Detecting major genetic loci controlling phenotypic variability in experimental crosses

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March 15, 2011

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Key Words: canalization, quantitative trait loci, epistasis, Collaborative Cross

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Abstract

Traditional methods for detecting genes that affect complex diseases in humans or animal models, milk production in livestock or other traits of interest, have asked whether variation in genotype produces a change in that trait’s average value. But focusing on differences in the mean ignores differences in variability about that mean. The robustness, or uniformity, of an individual’s character is not only of great practical importance in medical genetics and food production but also of scientific and evolutionary interest (e.g., blood pressure in animal models of heart disease, litter size in pigs, flowering time in plants). We describe a method to detect major genes controlling the phenotypic variance, referring to these as vQTL. Our method uses a double generalized linear model with linear predictors based on probabilities of line origin. We evaluate our method on simulated F2 and Collaborative Cross data, and on a real F2 intercross, demonstrating its accuracy and robustness to the presence of ordinary mean-controlling QTL. We also illustrate the connection between vQTL and QTLs involved in epistasis, explaining how these concepts overlap. Our method can be applied to a wide range of commonly used experimental crosses and may be extended to genetic association more generally.
INTRODUCTION
Quantitative trait locus (QTL) analysis has traditionally focused on detection of major genes controlling the expected mean of a phenotype. But there is substantial evidence that not only the mean but also the variance, that is, the stochastic variability of the phenotype about its average value, may itself be under genetic control. The identification of such variance-controlling loci, which we call vQTL, can be helpful in a variety of contexts, including selection of livestock for uniformity, evaluating predictability of response to medical treatment, identification of key biomolecular stabilizers, and assessment of population resilience in ecology and evolution.

One way of interpreting an increase in variability is as a decrease in stability. Waddington (1942) described the concept of canalization, whereby natural selection favors the relative constancy of some attributes, for example well-formed organs and limbs, and thereby leads to the evolution of heritable architectures that buffer the impact of environmental or background genetic variation that would otherwise cause development to go astray. These architectures create virtual “canals” down which developmental programs flow. For a canalized phenotype, which modern usage expands to include non-developmental traits, the “zone of canalization” is the range of underlying liability over which potentially disruptive variation may be absorbed without serious consequence to the expressed trait value (Lynch and Walsh 1998). A well-studied example of a stabilizing architecture is that provided by heat shock protein 90 (Hsp90), which buffers genetic and stochastic variation in the development of plants and flies (Rutherford and Lindquist 1998; Queitsch et al. 2002; Sangster et al. 2008).

But in absorbing variation, such stabilizing architectures also hide it from view, and a sensitizing change in the stabilizer that shifts liability outside the zone of canalization can have a dramatic effect on the phenotype. Such shifts release the combined effects of previously “cryptic” genetic variation: now decanalized, the phenotype is more sensitive to internal (including genetic) and external environment, and as a result varies more greatly
between individuals (Dworkin 2005; Hornstein and Shomron 2006). In this vein, de-
canalization has been proposed to explain why the genetic architectures of some diseases in
human populations seem more amenable than others to genetic dissection through genome
wide association (Gibson and Goldstein 2007). Specifically, whereas some disease pheno-
types in homogeneous populations may be heavily canalized and thereby harder to dissect,
others may have been decanalized by modern living conditions (e.g., inflammatory diseases)
or modern admixture, while yet others are simply too recent in evolutionary history for
buffering networks to have evolved (e.g., response to HIV).

Increased variability can also be adaptive. In natural populations disruptive selection
favors diversity, with increased “capacitance” (Rice 2008) or “bet-hedging” (Beaumont
recently proposed a heritable and selectable mechanism for this based on stochastic epige-
etic variation. In controlled populations, variability can be increased through directional
selection. For example, in a Drosophila selection experiment Clayton and Robertson
(1957) reported increased bristle number variance, which is consistent with the idea that
genotypes associated with higher environmental variance have a greater chance of being
selected under directional selection (Hill and Zhang 2004). Moreover, genetic differences
have been observed for phenotypic variability in body weight for chickens (Rowe et al. 2006)
and snails (Ros et al. 2004), and litter size in rabbits (Ibanez-Escriiche et al. 2008), sheep
(SanCristobal-Gaudy et al. 1998) and pigs (Sorensen and Waagepetersen 2003).

In natural populations with stabilizing selection we should expect to find alleles mini-
mizing variance for fitness traits (Lande 1980; Houle 1992), whereas directional selection
during domestication will favour alleles that increase variance. One may therefore expect
to find vQTLs in experimental crosses between wild and domestic animals (see Andersson
2001). Nonetheless, genetic buffering that leads to phenotypic robustness need not require
an evolutionary explanation to be observed, nor to be useful in medicine and agriculture.
Plainly, detecting vQTLs and inferring how they arose are separate questions, here we con-
centrate on the first.

Table 1 lists some sources of phenotypic variability in relation to the genetic groups studied. Unless otherwise qualified, we use “phenotypic variance” to describe the observed marginal variance of the phenotype in the population, and distinguish “phenotypic variability” as the residual variance after controlling for main effects of QTL and other anticipated or manipulated environmental covariates. Phenotypic variability is thus the byproduct of unmodeled interactions. Identifying major factors that influence variability requires defining groups between which variances would be contrasted (rows of Table 1). Our goal is to identify loci associated with differences in variance between such groups. For generality we concentrate on groups defined by the first row in Table 1, but note that the groupings defined in the remaining rows allow increasingly specific characterizations of vQTL effect. For instance, experimental crosses having multiple individuals within inbred lines will produce genetically identical individuals and the differences in phenotypic variability within each line are due to both environmental sensitivity and temporal fluctuation, but not epistasis.

[Table 1 about here.]

Few studies have explicitly looked for vQTL. Among the more recent, Ordas et al. (2008) studied morphological traits and flowering time in maize. They detected vQTL by contrasting the residual variance between genotypes in replicates of recombinant inbred lines (RILs; see 2nd row, Table 1). The effects were substantial, with alleles associated with a 30 – 40% increase over the average residual variance. Wittenburg et al. (2009) examined the sample variance of birth weight within pig litters as a gamma distributed trait among 3914 sows, estimating a heritability of 0.1 for this trait using a generalized linear mixed model. Sangster et al. (2008) used Levene’s test for detection of variance-controlling genes. In that test, the absolute values of the residuals are used as a response in an ANOVA (eg, Faraway 2004). Mackay and Lyman (2005) studied Drosophila bristle number and found substantial differences in the coefficient of variation (CV) between inbred lines, comparing
CV also using ANOVA. The methods used in these last two studies have the limitation of not being able to model confounding effects in the mean. Using residuals (as in Wittenburg et al. 2009; Sangster et al. 2008) can potentially incorporate covariates but involves conditioning on unknowns. There is thus considerable utility in a method that simultaneously estimates means and variances, flexibly accommodates covariates, applies to a wide range of experimental crosses, and is robust and fast enough for genomewide analyses.

Regression based models (Haley and Knott 1992; Martinez and Curnow 1992) have proven to be fast and powerful at detecting QTL controlling the mean of a complex trait in experimental crosses, and flexible since they are straightforwardly extended to include epistatic effects and interactions (Carlberg and Haley 2004). Mott et al. (2000) developed the haplotype reconstruction method HAPPY and its associated regression model, which allows for a variable number of strains and may therefore be applied to vQTL mapping in, eg, heterogeneous stocks (HS; Valdar et al. 2006) and multiparent advanced generation inbred cross resource populations (MAGIC lines; Cavanagh et al. 2008) such as the Collaborative Cross (CC; Churchill et al. 2004; Broman 2005; Valdar et al. 2006; Chesler et al. 2008) and the Arabidopsis recombinant inbred lines of Kover et al. (2009).

Our aim is to develop a regression model for detection of major genes controlling phenotypic variance that can be applied genomewide. The estimation uses double generalized linear models (DGLMs; Smyth 1989) and its parameterization is based on the HAPPY formulation of inferred haplotypes. The method fits ordinary QTL and vQTL simultaneously in the same model. We apply it to simulated data from an F$_2$ and the CC, and real data from an F$_2$ intercross of partially inbred lines.

MODELS AND METHODS

The standard regression model for interval mapping of a QTL uses the probability of line origin at a locus to describe its genetic state (Haley et al. 1994). In the simple case of mapping a single QTL in individuals arising from an F$_2$ intercross of inbred founder lines A
and B, the model is:

\[ y_i = \mu + x_i^T \beta + q_i^T \alpha + \varepsilon_i, \quad \varepsilon_i \sim N(0, \sigma^2), \]

where \( y_i \) is the phenotype of individual \( i \), \( x_i \) is the \( i \)th row in a design matrix of suitable non-genetic covariates, \( q_i \) represents the genetic state at the QTL, \( \varepsilon_i \) is the residual with variance \( \sigma^2 \), and \( \mu, \beta \) and \( \alpha \) are parameters estimated by the model. The QTL genotype \( g_i \) is typically unknown but, thanks to information from linked markers \( M \), its underlying haplotype pair is available indirectly as a probability distribution \( p_i = (p_{i1}, p_{i2}, p_{i3}) \), where \( p_{i1} = P(g_i = AA|M) \), \( p_{i2} = P(g_i \in \{AB, BA\}|M) \) and \( p_{i3} = P(g_i = BB|M) \). The regression predictor \( q_i \) in Eq 1 may therefore be formulated in terms of \( p_i \). For the additive genetic models considered here, \( q_i = (q_{iA}, q_{iB}) \), where \( q_{iA} = 2p_{i1} + p_{i2} \) and \( q_{iB} = 2p_{i3} + p_{i2} \) correspond to the expected “doses” of haplotypes A and B respectively, and \( \alpha \) corresponds to their estimated “dosage effects” on the phenotypic mean. In practice, to obviate the dependence induced by \( q_{iB} = 2 - q_{iA} \), the regression model is fitted using \( q_i = q_{iA} \), leading to a scalar effect \( \alpha = \alpha \) that estimates the dosage effect of A relative to B. The predictor \( q_i \) may be alternatively formulated to accommodate more general effects, eg, as \( q_i = p_i \), or represent observed or imputed genotypes of known variants (eg, Yalcin et al. 2005; Zheng et al. 2011). The QTL scan is usually summarized as a plot of the LOD score, F-statistic or, \(-\log_{10}(P\text{-value})\) (hereafter, logP), at each tested position along the genome. Chromosomal regions harboring QTL affecting the trait mean are located by the highest values of the test statistic above a suitable significance threshold (eg, Broman and Sen 2009).

QTL regression model for detection of major loci controlling phenotypic variability We consider the regression

\[ y_i = \mu + x_i^T \beta + q_i^T \alpha + \varepsilon_i, \quad \varepsilon_i \sim N\left(0, \sigma^2 \exp(z_i^T \gamma + q_i^T \theta)\right). \]

where \( z_i \) contains non-genetic covariates affecting the residual variance of the model, \( \gamma \) is their corresponding effects vector, and \( \theta \) is the dosage effect of each line on the residual variance,
ie, the additive vQTL effect. All other variables are defined as in eq 1. The regression in eq 2 is thus equivalent to eq 1 but with $\epsilon_i \sim N(0, \sigma_i^2)$ and $\log(\sigma_i^2) = \log(\sigma^2) + z_i^T \gamma + q_i^T \theta$, describing a model with separate effects for mean and variance.

Regression-based mapping of QTL (including vQTL) using eq 2 assumes that:

1. There are two founder lines.
2. The genetic state of the QTL is predicted accurately by marker data.
3. There is a single major QTL.
4. The QTL is fixed within each founder line.
5. The phenotype is Normally distributed, conditional on the QTL and covariate effects.
6. The observed values $y_i$ are exchangeable, conditional on the QTL and covariate effects.

We present a fitting procedure for eq 2 based on these assumptions and, thereafter, relax the assumptions one at a time to investigate the possibility of using eq 2 for vQTL detection in empirical studies. In the current paper we assess Assumptions 1-4 using both simulations on F$_2$ and the CC, and empirical results from a chicken F$_2$ cross. We give theoretical solutions for how to relax Assumptions 5 and 6 and discuss these in the Discussion section. Our fitting procedure is based on double generalized linear models (DGLMs; see Appendix A) and uses the *dglm* package (Dunn and Smyth 2009) in R (R Development Core Team 2009).

**Significance testing** A general method to calculate P-values applicable to different trait distributions is available in *dglm*. The R code to extract the P-values from the *dglm(.)* function is given in the Supplementary Material (Supplementary Theory, Section 1). We calculated 5% chromosome-wide significance thresholds by simulating chromosomes under a null model with no mean- and no variance-controlling QTL effects.
Estimation of line dosages  We estimated line dosages at each putative QTL using probabilities estimated by the haplotype reconstruction program HAPPY (Mott et al. 2000). Given genotype data on individual i and its h founders, HAPPY uses a hidden Markov model (HMM) to infer probabilistically the haplotype pair underlying the genotypes at each marker. For every interval between adjacent pairs of markers, it then calculates the expected diplotype composition: that is, the average proportion of diplotype AA, AB, etc, that would be expected across the interval, given the interval’s length and its descent either side. The diplotype composition is reported as an $h \times h$ matrix $D_i$ for each individual $i$, and the expected line dosages are calculated as the $h$-vector $q_i = 1^T(D_i + D_i^T)$. Because $q_i^T1 = 2$ always, $q_i$ has $h - 1$ degrees of freedom and so we typically omit the $h$th element during model fitting.

Relaxing Assumption 1: More than two founder lines  For experimental crosses with $h > 2$ founder lines, the predictors $q_i$, $\alpha$, and $\theta$ in eq 2 expand to have $h - 1$ elements each.

Relaxing Assumption 2: Uncertain genotype states  Uncertainty about the QTL genotype is most naturally modeled using a mixture distribution. When modeling QTLs affecting the mean only, the marginal likelihood for observation i (omitting covariates) is the mixture $L_i = \sum_{j=1}^{3} p_{ij} N(r_j \alpha, \sigma^2)$ where $p_i = (p_{i1}, p_{i2}, p_{i3})$ is defined as above for two founder lines, $N(.)$ is the normal density and $r = (-1, 0, 1)$. This is the likelihood used for maximum likelihood (ML) estimation in Interval Mapping (Lander and Botstein 1989). The regression approach to Interval Mapping (eq 1) treats $p_i$ as if it were an observed outcome, and as a result overestimates the residual variance for each observation by $v_i = \alpha^2(p_i^T(r)^2 - (p_i^T r)^2)$ (Xu 1995; Xu 1998; Feenstra et al. 2006). When modeling mean- and variance-controlling QTL based on eq 2, the marginal likelihood is

$$L_i = \sum_{j=1}^{3} p_{ij} N(r_j \alpha, \exp(r_j \theta) \sigma^2). \quad (3)$$
The regression approach for this model overestimates residuals further, by \( v_i^* = v_i + \exp(\theta)(p_i^T \exp(r) - \exp(p_i^T r)) \) (Supplemental Material, Theory Section 3). Although we could obtain ML estimates of the vQTL model using an EM-algorithm (Appendix B), as is done for Interval Mapping, we contend that the DGLM regression may be more useful. In particular, the EM-algorithm applied to this marginal likelihood is computationally slow, a marginal likelihood gives biased ML estimates for variances, and the regression approximation confers additional flexibility for modeling different distributions for the phenotype as well as different mean-variance relationships.

Relaxing Assumptions 1 & 2: Uncertain genotype states with more than two founders Uncertainty about line origin probability when there are multiple lines can produce multicollinearity among the genetic predictors in the regression framework. We overcome this technical problem using the dimension reduction approach described in Valdar et al. (2009), whereby the matrix of line dosage estimates from HAPPY is replaced by its informative eigenvectors.

Quantifying the effects of genotype uncertainty Regressing on expected dosages incorrectly models uncertainty in the predictors. At a mean-controlling QTL, individuals with less certain \( p_i \) will be less well predicted by the mean part of the model, which could lead to inflated estimates of \( \sigma_i^2 \) and, possibly, a false vQTL signal. We investigated this phenomenon empirically by monitoring the relationship between locus uncertainty and the proportion of false positive vQTL in simulations of the CC. At each marker interval in each individual we quantified the uncertainty in line-origin using the scaled selective information content (SIC), defined as follows. If \( P(g_i = j) \) is the prior probability that individual \( i \) is in genetic state \( j \) given no marker information, and \( P(g_i = j|M) \) is the posterior given marker data \( M \), as estimated by the HAPPY HMM, then a measure of the information provided by \( M \) about
the locus is the Kullback-Leibler divergence,

\[ I(M, i) = \sum_{j=1}^{J} P(g_i = j|M) \log \frac{P(g_i = j|M)}{P(g_i = j)}, \]

summed over all \( J \) possible states, with \( 0 \log(0) \equiv 0 \). Represent the states of the F\(_2\) cross as all possible phased founder diplotypes (ie, AA, AB, BA, and BB for the cross of strains A and B) and of the CC as the set of homozygote diplotypes (ie, AA, BB, .., HH; denoting founders by A-H), then \( P(g_i = j) = J^{-1}, \forall j \). Rescaling as \( \text{SIC} = I(M, i)/\log(J) \), the (scaled) selective information content at a locus for individual \( i \) ranges from 0, denoting equiprobable diplotypes and minimal certainty, to 1, denoting one diplotype with complete certainty.

**Relaxing Assumption 3: Multiple QTL** Multiple QTL can be fitted by including additional predictors in eq 2. Linked QTL may, however, affect the analysis if not included in the model. We therefore assessed, by means of simulations, the influence of additional independent and interacting (epistatic) QTL.

**Relaxing Assumption 4: QTL variation within lines** Model 2 assumes that both the mean- and variance-controlling QTL have been fixed within the founder lines, which is reasonable for crosses from highly inbred lines but not necessarily for divergent outbred lines. It is possible that non-fixation of a mean-controlling QTL may be detected as a spurious vQTL. We illustrate this phenomenon for the Growth 2 QTL on chicken chromosome 1, which was found not to have been fixed in a divergent F\(_2\) cross (Kerje et al. 2003; Rönnegård et al. 2008).

**SIMULATIONS AND DATA**

We assessed the performance of our method by applying it to simulated F\(_2\) and CC populations, and to a real Red Jungle Fowl × White Leghorn F\(_2\) dataset. Simulations were
generated with software used for Valdar et al. (2006) and Valdar et al. (2009) (See Supplementary Material, Software).

**Simulated F₂ intercross** Our simulated F₂ population included 800 individuals each comprising a single 100cM chromosome with 10 evenly spaced fully informative SNP markers. QTL, when simulated, were positioned midway between markers, at 45cM. The simulated population size of 800 was chosen to reflect a typically sized F₂ design such as the Red Jungle fowl × White Leghorn F₂ cross described further below.

**Simulated Collaborative Cross (CC)** The CC is a panel of recombinant inbred mouse lines (RILs) descended from 8 inbred founder strains: A/J, C57BL/6J, 129S1SvImJ, NOD/LtJ, NZO/H1LtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ (Churchill et al. 2004; Chesler et al. 2008). In keeping with previous simulations of this population (Valdar et al. 2006), we simulated 1,000 RIL individuals each generated from a separate breeding funnel. CC individuals comprised a single 100 cM chromosome with 1,000 SNP markers drawn from the Mouse Diversity Genotyping Array (Yang et al. 2009; see http://cgd.jax.org/tools/diversityarray.shtml) and chosen to be roughly equally spaced and informative among the 8 founders. QTL, when simulated were positioned at 28 cM and 68 cM, each midway between markers and in regions containing relatively informative markers.

**Detection of QTL in F₂ and CC with regression on known QTL genotypes** We simulated QTL in the F₂ and CC under four scenarios.

**Scenario 1:** No QTL effects

**Scenario 2:** Mean-controlling QTL effects

**Scenario 3:** Variance-controlling QTL effects

**Scenario 4:** Both mean- and variance-controlling QTL effects
Phenotypic values ($Y$) were generated as mean-controlling additive QTL effects ($Q$) plus a residual ($E$): 

$$Y = Q + E.$$ 

For mean controlling effects, $Q = 1$, 2 and 4 for QTL genotype aa, aA and AA respectively. These effects correspond to a QTL with a moderate effect explaining approximately 2% of the phenotypic variance. For the scenarios with no vQTL, 

$E$ was drawn from $N(0, \sigma^2)$ with $\sigma^2 = 100$. For the scenarios with vQTL, $\sigma^2 = 100, 125, 156.25$ for genotypes aa, aA and AA, such that the the A allele had additive effects on the log scale and increase variance by 25% for each copy. These effects are moderate in size. 

Ordas et al. 2008 found variance-controlling QTL in maize where the allele effects resulted in an increase of the residual variance of 30-40%. For each scenario, we generated 10,000 replicates of the F$_2$ simulation and 1,000 replicates of the CC (positioning the QTL at 28 cM only). Model fitting was implemented using dglm function in R (R Development Core Team 2009), applying the regression in eq 2 to the F$_2$ populations and that in eq 2 to the CC.

**Detection of QTL for F$_2$ and CC with regression on line dosage** We repeated the simulations above at each marker interval fitting the DGLM to line dosages inferred by the HAPPY HMM. The simulations from Scenario 1 (no effects) were used to obtain 5% chromosome-wise empirical significance levels.

**Fitting a single QTL in the presence of linked QTL and epistasis** To study a potential influence of linked QTL on estimated vQTL effects, we simulated three scenarios for the CC. DGLM regression was performed on line dosages as above, with 1,000 replicates simulated for each scenario.

**Scenario E1:** A single mean-controlling QTL at 28 cM

**Scenario E2:** Two linked mean-controlling QTL with additive effects at 28 cM and 68 cM

**Scenario E3:** Two linked mean-controlling QTL with epistatic effects at 28 cM and 68 cM
We generated phenotypes \((Y)\) as \(Y = Q + E\), with constant residual variance \(E \sim N(0, 100)\) throughout. For Scenario E1, \(Q = 0, 2\) or \(4\) for QTL genotype aa, aA or AA. For Scenario E2, the additive QTL effects were calculated as \(Q = Q_1 + Q_2\), where \(Q_k = 0, 2\) or \(4\) for aa, aA or AA at QTL number \(k\). For Scenario E3, QTL with interaction effects were simulated and \(Q\) was assigned values according to Table 2. For all three scenarios, we fitted a DGLM with line dosages as predictors for both mean and variance effects at the first QTL position only (28 cM).

[Table 2 about here.]

**Red Jungle fowl × White Leghorn F\(_2\) cross - a worst-case scenario to assess the effects of uncertainty in QTL genotype and non-fixation of QTL within founder lines** In an F\(_2\) cross between the chicken lines Red Jungle fowl and White Leghorn, Kerje et al. (2003) had previously detected two QTL affecting body weight at 200 days of age on chromosome 1 around 100 cM (Growth 1) and 490 cM (Growth 2). This trait was chosen because the QTL Growth 1 and Growth 2 have been thoroughly studied previously (Kerje et al. 2003; Rönnergård et al. 2008) where Growth 1 has a very large effect and Growth 2 was not fixed within the founder lines. The cross comprised four founder individuals (two from each line) and 756 F\(_2\) offspring. Although the QTL Growth 1 is easily shown to have a very strong effect on the mean (explaining 22% of the variance, Kerje et al. 2003), the analysis of Growth 2 is complicated by it being fixed within the Red Jungle fowl founders but variable within the White Leghorn line (Rönnergård et al. 2008), leading to additional uncertainty in the underlying QTL genotype. We use these data to explore the differences in estimates from the EM algorithm (Appendix B), which explicitly takes account of this uncertainty, and the DGLM estimation, which does not, when there is a very strong mean-controlling QTL and moderate marker information. Furthermore, we study the effect of a QTL not being fixed within the founder lines. A sex effect was included as a covariate in both the mean and variance parts of the model. Line dosages were calculated as Haley-
Knott probabilities (see Supplementary Material, Data) and models were fit as above using the regression in eq 2.

RESULTS

Detection of QTL for $F_2$ and CC with regression on QTL genotypes For the $F_2$ simulations the average of the estimated QTL effects were close to those simulated (Table 3). The false positive rate was close to 0.05 (Table 4) for both $F_2$ and CC, indicating that our DGLM approach produces the appropriate rate of false positives (Type I error) when applied to known QTL genotypes.

[Table 3 about here.]

[Table 4 about here.]

Detection of QTL for $F_2$ and CC with regression on line dosage For the $F_2$ simulations, the 5% chromosome-wide significance thresholds were $\log P = 2.02$ and $\log P = 2.01$ for the mean and variance parts of the model, respectively. Using these thresholds, the proportion of false positives was close to 0.05 (Table 5) both for Scenario 2 (mean-controlling QTL simulated) and Scenario 3 (vQTL simulated). The power to detect the mean-controlling QTL at a 5% genome-wide significance level was 91.7% (Table 5) and the power decreased slightly when a vQTL was added to the simulations due to the resulting increase in residual variance. The power to detect the vQTL was 77.0% (Table 5) and this was not substantially affected by including a mean-controlling QTL in the simulations (ie Scenario 4). For Scenario 4, with both ordinary QTL and vQTL being simulated, most QTL detected were positioned within, or close to, those simulated (Figure 1). The simulations gave similar results for Scenarios 2 and 3. The accuracy of the QTL position does not seem to be substantially affected if either a mean-controlling or a variance-controlling QTL is simulated versus the Scenario 4 where both effects are simulated (Table 6).
Marker informativeness was small (SIC around 0.1) for the F$_2$ simulations because markers were spaced 10cM apart, where these intervals are considerably larger than in most QTL studies today (see eg Kerje et al. 2003). For perfect information about QTL genotype the line dosage predictor $q_i$ is either 0, 1 or 2, whereas for low information it has an attenuated range and is centered around 1.0. As a result, the regression on line dosage overestimated QTL effect ($\alpha$ and $\theta$) for the F$_2$ simulations. The extent of over-estimation depends on the range of the line dosage (ie, $\max(q) - \min(q)$), which is 2 with complete information but was 0.348 in the simulations. In Table 3 we therefore report estimates from the line dosage model after division by $2/0.348 = 5.75$.

For the CC simulations the 5% chromosome-wide significance threshold for logP was 3.24 and 3.01 for the mean and variance parts of the model respectively. The power to detect the mean-controlling QTL at a 5% genome-wide significance level was 98.2% (Table 5) and, as above, this decreases slightly when a vQTL is added owing to the increased residual variance. The power to detect the vQTL was 80.8% (Table 5) and the power was not substantially changed when a mean-controlling QTL was included in the simulations. For the CC simulations, the proportion of QTL detected within the correct $\pm$0.3cM was highest for a vQTL when simulating both mean- and variance-controlling QTL (Table 6). The estimated QTL positions were well centered around the true simulated QTL position (Figure 1).

**Fitting a single QTL in the presence of linked QTL and epistasis**  Fitting the DGLM model of eq 2 to the simulated CC data with a single mean-controlling QTL (Scenario E1) resulted in 5.8% of replicates having P-values less than 0.05 in the variance part of the
model. This is consistent with the P-values for the vQTL being robust to the presence of moderate-effect ordinary QTL. Under Scenario E2 (two linked QTL with additive effects) the proportion of replicates having P-values less than 0.05 was 4.0%, and the rate of false positives (Type I error) did not seem to be substantially affected by an additional linked QTL acting additively. When there were two linked and interacting mean-controlling QTL (Scenario E3), 17.0% of the replicates had P-values for the variance submodel less than 0.05, indicating the proportion of false positives is substantially affected by linked QTL that act epistatically. By including interaction effects, between the two interacting loci, in the mean part of the model the false positive rate was reduced to 5.6%.

By increasing the mean-controlling QTL effect, under Scenario E1, from 2.0 to 20, the empirical Type I (false positive) error increased to 12.7%. Hence, vQTL detected close to mean-controlling QTL of large effect should be treated with caution and further analyzed using, eg, an EM-algorithm where the effects of marker uncertainty are accounted for (see Appendix B).

To investigate the effect of marker informativeness for the CC, we performed further simulations that repositioned the QTL at different locations in a 5cM-spaced ladder between 25cM and 70cM. For these simulations (200 replicates per QTL location), the SIC varied considerably both within and between the QTL locations, but there was no clear relationship between SIC and the logP-values for false vQTL (Figure 2) when an ordinary QTL was simulated with additive effect of 2.0.

[Figure 2 about here.]

Red Jungle fowl × White Leghorn F₂ cross - a worst-case scenario to assess the effects of covariate uncertainty and non-fixation of QTL within founder lines

In a preliminary model for chicken body weight without QTL effects, estimation of sex effects in a DGLM gave highly significant ($P < 10^{-6}$) estimates of 410.0 and 0.509 for mean and variance predictors respectively. The estimates being significant and having the same sign,
suggests a general mean-variance relationship. This was confirmed by applying the Box-Cox procedure (Box and Cox 1964), which suggested a square root transformation ($\hat{\lambda} = 0.55$, s.e. 0.11), and the extended quasi-likelihood (EQL) procedure of Nelder and Pregibon (1987), which indicated a linear mean-variance relationship ($\hat{\psi} = 1.2$, s.e. 0.2)(see Supplementary Material, Theory Section 4).

A chromosome scan using *dglm* revealed mean-controlling QTL (Figure 3) that were similar to those estimated by homoscedastic regression (Kerje et al. 2003). There were no large differences between the scans for ordinary QTL with Box-Cox transformed versus original body weight as response. However, there were substantial differences between the chromosome scans for vQTL (Figure 3, middle graph), although no vQTL reached 5% significance.

[Figure 3 about here.]

A moderate-sized peak was detected for the vQTL at approximately the same location as Growth 2 (nominal P-value=0.02, chromosome-wise P-value = 0.10). This peak is likely due to the fact that the QTL alleles of Growth 2 were not fixed within the founder line of domestic White Leghorn hens (Rönnergård et al. 2008). The reason for this is that, if an $F_2$ individual has an allele inherited from the domestic leghorn line then the residual variance will be greater than if the allele was inherited from the Red Jungle Fowl line since eq 2 assumes that the QTL alleles are fixed within founder lines. Consequently, the QTL around 490 cM is not a variance-controlling QTL but rather an effect of Growth 2 not being fixed within founder lines.

To assess spurious effects of uncertainty in genotype state, the additive effects for vQTL in eq 2 were calculated using an EM-algorithm (Appendix B) and were subsequently compared with the DGLM estimates (Figure 4). The estimates are given as percentage change in residual variance for allele substitutions, ie $100 \times (\exp(|\hat{\theta}|) - 1)$. The estimates are almost identical except for the positions around 115 cM where there is a strong mean-controlling
QTL and moderate marker information (logP = 57.3 and SIC = 0.7, Figure 3). Hence, no major improvement in the QTL detection was achieved by using the more theoretically correct EM-algorithm.

[Figure 4 about here.]

DISCUSSION

We have studied the potential of detecting variance-controlling QTL (vQTL) by fitting a double generalized linear model via a two stage procedure to simulated phenotype and marker information. The model can detect QTL that affect both the mean and the variance, or QTL that affect either the mean or the variance. Because we use line origin probabilities as predictors in the model, as calculated using the HAPPY HMM (Mott et al. 2000), our approach can be applied to a wide range of experimental crosses. There are, however, some important considerations to be emphasized.

Detecting vQTL can be more challenging in theory, practice and subsequent interpretation than detecting QTL controlling the trait mean. This is unsurprising: at the most basic level, variances are harder to estimate than means, typically requiring five times as many observations to achieve comparable precision (cf “Tukey’s rule of 5” in Lee and Nelder 2006). Indeed, Visscher and Posthuma (2010) demonstrate analytically that detecting small effect vQTL among unrelated humans using traditional method would require sample sizes in the 10,000s. More insidiously, inferences about differences in variances can be more sensitive to distributional assumptions than inferences about means. Specifically, it is common for raw phenotype measurements to exhibit a mean-variance relationship that naturally arises through the data generation process: for example, if body length is homoscedastic Gaussian then its square (eg, body area) will not be. In the detection of vQTL, it is therefore especially important to recognize such relationships at an early stage and apply suitable normalizations or explore parametric alternatives to the normal distribution in order to avoid QTL affecting the mean appearing also to affect the variance. In the Supplementary Material
(Theory Section 4) we illustrate this problem along with an effective remedy via the Box-Cox transformation. Moreover, we set forth guidelines for how to approach a conservative vQTL analysis of data that are likely to be approximately normal but only after an unknown transformation, and of data that are more suitably modeled by other members of the exponential family, eg, Poisson for count data, for which a known mean-variance relationship exists. Where it is felt such precautions may be inadequate or are impractical, we suggest resorting to the over-conservative strategy of prioritizing “pure vQTL”, that is, strong vQTL with negligible mean effect, subject to all the usual requirements of deconfounding that apply to the detection of ordinary QTL.

Regression on genotype probabilities or expected line dosages can lead to inflated estimates of the residual variance (see Models and Methods). In Supplementary Material (Theory Section 3), we describe theoretically the effect of uncertain genotype states on the risk of detecting false vQTL and conclude that detected vQTL can be trusted as long as the marker informativeness is high or, if it is not, that the vQTL is not close to a mean-controlling QTL. Our simulations show that the power of detecting a vQTL in genome scans is largely unaffected by whether the same locus also affects the trait mean (Table 5), and we note that this includes our F2 simulation with a modest degree of informativeness from markers spaced 10cM apart. In the analysis of chicken chromosome 1, we found that a mean-controlling QTL (Growth 1) having a very large effect (logP=57.3) together with moderate marker information (SIC=0.7) was needed to give any substantial bias in the vQTL estimation (Figure 4). We therefore conclude that the effect of inflated residual variance due to low marker information is not likely to be a major problem for our model.

We apply our model to populations of individuals who are genetically distinct and equally related, phenotyped in studies where genotype assignment is exchangeable across unmodeled environmental variation. A genetic locus whose genotype separates the population into groups with distinct variances is, by our definition, a putative vQTL. Table 1 illustrates how an effect detected in this way could arise from several different sources, each implying
a potentially distinct paradigm of vQTL action. For example, it could represent canalizing epistasis, whereby some vQTL genotypes cushion the impact of genetic variation on the phenotype. Our simulations demonstrated that a mean-controlling QTL under epistatic control may indeed be detected as a vQTL with our proposed method, and this result is consistent with recent work by Paré et al. (2010) and Struchalin et al. (2010). Alternatively, it could represent a differential sensitivity to environmental variation. Specifically, increased variability under one genotype may manifest as temporally stable phenotypes varying between individuals, phenotypes being highly fluctuant within an individual but on average similar between, little actual variation but a tendency for increased error of measurement, or some combination of these. Dissecting the components of the induced variability could be accomplished by applying our DGLM framework to a more focused experimental design, eg, incorporating replicates of the CC at different levels (eg, guided by column 1 of Table 1). In some cases, further statistical exploration on the same data may also be helpful. For example, if the mechanism is epistatic, vQTL-detection can be seen as a starting point for modeling the joint action of multiple interacting loci, and this is easily accommodated in our regression framework.

Levene’s test is a popular method for testing equality of variance. Paré et al. (2010) and Struchalin et al. (2010) used it to test for different variances between SNP genotypes. It is capable of modeling group effects and has the advantage of being quite insensitive to non-normality. The DGLM approach, however, is far more flexible: it allows us to model continuous predictors and more complex general relationships of covariates on the variance, which Levene’s test cannot. Levene’s test also does not account for possible imbalance in the data since the estimated residuals mask varying uncertainty among groups. The DGLM approach, by contrast, allows modeling of non-genetic effects in the variance part of the model, which may be important if for instance the variances are different between batches or contemporary groups in a designed QTL experiment. Moreover, DGLMs can also be extended to include random effects, due to family or polygenic effects, through hierarchical
generalized linear models (Lee and Nelder 1996; Lee et al. 2006; Rönneård et al. 2010) and double hierarchical generalized linear models (Lee and Nelder 2006; Rönneård et al. 2010). This modeling flexibility is necessary in QTL studies and gives a more general approach applicable to a wide range of experimental designs.

There are several possible extensions of our model. Dominance can be included similarly as in other QTL regression models (Haley et al. 1994), as can multiple loci (eg, Valdar et al. 2009). Because DGLM allows for any response distribution from the exponential family (Smyth 2002), our model is straightforwardly extended to binomial, Poisson or gamma distributed traits. In particular, several studies have focused on QTLs controlling the CV rather than the variance (eg Mackay and Lyman 2005; Ansel et al. 2008). Traits with a constant CV, given the explanatory variables, are naturally modeled using a gamma distribution (McCullagh and Nelder 1989), and the DGLM method can be adapted to model such traits by setting the dispersion to \( CV^2 \) (see Supplementary Material, Theory Section 2). If we do not know whether to search for variance-controlling or CV-controlling QTL (ie, whether the trait should be normal- or gamma-distributed), we can use the extended quasi-likelihood (EQL; Nelder and Pregibon 1987) to compare model fits (see Supplementary Material, Theory Section 4). Ideally, distributional assumptions should always be checked for a detected QTL using a QQ-plot for the deviance residuals (McCullagh and Nelder 1989) from the mean part of the DGLM.

We anticipate identification of vQTLs will be of interest in a wide range of genetics studies and applications. A clear application is in breeding systems, where vQTL detection could help selection for more robust livestock production (Mulder et al. 2007; Mulder et al. 2008). We believe there is also substantial scope for application in the study of animal models of human disease. For example, medical diagnosis of hypertension has traditionally focused on achieving reliable estimates of the underlying mean blood pressure. However, increasing evidence points to the dangers of temporal fluctuation about the mean (Parati et al. 2006; Rothwell et al. 2010; Brunelli et al. 2008). Our framework could be used...
to detect QTL affecting such variability in animal models, and could be adapted to use in suitably controlled human studies.

In conclusion, we have developed a regression model for detection of major loci controlling phenotypic variance, which can be applied on a wide range of experimental crosses such as backcross, F$_2$, and MAGIC lines such as the CC. We have studied the robustness of the model to varying marker information, misspecification of the response distribution, linked QTL and epistasis, we have proposed recommendations for its use, and discussed the meaning of detected vQTL and how they might be further dissected. We expect detection of vQTLs will have wide application in genetics.

ACKNOWLEDGMENTS

LR recognises financial support by the Swedish Research Council FORMAS. WV acknowledges partial support by NIMH/NHGRI grant P50 MH090338 and by a Career Development Award from the Medical Research Council, UK. Leif Andersson is acknowledged for providing data from the Red Jungle fowl × White Leghorn cross. Gary Churchill is acknowledged for helpful discussions regarding use of the EM algorithm.

APPENDIX A: DOUBLE GENERALIZED LINEAR MODEL THEORY

For known genotypes at the QTL, the maximum likelihood (ML) estimates of the effect parameters $\beta, \alpha, \gamma, \theta$ in eq 2 can be estimated using Fisher scoring. Smyth 1989 showed this to be equivalent to predicting the mean effects using a linear model (eq 1) and the squared residuals ($\varepsilon_i^2$) using a generalized linear model (GLM) with on a gamma distributed response and the log link.

ML gives biased variance component estimates and the estimates are also sensitive to influential observations with high leverages (Verbyla 1993). A restricted maximum likelihood (REML) approach, implemented in the dglm package, resolves these issues by incorporating leverage into the estimation (Smyth 2002; Lee et al. 2006). Specifically, the gamma GLM
is used to predict \( log(E(d_i)) = \log(\sigma^2) + z_i^T \gamma + q_i \theta \), where the response is now the weighted deviance component \( d_i = \frac{\varepsilon_i^2}{1 - h_{ii}} \). Here, the \( h_{ii} \) is the leverage of observation \( i \), equal to the \( i \)th diagonal element of the hat-matrix \( H = X_{all}(X_{all}^T V^{-1} X_{all})^{-1} X_{all}^T V^{-1} \), where \( X_{all} \) is the design matrix for all fixed effects in the mean submodel and \( V = \text{diag}(\sigma_1^2, \sigma_2^2, \ldots, \sigma_n^2) \).

Both the hat matrix and the associated leverages play an important role in ordinary regression theory (Hoaglin and Welsch 1978; Faraway 2004) and are utilized extensively in DGLM theory (Smyth 1989).

**APPENDIX B: AN EM-ALGORITHM FOR ESTIMATING VQTL FOR NORMALLY DISTRIBUTED TRAITS IN F2 CROSSES**

For an observation \( i \), the full likelihood for vQTL with genotype uncertainty is

\[
L_i = \sum_{j=1}^{3} p_{ij} N \left( \mu + x^T \beta + r_j \alpha, \sigma^2 \exp(z^T \gamma + r_j \theta) \right) = \sum_{j=1}^{3} p_{ij} f_{ij}
\]

with variables other than \( f_{ij} \) defined as for eq 3. Let \( l_i = \log(L_i) \), \( g_{ij} = \log(f_{ij}) \), and \( \psi \) be the parameter vector, and assume observations are independent given the parameters such that \( L = \sum_i^n L_i \). Then,

\[
\frac{\partial l_i}{\partial \psi} = \frac{1}{L_i} \sum_{j=1}^{3} p_{ij} \frac{\partial f_{ij}}{\partial \psi}
\]

where \( (\partial L_i/\partial \psi) = \sum_{j=1}^{3} p_{ij} (\partial f_{ij}/\partial \psi) \) and

\[
\frac{\partial L_i}{\partial \psi} = \sum_{j=1}^{3} p_{ij} \frac{\partial f_{ij}}{\partial \psi} = \sum_{j=1}^{3} p_{ij} f_{ij} g_{ij} \frac{\partial g_{ij}}{\partial \psi}
\]

\Rightarrow \frac{\partial l_i}{\partial \psi} = \frac{1}{\sum_{j=1}^{3} p_{ij} f_{ij}} \sum_{j=1}^{3} p_{ij} f_{ij} g_{ij} \frac{\partial g_{ij}}{\partial \psi}

Here \( g_{ij} \) is the log-likelihood given the genotypes, so the gradient and Hessian of \( g_{ij} \) are those obtained from the logarithm of the normal density function \( f_{ij} \). ML estimates of \( \psi \) are obtained by iterating the following EM steps until convergence:

1. Calculate the “weights” given the parameter estimates: \( w_{ij} = p_{ij} f_{ij} / (\sum_{j=1}^{3} p_{ij} f_{ij}) \)
2. Calculate the parameter estimates given \( w_{ij} \) from: 
\[
\frac{\partial l}{\partial \psi} = \sum_{i=1}^{n} \sum_{j=1}^{3} w_{ij} \left( \frac{\partial g_{ij}}{\partial \psi} \right) = 0
\]
which can be calculated using a Newton iterative method since we have the gradient and Hessian of \( g_{ij} \).

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Figure 2: Relationship between logP-values for false vQTL and marker information content (SIC) when simulating a mean-controlling QTL in a CC population. For each of 200 simulations, ordered along the x-axis by their most significant vQTL peak, the plot shows the mean and standard deviation of SIC for 1000 mice. The SIC statistics are stationary, indicating no apparent tendency for marker uncertainty to produce false vQTL signals.
Figure 3: Scan for QTL controlling the mean (top figure) and the variance (middle figure) of body weight at 200 days of age on chicken chromosome 1 in an F$_2$ cross between Red Jungle Fowl and White Leghorn with 756 F$_2$ offspring. Marker information contents (SIC) given in bottom figure. Genome-wide significance threshold calculated using 1000 permutations.
Figure 4: Estimates of variance-controlling QTL (vQTL) effects given as percentage change in residual variance for allele substitutions for body weight at 200 days of age on chicken chromosome 1 in an F_2 cross between Red Jungle Fowl and White Leghorn. Maximum likelihood estimates from the full marginal likelihood - solid line, and DGLM estimates - dashed line. Close up for 90-130 cM shown in bottom figure.
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Table 1: **Types of variance contributing to between-group differences in phenotypic variability**

<table>
<thead>
<tr>
<th>Variance Group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sources of Phenotypic Variability</th>
<th>Decanalization (Epistasis)</th>
<th>Environmental Sensitivity</th>
<th>Temporal Fluctuation</th>
<th>Measurement Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetically distinct individuals with same allele at a vQTL&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>●</td>
<td></td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Genetically identical individuals.</td>
<td></td>
<td></td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Same individual at different times.</td>
<td></td>
<td></td>
<td></td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Same individual at the same time.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>●</td>
</tr>
</tbody>
</table>

<sup>a</sup>The group in which variance is assessed, and between which variance is compared.<br>
<sup>b</sup>The variance groups compared here.
Table 2: Simulated epistatic effects for different QTL genotype combinations

<table>
<thead>
<tr>
<th>QTL 1 (28 cM)</th>
<th>aa</th>
<th>aA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTL 2 (68 cM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aa</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>aA</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 3: **Estimated QTL effects in simulated F$_2$.** Simulated value for mean-controlling QTL: 2.0, simulated value for variance-controlling QTL: 0.22

<table>
<thead>
<tr>
<th>Simulated effects</th>
<th>Regression on QTL genotypes</th>
<th>Regression on line dosages $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ordinary QTL</td>
<td>vQTL</td>
</tr>
<tr>
<td>No QTL</td>
<td>0.001</td>
<td>-0.003</td>
</tr>
<tr>
<td>Ordinary QTL</td>
<td>1.997</td>
<td>-0.003</td>
</tr>
<tr>
<td>vQTL</td>
<td>-0.010</td>
<td>0.2175</td>
</tr>
<tr>
<td>Ordinary and vQTL</td>
<td>1.999</td>
<td>0.2173</td>
</tr>
</tbody>
</table>

$^a$ Estimates from genome scan with regression on line dosages. Correction for shrunken line dosage estimates in HAPPY due to low marker information contents; corrected values = estimates from genome scan times half the range of line dosages.
Table 4: Power to detect QTL at a 5% nominal level for regression on QTL genotypes

<table>
<thead>
<tr>
<th>Simulated effects</th>
<th>( F_2 )</th>
<th>( \text{vQTL} )</th>
<th>CC</th>
<th>( \text{Ordinary QTL} )</th>
<th>( \text{vQTL} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No QTL</td>
<td>0.052</td>
<td>0.052</td>
<td>0.054</td>
<td>0.053</td>
<td></td>
</tr>
<tr>
<td>Ordinary QTL</td>
<td>0.997</td>
<td>0.051</td>
<td>1.000</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>vQTL</td>
<td>0.051</td>
<td>0.966</td>
<td>0.057</td>
<td>0.998</td>
<td></td>
</tr>
<tr>
<td>Ordinary and vQTL</td>
<td>0.984</td>
<td>0.963</td>
<td>1.000</td>
<td>0.998</td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Power to detect QTL at a 5% chromosome-wide significance level for regression on line dosages

<table>
<thead>
<tr>
<th>Simulated effects</th>
<th>$F_2$</th>
<th>Ordinary QTL</th>
<th>vQTL</th>
<th>Ordinary QTL</th>
<th>vQTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No QTL</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Ordinary QTL</td>
<td>0.917</td>
<td>0.055</td>
<td>0.982</td>
<td>0.053</td>
<td></td>
</tr>
<tr>
<td>vQTL</td>
<td>0.059</td>
<td>0.770</td>
<td>0.046</td>
<td>0.808</td>
<td></td>
</tr>
<tr>
<td>Ordinary and vQTL</td>
<td>0.808</td>
<td>0.808</td>
<td>0.928</td>
<td>0.808</td>
<td></td>
</tr>
</tbody>
</table>
Table 6: Proportion of QTL that were detected at a 5% chromosome-wide significance level and whose chromosomal position was estimated accurately<sup>a</sup>

<table>
<thead>
<tr>
<th>Simulated effects</th>
<th>Ordinary QTL</th>
<th>vQTL</th>
<th>Ordinary QTL</th>
<th>vQTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary QTL</td>
<td>0.462</td>
<td>-</td>
<td>0.452</td>
<td>-</td>
</tr>
<tr>
<td>vQTL</td>
<td>-</td>
<td>0.318</td>
<td>-</td>
<td>0.262</td>
</tr>
<tr>
<td>Ordinary and vQTL</td>
<td>0.442</td>
<td>0.341</td>
<td>0.372</td>
<td>0.272</td>
</tr>
</tbody>
</table>

<sup>a</sup> The chromosomal position was defined to be accurately estimated: i) for the F<sub>2</sub> cross if the QTL was detected within the correct marker interval, ii) for the CC if the estimated position was within the correct ±0.3cM.