $\mathbf{M}$ is the matrix of marker scores ($n_g \times r_\cdot$) with element $m_{i;kl}$ being the marker score for marker l on chromosome k for line i, using the results of Whittaker et al. (1996) we find in a similar manner to Verbyla et al. (2007) that

$$u_{gij} = \sum_{k=1}^{c}\sum_{l=1}^{r_k-1}(m_{i;kl}\lambda_{k;l,l} + m_{i;k,l+1}\lambda_{k;l+1,l})a_{j;kl} + u_{pij} \tag{3}$$

The terms $\lambda_{k;l,l}$ and $\lambda_{k;l+1,l}$ are functions of the recombination frequency for interval l on chromosome k, denoted by $\theta_{k;l,l+1}$ and the recombination frequency between the left marker defining the interval and the putative QTL, denoted by $\theta_{k;l}$. As in Verbyla et al. (2007) there are too many parameters ($\theta_{k;l}$) to estimate and $\lambda_{k;l,l}$ and $\lambda_{k;l+1,l}$ are replaced by their expected value (assuming the distance from the left flanking marker and the putative QTL is uniformly distributed). Thus both are replaced by

$$\lambda_{kl;E} = \frac{\theta_{k;l,l+1}}{2d_{k;l,l+1}(1-\theta_{k;l,l+1})} \tag{4}$$

where

$$d_{k;l,l+1} = -\frac{1}{2}\log(1-2\theta_{k;l,l+1})$$

is Haldane's distance between markers l and $l+1$ on chromosome k. If we form the matrix of genetic effects U_g ($n_g \times t$) we can write (3) as

$$\mathbf{U}_g = \mathbf{M}\Lambda_E\mathbf{A} + \mathbf{U}_p \quad (5)$$

where the matrix Λ_E is a block diagonal matrix (with blocks corresponding to chromosomes or linkage groups), with kth block Λ_k being $r_k \times r_k - 1$. The only non-zero elements of Λ_k are the two central diagonals, and the two values in each column are identical and given by (4). Note $\mathbf{A}$ is the $(r_\cdot - c) \times t$ matrix of QTL sizes $a_{j;kl}$ and $\mathbf{U}_p$ is the matrix of polygenic effects u_{pij}. If $\mathbf{M}_E = \mathbf{M}\Lambda_E$, in vector form (5) is given by

$$\mathbf{u}_g = (\mathbf{I}_t \otimes \mathbf{M}_E)\mathbf{a} + \mathbf{u}_p \quad (6)$$

The model for the QTL sizes $\mathbf{a}$ in (6) is a natural extension of the univariate specification given by Verbyla et al. (2007). Thus the QTL sizes are assumed $\mathbf{a} \sim N(\mathbf{0}, \mathbf{G}_a \otimes I_{r_\cdot - c})$, where $\mathbf{G}_a$ is a $t \times t$ variance–covariance matrix, allowing for the t variates. There may be applications in which $\mathbf{G}_a$ is non-negative definite, rather than positive definite, for example if a factor analytic model is used. The approach presented is applicable if $\mathbf{G}_a$ is not of full rank; see the Appendix.

The model for the polygenic effects $\mathbf{u}_p$ is $\mathbf{u}_p \sim N(\mathbf{0}, \mathbf{G}_p \otimes \mathbf{I}_{n_g})$. The matrix $\mathbf{G}_p$ may be an unstructured (and hence fully parameterized) $t \times t$ variance–covariance matrix, or it may take on another form. For example, factor analytic models have been used for the analysis of multi-environment trials (Smith et al. 2005) and can provide a very good and numerically stable approximation to the unstructured model.

Rule for selection of a putative QTL

The first step at each stage of possible selection of a QTL, is to determine if a multivariate QTL exists, that is if $\mathbf{G}_a \neq \mathbf{0}$. Thus we would like test the hypothesis $H_0 : \mathbf{G}_a = \mathbf{0}$ to establish if a QTL exists. If the test is rejected, there is evidence that at least one putative QTL exists and a process (described below) is used to select the most likely interval for the putative QTL. If the test is retained, the selection process concludes.

The test of $H_0 : \mathbf{G}_a = \mathbf{0}$ is non-standard, just as in the univariate case. From a practical point of view there are major difficulties with such a test. If a variance of a variate is zero, covariances or correlations with other variates are not defined. To overcome this problem, the approach taken is to initially fit a model with only variances for the multivariate sizes and test the significance of this so-called diagonal variance matrix. There is one additional complication. Fitting a correlated polygenic effect with a diagonal working model for the QTL random effect sizes distorts the null distribution of the test statistic. Thus at the stage of testing for putative QTL, the polygenic effects are also fitted using a diagonal variance model. In this case, if $\hat{\ell}$ is the maximized residual log-likelihood including the diagonal variance model for putative QTL and $\hat{\ell}_0$ is the maximized residual log-likelihood omitting the diagonal variance model for the QTL effects, the likelihood ratio test statistic is found by

$$X^2_{\mathrm{LR}} = 2(\hat{\ell} - \hat{\ell}_0) \quad (7)$$

and this statistic has an approximate distribution under the null hypothesis (zero diagonal variance matrix) that is a mixture of Chi-squared distributions. In fact, the mixture consists of Chi-squared distributions from zero to t degrees of freedom with approximate null distribution given by

$$X^2_{\mathrm{LR}} \sim \left(\frac{1}{2}\right)^t \sum_{k=0}^{t} \binom{t}{k} \chi^2_k \quad (8)$$

where χ^2_k represents a Chi-square distribution on k degrees of freedom. Thus a test of size α of the hypothesis that the diagonal $\mathbf{G}_a$ is zero is rejected if $X^2_{\mathrm{LR}} > c_{1\text{-}\alpha}$, where the critical value $c_{1\text{-}\alpha}$ is determined using (8). This establishes the presence of variation that is necessary for a QTL to exist. The use of (8) was investigated in a small simulation study.

Outlier detection and selection of QTL

If the hypothesis $H_0 : \mathbf{G}_a = \mathbf{0}$ is rejected, an outlier detection approach is used to select a putative QTL in a similar manner to Verbyla et al. (2007). The alternative outlier model (AOM) is again used, but on a transformed scale. Consider the Cholesky decomposition of $\mathbf{G}_a$, namely $\mathbf{G}_a = \mathbf{L}_a\mathbf{L}_a^T$ where $\mathbf{L}_a$ is a lower triangular matrix (that is having all elements above the diagonal equal to zero). Then

$$\mathbf{a} = (\mathbf{L}_a \otimes \mathbf{I}_{r_\cdot - c})\mathbf{f}_a$$

where $\mathbf{f}_a \sim N(\mathbf{0}, \mathbf{I}_t \otimes \mathbf{I}_{r_\cdot - c})$ are independent standard normal variates. The outlier model is based on $\mathbf{f}_a$ and matches the strategy used in the univariate case by Verbyla et al. (2007). The AOM is used for chromosomes in the first instance, and then for intervals within the selected chromosome. Thus the effects $\mathbf{f}_a$ are modified for chromosome k by (if intervals are nested within variates)

$$\mathbf{f}_{ak} = \mathbf{f}_a + (\mathbf{I}_t \otimes \mathbf{D}_k)\boldsymbol{\delta}_k \quad (9)$$

where $\boldsymbol{\delta}_k \sim N(\mathbf{0}, \sigma^2_{ak}\mathbf{I}_t \otimes \mathbf{I}_{r_k - 1})$ is a vector of departures for chromosome k. This outlier model provides for variance inflation for chromosome k via the variance component σ^2_{ak}. The matrix $\mathbf{D}_k$ is a $(r_\cdot - c) \times (r_k - 1)$ matrix with an identity matrix for the $r_k - 1$ intervals on chromosome k and zeros elsewhere, and therefore the additional random effects $\boldsymbol{\delta}_k$ are only added for intervals on chromosome k. The idea behind (9) is that if chromosome k has a QTL, the QTL sizes will exhibit variation, expressed in the AOM by $\boldsymbol{\delta}_k$. The aim is to detect this additional variation.

The AOM given by (9) results in QTL sizes

$$\mathbf{a}^{(k)} = (\mathbf{L}_a \otimes \mathbf{I}_{r_{.}-c})\mathbf{f}_{ak}$$

with a modified variance–covariance matrix of $(1 + \sigma^2_{ak})(\mathbf{G}_a \otimes \mathbf{I}_{r_k-1})$ for chromosome k. Thus the AOM allows for a rescaling of the underlying variate QTL size variance–covariance matrix and chromosomes indicating such an inflation are flagged as possibly containing a QTL.

As in Verbyla et al. (2007), the AOM does not have to be fitted. An outlier statistic is developed using an approach based on the score statistic evaluated at the null hypothesis that $\sigma^2_{ak} = 0$. Thus if $\mathbf{a}_{kl}$ is the $t \times 1$ vector of sizes for all variates for interval l on chromosome k, and $\tilde{\mathbf{a}}_{kl}$ the best linear unbiased predictor of $\mathbf{a}_{kl}$, it is shown in the Appendix that the score statistic is a function of

$$t^2_k = \frac{\sum_{l=1}^{r_k-1} \tilde{\mathbf{a}}^T_{kl}\mathbf{G}^-_a\tilde{\mathbf{a}}_{kl}}{\sum_{l=1}^{r_k-1} \mathrm{tr}\left(\mathbf{G}^-_a \mathrm{var}(\tilde{\mathbf{a}}_{kl})\right)} \qquad (10)$$

where $\mathbf{G}^-_a$ is a generalized inverse of $\mathbf{G}_a$ and tr() denotes the trace of the matrix argument. The statistic (10) is used to rank the chromosomes in terms of their outlier status. The largest statistic indicates the biggest departure from the null hypothesis ($\sigma^2_{ak} = 0$) and hence this chromosome is selected as being most likely to contain a putative QTL.

The same argument within the selected chromosome can be used to select the most likely interval using the statistic

$$t^2_{kl} = \frac{\tilde{\mathbf{a}}^T_{kl}\mathbf{G}^-_a\tilde{\mathbf{a}}_{kl}}{\mathrm{tr}\left(\mathbf{G}^-_a \mathrm{var}(\tilde{\mathbf{a}}_{kl})\right)} \qquad (11)$$

The selected interval is placed in the fixed effects part of the model as an interaction between the interval and the factor defining the variates. The process of selection continues until the stopping rule is invoked.

High-dimensional analysis

An important consideration is the computational efficiency of fitting the models and indeed of calculating the outlier statistics for QTL selection. Stranden and Garrick (2009) discuss the equivalence of computing algorithms for genomic predictions using markers and a marker based genomic relationship matrix. These ideas are used by Verbyla and Talor (2012) in an approach for univariate QTL analysis with dimension reduction from the number of intervals or markers to the number of genetic lines in the data. The same idea can be used to reduce the dimension of the problem in the multivariate context as well.

Firstly, (6) is a term that is fitted in the analysis using a mixed model. The term involving $\mathbf{a}$ has variance matrix

$$\mathrm{var}((\mathbf{I}_t \otimes \mathbf{M}_E)\mathbf{a}) = \mathbf{G}_a \otimes \mathbf{M}_E\mathbf{M}^T_E$$

An alternative model to (6) that leads to the same variance model is

$$\mathbf{u}_g = \{\mathbf{I}_t \otimes (\mathbf{M}_E\mathbf{M}^T_E)^{1/2}\}\mathbf{a}^* + \mathbf{u}_p$$

where $(\mathbf{M}_E\mathbf{M}^T_E)^{1/2}$ is the matrix square root of $(\mathbf{M}_E\mathbf{M}^T_E)$ and $\mathbf{a}^*$ is a $tn_g \times 1$ vector with distribution $N(\mathbf{0}, \mathbf{G}_a \otimes \mathbf{I}_{n_g})$. This reduces the dimension of the effects to be fitted from $t(r - c)$ for $\mathbf{a}$ to tn_g for $\mathbf{a}^*$.

If $\tilde{\mathbf{a}}$ and $\tilde{\mathbf{a}}^*$ are the best linear unbiased predictors (BLUPs) of $\mathbf{a}$ and $\mathbf{a}^*$ respectively, the former can be found from the latter using

$$\tilde{\mathbf{a}} = \left\{\mathbf{I}_t \otimes \mathbf{M}^T_E(\mathbf{M}_E\mathbf{M}^T_E)^{-1/2}\right\}\tilde{\mathbf{a}}^*$$

The outlier statistics for selection also require the variance of $\tilde{\mathbf{a}}$ and this is

$$\mathrm{var}(\tilde{\mathbf{a}}) = \left\{\mathbf{I}_t \otimes \mathbf{M}^T_E(\mathbf{M}_E\mathbf{M}^T_E)^{-1/2}\right\}\mathrm{var}(\tilde{\mathbf{a}}^*) \left\{\mathbf{I}_t \otimes (\mathbf{M}_E\mathbf{M}^T_E)^{-1/2}\mathbf{M}_E\right\}$$

These results mean that high-dimensional situations can be handled in MVWGAIM.

Final assessment of significance of QTL

Single multivariate QTL are determined using the above process using forward selection. Selected QTL are fitted as fixed effects as they are chosen. The fixed effects QTL model fitted depends on the context. For example, in a multi-trait situation, the QTL intervals are nested within traits and it is not meaninful to fit a main effect for the QTL interval. In a multi-environment or multi-treatment setting, a main effect for the QTL interval is fitted together with the interaction between the environment or treatment and the QTL interval. This is because the measurement is the same across environments and treatments and a common QTL is a sensible outcome. The final output of an analysis will therefore depend on the situation but will consist of individual Wald tests of the appropriate effects, be they main effects, interactions or a combination of both. The Wald tests are based on the methods discussed by Kenward and Roger (1997).

Unlinked markers

There are situations where it is desirable to include unlinked markers in an analysis. Thus consider the term for a single marker that occupies chromosome or linkage group $c + 1$. The QTL on linkage group $c + 1$ contributes a term

$q_{ij;c+1}a_{j;c+1}$

to (3). Given the marker scores $m_{i;c+1}$ on the marker for line i, the regression approach for single marker regression replaces $q_{ij;c+1}$ by

$$E\left(q_{ij;c+1}|m_{i;c+1}\right) = (1 - 2\theta_{c+1})m_{i;c+1} \tag{12}$$

where θ_{c+1} is the recombination fraction between the putative QTL and the marker. It is not possible with a single marker to estimate both the size and location (recombination fraction) of a QTL, and in the spirit of Verbyla et al. (2007) we integrate out the location or distance using a uniform distribution for the distance between the QTL and the marker. Thus if we use Haldane's distance, we have

$$\theta_{c+1} = \frac{1}{2}(1 - \mathrm{e}^{-2d_{c+1}})$$

where d_{c+1} is the distance between the marker and the QTL. Notice that

$$1 - 2\theta_{c+1} = \mathrm{e}^{-2d_{c+1}} \tag{13}$$

and we assume that d_{c+1} is uniform distributed over the range $(0, \infty)$. This uniform distribution is improper in the Bayesian sense (Gelman et al. 2004, page 61). We integrate out d_{c+1} in (12) using (13), namely

$$\int_0^\infty \mathrm{e}^{-2x}\mathrm{d}x = \frac{1}{2}$$

so that our regression model (12) becomes

$$E\left(q_{ij;c+1}|m_{i;c+1}\right) = \frac{1}{2}m_{i;c+1}$$

Thus the uncertainty in the position of the QTL down-weights the marker by a value of 2. Thus analysis with unlinked markers proceeds using the marker scores divided by two.

Estimation, prediction and computation

Estimation of the fixed effects and variance parameters in (1) is based on residual maximum likelihood (REML) as originally proposed by Patterson and Thompson (1971). Best linear unbiased prediction (BLUP) is used for the random effects; see Robinson (1991). This includes all modifications of (1) presented as part of MVWGAIM.

All computations were performed in R (R Development Core Team 2009) using the ASReml package (Butler et al. 2007), with multivariate QTL analysis using components of the wgaim package (Taylor et al. 2009). Code to carry out analysis is available in the online supplementary material and from the authors.

Simulation studies

Simulation study: null distribution and type I errors

A small simulation study was conducted to examine the null distribution for the stopping rule (the test of the diagonal variance matrix being zero) using (7) and (8). Data was simulated for population sizes (n_p) of 100, 200 and 400 lines with two replicates and four environments. The null model (no QTL) was given by ($i = 1, 2, \ldots, n_p; j = 1, 2, 3, 4; k = 1, 2$)

$$y_{ijk} = \mu_j + u_{pij} + e_{ijk}$$

where $\mu_j = 10$, the errors e_{ijk} were independent standard normal and the polygenic effects u_{pij} were simulated having zero mean and covariance matrix

$$\mathbf{G}_p = \begin{bmatrix} 1.0 & 0.9 & 0.7 & 0.5 \\ 0.9 & 1.0 & 0.7 & 0.3 \\ 0.7 & 0.7 & 1.0 & 0.5 \\ 0.5 & 0.3 & 0.5 & 1.0 \end{bmatrix} \tag{14}$$

for the four traits for each line. Note however, that only a diagonal variance matrix is fitted in examining the distribution of the residual likelihood ratio statistic. Two thousand (2,000) simulations were run for each population size.

The linkage map for each population size was generated as outlined in Verbyla et al. (2007) and consisted of 9 linkage groups of 11 markers, with an original spacing of 10 cM (the distances used in the simulation were estimated using the simulated marker data on the individuals).

Simulation study: power and false discovery rate

To examine the performance of the multivariate QTL analysis, an extension of the simulation experiment of Verbyla et al. (2007) was conducted. Population sizes were 100, 200 and 400 lines with 2 replicates. Nine chromosomes of 11 markers spaced at 10 cM were constructed with 7 QTL at locations as in Verbyla et al. (2007); thus QTL were placed at the midpoints of C1.4 (chromosome 1, interval 4), C1.8, C2.4, C2.8, C3.6, C4.4, and C5.1. Thus the two QTL on C1 and C2 are 40 cM apart. The simulated data involved four traits, with a generating model ($i = 1, 2, \ldots, n_p; j = 1, 2, 3, 4; k = 1, 2$)

$$y_{ijk} = \mu_j + \sum_{l=1}^{7} q_{il}a_{jl} + u_{pij} + e_{ijk} \tag{15}$$

where the means μ_j were 10, 11, 12, 13 for the 4 traits, the polygenic effects u_{pij} are defined as for the null distribution simulation, including (14), and e_{ijk} were assumed independent and identically distributed $N(0,1)$.

In the QTL component of (15), q_{il} gives the simulated allelic value (−1 or 1) for line i for QTL l. The effect size of this QTL for trait j is a_{jl}. The configuration of sizes is given in Table 1. Thus C1.4 and C1.8 are in repulsion for all traits, C2.4 and C2.8 are in coupling for all traits, while the QTL on chromosomes 3, 4 and 5 vary in their level of pleiotropy from 3 down to 1.

Two hundred (200) simulations were carried out for each population size.

For each population size five analyses were carried out for each simulated data set, namely 4 univariate WGAIM analyses, and a multivariate WGAIM analysis. Three sets of summaries were calculated for each set of 200 simulations, namely

1. the proportion of correct detection of each QTL for each trait, including proportions for the QTL in coupling and repulsion,
2. the proportion of false positives, both linked (on the same chromosome as actual QTL) and unlinked (on chromosomes not containing QTL), and
3. means of estimated sizes of QTL effects for simulations where the QTL was selected. Empirical standard deviations of the estimates were also found.

As in Verbyla et al. (2007), a QTL was considered detected, if the correct interval or intervals on either side of the correct interval were selected.

Materials

Late maturity α-amylase in wheat

Late maturity α-amylase (LMA) in wheat is a defect where potentially high levels of the enzyme pl α-amylase accumulates in the ripening grain. The expression of the enzyme and its accumulation in wheat grain has detrimental consequences for processing by end-users to produce value-added wheat products and usually results in downgrading of the grain quality and loss of premiums to farmers. LMA is a difficult trait to phenotype because it is induced by temperature changes. Experiments to investigate LMA are therefore complex and involve multiple phases.

Experimental details

A total of 194 doubled haploid (DH) lines derived from a cross between an advanced breeding line, WW1842, and the line Whistler, were used in the experiment. The phases of the experiment were growth, temperature induction, further growth and assaying of seeds using ELISA plates. A complete account of the experiment can be found in Tan et al. (2010) while the design of the experiment is discussed in Butler et al. (2009).

The first growth phase was conducted at Cobbity, NSW, Australia. Two micro-climate rooms were used, with 220 pots in each room, subdivided into two blocks, and two sides within each block of 55 pots, arranged in an 11 × 5 rectangular array. Randomization of parents and DH lines was restricted; 130 lines had two replicates while 60 lines had three replicates. Lines were assigned to pots so that each room contained either one (160) or two (30) pots of each line, randomized so that each side within each block contained only one pot of each of 55 lines. Four plants were grown in each pot and at anthesis spikes from healthy plants were tagged.

The induction phase was carried out 26–28 days after anthesis. Pots were assigned to induction cohorts (pots within many of the DH lines were induced on different days) for exposure to cool temperatures and were transferred to a cool temperature room. After 8–10 days the plants were returned to their original position in the micro-climate rooms until the plants reached harvest ripeness. This is the second growth phase, and at the commencement of this phase, the height of the plants in a pot was measured.

The aim was to assay approximately five grains from each primary tiller from each of the four plants per pot. Tillers from a total of 425 pots were deemed sufficiently healthy to produce a reliable result; only 1,375 tillers out of a potential 1,700 were used. Grain numbers per tiller varied from 1 to 62, with a median of 13.

Table 1 QTL effects in the simulation study

Trait	QTL						
	C1.4	C1.8	C2.4	C2.8	C3.6	C4.4	C5.1
1	0.38	−0.38	0.38	0.38	0.38	0.38	0.38
2	0.38	−0.38	0.38	0.38	0.38	0.38	0
3	0.38	−0.38	0.38	0.38	0.38	0	0
4	0.38	−0.38	0.38	0.38	0	0	0

The level of pleiotropy varied across traits for the single QTL on chromosomes 3, 4 and 5

Each ELISA plate had 96 wells arranged in a 12 column × 8 row array to which seeds were assigned. In the design, the actual number of seeds per pot varied from 1 to 22, with 25 % of pots having less than 19 seeds. With the additional requirement of at least one blank (negative control) per plate, the seeds for assay were ultimately distributed over 75 plates. Plates were grouped into 15 sets of 5, each group of plates being a near complete duplicate of each line, pot and plant.

Using the design, supernatant extract using a single seed (100 ml) was measured into the appropriate position of antibody-coated ELISA plates. Optical densities (OD), which characterize α-amylase activity, were measured at 450 nm with the micro-plate photometer (Multiskan Ascent, Thermo Scientific) and thus OD and height constitute the two traits of interest. A bivariate analysis of these two traits is preferable because LMA has been shown to be influenced by the height genes, *Rht1*, *Rht2* and *Rht3* (Mrva and Mares 1996).

Genetic information

A total of 697 DArT markers (http://www.triticarte.com.au/) and 101 polymorphic microsatellite (SSR) markers were genotyped on the WW1842 × Whistler DH population. A linkage map was constructed using MapManager QTXb20 (Manly et al. 2001).

For QTL analysis, redundant markers (coincident on the linkage map) were removed and the resultant map had a total of 437 markers (101 SSR and 336 DArT markers). The reduced linkage map was then checked using the `qtl` package (Broman et al. 2009) in the R environment (R Development Core Team 2009) using a combination of double cross-over statistics and likelihood based methods. This resulted in substantial changes to both the order of markers within linkage groups and to the groups themselves. The final map had 31 linkage groups with an additional 12 unlinked markers that were included for analysis. The total length of the linkage map was 3,160 cM, with an average spacing of 7.5 cM between markers.

Dough rheology

Mann et al. (2008) provide details of experiments that were conducted using the Kukri × Janz doubled haploid population in Queensland, Australia. Kukri has unique high dough strength while Janz is considered to have genes for "wide adaptation" and high yield in Australia. This cross was of interest to study the genetic basis of dough rheology, in particular dough strength and extensibility. The aim of the experiments was to broadly examine quality characteristics of wheat, from milling to final loaf characteristics.

Experimental details

Field trials were conducted at a number of sites. Two sites, Biloela and Lundavra, in the years 2001 and 2002 form the basis of the analysis presented here. At those two sites, two replicates of 156 doubled haploid lines (and the parents and other standard lines) were planted in two replicates of a Latinised row-column design, laid out in a two-dimensional array of up to 10 columns by 36 rows (32 rows at Biloela in 2001). A number of traits were measured in a series of multi-phase experiments. Grain samples were milled using a Buhler mill in an incomplete block design with milling days forming the blocks. For Biloela 2001, the milling consisted of 106 days by 9 samples per day, with each site processed as a separate block of days. For 2002, limited grain yield for the Lundavra site restricted laboratory duplication, so the Biloela and Lundavra plots were randomized within the milling design, with the majority of laboratory duplication coming from the Biloela site. The milling design was again an incomplete block design of 92 milling days by 10 samples per day. The milled grain was processed to obtain dough and the trait examined in this paper, maximum extensibility, was determined from two dough pieces of 150 g each from one mix, using the Extensograph (Brabender Duisburg, Germany). The extensograph testing was conducted in three laboratory sections over 59, 98 and 144 measurement days, respectively, for Biloela 2001, Lundavra 2001 and both sites in 2002 together. Some mixes of dough were duplicated.

Genetic information

A genetic linkage map consisting of 246 segregating loci spread over 21 linkage groups (chromosomes) and scored over 172 genetic lines was used in the analysis; only 156 of these lines were used in the field trials. The markers were mainly microsatellites analyzed by Syngenta Toulouse (France) and CSIRO Plant Industry (Australia) laboratories. The map was checked in the statistical software package `R` (R Development Core Team 2009) using the `qtl` library (Broman et al. 2009). The map length was 3,400 cM with an average spacing of 14 cM between markers.

Results

Simulation study: null distribution and type I errors

The simulation study to evaluate the type I error rates based on (7) and (8) resulted in empirical percentage points for the three population sizes given in Table 2. For all population sizes the empirical percentage points are less than or

Table 2 Estimated proportion of values out of 2,000 simulations of the residual likelihood ratio statistic exceeding the nominal critical value for levels 0.10, 0.05 and 0.01 for population sizes 100, 200 and 400

Nominal probability	Critical value	Population size		
		100	200	400
0.10	4.96	0.079	0.082	0.070
0.05	6.50	0.046	0.048	0.034
0.01	10.02	0.019	0.011	0.009

The simulation involved four variates

close to the corresponding nominal points, suggesting the mixture of Chi-squared distributions is a good approximation to the distribution under the null hypothesis.

Simulation study: power and false discovery rate

The results of the simulations to examine power of detection and false discovery rates are presented in Tables 3, 4, 5, 6 and 7. Each aspect is now discussed in turn.

Table 3 presents the proportion of simulations in which each QTL was successfully found together with the total number of QTL out of 7, 6, 5, and 4 for the univariate and 7 for the multivariate analyses. This shows that in the simulation study the multivariate approach detected more of the full complement of QTL than the individual univariate analyses for all populations sizes. For QTL that were pleiotropic, the multivariate approach again detected QTL more frequently. The QTL at C5.1 which was specific to trait 1 was detected more frequently using the univariate analysis. This shows that there is a price in using the multivariate approach, but that the multivariate approach increased the power of detection overall. This study also highlighted that the univariate approach detected fewer QTL as the number of true QTL decreased across the 4 traits.

For the two QTL in repulsion on C1, detection rates for the univariate analyses of traits 1 to 4 tend to decrease; see Table 4. The multivariate approach is best and for population size 400, both QTL were detected in all simulations. A similar pattern for QTL in coupling is presented in Table 5, although the detection rates compared to repulsion were lower.

Table 6 gives the proportion of false positives for the univariate and multivariate approaches. False positives can be linked (on the same chromosome as true QTL) or unlinked (on chromosomes where no QTL exist). In general, linked false positives were found more often than unlinked false positives for both univariate and multivariate analyses. For all methods the rate of false positives decreases with population size. The rates in the univariate analyses tend to decrease with the number of true QTL. The multivariate approach for population size 100 has rates of false positives of the order of the univariate analysis for trait 1, though the unlinked false positive rate is higher. For larger populations sizes, the multivariate approach has, in general, a lower rate of false positives.

One aspect of the univariate approach that was not examined by Verbyla et al. (2007) was bias in the estimated QTL sizes. It is well established that results of QTL analysis suffer from selection bias, see for example Beavis (1994, 1998), Melchinger et al. (1998) and Xu (2003). This

Table 3 Proportion of the 200 simulations in which the QTL was detected for individual WGAIM analyses of each trait and for the multi-trait or joint analysis (MVWGAIM)

Population	Trait	C1.4	C1.8	C2.4	C2.8	C3.6	C4.4	C5.1	Total
100	1	0.400	0.405	0.735	0.435	0.655	0.610	0.590	3.830
	2	0.350	0.375	0.730	0.470	0.660	0.620	–	3.205
	3	0.300	0.260	0.600	0.605	0.575	–	–	2.340
	4	0.215	0.200	0.505	0.620	–	–	–	1.540
	Multi	0.560	0.575	0.835	0.680	0.760	0.725	0.475	4.610
200	1	0.950	0.945	0.760	0.595	0.750	0.815	0.820	5.635
	2	0.940	0.895	0.700	0.510	0.715	0.825	–	4.585
	3	0.845	0.865	0.540	0.695	0.770	–	–	3.715
	4	0.735	0.620	0.650	0.585	–	–	–	2.590
	Multi	0.985	0.980	0.900	0.760	0.910	0.900	0.730	6.165
400	1	0.985	0.965	0.930	0.915	0.975	1.000	1.000	6.770
	2	0.985	0.960	0.950	0.900	0.970	0.985	–	5.750
	3	0.975	0.980	0.935	0.940	0.995	–	–	4.825
	4	0.925	0.940	0.925	0.920	–	–	–	3.710
	Multi	1.000	1.000	0.985	0.985	0.985	1.000	0.980	6.935

Table 4 Two way tables for the QTL in repulsion (C1.4 and C1.8) with proportions of the 200 simulations for each population size for the combinations of non-detected $\bar{D}$ and detected D QTL

Trait		Population size					
		100		200		400	
		$\bar{D}$	D	$\bar{D}$	D	$\bar{D}$	D
1	$\bar{D}$	0.500	0.100	0.015	0.035	0.010	0.005
	D	0.095	0.305	0.040	0.910	0.025	0.960
2	$\bar{D}$	0.555	0.095	0.035	0.025	0.010	0.005
	D	0.070	0.280	0.070	0.870	0.030	0.955
3	$\bar{D}$	0.635	0.065	0.035	0.030	0.010	0.015
	D	0.105	0.195	0.100	0.835	0.010	0.965
4	$\bar{D}$	0.730	0.055	0.240	0.025	0.040	0.035
	D	0.070	0.145	0.140	0.595	0.020	0.905
Multi	$\bar{D}$	0.345	0.095	0.005	0.010	0.000	0.000
	D	0.100	0.460	0.015	0.970	0.000	1.000

C1.4 is on the left and C1.8 on the top of each 2 × 2 table. The bottom right-hand corner of each 2 × 2 table is the proportion of simulations in which both QTL were detected

Table 5 Two way tables for the QTL in coupling (C2.4 and C2.8) with proportions of the 200 simulations for each population size for the combinations of non-detected $\bar{D}$ and detected D QTL

Trait		Population size					
		100		200		400	
		$\bar{D}$	D	$\bar{D}$	D	$\bar{D}$	D
1	$\bar{D}$	0.080	0.185	0.060	0.180	0.010	0.060
	D	0.485	0.250	0.305	0.415	0.075	0.855
2	$\bar{D}$	0.105	0.165	0.080	0.220	0.010	0.040
	D	0.425	0.305	0.410	0.290	0.090	0.860
3	$\bar{D}$	0.100	0.300	0.075	0.385	0.005	0.060
	D	0.295	0.305	0.230	0.310	0.055	0.880
4	$\bar{D}$	0.060	0.475	0.065	0.285	0.005	0.070
	D	0.320	0.185	0.350	0.300	0.075	0.850
Multi	$\bar{D}$	0.035	0.130	0.015	0.085	0.005	0.010
	D	0.285	0.550	0.225	0.675	0.010	0.975

C2.4 is on the left and C2.8 on the top of each 2 × 2 table. The bottom right-hand corner of each 2 × 2 table is the proportion of simulations in which both QTL were detected

was examined for both the univariate and multivariate approaches in the current simulation study and the results are given in Table 7. To provide a succinct summary, all relevant effects were averaged across traits (as the underlying sizes were all 0.38). The bias is clearly seen at population size 100 for the univariate (WGAIM) analyses. The bias decreases with population size. Variability in estimated sizes decreases with population size as expected.

The multivariate estimated sizes display less bias than the univariate analyses with comparable standard deviations. Notice that in the multivariate analysis, sizes for zero effects of some traits were also estimated, and the mean of the estimated sizes was close to the true zero value in all cases.

In summary, the multivariate approach is more powerful in detecting QTL, has lower false positive rates and reduced bias for the resulting estimates of QTL size.

Late maturity α-amylase

The height and optical density data were firstly analyzed separately, and subsequently together in a bivariate analysis. In all analyses the non-doubled haploid lines (the parental lines, Spica and the negative control) were omitted from the analysis. These lines are not of direct interest and in particular showed extremes in optical density that would have biased the results.

Table 6 Proportion of the 200 simulations in which false QTL were detected for individual WGAIM analyses of each trait and for the multi-trait or joint analysis

Trait	Population size					
	100		200		400	
	Linked	Unlinked	Linked	Unlinked	Linked	Unlinked
1	0.860	0.185	0.500	0.090	0.090	0.005
2	0.670	0.235	0.440	0.060	0.075	0.020
3	0.490	0.195	0.330	0.050	0.040	0.020
4	0.235	0.090	0.130	0.080	0.010	0.055
Multi	0.800	0.330	0.295	0.030	0.060	0.015

Both linked (selected QTL are on chromosomes with QTL) and unlinked (selected QTL are on chromosomes without QTL) are presented. The number of chromosomes in each group varies across the results for individual traits

Table 7 Mean estimated size of QTL effects with empirical standard deviations across the 200 simulations for each population size

Method	Interval	Number of traits	Population size		
			100	200	400
WGAIM	C1.4	4	0.521 (0.144)	0.446 (0.125)	0.389 (0.076)
	C1.8	4	−0.483 (0.139)	−0.466 (0.129)	−0.394 (0.076)
	C2.4	4	0.575 (0.148)	0.463 (0.118)	0.394 (0.078)
	C2.8	4	0.568 (0.151)	0.468 (0.126)	0.398 (0.084)
	C3.6	3	0.481 (0.089)	0.387 (0.068)	0.385 (0.056)
	C4.4	2	0.523 (0.131)	0.404 (0.081)	0.375 (0.054)
	C5.1	1	0.469 (0.089)	0.387 (0.059)	0.400 (0.056)
MVWGAIM	C1.4	4	0.426 (0.169)	0.412 (0.105)	0.385 (0.064)
	C1.8	4	−0.388 (0.173)	−0.447 (0.131)	−0.389 (0.066)
	C2.4	4	0.460 (0.167)	0.379 (0.132)	0.375 (0.072)
	C2.8	4	0.426 (0.180)	0.279 (0.111)	0.379 (0.067)
	C3.6	3	0.444 (0.121)	0.365 (0.086)	0.381 (0.057)
	C3.6	1	0.038 (0.123)	−0.004 (0.090)	0.013 (0.055)
	C4.4	2	0.469 (0.165)	0.389 (0.080)	0.373 (0.054)
	C4.4	2	0.028 (0.145)	0.003 (0.089)	−0.001 (0.054)
	C5.1	1	0.461 (0.097)	0.392 (0.063)	0.397 (0.053)
	C5.1	3	0.018 (0.118)	−0.087 (0.079)	0.024 (0.053)

The statistics are found by pooling across traits as appropriate; the column labelled Number of traits indicates how many effects were pooled. The statistics are presented for univariate WGAIM and the multivariate MVWGAIM for each QTL. For MVWGAIM, both the QTL and non-QTL traits provide estimates listed separately for intervals C3.6, C4.4 and C5.1. The sizes of all QTL were 0.38 in magnitude, zero for some traits for intervals C3.6, C4.4 and C5.1

Height

For height, the baseline mixed model without QTL effects is given symbolically by

$$\mathsf{ht} = \mathsf{1} + \mathsf{id} + \mathsf{Room} + \mathsf{Room.Block} + \mathsf{Room.Block.Side} + \mathsf{error} \quad (16)$$

reflecting the nested structure of the glasshouse experiment. ht is the height variable. This is a simple variance components model in which all terms apart from 1 are random. The terms in the model have their obvious meaning. The 1 represents the constant or mean height. The design or blocking factors in the glasshouse were Room, Block which is nested in room and Side which is nested in block within a room; all factors have two levels. The nesting of effects introduces the terms like Room.Block. The polygenic effects are given by the factor id and had 194 levels. The error was assumed independent and identically distributed. Fitting the baseline mixed model resulted in the variance parameter estimates given in Table 8, labelled 'Before QTL'. Note that the basic normality assumptions were examined using residual plots and no apparent departures were found.

Table 8 Variance component estimates for height for model without and with QTL effects for the LMA glasshouse experiment

Model	id	Room	Room. Block	Room. Block.Side	Error
Before QTL	81.91	27.00	0.00	10.99	35.90
After QTL	29.37	27.03	0.00	10.49	36.23

Using (16) as the baseline model, QTL were found using WGAIM. The parameter estimates after fitting all selected QTL are given in Table 8, labelled 'After QTL'. The polygenic component was considerably reduced after QTL were fitted and it was found that 64 % of the original polygenic variance was explained by the selected QTL.

The selected QTL effects are presented in Table 9. Four significant QTL were found for height; the interval on linkage group 4D is close to the height gene $Rht-D1$, while the interval on 4BL is consistent with the $Rht-B1$ gene in wheat.

Optical density or LMA

For LMA, the baseline model was given by

$$\begin{aligned}\mathsf{tod} = 1 &+ \mathsf{id} + \mathsf{Room} + \mathsf{Room.Block} + \mathsf{Room.Block.Side} \\ &+ \mathsf{Pot} + \mathsf{Pot.Tiller} + \mathsf{Induction} + \mathsf{GoSlide} \\ &+ \mathsf{Slide} + \mathsf{Slide.SlideRow} + \mathsf{Slide.SlideCol} + \mathsf{error} \end{aligned} \quad (17)$$

where tod is the transformed optical density ($-1/od^3$) which was used because of the highly skewed nature of the optical density *od*. All but the constant term 1 are random effects. Determination of optical density is a multi-phase process. Thus in addition to the variation through room, block and side, there is possible variation between Pots and tillers within pots, Pot.Tiller, through Induction group, and finally slide variation through groups of slides (GoSlide), Slides and variation as specified by rows and columns within slide (Slide.SlideRow and Slide.SlideCol). The error was assumed independent and identically normally distributed. Again the latter assumption for tod was examined using graphical means based on residuals and found to be a reasonable assumption. The variance parameter estimates fitting the baseline model are given in Table 10, labelled 'Before QTL' as in the height analysis.

After selecting QTL, the variance parameter estimates for tod are given under 'After QTL' in Table 10. Again the non-genetic variance components are stable, while the polgenic variance component is greatly reduced. In fact, 63 % of the polygenic variance was explained by the selected QTL.

Ten putative QTL were found using (17) as the baseline model. These are given in Table 11. Interestingly, the height QTL on 4B (in an adjacent interval) and 4D are also found for tod, but there are many QTL specific to tod.

Joint analysis of height and LMA

Joint analysis of ht and tod might be beneficial because of the connection of LMA with the height genes (Mrva and Mares 1996). The joint analysis of ht and tod is conducted by combining the models (16) and (17). In the combined model, correlation between height and transformed optical density is included in the model for genetic effects (id) and pot effects (Pot). The model is quite difficult to present succinctly, and the fitting process requires that laboratory effects be specified only for tod and not for ht; see the code given in the supplemetary material and note that the analysis is only possible because of the power of the package asreml. Note also that the residual error for ht is at the pot level. Examination of residuals indicated the assumption of bivariate normality of the residuals was reasonable.

The estimates of the variance parameters are given in Table 12 before and after QTL analysis. The polygenic effects for ht and tod decrease considerably after QTL analysis. The QTL selected using the multivariate approach explained 70 and 66 % of the polygenic variance for both ht and tod respectively. Note also that there is a strong correlation (negative because of the transformation of tod) between ht and tod before QTL analysis that decreases considerably after QTL effects are included. Remaining variance components are quite similar before and after QTL are included.

Nineteen QTL were selected in the joint QTL analysis and are presented in Table 13. To provide some insight, Fig. 1 is a graph of the chromosome or linkage group outlier statistics,

Table 9 Height QTL for the LMA glasshouse experiment

Linkage group	Left		Right			Wald	
	Marker	Dist (cM)	Marker	Dist (cM)	Size	Statistic	*P* value
3DL	gwm3	105.0	wPt-3863	118.4	−1.888	−3.44	0.0004
4BL	wPt-3908	90.7	wPt-6149	94.1	−3.803	−7.18	<0.0001
4D	wPt-0472	36.5	wPt-0710	38.7	6.761	12.59	<0.0001
6D	wPt-4830	0.0	barc146	3.4	1.435	2.66	0.0078

Table 10 Estimated variance components for transformed optical density for the models without and with QTL effects for the LMA glasshouse experiment

Term	Estimates ×100	
	Before QTL	After QTL
id	3.54	1.33
Room	0.00	0.00
Room.Block	0.00	0.00
Room.Block.Side	0.07	0.05
Induction	0.17	0.08
Pot	0.63	0.62
Pot.Tiller	1.93	1.94
GoSlide	5.42	5.42
Slide	6.88	6.86
Slide.SlideRow	2.31	2.32
Slide.SlideCol	1.04	1.04
error	11.42	11.42

t_k^2, plotted against the linkage group for selection of the second QTL (the first selection resulted in an outlier statistic of 26.82 for linkage group 4D, and this would mask patterns in any graph). The outlier statistics show several peaks with the largest being for 4BL. Note that several of the other peaks are consistent with QTL subsequently selected, but others are not. Forward selection of QTL had an impact on subsequent outlier statistics. Figure 2 is a graph of the outlier statistics, t_{kl}^2, for intervals on linkage group 4BL and these statistics show a maximum for interval 10. This interval was selected as the location of a putative QTL.

Returning to Table 13, in total, three QTL (on linkage groups 3B, 4BL and 4D) were common to both height and transformed optical density, 5 QTL were specific to height (on linkage groups 3DL, 5B, 5DS, 6D and 7A) and 7 QTL were specific to transformed optical density (on linkage groups 1A, 2A, 3A, 3AL, 3DL and an unlinked marker gwm301). Three were not significant when examined as fixed effects, even though they were selected, probably a consequence of the forward selection approach. These QTL are not significant given the other selected QTL, and from a practical point of view would not be very useful.

The QTL selected using separate analyses for height and transformed optical density and those selected using a joint analysis are presented in Table 14. Tick marks indicate selected QTL. There is some consistency between the two sets of analyses, with 10 of the 18 QTL being selected in both the univariate and bivariate analyses. The selected intervals are not always the same; often adjacent intervals are selected in the individual analyses while the joint analysis provides a selection that takes into account both traits simultaneously.

The code for the analysis is available as online supplementary material. Note however that the analyses involving transformed optical density are very time-consuming and require large amounts of memory. Fitting the baseline model took 5 minutes on a laptop with 3 Gb of memory. The complexity of the model seems to be the cause. When MVWGAIM was invoked, the full analysis took 8 h using the dimension reduction approach. This is in part because of complexity, but also because for each QTL selection, three models need to be fitted, two for testing if selection should proceed, and the third to facilitate the selection.

Dough rheology

Individual site QTL analyses

Individual site analyses are conducted before the joint multi-environment analysis that allows QTL × environment

Table 11 LMA QTL for the LMA glasshouse experiment for transformed optical density (tod)

Linkage group	Left		Right			Wald	
	Marker	Dist (cM)	Marker	Dist (cM)	Size	Statistic	*P* value
1AS	wPt-4384	14.54	wPt-3333	20.93	2.92	2.42	0.0155
3A	wPt-7217	4.41	wPt-2919	5.1	−3.76	−3.09	0.0020
3AL	rPt-9057	30.49	wPt-4077	44.61	5.12	4.01	0.0001
3B	barc147	124.28	rPt-7228	131.12	−4.95	−4.00	0.0001
3DL	wPt-0732	2.28	wPt-6262	20.5	4.83	3.62	0.0003
4BL	barc020	99.89	gwm113	106.02	−8.84	−7.18	<0.0001
4D	wPt-0472	36.53	wPt-0710	38.67	11.04	8.8	<0.0001
5BL	wPt-4791	54.98	barc232	56.88	2.92	2.41	0.0160
5DS	barc143	69.09	wPt-6225	70.63	−4.21	−3.45	0.0006
Unlinked	gwm301	0.00	–	–	−6.82	−2.8	0.0051

Table 12 Estimated variance components for height and transformed optical density for the bivariate models without and with QTL effects for the LMA glasshouse experiment

Factor	Trait	Estimates (×100 for tod)	
		Before QTL	After QTL
id	cor(ht,tod)	−0.54	−0.18
	ht	81.44	26.72
	tod	3.54	1.17
Room	ht	26.92	26.84
	tod	0.00	0.00
Room:Block	ht	0.00	0.00
	tod	0.00	0.00
Room.Block.Side	ht	10.79	10.83
	tod	0.08	0.08
Induction	tod	0.18	0.12
Pot	cor(ht,tod)	−0.12	−0.11
	ht	35.99	36.26
	tod	0.64	0.61
Pot.Tiller	tod	1.92	1.93
GoSlide	tod	5.42	5.42
Slide	tod	6.86	6.86
Slide.SlideRow	tod	2.32	2.33
Slide.SlideCol	tod	1.04	1.04
error	tod	11.42	11.42
	ht	0.00	0.00

Where the trait is listed, an estimated variance is presented. The term cor(ht,od) is the correlation between height and transformed optical density for the particular factor

interactions to be assessed. For the single site analyses the baseline model was of the form

$$\begin{aligned}\mathsf{ext} = {} & \mathsf{Type} + \mathsf{id} + \mathsf{Rep} + \mathsf{Rep.Block} + \mathsf{Column.Row} \\ & + \mathsf{MeasDay} + \mathsf{Labno} + \mathsf{error}\end{aligned}$$

where ext the maximum extensibility, Type is a factor with level DH for doubled haploid lines with other lines having their own level (this ensures that random genetic effects relate to the DH lines which are of interest), Rep and Block are design factors reflecting the blocking structure at the field level, Column.Row is a field plot effect, and MeasDay and Labno are factors for the measurement day and laboratory samples; in the latter case there were duplicate measures of extensibility for some samples. The factor id is the genetic effect due to doubled haploids. The error was adequately modelled using a constant variance. Informal diagnostics based on residuals suggest the assumptions of the model are not contradicted.

Having fitted a baseline model for each of the four sites, QTL analysis was conducted using wgaim in the R environment. Details are omitted, but the percentage of polygenic variance explained by the QTL found for each site was around 45 %.

Multi-environment QTL analysis

The model for the multi-environment analysis of extensibility was given by

$$\begin{aligned}\mathsf{ext} = {} & \mathsf{Expt} * \mathsf{Type} + \mathsf{fa}(\mathsf{Expt}, 1).\mathsf{id} + \mathsf{diag}(\mathsf{Expt}).\mathsf{Rep} \\ & + \mathsf{diag}(\mathsf{Expt}).\mathsf{Rep.Block} \\ & + \mathsf{diag}(\mathsf{Expt}).\mathsf{Column.Row} \\ & + \mathsf{diag}(\mathsf{Labsection}).\mathsf{MeasDay} \\ & + \mathsf{diag}(\mathsf{Labsection}).\mathsf{Labno} \\ & + \mathsf{diag}(\mathsf{Labsection}) + \mathsf{error}\end{aligned}$$

where the additional components in the joint analysis involve the site or environment factor Expt. The model is very similar to the single site form, with interactions or crossing with Expt. The genetic doubled haploid lines are correlated across environments using a first order Factor analytic model (fa(Expt, 1).id) as discussed by Smith et al. (2001). Other effects allow for separate (using a diagonal variance structure) variance components for field and laboratory effects in each environment; the dough was tested for extensibility in three laboratory sections which constitute the levels of the Labsection factor. This baseline model and a model with QTL by environment effects were fitted and the variance parameters were very similar for non-genetic effects before and after QTL analysis (not displayed). The estimated polygenic variances are given in Table 15 and show that The percentage of variance explained by the QTL by environment effects ranged from 42 to 58 %.

The selected QTL are given in Table 16. For this QTL by environment analysis it is possible to test for a common effect across environments for each QTL. These Wald tests are presented in Table 17 where the interval effects are represented symbolically; for example, `X.1A.4` is interval 4 on chromosome 1A, as listed in Table 16. For four QTL, the QTL by environment interactions were not significant and hence these QTL each provide a common contribution at those sites; these QTL are labelled by all under the Expt column in Table 16. The other QTL selected had significant QTL by environment effects, and hence had varying levels of expression across sites, from 2 to 4 sites showing significant association. This highlights the QTL by environment interaction for extensibility.

The consistency of QTL between individual and multi-site analyses is presented in Table 18. Five QTL are consistent across both the univariate and multivariate analyses, whereas seven are not. Of these seven QTL, one (on 3A) was a common QTL across all environments only found in the multi-environment QTL analysis, while another (on 4D) was found to be significant at two sites in the multivariate analysis. The remaining four inconsistent QTL were found using univariate analyses, and we saw in the

Table 13 Multivariate (height, ht, and transformed LMA, tod) QTL results for the LMA glasshouse experiment

Trait	Linkage group	Left		Right			Wald	
		Marker	Dist (cM)	Marker	Dist (cM)	Size	Statistic	*P* value
ht	1A	wPt-2384	0	wPt-5941	3.82	−0.05	−0.10	0.9203
tod						3.96	3.27	0.0011
ht	2A	wPt-1657	101.49	wPt-9320	108.70	1.01	1.92	0.0549
tod						−3.62	−3.00	0.0027
ht	3A	wPt-7217	4.41	wPt-2919	5.1	−0.59	−1.13	0.2585
tod						−4.12	−3.44	0.0006
ht	3AL	rPt-9057	30.49	wPt-4077	44.61	−0.58	−1.05	0.2937
tod						5.15	4.08	<0.0001
ht	3B	barc147	124.28	rPt-7228	131.12	−1.15	−2.10	0.0357
tod						−5.11	−4.08	<0.0001
ht	3DL	gwm341	0	wPt-0732	2.28	−0.26	−0.52	0.6031
tod						4.02	3.40	0.0007
ht	3DL	gwm3	105	wPt-3863	118.39	−2.08	−3.85	0.0001
tod						−0.64	−0.52	0.6031
ht	4BL	wPt-6149	94.14	barc020	99.89	−3.90	−7.50	<0.0001
tod						−8.58	−7.13	<0.0001
ht	4D	wPt-0472	36.53	wPt-0710	38.67	6.45	11.02	<0.0001
tod						11.51	8.47	<0.0001
ht	5B	wPt-6531	88.34	wPt-9300	119.19	−1.67	−2.85	0.0044
tod						−1.91	−1.41	0.1585
ht	5BL	wPt-4791	54.98	barc232	56.88	0.10	0.18	0.8572
tod						3.99	3.23	0.0012
ht	5DS	gwm190	31.5	barc143	69.09	3.65	2.35	0.0188
tod						−1.06	−0.30	0.7642
ht	5DS	barc143	69.09	wPt-6225	70.63	−2.21	−1.84	0.0658
tod						−3.78	−1.39	0.1645
ht	6D	wPt-4830	0	barc146	3.37	1.41	2.69	0.0071
tod						1.82	1.52	0.1285
ht	7A	wPt-0433	101.21	wPt-0556	120.96	−2.47	−4.32	<0.0001
tod						−2.15	−1.64	0.1010
ht	Unlinked1	wPt-0877	0			0.19	0.16	0.8729
tod		wPt-0877	0			−4.51	−1.64	0.1010
ht	Unlinked4	stm5tcacI	0			1.98	1.95	0.0512
tod		stm5tcacI	0			1.87	0.80	0.4237
ht	Unlinked5	gwm301	0			−1.09	−1.05	0.2937
tod		gwm301	0			−6.23	−2.61	0.0091

simulation study that the power to detect such QTL is lower for the multivariate analysis.

Finally, the code for the analyses of the multi-environment data is available online as supplementary material.

Discussion

There are many papers that utilize multivariate methods for QTL analysis. Multivariate methods can be more powerful than their univariate counterparts. Multivariate methods offer increased power for detection of QTL through correlation of the variates, provided the underlying assumptions hold. The main assumption is multivariate normality of the response vector and of components of the mixed model. If the assumption holds, multivariate methods allow pleiotropic and common QTL to be determined directly (for example in QTL by environment or QTL by treatment settings) rather than indirectly using univariate analyses.

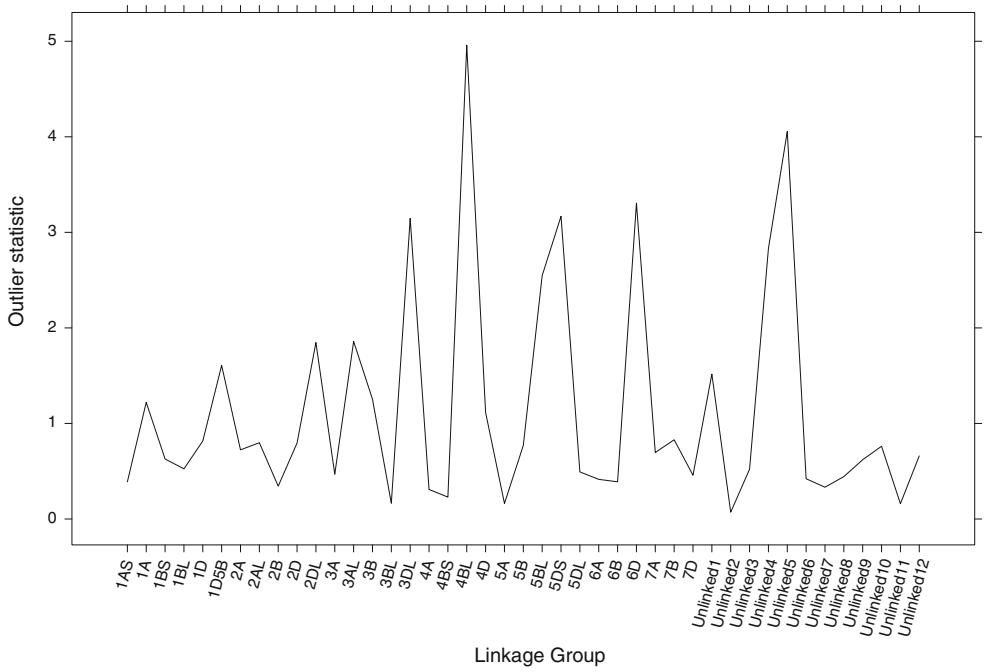

Fig. 1 Outlier statistics t_k^2 for linkage groups for selection of the second QTL in the joint QTL analysis of height and transformed optical density

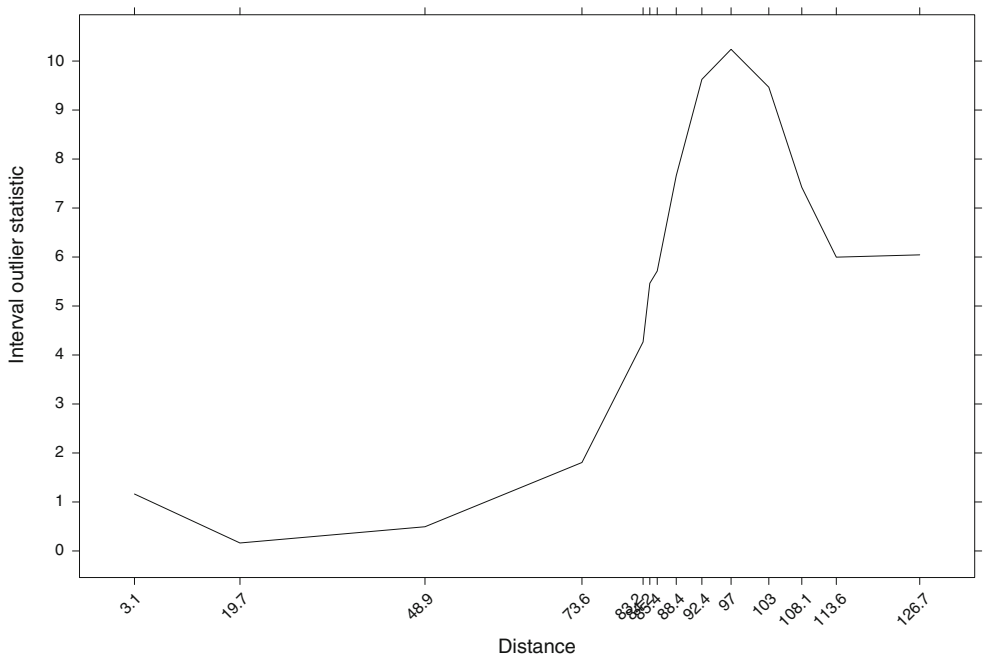

Fig. 2 Outlier statistics t_{kl}^2 for intervals (mid-points of the intervals in terms of genetic distance were used) on linkage group 4BL for selection of the second QTL in the joint QTL analysis of height and transformed optical density

We briefly consider some key papers and highlight the differences with the approach presented in this paper.

In a seminal paper on multivariate QTL analysis, Jiang and Zeng (1995) consider composite interval mapping (CIM) and examine several tests of hypotheses, for example QTL by environment interaction and pleitorpy versus close linkage. The use of CIM involves many analyses, iterative selection of putative QTL and determination of thresholds usually via permutation; these authors suggest an approach based on a Bonferonni as an alternative but as they indicate this is likely to be very crude. The use of permutation-based theresholds often necessitates a

Table 14 Summary of the selected QTL intervals for the models for each of height and transformed optical density and the joint analysis

QTL		Univariate		Joint	
Linkage group	Left marker	ht	tod	ht	tod
1AS	wPt-4384		✓		
1A	wPt-2384				✓
2A	wPt-1657				✓
3A	wPt-7217		✓		✓
3AL	rPt-9057		✓		✓
3B	barc147		✓	✓	✓
3DL	gwm341		✓[a]		✓
3DL	gwm3	✓		✓	
4BL	wPt-6149	✓[a]	✓[a]	✓	✓
4D	wPt-0472	✓	✓	✓	✓
5B	wPt-6531			✓	
5BL	wPt-4791		✓		✓
5DS	gwm190			✓	
5DS	barc143		✓	S	S
6D	wPt-4830	✓		✓	
7A	wPt-0433			✓	
Unlinked1	wPt-0877			S	S
Unlinked4	stm5cacI			S	S
Unlinked5	gwm301		✓		✓

The left hand marker for the interval selected in the joint analysis is given as an indicator. One interval was selected in a univariate analysis but was not selected in the joint analysis

[a] Interval selected in the univariate did not match the selected interval from the joint analysis but was on the same linkage group. Three QTL were selected (S) in the joint analysis but none of the effects was significant using a Wald test

two-stage analysis, with genetic line effects being estimated at the first stage, and QTL determined at the second stage using estimated genetic line effects as data. Importantly, Jiang and Zeng (1995) discuss the power that can be gained using multivariate methods, in particular when there are pleiotropic effects across traits or environments. This makes multivariate methods attractive.

Multiple interval mapping (MIM) for multivariate situations was considered by Korol et al. (1998) and Zeng et al. (1999). This is a stepwise procedure and there are difficult issues regarding the stopping rule as discussed in the latter paper. Again, it is likely two-stage methods will be used. In contrast, Boer et al. (2007) firstly develop a mixed model for the phenotype and then employ CIM for the QTL analysis. Thus while non-genetic effects are accommodated in a single stage analysis, many models for QTL effects need to be fitted using their approach.

Whole genome average interval mapping in which all markers are used simultaneously was shown to be superior to composite interval mapping in Verbyla et al. (2007). Increased power of detection of QTL incomparison to CIM was clear in the simulation study presented in that paper. The approach outlined in this paper is a natural extension of whole genome average interval mapping to the multivariate situation. As for the univariate approach, all markers are included in the analysis simultaneously. There is no need for genomic scans that are part of CIM and MIM and the number of models that need to be fitted is greatly reduced. Secondly, a simple stopping rule based on a likelihood ratio test is available and permutation-based methods are not required. An outlier detection technique is used to select the putative QTL and is based on simple t-statistics of the predicted QTL sizes for every interval. This stage does not involve testing hypotheses, it involves ranking the evidence of putative QTL for each interval. The method does involve forward selection to ensure simplicity but it was shown in the simulation study to work remarkably well. In the multi-environment situation, common QTL can be determined across the multivariate specification at the final stage of analysis. The approach has been implemented in `R` and code is available in the online supplementary material. The advantage of using `R` is the ability to manipulate outcomes of the analysis both numerically and graphically.

Some key results come from the simulation studies. A simulation study without QTL showed the null distribution of the likelihood ratio statistic that precedes selection of QTL is well approximated by a mixture of Chi-squared distributions. A further simulation study showed that using a multivariate approach results in an increase in power of detection of QTL that are pleiotropic or common to several environments or treatments, but that QTL on single traits or environments can sometimes be more difficult to detect using the multivariate approach. The false positive rate for population size 100 was sufficient to suggest that both univariate and multivariate methods could be recommended for population sizes above 200. At this point, the multivariate approach is likely to detect almost all QTL,

with a low rate of false positives. Bias in the estimated QTL effects using the multivariate approach is also low.

Table 15 Estimated polygenic variances for models without and with QTL effects and the percentage of genetic variance explained by the QTL for each of the 4 experiments on the Kukri × Janz doubled haploid population

Polygenic variance	2001		2002	
	Biloela	Lundavra	Biloela	Lundavra
Without QTL	3.33	3.12	2.37	3.23
With QTL	1.40	1.59	1.15	1.87
% Explained	58	49	51	42

The estimates are based on the multi-environment or joint analysis

A key aspect of the approach is the ability to include and hence allow for non-genetic sources of variation in the analysis. It is conjectured that this will reduce the likelihood of false positives due to omission of such effects. The examples illustrate the incorporation of such effects. As indicated above, a more common approach is to use a two-stage analysis. At the first stage estimated genetic line effects are obtained and these effects are then used at the second stage to find putative QTL. In general these methods use simple means of raw genetic line data, thereby ignoring experimental design and other non-genetic effects, or use best linear unbiased predictions (BLUPs) of the genetic line effects. In both cases the resulting effects are estimates that can be correlated and also that have an standard error associated with them. This standard error is

Table 16 Multi-environment QTL for the extensibility: Four common QTL across environments and 4 QTL by environment interactions

Expt	Chromosome	Interval	Left		Right			Wald	
			Marker	Dist (cM)	Marker	Dist (cM)	Size	Statistic	*P* value
01.B	1A	4	cfd021a	8.38	NW2343	11.42	−0.591	−4.73	<0.0001
01.L							−0.235	−1.92	0.0549
02.B							−0.248	−2.30	0.0214
02.L							−0.223	−1.50	0.1336
01.B	1B	14	Bx7	73.91	NW2242	78.31	0.997	7.95	<0.0001
01.L							0.297	2.46	0.0139
02.B							0.433	4.05	0.0001
02.L							0.545	3.73	0.0002
01.B	1D	6	GluD1	83.85	cfd048	85.92	−0.378	−3.07	0.0021
01.L							−1.066	−8.79	<0.0001
02.B							−0.728	−6.83	<0.0001
02.L							−0.976	−6.67	<0.0001
all	3A	2	NW0976	14.18	NW2237	63.18	0.41	3.74	0.0002
01.B	4D	1	Rht2	0	cfd071	21.15	−0.065	−0.47	0.6384
01.L							−0.443	−3.33	0.0009
02.B							−0.549	−4.69	<0.0001
02.L							−0.172	−1.07	0.2846
all	4D	7	NW2208	132.12	gwm609	142.6	−0.266	−3.01	0.0026
all	5D	1	cfd018	0	NW2160	3.55	−0.384	−4.50	<0.0001
all	7A	9	NW2062	232.49	FC1	250.9	0.413	4.29	<0.0001

The label all under Expt signifies a common QTL effect across all environments

Table 17 Wald statistics for environment by QTL interactions

Term	*df*	den *df*	*F*	*P* value
Expt:X.1A.4	3	177.1	3.52	0.0162
Expt:X.1B.14	3	176.3	7.03	0.0002
Expt:X.1D.6	3	174.7	6.80	0.0002
Expt:X.3A.3	3	174.8	0.26	0.8541
Expt:X.4D.1	3	177.1	4.90	0.0027
Expt:X.4D.7	3	174.0	0.57	0.6377
Expt:X.5D.1	3	175.5	1.44	0.2327
Expt:X.7A.9	3	173.7	2.62	0.0522

Conditional *F* statistics were calculated (labelled *F*) with denominator degrees of freedom (den *df*) estimated using Kenward and Roger (1997). The *P* values suggest four QTL do not interact with the environment

Table 18 Summary of the selected QTL intervals for the models for each site both from individual site analyses and the multi-environment analysis

QTL		Single site analysis				Multi-site analysis			
Chromosome	Left marker	01B	01L	02B	02L	01B	01L	02B	02L
1A	cfd021a	✓				✓			
1B	Bx7	✓		✓[a]	✓[a]	✓	✓	✓	✓
1D	GluD1	✓[a]	✓[a]	✓	✓	✓	✓	✓	✓
3A	NW0976					✓	✓	✓	✓
4A	NW2277				✓				
4B	Rht1	✓							
4D	Rht2						✓	✓	
4D	NW2208	✓	✓[a]	✓[a]		✓	✓	✓	✓
5D	cfd018	✓	✓			✓	✓	✓	✓
5D	barc110	✓							
7A	NW2062		✓[a]			✓	✓	✓	✓
7B	barc065				✓				

The left hand marker for the interval selected in the joint analysis is given as an indicator. Four intervals were selected in univariate analyses but were not selected in the joint analysis

[a] Interval selected did not match the selected interval from the joint analysis

typically ignored in the second stage of analysis and leads to inefficient selection of putative QTL. In addition, if BLUPs are used, the estimated genetic line effects are shrunken versions of the fixed effects equivalents and hence biased downwards. This potentially reduces the chance of QTL detection. It is best to avoid two-stage analyses if possible. Thus the methods discussed in this paper provide a comprehensive approach to multivariate QTL analysis.

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Appendix: The score statistic under the alternative outlier model

The full (marginal) model for $\mathbf{y}$ under the AOM (9) can be written as

$$\mathbf{y} \sim N(\mathbf{X}\tau,\ \mathbf{H}) \tag{18}$$

where if $\mathbf{M}_{E,k} = \mathbf{M}_E\mathbf{D}_k$, the selected columns of $\mathbf{M}_E$ corresponding to the kth chromosome, the variance matrix $\mathbf{H}$ is given by

$$\begin{aligned}\mathbf{H} = \mathbf{R} + \mathbf{Z}_0\mathbf{G}_0\mathbf{Z}_0^T + \mathbf{Z}\left\{(\mathbf{L}_a\mathbf{L}_a^T) \otimes \mathbf{M}_E\mathbf{M}_E^T\right\}\mathbf{Z}^T \\ + \mathbf{Z}\left\{(\sigma^2_{ak}\mathbf{L}_a\mathbf{L}_a^T) \otimes \mathbf{M}_{E,k}\mathbf{M}_{E,k}^T\right\}\mathbf{Z}^T\end{aligned}$$

As in Verbyla et al. (2007), an outlier statistic is developed using the score for σ^2_{ak} under the null hypothesis $H_0:\ \sigma^2_{ak} = 0$. If $\mathbf{P} = \mathbf{H}^{-1} - \mathbf{H}^{-1}\mathbf{X}(\mathbf{X}^T\mathbf{H}^{-1}\mathbf{X})^{-1}\mathbf{X}^T\mathbf{H}^{-1}$, the REML score for σ^2_{ak} evaluated at zero is

$$\begin{aligned}U_k(0) &= -\frac{1}{2}\left\{\mathrm{tr}\left(\mathbf{PZ}(\mathbf{L}_a\mathbf{L}_a^T \otimes \mathbf{M}_{E,k}\mathbf{M}_{E,k}^T)\mathbf{Z}^T\right)\right. \\ &\quad \left. -\mathbf{y}^T\mathbf{PZ}(\mathbf{L}_a\mathbf{L}_a^T \otimes \mathbf{M}_{E,k}\mathbf{M}_{E,k}^T)\mathbf{Z}^T\mathbf{Py}\right\} \\ &= -\frac{1}{2}\left\{\mathrm{tr}\left(\mathbf{PZ}(\mathbf{G}_a \otimes \mathbf{M}_{E,k}\mathbf{M}_{E,k}^T)\mathbf{Z}^T\right)\right. \\ &\quad \left. -\mathbf{y}^T\mathbf{PZ}(\mathbf{G}_a \otimes \mathbf{M}_{E,k}\mathbf{M}_{E,k}^T)\mathbf{Z}^T\mathbf{Py}\right\}\end{aligned} \tag{19}$$

Let $\mathbf{a}_k$ be the vector of sizes for all variates for all intervals on chromosome k, with individual intervals having sizes given by $\mathbf{a}_{kl}$. Then the BLUP for $\mathbf{a}_k$ is

$$\tilde{\mathbf{a}}_k = (\mathbf{G}_a \otimes \mathbf{M}_{E,k})^T\mathbf{Z}^T\mathbf{Py} \tag{20}$$

with variance

$$\mathrm{var}(\tilde{\mathbf{a}}_k) = (\mathbf{G}_a \otimes \mathbf{M}_{E,k})^T\mathbf{Z}^T\mathbf{PZ}(\mathbf{G}_a \otimes \mathbf{M}_{E,k}) \tag{21}$$

It may be that $\mathbf{G}_a$ is non-negative definite, rather than positive definite. Thus $\mathbf{G}_a$ may be singular. If $\mathbf{G}_a^-$ is a generalized inverse of $\mathbf{G}_a$, $\mathbf{G}_a$ can be replaced in (19) by $\mathbf{G}_a\mathbf{G}_a^-\mathbf{G}_a$, and hence using (20), (21) and properties of the trace, (19) can be written as

$$\begin{aligned}U_k(0) &= -\frac{1}{2}\left[\mathrm{tr}\left((\mathbf{G}_a^- \otimes \mathbf{I}_{r_k-1})\mathrm{var}(\tilde{\mathbf{a}}_k)\right) - \tilde{\mathbf{a}}_k^T(\mathbf{G}_a^- \otimes \mathbf{I}_{r_k-1})\tilde{\mathbf{a}}_k\right] \\ &= -\frac{1}{2}\sum_{l=1}^{r_k-1}\left[\mathrm{tr}\left(\mathbf{G}_a^-\mathrm{var}(\tilde{\mathbf{a}}_{kl})\right) - \tilde{\mathbf{a}}_{kl}^T\mathbf{G}_a^-\tilde{\mathbf{a}}_{kl}\right] \\ &= \frac{1}{2}\left(\sum_{l=1}^{r_k-1}\mathrm{tr}\left(\mathbf{G}_a^-\mathrm{var}(\tilde{\mathbf{a}}_{kl})\right)\right)(t_k^2 - 1)\end{aligned}$$

where t_k^2 is given by (10). Thus t_k^2 indicates the departure from $U_k(0) = 0$. This statistic therefore provides evidence that σ^2_{ak} departs from zero for chromosome k. The

chromosome most likely to contain a QTL is the one with largest t_k^2.

The statistic t_k^2 is made up of components that relate to the intervals on chromosome k. Hence using a similar argument, the outlier statistics for individual intervals is given by t_{kl}^2 in (11).

A fully parameterized $\mathbf{G}_a$ requires many parameters for larger multivariate problems and an approximation becomes both sensible and necessary. It is possible to use a factor analytic approximation for the full covariance model $\mathbf{G}_a$ which mirrors the use of FA models in the analysis of multi-environment trials.

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