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Power studies in the estimation of genetic parameters and the localization of quantitative trait loci for backcross and doubled haploid populations

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Abstract. A statistical model for doubled haploids and backcrosses based on the interval-mapping methodology has been used to carry out power studies to investigate the effects of different experimental designs, heritabilities of the quantitative trait, and types of gene action, using two test statistics, the F of Fisher-Snedecor and the LOD score. The doubled haploid experimental design is more powerful than backcrosses while keeping actual type I errors similar to nominal ones. For the doubled haploid design, individual QTLs, showing heritabilities as low as 5% were detected in about 90% of the cases using only 250 individuals. The power to detect a given QTL is related to its contribution to the heritability of the trait. For a given nominal type I error, tests using F values are more powerful than with LOD scores. It seems that more conservative levels should be used for the LOD score in order to increase the power and obtain type I errors similar to nominal ones.

Key words: QTL – LOD score – Genetic markers – Interval mapping – RFLPs

Introduction

Molecular marker techniques assisted by specific statistical methods provide the means to locate the genetic factors involved in variation for the expression of a quantitative trait (QTL). Four types of experimental design are generally used in these studies: F_2s, backcrosses, recombinant inbred lines, or doubled haploid lines. The first two designs are the most frequently used for gene mapping mainly due to the less time involved, but the last two allow unlimited replications. Studies on the power involved in the detection of the linkage between marker loci and QTLs and on the factors influencing this power are critical for the efficient design of future experiments. Soller et al. (1976) compared the sample size that would be required to detect a given difference between the marker classes in backcross and F_2 populations. Later, Soller and Genizi (1978) used the same concept in other populations, such as half-sibs and full-sibs, while Beckmann and Soller (1988) applied the concept to crosses between segregating populations. Weller (1986) presented some simulation data that indicated the good agreement between predicted effects and actual values in ten replications of 2,000 F_2 individuals. His method was more effective for codominant than for dominant genes. Luo and Kearsey (1991) showed that doubled haploid family means can yield a more accurate estimate of the recombination fraction for a fixed sample size than can a backcross. In none of the above simulation studies, the relative merits of the crossing designs, in terms of their ability to detect the linkage of QTLs while avoiding for false positives, were studied. Soller and Beckmann (1990) gave the relative number of F_3 and F_4 lines, vegetative clones, recombinant inbred lines, and doubled haploid lines, required for a given power as compared to the number of F_2 individuals in the case of codominance at all QTLs. Simpson (1989) used simulated data to test the utility of maximum likelihood and a comparison of marker genotype means to detect linkage. All these studies are based on individual markers and none of them have investigated the actual probabilities of declaring false associations between the markers and the QTLs. This is an important issue given its practical and economical implications. Lander and Botsein (1989) demonstrated that the interval-mapping methodology was more

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powerful than the comparison of means using individual markers. However, it is necessary to investigate the effects influencing both the power of QTL mapping using the interval-mapping methodology and the actual type I errors involved in the tests. Very recently, Carbonell et al. (1992) presented a method based on interval mapping to estimate the genetic parameters of a non-additive QTL and its association with pairs of markers using F_2 populations. The present paper presents an extension to backcross and doubled haploids, evaluates type I error probabilities and studies the effects of different experimental variables on the power of the approach. Situations involving different types of gene action, heritabilities, contributions of individual QTLs to the total phenotypic variance, and crossing designs under the assumption that several QTLs are responsible for the expression of the quantitative trait, are investigated using two different test statistics. The problems associated with the case of two linked QTLs are also studied.

The model

Consider a backcross (or a doubled haploid) population derived from a cross between two inbred lines, and two linked marker loci involved in the interval mapping each having two codominant alleles; p being the recombination fraction between them independent of the sex of the gametes produced. Let Q be a locus involved in the expression of a quantitative trait (QTL) with two alleles Q_1 and Q_2. If the QTL is located within the interval defined by the two markers, we denote r and r′ the recombination fractions to the left and right markers, respectively. Allowing for double crossing-over and assuming the Haldane function under no interference, it follows that $p = r + r' - 2rr'$. Defining a and d after Falconer (1960) as the genotypic values of the homozygote and the heterozygote genotypes, then for a backcross obtained by crossing the F_1 with the "low producing" inbred line:

Q_1Q_2 ..... d
Q_2Q_2 ..... −a

and for doubled haploids:

Q_1Q_1 ..... +a
Q_2Q_2 ..... −a

For backcrosses, the d and a parameters cannot be estimated separately but rather as (a + d), the difference between both genotypes. Hence, in what follows we will use a to actually designate (a + d) in Falconer's notation in order to be consistent with the double haploid case and with the F_2 population case described by Carbonell et al. (1992). Thus, after an adequate change of scale, the phenotypic value of an individual i could be expressed for a QTL as:

$$y_i = \mu + a x_i + \varepsilon_i, \qquad i = (1, \ldots, n)$$

were x is a coded variable taking values of 0 (for the Q_1Q_2 genotype) and −1 (for Q_2Q_2) for backcross, and 1 (for Q_1Q_1) and −1 for doubled haploids; ε is a random variable normally distributed with mean 0 and variance σ^2. If the phenotypic values are not related to the number of Q_1 alleles, then $y_i = \mu + \varepsilon_i$ and a = 0. Hence, a way to detect the presence of a putative QTL in the interval will be given by the significance of the linear model by the Fisher-Snedecor F statistic. Details of the statistical procedures to estimate the genetic effects and the most likely position of the QTL are given by Carbonell et al. (1992).

Power studies data

The model was applied to 100 replicated sets of 250 simulated individuals having eight linkage groups, with six markers each separated by 20 cM. Six QTLs involved in the expression of a quantitative trait, with different gene action according to the parameters defined in Tables 1 (dominance in some QTL) and 2 (complete additive trait), were studied under both backcross and double haploid crossing schemes. For the dominance case (Table 1), two different heritabilities of the trait, 50% and 20%, were investigated. The parameters of the simulations were purposely chosen to reflect different experimental conditions to allow comparisons of interest. Sample size was kept to a small value in order to allow differences in the comparisons to show up. Phenotypic values of the simulated quantitative trait were calculated by adding to the genotypic values a random environmental error which is normally distributed with mean 0 and a variance such that the predetermined heritability was obtained. To avoid false positives by repeatedly using non-independent tests on the same data, the Bonferroni correction was used; then, the significance level was chosen to give an overall probability

Table 1. Parameters used in the first simulation (dominance case), percentage of the total phenotypic variances (V_p for $h^2 = 0.50$ and V'_p for $h^2 = 0.20$) attributable to the genotypic variance of each QTL, and heritability of each individual QTL (h_j^2 for $h^2 = 0.50$ and $h_j'^2$ for $h^2 = 0.20$)

Linkage group	a	d	Location	Percentage of		h_j^2	$h_j'^2$
				V_p	V'_p		
1	1.5	0.0	0.82	14.76	5.91	14.76	5.91
2	1.5	1.5	0.62	22.25	8.86	14.76	5.91
3	1.0	0.0	0.70	6.56	2.62	6.56	2.62
4	1.0	1.0	0.70	9.84	3.94	6.56	2.62
5	0.75	0.75	0.41	5.51	2.20	3.67	1.47
6	0.75	0.75	0.50	5.51	2.20	3.67	1.47
7	0.0	0.0	0.0				
8	0.0	0.0	0.0				

Table 2. Parameters used in the second simulation (complete additivity case), percentage of the total phenotypic variance (V_p) attributable to the genotypic variance of each QTL for $h^2 = 0.50$

Linkage group	a	d	Location	Percentage of V_p [a]
1	1.5	0	0.82	5.42
2	3.0	0	0.62	21.68
3	1.0	0	0.70	2.41
4	2.0	0	0.70	9.64
5	1.5	0	0.41	5.42
6	1.5	0	0.50	5.42
7	0.0	0	0.00	
8	0.0	0	0.00	

[a] In this case, given that there is no dominance, the value of V_p is equivalent to the heritability attributable to each individual QTL (h_j^2)

Table 3. Mean and standard error of the predicted biometric parameters, and location and number of significant replications (N) using LOD (first row) or F values (second row) as the test statistic, averaged over 100 replications for Table 1 data from backcross populations. Subindex 5 is for $h^2=0.50$ and subindex 2 for $h^2=0.20$. Actual values used in the simulations are included for comparison purposes

Linkage group	r	$\hat{r}_5$	$\hat{r}_2$	a+d	$\widehat{(a+d)}_5$	$\widehat{(a+d)}_2$	N_5	N_2
1	0.82	0.80±0.007 0.81±0.009	0.75±0.020 0.74±0.022	1.5	1.55±0.04 1.60±0.04	1.63±0.07 1.66±0.08	89 91	32 44
2	0.62	0.62±0.003 0.64±0.006	0.61±0.006 0.63±0.008	3.0	3.05±0.04 3.14±0.04	3.19±0.06 2.29±0.07	100 100	98 99
3	0.70	0.65±0.023 0.66±0.021	0.62±0.029 0.61±0.029	1.0	1.01±0.05 1.04±0.05	1.08±0.08 1.10±0.08	34 43	8 12
4	0.70	0.70±0.009 0.71±0.010	0.69±0.017 0.69±0.017	2.0	2.00±0.04 2.02±0.04	2.05±0.05 2.09±0.05	99 99	59 65
5	0.41	0.42±0.009 0.43±0.010	0.44±0.019 0.44±0.019	1.5	1.62±0.04 1.68±0.04	1.71±0.08 1.76±0.08	90 94	40 51
6	0.50	0.51±0.012 0.50±0.010	0.52±0.018 0.52±0.018	1.5	1.56±0.03 1.58±0.03	1.67±0.06 1.71±0.06	82 87	33 47
7							1 3	1 3
8							1 1	2 4

Table 4. Mean and standard error of the predicted biometric parameters, and location and number of significant replications (N) using LOD (first row) or F values (second row) as the test statistic, averaged over 100 replications for Table 1 data from doubled haploid lines. Subindex 5 is for $h^2=0.50$ and subindex 2 for $h^2=0.20$. Actual values used in the simulations are included for comparison purposes

Linkage group	r	$\hat{r}_5$	$\hat{r}_2$	a	$\hat{a}_5$	$\hat{a}_2$	N_5	N_2
1	0.82	0.81±0.003 0.83±0.007	0.81±0.005 0.82±0.008	1.5	1.51±0.02 1.56±0.02	1.57±0.03 1.62±0.03	100 100	96 96
2	0.62	0.62±0.004 0.64±0.007	0.62±0.005 0.63±0.007	1.5	1.52±0.02 1.57±0.02	1.59±0.03 1.64±0.04	100 100	95 98
3	0.70	0.71±0.007 0.71±0.008	0.69±0.016 0.69±0.016	1.0	1.01±0.02 1.02±0.02	1.03±0.04 1.04±0.04	90 95	54 68
4	0.70	0.71±0.010 0.71±0.011	0.65±0.020 0.65±0.020	1.0	1.00±0.02 1.02±0.02	1.01±0.04 1.03±0.04	94 96	52 66
5	0.41	0.42±0.010 0.42±0.011	0.45±0.019 0.45±0.019	0.75	0.83±0.02 0.85±0.02	0.83±0.04 0.83±0.05	74 80	34 44
6	0.50	0.51±0.015 0.52±0.015	0.53±0.018 0.52±0.019	0.75	0.79±0.02 0.80±0.02	0.85±0.03 0.86±0.03	62 74	30 40
7							5 6	3 5
8							0 1	1 1

of rejection of 0.05. According to the number of intervals being tested and supposing a "sparse-map" situation (Lander and Botsein 1989), this was equivalent to a nominal significance level of 0.001.

Results

For the dominance case (Table 1 data), backcrosses and heritabilities of 0.50 and 0.20, Table 3 presents the estimates of the genetic parameters and their standard errors and the location of the QTL predicted by the model for each of the eight simulated linkage groups averaged for all 100 replications, and the number of sets that showed significant LOD or F values. This number for linkage groups 1 through 6 is an indication of the power involved in the detection of a given QTL, and for groups 7 and 8 represents the probability of detection of false positives,

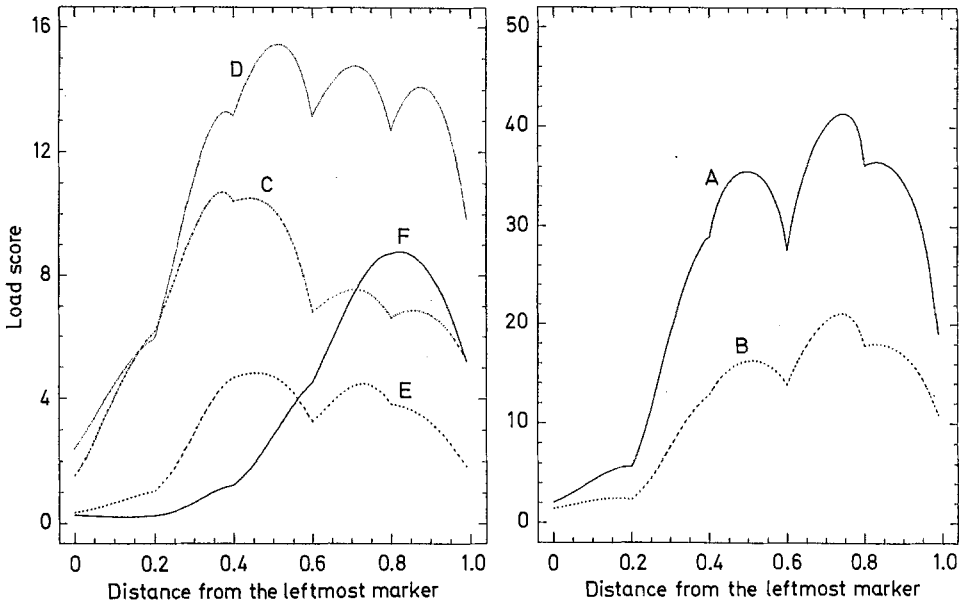

Fig. 1. Typical distributions found with two QTLs located in the same linkage group for doubled haploids (*A*) and backcrosses (*B*) and a heritability of 0.50 (right side), and for doubled haploids and a heritability of 0.08 (left side). See text for explanations of the symbols.

Table 5. Mean and standard error of the predicted biometric parameters, and location and number of significant replications (N) using LOD (first row) or F values (second row) as the test statistic, averaged over 100 replications for Table 2 data from backcross populations (subindex b) and doubled haploid lines (subindex d) and $h^2 = 0.50$. Actual values used in the simulations are included for comparison purposes

Linkage group	r	$\hat{r}_b$	$\hat{r}_d$	a	$\hat{a}_b$	$\hat{a}_d$	N_b	N_d
1	0.82	0.76±0.018	0.80±0.007	1.5	1.60±0.07	1.55±0.04	44	89
		0.76±0.018	0.81±0.009		1.64±0.07	1.60±0.04	58	92
2	0.62	0.62±0.004	0.62±0.003	3.0	3.15±0.06	3.05±0.04	100	100
		0.63±0.008	0.64±0.006		3.25±0.06	3.14±0.04	100	100
3	0.70	0.61±0.029	0.65±0.023	1.0	1.06±0.07	1.00±0.05	9	35
		0.62±0.029	0.66±0.021		1.09±0.07	1.04±0.05	23	43
4	0.70	0.69±0.016	0.71±0.009	2.0	2.03±0.05	1.99±0.04	68	99
		0.69±0.016	0.71±0.010		2.06±0.05	2.01±0.04	83	99
5	0.41	0.45±0.016	0.42±0.008	1.5	1.71±0.06	1.62±0.04	52	90
		0.44±0.016	0.43±0.010		1.78±0.06	1.68±0.04	62	94
6	0.50	0.52±0.016	0.51±0.012	1.5	1.64±0.05	1.56±0.03	49	82
		0.51±0.017	0.50±0.010		1.68±0.05	1.58±0.03	57	87
7							1	1
							3	4
8							1	1
							4	3

i.e., the type I error. Similar information for doubled haploid lines is shown in Table 4. For the complete additivity case (Table 2 data), Table 5 presents the results for both backcrosses and doubled haploids.

Good agreement between simulated and predicted values was obtained using the proposed model. When the QTL shows dominance, the estimates of the additive effect for backcrosses is the sum of both additive and dominant components as expected from the definition of the model. Dominance did not affect the estimates of the additive effect for doubled haploids.

The highest scores were obtained for the QTL with the largest effects. When two QTLs had similar effects (as those in linkage groups 5 and 6), the one closer to a

marker consistently showed a higher power. The lower the effect of the QTL the larger the standard deviation and the less accurate the prediction of the location.

There was a drastic loss of power for the complete additive trait (Table 2 data) as compared with the case when the trait had some QTLs showing dominance (Table 1 data) for the backcross. The power of the analyses is much higher for doubled haploids than for backcrosses while keeping actual Type I errors at similar values. The estimates of the prediction of the QTL location and of the additive effect are more accurate for doubled haploids than for backcrosses. For a given type of gene action, the standard errors of the estimates increase as the heritability of the trait decreases for both types of crossing schemes.

Using the F value as the test statistic, Type I errors are similar to the nominal ones; on the other hand, the LOD score gives much lower Type I error than expected and also less powerful analyses.

To study the effect of several QTLs in the same linkage group, additional simulations were performed using just two QTLs located at 42 and 82 cM from the extreme left of the first linkage group. Both QTLs had the same additive value of 1.5 and no dominance. Two heritabilities of the trait, 0.08 and 0.50, were investigated for doubled haploids but only 0.50 for backcrosses. In Fig. 1, some typical situations are depicted. Results from 100 simulations consistently gave a bimodal distribution for the higher heritability (curve A for doubled haploids and B for the backcross). In a few cases, a highly significant unimodal distribution (curve type C) or a plateaued or multimodal (curve type D), and more frequently a bimodal (curve type E), distribution was found for the lower heritability case. For the bimodal distribution shape, the predicted locations of the QTLs were biased in the sense that the leftmost QTL was shifted to the right and the rightmost one to the left. Curve type F using only one QTL (that at 82 cM) is included for comparison purposes.

Discussion

The basic assumptions of the model given by Carbonell et al. (1992) also apply here.

Doubled haploid populations allow experiments to be conducted with smaller sample sizes because they show much higher power than backcrosses (see Table 5 where the absence of cominance does not mask the comparisons). Moreover, they give more accurate estimates of the location of the QTL and with less variance. This result could be expected from the fact that the regression model uses more spread-out values for doubled haploids ($+1$ and -1) than for backcrosses (0 and -1) and, as Luo and Kearsey (1991) pointed out, from the fact that the absolute difference between the means of both homozygous genotypes is larger than that between the heterozygous and the recessive homozygous genotypes.

If dominance is present backcrosses not only give biased estimation of the effects, because additive and dominance are completely confounded, but also some QTLs could not be detected if the genetic values of the homozygote and the heterozygote at those loci are the same. This could be the case for complete dominance when the F_1 population is backcrossed with a "high producing" parental and also when, independently of the direction of the backcross, the recurrent parental line had the "high" allele at some loci.

For doubled haploids, the power of detecting a given QTL is clearly related to its heritability h_j^2 (i.e., proportional to a^2); the higher its contribution to the total heritability of the trait, the higher the probability of being detected. For backcrosses, the ranking in power is determined by the proportion of $(a+d)^2$. Even QTLs having small effects were identified by the model; for doubled haploids, the power of the test was about 90% for heritabilities as low as 0.05. To obtain a similar power for backcrosses, the heritability attributable to an individual QTL should be around 14%.

For a given type of gene action, the comparison with previously reported findings by Carbonell et al. (1992) is difficult because two different criteria were used with F_2 populations. If one chooses the one degree of freedom test that gives type I errors similar to nominal ones, doubled haploids have a similar or slightly higher power than the F_2; hence, both experimental designs are very efficient to detect QTLs by linkage studies. However, if dominance is present, doubled haploids will only detect the additive component of a particular QTL. This fact could be of extreme importance in a marker-assisted selection scheme in order to exploit the non-additive variation shown by the trait but hidden in some QTLs. The relative merit of the backcross as compared with the F_2 experimental design depends on the amount of dominance as Soller et al. (1976) indicated. Under no dominance, the F_2 has a higher power, but under complete dominance it seems that backcrosses are better; however, as mentioned above, some QTLs might remain undetected when using this crossing scheme.

For a given nominal type I error, tests using F values are more powerful while keeping the realized type I to values similar to nominal ones because the LOD score results in a much lower Type I error than expected and consequently less powerful analyses. It seems that the nominal significance level used to cope with the fact of repeatedly using non-independent tests with the same data, was too low for LOD and a more conservative test should be used in this case. On the other hand, it appears that the F test finds a maximum near the center of the interval giving a biased estimation of the location of the QTL.

The method here presented, based on the interval-mapping concept, estimates more accurately the effects and location of the QTL and is more powerful than methods based on a comparison of marker means, as Lander and Botsein (1989) demonstrated, and also than methods based on maximum-likelihood estimation using "individual markers". Recombination frequencies obtained using the nonlinear-model analysis proposed by Knapp et al. (1990) are less efficiently estimated because information is limited to some specific marker genotypic classes. Luo and Kearsey (1991), using 500 simulated individuals replicated 20 times and with a heritability of a single QTL of 10%, found that the standard errors of the prediction of the location were about 0.03 at the minimum, for doubled haploids. In our case, comparable heritabilities (like that in linkage group 4 of Table 4), gave lower standard errors using half as many individuals. Weller (1986) used a procedure that requires a significant investment in mainframe computing time; in a simulation with 2,000 individuals replicated ten times it was shown to be useful only for QTLs with effects greater than a 1.0 phenotypic standard deviation and did not yield meaningful results for loci of smaller effect in F_2 populations. No comparisons of the power involved in their approaches can be made because the number of replications employed was very small.

The above studies involved only one QTL per trait; in our simulation, a more realistic approach considered several QTLs acting together to result in the final phenotypic value of the trait. In order to be fully comparable with their approach, additional simulations were performed using only one QTL instead of six, that one corresponding to the first linkage group (unpublished data). For doubled haploids, using heritabilities as low as 5%, the QTL was located in 95 out of 100 simulations using only 250 individuals. Backcrosses, as previously shown, are much less powerful; only 51% of the simulations gave significant results for the same heritability. In order to obtain a similar power, the heritability of the single QTL should be around 15%.

When several QTLs are located in the same linkage group, results from a single experiment could be misleading. One could infer the presence of a single QTL with a large effect (about the sum of the effects of the QTLs involved), placed in a location intermediate between both QTLs, if the heritability is low and the number of tested individuals small. Nonetheless, most of the time either a plateaued or a multimodal-shape distribution will be obtained. This will be an indication that several QTLs are located in the same linkage group and a more detailed study should be performed. Lander and Botsein (1989) discussed such a case and, in their example, both QTLs were located as far as 80 cM from each other; this would explain the good agreement between predicted and actual location. However, if the QTLs are more closely located, as in our case, one could obtain a biased estimation of the genetic effects and even of predicting a location in a different interval. These findings are in agreement with results obtained by Knapp (1991) using 250 simulated doubled haploid lines. This fact may have important implications if applied to a breeding program for marker-assisted selection. Hence, even though the method here presented has been proven superior in detecting and estimating the effects of several QTLs while keeping type I errors similar to the nominal ones, one should be cautious in actual experiments given that the assumptions underlying present statistical models are still somewhat restrictive. Further theoretical studies are needed in order to make them of wider applicability and to clearly establish the grounds for experimenters to set up their work.

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