Theor Appl Genet (1997) 95:384–392

S. M. Kaeppler

Quantitative trait locus mapping using sets of near-isogenic lines: relative power comparisons and technical considerations

Received: 12 December 1996 / Accepted: 21 March 1997

Abstract The power to detect QTL using near-isogenic line (NIL) comparisons versus recombinant inbred (RI) populations was assessed. The power to detect QTL was found to be generally greater when using RI populations than when using NIL contrasts. Power to detect QTL with NIL contrasts never exceeded that of RI populations when the number of RI lines is maximized relative to replication of lines for a given number of experimental units. The relative power of NIL contrasts is highest for traits with high heritability and when a gain in precision is realized due to increased replication of entries. Although NIL populations are generally less powerful than RI populations of similar size, some practical considerations may enhance the value of these materials. Availability of NILs allows the genetic effect of a specific chromosome region to be determined by comparing two lines; all RI lines in a population need to be rescored for each new trait even if the effect of a specific chromosome region is suspected. NIL comparisons may allow genetic differences to be detected by visual inspection; genetic effects can only be expressed as means and variances with recombinant inbred populations. In summary, RI populations generally, and in some cases, substantially, provide better power for QTL detection than NIL comparisons. Practical considerations, however, indicate that many factors need to be considered when choosing a population structure to meet an experimental objective.

Key words Backcross-derived line · Near-isogenic line · Quantitative trait · Statistical power

Communicated by G. E. Hart

S. M. Kaeppler (✉)
Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison WI 53706, USA

Abbreviations *BDL* Backcross-derived line · *NIL* near-isogenic line · *QTL* quantitative trait locus · *RI* recombinant inbred line · *RP* recurrent parent · SB substitution line

Introduction

Near-isogenic lines (NILs) derived by backcrossing or selfing are a valuable resource for quantitative trait analysis in plants. Chromosome substitution lines (SB) in wheat, which are near-isogenic to the recurrent parent for 20 chromosome pairs and divergent for 1 chromosome pair, are useful for dissecting quantitative traits, especially when polymorphic molecular markers are not available. This method has been used to determine chromosome effects for a wide variety of traits including plant height, maturity, grain quality parameters, and yield and yield components (Law 1966a, b; Mansur et al. 1990; Berke et al. 1992).

The inbred-backcross approach was proposed by Wehrhahn and Allard (1965) and used to study quantitative inheritance in wheat. This procedure was designed to isolate major genes (effective factors) in a relatively uniform genetic background. The utility of the method was shown for the trait 'heading date'. Baker (1978) subsequently demonstrated that the inbred-backcross approach is less effective if alleles with major effects are not segregating in the population under study.

Analysis of NILs using molecular markers has been shown to be an effective method to detect quantitative trait loci (QTL) (Osborn et al. 1987; Phillips et al. 1992; Koester et al. 1993). In these studies, backcross-derived lines (BDLs) were produced by selection for a specific trait during the backcrossing process. Molecular markers were used to identify portions of the donor parent genome remaining in one or more of the BDLs, and then to determine the effect of these regions on the trait of interest. This approach to quantitative trait

analysis is a "trait-based" approach, where markers are used to assess population or line divergence due to selection.

Most quantitative trait locus mapping studies currently conducted are "marker-based" studies, where phenotypic or genetic variation is partitioned based on molecular marker genotypes. Marker-based analysis has been done on numerous types of populations including single individual F_2 populations, populations of F_2-derived F_3 families, and recombinant-inbred-line (RI) populations. With all of these population structures, QTL detection is markedly affected by population size, QTL effect, heritability, and QTL number (Beavis 1994). In many scenarios, the power to detect a QTL is most affected by other QTL segregating in the population (Knapp and Bridges 1990). Because of the importance of background genetic variation in QTL detection, NILs have been suggested or utilized as a resource to theoretically improve QTL detection and estimation.

Deriving populations by backcrossing effectively homogenizes the genomes of population members, reducing variability among lines and minimizing the effect of background genetic variation on QTL detection. The development of an introgression line population by Eshed and Zamir (1995) was inspired, in part, by the desirable reduction in background genetic variation in the population. The population was shown by the authors to be useful in detecting quantitative trait loci for several traits in tomato. The number of QTL detected for soluble solids and fruit mass was reported to be twice that detected in other populations of similar parentage.

Power comparisons for F_2-derived, single backcross, and RI populations are well-documented (Knapp and Bridges 1990). The objective of this study was to determine the theoretical relative effectiveness of NILs in QTL detection studies. Three scenarios are documented. First, the power to detect QTL using sets of NILs is compared to QTL detection using RI populations. This scenario is designed to assess the relative utility of sets of NILs (eg. introgression lines) for initial detection of QTL. Second, the relative power to confirm a QTL using a specific NIL pair [e.g., a BDL versus its recurrent parent (RP)] is compared to the power to detect the same QTL in an RI population. This scenario is designed to test the logic that production of NILs is the preferred method to confirm QTL presence. Finally, power to detect QTL using populations of chromosome substitution lines, recombinant chromosome lines (derived from substitution lines) and RI lines is compared. This scenario is designed to determine the relative effectiveness of chromosome substitution line populations in an era where other population structures may now be reasonably considered. The substitution line concept is analogous to the first objective, but since this population structure is relatively unique, it is dealt with separately.

Theory and methods

The linear model for the RI analysis is:

$$y_{ijk} = \mu + \tau_i + \gamma_{ij} + e_{ijk}$$

where y_{ijk} is the phenotypic value of the *k*th replication of the *j*th line at the *i*th marker genotype, μ is the population mean, τ_i is the effect of the *i*th marker genotype (i = 1 to 2), γ_{ij} is the effect of the *j*th line in the *i*th marker genotype (j = 1, 2, … , number of lines), and e_{ijk} is residual error (k = 1, 2, … number of replications).

The mean square for variation among lines can be calculated and further partitioned into variation due to marker genotypes and variation due to lines within marker genotypes (Table 1). Variation due to marker genotypes is a fixed effect and is equal to variation due to a QTL if no recombination occurs between the marker and the QTL. Significance of this effect can be tested by analysis of variance using lines within marker genotype mean square as the denominator of the *F*-test. A significant difference among means of marker genotypes indicates the linkage of the marker and a QTL.

The linear model for analysis of NIL pairs is:

$$y_{jk} = \mu + \gamma_j + e_{jk}$$

where y_{ik} is the phenotypic value of the *k*th replication of the *j*th line, μ is the mean of the two lines, γ_j is the effect of the *j*th line (j = 1 to 2), and e_{jk} is residual error (k = 1, 2, … , number of replications).

Analysis of variance is used to test for equality of NIL means (Table 1). QTL presence is indicated by a significant difference between the means. Individual single-factor analyses of variance are done on the same data set for each marker scored on an RI population. Tests of significance for NIL pairs require at least two replications. The replication of lines is not necessary to test differences among marker genotype means when using RI populations.

Heritability, for the purposes of this study, was defined as the ratio of genetic to total variation in the reference RI population. Relationships between heritability, variance in an RI population, and variance among an NIL pair were established for this comparison based on the above linear models. Genotype x environment interactions were not included in the models. Each QTL was considered to be coincident with a marker locus and assumed to segregate independently. Relationships were defined as follows:

Heritability $= \sigma_g^2/\sigma_g^2 + \sigma_e^2$, where

σ_g^2 = genetic variance, and

σ_e^2 = experimental error variance

Genetic variance (σ_g^2) is the sum of the variance due to each QTL assuming independence and no epistasis. To accommodate analysis

Table 1 ANOVA tables for analysis of RI populations and NIL contrasts. Variance components were based on parameters for the RI populations and related, as shown, to the NIL comparison

Source of variation	Degrees of freedom	Expected mean square
ANOVA for recombinant inbred analysis		
Replications	$r - 1$	$\sigma_e^2 + 1\sigma_r^2$
Marker genotypes	$m - 1$	$\sigma_e^2 + r\sigma_{1:m}^2 + rl\Phi_q^2$
Lines : marker genotypes	$(l - 1)m$	$\sigma_e^2 + r\sigma_{1:m}^2$
Residual	$(r - 1)(l - 1)$	σ_e^2
ANOVA for analysis of NIL contrasts		
Replications	$r - 1$	$\sigma_e^2 + 1\sigma_r^2$
BDL vs RP	1	$\sigma_e^2 + r\Phi_q^2$
Residual	$r - 1$	σ_e^2

of variance tests among RI lines or NIL pairs, genetic variance was further partitioned as follows:

$\sigma_g^2 = \Phi_q^2 + \sigma_{1:m}^2$, where

Φ_q^2 = fixed effect of the QTL in the genomic region under analysis, and

$\sigma_{1:m}^2 = (\sigma_g^2 - \Phi_q^2)$

= variation due to all QTL independent of the QTL under analysis.

All genetic variation was assumed to be additive genetic variation in the two models described since the populations are homozygous and epistasis assumed absent.

Power comparisons between RI populations and NIL pairs were made based on the preceding definitions. Heritability values ranging from 0.1 to 0.9 were chosen to define the proportion of genetic versus non-genetic variation in the RI population. The variance due to the QTL under analysis (Φ_q^2) was defined as a percentage of the total genetic variation, with the remaining genetic variation partitioned as $\sigma_{1:m}^2$. Genetic variance among an NIL pair is due to the introgressed QTL and, therefore, is defined as Φ_q^2 for purposes of comparison. Total variation was given a value of 1, so experimental error was defined as (1 − heritability) for the RI population, and this value was used to define experimental error for the NIL contrast.

Comparisons of methods were made using balanced models. The critical test for the RI mapping experiment is H_o:$\mu_{11} = \mu_{22}$, where μ_{11} and μ_{22} are the means of marker genotypes 11 and 22, respectively. The F-statistic for testing this hypothesis is $F = MS_m/MS_{1:m}$, and the non-centrality parameter is:

$$\lambda = \frac{E(SS_m)}{E(M_{1:m})} = \frac{\sigma_e^2 + r\sigma_{1:m}^2 + rl\Phi_q^2}{\sigma_e^2 + r\sigma_{1:m}^2}$$

The critical test for the NIL contrast is H_o: $\mu_{RP} = \mu_{BDL}$, where μ_{RP} and μ_{BDL} are the means of the recurrent parent (RP) and BDL, respectively. The F-statistic for testing this hypothesis is $F = MS_{RP\ vs\ BDL}/MS_e$, and the non-centrality parameter is:

$$\lambda = \frac{E(SS_{RP\ vs\ BDL})}{E(M_e)} = \frac{\sigma_e^2 + r\Phi_q^2}{\sigma_e^2}$$

SAS (1987) functions PROBF and FINV were used to calculate critical values and probabilities for F-distributions.

Comparison of QTL detection using recombinant inbred populations versus sets of near-isogenic lines

The power to detect QTL was determined for an RI population f 200 individuals versus 100 NIL pairs. This comparison was chosen to approximate mapping in a species with a genome the size of maize (roughly 2000 cM on 10 chromosomes) with each BDL containing a different 20 cM of the donor parent genome. The comparison assumes that each BDL is grown in a two-entry experiment with the other entry being the RP. An NIL pair is constituted by a BDL and its RP. Based on the choice of 100 NIL pairs, a population size of 200 recombinant inbred lines was chosen to represent an RI line experiment with an equal number of entries for comparison.

Comparisons were made using a factorial of heritabilities from 0.1 to 0.9 by 0.1, replications from 2 to 20 by 2, and QTL percentage of genetic variation of 1%, 5%, 10%, and 20%. A Type-I error rate of $\alpha = 0.01$ was used for all comparisons. Experiment-wise error rates would be approximately the same for the two procedures if it is assumed that 100 markers were used in the recombinant inbred mapping experiment. Since there may be some gain in precision due to the smaller block size of the NIL contrast relative to the RI population, analyses were also done with σ_e^2 in the NIL contrast set at varying proportions of σ_e^2 in the RI line experiment.

Analysis of power to confirm presence of a QTL using a near-isogenic line/recurrent parent contrast relative to detection of the QTL in a recombinant inbred population

Power comparisons were done as described, with one modification. QTL detection in a recombinant inbred experiment usually involves multiple significance tests on the same data set. In the previous analyses, the number of significance tests (markers) was 100. QTL detection in a specific NIL contrast involves only one significance test. Therefore, power values were determined at different α levels to facilitate comparisons based on equivalent experiment-wise error rates in the scenarios compared.

Implementation of theory to assess QTL detection using chromosome substitution lines, recombinant chromosome lines, and recombinant inbred lines

For this example, the following parameters were chosen. Forty unlinked QTL, each accounting for 2.5% of the genetic variation, are present in the genome. Heritability in the reference recombinant inbred population is 0.5. The substituted chromosome in the substitution line contains 2 QTL, and thus accounts for 5% of the genetic variation in the recombinant inbred population. Based on parameters from the recombinant inbred population, the heritability in the recombinant chromosome population is approximately 0.05, and the QTL under study accounts for 50% of the genetic variation. The recombinant inbred population is comprised of 200 lines, the SB vs. RP contrast is a two-entry experiment, and recombinant chromosome populations contained 50, 100, or 500 individuals.

Results and discussion

Comparison of QTL detection using recombinant inbred populations versus sets of near-isogenic lines

The power to detect a specific QTL was higher, across the heritabilities and replications analyzed, for RI populations than NIL contrasts. This statement is based on power estimates for an RI population of 200 relative to an NIL pair, and equal σ_e^2 in both analyses. Figure 1a versus 1b illustrates this power comparison for a QTL accounting for 1% of the genetic variation, heritabilities of 0.1–0.9, and 2–20 replications. Power using the RI population exceeded that for the NIL contrast across the entire range of parameters shown. Trends are the same for QTL accounting for larger percentages of the genetic variation.

The RI population size of 200 lines was chosen based on the prediction that 100 BDLs would cover an entire genome of a crop such as maize. The RI population would be grown in one experiment with a maximum complete block size of 200 entries. Each of the 100 NIL pairs, however, would be grown as a separate experiment. Depending upon the variability present in the field where the experiment is grown, a substantial gain in precision could be realized due to the reduction in block size from 200 to 2. Power estimates were therefore made for different simulated values of σ_e^2 proportionately assigned in the two cases. Figure 1b shows power estimates for an NIL comparison over a range of

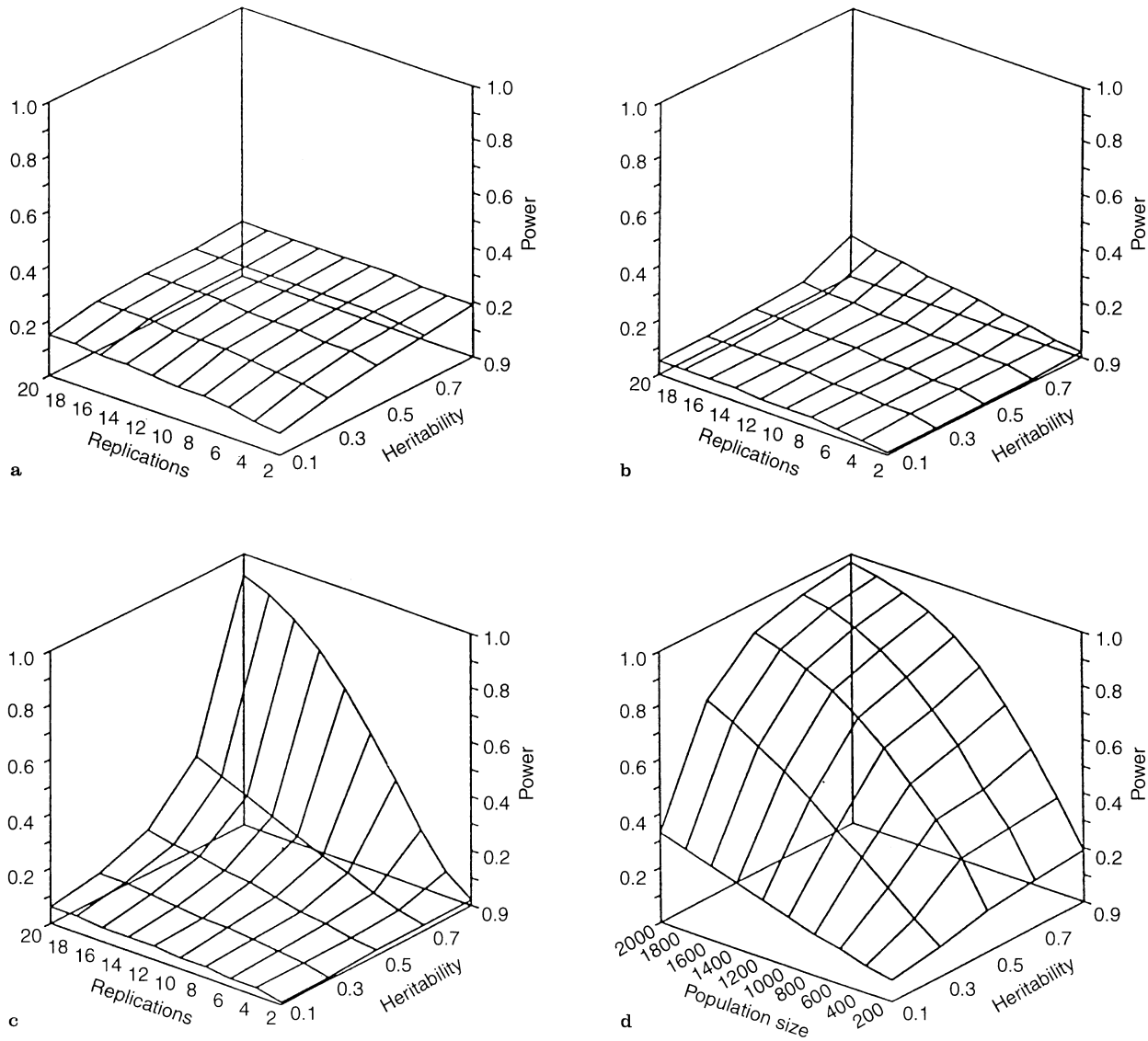
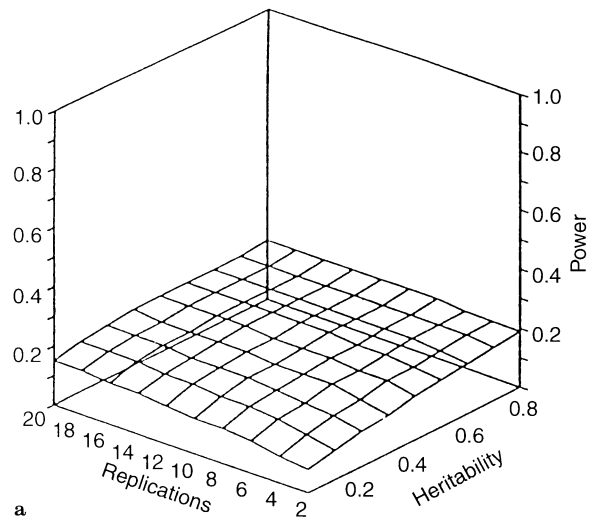


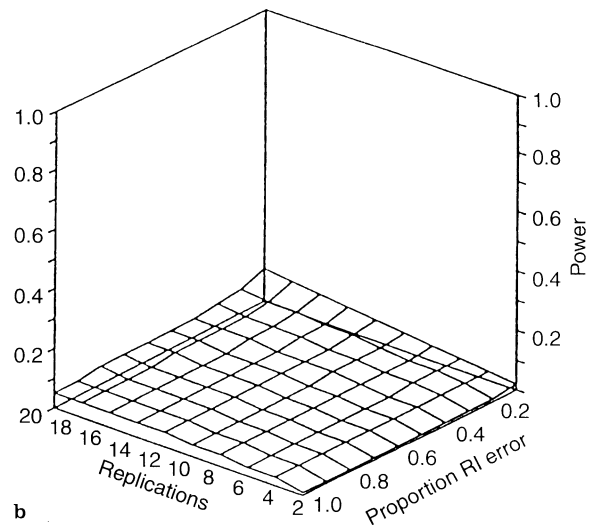
Fig. 1a–d Plots of power estimates for RI populations (**a** and **d**) and b NIL comparisons (**b** and **c**) over a range of heritabilities and replications. Parameters were a QTL accounting for 1% of the genetic variation and a Type-I error rate of $\alpha = 0.01$. The value of experimental error (σ^2e) used in **1c** is one-tenth of that used in **1b**. **a** is based on a population size of 200 individuals, **b** and **c** are based on the comparisons of a backcross-derived line and its recurrent parent, and **d** is based on variable population sizes with two replications. The Y-axis values of **a** and **d** (replications and population size, respectively) relate to equal numbers of experimental units at each point. The comparison of **a** and **d**, therefore, allows visualization of power differences for recombinant inbred populations when the number of lines (**d**) is maximized relative to replication of lines (**a**) for a given number of experimental units

heritabilities and replications when σ_e^2 in the NIL and RI experiment are equal. Figure 1c shows the same power estimates when σ_e^2 for the near-isogenic line/recurrent parent comparison was set at one-enth the value of the recombinant inbred population analysis.

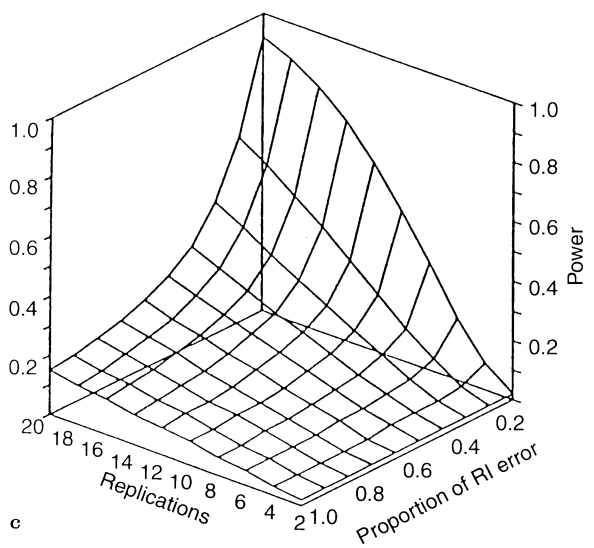
The power to detect QTL was higher for NIL populations than RI populations when a substantial reduction in σ_e^2 was simulated for the NIL population (Fig. 1c vs. 1a). This reduction was included in the analysis since the smaller block size for the NIL contrast relative to the RI population would potentially affect the relative magnitude of σ_e^2 in the two populations. In the figures presented, power of the NIL contrast exceeded that of the RI analysis for heritabilities greater than 0.5 and with 6 or more replications. At higher heritabilities, the variation due to unlinked QTL becomes a proportionately larger fraction of the denominator for the critical RI population *F*-test. The homogeneous genetic background in the NIL comparison results in the power advantage at higher heritabilities *if* accompanied by a relative reduction in σ_e^2 in the NIL comparison. A reduction in experimental error of 90% would likely be a practical maximum. As seen in Fig. 2b and 2c, the power response to relative error is


1.0
0.8
0.6
0.4
0.2
Power
20 18 16 14 12 10 8 6 4 2
Replications
0.2 0.4 0.6 0.8
Heritability

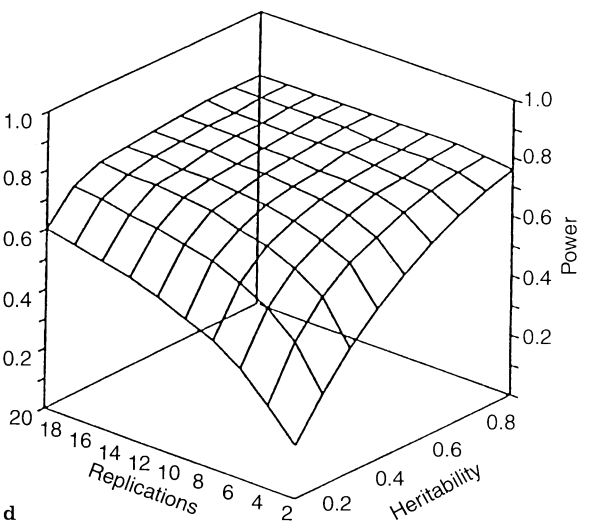
a


1.0
0.8
0.6
0.4
0.2
Power
20 18 16 14 12 10 8 6 4 2
Replications
1.0 0.8 0.6 0.4 0.2
Proportion RI error

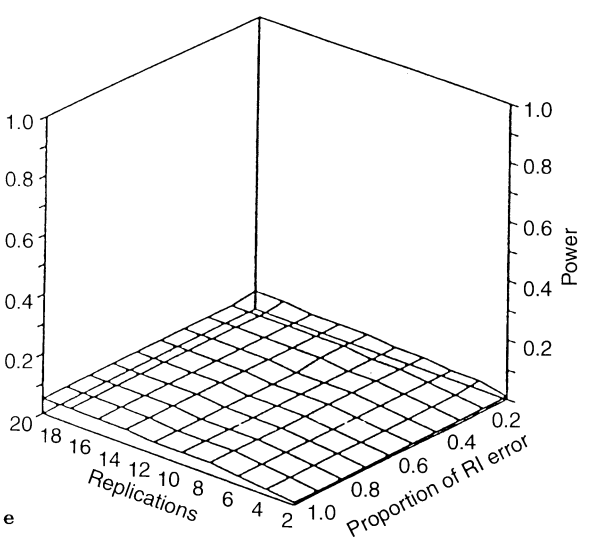
b


1.0
0.8
0.6
0.4
0.2
Power
20 18 16 14 12 10 8 6 4 2
Replications
1.0 0.8 0.6 0.4 0.2
Proportion of RI error

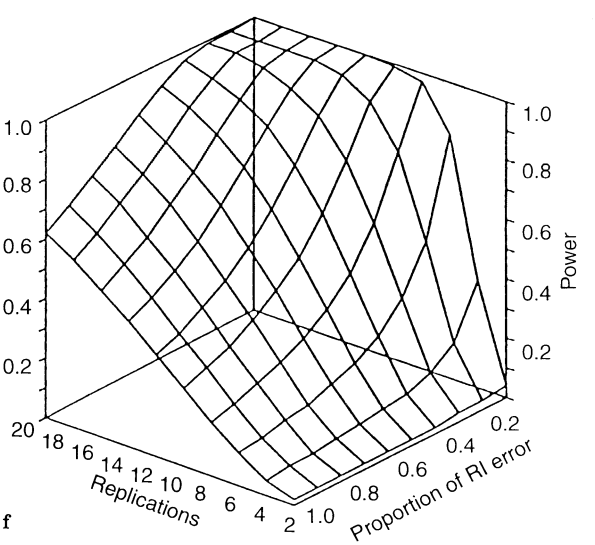
c


1.0
0.8
0.6
0.4
0.2
Power
20 18 16 14 12 10 8 6 4 2
Replications
0.2 0.4 0.6 0.8
Heritability

d


1.0
0.8
0.6
0.4
0.2
Power
20 18 16 14 12 10 8 6 4 2
Replications
1.0 0.8 0.6 0.4 0.2
Proportion of RI error

e


1.0
0.8
0.6
0.4
0.2
Power
20 18 16 14 12 10 8 6 4 2
Replications
1.0 0.8 0.6 0.4 0.2
Proportion of RI error

f

inversely curvilinear so that an error reduction of 50% or more is required to realize substantial improvement in power.

The preceding discussion has considered a RI population of fixed size with variable numbers of replications. As described by Knapp and Bridges (1990), the power to detect QTL with RI populations is generally increased more by maximizing the number of lines (n), rather than replicating (r) a fixed set of lines, although the importance of r and n become similar when a substantial proportion of the genetic variation is explained by markers. The relative relationship between r and n is depicted in Fig. 1a versus 1d for a QTL explaining 1% of the genetic variation. In these figures, the number of experimental units is consistent at each point along the Y-axis (1a – population size is fixed at 200; 1d – replications are fixed at 2). The advantage of maximizing lines relative to replications is obvious and increases at higher heritabilities. Power estimates for NIL comparisons were obtained for heritabilities of 0.1–0.9, replications from 2–20, and with σ_e^2 values ranging from 1.0 to 0.1 times those of the relevant RI population. Within the parameters of our analysis and given a fixed number of experimental units, RI populations which maximized the number of lines relative to replication of lines always had greater power to detect QTL than NIL contrasts based on the same heritability and QTL effect.

Figure 2 gives examples of power estimates for QTL accounting for 1% (2a–c) and 5% (2d–f) of the genetic variation. Acceptable power for NIL comparisons relative to RI populations requires four or more replications, and the utility of the comparisons is realized for traits with higher heritabilities. Excellent power can be realized for detecting QTL with small effects if error can be minimized.

As discussed, the potential benefit of QTL analysis using NILs is due to a reduction in background genetic variation. Statistical methods to account for background genetic variation have been proposed and shown to be useful in enhancing QTL detection (Jansen and Stam 1994; Zeng 1994). These methods implement multiple marker models, partitioning variation to multiple loci simultaneously. Use of these types of analyses would be expected to further enhance QTL detection in recombinant inbred populations, making NIL contrasts comparatively less attractive.

In summary, RI populations are preferred over sets of NILs for QTL detection in all situations if the number of lines is maximized relative to replication of lines and if multiple marker analyses are used. This is true even if a substantial reduction in error variation is realized due to a reduction in experimental block size. This statement assumes the absence of epistasis.

Fig. 2 Plots of power estimates for QTL accounting for 1% of the genetic variation (**a–c**) and 5% of the genetic variation (**d–f**). Recombinant inbred populations of 200 lines (**a** and **d**) are compared to NIL contrasts with heritabilities of 0.1 (**b** and **e**) and 0.9 (**c** and **f**). Variable values for σ_e^2 were used for the NIL comparisons, and were based on σ_e^2 from the RI population

Analysis of power to confirm presence of a QTL using an NIL contrast relative to detection of the QTL in an RI population

Near-isogenic lines derived by backcrossing or selfing have been proposed as a resource to confirm the effect of QTL detected in populations, such as RI populations. I have calculated power estimates for a number of scenarios to explore this hypothesis (Table 2). The power estimates shown assume equal experimental error in the RI experiment and the NIL comparison.

Power estimates are shown for different Type-I error levels to facilitate comparison of methods at equivalent experiment-wise Type-I error rates. For example, analysis of 100 markers on an RI population would involve 100 significance tests on the same data set. To approximate an experiment-wise Type-I error rate of $\alpha = 0.05$, individual significance tests would have to be declared significant at $P < 0.0005$. Since the NIL contrast involves one test, an equivalent Type-I error rate would be $\alpha = 0.05$. In reality, all loci are not independent, and most researchers do not use α levels as stringent as $P = 0.0005$.

A reasonable example may be as follows. A QTL accounting for 5% of the genetic variation is present in an RI population, and the population, is replicated twice. The power to detect this QTL in an RI population at $\alpha = 0.01$ given a heritability of 0.5 is 0.583. The probability of detecting this same QTL using an NIL contrast at $\alpha = 0.05$ is 0.075, 0.195, and 0.269 for 2, 10, and 20 replications, respectively. In this circumstance, there is less power to detect a QTL in the NIL contrast than in the original RI population, an unattractive prospect for an approach meant to confirm the presence of a QTL. Surprisingly, NIL contrasts generally have less power to detect a QTL than the original RI population for populations of 200 or greater. Evidently the replication of marker genotypes across lines overcomes the disadvantage of background genetic variation in the error term. As stated above, power is increased using RI populations if the population size is maximized relative to replication of lines and if multiple marker analyses are implemented.

The effectiveness of NIL contrasts relative to RI populations is greatest for QTL with small effects and with high heritability. In these cases, substantial replication of the NIL lines can enhance the power of QTL detection. The power to confirm QTL is increased if a reduction in σ_e^2 is realized, but the response curve is inversely curvilinear so only large proportionate reductions in error result in appreciable gains in power.

Table 2 Power estimates for a factorial set of QTL effects, heritabilities, and replications. The RI populations consist of 200 lines, and the NIL contrast is a two-entry experiment. Power estimates provided for several values of α (Type-I error rate)

QTL			Recombinant inbred population				NIL contrast	
Effect (%)	Heritability	Replications	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.001$	$\alpha = 0.0005$	$\alpha = 0.05$	$\alpha = 0.01$
1	0.1	2	0.213	0.078	0.016	0.010	0.073	0.015
1	0.1	10	0.298	0.124	0.030	0.019	0.147	0.041
1	0.1	20	0.337	0.148	0.039	0.025	0.161	0.050
1	0.5	2	0.331	0.145	0.037	0.024	0.073	0.015
1	0.5	10	0.388	0.182	0.052	0.034	0.156	0.044
1	0.5	20	0.398	0.189	0.054	0.036	0.180	0.058
1	0.9	2	0.397	0.189	0.054	0.036	0.077	0.015
1	0.9	10	0.406	0.195	0.057	0.038	0.235	0.076
1	0.9	20	0.408	0.196	0.057	0.038	0.356	0.148
5	0.1	2	0.388	0.182	0.052	0.034	0.073	0.015
5	0.1	10	0.712	0.474	0.214	0.161	0.151	0.043
5	0.1	20	0.811	0.583	0.317	0.251	0.170	0.054
5	0.5	2	0.799	0.583	0.302	0.238	0.075	0.015
5	0.5	10	0.898	0.739	0.464	0.387	0.195	0.060
5	0.5	20	0.910	0.760	0.492	0.415	0.269	0.094
5	0.9	2	0.909	0.760	0.489	0.412	0.091	0.018
5	0.9	10	0.919	0.780	0.516	0.438	0.553	0.259
5	0.9	20	0.921	0.783	0.520	0.442	0.851	0.581
10	0.1	2	0.579	0.335	0.125	0.089	0.073	0.015
10	0.1	10	0.934	0.809	0.556	0.479	0.157	0.045
10	0.1	20	0.977	0.903	0.736	0.668	0.183	0.058
10	0.5	2	0.973	0.903	0.714	0.644	0.077	0.015
10	0.5	10	0.995	0.975	0.891	0.849	0.245	0.080
10	0.5	20	0.997	0.981	0.910	0.874	0.376	0.148
10	0.9	2	0.997	0.981	0.908	0.871	0.107	0.021
10	0.9	10	0.998	0.985	0.924	0.892	0.803	0.504
10	0.9	20	0.998	0.986	0.926	0.894	0.985	0.912

Implementation of theory to assess QTL detection using chromosome substitution lines, recombinant chromosome lines, and recombinant inbred lines

Wheat is a polyploid crop with a wealth of well-characterized cytogenetic stocks. These stocks have been used to create chromosome substitution lines in which a single, non-recombinant chromosome of one genotype has been substituted into a recurrent parent background. Substitution lines can, furthermore, be crossed to the recurrent parent and the F_1's manipulated to produce recombinant chromosome populations containing lines detectably recombinant for only the substituted chromosome. This example will compare the power to detect a QTL in a recombinant inbred line population relative to a substitution line/recurrent parent contrast or a recombinant chromosome line population.

Power to detect QTL is substantially higher with the RI population than the substitution line/recurrent parent (SB/RP) contrasts (Table 3). The power comparison is a real-life situation and thus relevant, however equal numbers of experimental units are not compared. A set of SB/RP contrasts would contain 21 pairs of lines, with a total entry number of 42; the recombinant inbred population has 200 individuals. In addition, two QTL were relevant in the SB/RP contrast, but the power to detect only one was considered in the RI or recombinant chromosome population analyses.

Power values are shown for both $\alpha = 0.05$ and 0.01. When multiple comparisons are made, such as multiple markers in the recombinant inbred populations or multiple substitution line/recurrent parent contrasts, an α value of 0.01 or higher is necessary to control the experiment-wise Type-I error rate.

Power to detect QTL in the recombinant chromosome populations was very high, greater than 0.9 in many of the examples presented. Increasing lines relative to increasing replications for a given number of experimental units resulted in higher power values, analogous to the RI population comparisons shown in Fig. 1. The heritability for the recombinant chromosome population was approximately 0.05, and the effect of each QTL was 50% of the genetic variation. These calculations show that QTL can be detected in populations which have low heritability values if the QTL account for a large portion of the genetic variation.

Several points can be summarized from the example. (1) Recombinant inbred populations will generally provide more power to detect QTL than substitution line/recurrent parent contrasts. This observation was

Table 3 Example of application of theoretical discussion in three population structures in wheat. Power estimates provided for specific population sizes and replications at two Type-I error rates. Parameters were based on a reference RI population with 40 unlinked QTL each accounting for 2.5% of the genetic variation, and with a heritability of 0.5. The substitution line contains 2 QTL and thus contained 5% of the genetic variation found in the reference RI population. Parameters determined for the recombinant chromosome population relate to detection of 1 of the 2 QTL segregating in that population

Experimental structure	Population size	Replications	Power	
			$\alpha = 0.01$	$\alpha = 0.05$
Recombinant inbred population	200	10	0.41	0.90
Substitution line vs. recurrent parent				
$\sigma_e^2 = \sigma_{e(RI)}^2$	2	10	0.06	0.20
$\sigma_e^2 = 0.1\sigma_{e(RI)}^2$	2	10	0.29	0.59
Recombinant chromosome population	200	10	0.99	1.00
	50	10	0.78	0.92
	250	2	0.86	0.96
	100	10	0.98	1.00
	500	2	1.00	1.00

theoretically predicted above for NILs which are basically lines with small substituted chromosome regions. (2) Substitution line x recurrent parent-derived populations (recombinant chromosome populations) enhance the power to detect QTL on specific chromosomes or in specific chromosome regions. Evidently, the benefits of genome homogenization through backcrossing for QTL detection are not realized in the comparison of the substitution lines with the recurrent parent. Rather, the benefits can be realized in subsequent crosses with the recurrent parent or of related lines. (3) The wheat example also illustrates that line development time must also be considered. Substitution lines can take many years to produce and undoubtedly require more effort than the development of RI populations. In an outcrossing species such as corn, backcross and RI populations can be produced with similar amounts of effort. In a selfing species such as wheat, RI populations require much less effort to produce than backcross-derived populations.

Summary of statistical issues

Power to detect QTL is generally higher using recombinant inbred populations than sets of NILs. For a given number of experimental units, greatest power is realized when the number of RI lines is maximized relative to more replication of fewer lines. Multiple marker analyses will further enhance the advantage of QTL detection in RI populations over NIL/RP contrasts. The power to confirm QTL is often less in the NIL comparison than in the population in which it was originally detected. Populations derived from near-isogenic line × recurrent parent crosses or crosses of related lines provide good power to detect QTL using relatively small population sizes.

It should be noted that all of the conclusions drawn to this point are based on the absence of epistasis. Detection and quantification of the magnitude of epistasis has been a particularly troublesome area of quantitative genetics. If epistasis is of substantial importance, especially multi-locus epistasis, then the assessment of QTL in a reference genetic background is likely preferred. An example would be the assessment of QTL from wild species in elite or adapted lines (e.g. Eshed and Zamir 1995). NILs may also be a preferred resource for better assessing di- or tri-genic interactions. Therefore, the presence of epistasis will affect population structure choice for reasons other than statistical power.

Practical considerations in the use of near-isogenic lines for QTL analysis

The preceding discussion has focused primarily on statistical issues related to QTL detection using NIL comparisons relative to RI populations. However, some issues regarding the utility of NILs do not relate specifically to power comparisons.

NILs allow the effect of a specific chromosome region to be studied in a two entry experiment; the entire recombinant inbred population needs to be rescored each time the effect of a new trait is determined, even if a suspected chromosome region is involved. Specific NILs could be chosen to examine the effect of a specific chromosome region when a new trait is studied. For example, effect of characterized resistance loci could be assessed for a new strain of a pathogen without analyzing an entire population.

NIL comparisons may allow the effect of specific chromosome regions to be observed directly by eye. Experiments using recombinant inbred populations will only allow measurement of a trait in terms of

means and variances. Some traits can be quite easily visualized when two lines are grown side by side but may be much harder to define on a quantitative scale. For example, disease symptoms are often very characteristic on specific genotypes, but devising a scoring system to quantify obvious but variable observations over a whole population can be quite difficult.

Crosses between NILs will likely allow better studies of epistasis to be done, as the interactions of two or three specific loci can be studied in a uniform background. Problems of collinearity due to sample size are increased when interactions of multiple variables are studied in RI populations. As depicted in the wheat example above, populations derived from substitution line x recurrent parent crosses are very useful for high-resolution mapping and the analysis of specific chromosome regions.

Finally, NILs are a useful resource for map-based cloning and high-resolution mapping studies. As with many genetic mapping studies, future goals must be considered in concert with theoretical realities when identifying the best population structure and experimental approach for mapping studies.

In summary, backcross-derived or near-isogenic lines are not the most statistically powerful population structure for QTL detection. Implementation of NILs in the analysis of quantitative traits must be justified by practical issues since other population structures provide more power for QTL detection and may be easier to construct. The importance of epistasis in controlling the trait of interest will affect decisions on the use of near-isogenic lines. For QTL with large effects, relative power may not be as important as QTL map resolution or objectives facilitated by the availability of pairs of NILs. In this case, the benefits of NILs may outweigh the relative reduction in statistical power.

Acknowledgements Appreciation is expressed to Dr. S. Openshaw, Pioneer Hi-Bred, Int, for valuable comments on the manuscript.

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