

Supplementary data file S3:

Multi-allelic QTL mapping in autopolyploids

In the main text we simulated an extensive set of bi-allelic QTL to help answer some questions regarding autopolyploid QTL analysis. The “functional” QTL allele was simulated to have an effect on the phenotypes, with the remaining alleles assigned a functional effect of 0. However, in many real settings it might be expected that QTL loci would carry more than two allelic variants, even within a bi-parental population where the number of alleles is constrained to be at most $2 \times ploidy$. These QTL effects might also be antagonistic, with positive and negative-effect alleles being inherited from one or both parents. Therefore, in order to translate our results to include multi-allelic QTL scenarios, we ran a smaller simulation study involving multi-allelic loci.

Simulation description

We simulated multi-allelic QTL datasets with at least 2 and at most 8 functional QTL alleles in the context of an autotetraploid biparental F_1 population. The number of alleles at each QTL was randomly assigned by sampling from a Poisson distribution with $\lambda = 2.5$, where the tails of the distribution were removed so that each sample x lay within the range $2 \leq x \leq 8$. In situations with less than 8 functional alleles, the remaining alleles were assigned an effect size of 0. Each QTL had a randomised configuration (parental homologues where the allele originated from), randomised cM position (to 2 decimal places), and random “heritability” ($0 < h^2 < 1$) as described in the main text. For the mode of QTL action, we simulated both additive and dominant QTL, where dominance could either be simplex-dominant or duplex-dominant (Rosyara et al., 2016). In the case of simplex-dominant, a single copy of the QTL allele conferred the full QTL effect (regardless of dosage), *i.e.* $QQQQ = QQQq = QQqq = Qqqq$ and $qqqq = 0$. For a duplex-dominant it was assumed that two copies of the dominant allele were required to produce the full effect, *i.e.* $QQQQ = QQQq = QQqq$ and $Qqqq = qqqq = 0$. Therefore, only additive-effect QTL were actually multi-allelic (in the sense of having more than two alleles of different effect at the same locus).

We incorporated variable allele dosages by constraining the QTL allele effects to a relatively small set of possible allele effects (-100, -80, ..., -20, 20, ..., 80, 100) and sampled from this list (with replacement), so that *e.g.* a QTL segregation type AABC x ADDD was possible, and not just ABCD x DDDD (where the D allele is understood here to be the “null effect” allele of effect 0). In the case of dominant QTL, we selected the first assigned QTL allele as the dominant allele (which was not always in simplex condition). The remaining alleles were assumed to have no effect on the phenotype (*i.e.* complete dominance). In our simulation, QTL alleles with the same effect were taken to be the same allele.

We produced a dataset of 1000 random QTL for a simulated population of size $N = 200$ (simulated using PedigreeSim (Voorrips and Maliepaard, 2012)) for each of three rates of quadrivalent pairing ($q = 0, 0.5$ and 1) with marker coverage based on the potato chromosome 12 linkage map of Hackett et al. (2013), and tested them using our QTL detection procedure as described in the main text. The only modification we made from our initial analysis was in significance-threshold setting, where we implemented the procedure of (Nettleton and Doerge, 2000) as also suggested by (Hackett et al., 2014), which allowed us to generate confidence intervals (CI) around significance thresholds using fewer permutations. In most cases, these CI's were narrow enough to enable a decision to be made on whether the QTL was significant or not after 100 permutations (the minimum recommended number of permutations for $\alpha = 0.05$ (Nettleton and Doerge, 2000)). Similar to the description in the main text, we defined QTL detection as situations where the LOD score at the true QTL position exceeded the significance threshold. Since the QTL were simulated independently of marker data, we used cubic splines to determine an approximate LOD score at the precise QTL location. In cases where the LOD score fell within the CI (*i.e.* unresolved) we increased the number of permutations to increase resolution, up to a maximum of 1000 permutations (using the procedure described in (Nettleton and Doerge, 2000)). All QTL were resolved as either significant or not in this way.

Among detected QTL, we subsequently calculated the Bayesian Information Criterion (BIC) at the QTL peak position (*i.e.* at the location of highest LOD), testing for bi-allelic and multi-allelic additive models and for simplex and duplex dominant models (in total, 676 competing QTL models were compared). A QTL was declared correctly identified if the correct QTL model was the model with the minimum BIC (however, without requiring this minimum to be unique). Non-unique minima can occur for example between a QTL with a duplex allele QQqq x qqqq (configuration 1,2) which cannot be distinguished from qqQQ x qqqq (configuration 3,4). Note that for the bi-allelic QTL study (main text), we removed all possible duplications to maximise computational efficiency (Supplementary Table1).

Simulation Results

QTL detection power

As before, we found that the power of QTL detection dropped slightly at higher rates of quadrivalent pairing, with the DR model performing significantly better ($p < 0.0001$) when the rate of quadrivalents was 1 (Figure S3.1). A marginal improvement in performance was also detected at $q = 0.5$.

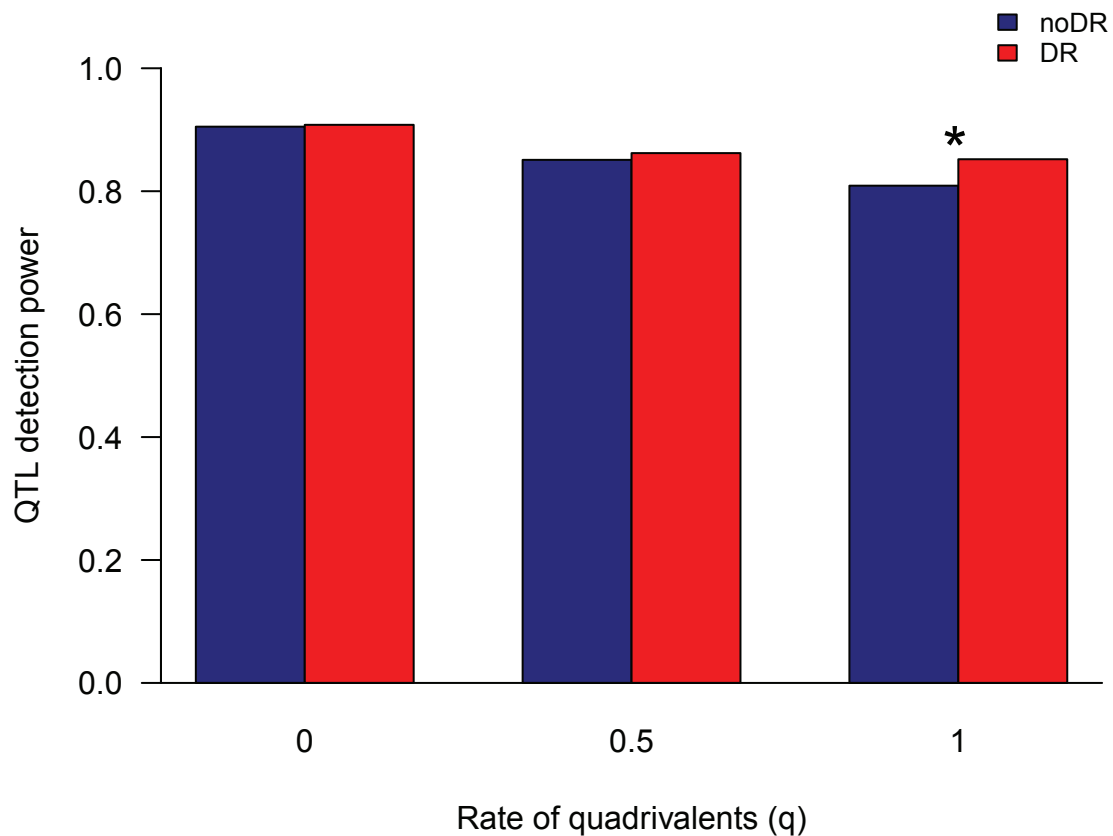


Figure S3.1 QTL detection power for QTL simulated in populations of variable rates of quadrivalent pairing (q) described previously. Results of QTL analysis using a model that assumes only bivalent pairing (noDR) were compared to those of a general autotetraploid model that also accommodates quadrivalent pairing and double reduction (DR).

Significance codes from paired t-tests (1000 samples): $p < 0.05$ (*)

Detection of dominant QTL

The power of detection of simplex dominant QTL and duplex dominant QTL was equal when bivalent pairing occurred exclusively ($q = 0$, Figure S3.2). However, when quadrivalent pairing also occurred, the rate of detection of duplex dominant QTL dropped considerably, although markedly more so for the noDR model than the DR model. There were unexpected fluctuations in the results for $q = 0.5$ and $q = 1$ for the DR model, although it is hard to know whether this is attributable to random variation of the simulation itself or whether it was the result of some systematic effect between these two rates of quadrivalent pairing (Figure S3.2).

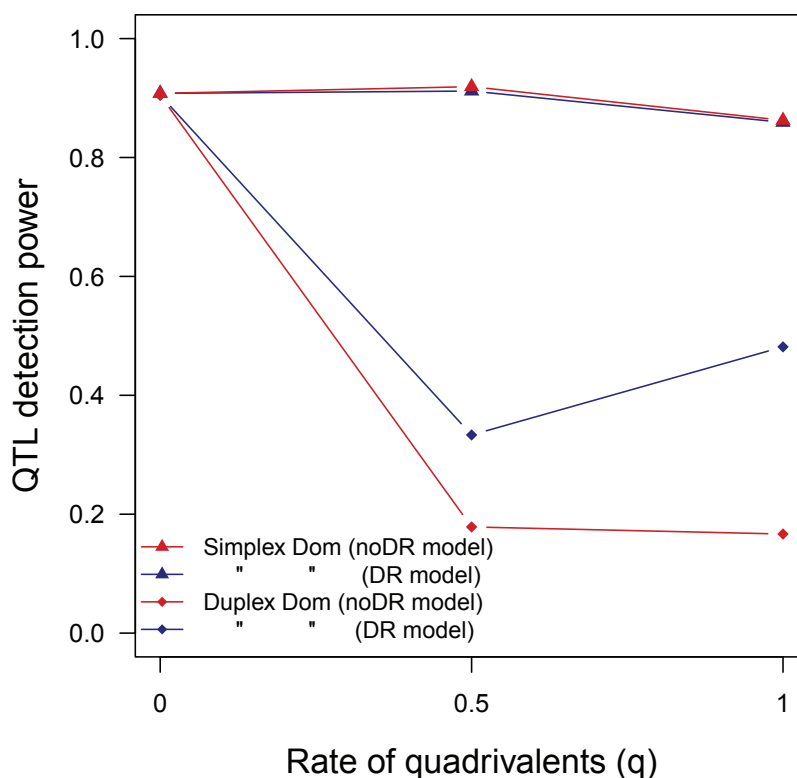


Figure S3.2 QTL detection power for dominant QTL simulated in populations of variable rates of quadrivalent pairing (q). Results of QTL analysis using a model that assumes only bivalent pairing (noDR model, red lines) were compared to those of a general autotetraploid model that also accommodates quadrivalent pairing and double reduction (DR model, blue lines). Simplex Dom = simplex-dominant QTL gene action, and Duplex Dom = duplex-dominant QTL gene action.

Effect of the number of QTL alleles

We checked whether there was any effect of the number of simulated alleles on the ability to detect QTL, but found that in general multi-allelic QTL were about as likely to be detected as bi-allelic QTL (Figure S3.3). There did not appear to be any consistent pattern between the QTL detection rates and the rate of quadrivalent pairing (q) in the simulation, with the order of detection power reversing between $q = 0, 0.5$ and 1 at different rates of simulated alleles. However, the high level of variance associated with these data points means that such differences are most likely due to random variation about the means.

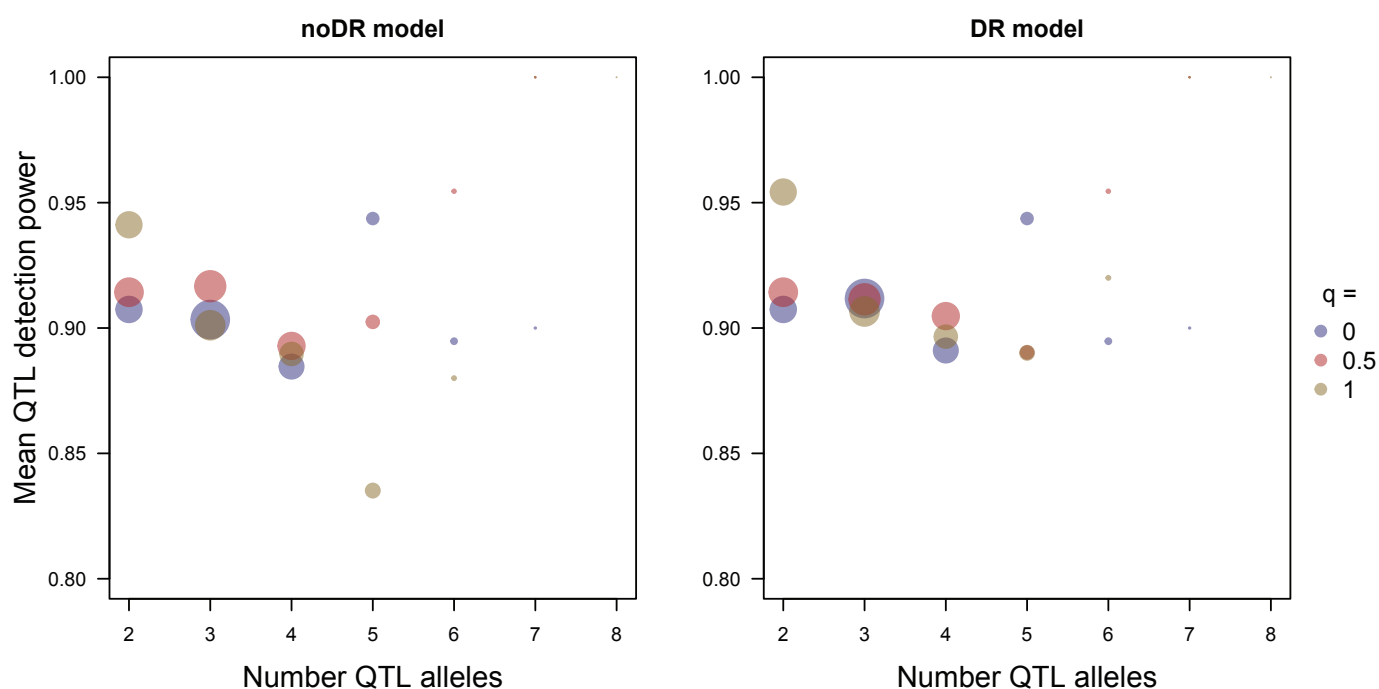


Figure S3.3 Effect of the number of QTL alleles on mean QTL detection power (additive simulation only). Results of QTL analysis using a model that assumes only bivalent pairing (noDR model, left-hand figure) were compared to those of a model that also accommodates quadrivalent pairing and double reduction (DR model, right-hand figure). Points are coloured according to the rate of quadrivalent pairing in the simulation, with the size of each point drawn relative to the number of simulated QTL.

Effect of heritability

QTL with a higher associated heritability were detected more often than those with a lower associated heritability (Figure S3.4). With purely bivalent pairing ($q = 0$), the difference in the range of heritabilities between detected and undetected QTL was quite clear. At higher levels of quadrivalent pairing, we found that QTL with higher associated heritabilities were also being missed / undetected, although we observed a bigger spread of these h^2 values if the noDR model was used instead of the DR model.

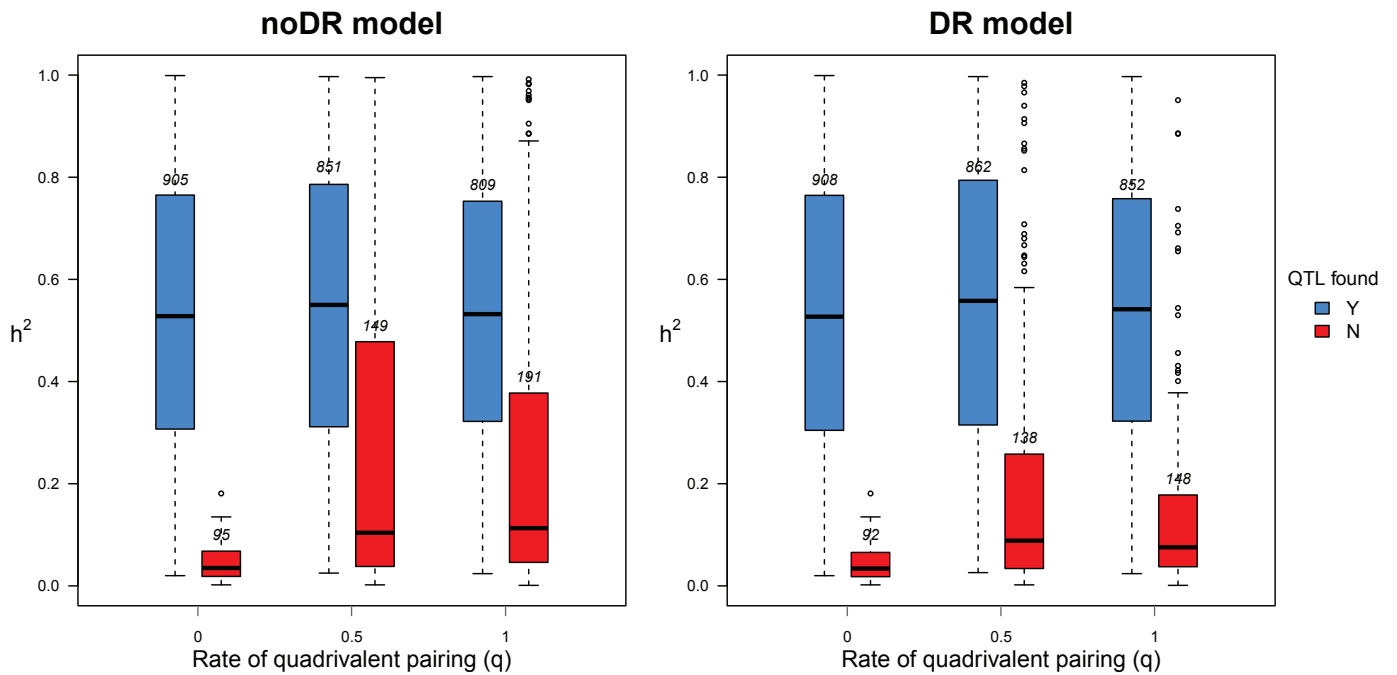


Figure S3.4 Range of heritabilities (h^2) associated with detected (blue boxes) and undetected QTL (red boxes). The results from the analysis using the noDR model (no double reduction) are shown on the left, with the DR model results (including double reduction in the QTL model) are shown on the right. Datasets from populations simulated with three different rates of quadrivalent pairing ($q = 0, 0.5$ and 1) were used, with the number of data points printed above the box (*i.e.* above the 75th percentile).

Performance of the BIC

We tested the performance of the Bayesian Information Criterion (BIC) in correctly determining the QTL configuration (parental origin of the functional QTL alleles) and the QTL mode of action (additive or dominant). On the whole, the performance of the BIC approach to QTL model detection was relatively poor, particularly in correctly determining the QTL configuration (Figure S3.5). This contrasts with the high accuracies achieved in the bi-allelic QTL setting (main text). Only at the highest level of multivalent pairing ($q = 1$, *i.e.* only multivalent pairing) was there any significant difference found, with the DR model performing significantly better.

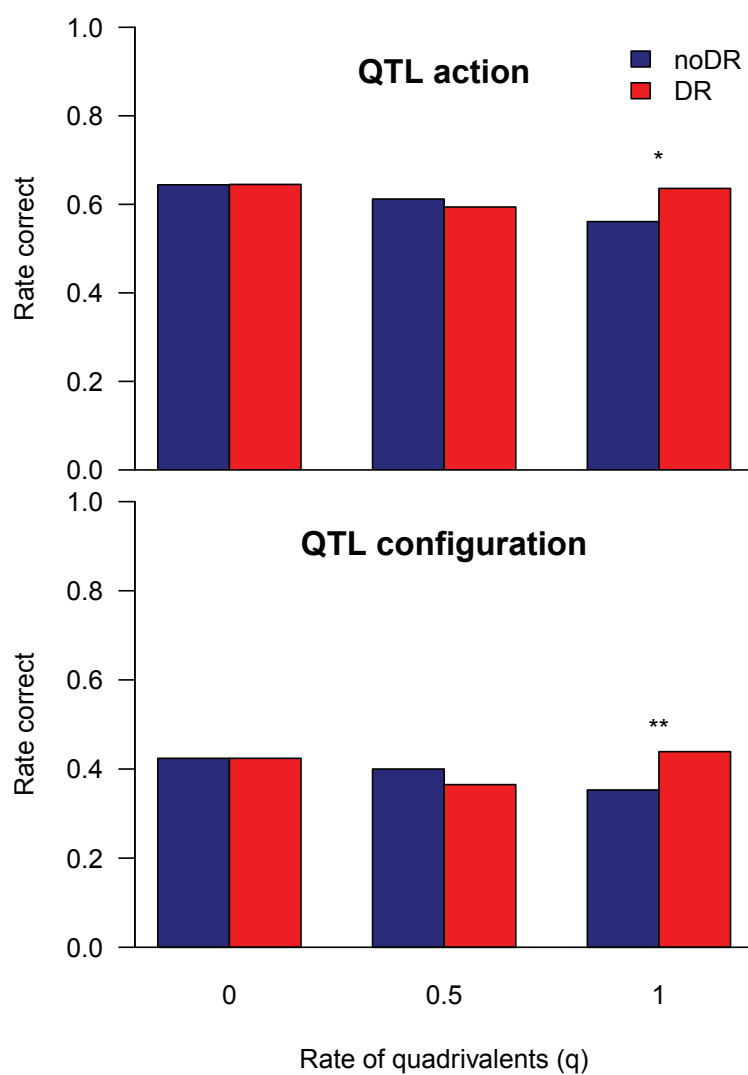


Figure S3.5 Performance of the Bayesian Information Criterion (BIC) in correctly establishing the QTL mode of action (additive, simplex-dominant or duplex-dominant) and QTL configuration (parental origin of the causative QTL alleles). Significance codes using Welch's two-sample t-test: $p < 0.05$ (*); $p < 0.01$ (**).

Influence of the number of QTL alleles on BIC performance

We were also interested in seeing whether there was a connection between the number of QTL alleles and the performance of the BIC model selection procedure. For this we defined a QTL as correctly diagnosed if *both* the QTL configuration (parental origin of QTL alleles) and the mode of action (additive / simplex-dominant / duplex-dominant) were correctly identified. Judging from the results shown in Figure S3.6, it is possible that the rate of correct identification drops somewhat as the QTL complexity increases, although this is most likely a minor effect. Note that there were fewer data points at the higher levels of QTL alleles (to reflect the decreasing likelihood that *e.g.* an octo-allelic QTL would be present in a bi-parental F_1 population, we used a Poisson distribution with $\lambda = 2.5$ to simulate the number of different QTL alleles).

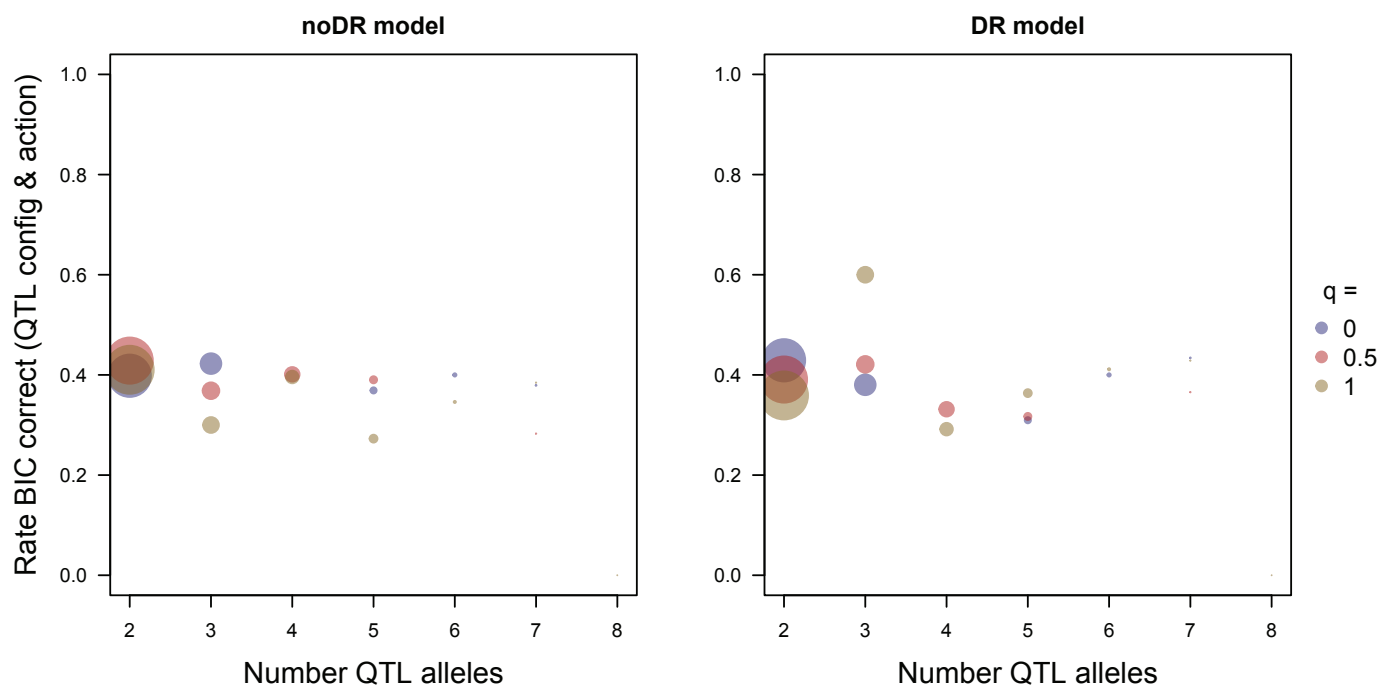


Figure S3.6 Effect of the number of different QTL alleles on the performance of the Bayesian Information Criterion (BIC) in correctly establishing the QTL mode of action (additive, simplex-dominant or duplex-dominant) and QTL configuration (parental origin of the functional QTL alleles). Points are coloured according to the rate of quadrivalent pairing in the simulation, with the size of each point drawn relative to the number of simulated QTL per scenario. Note that results from dominant QTL are also included, ncreasing the frequency of QTL with two alleles.