Genet. Res., Camb. (2001), **78**, *pp.* 303–316. *With 2 figures.* © 2001 Cambridge University Press
DOI: 10.1017/S0016672301005365 Printed in the United Kingdom

Statistical power of QTL mapping methods applied to bacteria counts

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(*Received 12 February 2001 and in revised form 22 June and 7 August 2001*)

Summary

Most QTL mapping methods assume that phenotypes follow a normal distribution, but many phenotypes of interest are not normally distributed, e.g. bacteria counts (or colony-forming units, CFU). Such data are extremely skewed to the right and can present a high amount of zero values, which are ties from a statistical point of view. Our objective is therefore to assess the efficiency of four QTL mapping methods applied to bacteria counts: (1) least-squares (LS) analysis, (2) maximum-likelihood (ML) analysis, (3) non-parametric (NP) mapping and (4) nested ANOVA (AN). A transformation based on quantiles is used to mimic observed distributions of bacteria counts. Single positions (1 marker, 1 QTL) as well as chromosome scans (11 markers, 1 QTL) are simulated. When compared with the analysis of a normally distributed phenotype, the analysis of raw bacteria counts leads to a strong decrease in power for parametric methods, but no decrease is observed for NP. However, when a mathematical transformation (MT) is applied to bacteria counts prior to analysis, parametric methods have the same power as NP. Furthermore, parametric methods, when coupled with MT, outperform NP when bacteria counts have a very high proportion of zeros (70·8%). Our results show that the loss of power is mainly explained by the asymmetry of the phenotypic distribution, for parametric methods, and by the existence of ties, for the non-parametric method. Therefore, mapping of QTL for bacterial diseases, as well as for other diseases assessed by a counting process, should focus on the occurrence of ties in phenotypes before choosing the appropriate QTL mapping method.

1. Introduction

Infectious diseases cause important economic losses in livestock production (Adams & Templeton, 1998). A major objective in current animal breeding strategies is therefore to improve animal health, but this is difficult to achieve by traditional breeding methods. There are many documented instances of breed or individual differences in genetic disease resistance. Thus, the identification of chromosomal regions implied in disease resistance, i.e. disease resistance loci (DRL), might have important implications for the development of strategies for the control of diseases in livestock. Statistical methods have been developed for the mapping of genes that contribute to the variation of quantitative traits (e.g. Beckmann & Soller, 1988; Lander & Botstein, 1989; Weller *et al.*, 1990). Such genes are called QTL, for quantitative trait loci. QTL mapping methods use marker information to identify chromosomal segments likely to contain QTL. For disease resistance traits that show continuous variation, QTL mapping methods have already proved to be effective for the identification of DRL (e.g. Kemp *et al.*, 1997; Vallejo *et al.*, 1998; Heyen *et al.*, 1999).

Most QTL mapping methods share a common assumption: the phenotype follows a normal distribution. However, there are many instances of disease resistance phenotypes that are not normally distributed. Examples include counts (such as number of bacteria in spleen or nodes), truncated data (such as

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https://doi.org/10.1017/S0016672301005365 Published online by Cambridge University Press

survival times in a censored experiment) and qualitative data (such as severity grades assigned upon histological examination). QTL mapping methods based on parametric assumptions cannot be directly applied in such cases. On the one hand, least-squares and ANOVA based methods (Haley & Knott, 1992; Martinez & Curnow, 1992; Weller *et al.*, 1990) assume that residual errors, i.e. residuals within QTL genotype classes, are normally distributed. Such methods are commonly said to be robust against non-normality; however, robustness against any type of non-normality in the context of QTL mapping methods has, to our knowledge, never been established. On the other hand, maximum-likelihood based methods (Lander & Botstein, 1989) use the normal density function for the building of the likelihood itself. Quality of estimations is therefore very dependent on the normality of the phenotype. One approach to circumvent the assumption of normality is to attempt to find a mathematical transformation that will convert the trait into an approximately normal variable. An alternative approach is to apply non-parametric (or distribution-free) methods to QTL mapping. Recently, Kruglyak & Lander (1995*a*) described a non-parametric interval mapping approach based on the Wilcoxon rank-sum test applicable to experimental crosses. Coppieters *et al.* (1998) adapted this method to half-sib pedigrees in outbred populations.

Natural resistance to bacterial diseases was observed a long time ago (e.g. Roberts & Card, 1926; Cameron *et al.*, 1943 [cited by Adams & Templeton, 1998]). More recently, studies were carried out on resistance to *Salmonella enteriditis* in chicken (Guillot *et al.*, 1995; Protais *et al.*, 1996), and differences in susceptibility between lines were highlighted. Typically, measures of resistance to bacterial diseases are counts such as colonization levels in organs (CFU, i.e. colony-forming units). This type of data presents an extremely asymmetric distribution with, sometimes, a spike at zero. In order to study the robustness of current QTL mapping methods to this type of highly asymmetric distributions, data were obtained from a bacterial infection which was performed experimentally in a sheep flock of 30 sires families of 40 half-sibs each (Frédéric Lantier, personal communication; Moreno *et al.*, 2001).

A first aim of the present study is to compare the current QTL mapping methods in terms of statistical power when applied to bacteria counts. The second is to assess the interest of applying a mathematical transformation to bacteria counts prior to QTL analysis. By use of simulations, the efficiency of four QTL mapping methods in a half-sib design are compared: (1) least-squares (LS) analysis, (2) maximum-likelihood (ML) analysis (Knott *et al.*, 1996), (3) non-parametric (NP) mapping set up by Kruglyak & Lander (1995*a*) and adapted to half-sib designs by Coppieters *et al.* (1998) and (4) nested ANOVA (AN). Methods are compared according to their efficiency for detecting QTL of various effects, either when a single marker and a single QTL sharing the same location are simulated, or when chromosome scans are simulated.

2. Materials and methods

All the simulations are based on the same design: 30 families of 40 half-sibs. Each sire is randomly mated to 40 unrelated dams and the trait is measured on a single offspring per mating, which amounts to 1200 measured individuals. Heritability of the trait is set to 0·25 in all simulations. Either single positions (1 position, 1 marker) or chromosomes scans (100 cM, 11 markers) are simulated. Simulations are carried out either under the null hypothesis (H_0) or under the hypothesis of one QTL segregating (H_1). In single-position simulations and under H_1, a single marker and a single QTL share the same location on a chromosome. In chromosome scans, the 11 markers are evenly spaced (interval 10 cM) on a 100 cM chromosome segment. For simulations under H_1, a single QTL is positioned at 35 cM on the 100 cM chromosome segment (between the fourth and the fifth marker). In all simulations, the number of alleles at the markers is equal to 16, occurring with equal frequency. Such a number of alleles was chosen to mimic a fully informative situation. Furthermore, the dam allele is specifically coded to be always identifiable. For simulations under H_1 (one QTL segregating), the number of alleles at the QTL is equal to 2 with equal frequency (0·5). The value of the QTL effect is given according to Falconer & Mackay's (1996) definition of substitution effect (a), i.e. half the difference between the mean trait values for the two alternative homozygotes at the QTL (in phenotypic standard deviation unit). Conversion to heritability due to the QTL can be achieved using the following equation:

$$h^2_{\mathrm{QTL}} = 2\,p\,q\,a^2 \qquad (1)$$

where p and q are the frequencies of alleles A and B of the QTL. Using this equation and assuming that $p =$ 0·5, values of 0·2, 0·4 and 0·6 of Falconer & Mackay's substitution effect are respectively equal to 0·02, 0·08 and 0·18 in terms of heritability due to the QTL, i.e. 2%, 8% and 18% of the total phenotypic variance. In chromosome scans, only one level of the QTL effect is simulated, i.e. a QTL accounting for 8% of the total phenotypic variance.

(i) *Simulation process*

The simulation process is based on an algorithm developed by Baret *et al.* (1998). The QTL has two

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alleles that are assigned at random to the sire haplotypes depending on their frequency. For each offspring, the haplotype inherited from the sire (taking into account recombination events) is determined by a binomial distribution draw between the two sire haplotypes. The QTL allele inherited from the dam is drawn from a binomial distribution.

Normally distributed values of offspring phenotypes are simulated as detailed below. Sire breeding value is equal to:

$$A_s = A_{QTL_s} + A_{inf_s} \quad (2)$$

where A_{QTL_s} is the sum of the effects of QTL alleles allocated to the sire; and A_{inf_s} is the contribution of other chromosomes to the trait and is randomly simulated by a normal distribution assuming the infinitesimal model $N(0, [h^2 \sigma_P^2])$. Offspring phenotypic value is equal to:

$$P_{offspring} = A_{QTLsire} + A_{QTLdam} + \tfrac{1}{2} A_{inf_s} + I + E \quad (3)$$

where $A_{QTLsire}$ is the effect of the QTL allele inherited by the offspring from the sire; A_{QTLdam} is the effect of the QTL allele inherited by the offspring from the dam; I is the remaining polygenic effect simulated by a normal distribution $N(0, [0{\cdot}75h^2 \sigma_P^2])$ – this term includes dam infinitesimal effects; and E is the environmental noise simulated by a normal distribution $N(0, [(1-h^2)\sigma_P^2])$.

In order to simulate non-normally distributed phenotypes (bacteria counts), an approach used in geostatistics and referred to as *normal score back-transformation* is applied (Goovaerts, 1997; Deutsch & Journel, 1998). The rationale of this transformation is explained below.

Let $G(z)$ be the cumulative density function (*cdf*) of the standard normal random variable Z (the one of the simulated normal phenotypes); let $F(y)$ be the *cdf* of the non-normal random variable Y (i.e. the non-normally distributed phenotype):

$$G(z) = \Pr(Z \leqslant z) \quad (4)$$

$$F(y) = \Pr(Y \leqslant y) \quad (5)$$

The *normal score back-transformation* can be seen as a correspondence table between the p-quantiles z_p and y_p of the two different distributions; in other words, z_p and y_p correspond to the same cumulative probability p:

$$F(y_p) = F[F^{-1}(G(z_p)] = G(z_p) = p \quad \forall p \in [0, 1] \quad (6)$$

In practice, the cumulative density function of the non-normal phenotype is not available. Instead, one uses a sample of the distribution, called the conditional cumulative density function (*ccdf*), since it depends on the number of observations in the data set. Let $F(y|N)$ and $G(z|N)$ be the *ccdf* of the non-normal random variable Y and the standard normal variable function Z; the transformation of a normally distributed phenotypic value z_i into a non-normally distributed phenotypic value y_i is given by:

$$y_i = F^{-1}[G(z_i|N)] \quad (7)$$

where $G(z_i|N)$ is the probability value of the phenotypic value z_i in the standard normal *ccdf*. The value of the non-normally distributed phenotype is obtained by reversing the non-normal *ccdf* $F(y|N)$. Simulation of a non-normally distributed phenotype is therefore performed by applying this transformation to all simulated values of the normally distributed phenotype.

In most cases the simulated z_i values will not correspond exactly to an original value of the standard normal *ccdf*; therefore, to get y values of the non-normally distributed phenotype, some interpolation between the original y values or extrapolation beyond the smallest and largest y value of the non-normal *ccdf* will be required. Interpolation between two known values is chosen to be linear. For the treatment of the lower and upper tails, no extrapolation is used: the simulated z_i values lower or greater than the minimum or the maximum values of the standard normal *ccdf* are respectively set to the minimum (i.e. zero) and to the maximum values of the non-normal *ccdf*.

(ii) *Data set*

Distributions of bacteria counts (i.e. non-normal *ccdf* $F(y|N)$) were obtained from the study of Frédéric Lantier (personal communication) who performed an artificial infection with a live vaccine in sheep, using 30 sires families of 40 half-sibs. Lambs weighing approximately 36 kg (males) or 32 kg (females) were infected intravenously with the vaccinal *Salmonella abortusovis* strain Rv6. Their blood, spleen and lymph nodes were sampled at slaughtering (10 days later) for the assessment of bacterial colonization and antibody responses, and for DNA typing. Bacteria counts (per gram of the organ dry weight), i.e. CFU, were measured in the spleen and in left and right lymph nodes.

Even though pooling of the counts from left and right lymph nodes is controversial since vaccination was done on the right side of the lambs (which showed higher values than the left lymph node), counts are pooled to take advantage of the resulting distribution. Indeed, bacteria counts present an extremely asymmetric distribution which is bimodal: a spike at zero (8·5 % of zeros for the sum of both lymph nodes, 20·0 % and 16·9 % of zeros in right and left lymph nodes respectively, 70·8 % of zeros in spleen) and the rest distributed as an exponential distribution. As our objective is to study a representative range of zero values, three sample distributions from the study of Frédéric Lantier (personal communication) are

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chosen: bacteria counts for the sum of both lymph nodes ($n = 1170$, 8·5% zeros), in the right lymph node ($n = 1171$, 20·0% zeros) and in the spleen ($n = 1169$, 70·8% zeros). A fourth distribution was generated by suppressing zero-valued data in the first sample distribution of bacteria counts; the resulting distribution will be referred to as bacteria counts with 0·0% of zeros ($n = 1071$).

(iii) *Methods of analysis*

Four methods of analysis are compared: three interval mapping methods – two based on parametric assumptions concerning the distribution of the phenotype (least-squares and maximum likelihood interval mapping) and one that makes no assumptions (non-parametric interval mapping); and one QTL detection test commonly used for a preliminary genome scan (e.g. Vallejo *et al.*, 1998; Yonash *et al.*, 1999; Heyen *et al.*, 1999), which will be referred to as single-marker analysis.

(a) *Interval mapping analysis.* In the interval mapping approach (Lander & Botstein, 1989), for given positions (e.g. 1 cM intervals) in a linkage group (i.e. chromosome), the probability of an offspring inheriting one or the other of its parents' haplotypes (gametes) at that position is calculated conditional on its marker genotype. As applied to a half-sib design, where little or no information on QTL/marker linkages can come from the dam, it is of interest to calculate these probabilities only for the sire gamete. Once these probabilities have been calculated, they can be incorporated into either a least-squares (LS), a maximum-likelihood (ML) or a non-parametric (NP) analysis.

The LS approach is based on a regression of the value of the trait on the probabilities of inheriting a given gamete from the sire (see Knott *et al.*, 1996). The evidence in favour of a segregating QTL, at a given chromosome position is assessed by an F-test: the ratio of the between-marker alleles within-sire mean square to the residual mean square. Under the null hypothesis of no QTL at the corresponding chromosome position, the test statistic is assumed to follow an F distribution with s and $s(n-2)$ degrees of freedom, s being the number of sires heterozygous at the marker and n the number of offspring per family.

The ML analysis is based on the approximate model proposed by Knott *et al.* (1996). The ML at each position is entirely defined by three parameters: the proportion of sires homozygous at the QTL (h), Falconer & Mackay's (1996) substitution effect of the QTL (a) and the within-marker within-sire residual variance (σ^2_w) (see likelihood equation in Knott *et al.*, 1996). The statistic is a likelihood ratio test (LRT): the ratio of the ML fitting a QTL and of the ML when the QTL is omitted. As shown by Baret *et al.* (1998), the distribution of this LRT is assumed to be close to a mixture of two χ^2 distributions of degrees of freedom 0 and 1 ($\frac{1}{2}\chi^2_{(0)} + \frac{1}{2}\chi^2_{(1)}$).

The NP interval mapping approach based on the Wilcoxon rank-sum test was described by Kruglyak & Lander (1995*a*). Coppieters *et al.* (1998) adapted this method to half-sib pedigrees in outbred populations. Principles of this approach were extensively presented in Coppieters *et al.* (1998). Briefly, to measure the evidence in favour of a QTL at a given map position, the following statistic is used (illustrated for an half-sib design):

$$Z_K(p) = Y_K(p)/\sqrt{\langle \sigma^2_{Y_K(p)} \rangle} \tag{8}$$

where

$$Y_K(p) = \sum_{j=1}^{n_i} [n_i + 1 - 2\,\text{rank}(j)]\,(P_{ij}(A) - P_{ij}(B)) \tag{9}$$

in which n_i is the number of progeny of sire i; rank(j) is the rank by phenotype of progeny j; $P_{ij}(A)$ (and $P_{ij}(B)$) are the probabilities – conditional to marker information – that offspring j inherits gamete A (or B) from sire i at the position being considered (equivalent to the notation $P[g_{i,A}(p) \mid g_{i,L}, g_{i,R}]$ in Coppieters *et al.*, 1998); and

$$\sqrt{\langle \sigma^2_{Y_K(p)} \rangle} \tag{10}$$

is the standard deviation of $Y_K(p)$, expected under the null hypothesis of no QTL over all possible sets of genotypes. Under the null hypothesis of no QTL, Z_K is shown to behave asymptotically as a standard normal variable that reduces to a Wilcoxon rank-sum test at the marker positions. As demonstrated by Kruglyak & Lander (1995*a*), the expected variance of $Y_K(p)$ is:

$$\langle \sigma^2_{Y_K(p)} \rangle = \left(\frac{n^3 - n}{3} \right) \langle \sigma^2_{(P_{ij}(A) - P_{ij}(B))} \rangle \tag{11}$$

where $\langle \sigma^2_{(P_{ij}(A)-P_{ij}(B))} \rangle$ is the expected value of $\sigma^2_{(P_{ij}(A)-P_{ij}(B))}$ over all possible genotypes. In outbred designs, it depends on the marker allele frequencies (among the dams) and on the genotype of the founder sire. Coppieters *et al.* (1998) calculated the value of $\langle \sigma^2_{(P_{ij}(A)-P_{ij}(B))} \rangle$ for each half-sib pedigree by simulating all possible offspring and by calculating a frequency-weighted variance of $P_{ij}(A) - P_{ij}(B)$. By definition, the within-family ratio of the observed variance of $Y_K(p)$ to the expected variance $\langle \sigma^2_{Y_K(p)} \rangle$ is the information content at position p.

When doing across-family analysis, the different sibships cannot be assumed to segregate for the same alleles within the QTL or even for the same QTL; i.e. one cannot assume locus and allelic homogeneity across families. Instead of analysing every half-sib family separately, Coppieters *et al.* (1998) suggested squaring and summing the individual $Z_K(p)$ scores

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Table 1. *Summary of the characteristics of the three interval mapping methods and of the single-marker method*

Method	Test statistic	Theoretical distribution	Estimates
LS	$F = \frac{MS_m}{MS_\epsilon}$	$F[s;s(n-2)]$[a]	Position Within-sire effect
ML	$\text{LRT} = -2\log\left(\frac{ML_{\text{reduced}}}{ML_{\text{QTL}}}\right)$	$\frac{1}{2}\chi^2_{(0)} + \frac{1}{2}\chi^2_{(1)}$	Position Experiment-wise effect Frequency of heterozygous sires
NP	$\chi^2_{(s)} = \sum_{i=1}^{s} Z^2_K(p)_i$	$\chi^2_{(s)}$	Position
AN	$F = \frac{MS_m}{MS_\epsilon}$	$F[s;s(n-2)]$	Marker associated with the QTL

[a] s is the number of sires heterozygous at the marker and n is the number of offspring per family.

over all s families to yield a χ^2 statistic with s degrees of freedom:

$$\chi^2_{(s)} = \sum_{i=1}^{s} Z^2_K(p)_i. \tag{12}$$

A slightly modified version of the computer program developed by Coppieters *et al.* (1998) is used in our analysis to implement this non-parametric approach. The first modification concerns the most likely linkage phase, which is calculated in Coppieters's computer program using a computationally demanding algorithm based on a maximum-likelihood method described by Georges *et al.* (1995). Instead, the most likely linkage phase is calculated by choosing for every pair of markers the most frequent combination of adjacent marker alleles among all offspring from the sire. In this way, all interval mapping methods (LS, ML and NP) receive the same conditional probabilities, i.e. $P_{ij}(A)$ and $P_{ij}(B)$, which allows us to compare them on the same basis.

The second modification concerns the method of calculation of the information content, which is required by the non-parametric method. As described above, Coppieters *et al.* (1998) calculated within-family values of information content at each position by simulating all possible offspring and calculating a frequency-weighted variance of $P_{ij}(A) - P_{ij}(B)$. If the true descent (maternal or paternal) of every centimorgan of DNA were known, values of QTL conditional probabilities would be either 0 or 1. The distribution of these QTL conditional probabilities has mean 0·5 and variance 0·25. Under the design used in our study, the dam allele is specifically coded to be always identifiable, and therefore, at a marker location, the allele inherited by every offspring from its sire can always be identified. Consequently, at a marker location, true descent applies in our study. It is therefore a realistic assumption to take a distribution of the QTL conditional probabilities with mean 0·5 and variance 0·25. Outside a marker location, this variance would take lower values. Following similar applications by Kruglyak & Lander (1995*b*) and Spelman *et al.* (1996), for each position, the within-family ratio of the observed variance of QTL conditional probabilities to an expected maximum value of 0·25 is used as a measure of the information content.

(b) *Single-marker analysis.* The principles of using analysis of variance (AN) to detect association between a marker and a QTL have been previously described (Neimann-Sørensen & Robertson, 1961; Weller *et al.*, 1990). For each marker in turn, informative (i.e. heterozygous) sires and offspring in which the allele inherited from the sire can be identified are selected; the test performed is an F-test: the ratio of the between-marker allele within-sire mean square to the residual mean square. As for the F-test applied in LS interval mapping, under the null hypothesis of no QTL at the corresponding marker, the test statistic is assumed to follow an F distribution with s and $s(n-2)$ degrees of freedom.

The principal characteristics of the three interval mapping methods and of the single-marker method are summarized in Table 1.

(c) *Mathematical transformation.* A classic way to cope with non-normally distributed data such as bacteria counts is to apply a mathematical transformation. After tests (data not shown), the logarithmic transformation was chosen; a constant 1 was added to the phenotypic values prior to transformation to avoid negative infinity problems due to the log of zero.

(iv) *Comparison of methods*

Using parameters described above, single-position simulations as well as chromosome scans are achieved either under the null hypothesis of no QTL (10000 or

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1000 replicates, respectively, for single-position simulations or chromosome scans), for the determination of empirical thresholds. Simulations are also achieved under the hypothesis of one QTL segregating (1000 replicates), for the determination of power estimates. Methods are compared according to their power to detect the simulated QTL, either when the phenotype is normally distributed or when the phenotype is distributed as bacteria counts. For chromosome scans, mean estimates of QTL location over all replicates are compared.

In the single-position context, AN and LS are statistically identical and give the same results; only LS, ML and NP are therefore compared in single-position simulations, while all four methods are compared in chromosome scans. In chromosomes scans, analysis is repeated at fixed location (every 1 cM); a grid search increment of 0·25 is used in the ML optimization (see Baret *et al.*, 1998).

(a) *Empirical thresholds*. Each simulated data set is analysed using three or four methods according to the type of simulation (LS, ML and NP for single-position simulations, or AN, LS, ML and NP for chromosome scans).

In the context of the single-position simulations, degrees of freedom of F ratios (LS) vary from one replicate to another since sires homozygous at the marker are dropped from the analysis. A sire has a 1/16 chance of being homozygous at the single marker. Therefore, once a sire is homozygous, 1 degree of freedom is lost for the numerator and $(n-2)$ for the denominator of the F-test (n being the number of sons in the half-sibship). Hence, direct comparison of F ratios may not be possible as they do not belong to the same distribution and, instead, the P value of each F ratio is used as proposed by Knott *et al.* (1996). In this way, all analyses can contribute to a single distribution of the test statistic. Since the χ^2 statistic of the NP analysis is dependent on s (the number of heterozygous sires), the values of the test statistics are also converted into P values. To aid comparison, however, rather than using values of the LRT, P values of the LRT are calculated, assuming a mixture of two χ^2 distributions: $\frac{1}{2}\chi^2_{(0)}+\frac{1}{2}\chi^2_{(1)}$ (Baret *et al.*, 1998). Over the 10000 replicates, for all three methods, 5% and 1% significance thresholds are determined by ranking P values of the observed test statistics under the null hypothesis. In this way, the values of significance thresholds that are used are threshold values of P values to reach an overall 5% or 1% error rate.

In chromosome scans, for LS, ML and NP, maximum values of the test statistics are ranked to determine chromosome-wise 5% and 1% significance thresholds. Indeed, unlike the single-position case, degrees of freedom of F-tests for LS will not differ between markers for two reasons: firstly, because the probability of a sire being homozygous at all markers is very low, i.e. $(1/16)^{11}$ (11 markers, with 16 alleles each); secondly, because when a marker is homozygous, information at that position will come from flanking markers. Consequently, the number of degrees of freedom of F ratios will be constant throughout the chromosome, and F values will be used for the computation of significance thresholds. Similarly, for NP and ML analysis, actual values of the χ^2 statistics will be used. Conversely, as stated by Knott *et al.* (1996), degrees of freedom of F ratios in AN will vary from marker to marker. To allow for this and similarly to single-position simulations, the P value of the F ratio for each marker is determined and the most significant marker selected as providing the best estimate of the closest marker to any QTL.

(b) *Power calculations*. Simulations under the hypothesis of one QTL segregating are achieved by generating data sets with a QTL (at marker location in single-position simulations and at 35 cM in chromosome scans). Power estimates are obtained using empirical thresholds for the three levels of QTL effect in single-position simulations (2%, 8% and 18% of phenotypic variance) and for one level of QTL effect in chromosome scans (8%). Power estimates are considered as different if the difference is higher than $1{\cdot}96\sqrt{u(1-u)(1/n_1+1/n_2)}$, where u is the proportion of runs above the significance threshold pooled across methods, and n_1 and n_2 are the number of runs for each method. As power is calculated in parallel on the same simulated data, the results of the methods of analysis are positively correlated and this test is conservative (Baret *et al.*, 1998).

3. Results

(i) *Simulated data*

Using the approach described in Section 2, we use the *normal score back-transformation* to simulate bacteria counts with various proportions of zeros (Fig. 1). Average percentages of zeros (mean ± SD) in simulated bacteria counts (for the 10000 replicates of single-position simulations) were 8·0 ± 1·1%, 19·4 ± 1·7% and 71·1 ± 2·1%, respectively, for bacteria counts in both lymphe nodes together, in the right lymphe node and in the spleen. Such values are very close to the initial parameters of the simulation: 8·5%, 20·0% and 70·8%.

(ii) *Empirical thresholds*

As expected, degrees of freedom of F ratios vary between replicates (from F[21;798] to F[30;1140]). This confirms the interest of taking into account homozygous sires for the determination of empirical

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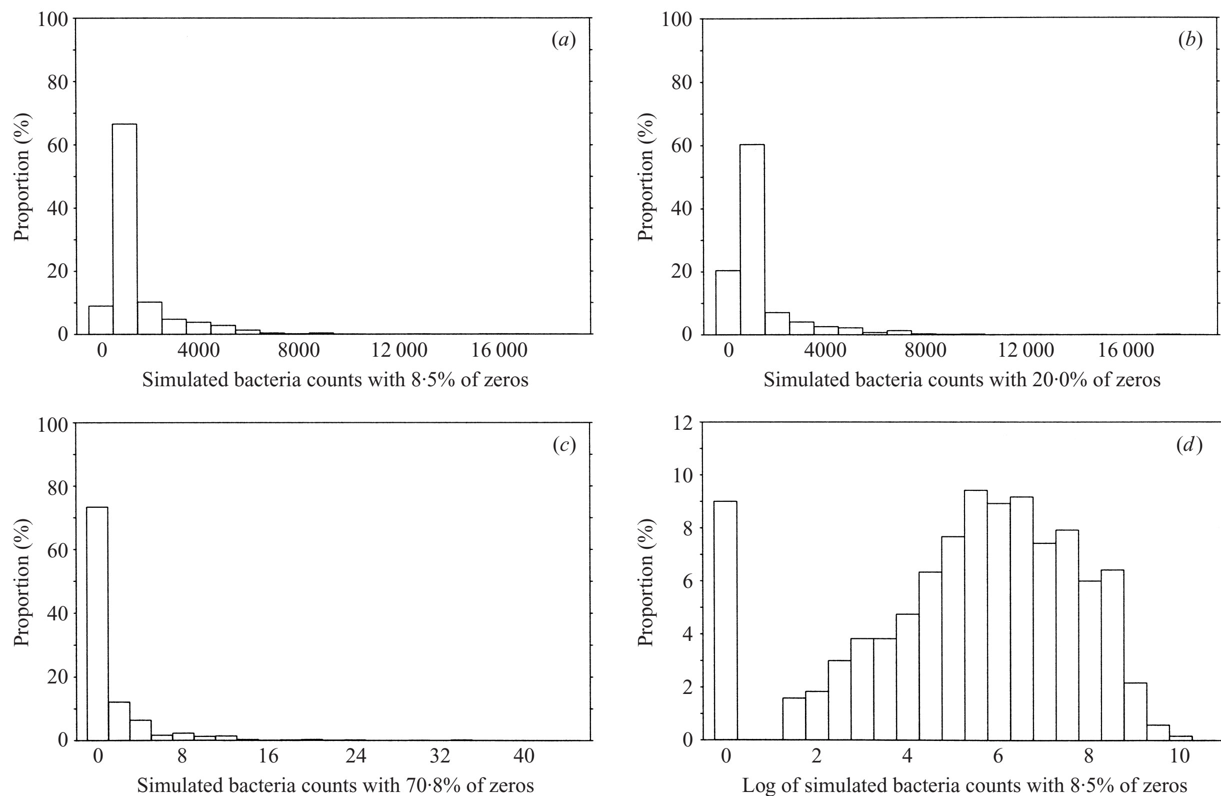

Fig. 1. Distribution of simulated bacteria counts in CFU units with (*a*) 8·5% of zeros, (*b*) 20·0% of zeros, (*c*) 70·8% of zeros (data of bacteria counts with 0·0% of zeros are not shown since the graph is very similar to graph (*a*)); (*d*) distribution of bacteria counts with 8·5% of zeros after mathematical transformation (other transformed data not shown); one replicate used ($n = 1200$). Fixed boundaries are used for histograms: the first bar is the proportion of zero values, and the rest are intervals of 1000 for (*a*) and (*b*), 2 for (*c*) and 0·5 for (*d*).

Table 2. *Empirical 5% significance thresholds of P values for single-position simulations and for chromosome scans*

	Phenotype				
		Bacteria counts			
		Proportion of zeros			
Method	Normal	0·0%	8·5%	20·0%	70·8%
Single-position simulations					
LS	0·05005[a]	0·03692[a,b]	0·03587[a,b]	0·03365[b]	0·03092[b]
ML	0·03767[a]	0·00036[b]	0·00030[b]	0·00018[b]	0·00006[c]
NP	0·04343[a]	0·04327[a]	0·04115[a]	0·04074[a]	0·04481[a]
Chromosome scans					
LS	0·00575[a]	0·00122[a]	0·00131[a]	0·00105[a]	0·00086[a]
ML	0·00388[a]	1·348 E-6[b]	7·044 E-7[b]	4·309 E-7[b,c]	8·391 E-8[c]
NP	0·00397[a]	0·00421[a]	0·00409[a]	0·00375[a]	0·00362[a]
AN	0·00620[a]	0·00226[a]	0·00234[a]	0·00165[a]	0·00114[a]

Based on 10000 replicates for single-position simulations, and on 1000 replicates for chromosome scans, under the null hypothesis of no QTL. Threshold values are presented as the probability of the relevant test statistic (i.e. *P* value), since degrees of freedom of test statistics (LS and NP in single-position simulation and AN in chromosome scans) vary between replicates.

[a] Comparison of *P* value distributions between phenotype within methods (LS, ML, NP or AN; Mann–Whitney *U*-test); values with the same superscript are not significantly different; $P > 0{\cdot}005$.

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Table 3. *Power (%) at the 5% significance level in single-position simulations*

QTL effect (%)	Phenotype	Proportion of zeros (%)	Mathematical transformation: No			Yes		
			LS	ML	NP	LS	ML	NP
2[a]	Normal		20	20	18	20	20	18
	Bacteria counts	0·0	11	7	18	19	18	18
		8·5	11	7	17	18	17	17
		20·0	10	7	17	16	16	17
		70·8	9	7	10	13	9	10
8	Normal		78	76	74	78	76	74
	Bacteria counts	0·0	45	16	74	77	75	74
		8·5	43	15	73	75	73	73
		20·0	36	13	71	73	73	71
		70·8	32	11	30	51	33	30
18	Normal		99	99	99	99	99	99
	Bacteria counts	0·0	85	47	99	99	99	99
		8·5	82	43	98	98	98	98
		20·0	75	30	98	98	98	98
		70·8	67	20	69	91	80	69

Power estimates based on 1000 replicates of each alternative using 5% significance thresholds determined from 10000 simulations under the null hypothesis of no QTL. Heritability of the trait was 0·25.
[a] QTL effect is expressed in terms of heritability due to the QTL (h^2_{QTL}); conversion to Falconer & Mackay's (1996) substitution effect amounts to values of 0·2, 0·4 and 0·6 respectively for values of 2%, 8% or 18% of h^2_{QTL}.

thresholds of LS and NP methods, in the context of our single-position simulations, and of AN in the context of our chromosome scans (Table 2).

In single-position simulations, when the normal phenotype is analysed, the 5% thresholds for ML and NP are lower than the expected 5% value (i.e. more conservative). The same pattern was observed for 1% thresholds (data not shown). Conversely, the LS method has threshold values close to the expected level for a single test. When bacteria counts are analysed, the significance thresholds decrease as the proportion of zeros in the phenotype increases (i.e. to be significant, the *P* value of the test statistic obtained for a single analysis has to be more extreme). The threshold values of parametric tests obtained for bacteria counts are lower than the threshold values obtained with the normal phenotype, especially with ML. Unlike parametric methods, threshold values of the non-parametric test are not influenced by the proportion of zeros in bacteria counts. When a mathematical transformation is applied to bacteria counts prior to QTL analysis (data not shown), threshold values are close to the one obtained with the normally distributed phenotype, except for ML when bacteria counts have a high proportion of zeros (70·8%): the value of the 5% significance threshold is equal to 0·7%.

In chromosome scans, 5% thresholds are lower than the expected 5% value and are close to those that would be obtained using a Bonferroni adjustment for the number of intervals on the chromosome. All significance thresholds decrease when bacteria counts are analysed, but this decrease is only significant for ML.

(iii) *Power estimates*

In single-position simulations, power estimates at the 5% level (and at the 1% level; data not shown) are not significantly different between methods when the normally distributed phenotype is analysed and within each QTL effect (Table 3) (except at the 1% level and for a QTL effect of 18% for which the power of LS (97%) is significantly higher than the power of NP (95%); data not shown). However, when bacteria counts are analysed, at the 5% level, all power estimates of parametric methods are significantly decreased. Conversely, the power of NP is not significantly decreased when bacteria counts are analysed, except when the proportion of zeros in bacteria counts is very high (70·8%) and for a QTL effect of 8% and 18%. Even when bacteria counts have 20% of zeros, at the 5% level, the non-parametric method detects the simulated QTL of 8% in 71% of the replicates, compared with 36% of the replicates for *F* ratio based methods. At the 1% level, the same decrease is observed when bacteria counts are analysed, except for a small QTL effect (2%) for which this decrease is not significant (data not shown).

At the 5% level and for bacteria counts with 0·0%, 8·5% and 20·0% of zeros, the power of the non-parametric method is significantly higher than the

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Table 4. *Power (%) at the 5% significance level in chromosome scans*

Phenotype	Proportion of zeros	Method							
		LS	MT[a]	ML	MT	NP	MT	AN	MT
Normal		61	–	60	–	52	–	59	–
Bacteria counts	0·0%	19	59	8	58	53	52	23	56
	8·5%	19	55	7	54	53	53	22	54
	20·0%	15	58	6	54	50	51	16	51
	70·8%	13	30	6	21	16	16	13	28

Power estimates obtained using 5% significance thresholds determined from the ranking of maximum values of 1000 simulations under the null hypothesis of no QTL. Heritability of the trait was 0·25. Heritability due to the QTL was set to 8% of the total phenotypic variance.
[a] Mathematical transformation (MT) of bacteria counts prior to analysis.

power obtained by other methods except for the lowest QTL effect (2%). For the highest proportion of zeros in bacteria counts (70·8%), the power of NP is equal to the power of F ratio based methods and higher than the power of ML. At the 1% level, the same pattern is observed, except for the highest QTL effect (18%) and the highest proportion of zeros, where the power of NP is significantly higher than the power of LS, i.e. 49% versus 39% (data not shown).

Among the parametric methods, ML is the most affected by the analysis of bacteria counts: power estimates are strongly decreased compared with the power estimate when the normally distributed phenotype is analysed. This decrease is higher for a QTL accounting for 8% of phenotypic variance than for small (2%) and high (18%) QTL effects (Table 3).

Unlike single-position simulations, there are differences between power estimates in chromosome scans when the normally distributed phenotype is analysed: the power of parametric methods is significantly higher than the power of NP (Table 4). As expected, the power of the single-marker method (AN) is lower than the power of LS, but not significantly. Except for NP, all power estimates are significantly decreased when bacteria counts are analysed. Indeed, the power estimate of NP is not significantly decreased for bacteria counts with low and intermediate proportions of zeros (0·0%, 8·5% and 20·0%).

(iv) *Mathematical transformation*

In single-position simulations, when a logarithmic transformation is applied on simulated bacteria counts before analysis, the power of all parametric methods is significantly increased compared with the analysis of raw bacteria counts, except for the smallest QTL effect for LS (Table 3). For NP, the power is unchanged when data are mathematically transformed. For low and intermediate proportions of zeros (8·5% and 20·0%), the power of LS and ML is slightly higher than the power obtained by NP (non-significant), when mathematically transformed bacteria counts are analysed. For the highest proportion of zeros (70·8%) with QTL effect of 8% and 18%, LS outperforms NP by 21% and 22% of power respectively (significant) (Table 3). Within each method, all the differences in power between the analyses of normal phenotypes and of mathematically transformed bacteria counts are non-significant except for the highest proportion of zeros and with a QTL effect of 8% and 18%.

In chromosome scans, the power of all parametric methods is increased when a logarithmic transformation is applied to bacteria counts (Table 4). Values of power estimates are not significantly different from the power obtained when the phenotype is normally distributed, except for ML and AN for 20·0% of zeros, and for all four methods for the highest proportion of zeros, for which power estimates are significantly lower. The LS method coupled with a mathematical transformation of bacteria counts yields higher power estimates than the non-parametric method for all proportions of zeros in bacteria counts: 59% versus 53%, 55% versus 53%, 58% versus 50% and 30% versus 16% respectively from 0·0% to 70·8% of zeros (Table 4). This difference is significant only for intermediate and high proportion of zeros (20·0% and 70·8%). Other parametric methods (ML and AN) give significantly higher power estimates than NP when the proportion of zeros is equal to 70·8%.

(v) *Mean values of test statistics along the chromosome*

All interval mapping methods clearly yield very similar curves when the normally distributed phenotype is analysed (Fig. 2). However, there are differences between methods in terms of sensitivity to bacteria counts. Interval mapping using ML is the method most affected, showing a very flat curve for the three proportions of zeros. Mean values of F ratios, along

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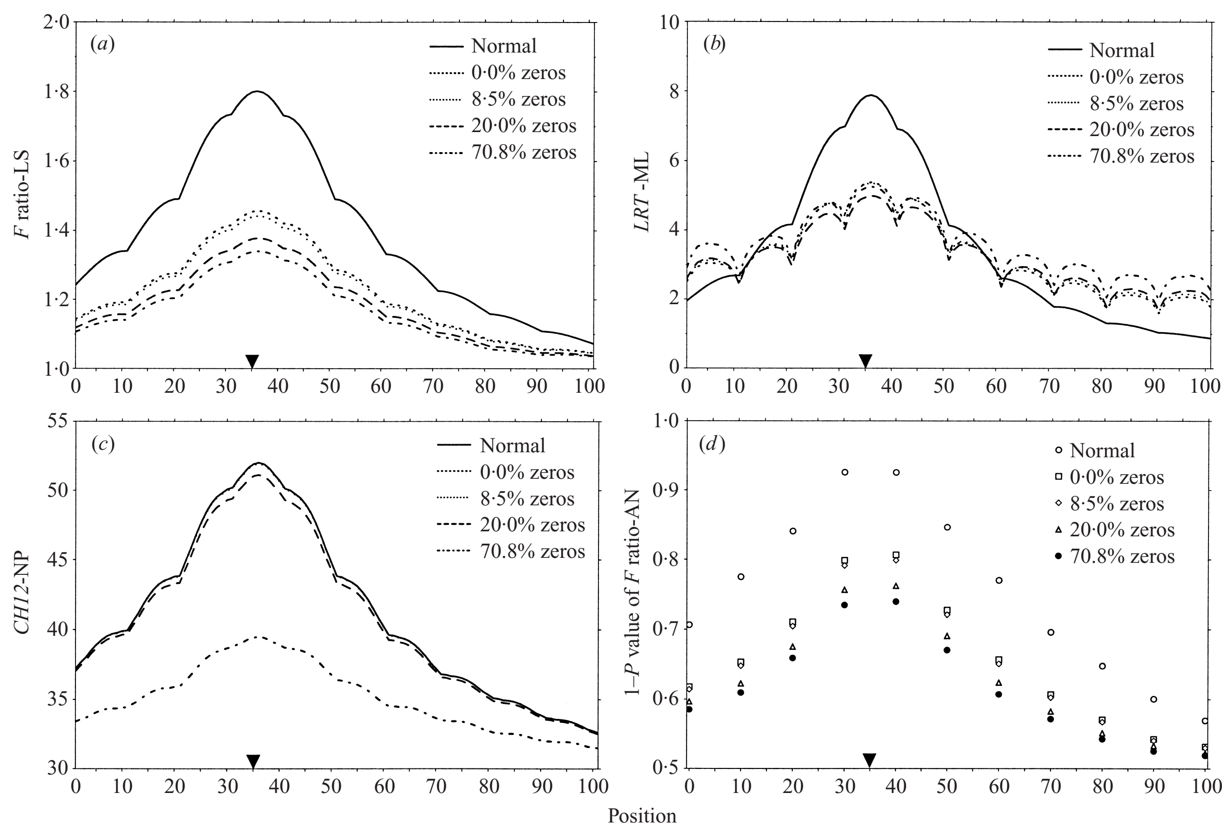

Fig. 2. Mean test statistic values (1000 replicates) along the chromosome for the four QTL mapping methods when a normally distributed phenotype and bacteria counts (0·0 %, 8·5 %, 20·0 % or 70·8 % of zeros) are analysed. For the single-marker method (AN), a test is performed only at marker location (positions 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100); instead of F ratio values, $(1-P)$ values of the F ratios are presented, since degrees of freedom vary between markers and replicates. Arrowheads indicate the location of the simulated QTL.

Table 5. *Mean estimates* ($\pm SE$) *of QTL location in chromosome scans* (*based on 1000 replicates of each alternative*)

Phenotype	Proportion of zeros	Method LS	ML	NP	AN
Normal		$38·6 \pm 0·6^{a,b}$	$38·7 \pm 0·6^{a}$	$38·4 \pm 0·6^{a}$	$38·8 \pm 0·6^{a}$
No mathematical transformation					
Bacteria counts	0·0 %	$41·7 \pm 0·8^{b,c}$	$43·5 \pm 0·9^{b}$	$38·5 \pm 0·6^{a}$	$41·3 \pm 0·8^{a,b}$
	8·5 %	$41·7 \pm 0·8^{b,c}$	$43·5 \pm 0·9^{b}$	$38·5 \pm 0·6^{a}$	$41·7 \pm 0·8^{a,b}$
	20·0 %	$42·7 \pm 0·9^{a}$	$44·1 \pm 0·9^{b}$	$38·7 \pm 0·6^{a}$	$42·6 \pm 0·9^{b}$
	70·8 %	$43·7 \pm 0·9^{a}$	$45·5 \pm 1·0^{b}$	$43·0 \pm 0·9^{b}$	$42·9 \pm 0·9^{b}$
Mathematical transformation					
Bacteria counts	0·0 %	$38·2 \pm 0·6^{a}$	$38·8 \pm 0·6^{a,b}$	$38·4 \pm 0·6^{a}$	$38·6 \pm 0·6^{a}$
	8·5 %	$38·1 \pm 0·6^{a}$	$38·6 \pm 0·6^{a}$	$38·5 \pm 0·6^{a}$	$38·6 \pm 0·6^{a}$
	20·0 %	$38·5 \pm 0·6^{a,b}$	$38·1 \pm 0·6^{a}$	$38·8 \pm 0·6^{a}$	$38·4 \pm 0·6^{a}$
	70·8 %	$40·9 \pm 0·8^{a,b,c}$	$42·4 \pm 0·8^{b,c}$	$43·0 \pm 0·9^{b}$	$41·5 \pm 0·8^{a,b}$

Heritability of the trait was 0·25. Heritability due to the QTL was set to 8 % of total phenotypic variance. One QTL was simulated in position 35·0 cM.

[a] Comparison of mean estimates of QTL location between phenotype within methods (LS, ML, NP or AN, Bonferroni t-tests); values with the same superscript are not significantly different; $P > 0·05$.

the chromosome (LS) and at marker location (AN), are strongly decreased when bacteria counts are analysed, especially with the two highest proportions of zeros (20·0 % and 70·8 %). Except with the highest proportion of zeros (70·8 %), the mean value of the NP test statistic is not decreased compared with the mean value of the test statistic when the normally distributed phenotype is analysed.

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When bacteria counts are mathematically transformed prior to analysis, mean values of test statistics are close to values of the normally distributed phenotype, except with the highest proportion of zeros (data not shown).

(vi) *Mean estimates of QTL location*

For all QTL mapping methods, when a normally distributed phenotype is analysed, the estimated QTL location tends to be biased towards the centre of the chromosome as expected (Table 5). This bias increases as the proportion of zeros in bacteria counts increases, except for NP and at a low proportion of zeros (0·0%, 8·5% and 20·0%), for which the mean location estimates are between 38·5 and 38·7 cM. Similarly, as the proportion of zeros increases, the mean location estimates become less precise, i.e. the standard errors (SE) of the estimates increase. This pattern is observed for all methods except for NP, for which this increase in the SE is only observed with 70·8% of zeros in bacteria counts.

For low and intermediate proportions of zeros (0·0%, 8·5% and 20·0%), when a mathematical transformation is applied to bacteria counts before analysis, mean estimates of QTL location are closer to the mean estimate when the normal phenotype is analysed (non-significantly different for all methods). Furthermore, the standard errors of the mean estimates are decreased. For the highest proportion of zeros (70·8%), the mean estimates of QTL location for ML and NP are significantly more biased than the mean estimates of the normal phenotype. Mean estimates of QTL location for LS and AN are also more biased to the centre of the chromosome but not significantly: this bias is lower for the interval mapping test (LS) than for the association test (AN): $40·9 \pm 0·8$ versus $41·5 \pm 0·8$.

4. Discussion

To our knowledge, this is the first study in which three interval mapping methods and one association test are studied in parallel in the context of non-normal phenotypes and outbred half-sib populations. Other studies on non-normality in QTL mapping focused on artificial distributions (rarely met in the field) which were simulated from random functions (Doerge & Rebaï, 1996; Rebaï, 1997; Coppieters *et al.*, 1998). Studies by Doerge & Rebaï (1996) and Rebaï (1997) were dedicated to backcross and F2 experiments. Coppieters *et al.* (1998) compared LS and NP methods in the context of half-sib designs. In the present study, real data (bacteria counts) from a case study by Frédéric Lantier (personal communication) are used to simulate non-normality by means of the *normal score back-transformation* used in geostatistics. This transformation is a very efficient tool to simulate any type of non-normality. When it is coupled with the simulation algorithm developed by Baret *et al.* (1998), it enables simulation of half-sib populations with a segregating QTL for both normally and non-normally distributed phenotypes.

In this study we showed that the power of parametric QTL mapping methods is strongly reduced if raw bacteria counts are analysed. This reduction was observed for single-position simulations (1 marker, 1 QTL) as well as for simulations of chromosome scans (11 markers, 1 QTL). For example, in single-position simulations, in the situation of a moderate QTL effect (8% of the total phenotypic variance), and when bacteria counts with 20·0% of zeros are analysed, we observed a loss of power of 42% for LS at the 5% significance level, when compared with the analysis of a normally distributed phenotype. This loss of power reached 63% in the case of ML analysis. In chromosome scans, the loss of power using LS and ML was respectively 46% and 54%. Conversely, no significant loss of power was observed with the non-parametric method (3% and 2% respectively for single-position and chromosome scan simulations, at the 5% significance level).

LS, as well as AN, had the same power as NP for low proportions of zeros in bacteria counts when it is coupled with a logarithmic transformation of bacteria counts before analysis. The power of LS was even significantly higher than the power of NP for bacteria counts with 20·0% and 70·8% of zeros. For example, for 70·8% of zeros, in the situation of a moderate QTL effect (8%), the power of LS was 21% and 14% higher than the power of NP for single-position and chromosome scan simulations respectively.

Losses of power encountered by either parametric or non-parametric methods when mapping QTL for bacteria counts can be explained by two distinct causes: the non-normality of bacteria counts, and the occurrence of tied values (zero values) in bacteria counts.

Non-normality: Our results showed that parametric methods are not as robust as expected to non-normality. Even though least-squares methods and ANOVA are commonly said to be robust against non-normality, we showed that their robustness against bacteria counts is low. As expected, robustness of maximum-likelihood QTL mapping to non-normality was very poor. Indeed, the ML method uses the normal density function for the building of the likelihood. The poor performance of ML is therefore explained by the fact that the distribution of bacteria counts is closer to an exponential distribution than to a normal distribution. Indeed, bacteria counts are characterized by some high values (highly infected individuals) which can be considered as outliers as opposed to the normal distribution (see Fig. 1). For

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example, bacteria counts in both lymph nodes together, when zero-valued data are suppressed, range between 3 and 22 628 while 50% of the observations are below the value of 318 bacteria. These outliers contribute excessively to the residual variation, and therefore decrease the power of parametric tests. As stated by Coppieters *et al*. (1998), when using the NP method, the contribution of outliers to the residual variation is tempered, since ranks are used rather than the actual phenotypes.

From a probabilistic point of view, distributions of bacteria counts are neither continuous nor categorical. Indeed, these distributions are usually composed of a spike at zero and a continuous right tail (also known as 'tobit' distributions in econometrics). One approach could be to transform bacteria counts into either a dichotomous trait (resistant vs. sensible) or a polychotomous trait (discrete levels of infection). This would allow the existence of outliers to be circumvented and would imply the use of methods adapted to threshold traits such as those proposed by Visscher *et al*. (1996) and Hackett & Weller (1995). However, such an approach would lead to a loss of information since the intensity of the colonization would be omitted. Another approach would be to consider the use of Poisson models to analyse bacteria counts, by including the Poisson density function in a likelihood. Up to now, the use of Poisson models for QTL mapping was restricted to traits presenting a small number of categories, such as litter size (e.g. Kayis *et al*., 1999). Extensions to distributions with very large values – up to 22000 in our data – should be studied.

Occurrence of tied values: When a mathematical transformation was applied to bacteria counts before analysis, the power of parametric methods was increased. Such a result was observed even if there was a high proportion of tied values (zeros). This confirms that the robustness problems of parametric methods can be explained by the non-normality of bacteria counts (and therefore by the existence of outliers) rather than by the occurrence of tied values. Conversely, loss of power of NP when bacteria counts with a high proportion of zeros (70·4%) were analysed, could be explained by this high occurrence of tied values. As suggested by Kruglyak & Lander (1995*a*), random ranking of ties was applied in our study. This approach has the merit of simplicity but obviously sacrifices some information contained in the data (i.e. the existence of ties). Furthermore, when the number of ties is very high, this random ranking of tied values has the drawback of adding new information in the data: individuals with the same phenotypic value but with different genotypes turn out to be hierarchically classified even though they should have the same rank. Conversely, when using parametric methods, i.e. when using actual values of phenotypes rather than the ranks, the information of tied values is kept, and individuals with the same phenotypic value are considered as equivalent. The problem of the random ranking of tied individuals highlighted in this study is restricted to phenotypes presenting a high number of ties (e.g. bacteria counts, lesion scores). For continuous phenotypes where the occurrence of ties is rare, one should not observe a loss of efficiency when using a non-parametric test (as shown in this study for small proportions of zeros). Further research on NP interval mapping could therefore focus on differences in power when tied values are ranked at random, compared with the method of attributing to each of the tied observations the average of those tied.

Except for the NP method, probability values of empirical thresholds were strongly reduced (i.e. actual values increased) when bacteria counts were analysed. This confirms the interest of using empirically determined thresholds, e.g. simulation-based or permutation-based thresholds (e.g. Churchill & Doerge, 1994), instead of using theoretical thresholds when analysing non-normally distributed traits. Indeed, theoretical thresholds could lead to an increase in the false positive rate. Contrary to Coppieters *et al.* (1998), we did not observe a gain in power of NP when analysing non-normal traits compared with the analysis of a normally distributed trait. The maximum power of NP was obtained when the phenotype was normally distributed or when bacteria counts with no ties were analysed. This could be explained by differences in the computation of significance thresholds: permutation-based for Coppieters *et al.* (1998) versus simulation-based in our approach.

The simulation of the non-normal phenotype was achieved using a simple correspondence table between a normal distribution and an observed distribution (*normal score back-transformation*). We could have simulated separately the QTL effect, polygenic background and residual variation that underlie the bacteria counts phenotype. Such an approach would have implied that the properties of the distributions of these three components were known or inferred.

In the context of our chromosome scan simulations, interval mapping methods (LS, ML and NP) and the nested ANOVA (single-marker analysis) were compared simultaneously. However, AN is not exactly a QTL mapping method and should be considered more as an association test. Our results showed that the power of AN was very close to the power of interval mapping methods; furthermore, mean estimates of QTL location were very similar. However, in a less informative situation (lower number of alleles per marker and with adjacent markers of varying information content), interval mapping methods would outperform AN in terms of power and precision of QTL location as shown by Rebaï *et al*. (1995) and Knott *et al*. (1996). Therefore, the use of AN for QTL detection should be restricted to a first screening of

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chromosome regions susceptible to containing QTL (when a small number of markers are typed), before DNA typing of a larger number of markers. Putative association should always be confirmed by an interval mapping analysis.

This study showed that the use of standard QTL mapping methods for the analysis of bacteria counts data may lead to a significant decrease in statistical power. A QTL analysis of bacteria counts should either use a non-parametric test or transform the observed phenotype by use of the appropriate mathematical transformation prior to using a parametric test. However, it needs to be borne in mind that parametric methods (LS and ML) provide a direct estimate of phenotypic effect of the QTL, whereas the non-parametric method simply tests for the presence of a QTL. Consequently, when there is evidence of non-normality, both non-parametric and parametric approaches should be used as recommended by Kruglyak & Lander (1995*a*). Our results could be extended to other disease traits implying a counting process, e.g. fecal egg counts (FEC) in parasitic diseases or plaque-forming units (PFU) in viral diseases.

We are grateful to Patrick Bogaert, Xavier Draye and Michel Georges for helpful suggestions, and to Eric Le Boulengé and Peter Visscher for their comments on earlier versions of the paper. We thank three anonymous reviewers for their helpful comments and criticisms on an earlier version of the manuscript. We thank Emmanuel Tilquin for language editing. Financial support is acknowledged from the Université catholique de Louvain and from the European Union (FAIR CT-98-4311).

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https://doi.org/10.1017/S0016672301005365 Published online by Cambridge University Press