

Letter to the Editor

Orr's Quantitative Trait Loci Sign Test Under Conditions of Trait Ascertainment

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ORR (1998) presents two statistical tests for testing whether a quantitative trait has evolved neutrally. His quantitative trait loci (QTL) sign test (QTLST) rejects the null hypothesis of neutrality if the number of QTL acting in a positive direction is improbably high given the magnitude of the trait difference between the two lines studied. His second test, the QTL sign test with equal effects (QTLST-EE), rejects neutrality when the number of QTL acting in a positive direction is improbably high given only that the trait difference is positive between the two lines. Orr points out that, although the QTLST-EE is not as biologically realistic as the QTLST, it is still preferable to a simple sign test. However, the statistical properties of the QTLST-EE are not clear from ORR (1998). The recent publication of a report (RIESEBERG *et al.* 2002) applying the QTLST-EE to numerous traits and rejecting the neutral evolution hypothesis for many of them prompted us to investigate the statistical behavior of the QTLST and QTLST-EE. Of particular concern is whether the type I error rate of either test is greater than the reported P value when the test is applied to traits that are particularly divergent between the "high" and the "low" lines. This is important because, in practice, the traits to which the test is applied tend to be more divergent than average. Such traits are said to have been "ascertained" for study. The actual ascertainment process is typically unknown; nonetheless, it is possible to simulate traits under simple ascertainment schemes. Applying the QTLST and the QTLST-EE to these simulated traits provides insight into how robust the tests are to ascertainment bias.

We simulated traits under a simple ascertainment scheme in which the traits selected for study were the maximally different of T identically and independently distributed traits. Larger values of T correspond to more extreme ascertainment of traits. Applying the two tests to these simulated traits, we find that the QTLST is conservative—the risk of falsely rejecting the null hypothesis using the QTLST is lower than the reported P

value. Further, the QTLST remains conservative even when T is as high as 50. However, our results indicate that the QTLST-EE does not enjoy this insensitivity to the ascertainment scheme and may lead one to regularly reject the hypothesis of neutral evolution of an ascertained trait, even when that hypothesis is true.

We first simulated traits under the neutral evolution model assumed for the QTLST with $n = 10$ loci and assuming that there was no threshold for detection of the effect of a locus. Under this model, the t th simulated trait ($t = 1, \dots, T$) has trait difference $R_t = \sum_{i=1}^n 2\delta_i G_i$, where each G_i is an independent realization from an exponential distribution with mean 1, each δ_i is independently 0 or 1 with probability 1/2, and only the positive values of R_t are considered. The maximal value of R_1, \dots, R_T was chosen, and the QTLST was performed on the number of plus factors ($n_{\text{obs}}^+ = \sum_{i=1}^n \delta_i$) associated with that maximal value, assuming that the shape and scale parameter were known to both be 1 and that there was no threshold for QTL detection. The test was implemented using the software described in ORR (1998).

This testing procedure was completed 1000 times for each of four different values of $T = 1, 10, 25, 50$, and the resulting P values of the test were recorded. The results are shown in the plots of R vs. P value in Figure 1. In the figure, a dotted line appears at the P value of 0.05. For a test of size 0.05 one would expect 50 of 1000 data sets simulated under the null hypothesis to fall beneath this line. For all levels of ascertainment, far fewer of the data sets are rejected: 2 for $T = 2$, 8 for $T = 10$, 10 for $T = 25$, and 12 for $T = 50$. Thus, while increasing the ascertainment intensity does increase the type I error rate, the error rate still remains much smaller than the nominal P value. The QTLST, at least when the shape and scale parameter of the effect size distribution are known, appears to be conservative and is little affected by trait ascertainment.

We performed similar simulations and applied the QTLST-EE to them. With 10 loci, if $n_{\text{obs}}^+ \geq 9$, then the QTLST-EE will reject the null hypothesis of neutral evolution at $P = 0.0285$. First, we simulated traits under the assumptions of the QTLST-EE with $n = 10$ loci of equal and arbitrary effect size a . In this case $R_t = 2a\sum_{i=1}^n \delta_i$,

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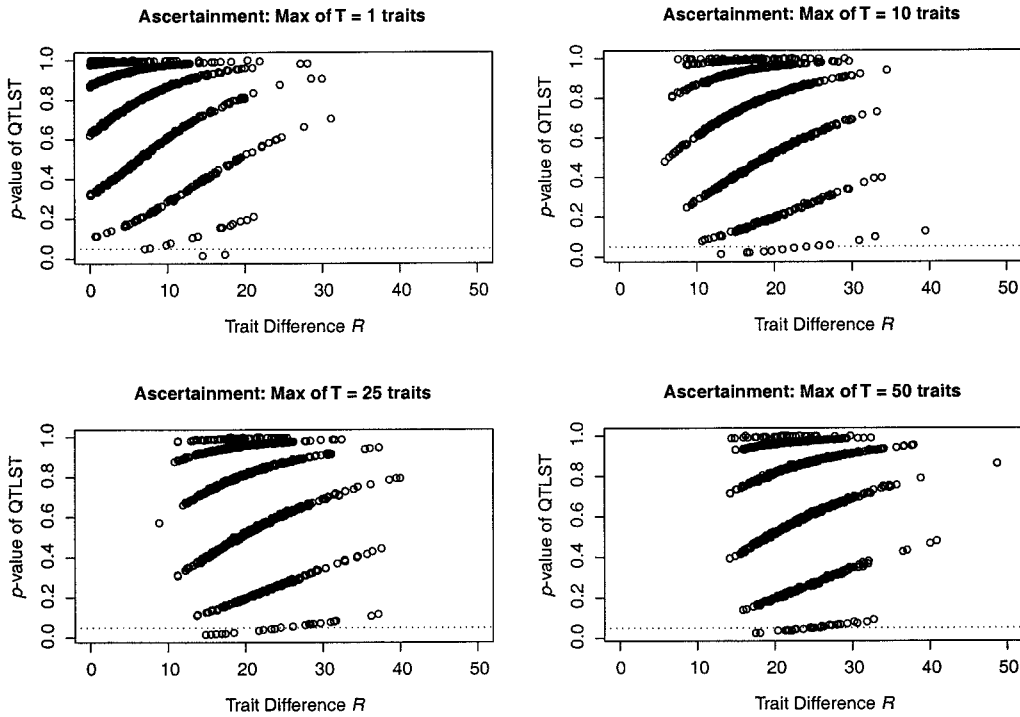


FIGURE 1.—Plots of R vs. P value for 1000 ascertained traits. Each circle represents a trait simulated as described in the text. Each distinct curve upon which the points fall is composed of traits having the same value of n_{obs}^+ , starting from 10 at the bottom and growing smaller for curves higher in the graph.

when the sum is >0 , and, once again, ascertainment was imposed by applying the QTLST-EE to the trait that was the greatest of R_1, \dots, R_T for $T = 1, 10, 25$, and 50 . We performed this test 10,000 times for each value of T . The results appear in Table 1. It is clear that ascertainment of traits leads to a type 1 error rate much higher than the reported P value of the test. For example, with $T = 25$, the rate of rejection of the null hypothesis is $>50\%$, but the reported P value of the test is 0.0285. The final column of Table 1 gives the type 1 error rate of the QTLST-EE when the data are simulated not under the equal-effects model, but under the model in which locus effects are exponentially distributed as described above. Here, the effect of ascertainment bias is not as great, but is still considerable. At $T = 25$ the type 1 error rate is 0.1570—still 5.5 times higher than the reported P value of 0.0285.

TABLE 1

Monte Carlo estimates of type 1 error rate α for the QTLST-EE under different ascertainment intensities, T , and a nominal P value of 0.0285

T	α under EEM	α under EXP
1	0.0276	0.0187
10	0.2577	0.1032
25	0.5219	0.1570
50	0.7629	0.2013

Data were simulated under either the equal-effects model (EEM) or a model in which the effect of each locus was drawn from an exponential distribution with mean 1 (EXP). Estimates are based on 10,000 simulated replicates.

The process by which traits come to be studied by quantitative genetics techniques is complex, involving psychological and historical factors that we may never hope to model explicitly. However, it seems evident that traits selected for study are not a random sample of all possible traits that one could investigate. Rather, in the search for QTL, investigators will focus on traits that show marked differences between lines. In this way, our simple ascertainment simulations mimic reality—when one considers the many traits available for study, ascertainment of the greatest trait difference of 50 traits does not seem extreme.

From our simulations, it appears that the QTLST, by conditioning upon a trait difference of R or greater, appropriately controls for trait ascertainment. It is clear, however, that the QTLST-EE is not robust to trait ascertainment. Accordingly, any conclusions from the QTLST-EE regarding the neutral evolution hypothesis must be regarded with caution.

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