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Disequilibrium Mapping of a Quantitative-Trait Locus in an Expanding Population

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Summary

Linkage disequilibrium (LD) mapping can be successful if there is strong nonrandom association between marker alleles and an allele affecting a trait of interest. The principles of LD mapping of dichotomous traits are well understood, but less is known about LD mapping of a quantitative-trait locus (QTL). It is shown in this report that selective genotyping can increase the power to detect and map a rare allele of large effect at a QTL. Two statistical tests of the association between an allele and a quantitative character are proposed. These tests are approximately independent, so information from them can be combined. Analytic theory is developed to show that these two tests are effective in detecting the presence of a low-frequency allele with a relatively large effect on the character when the QTL is either already a candidate locus or closely linked to a marker locus that is in strong LD with the QTL. The latter situation is expected in a rapidly growing population in which the allele of large effect was present initially in one copy. Therefore, the proposed tests are useful under the same conditions as those for successful LD mapping of a dichotomous trait or disease. Simulations show that, for detection of the presence of a QTL, these tests are more powerful than a simple *t*-test. The tests also provide a basis for defining a measure of association, γ, between a low-frequency allele at a putative QTL and a low-frequency allele at a marker locus.

Introduction

Linkage disequilibrium (LD) mapping has been successfully applied to several disorders in populations, such as the Finnish population, that have grown rapidly in relative isolation (Peltonen et al. 1995). LD mapping is effective when a single copy of a disease-associated allele was present in the founding population and low-frequency alleles at marker loci were in perfect LD with the disease-associated allele. LD between a marker allele and the disease-associated allele decays as an exponential function of the product of time and the recombination rate, so markers currently in strong LD with a disease phenotype are likely to be very closely linked to the causative locus. LD mapping is particularly useful in a rapidly growing population, because the effects of genetic drift are minimized. Several recent theories have examined the statistical and population-genetic aspects of LD mapping (Thompson and Neel 1997; Xiong and Guo 1997; Rannala and Slatkin 1998). All of these theories assume a dichotomous trait—individuals are classified as diseased or not.

The same logic applies to an allele that has a substantial effect on a quantitative character (Laitinen et al. 1997). A founder allele will remain in strong LD with closely linked marker alleles that were on the same ancestral chromosome, and hence those marker alleles will tend to be associated with larger or smaller values of the character. Although this idea is sound in principle, the question is how best to implement it and to understand the conditions under which it will be useful. One approach, used by Laitinen et al. (1997), is to dichotomize the quantitative trait. In their case, the quantitative trait was serum immunoglobulin E concentration, which is elevated in asthma patients. In a group of asthma patients, Laitinen et al. classified individuals with concentrations >100 kU/liter as high and classified the rest as low, and then they tested for a significant association between marker alleles and the dichotomized trait.

Here I suggest a different approach, based on two statistical tests for the presence of a quantitative-trait locus (QTL). Both tests require "selective genotyping" (Lander and Botstein 1989; Lynch and Walsh 1998): some individuals are members of a sample selected because of unusually high or low values of the quantitative character. The first test is for a significant allele-frequency difference between a random sample from the

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population and the selected sample, and the second test is for significant heterogeneity within the selected sample. The second test is approximately independent of the first. These tests differ from a simple *t*-test for the difference between the mean values of the character in individuals who carry or do not carry a particular marker allele, because they focus on the selected sample in which the frequency of an allele of large effect is in much higher frequency than it is in the whole population.

These two tests can be applied to any locus and any character, but they probably will be useful only in two cases, both of which are of some practical interest. First, if the locus is itself a candidate QTL, then these tests can be used to determine whether a low-frequency allele has a significant effect on the character of interest. Candidate loci are often the objects of study in pharmacogenetics because many of the loci involved in drug metabolism are known (Krynetski and Evans 1998). Second, when the conditions for LD mapping are met—that is, when there was a single founder allele of large effect in a rapidly growing population—these tests can determine whether a significant association exists between a low-frequency allele at a marker locus and the character. A significant positive result would indicate the presence of a QTL in the region of the marker locus. In that case, a measure of the relative association between a marker allele and the character—an association defined below (in the section A Measure of Marker Association with a Quantitative Character) as γ—would be useful for mapping the QTL.

If one of the alleles at the QTL is at low frequency, that locus explains only a small fraction of the genetic variance in the quantitative character in the study population. Hence, the method described in this article differs from most methods of QTL mapping that focus on QTLs that account either for a significant fraction of the heritable variation within a population or for substantial differences in average values between populations or species (Lynch and Walsh 1998). LD mapping of the kind described in this article can take advantage of the opportunity created by isolated rapidly growing populations to study QTLs that otherwise might be difficult to detect and map. Identification of alleles that have a large effect on a character would be useful both for understanding genetic factors that influence the character and for providing a way to survey for other variants at the same locus.

In the following sections, I will introduce the statistical tests and investigate their performance both with a candidate locus and with a closely linked marker locus. Finally, I will define a measure of LD for a QTL and will discuss how this measure can be used for LD mapping.

Statistical Tests

The quantitative trait is measured by a value x. The QTL is assumed to have two alleles, A and a, in frequencies p and $1 - p$, respectively, with p being sufficiently small that, to a first approximation, AA homozygotes can be ignored. The units of measurement of the character are chosen so that distribution of x in aa individuals is normal with mean 0 and variance 1 and so that the distribution in Aa individuals is normal with mean ϵ and variance 1. To simplify discussion, I assume $\epsilon > 0$. With Hardy-Weinberg proportions of the three genotypes at this locus, the distribution of x in the population is a mixture of two normal distributions, weighted by $2p$ and $1 - 2p$, and hence will not be exactly normal. If p is small, deviations from normality will not be apparent, and the A locus will account for a very small proportion, $\sim 2p\,\epsilon^2$, of the total variance.

Assume that we can assess the genotype at a diallelic locus with alleles B and b. This locus could be either the QTL itself or a marker locus closely linked to the QTL. In either case, assume that the B allele is at low frequency, q, in the population, so we need distinguish only bb homozygotes from Bb heterozygotes. All of the analysis will be of two groups of individuals who can be measured for the character and whose genotype at the B/b locus can be determined. One group, containing n_1 individuals, is chosen from the population at random and independently of x. This group is the "population sample." The other group, containing n_2 individuals, is chosen because of unusually large values of x. This group is the "selected sample." The population sample will probably contain individuals who could be included within the selected sample, but, for statistical analysis, an individual should not be in both groups.

Let $w(x)$ be the selection function representing the relative probability that an individual with phenotype x is included in the sample. It is known from the theory of quantitative genetics (Lynch and Walsh 1998) that the most efficient method of selection is truncation selection; $w(x) = 1$ if $x > \beta$ and 0 otherwise. In some cases, however, selection will occur because individuals with larger x tend to have a disease or a medical condition that causes them to become part of a study group, as in the asthma example discussed above. Then, selection is probably less efficient than truncation selection. The theory will be developed for arbitrary $w(x)$, but the numerical examples will be based on truncation selection. Lander and Botstein (1989) caution against the selection of too small a fraction of the population, because individuals having very extreme values of the character might be aberrant for other reasons and, therefore, not be useful for detection of QTLs. They recommend that individuals beyond the upper or lower 5%—corresponding, in this case, to values of β that are slightly >2—not

be used. In the present context, the selected sample could be further subsampled, and a second set of tests could be performed on the new selected sample.

Selecting a sample fits the design of case-control studies. The selected sample corresponds to the cases, and the population sample corresponds to the controls. It is not necessary to match each individual in the population sample with an individual in the selected sample, but the population sample as a group must be comparable—in age, sex, and other factors that might affect the value of x—to the selected sample The number of individuals in the selected sample and the number of individuals in the population sample need not be the same.

The first statistical test (test 1) tests for a significant difference in the numbers of *Bb* individuals in the selected and population samples. Testing for significance is straightforward. In what follows, I assume that sample sizes are large enough that a χ^2 test applied to the appropriate 2 × 2 contingency table can be used. The Appendix shows an example of the calculations. The result of the χ^2 test is a probability, P_1, of rejection of the null hypothesis that there is no difference in the numbers of *Bb* individuals. In particular applications, the number of *Bb* individuals in the population sample might be small enough that Fisher's exact test is more appropriate.

The second test (test 2) depends only on the selected sample. Assume that k of the n_2 individuals in that sample are *bb* homozygotes with phenotypes $x_1, \ldots, x_k$ and that m individuals are *Bb* heterozygotes with phenotypes $y_1, \ldots, y_m$. To test for significant differences between groups, either a nonparametric test, such as the Mann-Whitney U-test, a randomization test, or a test tailored to this particular situation (samples from truncated normal distributions) could be used. Student's t-test would not be appropriate for small sample sizes, because the distributions of the x_i and y_j are likely to be far from normal.

To get an idea of the power of test 1, I assume that sample sizes are large enough that asymptotic results apply. Although x_i and y_j are not themselves normally distributed, the central-limit theorem ensures that their average values will be. Hence, under the null hypothesis that $\epsilon = 0$, the statistic

$$z = \frac{\bar{y} - \bar{x}}{\sqrt{\frac{V_x}{k} + \frac{V_x}{m}}}$$

will be normally distributed with mean 0 and variance 1, where $\bar{x}$ and $\bar{y}$ are the observed average values and where V_x and V_y are unbiased estimates of the variances (Bulmer 1979). The value of z and a table of the standard normal distribution give a probability, P_2, of rejection of the null hypothesis. The Appendix illustrates the calculations.

When both tests can be performed, the results are asymptotically independent of each other. The dependence between them arises only because the values of k and m are needed for test 1 and are also part of the definition of z. As n_2 becomes large, k and m approach their expectations, $(1 - p')n_2$ and $p'n_2$, and hence the two tests become independent. We can take advantage of the approximate independence to use Fisher's method for the combining of probabilities (Sokal and Rohlf 1980). The resulting test is test 3. According to Fisher's method, $v = -2\ln(P_1) - 2\ln(P_2)$ has a χ^2 distribution with 4 df under the null hypothesis. This test results in a third probability of rejection of the null hypothesis ($\epsilon = 0$), P_3. If v_c is the critical value for a level-α test ($v_c = 9.49$ for $\alpha = .05$ and $v_c = 18.47$ for $\alpha = .001$), then we would reject the null hypothesis at level α if $P_1 P_2 e^{-v_c/2}$. Combining results from the two tests can lead to rejection of the null hypothesis even if neither test alone does; for example, if $P_1 = .1$ and $P_2 = .08$, then $v = 9.66$, allowing rejection of the null hypothesis at the 5% level.

In the simulations discussed below, I compare the performance of these three tests to Student's t-test applied to a population sample size of $n_1 + n_2$. This sample size for the t-test is chosen so that the same number of individuals are genotyped. In this sample, i individuals are *Bb* heterozygotes, and $n_1 + n_2 - i$ are *bb* homozygotes. Student's t-statistic is computed in the usual way (Bulmer 1979), and its value is compared with a two-sided t distribution with $n_1 + n_2 - 2$ df, to yield the probability of rejection of the null hypothesis under this test, P_t.

Candidate Locus

If A is a candidate allele, then, in the above notation, $q = p$, and we do not have to consider the B locus separately. Selection of the sample increases the frequency of A—from p to a larger value, p'. The frequency of A in the selected sample is a random variable with expected value

$$p' = p + p\frac{\int w(x)[f_{Aa}(x) - f_{aa}(x)]dx}{\bar{w}}, \qquad (1)$$

where $f_{Aa}(x)$ is the distribution of x among *Aa* individuals (a normal distribution with mean ϵ and variance 1), $f_{aa}(x)$ is the distribution of x among *aa* individuals (a normal distribution with mean 0 and variance 1),

$$\bar{w} = (1 - 2p)\int w(x)f_{Aa}(x)dx + 2p\int w(x)f_{aa}(x)dx,$$

and, throughout, integrals will be taken over all possible values of x.

The interpretation of equation (1) is simple: the numerator in the expression on the right-hand side is the difference between the expected fractions of the *Aa* and *aa* individuals in the selected sample, and the denominator, $\overline{w}$, is the expected fraction of the population in the selected sample. Therefore, if the selection function $w(x)$ were unknown, the expected increase in the frequency of *A* in the selected sample could be found empirically by estimation of the relative probabilities that *Aa* and *aa* individuals will be included within the selected sample.

In the particular case of truncation selection, the integrals can be expressed in terms of error functions, but, since those have to be evaluated numerically, it is just as easy to use numerical integration directly, to obtain quantitative results. Figure 1 shows p' as a function of ϵ, for $\beta = 2$ and $\beta = 3$, corresponding to sampling of the upper 7.8% and the upper 1.7% of the population, respectively, with $p = .005$ in both cases. If ϵ is large enough, truncation sampling can increase the expected frequency of *A* by an order of magnitude or more, ensuring that an allele that is rare in the population will be represented in substantial numbers in the selected sample.

When *A* is a candidate allele, the question is whether there is evidence that *A* has a significant effect on the character. Figure 2 shows the power of test 1 as a function of ϵ for $\beta = 2$ and $\beta = 3$. These results were obtained by assuming binomial distributions with probabilities $2p$ and $2p'$ for the numbers of *Aa* individuals in, respectively, the population sample and the selected sample. Then, for each configuration of the numbers of copies, a χ^2 value was calculated, and the probability of that configuration was accumulated if it led to a rejection of the null hypothesis at level $\alpha = .05$. Even with only 50 individuals each in the population sample and the selected sample, there is considerable power to reject the null hypothesis.

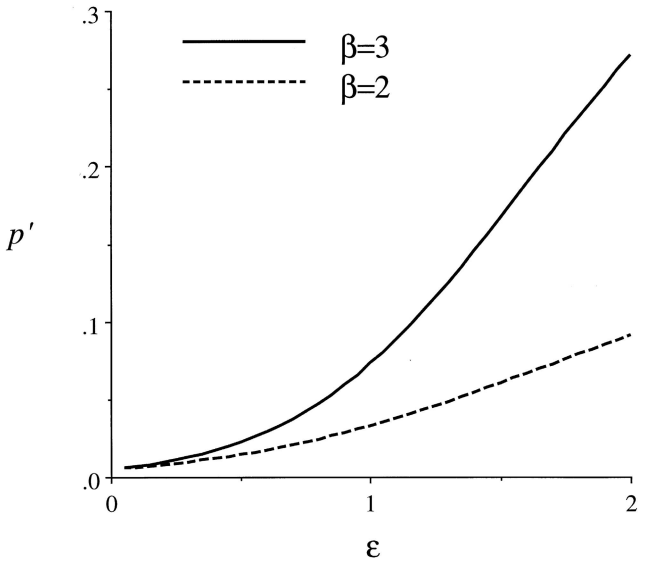

Figure 1 Graphs of p', the frequency of *A* in the selected sample, computed from equation (1) in the text, under the assumption of truncation selection with $\beta = 2$ and $\beta = 3$. In both cases, p, the population frequency of *A*, is .005.

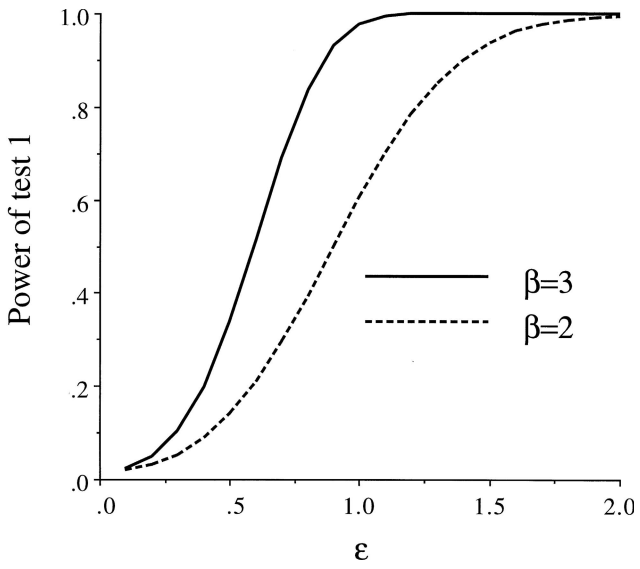

Figure 2 Graphs of the power of test 1 (the test for significant difference, between the population sample and the selected sample, in the number of *Aa* heterozygotes); the vertical axis is the probability of rejection of the null hypothesis that there is no difference in the numbers of *Aa* individuals. These results were obtained under the assumption that p is given by equation (1) (applying truncation selection with $\beta = 2$ and $\beta = 3$) and that $n_1 = n_2 = 50$. In both cases, p, the population frequency of A, is .005. Under the assumption of binomial distributions with means $2p$ and $2p'$ of the number of *Aa* individuals in the two groups of 50 individuals, the overall probability of rejection of the null hypothesis, by use of a χ^2 test with 1 df, was computed by averaging over the distribution of the numbers of *Aa* individuals in both groups.

We can obtain a rough idea of the power of test 2 under the present model by approximating the expectation of z as a function of β and ϵ. The expectations of $\bar{x}$ and $\bar{y}$ are, respectively,

$$E(\bar{x}) = \frac{\int x w(x) f_{aa}(x)\,dx}{\int w(x) f_{aa}(x)\,dx} \qquad (2)$$

and

$$E(\bar{y}) = \frac{\int x w(x) f_{Aa}(x)\,dx}{\int w(x) f_{Aa}(x)\,dx} \quad . \qquad (3)$$

The expected values of the variances are found from

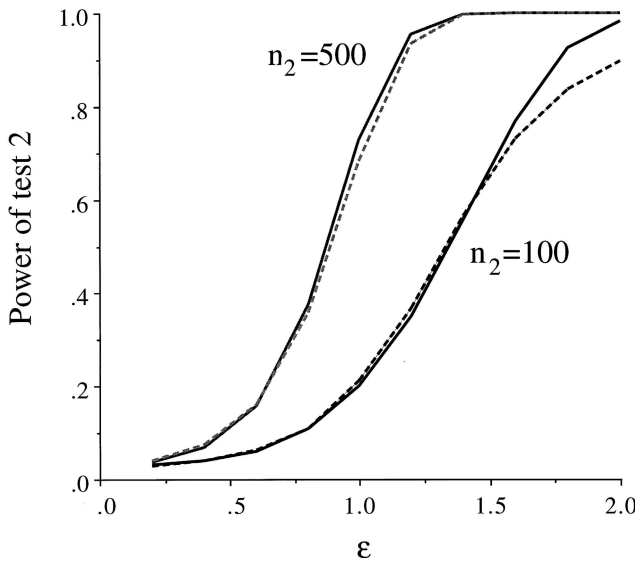

Figure 3 Graphs of the approximate power of test 2 (the test for significant heterogeneity in the values of x among Aa and aa individuals in the selected sample containing n_2 individuals); the vertical axis is the probability of rejection of the null hypothesis that there is no heterogeneity. These results were obtained under the assumption that the selected sample contains n_2 individuals and that the proportion of Aa individuals was $2p'$, with p' given by equation (1). The value of the test statistic z (defined in the text) was computed under the assumption that the means and variances of the character in the selected sample took their expected value. The value of P_2 is then obtained from a standard normal distribution with mean 0 and variance 1, for that value of z.

similar integrals for the second moments. On the basis of these expectations and variances, we find the expected value of z and then use a table of a standard normal distribution to obtain the probability of rejection of the null hypothesis under this model. The results shown in figure 3 are only approximations to the power, because this method does not account for random variation in z.

Figure 3 shows some results for two sample sizes ($n_2 = 100$ and $n_2 = 500$) under the assumption that the frequency of A in the selected sample is p'. We can see that, for $n_2 = 100$, this test has less power than test 1 but that, with a larger sample size, $n_2 = 500$, the power increases substantially. In this test, β, the threshold value of x, makes much less difference, for most values of ϵ, than it does for test 1.

Simulation Test for a Candidate Locus

The analysis in the preceding section suggests that these two tests have some power to detect the presence of an allele having a strong effect on a quantitative character. To verify that and to compare their performance with that of the t- test described above, a simulation program was used to generate hypothetical data according to the model described in the first section (Statistical Tests). In each replicate, the population sample of n_1 individuals was generated by assuming that there is a mixture of normal distributions, with means 0 and ϵ unit variances, with proportions $1-2p$ and $2p$. The selected sample of n_2 individuals was generated by use of a rejection scheme in which randomly chosen individuals from the population were retained only if x exceeded β. A χ^2 test with 1 df was used to provide the probability P_1 of rejection of the null hypothesis. Then test 2, for significant heterogeneity within the selected sample, based on the z statistic defined above (in the Statistical Tests section), was performed, providing the value of P_2. The values of P_1 and P_2 were combined according to Fisher's method described above, to provide a value of P_3. Student's t-test was performed on a randomly generated population sample containing $n_1 + n_2$ individuals, to give the value of P_t. At the end of a set of 100 replicate simulations, the numbers of replicates in which the null hypothesis could be rejected at the 5% level were reported for each of the four tests.

Table 1 shows some results for a candidate locus. The parameter values for each case are p, ϵ, β, n_1, and n_2. These results confirm the conclusions based on the approximate calculations in the preceding section. Test 1 is more powerful in rejecting the null hypothesis than is test 2, and hence, for both tests to be comparable in power, the number of individuals in the selected sample, n_2, must be larger than the number in the population sample, n_1. For $\beta = 2$ and especially for $\beta = 3$, these

Table 1

Results of Simulations of Samples Genotyped at a Candidate Locus

Value of Parameter				No. of Replicates Significant at 5% Level, for[a]			
ϵ	β	n_1	n_2	P_1	P_2	P_3	P_t[b]
.5	2	100	100	31	12	34	20
.5	3	100	100	54	12	50	20
.5	2	200	200	49	14	49	28
.5	2	300	300	74	14	70	43
.5	2	50	200	11	9	21	13
.5	3	50	200	32	13	31	13
1.0	2	100	100	89	38	88	53
1.0	3	100	100	100	68	100	53
1.0	2	200	200	98	52	97	83
1.0	2	300	300	100	61	100	90
1.0	2	50	200	65	55	84	55
1.0	3	50	200	100	74	100	55

[a] Data are no. of replicates for which the null hypothesis could be rejected at the 5% level. In all cases, 100 replicates were run and $p = .01$.

[b] The t-test was done on a population sample of size $n_1 + n_2$. Since the results of the t-test do not depend on β, they are the same for cases that differ only in the value of β.

tests were more effective that a *t*-test, in the detection of a significant effect at a candidate locus.

Linked Marker Locus

If there is no candidate gene but, instead, a candidate region containing a biallelic marker locus with alleles *B* and *b*, then the goal is to find evidence for nonrandom association between a marker allele and the putative QTL. Disequilibrium mapping of *A* is feasible only if the allele at the marker locus on the ancestral *A* chromosome was and is rare in the population. Assume that *B* was on the ancestral chromosome, that its frequency in the population at the time of sampling is q, and that the LD coefficient between the two loci is D.

Both of the tests described for candidate alleles can be used with marker alleles, but there are two confounding factors that reduce statistical power. Some of the copies of *B* in the population are not on chromosomes carrying *A*, because they are not descendants of the copy of *B* that was on the ancestral *A* chromosome; and some of the copies of *B* that descend from the copy on the ancestral *A* chromosome are now on chromosomes carrying *a*, because of recombination between the two loci. The problem is to find how much power is lost because of these two effects.

The first step is the same: select individuals with large x, for genotypic analysis. As in the preceding section, $w(x)$ is the selection function, and, in the numerical examples, truncation selection with truncation value β will be used. In the selected sample, the expected frequency of *A* will still be given by equation (1). In the population sample *B* has expected frequency q, and in the selected sample its expected frequency is

$$q' = q + \frac{(p' - p)D}{p(1 - p)} \quad . \tag{4}$$

We are concerned with the case in which *A* and *B* are both at low frequency but with $q > p$ and positive disequilibrium ($D > 0$ implies that there is an excess of *AB* chromosomes). We would expect q to exceed p, because the concern is with the case in which *A* arose in the recent past in a population in which *B* was already present at some frequency. In this case, the maximum value of D is $p(1 - q)$, and it is convenient to replace D by Lewontin's (1964) D', defined to be the ratio of D to its maximum possible value. Substituting for D, we find that

$$q' - q = \frac{1 - q}{1 - p} D'(p' - p) \cong D'(p' - p) \quad . \tag{5}$$

The proportional increase in the frequency of *B* decreases with D'. If *A* arose as a mutant on a *B* chromosome, then $D' = 1$ initially. The expected value of D' decays exponentially with time: $D' = e^{-cg}$, where c is the recombination rate between the loci and g is the time in generations.

Figure 4 shows the dependence of the power of test 1 on q, for one of the sets of parameter values used for figure 2, assuming $D' = .95$. We can see that, when $q \leq .1$, P_1 is reduced by almost a factor of 2 but that, for much smaller values, there is a much smaller effect on P_1. This is the pattern found in other numerical results as well. Changing the value of D' has a similar effect, which can be anticipated from equation (5).

We can also test for heterogeneity within the selected sample. In this case, the expected value of $\bar{x}$ is given not by equation (2) but, instead, by a mixture of equations (2) and (3) weighted by the fractions of *Bb* individuals that are *Aa* and *aa*. Similarly, $\bar{y}$ is given by a mixture of equations (2) and (3) weighted by the fractions of *bb* individuals that are *Aa* and *aa*. The loss of power results from the incorrect identification of *Bb* individuals as *Aa* individuals and from the incorrect identification of *bb* individuals as *aa* individuals. The expected fraction of *bb* individuals who are actually *Aa* is approximately $2p - D$, which is so small that to a first approximation it can be ignored. The expected fraction of *Bb* individuals who are actually *aa* is approximately $1 - pD'/q$. Even if $D' = 1$, the difference between the frequencies

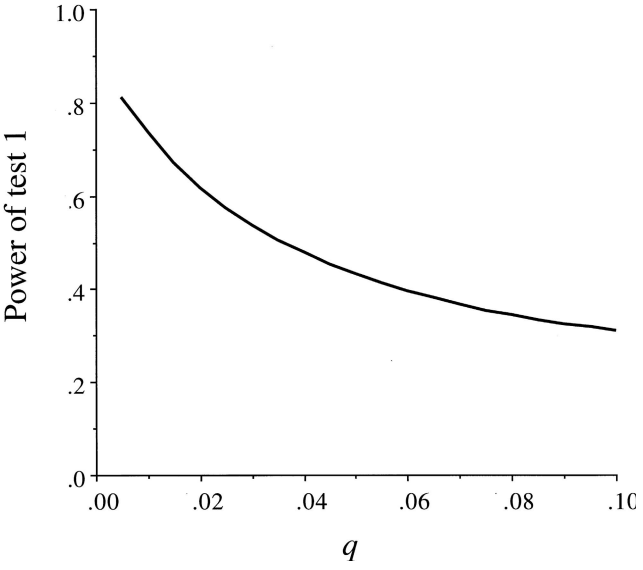

Figure 4 Graphs of the power of test 1 (the test for significant differences, between the population sample and the selected sample, in the numbers of *Aa* heterozygotes). The method for obtaining these results is the same as that described in the legend to figure 2, except that p and p' are replaced by q and q', the frequencies of *B*, a marker allele linked to *A*, in the population sample and the selected sample. The results shown are for $\epsilon = 1$, $\beta = 2$, $D' = .95$, and $p = .005$.

of B and A causes some Bb individuals to be aa. The effect on test 2 is roughly equivalent to the multiplication of ϵ by $1 - pD'/q$. Therefore, we can expect the results of test 2 to be more sensitive to differences between p and q than is test 1.

Simulation Test for a Marker Locus

The simulation program for a candidate locus was modified to allow for a marker locus. The two additional parameters of the simulation model are q and D'. The same four tests were applied. Table 2 shows results related to one of the table 1 cases that was of particular importance because it showed the potential utility of combining the results of tests 1 and 2. As expected, the performance of every test deteriorates as q increases and D' decreases. Also as expected, test 2 is more sensitive to changes in the value of D' than is test 1. The performance of the t-test is sensitive to changes in either q or D', because it depends on the correct identification of the few Bb individuals in the population sample as being Aa individuals. Even a single incorrect identification can affect the result. In these simulations, $D' = .95$ corresponds to a recombination rate of $c = -\ln(.95)/g$, or $c = .0005$ for a population founded 100 generations in the past, and $D' = .90$ corresponds to $c = .001$.

A Measure of Marker Association with a Quantitative Character

The statistical tests described in the preceding sections provide a way to test for the presence of a QTL. If a QTL is in a chromosomal region, then equation (4) suggests an appropriate measure of association between a marker allele and an allele of large effect at a QTL. Given the criterion for selection of the sample, $w(x)$, the quantity γ, defined by $\gamma = [(q' - q)/(1 - q)]$, is analogous to the quantity δ defined by Bengtsson and Thomson (1981), which Devlin and Risch (1995) showed to be useful in the context of disequilibrium mapping. Equation (5) tells us that $\gamma \cong D'(p' - p)$, and hence γ depends on both D' between the marker and the QTL and the extent to which selection function increases the frequency of A. If genotypes of several marker loci in the vicinity of the hypothesized QTL can be surveyed, γ would be expected to be largest for the locus or loci most closely linked. The maximum value of γ would depend on $w(x)$, but the relative values would depend on only D'.

The statistical problems associated with practical application of γ to a data set with one or more linked marker loci are very similar to the problems of disequilibrium mapping of a dichotomous trait. With a single marker, the estimation of recombination rates by use of maximum likelihood is relatively straightforward (Rannala and Slatkin 1998). The only addition necessary for the use of maximum likelihood for the mapping of the location of a QTL is a step accounting for the randomness in selection of the sample. At the present time, the simultaneous incorporation of information from several marker loci is difficult, and a complete maximum-likelihood method for a dichotomous trait is not yet available.

Because marker alleles of interest are at low frequency, resolution of multilocus genotypes into haplotypes is not necessary. Low-frequency marker alleles that will be informative for LD mapping would have to be in strong LD with each other and with A, because of their presence on the ancestral A chromosome. The lack of strong association should be apparent from low values of γ. Of course, having haplotypes instead of genotypes at marker loci allows the detection of shared chromosomal segments associated with the QTL, as was tested for by Laitinen et al. (1997), but resolution of haplotypes requires considerable additional effort and may not be possible in all cases. The statistical tests and the measure of association described here do not depend on knowledge about the haplotypes. Finding suitable low-frequency marker alleles may be quite difficult and might well be the limiting factor for the utility of the methods described in this article.

Table 2

Results of Simulations of Samples Genotyped at a Marker Locus Linked to a QTL

Value of Parameter				No. of Replicates Significant at 5% Level, for[a]			
q	D'	ϵ	β	P_1	P_2	P_3	P_t[b]
.01	1.0	1.0	2	65	48	81	55
.02	1.0	1.0	2	55	54	74	44
.02	.95	1.0	2	45	52	71	33
.02	.90	1.0	2	32	34	52	41
.02	.95	1.0	3	97	48	96	33
.02	.90	1.0	3	94	44	99	41
.02	.85	1.0	2	32	37	63	37
.02	.50	1.0	2	13	16	22	22
.02	.25	1.0	2	11	8	14	11
.03	.90	1.0	2	26	46	58	33
.03	.95	1.0	2	38	41	63	34

[a] Data are no. of replicates for which the null hypothesis could be rejected at the 5% level. In all cases, 100 replicates were run, $p = .01$, $n_1 = 50$, and $n_2 = 200$.

[b] The t-test was done on a population sample of size $n_1 + n_2$. Since the results of the t-test do not depend on β, they are the same for cases that differ only in the value of β.

Conclusion

The analysis in this article is intended to show that the detection and disequilibrium mapping of a rare allele of relatively large effect at a QTL is possible if appro-

priate variation is present in populations that have grown rapidly from a small founder population. The results presented here show that it is possible to detect the presence of an allele at a QTL that would account for as little as 25% of the genetic variance of a trait ($\epsilon = 1$) if it reached a frequency of .5—and that, under many conditions, there is some chance of detection of a rare allele with a much smaller effect ($\epsilon = .5$). The conditions for successful mapping are the same as those for the successful mapping of a locus affecting a dichotomous trait: a single copy of the allele was present in the ancestral population and was closely linked to a low-frequency allele at a marker locus. Rapid growth of the population after the founding event ensures that the effect of genetic drift is minimal and that the LD signal is clear.

Whether genetic variants suitable for LD mapping exist for many quantitative characters is currently unknown. Alleles of relatively large effect have been found in many studies of QTLs, but methods for detection of QTLs are biased toward the finding of alleles of large effect. At present, it is an open question whether such alleles are typical for an arbitrarily chosen quantitative character. Some models of the maintenance of variation of quantitative characters (Lynch and Walsh 1998, chap. 12) assume the presence of many such low-frequency alleles, so, if those models are valid, the conditions for successful LD mapping of QTLs might well be common. Because of their relatively low frequency, alleles of large effect would not announce themselves by means of obvious deviations from a normal distribution, although their presence might be suspected if very large or very small values of the character are associated with a disease that is present at unusually high frequency in the population of interest.

The approach described here assumes that measurement of the quantitative character involves much less effort than characterization of genotypes does. In that case, the selection of a sample of individuals with unusually large or unusually small values for the character chosen for genetic analysis is more efficient than the genotyping of all individuals in the study population. The theoretical analysis shows that, all else being equal, the more strongly selected a sample is, the more likely it is to detect the presence of a low-frequency allele of large effect; but the caution suggested by Lander and Botstein (1989) should be borne in mind. Extreme outliers may be so for nongenetic reasons. On the other hand, if measurement of the quantitative character is more difficult than characterization of genotypes, then every individual measured for the character should be genotyped. The simulation analysis in table 2 shows that relatively large sample sizes are needed in order to find evidence for the presence of a low-frequency allele of large effect. Selective genotyping increases the frequency of rare alleles sufficiently that they are much more easily detected, and very strong selection ensures that such an allele will be detected with high probability if it is present in the genome region examined.

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Appendix

Sample Calculation of Tests 1–3

The sample sizes were chosen for illustrative purposes and are too small for asymptotic theory to apply. Note that the values of the character in the population sample do not affect the results.

Population sample:

2 *Bb* individuals and 8 *bb* individuals; $n_1 = 10$, $i = 1$, $j = 8$

Selected sample:

x_i (*bb* individuals), 2.33, 3.14, 2.03, 2.19; y_i (*Bb* individuals), 2.04, 2.29, 3.47, 2.76, 2.16, 3.02; $k = 4$, $m = 6$, $n_2 = 10$

Test 1:

χ^2 value for 2×2 contingency table $(i,j,k,m) = 10/3$, which implies $P_1 = .0688$

Test 2:

$\bar{x} = 2.422$, $\bar{y} = 2.623$, $V_x = .247$, $V_y = .314$, $z = .595$, which implies $P_2 = .276$

Test 3:

$v = -2\ln(P_1P_2) = 7.942$, which implies $P_3 = .0937$

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