

A comparison of bootstrap methods to construct confidence intervals in QTL mapping

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Summary

The determination of empirical confidence intervals for the location of quantitative trait loci (QTLs) by interval mapping was investigated using simulation. Confidence intervals were created using a non-parametric (resampling method) and parametric (resimulation method) bootstrap for a backcross population derived from inbred lines. QTLs explaining 1%, 5% and 10% of the phenotypic variance were tested in populations of 200 or 500 individuals. Results from the two methods were compared at all locations along one half of the chromosome. The non-parametric bootstrap produced results close to expectation at all non-marker locations, but confidence intervals when the QTL was located at the marker were conservative. The parametric method performed poorly; results varied from conservative confidence intervals at the location of the marker, to anti-conservative intervals midway between markers. The results were shown to be influenced by a bias in the mapping procedure and by the accumulation of type 1 errors at the location of the markers. The parametric bootstrap is not a suitable method for constructing confidence intervals in QTL mapping. The confidence intervals from the non-parametric bootstrap are accurate and suitable for practical use.

1. Introduction

In the mapping of quantitative trait loci (QTLs) it is valuable to have some idea about how accurately a locus is mapped. For example, molecular biologists require a precise estimate of location for positional cloning and breeders who may wish to incorporate genes need to know the optimum length of chromosome to introgress. In recognition of this problem it is common to give confidence intervals stating the probability (P) that an interval on the chromosome contains the QTL. It is of fundamental and economic importance to those wishing to use this information that the true probability an interval contains a QTL is close to the probability stated, i.e. a 90% confidence interval should contain a QTL in 90% of all cases.

Methods of calculating confidence intervals vary. The suggestion of Lander & Botstein (1989) was a one LOD support interval (defined by the points on the genetic map at which the likelihood ratio has fallen by

a factor of 10 from the maximum). Van Ooijen (1992), by studying support intervals for four different values of LOD drop, showed that a 95% confidence interval could require up to a two LOD drop for the simulated situations tested (population size = 200 or 400, heritability of QTL = 5% or 10%, backcross and F_2 populations considered). The size of the LOD drop to produce the confidence interval varied with different parameter settings, and the size of confidence intervals produced by the two LOD drop were, in the view of Van Ooijen (1992), large and variable.

Mangin *et al.* (1994) showed the 90% and 95% confidence intervals calculated by a one LOD drop to be biased when the QTL effect was small. Bias was downward, i.e. the proportion of intervals containing the QTL location at the 90% level was less than 90%. Mangin *et al.* (1994) also derived a complex formula for calculating confidence intervals, assuming normality of residuals.

Viisscher *et al.* (1996) suggest a non-parametric bootstrap method (Efron, 1982, chapter 10) applied to QTL mapping and compared it with the LOD drop

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method. The method performed well in comparison with the LOD drop, but generally the estimates were slightly conservative, i.e. the 90% confidence interval contained over 90% of the QTL, particularly for smaller QTL effects. Confidence intervals produced by the method were larger than those produced by the one LOD drop, with intervals under 20 cM in length produced only when population size and QTL effect were high.

Several questions regarding the creation of confidence intervals remain unanswered. It is clear that the LOD drop method is unsatisfactory in populations the size of those used in practice. The non-parametric bootstrap does seem suitable although slightly conservative. However, many QTL mapping populations use more complex pedigrees than the standard backcross or F_2 populations mentioned by Visscher *et al.* (1996). In these more complex pedigrees it is unclear how the non-parametric bootstrap can be performed without dissolving the population structure. Would an alternative method or variation of current methods provide more suitable techniques and results?

Visscher *et al.* (1996) mention that there are many possible bootstrap strategies, of which the non-parametric bootstrap is only one. There are also no fixed guidelines on how to perform bootstrapping under linear models. This gives many options to address the problems of creating confidence intervals and developing the bootstrapping method(s) to create a suitable process for their calculation.

We have investigated, a parametric bootstrap method (Efron, 1982, chapter 5) to determine approximate confidence intervals for the position of a QTL and to study the performance of the non-parametric bootstrap method in more detail. The parametric bootstrap has a large advantage over the non-parametric method in that it can be applied to any population, because the re-simulations can simply mimic the original data structure. The aims of the study are to test, by simulation, how well the parametric bootstrap method works in QTL mapping experiments with experimental populations and to compare the method with the non-parametric bootstrap proposed by Visscher *et al.* (1996). In particular we are interested in the accuracy of the two methods, i.e. whether the QTL appears in the 90% or 95% confidence interval in 90% or 95%, respectively, of the replicates. A secondary consideration is the size of the 90% and 95% confidence intervals, which ideally would be small.

2. Materials and methods

(i) Simulation

Data for N individuals ($N = 200$ or 500) from a backcross population derived from two inbred lines

were simulated. Each individual was assigned a single chromosome of length 100 cM with m evenly spaced markers ($m = 6$ or 11) corresponding to a marker spacing of Δ cM ($\Delta = 10$ or 20). The chromosomes contained a single QTL with an additive effect such that heritabilities of 1%, 5% and 10% were obtained in the backcross population. The position of the QTL (d) was at 45 or 85 cM from one end of the chromosome in the initial analyses, then at all locations between 0 and 50 inclusive in the subsequent work. Haldane's mapping function (Haldane, 1919) was used throughout.

(ii) Model

Data were analysed with the Whittaker regression method (Whittaker *et al.*, 1996). The method performs a multiple regression of phenotypes on pairs of flanking markers and transforms the estimated effects of the two markers, in each regression, to estimates of the QTL effect and its location. This method is preferred to the regression method of Haley & Knott (1992) because of the speed of calculation, but produces equivalent results. Only a single QTL was fitted in the analyses.

(iii) Non-parametric bootstrap

Sampling, with replacement, N individual observations (a single individual's genotype and phenotype maintained together) from a population of size N generated non-parametric bootstrap samples (Visscher *et al.*, 1996). Each sample was analysed and the best estimate of the position of the QTL recorded. After R bootstrap samples (where R is the number of 'new' resampled populations and hence the number of estimates of QTL position) the empirical central 90% and 95% confidence intervals of the QTL position were determined. This was achieved by ordering the R estimates and taking the top and bottom 5th and 2.5th percentile, respectively.

(iv) Parametric bootstrap

The parametric bootstrap uses a Monte Carlo simulation method with original parameter estimates. Samples were generated by analysing the data from a population of size N , recording the estimates of parameters for the best position of the QTL, mean, standard deviation and QTL effect. This information was used to create two normal distributions of individuals with these parameters (one distribution with the QTL and one without). N individuals were randomly assigned a marker genotype; phenotypes were drawn from one of the distributions conditional upon the marker genotype of the individual. This generates a parametric bootstrap sample. Each 'new'

population was analysed and the best estimated position recorded. After R parametric bootstrap samples the empirical central 90% and 95% confidence intervals were calculated as described previously. For each parameter set, there were R re-simulated populations after the initial analysis for each of the 1000 replicates and therefore 1000 ($R+1$) QTL mapping analyses were done.

The difference between the two methods is that the non-parametric method samples from the original data whereas the parametric method samples from the distributions inferred from the analysis of the data.

3. Results

The results for the initial analyses for the non-parametric and parametric bootstraps are shown in Table 1. The analyses of the non-parametric bootstrap have been previously published with these population parameters (Visscher *et al.*, 1996). Our results are in close agreement with this work.

(i) Number of bootstrap samples

Changing the number of bootstrap samples had very little effect on the results. No significant changes in

either confidence intervals or probabilities occurred when R varied from 100 to 1000 (results not shown). For all subsequent analyses $R = 200$ to allow a direct comparison between the results of Visscher *et al.* (1996) and the results for the parametric bootstrap. Although this is suitable for simulation studies because of the large numbers of replicates, real data analysis would preferably use larger bootstrap samples (Efron & Tibshirani, 1993).

(ii) Population size

Comparing top and bottom halves of Table 1 illustrates that increasing population size creates smaller confidence intervals. When $N = 500$ and $\Delta = 10$, the intervals are anti-conservative when the heritability is high for the non-parametric bootstrap, i.e. the proportion of 90% confidence intervals containing the simulated QTL was under 0.9. The differences in confidence interval size with population size are consistent with other studies (Van Ooijen, 1992; Darvasi *et al.*, 1993).

(iii) Heritability

Increasing the heritability of the QTL produces smaller intervals. The probability of detecting the simulated

Table 1. Effect of marker spacing, heritability and population size on confidence intervals for various different bootstrap methods

N	Δ	d	h^2	Non-parametric bootstrap				Parametric bootstrap			
				CI90 ^a	P90 ^b	CI95	P95	CI90	P90	CI95	P95
200	10	45	0.01	84	0.99	92	0.99	84	0.97	93	0.99
			0.05	57	0.94	69	0.97	50	0.91	66	0.97
		85	0.1	37	0.92	47	0.96	27	0.87	40	0.96
			0.01	86	0.91	93	0.97	86	0.90	94	0.97
		—	0.05	61	0.91	74	0.96	52	0.90	68	0.95
			0.1	36	0.91	47	0.96	26	0.87	37	0.95
	20	45	0.01	87	0.98	94	0.99	87	0.98	94	0.99
			0.05	59	0.94	71	0.98	53	0.94	69	0.98
		85	0.1	37	0.91	47	0.95	32	0.94	43	0.97
			0.01	88	0.91	94	0.96	87	0.92	94	0.97
		—	0.05	64	0.89	75	0.94	54	0.94	69	0.98
			0.1	38	0.90	50	0.95	30	0.94	41	0.97
500	10	45	0.01	75	0.97	85	1.00	70	0.95	84	0.98
			0.05	33	0.92	42	0.97	22	0.85	32	0.93
		85	0.1	16	0.89	21	0.94	12	0.79	15	0.86
			0.01	78	0.92	87	0.97	72	0.89	85	0.95
		—	0.05	32	0.92	42	0.96	21	0.85	31	0.94
			0.1	15	0.89	20	0.95	12	0.81	15	0.88
	20	45	0.01	78	0.97	88	0.99	73	0.95	86	0.98
			0.05	32	0.91	42	0.96	27	0.91	36	0.95
		85	0.1	18	0.89	22	0.94	16	0.88	20	0.97
			0.01	82	0.91	90	0.96	74	0.93	86	0.96
		—	0.05	31	0.88	41	0.94	25	0.93	33	0.96
			0.1	16	0.89	20	0.93	15	0.88	19	0.96

^a CI x is the mean width of the x % confidence interval (in cM).

^b P x is the proportion of the x % confidence intervals that contain the QTL.

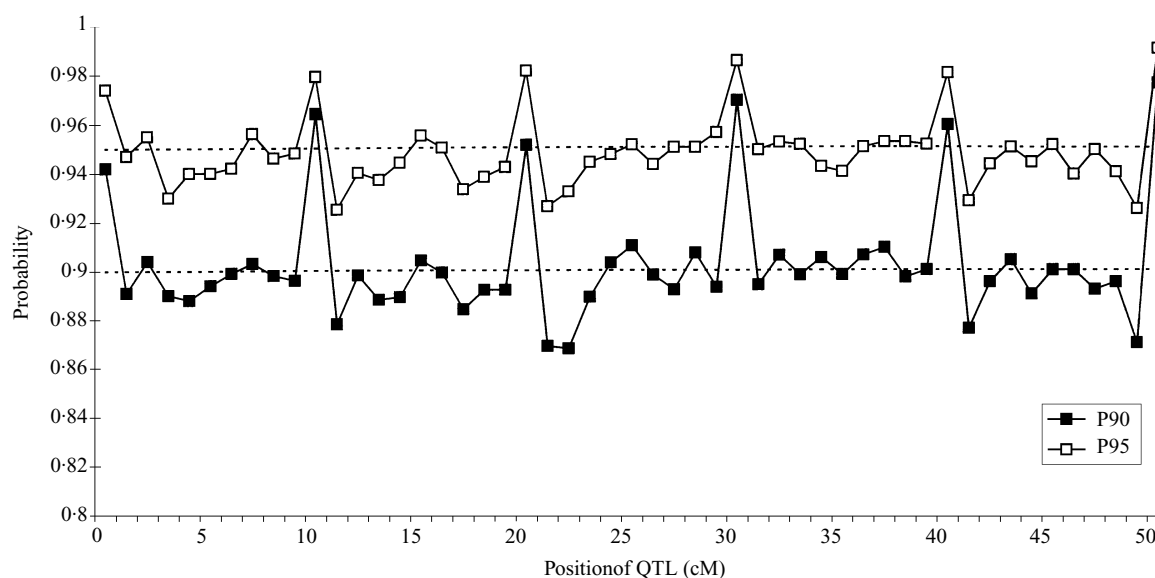


Fig. 1. $N = 500$, $h^2 = 0.1$, $\Delta = 10$. The probability a confidence interval contains a QTL at different simulated locations on a chromosome using the non-parametric bootstrap.

QTL when $h^2 = 0.01$ is low and the number of type 1 errors is high. A type 1 error does not detect the simulated QTL but still declares the best position of a QTL at a point on the chromosome. In these cases the confidence interval is chiefly created by type 1 errors and hence the interval, by chance, is large and more likely to cover central regions of the chromosome. This explains the higher probabilities for $d = 45$ compared with $d = 85$ when $h^2 = 0.01$ and is in agreement with the findings of Paterson *et al.* (1991).

(iv) Parametric versus non-parametric bootstrap

The parametric bootstrap produces smaller intervals in comparison with the non-parametric equivalent when heritabilities exceed 0.05. However, these intervals are anti-conservative when $N = 500$, for the parametric bootstrap. When the heritability was 0.01 the two methods produced similar results.

(v) QTL position

From Table 1 it would appear that the parametric bootstrap produces consistent results independent of the position of the QTL on the chromosome. This is the same conclusion as Visscher *et al.* (1996) derived for the non-parametric bootstrap, but this preliminary study and the work of Visscher *et al.* both simulated only two QTL positions on a 100 cM chromosome.

(vi) Marker spacing

Table 1 shows that less dense marker maps create slightly narrower confidence intervals. Results for the parametric bootstrap show that when $N = 500$ and

$\Delta = 10$, confidence intervals are anti-conservative. When $N = 500$ and $\Delta = 20$ the 90% and 95% confidence intervals contain the simulated QTL in approximately 90% and 95% of all replicates respectively. From this we would conclude that a denser marker map creates anti-conservative results. This goes against expectation, as more information should increase the accuracy of the results.

By studying only two positions (45 and 85 cM), the initial study fails to examine fully the effect of position of the QTL on the chromosome and position within the interval upon the accuracy of the confidence intervals created. The results for QTL at locations 0–50 cm for the two marker map densities allow these two situations to be investigated. The chromosome is assumed to show symmetrical results around the midpoint; hence, to reduce running time of computer simulations, locations 0–50 cM were investigated.

The non-parametric bootstrap performs reasonably well in most cases, i.e. the 90% confidence interval generally includes the QTL 90% of the time. A bias occurs when the QTL is simulated at the same position as a marker (Fig. 1). At these locations the confidence interval is conservative, e.g. in Fig. 1 the probability the 90% confidence interval contains the QTL at 0, 10, and 20 cM is 0.942, 0.964 and 0.951 respectively.

The parametric bootstrap performs substantially less well than the non-parametric equivalent. Fig. 2 illustrates the pattern seen as the QTL is placed at different locations along the chromosome. Large waves are produced peaking at the position of the markers and reaching a minimum midway between markers. At marker positions the parametric bootstrap is more conservative than the non-parametric

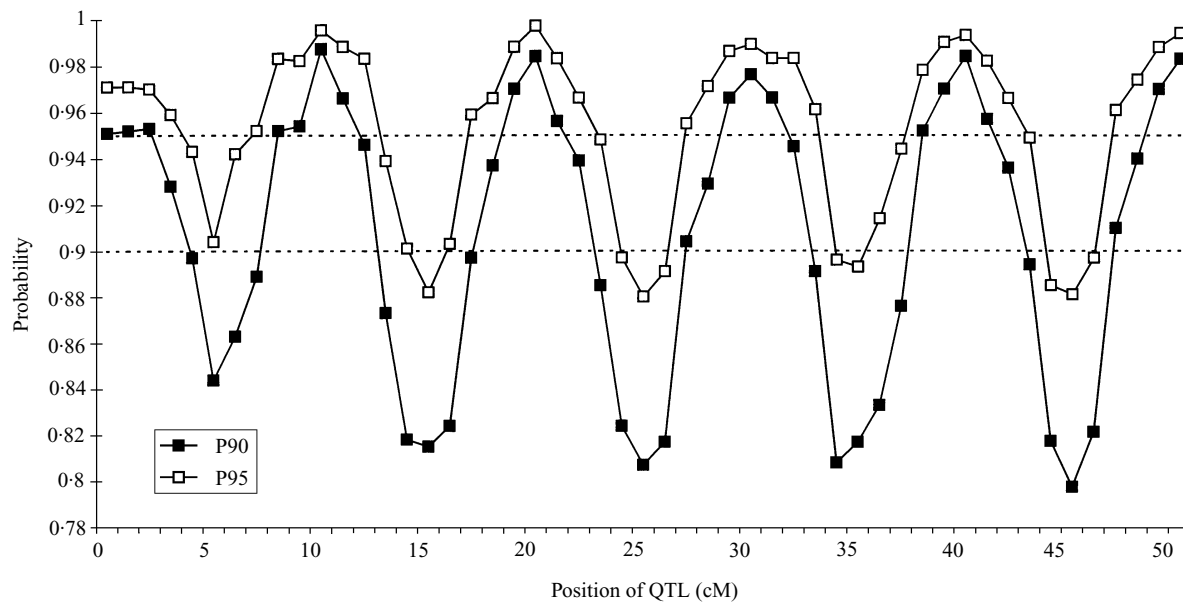


Fig. 2. $N = 500$, $h^2 = 0.1$, $\Delta = 10$. The probability a confidence interval contains a QTL at different simulated positions on the chromosome using the parametric bootstrap.

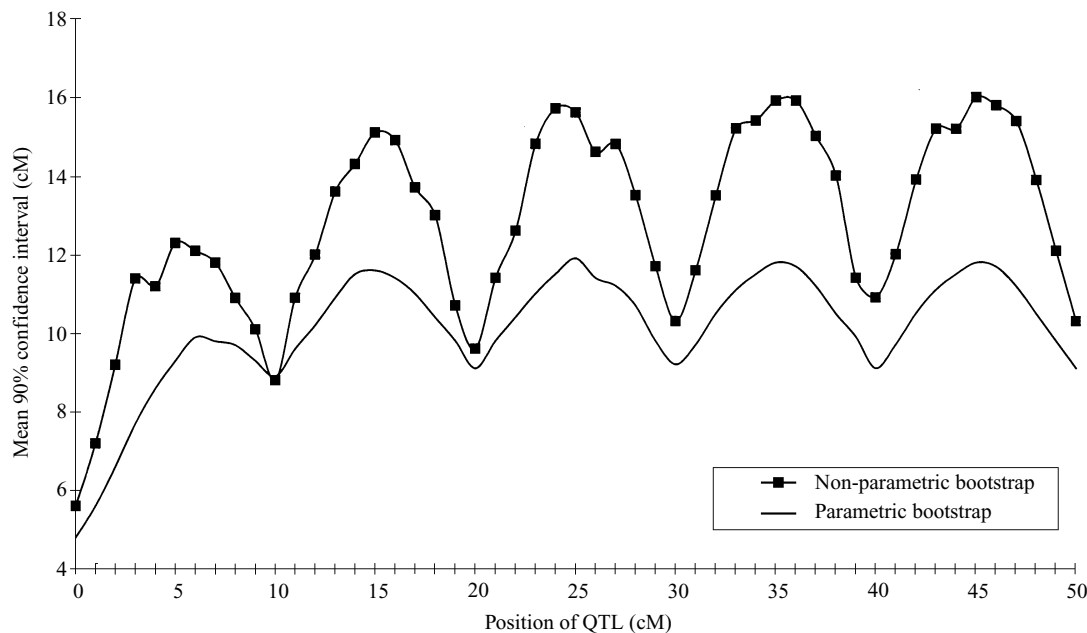


Fig. 3. $N = 500$, $h^2 = 0.1$, $\Delta = 10$. The size of CI90 at different simulated positions on the chromosome.

and at intermediate positions it is substantially anti-conservative. Decreasing the number of markers increases the wavelength and decreasing the heritability of the QTL decreases the amplitude of the waves (results not shown).

The sizes of the confidence intervals produced by the two methods, over the length of the chromosome, are shown in Fig. 3. The parametric bootstrap produces smaller intervals than the non-parametric method. Confidence intervals are largest when the QTL is simulated equidistant between two markers and therefore the maximum distance away from a marker. Conversely, the smallest intervals are found

when the QTL and marker lie at the same location. It is evident that the end of the chromosome causes a truncation of the confidence interval, for when the QTL is simulated at 0–10 cM the confidence interval is smaller than the equivalent positions in other intervals. The non-parametric bootstrap shows greater variation in the size of confidence interval. Intervals differ by approximately 6 cM when comparing the size of interval when the QTL is on the marker with the size of interval produced when the QTL is equidistant between two markers. The same comparison with the parametric bootstrap shows a difference of 2 cM.

4. Discussion

From the results it is clear that the parametric bootstrap is unsuitable for use with experimental data because of the variation with QTL position in the probability that a 90% or 95% confidence interval contains the actual QTL position. The reasons for these large fluctuations are not completely clear. The patterns seen are consistent with the hypothesis that the waves are produced as an amplification of the peaks seen in the non-parametric analysis. This arises because the mapping procedure shows a small bias towards placing a best estimate at the location of a marker. This bias can be calculated (Visscher *et al.*, in preparation) using the equations from Whittaker *et al.* (1996).

This bias can be visualized in Fig. 4. It is clear from the test statistic that the best estimate of the location of the QTL in the analysis of the original data set is approximately at the middle of the chromosome. The distribution of positions of best estimates of QTL position from the bootstrap examples might be expected to show an approximation of the distribution of the test statistic. The distribution of the best position estimates follows the distribution of the test statistic well except at marker positions, where the number of best estimates is large in comparison with the other locations. Note, however, that the mean test statistic is significantly lower. This implies that when a marker position is the best estimate of a QTL location, the evidence for a QTL is less than when the QTL is at a non-marker position.

One hypothesis explaining the presence of the marker peaks could be the accumulation of type 1

errors. The method used did not select a subset of the bootstrap re-samples. In practice, if the test statistic in the initial analysis did not exceed a set threshold, no QTL would be declared. The non-selective bootstrap analyses each bootstrap re-sample and records the best estimated position, but does not assess whether the data support the hypothesis that a QTL is located at that position. These replicates with less evidence for a QTL accumulate in the analysis, particularly when the chance of detecting the QTL, the power of the experiment, is low, e.g. a QTL with small effect. Fig. 5 shows the histogram of best estimated positions of QTL from 100 000 replicates when no QTL was simulated ($N = 200$, $\Delta = 20$, $h^2 = 0$). This shows that the method, when analysing type 1 errors, tends to place the best estimated position of the QTL on a marker. In Fig. 5 over 50% of replicates were placed at markers, which compares with an expectation of under 6% if the distribution in Fig. 5 was uniform.

Type 1 errors, analogous to the replicates with less evidence for a QTL, cannot explain all the peaks at markers in Fig. 4, as the power of the analysis for such a configuration is high and therefore type 1 error rate is generally low. If the power was assumed to be 0.9 for the situation in Fig. 4 (the true value is slightly larger) approximately 100 000 replicates would be type 1 errors. Since Fig. 5 shows that approximately 13% of type 1 errors are placed at 0 or 100 cM and 8% at the location of each marker, type 1 errors could explain peaks in the histogram of up to 13 000 at positions 0 or 100 cM and 8000 at 20, 40, 60 and 80 cM. Type 1 errors could therefore account for the small accumulation of replicates in Fig. 4 positioned at 20, 80 and 100 cM, since the numbers of replicates ac-

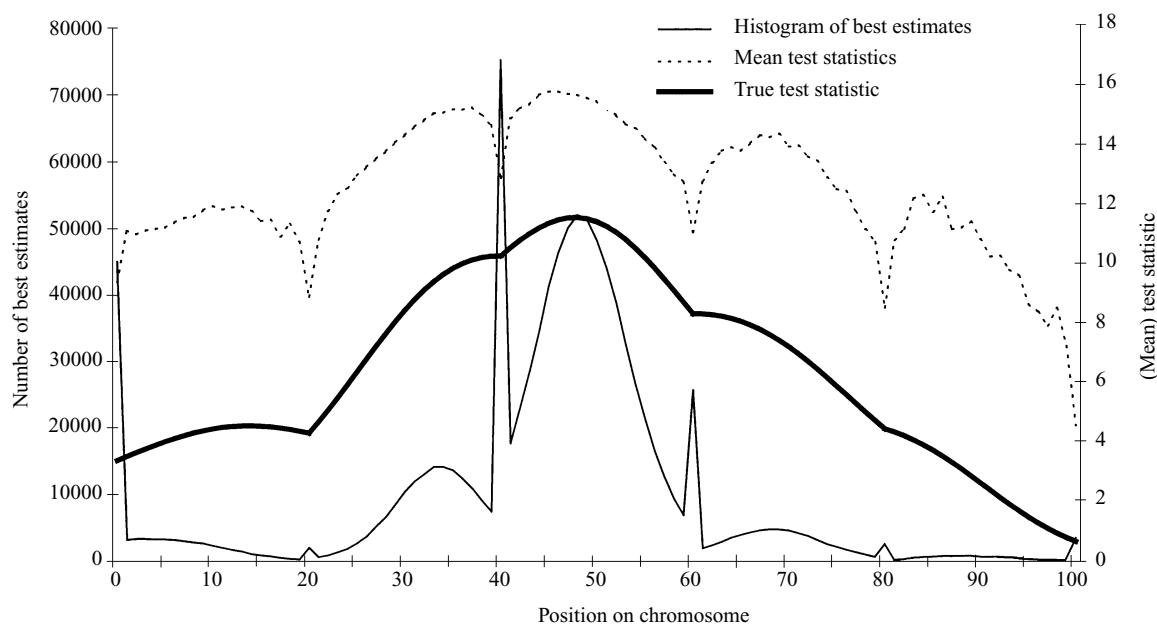


Fig. 4. One million non-parametric bootstraps for one replicate ($N = 200$, $\Delta = 20$). The mean test statistic was calculated for each point only when that location was the best estimated position of the QTL.

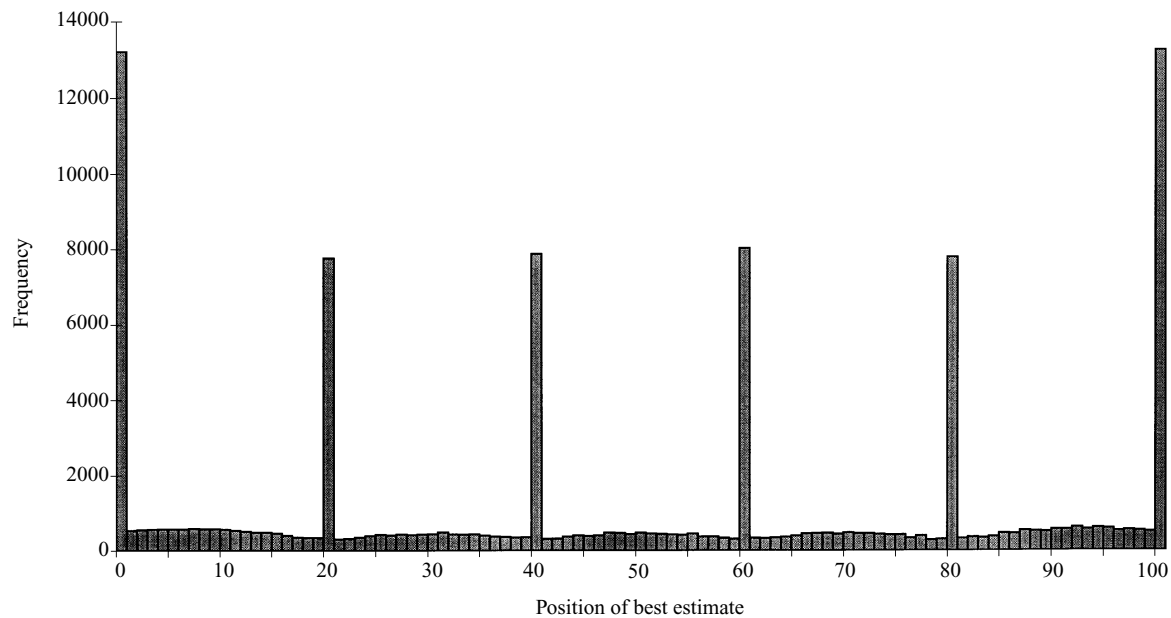


Fig. 5. Histogram of best estimated positions from 100000 replicates with no QTL simulated ($N = 200$, $\Delta = 20$).

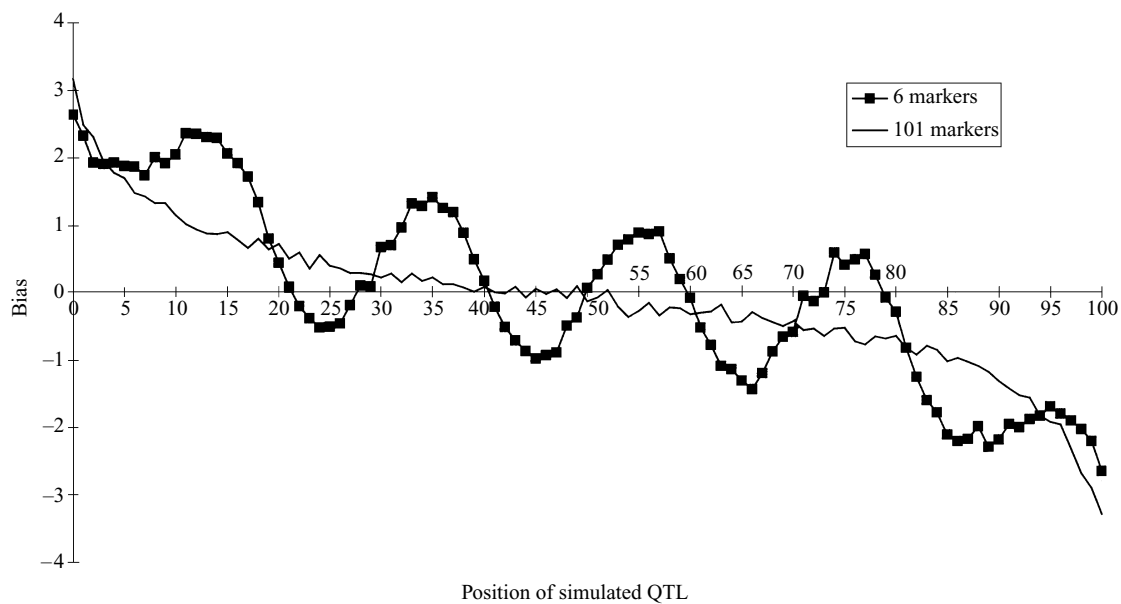


Fig. 6. Bias of position in QTL mapping. Each point was calculated by simulating 1000 replicates at point d ($N = 200$, $h^2 = 0.1$). Mean estimated position from 1000 analysed replicates subtracted from true simulated position gives bias at point d . Thus if the position 8 cM from the left of the chromosome maps, on average, at 10 cM the bias is $10 - 8 = +2$. If, however, the same point maps, on average, at 5 cM the bias is $5 - 8 = -3$.

cumulating at these positions is small. It is unable to account for the larger collection of replicates at 0, 40 and 60 cM where additional bias must exist over that caused by the type 1 error rate.

To explain these larger peaks the precision of the mapping procedure itself has to be studied. Fig. 4 implies there is a distinct bias for the mapping method to place best estimates of positions on markers. This can be studied by looking at mean estimated position of a QTL when simulated at position (d) over replicates. Fig. 6 shows this for a chromosome with

$\Delta = 20$. Bias is calculated as mean position over replicates subtracted from the true simulated position. It is apparent from the oscillating waves of bias in the estimated QTL position that the markers influence the results; this is confirmed in other plots for $\Delta = 10$ and $\Delta = 25$ (not shown). In fact the results produced contain two types of bias in the mapping procedure. The first bias is that of the markers, which can be seen by the oscillating waves in Fig. 6. The second type is caused by the symmetrical placement of type 1 errors (i.e. replicates that by chance contain a significant

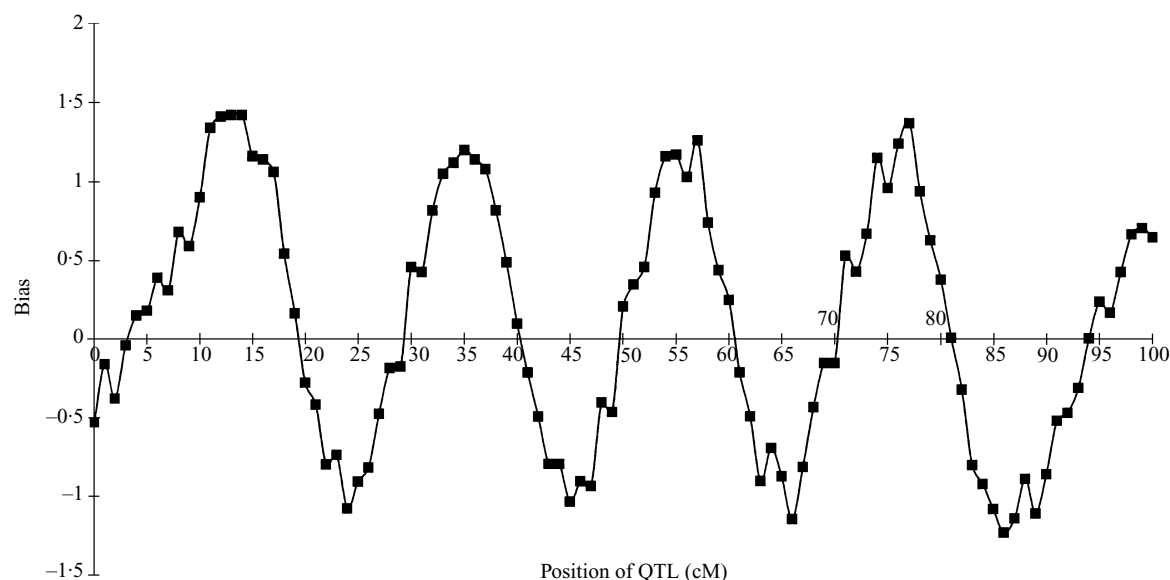


Fig. 7. The influence of markers alone on the positional mapping of QTL. Figure were produced by subtracting values in Fig. 6 for 101 markers from the equivalent point for 6 markers.

QTL effect at a location other than that simulated), around the centre of the chromosome. This pushes the mean towards the centre (in this case 50 cM) because this would be the mean position if no QTL was simulated, assuming a uniform probability distribution of positioning across the chromosome. This can be seen in Fig. 6, where marker effect is minimized by placing a marker at 1 cM intervals.

The plot illustrates the general 'pull' towards the centre of the chromosome, the 'pull' being largest at the ends of the chromosome where the mapping procedure places the QTL, on average, approximately 3 cM closer to the centre of the chromosome than the true position. Only the outer regions of the chromosome show a large bias; the majority of the chromosome (11 cM to 86 cM) has only a small bias (< 1 cM). Hyne *et al.* (1995), for a population of 300 and a QTL with heritability of 10%, previously reported this bias due to the asymmetrical location of the QTL in an F_2 population.

By subtracting the bias when $\Delta = 1$ from the equivalent position when $\Delta = 20$ it is possible to see the influence of the markers alone (Fig. 7). Removing the bias due to overall chromosomal position leaves the bias due to position in relation to markers. Fig. 7 shows that each marker has an equal effect with the general trend being a pull towards the marker. Midway between markers the bias disappears, because the effect of each marker is equal. At other locations in the interval the bias depends on the position of the QTL in relation to the two flanking markers. Bias is largest one-quarter from the left marker (acting towards the left marker) and three-quarters from the left marker (acting towards the right marker). The bias is not large, however, never exceeding ± 1.5 cM.

With this pull towards markers, as well as the tendency of type 1 errors to estimate QTL position on markers, a large number of bootstrap re-samples have best estimated positions at markers, so many confidence intervals either start and/or finish at the location of a marker. Since intervals that start or finish at the location of the QTL are considered to contain the QTL, the method is conservative when the QTL lies at the same location as the marker.

A selective bootstrap (selecting 'significant' replicates) more relevant to actual practice was tested by removing all replicates with a maximum test statistic that did not exceed a threshold of 6.08. The threshold of 6.08 was set as the 95th percentile of the test statistic calculated for the single chromosome from 1 000 000 simulated populations with no QTL present. The implementation of this threshold in the analysis removes 95% of all type 1 errors. This would indicate whether type 1 errors were exclusively the cause of the conservative results when the QTL is at the marker. The results of the selective bootstrap showed only a small difference from the non-selected method (results not presented). Confidence intervals produced were smaller but still conservative when the QTL was located at a marker. When $N = 200$, $\Delta = 20$, $h^2 = 0.1$ and $d = 20$ the 90% confidence interval was 28.1 cM when selecting replicates and 29.2 cM when no selection was used, but the probability of the interval containing the QTL did not change (0.974 and 0.970 respectively). A double selective bootstrap (selecting replicates and then only significant bootstrap re-samples exceeding the same threshold of 6.08) also failed to correct the situation at the site of the marker, although having an obvious effect at other locations. Confidence intervals remained conservative at the

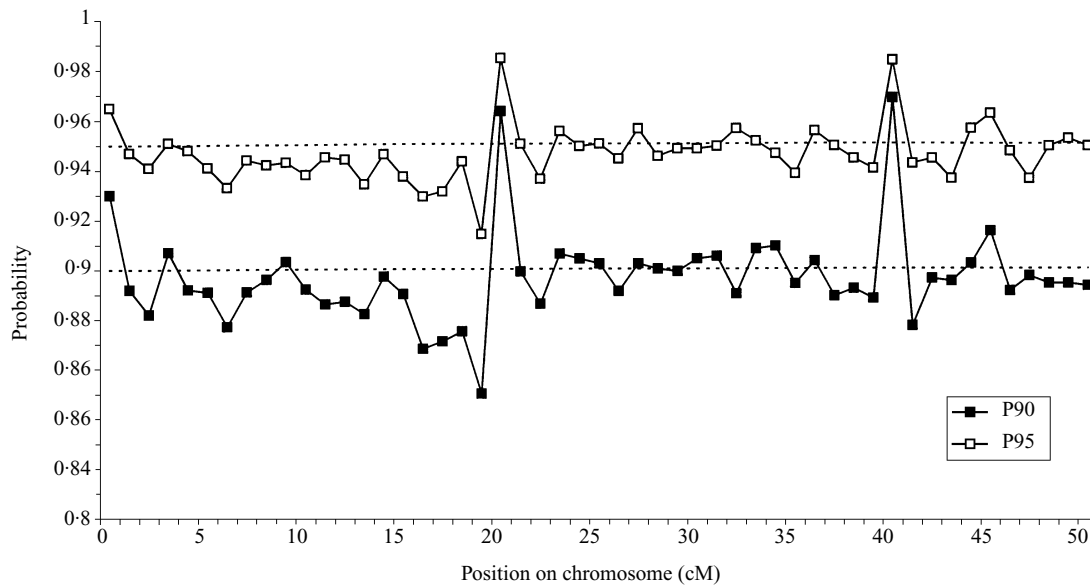


Fig. 8. $N = 200$, $\Delta = 20$, $h^2 = 0.1$. The probability that a confidence interval contains a QTL at different simulated locations on the chromosome using the non-parametric bootstrap. Replicates and bootstrap resamples were both selected using a threshold of 6.08.

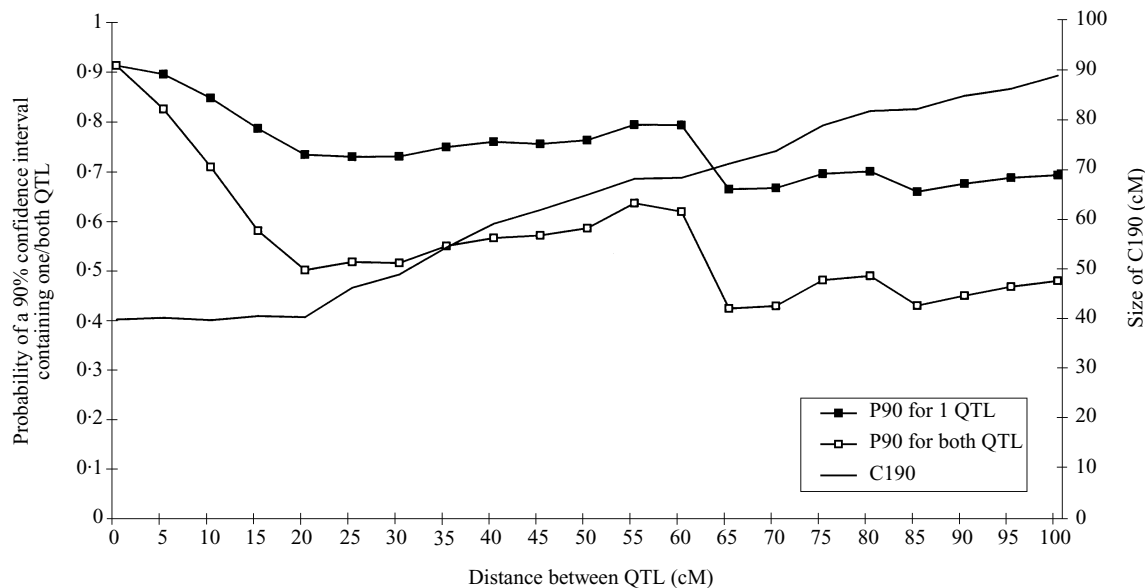


Fig. 9. The probability that a 90% confidence interval contains (i) one QTL and (ii) both QTLs when two equally sized QTL ($h^2 = 0.05$) are simulated varying distances apart symmetrically around the centre of the chromosome ($N = 200$, $\Delta = 20$, 1000 replicates per point).

marker but were anti-conservative in the outer interval (Fig. 8), i.e. in this example between 0 and 20 cM since $\Delta = 20$. When $N = 200$, $\Delta = 20$, $h^2 = 0.1$ and $d = 20$ the 90% confidence interval was 26.3 cM and $P = 0.963$, but when $d = 17$ the size was 29.3 cM with $P = 0.871$. From this work it is apparent that the type 1 errors are not the sole reason for conservative confidence intervals by the non-parametric bootstrap.

The method used fitted only one QTL. In practice it is likely that the data would be analysed to see whether they supported the hypothesis for two or

more QTLs present. If more than one QTL was detected, the progression of the method would be different from that applied in the methods used in this example. To examine the effect of a second QTL on the chromosome, two QTLs of equal size ($h^2 = 0.05$ each) were placed symmetrically around the centre of a 100 cM chromosome, different distances apart. These data were analysed with the same program as was previously used and results are summarized in Fig. 9. The confidence interval remained the same size as obtained for one QTL of combined effect when

both were within the same interval; but when in different intervals and as the QTLs moved further apart, the confidence interval increased in size. Confidence intervals were anti-conservative for either QTL when they were over 5 cM apart. It is clear that if, by chance, a second 'ghost' QTL (Martinez & Curnow, 1992) was simulated in some of the replicates it would be more likely to contribute to anti-conservative confidence intervals.

Since the mapping procedure is used twice in the parametric bootstrap, the bias is amplified. This causes the confidence intervals midway between two markers to be anti-conservative because best estimated positions have been placed closer to markers and away from the true position of the QTL. When the QTL lies at the same location as the marker, however, the mapping method places too many best estimates at the location of the marker. This causes the confidence interval to be conservative. There appears to be no simple correction that can be made to the results to remove the effects of the bias. Trying to develop the parametric bootstrap seems to be unnecessary when the non-parametric bootstrap works satisfactorily in the majority of cases.

In summary, the non-parametric bootstrap performed well. Between markers the probability that a confidence interval contained the true QTL position was generally close to the expected value under all parameter combinations tested. Further to the work of Visscher *et al.* (1996), this study tests the non-parametric bootstrap thoroughly and shows that results are conservative when a QTL is simulated on a marker. In practice this at least errs on the side of caution. The work goes further to show the parametric bootstrap has significant failings and no appreciable advantages over the non-parametric bootstrap. The bias caused by the positioning of markers has not been previously reported and when coupled with the bias from the asymmetrical positioning of the QTL on the chromosome (Hyne *et al.*, 1995), contributes to error in the estimated position of the QTL.

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