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How accurate are the marker orders in crop linkage maps generated from large marker datasets?

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Abstract. Marker ordering during linkage map construction is a critical component of QTL mapping research. In recent years, high-throughput genotyping methods have become widely used, and these methods may generate hundreds of markers for a single mapping population. This poses problems for linkage analysis software because the number of possible marker orders increases exponentially as the number of markers increases. In this paper, we tested the accuracy of linkage analyses on simulated recombinant inbred line data using the commonly used Map Manager QTX (Manly *et al*. 2001: *Mammalian Genome* 12, 930–932) software and RECORD (Van Os *et al*. 2005: *Theoretical and Applied Genetics* 112, 30–40). Accuracy was measured by calculating two scores: % correct marker positions, and a novel, weighted rank-based score derived from the sum of absolute values of true minus observed marker ranks divided by the total number of markers. The accuracy of maps generated using Map Manager QTX was considerably lower than those generated using RECORD. Differences in linkage maps were often observed when marker ordering was performed several times using the identical dataset. In order to test the effect of reducing marker numbers on the stability of marker order, we pruned marker datasets focusing on regions consisting of tightly linked clusters of markers, which included redundant markers. Marker pruning improved the accuracy and stability of linkage maps because a single unambiguous marker order was produced that was consistent across replications of analysis. Marker pruning was also applied to a real barley mapping population and QTL analysis was performed using different map versions produced by the different programs. While some QTLs were identified with both map versions, there were large differences in QTL mapping results. Differences included maximum LOD and R^2 values at QTL peaks and map positions, thus highlighting the importance of marker order for QTL mapping.

Additional keywords: linkage analysis, marker ordering, accuracy, weighted accuracy of marker order scores, QTL mapping, quality, hardness.

Introduction

Today linkage maps exist for the majority of crop species, and the scientific literature consists of the thousands of reports of quantitative trait loci (QTLs) that have been identified for important traits. QTL mapping data may provide the foundation for using markers in marker assisted selection (MAS) breeding schemes and map-based cloning of genes (Francia *et al*. 2005).

QTL mapping involves two main stages: linkage map construction and QTL analysis (Collard *et al*. 2005). Map construction involves producing a population such as F_2, backcross, or recombinant inbred (RI) populations that segregate for at least one trait of interest. This mapping population is genotyped with molecular markers. The marker data are later analysed to generate a genetic map consisting of linkage groups, each containing a group of markers in a defined order, with genetic distances in centiMorgans (cM) calculated between markers. The linkage map is then used to identify genomic regions associated with a trait, using any of the available QTL analysis methods.

The most commonly used programs for linkage map construction include Mapmaker/EXP (Lander *et al*. 1987; Lincoln *et al*. 1993), MapManager QTX (MMQTX; Manly *et al*. 2001), and JoinMap (Stam 1993). In wheat and barley molecular genetic research in Australia, MMQTX is the most commonly used program because it is freely available, user-friendly, and offers many options for both linkage and QTL analysis (e.g. special issues of *Australian Journal of Agricultural Research* Vol. 52 (2001) and 54 (2003) for wheat and barley, respectively).

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The mapping populations that have typically been used consist of 90–200 individuals derived from a bi-parental cross. The number of markers used to construct a map varies according to the purpose of the study, ranging from ~100 markers to several thousand markers. However, the number of markers required for even genome coverage will depend on the number of chromosomes and length of chromosomes for a given species. For the construction of framework linkage maps, markers that are spaced every 10–20 cM are adequate for QTL mapping (Darvasi *et al*. 1993). In more recent years, however, high-throughput genotyping techniques such as amplified length fragment polymorphism (AFLP, Vos *et al*. 1995) and diversity arrays technology (DArT, Jaccoud *et al*. 2001) have been used for rapid map construction.

One problem associated with map construction using datasets containing large numbers of markers scored on relatively modest population sizes is the difficulty in accurately determining marker order, which is critical for accurate QTL analysis. Recombination events (or crossovers) are the critical information required for estimating marker order and distances. The amount of information from recombination events from within a given population is determined by the population size, population structure, and marker inheritance. Larger populations of the same type provide more opportunities to sample recombinants between particular markers. Populations developed from multiple rounds of recombination, such as RI, also accumulate more recombination events in each individual with F_2 or backcross 1 F_1 (BC_1F_1). In the case of markers where heterozygous individuals are present, the inheritance patterns of the markers (co-dominant *v*. dominant) reduce the amount of information that can be obtained from each recombination event.

The complexity of the marker ordering problem is related to the number of markers in a linkage group, the length of the linkage group in cM, and the amount of information available about recombination (i.e. recombination events or crossovers). The number of possible marker orders is given by $n!/2$ where n equals the number of markers in the linkage group (Liu 1998). Larger amounts of information (recombination events) are available to order more distantly linked markers because as markers are more closely linked, the number of recombination events sampled in a population decreases while the number of markers to be ordered increases.

To deal with this problem of closely linked markers, several new marker ordering algorithms have been developed and implemented in programs such as JoinMap 4.0 (Jansen *et al*. 2001), RECORD (Van Os *et al*. 2005), and Multipoint (Mester *et al*. 2003, 2004). We have observed that the stability of marker orders (i.e. consistency of orders after repeated analysis of an identical dataset) is related to the number of markers in a mapping dataset, a feature that has not received much attention in the literature.

The aims of this study were to: (*1*) evaluate the accuracy of marker orders by analysing simulated datasets using different software (MMQTX and RECORD); (*2*) use two calculations including a weighted rank accuracy of marker order (WRAMO) score to measure marker order accuracy in comparison with a known order; and (*3*) evaluate the effect of reducing the size of the marker datasets (by pruning closely linked markers) on the accuracy and stability of marker orders. We applied these principles to a real barley mapping population and demonstrate the effect on QTL detection.

Materials and methods

Simulated populations

Ten simulated marker datasets consisting of 60 or 61 markers within a single chromosome were generated by QU-GENE (Podlich and Cooper 1998). The mean of map lengths was 96.9 cM and the mean marker spacing was 1.6 cM. Each dataset was simulated for 200 recombinant inbred individuals (with no residual heterozygosity). Marker nomenclature consisted of a 2-digit prefix indicating sample set number (01–10), 'M' for marker, and a 4-digit number indicating marker order. True marker orders were defined in ascending numerical order.

Linkage analysis

Linkage maps for the simulated datasets were constructed using MapManager QTX (MMQTX; version b20) using the Kosambi mapping function (Manly *et al*. 2001). A linkage evaluation criterion of $P = 10^{-6}$ was used and the population type was set as 'self RI'. For RECORD analysis, default settings were used. Three replicates of data analysis using the identical input file were performed in all cases.

Barley mapping population

The real barley mapping population was an F_6-derived RI population produced from a Patty/Tallon cross (hereafter referred to as PT), which consisted of 95 individuals. Marker genotyping for this population using microsatellite (SSR), AFLP and DArT markers and linkage analysis has been previously reported (Wenzl *et al*. 2006; Fox *et al*. 2007). Linkage analysis using MMQTX and RECORD was performed using the settings described above except that a linkage evaluation criterion of $P = 0.0001$ (logarithm of odd (LOD) value = 3.0) was used in MMQTX. 'Distribute' and 'Ripple' functions were used to incorporate unlinked markers into existing linkage groups and improve marker orders. Using RECORD, 'count' data were used to select the 'best' order of replicate runs (i.e. the order with the lowest number of recombination events). SSR and DArT marker locations as identified from the DArT consensus map (Wenzl *et al*. 2006) were used to assign linkage groups to barley chromosomes.

Measurement of accuracy of marker orders

Two measurement scores were used to compare marker orders: % correct marker positions (referred to as % accuracy), and a novel, weighted rank accuracy marker order (WRAMO) score. The first measurement score was calculated as the percentage of correct marker positions and divided by the total number of markers. Calculations were performed by comparing the true order with the observed marker order, using Microsoft Excel 'IF' or 'EXACT' functions. The WRAMO score was calculated from the sum of absolute values of true minus observed ranks (of marker positions) divided by the total number of markers within the linkage group. A perfect marker order has a WRAMO score of zero. From non-zero

values (which can be calculated when marker orders are not perfect), averages, maximum, and minimum values were also calculated in order to provide an intuitive measure of the extent of error of markers from their true location. Duplicate or binned markers (i.e. those with identical segregation patterns) were excluded from the analysis. MapChart (Voorrips 2002) was used to produce graphical comparisons of marker orders using the 'show homologues' setting. Binned markers were retained in graphical comparisons.

Additional criteria for evaluating maps

In addition to the accuracy of marker order, other characteristics were calculated to evaluate their correlation with marker accuracy. These included number of crossovers, map length, and sum of adjacent LOD values (SALOD). SALOD is a commonly calculated parameter in linkage analysis and is used as an optimisation criterion in MMQTX. These characteristics were taken directly from the Stat Window of MMQTX or by importing Stat Windows into Excel and using basic functions.

Marker pruning

Marker datasets were manually reduced or pruned by using the Stat Window in MMQTX. For the simulated data, 3 levels of pruning were performed: level 1, deleting markers with identical segregation patterns and markers with 0 or 1 crossover between flanking markers (<0.3 cM); level 2, markers with 2 or less crossovers were deleted (0.5 cM

Table 1. Comparison of true, MMQTX and RECORD maps produced from simulated data

No. markers, number of markers in linkage group; Xovers, crossovers or recombination events; map length, calculated using Kosambi mapping function within MMQTX; SALOD, scores from Stat Window in MMQTX

Dataset[A]	No. of markers	% accuracy[B]	WRAMO score[B]	Ave. non-zero[B]	Max. non-zero[B]	Min. non-zero[B]	Xovers	Map length (cM)	SALOD
				TRUE					
1	60	100	0	–	–	–	339	92.2	2947
2	61	100	0	–	–	–	279	74.3	3081.3
3	60	100	0	–	–	–	354	97.3	2935.2
4	61	100	0	–	–	–	332	89.1	2999.2
5	60	100	0	–	–	–	458	126.5	2775.5
6	60	100	0	–	–	–	326	87.8	2961.5
7	61	100	0	–	–	–	360	98	2973.8
8	60	100	0	–	–	–	306	81.5	2979.9
9	61	100	0	–	–	–	377	103.2	2946
10	61	100	0	–	–	–	433	118.7	2861.8
Mean	60.5	100	0	–	–	–	356.4	96.9	2946.1
				MMQTX					
1	60	64.7	2.86	7.1	16	1	487	179.1	2860.9
2	61	64.9	3.51	9.1	18	1	421	121.2	2942.4
3	60	3.8	12.31	12.3	33	1	552	167.9	2767.6
4	61	94.6	0.07	1.3	2	1	328	85.9	3006.5
5	60	30.9	11.82	17.1	35	1	637	270.9	2680.1
6	60	80	0.68	3.4	7	2	354	94.5	2934.7
7	61	100	0	–	–	–	360	95.3	2973.8
8	60	77.8	0.81	3.7	7	1	346	94.2	2946.6
9	61	74.1	0.41	1.6	3	1	391	104.5	2934.2
10	61	64.3	1.46	4.1	9	1	520	149	2794.6
Mean	60.5	65.5	3.39	6.6	14.4	1.1	439.6	136.3	2884.1
				RECORD					
1	60	96.1	0.039	1	1	1	339	89.8	2949.9
2	61	96.4	0.036	1	1	1	279	72.7	3080.8
3	60	96.1	0.039	1	1	1	354	94.2	2935.2
4	61	94.6	0.071	1.3	2	1	328	86.1	3006.5
5	60	100	0	–	–	–	458	122.2	2775.5
6	60	96	0.04	1	1	1	322	84.8	2967.3
7	61	100	0	–	–	–	360	95.3	2973.8
8	60	100	0	–	–	–	306	80	2979.9
9	61	89.7	0.138	1.3	2	1	377	100	2946
10	61	100	0	–	–	–	433	115.4	2861.8
Mean	60.5	96.9	0.0363	1.1	1.3	1.0	355.6	94	2947.7

[A]Simulated RI datasets based on true, MMQTX, and RECORD marker orders.
[B]Full details explained in Materials and methods.

resolution); and level 3, markers with 3 or less crossovers were deleted (1 cM resolution). For the PT population, markers were pruned at the 1 cM resolution level because the population size was <100 individuals (level 3 pruning) and hence the map could not be resolved below 1 cM. Markers were prioritised for pruning based on marker type and level of missing data.

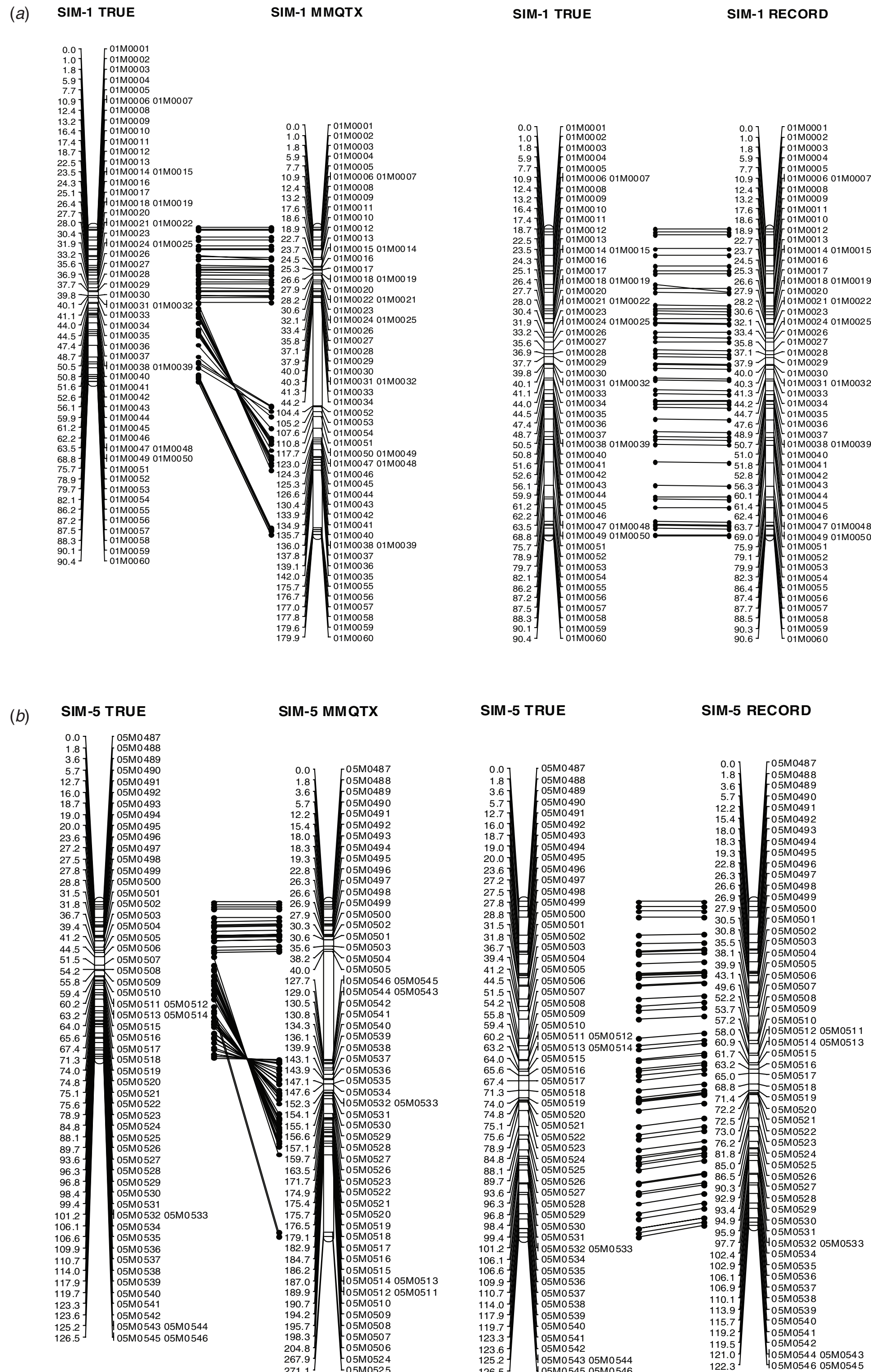

Fig. 1. Graphical comparison of marker orders between MMQTX and RECORD. (*a*) Dataset #1. (*b*) Dataset #6. Map distances are given on the left-hand side of each LG.

For marker type, markers were ranked in order of preference for being retained as SSRs, DArTs, and AFLPs, listed in order of preference from high to low. Within DArT markers, the marker quality (Q) value reflecting the confidence of genotype calling was used for pruning; DArTs with lower quality were given the first preference to be deleted.

Trait data and QTL analysis

QTL analysis was performed using Windows QTL Cartographer version 2.5 (Wang *et al*. 2007). Marker and trait data were exported from MMQTX into Windows QTL Cartographer. The 'hide redundant loci' function was applied to exclude redundant markers from the QTL analysis within MMQTX before exporting. Composite interval mapping was performed using a forward regression model, and a LOD threshold of 2.5 was used to declare significance of a QTL.

Ten quantitative quality traits were used for QTL analysis. These traits concerned particle size index (PSI), near-infrared reflectance (NIR) spectroscopy estimates of Montana State University Particle Size (PS), and hardness (single-kernel characterisation system; SKCS). Trait data were collected at two sites in 2002 and/or 2003 and full details were previously reported by Fox *et al*. (2007).

Results and discussion

Marker ordering is of critical importance for QTL mapping but also for subsequent research such as comparative mapping, map-based cloning, or MAS. Despite the importance, testing the effects of different algorithms and analysis settings on marker ordering in crops has received little attention in the literature, with some notable exceptions (Ruiz and Asins 2003; Wu *et al*. 2003; Lehmensiek *et al*. 2005). In linkage map construction involving *Citrus* spp., variation in marker orders was caused by the inclusion of new markers, lower LOD thresholds for mapping, and mapping software (Ruiz and Asins 2003). In a map curation project of Australian wheat maps, data checking and re-analysis led to revisions in map lengths and marker orders, which subsequently affected QTL analysis (Lehmensiek *et al*. 2005).

Several methods have been adopted for linkage analysis. These include minimising the sum of adjacent recombination fractions (SARF) or the product of adjacent recombination

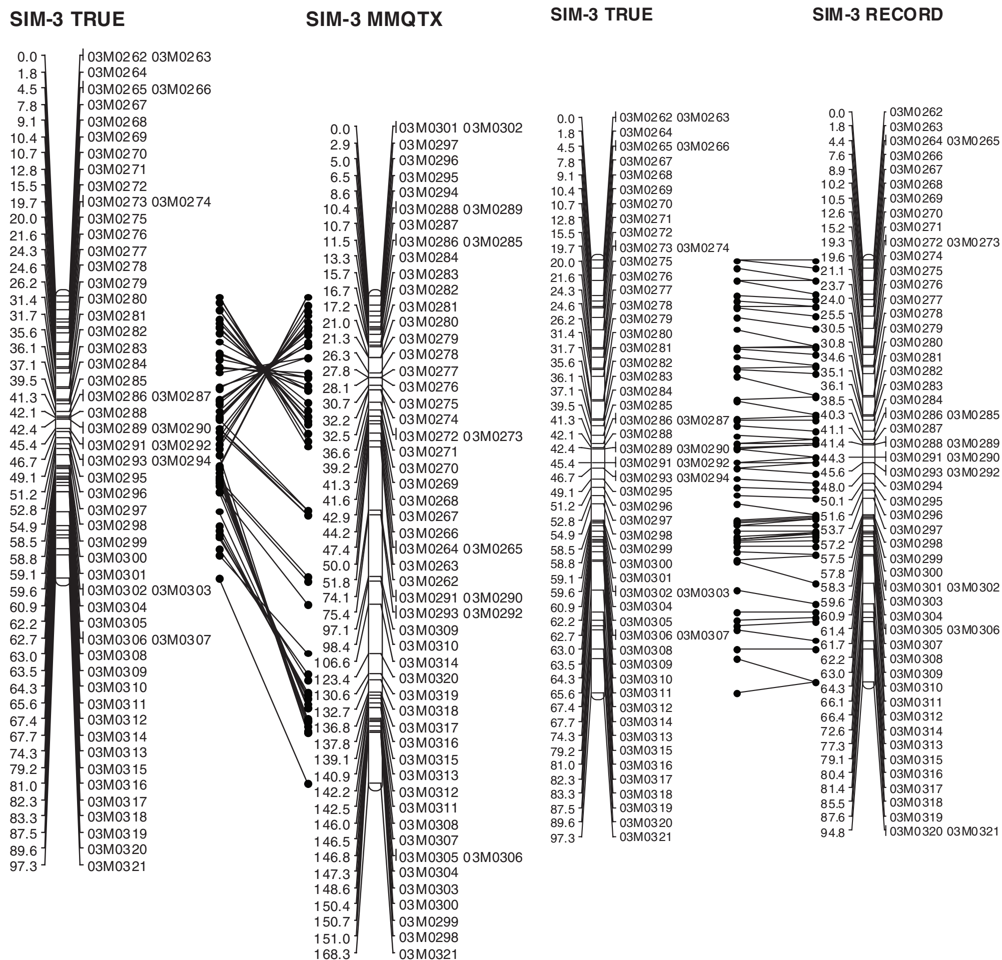

Fig. 2. Comparison of the true marker order with maps generated by MMQTX and RECORD based on simulated dataset #3. Map distances are detailed on left-hand side of each LG.

fractions (PARF), maximising the sum of adjacent LOD scores (SALOD), maximum likelihood, and seriation (Liu 1998). Based on simulated data for doubled haploid populations, 5 different methods (maximum likelihood, SALOD, SARF, PARF, and seriation) were found to have almost identical ordering power for marker spacings of 5, 10, and 15 cM (Wu *et al*. 2003).

Accuracy of marker order

In this paper, we were predominantly concerned with marker order accuracy in large marker datasets (i.e. >50 markers per LG). Our previous empirical observations have indicated that MMQTX and RECORD produce virtually identical marker orders when the marker datasets are relatively small (<30 markers per LG), with on average relatively wide marker spacing (e.g. 5 cM). Table 1 summarises the features of true maps (based on simulated data) and maps generated with MMQTX and RECORD. The % accuracy of marker orders generated by MMQTX ranged from 3.8 to 100% whereas the accuracy of RECORD maps ranged from 89.7 to 100%. Using RECORD, 4 out of 10 maps were 100% accurate and 5 of the 10 were >94% accurate. For two datasets (#4 and #7), the maps produced by MMQTX and RECORD were identical.

Comparisons of marker orders of true *v.* MMQTX maps indicated frequent segments of 'flipped' markers in which the marker order sequence was inverted compared with the true order. Examples of this are shown in Fig. 1. It is worth noting that although the calculation of marker order accuracy can be extremely low, there may still be considerable colinearity between maps, due to the formula used for calculation of marker accuracy (Fig. 2). To complement this accuracy measure, we formulated the weighted rank accuracy of marker order (WRAMO) score, which takes into account the extent to which markers deviate from their true position. WRAMO scores tend to zero as the observed marker order approaches the true order. In other words, the smaller the WRAMO score, the more accurate the marker order; the perfect marker order equals zero. Averages, maximum values, and minimum values from non-zero provide a measure of the extent of error of markers from their true location and also the magnitude of deviations in regions containing errors in marker order (maximum and minimum). These scores can be multiplied by the average distance between flanking markers in order to provide an intuitive measure of the extent of the error of marker order in a map distance. For example, MMQTX maps #1, 2, and 10 have very similar % accuracy scores but different WRAMO scores (Table 1). MMQTX maps #1 and #2 each contain a large flipped segment whereas MMQTX map #10 contains two smaller flipped segments (Figs 1*a* and 3). In our opinion, MMQTX map #10 may be considered the more accurate marker order of the three maps because it has the lowest WRAMO score and the least severe deviations from the true order. Inspection of the average, maximum, and minimum non-zero scores indicates

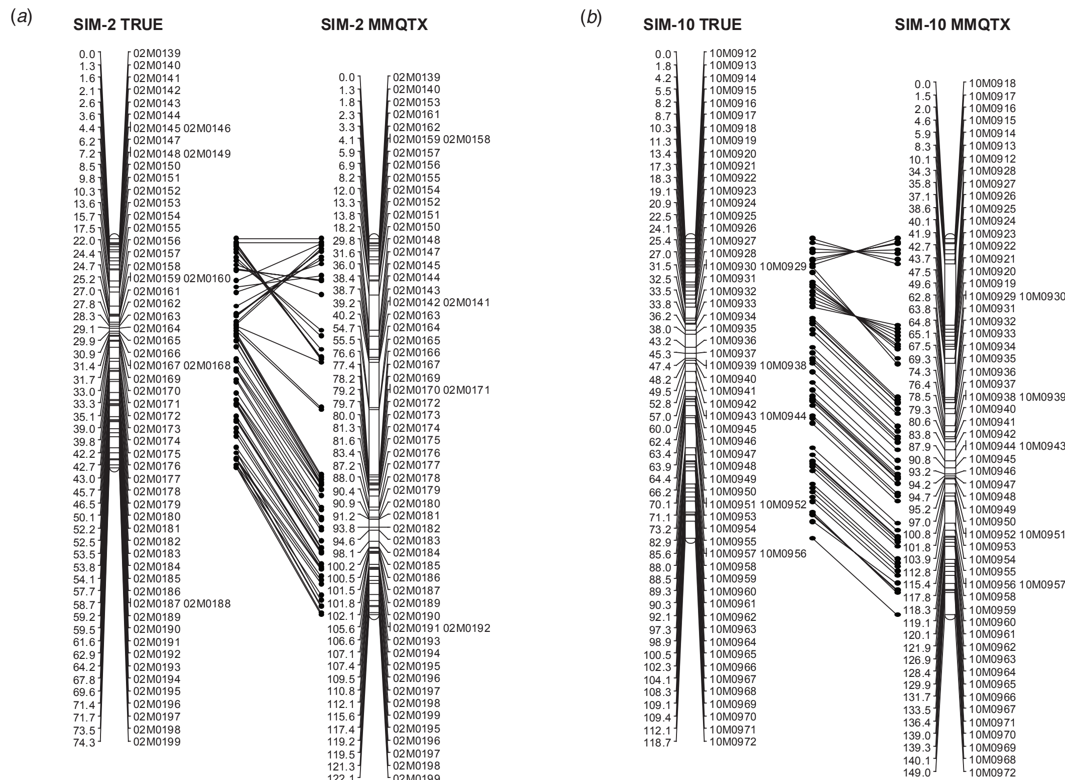

Fig. 3. Graphical comparison of true marker order *v.* marker order generated by MMQTX for simulated datasets #2 and #10. The MMQTX maps have % accuracy scores of 64.9 and 64.3, respectively.

that MMQTX #10 has the smallest error of marker order on average, and the smallest deviation from the true marker order in erroneous regions. We propose that the WRAMO scores can be used in conjunction with % accuracy scores in order to evaluate observed marker orders in relation to known marker orders.

Additional map evaluation criteria

Several additional criteria were used to determine if there was a correlation with the true marker order. Number of crossovers forms the basis for RECORD since the algorithms minimise the total number of recombination events (Van Os *et al*. 2005). Interestingly, RECORD produced two marker orders that had fewer recombination events compared with the true maps (see datasets #4 and #6). However, for 8 out of 10 datasets, the number of crossovers calculated by RECORD was identical to the numbers derived from the true maps. Generally, there was a negative correlation between the number of crossovers and the accuracy of marker order (Table 1).

Total map length has been used to evaluate different marker orders, and generally the shorter the map (produced from the datasets under investigation) the more accurate the marker order. In two cases using simulated data, the map length for MMQTX maps was smaller than the true maps (#4 and #7); however, in the other cases, map lengths were greater. Four out of the 10 MMQTX maps were only <10 cM longer compared with the true maps, however 5 MMQTX maps (#1, 2, 3, 5, and 10) were >30 cM longer. The largest difference was observed for dataset #5, for which the MMQTX map was 144 cM longer than the true map. Interestingly, 3 RECORD maps were <1.5 cM shorter than the true maps (i.e. non-identical), which indicates that true datasets may not always produce the shortest maps with the smallest possible number of crossovers (Table 1). The shorter RECORD maps can be explained in terms of sampling of recombination events in the mapping populations and the nature of the algorithm, since the RECORD algorithm seeks to minimise the number of crossovers to produce a marker order. In most cases the real order is the optimal solution in terms of number of crossovers and map length; however, rare events do occur and it is possible (as demonstrated here) that a true map may be larger than the estimated map. In general, these differences will be of minor importance for QTL mapping, although they could be more important in fine mapping.

The differences in marker orders were attributable to the ordering algorithms and optimality criteria used by the software MMQTX and RECORD. The optimality criterion used in MMQTX is based on SALOD (Manly *et al*. 2001), whereas in RECORD, the optimality criterion is based on COUNT, the number of recombination events (Van Os *et al*. 2005). SALOD is a commonly used algorithm for producing marker orders and is used by MMQTX (Olson and Boehnke 1990). The higher the SALOD score, the more accurate the

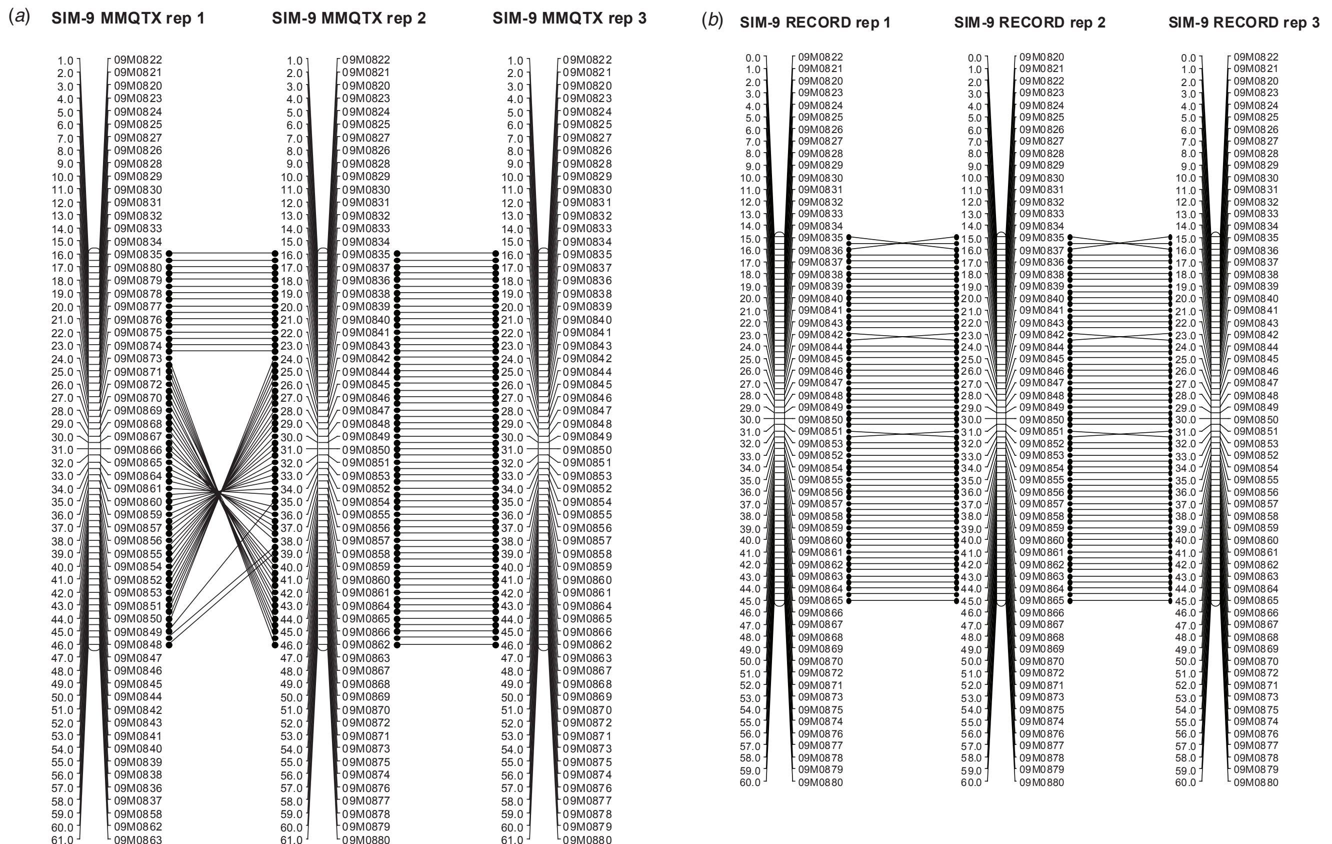

Fig. 4. Comparison of three independent linkage analyses with identical datasets using (*a*) MMQTX and (*b*) RECORD.

marker order of a map. In our results, SALOD did not always reliably predict the accuracy of marker order although the MMQTX maps (which were less accurate than the true or RECORD maps) had lower SALOD values. For example, MMQTX map #4 and RECORD map #4 both had higher SALOD values than the true map. Comparison of SALOD with number of crossovers between true, MMQTX, and RECORD maps clearly indicated that the minimum number of

Table 2. Comparison of maps generated from full and pruned marker data

Level 1 indicates pruning to remove markers with either 0 or 1 crossovers between adjacent pairs. Level 2 indicates pruning to remove markers with <3 crossovers between adjacent pairs (0.5 cM resolution). Level 3 indicates pruning to remove markers with <4 crossovers between adjacent pairs (1 cM resolution)

Dataset	No. of markers	% accuracy	WRAMO score	Ave. non-zero	Max. non-zero	Min. non-zero	Map length (cM)	Ave. spacing (cM)	Instability
				Full dataset					
1	60	96.1	0.039	1	1	1	89.8	1.6	*
2	61	96.4	0.036	1	1	1	72.7	1.2	*
3	60	96.1	0.039	1	1	1	94.2	1.6	
4	61	94.6	0.071	1.3	2	1	85.9	1.5	
5	60	100	0	–	–	–	122.2	2.1	*
6	60	96	0.04	1	1	1	84.8	1.5	*
7	61	100	0	–	–	–	95.3	1.6	
8	60	100	0	–	–	–	80	1.4	
9	61	89.7	0.138	1.3	2	1	100	1.7	*
10	61	100	0	–	–	–	115.4	2	*
Mean	60.5	97.3	0.0363	1.1	1.3	1.0	94	1.6	
				Pruning – level 1					
1	50	100	0	–	–	–	87.6	1.8	
2	54	100	0	–	–	–	72.8	1.4	
3	50	96	0.04	1	1	1	94.2	1.8	*
4	55	94.5	0.073	1.33	2	1	85.9	1.7	*
5	53	100	0	–	–	–	122.3	2.4	
6	49	95.9	0.041	1	1	1	84.1	1.8	
7	52	100	0	–	–	–	95.3	1.9	
8	54	100	0	–	–	–	80	1.6	*
9	54	92.6	0.074	1	1	1	98	1.9	*
10	55	96.4	0.036	1	1	1	114.9	2.1	
Mean	52.6	97.5	0.0264	1.066	1.2	1	93.5	1.8	
				Pruning – level 2					
1	44	100	0	–	–	–	87.4	2	
2	46	100	0	–	–	–	72.9	1.6	
3	48	100	0	–	–	–	84.6	2.3	
4	52	100	0	–	–	–	85.5	1.7	
5	50	100	0	–	–	–	122.3	2.5	
6	49	95.7	0.041	1	1	1	84.1	1.9	
7	49	100	0	–	–	–	95.3	2	
8	52	100	0	–	–	–	80	1.7	
9	53	100	0	–	–	–	98	2	
10	53	96.2	0.038	1	1	1	114.9	2.2	*
Mean	49.6	99.2	0.0079	1	1	1	92.5	2	
				Pruning – level 3					
1	38	100	0	–	–	–	87.4	2.4	
2	34	100	0	–	–	–	72.7	2.2	
3	36	100	0	–	–	–	85	2.4	
4	41	100	0	–	–	–	85.6	2.1	
5	43	100	0	–	–	–	122.6	2.9	
6	40	100	0	–	–	–	84.3	2.2	
7	40	100	0	–	–	–	95.5	2.4	
8	39	100	0	–	–	–	78.5	2.1	
9	38	100	0	–	–	–	97.7	2.6	
10	45	100	0	–	–	–	114.1	2.6	
Mean	39	100	0	–	–	–	92.3	2.4	

*Indicates that differences were obtained between 3 replications of RECORD analysis using identical dataset.

crossovers is a far superior determinant of marker order accuracy compared with SALOD. Furthermore, in this study, SALOD values of true maps were sometimes higher than those generated by the software programs used for linkage analysis (e.g. RECORD maps #1 to #4 *v.* the true maps).

Effect of reducing marker density by pruning on marker order accuracy

An interesting finding from this study was that marker orders differed between independent analyses of the identical marker data input file (using identical analysis parameters). This has been seldom reported in the literature but is not unexpected given that even in datasets without errors there may be several marker orders that are equally likely, or where the software invokes an iterative process, the number of iterations may be insufficient to identify the optimal order from a large number of highly likely orders. Van Os *et al*. (2005) reported that when good quality data were used, the replicate solutions produced by RECORD were all identical. However, with the perfect simulated marker data used in this study (i.e. no missing data and no genotyping errors), differences between replicates typically occurred in regions where the markers were tightly linked. These regions included flanking markers with only a few crossovers (<1 cM spacing). We refer to this as marker order instability. Using MMQTX, differences between repetitions of the same marker data input file with the same analysis parameters could be quite drastic, whereas using RECORD, minor differences between repetitions also occurred, frequently in regions containing markers with identical segregation patterns and areas where markers were tightly linked with only a few crossovers between adjacent markers (Fig. 4). This can be explained by the RECORD algorithm used, which begins with a randomly chosen pair of markers and adds one marker at a time to determine marker order (Van Os *et al*. 2005).

Our previous empirical observations from linkage analysis indicated that marker orders from marker datasets with lower marker densities were stable and did not differ between replicated analyses. This enhanced marker order stability in less dense datasets is the expected result of the greater amount of information available to order these markers compared with closely linked markers. To demonstrate the effect of reducing marker density on the stability of marker orders, we pruned the simulated datasets at different levels and determined the effect on marker order accuracy and stability.

The results indicated that the accuracy and stability of the marker order generally improved with more widely spaced marker datasets (Table 2). This is in spite of the fact that comparisons of marker order accuracy between datasets may not always be reliable because % accuracy is calculated as the percentage of incorrect matches divided by the total number of markers in the linkage group (which is smaller for pruned datasets). Nevertheless, the results clearly indicated that the mean marker order accuracy increased as the marker density was reduced by pruning. Interestingly, the map lengths for each dataset were very similar after pruning, despite the fact that >25% of markers were pruned from the original dataset.

Effect of marker order on QTL mapping

The effect of map accuracy on QTL analysis has been previously investigated using simulated data. In the study by Dodds *et al*. (2004), the accuracy of maps had little or no effect on the ability to detect QTLs, significance levels with which QTLs were detected, and relative placement of QTLs. This was in contrast to the study by Lehmensiek *et al*. (2005) who discovered that QTL peaks and LOD scores were drastically affected by marker order, using real mapping data from wheat populations.

The results in this study, which were based on 10 traits in the PT population, also suggest that QTL analysis may be greatly affected by the accuracy of marker order within a linkage map. Although some QTLs were consistently detected with both MMQTX and RECORD maps, the maximum LOD values at QTL peaks and map position of QTLs differed considerably. In some cases, QTLs were detected on some maps but not detected on others.

A comparison of linkage maps for PT linkage group 5H-2 (which corresponds to 5HL but could not be linked to 5HS) is shown in Fig. 5, and QTL mapping results are shown in Fig. 6. QTL analysis of linkage group 5H-2 indicated QTLs for three traits (2, 5, and 6) using the MMQTX map. For trait 2, a QTL of LOD 5.6 was detected using the MMQTX map; however, the position and maximum LOD value dropped markedly to LOD 3.7 using both RECORD chromosome maps. For trait 5, a QTL of LOD 7.1 was detected using the MMQTX map; however, the position and maximum LOD value

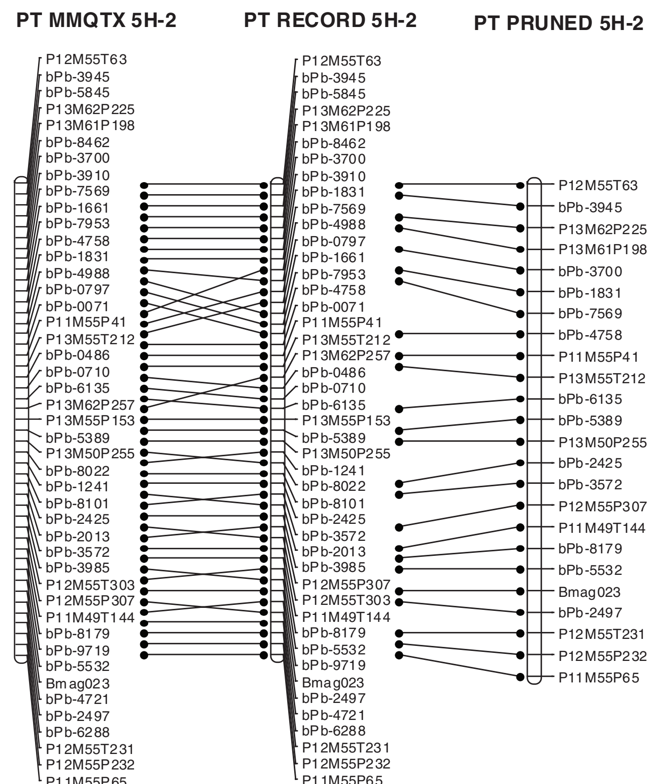

Fig. 5. Graphical comparison of marker order in chromosome 5H-2 in the PT mapping population.

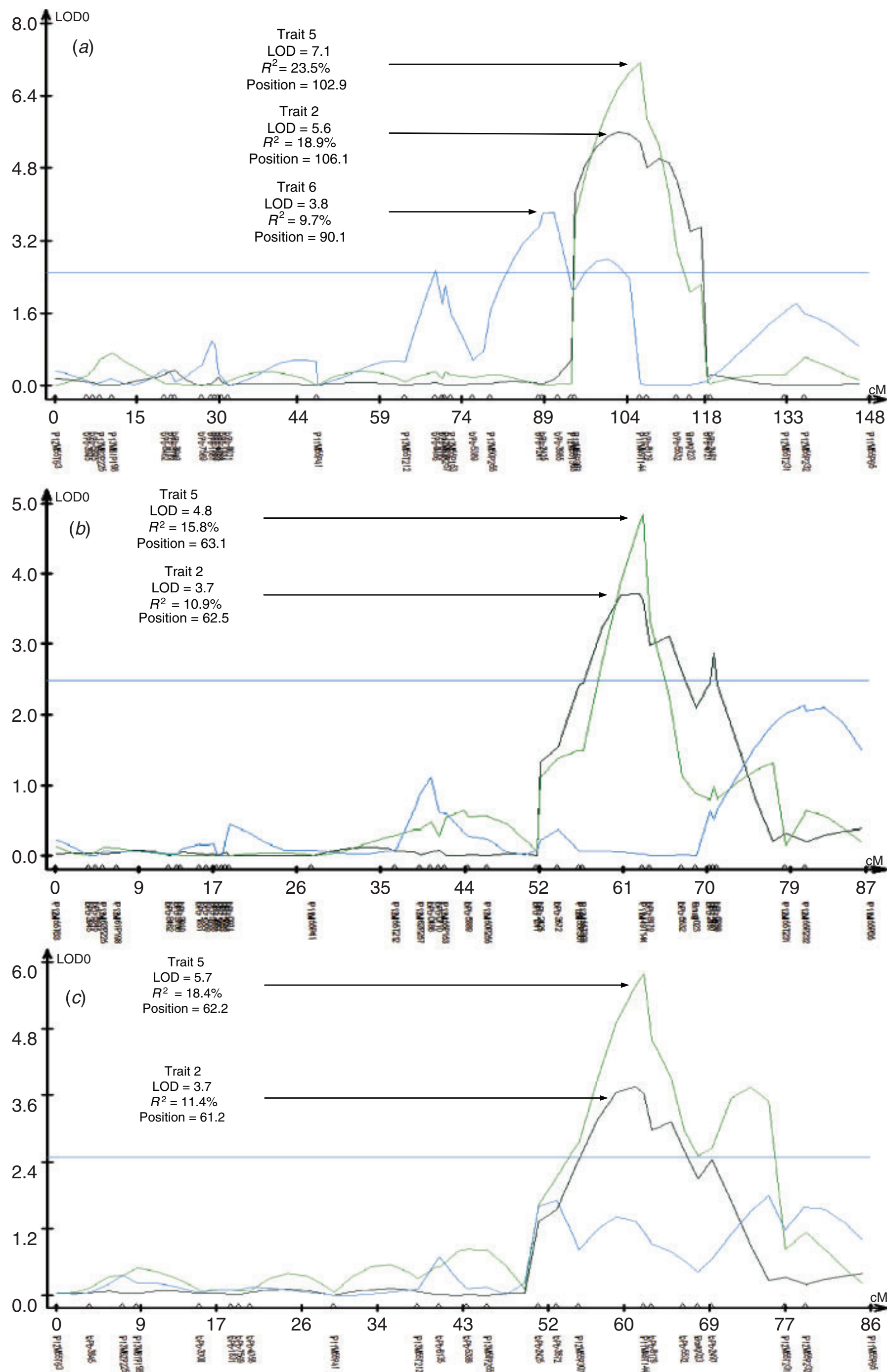

Fig. 6. LOD plots showing differences in composite interval mapping profiles between (*a*) MMQTX, (*b*) RECORD and (*c*) pruned RECORD maps for LG 5H-2 in the PT mapping population.

dropped markedly to below 6 using RECORD chromosome maps. The QTL for trait 6 detected at LOD 3.8 using the MMQTX map was not detected as being significant (i.e. <LOD 2.5) using RECORD maps. Furthermore, the R^2 values reflecting the proportion of the phenotypic variance explained by a QTL were considerably higher for the QTLs detected using the MMQTX map. These results clearly illustrate the importance of marker order on the accuracy of QTL analysis.

It is also highly likely that the occurrence of flipped segments may split a single QTL into two if a QTL is located within the flipped segment. Thus, a fake QTL could be detected. Empirical observations in sorghum QTL mapping have shown that more QTLs were detected using preliminary

maps than improved maps generated by RECORD, which supports this idea.

In general, marker pruning had little effect on QTL detection (at least in the case of RECORD maps), which is consistent with the findings by Darvasi *et al*. (1993). We believe, however, that the QTL results obtained with the pruned RECORD maps may be the most accurate because the marker order is accurate. The full results of genetic mapping and QTL analysis of predominantly quality traits in the PT population will be reported elsewhere.

Concluding remarks

We have demonstrated the potential differences that can occur in linkage analysis involving large datasets for RI populations based on two different mapping programs. This has important implications for the validity of many previously published QTL mapping results.

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