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Application of non-parametric bootstrap methods to estimate confidence intervals for QTL location in a beef cattle QTL experimental population

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Summary

Empirical confidence intervals (CIs) for the estimated quantitative trait locus (QTL) location from selective and non-selective non-parametric bootstrap resampling methods were compared for a genome scan involving an Angus × Brahman reciprocal fullsib backcross population. Genetic maps, based on 357 microsatellite markers, were constructed for 29 chromosomes using CRI-MAP V2.4. Twelve growth, carcass composition and beef quality traits ($n = 527$–602) were analysed to detect QTLs utilizing (composite) interval mapping approaches. CIs were investigated for 28 likelihood ratio test statistic (LRT) profiles for the one QTL per chromosome model. The CIs from the non-selective bootstrap method were largest (87·7 cM average or 79·2% coverage of test chromosomes). The Selective II procedure produced the smallest CI size (42·3 cM average). However, CI sizes from the Selective II procedure were more variable than those produced by the two LOD drop method. CI ranges from the Selective II procedure were also asymmetrical (relative to the most likely QTL position) due to the bias caused by the tendency for the estimated QTL position to be at a marker position in the bootstrap samples and due to monotonicity and asymmetry of the LRT curve in the original sample.

1. Introduction

The confidence interval (CI) for quantitative trait locus (QTL, chromosomal region where a gene responsible for variation in a quantitative trait resides) location is important because it influences tests for close linkage versus pleiotropy, QTL fine-mapping, marker-assisted selection, marker-assisted introgression and candidate gene selection. The one LOD drop-off method (Lander & Botstein, 1989) has been widely used to provide CIs for QTL location. However, this method has been shown to be biased, with the degree of bias depending on sample size and experimental design (F2 or backcross), QTL location and magnitude of effect and marker map density (van Ooijen, 1992; Mangin *et al*., 1994; Visscher *et al*., 1996).

Visscher *et al*. (1996) presented an empirical non-parametric bootstrap method for the construction of CIs. This approach seems to be practical, because the generation of CIs is based on prior knowledge of QTL existence and consideration of the real data configurations (sample size, phenotypes, tested genome size, marker map density, missing genotype pattern, etc.). They found that the bootstrapped CIs for QTLs with moderate sizes of effect and experiment were less biased than using the LOD drop-off method and were robust to QTL location and marker spacing. This empirical bootstrapping method has also been applied for CI estimation in outbred livestock populations (de Koning *et al*., 1998; Zhang *et al*., 1998; Walling *et al*., 2000). To obtain improved estimates of CIs that have small size and range with unbiased coverage (i.e. the proportion of 95% CIs that contain the true QTL is 0·95), Lebreton & Visscher (1998) proposed a selective bootstrapping method in which only the bootstrap samples that met selection criteria related to the estimated QTL characteristics were retained. They concluded that the non-selective bootstrap method produced larger CIs and that the selective bootstrapped CIs were either unbiased or minimally biased when the QTL was situated near the middle of the chromosome.

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The purpose of this study was to evaluate and compare confidence intervals for QTL position produced by the non-selective and selective bootstrapping methods as well as the LOD drop-off method for the QTLs detected in a project designed to localize genes influencing economically important traits in beef cattle.

2. Materials and methods

(i) *Resource family structure and genetic map construction*

The Texas A&M University's three-generation beef cattle population includes 614 progeny from 14 Angus backcross (47·5%), 15 Brahman backcross (43·6%) and three F2 (8·9%) fullsib families produced by embryo transfer from 80 Brahman, Angus and F1 parents and grandparents. The average number of progeny per family was $19{\cdot}1 \pm 6{\cdot}5$. The traits in this study were 12 growth, carcass composition and beef quality traits, and measurements of these traits have been described in detail in Kim (1999) and Kim & Taylor (2001). All traits were analysed as phenotypes after adjustment for fixed effects (year-season of birth, gender, cross type (two double reciprocal backcross and F2)), a random effect (family nested within cross type) and appropriate covariates for each trait.

Three hundred and fifty-seven genetic markers, mainly microsatellites, were scored for the construction of linkage maps and progeny genotypes were assigned for each chromosome as being Angus (A) or Brahman (B) in origin using a three-generation pedigree, parental breed information and identity by descent data from the CHROMPIC option of CRIMAP V2.4 (Green *et al.*, 1990). The final map comprised 29 autosomes with an average length of 91 cM (98 cM for test chromosomes), an average of 12·3 markers (maximum 31, minimum 2) and 48% (79%) average (multi-point) informative meioses for markers on each chromosome, and the sex-average map spanned 2642·5 Kosambi cM with an average intermarker distance of $8{\cdot}1 \pm 7{\cdot}1$ cM.

(ii) *Detection of QTL on the test chromosome*

Interval mapping (IM) and composite interval mapping (CIM) approaches under a line cross model in which alternate breed QTL alleles are assumed to be fixed were applied to QTL detection using QTLCartographer (Lander & Botstein, 1989; Basten *et al.*, 1994; Zeng, 1994). In the CIM approach, which was applied only to chromosomes in which QTLs were detected from IM in the one QTL model, the 10 most significant markers outside of the test chromosome were utilized as cofactors (background markers) by the stepwise regression procedure. This number of background markers was finally arrived at empirically in an attempt to account for background genetic variation due to QTL on other chromosomes, while avoiding the inclusion of markers that were spuriously correlated with phenotype due to chance. The fitted linear model included breed additive (a) and dominance (d) effects for a putative QTL, and the likelihood ratio test statistic (LRT) was estimated as the position of a putative QTL was moved at 1 cM increments along the chromosome. To determine the appropriate significance threshold values for QTL detection, 'nominal' (0·05 and 0·03 chromosome-wide linkage levels for IM and CIM, respectively), 'suggestive' (0·035 and 0·012 chromosome-wide linkage levels for IM and CIM, respectively) and 'significant' (0·05 genome-wide linkage level) levels of linkage evidence were used (Lander & Kruglyak, 1995; Kim, 1999). Permutation tests were performed with 1000 replicates to determine the significance thresholds (Churchill & Doerge, 1994).

To determine whether there was evidence for more than one QTL on each chromosome, a one versus two QTL test was performed. A second QTL with maximum LRT value (Ha: two QTLs versus Ho: one QTL) that was located outside the marker interval containing the most likely position for a single QTL was estimated while fitting the best single QTL in the model (MImapqtl option in QTLCartographer). Permutations ($n = 1000$) were performed to generate empirical LRT values, while fitting the best single QTL across the permuted samples. If the maximum LRT value was greater than a threshold LRT value at the 0·1 chromosome-wide significance level, then it was concluded that two QTLs resided on the test chromosome and these QTLs were excluded for CI evaluation.

(iii) *Methods to generate confidence intervals for QTL location*

Two methods for constructing CIs for QTL location were used: the LOD drop support interval and empirical non-parametric bootstrap methods. A two LOD drop (LRT = 9·2) was used to generate 95% CIs that were expected to be unbiased, at least for QTLs with modest effects in a sample of this size (van Ooijen, 1992). For the empirical non-parametric bootstrapping approach, selective and non-selective resampling methods were used according to the protocol of Lebreton & Visscher (1998). In the non-selective method (Non-Sel), bootstrap samples were created by sampling with replacement n observations from the pool of n original observations ($n =$ 527–602). In the selective bootstrap method where only bootstrap samples providing statistical significance for the QTL were resampled, two procedures were applied. The first (Sel-I) chose bootstrap samples where the maximum LRT values exceeded the

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corresponding statistical level of evidence for linkage (i.e. nominal significance level for QTL detected at the nominal level of linkage evidence). The second (Sel-II) chose samples where the estimated mode of QTL gene action and signs of additive and dominance effects were the same as those found in the original sample at the most likely QTL position after the samples were chosen using the Sel-I strategy. Three hundred bootstrap samples were obtained for each procedure and the empirical symmetrical confidence interval of 95% for QTL location was determined by ordering the estimates from each bootstrap sample and taking the 2.5^{th} and 97.5^{th} percentiles. For the CIM analyses, the identities of the background markers fitted in the model were maintained across the bootstrap samples.

3. Results and discussion

Four, 12 and 12 QTLs detected under the one-QTL model possessed significant, suggestive and nominal levels of linkage, respectively. The average proportions (%) of phenotypic variance due to the QTL were $7{\cdot}1 \pm 3{\cdot}4$, $5{\cdot}0 \pm 3{\cdot}5$ and $4{\cdot}0 \pm 2{\cdot}8$ for the respective statistical levels based on the assumption of equal frequency of alternate breed alleles in the experimental population.

In general, CIs produced by non-selective bootstrapping were much larger than those from the selective and the LOD drop methods. The average CI generated by Non-Sel was $87{\cdot}7 \pm 26{\cdot}7$ cM ($79{\cdot}2 \pm 20{\cdot}4$% of the test chromosome coverage), and some CIs spanned the entire length of the chromosome as in Fig. 1*e*. Average CI lengths from Sel-I and Sel-II were $73{\cdot}5 \pm 29{\cdot}0$ cM ($66{\cdot}5 \pm 24{\cdot}6$%) and $42{\cdot}3 \pm 26{\cdot}7$ cM ($37{\cdot}7 \pm 23{\cdot}3$%), respectively. The CIs from Sel-II were smaller than those from the two LOD drop ($60{\cdot}1 \pm 29{\cdot}4$ cM average) approach. However, CI lengths produced by the Sel-II approach were more variable (coefficients of variation of the CIs from Sel-II and the two LOD drop approach were 63·3% and 46·0%, respectively).

Lengths of CIs from Sel-I were generally smaller and those from Sel-II were much smaller (50% smaller) than CIs produced by Non-Sel. These results are consistent with the simulation results and the assertion of Lebreton & Visscher (1998) that to obtain better (unbiased and narrow) CIs the resampled data should produce QTL estimates with the same mode of gene action as the QTL detected in the original sample. However, in some cases, the CI length was not significantly reduced after applying the Sel-I (Fig. 1*b*, *e*, *i* and *l*), or Sel-II procedures (Fig. 1*a* and *b*). These results might, in part, be due to the existence of other undetected (minor) QTLs on the test chromosome that the one-QTL versus two-QTL test failed to detect. A QTL that was not detected in the original sample due to its small effect might be detected in some bootstrap samples where a different subset of the original sample may provide more detection power for that QTL relative to the QTL detected in the original sample. In within-family interval analyses, Kim (1999) found evidence for additional QTL in the centromeric and telomeric thirds of the chromosome, which may have influenced the lengths of the bootstrap CIs for the QTL in the centre of this chromosome in Fig. 1*b*.

Lebreton & Visscher (1998) reported that the selective bootstrap method produced relatively unbiased and smaller CIs that ranged at least 40 cM. In this study, the Sel-II procedure generated 95% CI sizes that were very small for some of the detected QTLs (Fig. 1*g*, *i*, *j* and *k*: 13, 17, 8 and 11 cM, respectively). Also, the LRT values at the most likely positions of two QTLs (Fig. 1*i* and *j*) were not sufficiently large to provide such small CI widths in the bootstrapping method (Visscher *et al*., 1996). Because there is a positive relationship between CI size and the probability of a randomly selected interval containing the true QTL (van Ooijen, 1992; Visscher *et al*., 1996), it is likely that these CIs do not contain the true QTLs.

As there is a bias toward the marker positions for the estimate of most likely QTL position in bootstrapped samples, some CI ranges may start, or end, at marker positions where the QTL positions were estimated in the original sample (Walling *et al*., 1998). Fig. 1*d*, *g*, *j*, *k* and *l* show asymmetrical CI ranges relative to the most likely QTL position located on, or near, the marker positions from the selective procedures, which may cause a serious bias in determining the confidence interval if the true QTL is located at the opposite side of the asymmetric CI. This bias was also found in the simulation study of Walling *et al*. (1998), where significantly fewer than 95% of the CIs estimated according to a 5% Type I error rate contained the QTL, when the QTL was simulated at locations close to (but not exactly at) a marker position.

Another mode of asymmetry was found in bootstrapped CIs of some QTLs, for which the CI range depends on the LRT curve shape. The CI ranges included a chromosomal region for which the LRT profile contains small peaks (Fig. 1*c*, *e*, *f* and *h*). This sensitivity of CI range to problems of smoothness has been pointed out for some statistics that are based on the tails of the empirical distributions (Efron & Tibshirani, 1993), while the simulation results of Lebreton & Visscher (1998) suggested that bootstrapped CIs were robust to this problem.

In conclusion, the selective non-parametric bootstrap methods produced smaller confidence intervals for QTL position than the non-selective method.

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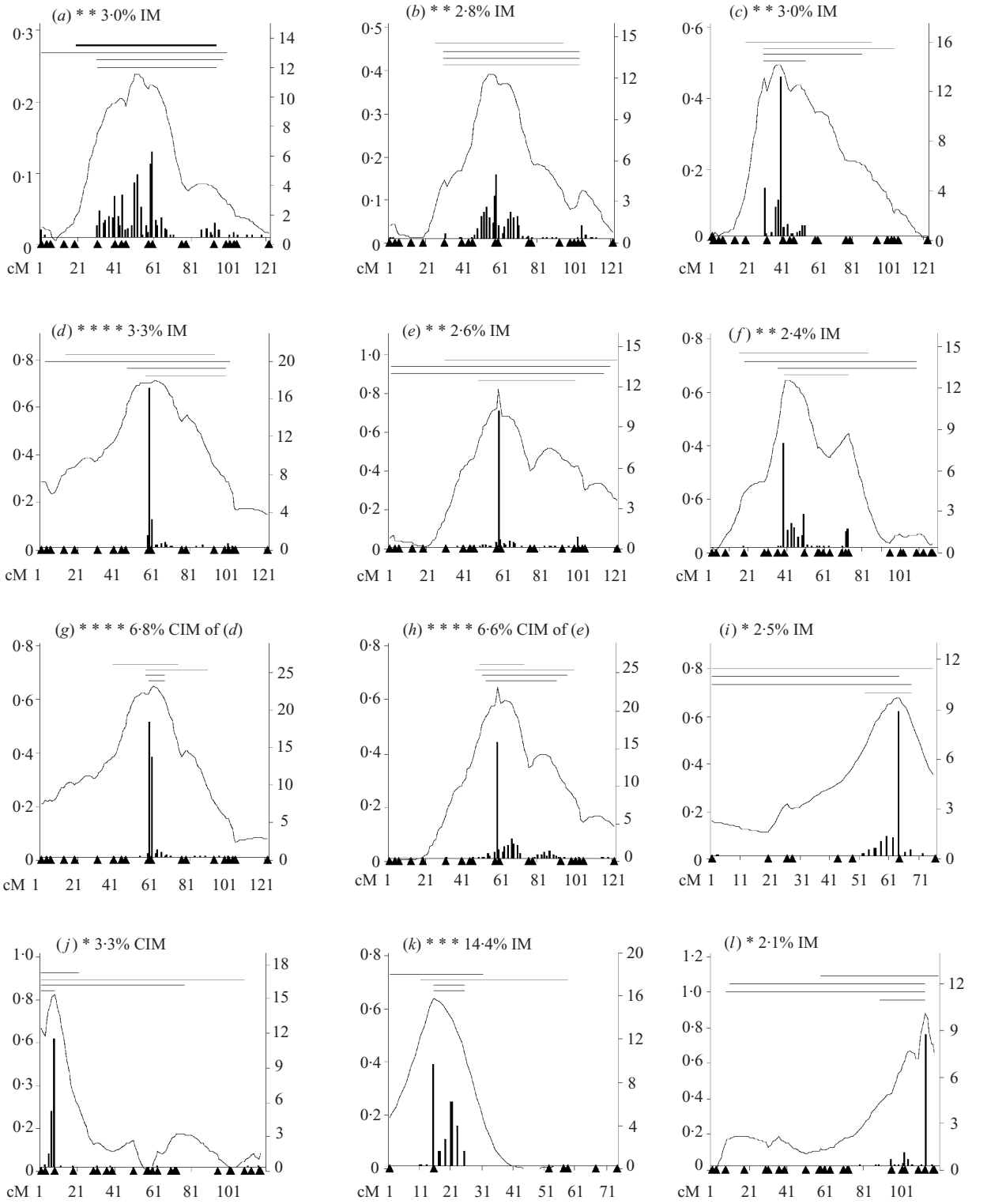

Fig. 1. LRT profiles (right *Y*-axis) and empirical frequency distributions (left *Y*-axis) for the estimated positions of the QTL from the Selective II bootstrapped samples ($n = 300$). *, nominal level of linkage evidence; **, suggestive level of linkage evidence; ***, highly suggestive ($P < 0{\cdot}1$ genome-wide) level of linkage evidence; ****, significant level of linkage evidence. %, proportion of phenotypic variance due to the QTL at the most likely position on the assumption of equal frequency of alternate breed alleles; IM, interval mapping analysis; CIM, composite interval mapping analysis. Lines in order indicate confidence interval range from the two LOD drops (top), non-selective, Selective I and Selective II bootstrapping methods (bottom), respectively. Arrows along the *X*-axis indicate marker positions. The lengths of some chromosomes were standardized to 120% and the corresponding CI lengths and ranges were also modularized accordingly.

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However, this study suggests that the use of selective bootstrap methods could lead to inappropriately small CIs and asymmetry of the CIs due to the bias caused by the tendency for the estimated QTL position to be at a marker position in the bootstrap samples and due to the asymmetry and smoothness of the LRT curve in the unpermuted sample.

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