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Non-parametric interval mapping in half-sib designs: use of midranks to account for ties

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Summary

In QTL analysis of non-normally distributed phenotypes, non-parametric approaches have been proposed as an alternative to the use of parametric tests on mathematically transformed data. The non-parametric interval mapping test uses random ranking to deal with ties. Another approach is to assign to each tied individual the average of the tied ranks (midranks). This approach is implemented and compared to the random ranking approach in terms of statistical power and accuracy of the QTL position. Non-normal phenotypes such as bacteria counts showing high numbers of zeros are simulated (0–80% zeros). We show that, for low proportions of zeros, the power estimates are similar but, for high proportions of zeros, the midrank approach is superior to the random ranking approach. For example, with a QTL accounting for 8% of the total phenotypic variance, a gain from 8% to 11% of power can be obtained. Furthermore, the accuracy of the estimated QTL location is increased when using midranks. Therefore, if non-parametric interval mapping is chosen, the midrank approach should be preferred. This test might be especially relevant for the analysis of disease resistance phenotypes such as those observed when mapping QTLs for resistance to infectious diseases.

1. Introduction

Most quantitative trait locus (QTL) mapping methods share a common assumption: that the phenotype follows a normal distribution. However, many phenotypes of interest are not normally distributed. Examples include: counts, such as bacteria counts or colony-forming units in infected organs (CFU) (Berthelot *et al.*, 1998), faecal egg counts (FEC) (Bouix *et al.*, 1998), plaque-forming units (PFU) in peripheral blood cells and number of tumors (Vallejo *et al.*, 1998), and somatic cell counts (SCC) (Schrooten *et al.*, 2000); survival times and qualitative data (severity grades assigned upon histological examination). Traditional QTL mapping methods cannot be directly applied in such cases. On the one hand, ANOVA and least-squares-based methods (Haley & Knott, 1992; Martinez & Curnow, 1992; Weller *et al.*, 1990) assume that residuals within QTL genotype classes are normally distributed. Such methods are commonly said to be robust against non-normality. However, the limits of this robustness in the context of QTL mapping methods have only been explored in specific conditions (e.g. Hackett & Weller, 1995; Coppieters *et al.*, 1998). On the other hand, maximum-likelihood-based methods use the normal density function for the structure of the likelihood itself (Lander & Botstein, 1989). Quality of estimations is therefore very dependent on the normality of the phenotypic distribution. One approach to circumvent the assumption of normality is to apply a mathematical transformation that will convert the trait into an approximately normal variable. If no mathematical transformation is available, such as for lesion scores (e.g. Roberts *et al.*, 1997), an alternative approach is to use distribution-free methods. Kruglyak & Lander (1995) described a non-parametric interval mapping

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approach based on the Wilcoxon rank-sum test that is applicable to experimental crosses. Coppieters *et al.* (1998) adapted this method to half-sib pedigrees in outbred populations.

In a recent study, Tilquin *et al.* (2001) compared three different QTL mapping methods in the context of resistance to bacterial diseases: (1) least-squares analysis; (2) maximum-likelihood analysis; and (3) non-parametric mapping. When searching for QTLs implicated in resistance to bacterial diseases, the distribution of the trait under study (bacteria counts) is extremely skewed to the right and can present a high frequency of zero values. From a statistical point of view, those zero values are ties. Tilquin *et al.* (2001) showed that, under these conditions, losses of power in parametric and non-parametric methods can be explained by two distinct causes: the asymmetry of the phenotypic distribution and the existence of ties.

Two methods of treating ties have been discussed in the literature. The first is to order tied observations at random (random ranking approach). The second method is to attribute to each of the tied observations the average of the tied ranks; that is, the mean of the ranks that the observations would have if they were not tied (midrank approach).

In order to maintain the null distribution of their interval mapping test statistic, which is asymptotically normal, Kruglyak & Lander (1995) and Coppieters *et al.* (1998) choose to rank tied individuals at random. This approach has the benefit of simplicity, because no new theory is necessary: the variance of the test statistic is unchanged in presence of ties. However, when dealing with phenotypes presenting a high number of ties, the information on ties is ignored and, furthermore, new information is added into the data (i.e. individuals are ordered even though they were tied). As stated by Lehmann (1975) for the classic Wilcoxon rank-sum test, when attributing midranks instead of random ranking, the variance of the test statistic now depends on the number and type of ties observed, and the asymptotic normal distribution of the test statistic no longer holds. Consequently, if midranks are preferred, it is necessary to modify the test statistic of Coppieters *et al.* (1998) and to reformulate its asymptotic properties.

Therefore, the aim of the present study is to obtain a non-parametric test statistic using midranks, and to compare it with the random ranking approach in the presence of ties (counts data). Comparisons will be carried out in terms of statistical power and of the accuracy of the estimated QTL location.

2. Methods

After recalling briefly the principle of the non-parametric interval mapping test, a modification of the test statistic to account for high number of ties by attributing midranks is presented. By means of simulations, the new interval mapping test is compared to the non-parametric interval mapping test using random ranks and to the classical regression interval mapping test.

(i) *Non-parametric interval mapping*

The Wilcoxon rank-sum test was adapted to the interval mapping problem by Kruglyak & Lander (1995). The principles of this approach have been extensively reviewed (Georges & Coppieters, 2000; Coppieters *et al.*, 1998). The Wilcoxon rank-sum test can easily be applied to QTL mapping in a single half-sib pedigree by considering that the treatments are either to receive homologue A from the sire at map position p or to receive homologue B from the sire. Kruglyak & Lander (1995) define the statistic $Y_W(p)$ for the backcross, and Coppieters *et al.* (1998) for outbred half-sib pedigrees. For the sake of simplicity, only the statistic adapted to outbred half-sib pedigrees is detailed here

$$Y_k(p) = \sum_{j=1}^{N} [N+1-2\,rank_j](P_j(A)-P_j(B)), \qquad (1)$$

where N is the number of progeny in the half-sibship, $rank_j$ is the rank by phenotype of progeny j and $P_j(A)$ and $P_j(B)$ are the probabilities – conditional on marker information – that offspring j inherits homologue A or B from its sire at the position p being considered (equivalent to the notation $P[g_{i,A}(p)|g_{i,L},g_{i,R}]$ of Coppieters *et al.* (1998)). At the marker location, $P_j(A)$ and $P_j(B)$ take values 0 or 1. Under the null hypothesis of no QTL and for large values of N, $Y_K(p)$ can be shown to be normally distributed with mean 0 and variance given by Eqn 2

$$Var(Y_K(p)) = \left(\frac{N^3-N}{3}\right) Var(P_j(A)-P_j(B)), \qquad (2)$$

where $Var(P_j(A)-P_j(B))$ is the expected variance of $P_j(A)-P_j(B)$ over all possible marker genotypes. Therefore, one can define the statistic $Z_k(p)$

$$Z_K(p) = \frac{Y_K(p)}{\sqrt{Var(Y_K(p))}}, \qquad (3)$$

which is distributed as a standard normal variable and allows the significance of the QTL effect to be tested at map position p. This test statistic reduces to a classic Wilcoxon rank-sum test at fully informative markers. As in Tilquin *et al.* (2001), the variance of the differences between observed values of $P_j(A)$ and $P_j(B)$ was used to calculate $Var(P_j(A)-P_j(B))$ in Eqn 2. To perform an across-family analysis, Coppieters *et al.* (1998) proposed to square and sum the individual $Z_K(p)$ scores over all s families to yield a χ^2 statistic with s degrees of freedom. This procedure

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was originally proposed by Neimann-Sørensen & Robertson (1961).

(ii) *Modification to use midranks*

In the presence of ties, the null distribution of the ranks (and consequently of the sum of ranks) no longer holds. The mean is unchanged but the variance of the ranks decreases as the number of ties in the data increases (Lehmann, 1975). To maintain the null distribution of the ranks, tied individuals can be ranked at random. Another approach is to give to all tied individuals the average of the tied ranks (a midrank) (see for example Lehmann, 1975). This latter approach is somewhat more correct because no new information is added into the data: individuals with the same phenotypic value get the same rank. However, it has the drawback that hypotheses of the Wilcoxon rank-sum test no longer hold (the variance of the test statistic is changed). The null distribution of the interval mapping statistic ($Z_K(p)$) also depends on the number and pattern of ties: the test statistic within a single family is composed of $Y_K(p)$ divided by its expected standard deviation $\sqrt{Var(Y_K(p))}$, which uses the variance of ranks in its formula (data not shown).

A correction for $Var(Y_K(p))$ is easily found by introducing into Eqn 2 a correction for the variance of ranks proposed by Lehmann (1975). Suppose that the N observations take on e distinct values, and that d_1 of the N observations are equal to the smallest value, d_2 to the next smallest and so forth, and d_e to the largest. With this notation:

$$Var(Y_K^*(p)) = \frac{1}{3}\left[(N^3 - N) - \sum_{i=1}^{e} d_i(d_i^2 - 1)\right] \times Var(P_j(A) - P_j(B)), \quad (4)$$

which reduces to the previous variance formula in the absence of ties. Therefore, one can define the following new statistic corrected for the presence of ties

$$Z_K^*(p) = \frac{Y_K^*(p)}{\sqrt{Var(Y_K^*(p))}}, \quad (5)$$

which is distributed as a standard normal variable and allows a test of significance of the QTL effect at map position p. As the previous $Z_k(p)$, this test statistic reduces to a classic Wilcoxon rank-sum test at the marker positions and, if squared and summed over all s families, yields a χ^2 statistic with s degrees of freedom.

(iii) *Interval mapping by regression*

Both non-parametric tests (using random ranking of ties or using midranks, hereafter referred to as NP-RA and NP-MI, respectively) were compared with regression interval mapping (RIM). The RIM approach is based on a regression of the value of the trait on the probabilities of inheriting a given gamete from the sire (Knott *et al*., 1996).

(iv) *Comparison of methods*

(a) *Simulated dataset*

We simulated the segregation of a QTL in a half-sib design. Each of 30 sires were randomly mated to 40 unrelated dams and the trait was measured on a single offspring per mating, totalling 1200 measured individuals. The heritability of the trait was set to 0·25 in all simulations. Simulations were carried out under the hypothesis of one QTL segregating (H_1) and were repeated 1000 times. 11 markers were evenly spaced (interval 10 cM) on a 100 cM segment. The QTL was positioned in the centre of the fourth interval (position 35 cM on a 100 cM chromosome). In all simulations, the number of alleles at the markers was 16, occurring with equal frequency. This number of alleles was chosen to mimic a fully informative situation. Furthermore, the dam allele was specifically coded to be always identifiable. The number of alleles at the QTL was equal to 2 with equal frequency (0·5). Seven levels of heritability owing to the QTL were simulated: 0·5%, 2%, 4·5%, 8%, 12·5%, 18% and 24·5% of the total phenotypic variance (i.e. 0·1, 0·2, 0·3, 0·4, 0·5, 0·6 and 0·7 in terms of Falconer & Mackay's substitution effect).

The simulation process was based on an algorithm already described by Baret *et al*. (1998). A normally distributed phenotype was simulated and used as a reference. Non-normally distributed phenotypes presenting various proportions of ties were simulated according to an approach used in geostatistics and referred to as normal score back transformation; see Tilquin *et al*. (2001) for the rationale for this transformation.

In order to compare both non-parametric statistics in the presence of ties, zero-inflated continuous phenotypes were simulated with increasing proportions of zeros (ties) – bacteria counts presenting 0%, 8·5%, 20%, 35%, 50%, 65% and 80% zeros. The distribution of bacteria counts with 8·5% zeros was simulated using an observed distribution from the study of Frédéric Lantier (personal communication), who performed an artificial infection in sheep with a vaccinal *Salmonella* strain (Tilquin *et al*., 2001; Moreno *et al*., 2003). This distribution was used as a reference for our simulations. Other distributions of bacteria counts used for the simulations were artificially generated by suppressing zero values (yielding 0% zeros) or adding zero values to the original distribution of bacteria counts with 8·5% zeros.

Because the RIM method requires normally distributed residuals, a logarithmic transformation was applied to bacteria counts prior to analysis for this method. A constant 1 was added to the phenotypic

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Table 1. *Power (%) at the 5% significance level and mean bias (±s.e.) of the estimated QTL location (cM) for a range of proportions of zeros in bacteria counts and a QTL substitution effect of 0·4* ($h_{QTL}^2 = 0{\cdot}08$). *Power estimates are based on 1000 replicates of each alternative (phenotype and method), using 1000 permutations to obtain the significance level of each replicate. Heritability of the trait was 0·25*

		Power			Mean bias		
Phenotype	Zeros	NP-RA	NP-MI	log+RIM	NP-RA	NP-MI	log+RIM
Normal		55[a]	55[a]	60[a]	3·4±0·6[a]	3·5±0·6[a]	3·6±0·6[a]
Bacteria counts	0·0%	55[a]	55[a]	59[a]	3·5±0·6[a]	3·5±0·6[a]	3·3±0·6[a]
	8·5%	55[a]	55[a]	57[a]	3·5±0·6[a]	3·4±0·6[a]	3·1±0·6[a]
	20·0%	54[a]	54[a]	56[a]	3·8±0·6[a]	3·5±0·6[a]	3·5±0·6[a]
	35·0%	49[a]	51[a]	53[a]	4·8±0·7[a]	4·1±0·6[a]	3·9±0·6[a]
	50·0%	38[a]	46[b]	47[b]	5·0±0·7[a]	3·9±0·7[a]	4·1±0·6[a]
	65·0%	26[a]	37[b]	37[b]	6·9±0·8[a]	5·3±0·8[a]	5·7±0·7[a]
	80·0%	13[a]	24[b]	25[b]	10·6±0·9[a]	6·6±0·8[b]	7·0±0·8[a,b]

[a,b] Comparison of power estimates and mean bias between methods within phenotype (tests for the comparison of two proportions based on a normal approximation and Wilcoxon two-sample tests respectively for power estimates and mean bias); values with the same superscript are not significantly different, $P > 0{\cdot}0167$ (Bonferroni correction of $P > 0{\cdot}05$).

values prior to transformation to avoid problems caused by the log of zero.

(b) *Significance thresholds and power estimates*

For both non-parametric methods (NP-RA and NP-MI) and for RIM, significance thresholds were determined using permutations of the phenotypes (or ranks) as suggested by Churchill & Doerge (1994). For each simulation, 1000 permutations were carried out of phenotypes within family. For each permutation, the highest value of the test statistic over the entire chromosome was retained to yield chromosome-wise distributions of the maximum test statistics under the null hypothesis and under the specific conditions of each simulation. A simulation was declared to be significant when its test statistic value was higher than the 5% chromosome-wise threshold obtained by permutations. Among the 1000 simulations, the proportion of significant simulations was used as a power estimate. Power estimates were considered to be statistically different if their difference was higher than $z_{1-\alpha/2}\sqrt{(u(1-u)(1/n_1+1/n_2))}$, where $z_{1-\alpha/2}$ is the $1-\alpha/2$ quantile of the standard $N(0, 1)$, u is the proportion of significant runs pooled across methods and n_1 and n_2 are the number of runs for each method (Snedecor & Cochran, 1967, p. 220). The significance level of this test was adjusted for multiple comparisons using the Bonferroni method.

To compare the accuracy of the three QTL mapping methods, the mean bias (±standard error (s.e.)) of the estimated QTL location was computed over the 1000 simulations. For each simulation, the estimated QTL location was given by the position maximizing the test statistic along the chromosome. The bias of the estimated QTL location was computed by subtracting 35 (the simulated location of the QTL) from the estimated position. Mean bias values were compared using Wilcoxon two-sample tests. Furthermore, empirical 95% confidence intervals (mean, 2·5% and 97·5% percentiles) were computed for the mean estimated QTL location.

3. Results

(i) *Simulated data*

Bacteria counts with various proportions of zeros were simulated. Average percentages of zeros (mean±s.e.) in simulated bacteria counts over the 1000 replicates and with a QTL substitution effect of 0·4 were 8·2±0·03%, 19·6±0·4%, 34·7±0·8%, 49·9±1·3%, 65·1±1·8% and 80·3±2·3%, respectively, for bacteria counts with 8·5%, 20%, 35%, 50%, 65% and 80% of zeros. When other values of QTL substitution effect were simulated, proportions of zeros in simulated bacteria counts were very similar to the proportions observed with a QTL effect of 0·4 (data not shown).

(ii) *Power estimates*

For the three QTL mapping methods, the power was estimated at various levels of QTL substitution effect (a) and for increasing proportions of zeros (ties) in the phenotype. Full results are presented for a QTL substitution effect of 0·4 (8% of total phenotypic variance) (Table 1) and summarized according to the QTL effect and for increasing proportions of zeros by means of power curves (Fig. 1).

Up to 20% zeros, there were no differences in power between the random ranking and the midranks approaches. From 35% to 80% zeros, the advantage of using the midranks approach increased from 1% to

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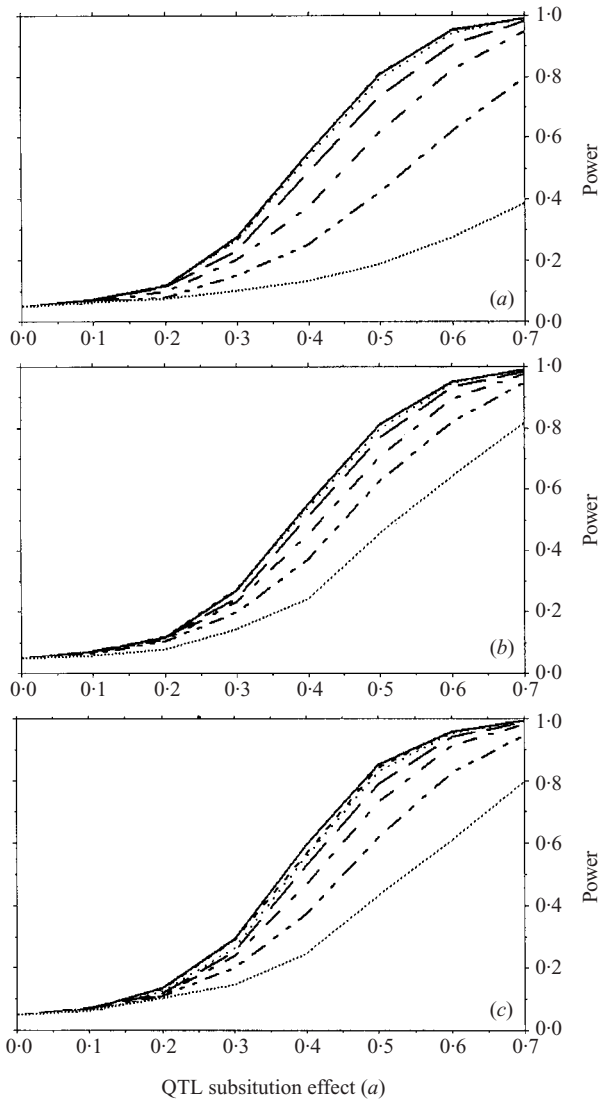

Fig. 1. Power (%) of NP-RA (a), NP-MI (b) and log+RIM (c) methods as a function of the QTL substitution effect (*a*) and of the proportion of zeros in bacteria counts: 0% (solid line), 8·5% (large dots), 20% (small dots), 35% (long dashes), 50% (long–short dashes), 65% (long–short–short dashes) and 80% (short dashes).

43% according to the QTL substitution effect. For a QTL effect of 0·4 (8% of total phenotypic variance), the gains in using the midranks approach for phenotypes with 35%, 50%, 65% and 80% zeros were 2%, 8%, 11% and 11% of power respectively (gains were significant for 50%, 65% and 80% of zeros). The power of NP-MI was always significantly higher than the power of NP-RA when searching for QTL with effects higher than or equal to 0·4 (8% of total phenotypic variance) and for a high proportion of zeros (50%, 65% and 80% of zeros). The gain in power using NP-MI compared to NP-RA therefore depends on the levels of the QTL substitution effect (*a*) and of the proportions of zeros (ties) in the phenotype.

For phenotypes producing 80% zeros, non-significant differences were observed between power estimates of NP-MI and RIM methods. For QTL effects of 0·1, 0·2 and 0·4, the power of RIM was respectively 1%, 3% and 1% higher than the power of NP-MI, whereas, for QTL effects of 0·5, 0·6 and 0·7, the power of NP-MI was 2%, 3% and 2% higher than the power of RIM (Fig. 1). The power of RIM was significantly higher (4%) than the power of NP-MI only when analysing the normal phenotype for a QTL substitution effect of 0·5 (12·5% of total phenotypic variance).

(iii) *Position estimates*

The mean bias (±s.e.) of the estimated QTL location was computed over the 1000 replicates and for alternative set of simulations. Full results are presented for a QTL substitution effect of 0·4 (8% of total phenotypic variance) (Table 1).

For all QTL mapping methods and for all phenotypes and QTL effects, the bias was positive, meaning that the estimated position tends to be biased towards the centre of the chromosome, as already observed by other authors (e.g. Walling *et al.*, 2002). The mean bias increases as the proportion of zeros in the phenotypic distribution increases. This increase is higher for the non-parametric test using random ranking of ties (NP-RA) than for the test using midranks (NP-MI). Furthermore, for a high proportion of zeros (⩾50%), the mean bias of the NP-MI method is also smaller than the mean bias of the RIM method, but the differences were not significant.

The variation of the estimated QTL position according to the QTL effect and for the three QTL mapping methods is depicted by use of empirical 95% confidence intervals of the mean position estimate over the 1000 replicates, and for three proportions of zeros in the phenotype: 20%, 50% and 80% (Fig. 2). For QTL effects less than 0·4, the empirical 95% confidence intervals were not depicted because they included the entire chromosome.

There is a bias towards the centre of the chromosome when the QTL effect is small: for $a=0{\cdot}1$ or 0·2, the mean estimated QTL position is shifted to the centre of the chromosome (50 cM) (data not shown). This bias decreases to zero as the effect of the simulated QTL increases. The size of the confidence interval is also influenced by the QTL effect: the larger the effect, the smaller the interval. For QTL effects larger than 0·2, the mean estimated position of the QTL using the NP-MI method was closer to the simulated position than using the NP-RA method. This result was especially observed for 80% of zeros (Fig. 2), for which the mean location estimate of the NP-RA method was more biased towards the centre of the chromosome than the NP-MI and RIM methods. For proportions of zeros larger than 20% and for QTL effects larger than 0·4, the length of the confidence

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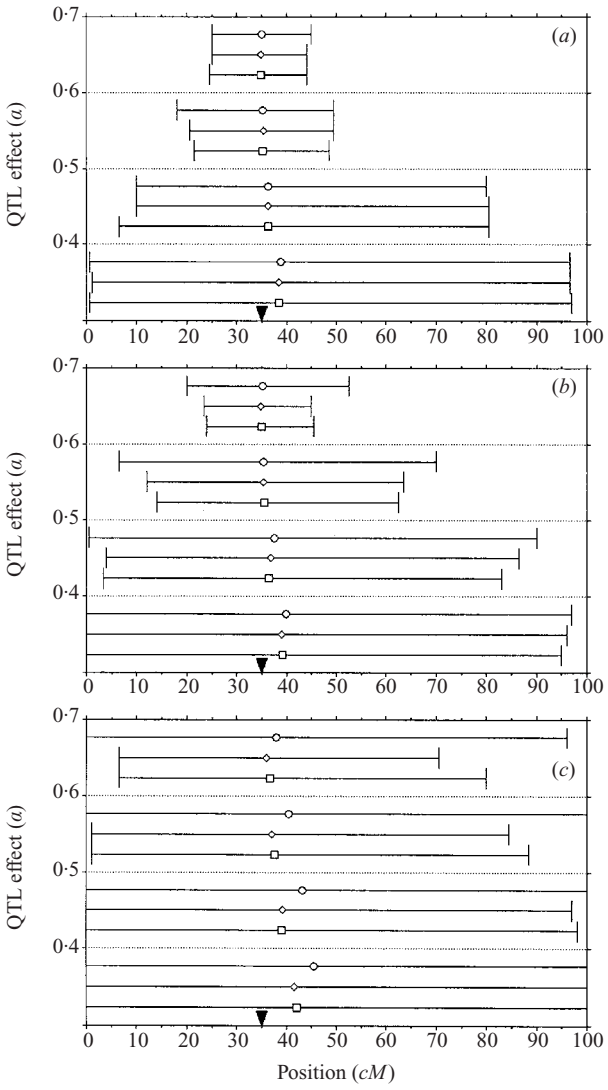

Fig. 2. Empirical 95% confidence intervals for the mean estimated QTL position (mean, 2·5% and 97·5% percentiles) over the 1000 replicates as a function of the QTL substitution effect and for the three QTL mapping methods (NP-RA, circles; NP-MI, diamonds; log + RIM, squares). Three proportions of zeros in bacteria counts are depicted: 20% (a), 50% (b) and 80% (c). Arrowheads indicate the location of the simulated QTL.

interval was always shorter for the NP-MI than for the NP-RA method.

4. Discussion

In this study, we have adapted the non-parametric QTL mapping method based on the sum of ranks that was described previously by Kruglyak & Lander (1995) and Coppieters *et al.* (1998) to the analysis of aggregated or discontinuous phenotypes (producing a high number of ties). This is particularly relevant for the analysis of disease-resistance phenotypes. Indeed, the assessment of the disease-resistance status of animals to bacterial, parasitic or viral diseases is often based on a counting process. The distribution of this type of trait is right-skewed with a long tail, and a high frequency of zero values is often observed (e.g. Bishop & Stear, 2001). Such a feature comes from the difficulty of appropriately choosing the moment for sampling individuals, in order to have the most informative data set. Because of the high costs of such experiments, data cannot be thrown away and should be analysed, even if there is a high frequency of zeros in the phenotypic distribution.

Such distributions are neither continuous nor categorical. Furthermore, because of the excess of zeros and because of the high values taken by those counts, a Poisson structure cannot generally be assumed. In the context of parasitology, Wilson & Grenfell (1997) have proposed the use of generalized linear modelling with a negative binomial structure of errors for this type of distribution. However, to our knowledge, no QTL mapping methods have yet been developed with such a feature.

By simulating increasing proportions of ties, we showed that power and precision were both increased using midranks instead of random ranks. For a QTL substitution effect of 0·4 (8% of phenotypic variance), a gain from 8% to 11% of power can be obtained. For higher levels of QTL effects and when there are 80% of zeros in the phenotype, this gain can reach the values of 27%, 37% and 43% respectively for QTL substitution effects of 0·5, 0·6 and 0·7 (Fig. 1).

This gain can easily be explained by the fact that, when there are many ties in the phenotype, new information is added in the data if ties are ranked at random: individuals with the same value get different ranks although they should have the same rank. As noted by Kendall & Stuart (1979), random ranking of ties has the merit of simplicity and needs no new theory but obviously sacrifices information contained in the observations and might be expected to lead to loss of efficiency compared with the midranks approach. Other studies on the Wilcoxon rank-sum statistic have already shown that, when random ranks are used, the efficiency of the test is reduced (Putter, 1955; McNeil, 1967).

However, if the chance of having a group of ties in the phenotype is very low (e.g. true continuous distributions in which ties would occur only by chance, for example by the rounding of values), the gain by using midranks would be very small. Indeed, the magnitude of the correction factor in the variance of the test statistic depends on the number of tied values and on the distribution of these values in different groups. For a given number of tied values, the contribution to the correction factor is bigger if they are all in the same group. This was stated by Siegel & Castellan (1988) for the classic Wilcoxon rank-sum statistic.

For high proportions of zeros in the phenotype, results for the non-parametric interval mapping method using midranks were not significantly different

from the results of the regression interval mapping method (Knott *et al*., 1996), whereas results for the non-parametric interval mapping using random ranks were significantly lower. Even though the RIM method performed equivalently to the newly modified NP-MI, there are situations in which the use of a non-parametric method should be preferred. Indeed, one can sometimes fail to find the appropriate mathematical transformation to normalize the data. Furthermore, other assumptions (such as homogeneity of variances) are not always fulfilled when a mathematical transformation is applied, and parametric tests are more sensitive than non-parametric methods to the presence of outliers.

Instead of simulating increasing proportions of zeros, one group of ties at the end of the distribution could have been simulated by giving the same value to individuals with a phenotypic value higher than a specified threshold. Because it is the size of the group of ties that determines the correction factor of the variance of the test statistic, this would have had the same effect as one group of ties at the beginning of the distribution. Another alternative would have been to simulate one group of ties randomly positioned in the distribution by giving the same value to individuals having a phenotypic value in a specified window, but such a random group of ties would have had no obvious biological basis. The simulation of some small groups of ties randomly distributed in the phenotype, as observed when observations are rounded, was not considered because their contribution to the correction factor of the variance of the test statistic would have been too small. Finally, no categorical nor ordinal traits were simulated because there are specific methods to deal with this type of trait (Hackett & Weller, 1995; Visscher *et al*., 1996).

In conclusion, the midranks approach is easy to implement (Fortran code is available upon request). Therefore, in practice, if non-parametric interval mapping is chosen, one should favour the use of midranks to deal with tied values.

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