The Power and Deceit of QTL Experiments: Lessons from Comparative QTL Studies

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INTRODUCTION

Quantitative genetic theory has been developed on the premise that the variability in expression of a quantitative trait is the net result of multiple segregating genetic loci interacting with the environment, (Fisher, 1918). Plant breeders, trained in classical quantitative genetic theory, tend to classify agronomically important traits as simple, oligogenic, or polygenic. Such a classification scheme is based on the perceived numbers and magnitudes of segregating genetic factors affecting the expression of the trait. The waxy and shrunken phenotypes of maize grain exhibit Mendelian inheritance with major effects that are "simple" to classify. Most disease and insect resistance traits are perceived as oligogenic, (three to five loci) with fairly large genetic effects at each locus (Melchinger, 1989). Traits related to plant stature, maturity and yield are perceived as polygenic, (>5 loci) with relatively small genetic effects at each locus. Furthermore, there is a tendency to distinguish among polygenic traits. For example, the number of genetic loci thought to be involved with the variability of plant height from a cross of adapted germplasm is generally regarded as being five to ten, whereas the number of genetic loci affecting variability in grain yield (Mg/ha) could be more than 20. Although, yield is thought to have many more genes with smaller effects than plant height, most of the evidence is anecdotal. There is little empirical evidence for the number of genetic loci affecting most quantitatively expressed traits in plant species. It can be shown that a normal distribution of phenotypes

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in a segregating population can be the result of as few as three independently segregating loci (Thoday and Thompson, 1976). Most biometric techniques designed to estimate the number of underlying genetic factors responsible for the variability of a continuously expressed trait require population structures and sizes that are seldom used by plant breeders (Zeng, 1992).

The development of polymorphic genetic markers made it possible for plant breeders and geneticists to investigate what Edwards et al. (1987) referred to as the numbers, magnitudes and distributions of quantitative trait loci (QTL). Anticipating the development of such genetic markers, Soller et al, (1976) showed that if our assumptions about the magnitude of genetic effects underlying quantitative traits were correct, then it would take about 1000 progeny to have even odds of detecting these QTL. Based on such theoretical considerations, QTL studies that used isozyme markers evaluated from 1500 to 1800 F_2 progeny (Edwards et al., 1987; Weller, 1987). These studies were able to detect QTL with small genetic effects, but also detected QTL with larger estimated effects that could have been identified with fewer progeny (Edwards et al., 1992).

Since 1987, molecular genetic markers (e.g., RFLPs) that span the genome have been developed and these have been used to identify plant height and yield QTL with as few as a hundred progeny. Due to it's economic importance, many of the maize QTL studies have been conducted by commerical seed companies. Although the results of many of these studies are not generally known, studies that Charlie Stuber has reported at this conference (1992, 1994) indicate that grain yield can be improved through identification and selection of a few QTL. While these results are very encouraging for the development and application of molecular markers, the results of the reported QTL studies appear to challenge the conventional wisdom concerning polygenic quantitative traits. In most reported studies, less than ten QTL with relatively large estimated genetic effects have been identified for all quantitative traits. Two traits that have been routinely studied and reported in maize QTL experiments are plant height and grain yield, (Beavis et al., 1991; Edwards et al., 1992; Stuber et al., 1992; Stuber and Sisco, 1991; Veldboom et al., 1992; Schon et al., 1994; Beavis et al., 1994), and a comparison of the results from these studies can be useful to derive inferences about the quantitative genetics underlying these complex traits.

COMPARISONS OF PLANT HEIGHT AND YIELD QTL

The estimated numbers and magnitudes of genetic effects from reported plant height QTL are summarized in Table 1. The number of progeny evaluated in these experiments has ranged from about 100 to 400 and the types of progeny evaluated were F_2 derived lines evaluated *per se*, backcrossed to the inbred parents, and topcrossed to inbred testers. The estimated number of QTL identified ranged from three to seven and the estimated magnitude

Table 1—Estimated numbers and magnitudes for plant height QTL in maize

Population[1]	Number of Progeny	Number of QTL	Magnitude of Effects[2]	
			Minimum	Maximum
B73xMo17($F_{2:4}$)[a]	112	6	12	23
B73xG35($F_{2:4}$)[a]	112	4	11	24
K05xW65($F_{2:4}$)[a]	144	3	12	23
J90xV94($F_{2:4}$)[a]	144	3	17	25
C0159xTx303(F_2)[b]	187	4	12	27
B73xMo17($F_{2:3}$)xB73[c]	264	4	4	21
B73xMo17($F_{2:3}$)xMo17[c]	264	6	6	12
B73xMo17($F_{2:3}$)[d]	100	5	13	30
Mo17xH99($F_{2:3}$)[e]	150	5	6	40
B73xMo17($F_{2:4}$)xV78[f]	112	5	6	14
KW1265xD146($F_{2:3}$)xKW4115[g]	380	7	4	17
KW1265xD146($F_{2:3}$)xKW5361[g]	380	4	5	32

[1]Population indicated by the original parents and type of progeny in which the traits were evaluated:
a. Beavis et al., 1991.
b. Edwards et al., 1992.
c. Stuber et al., (personal communication).
d. Beavis, Hallauer and Lee (unpublished).
e. Veldboom et al., 1994.
f. Beavis et al., 1994.
g. Schon et al., 1994.
[2]Magnitude of QTL effects are reported as the minimum and maximum percent of phenotypic variability explained by the significant QTL.

of the genetic effects, as expressed by the amount of phenotypic variability among the progeny explained by any one of the QTL ranged from about 5% to almost 40%, although the maximum for most of the studies is about 25%. A feature of these studies not shown in this summary table is the distribution of the estimated magnitude of the genetic effects. In most of the studies the distribution is characterized by one locus with a large estimated genetic effect and several additional QTL that explained a relatively small amount of the phenotypic variability.

The estimated numbers and magnitudes of genetic effects for yield QTL are summarized in Table 2. The numbers of progeny used to evaluate the grain yield were in the range of 100 to 250 and the types of progeny included F_2 derived lines evaluated *per se*, backcrossed to the inbred parents or top-crossed to an inbred tester. The estimated number of QTL identified ranged from two to eight and the estimated magnitude of the genetic effects for any single QTL ranged from 5 to 25% of the phenotypic variability. These values are essentially the same as those reported for plant height. As with the plant height QTL the distribution of the estimated genetic effects for yield QTL in all of the studies is characterized by one locus with large estimated effects

Table 2—Estimated numbers and magnitudes of grain yield QTL in maize

Population[1]	Number of Progeny	Number of QTL	Magnitude of Effects[2]	
			Minimum	Maximum
Oh43xTx303($F_{2:3}$)xB73[a]	216	6	NR[3]	NR[3]
Oh43xTx303($F_{2:3}$)xMo17[a]	216	6	NR[3]	NR[3]
C0159xTx303(F_2)[b]	187	3	6	17
B73xMo17($F_{2:3}$)xB73[c]	264	6	6	18
B73xMo17($F_{2:3}$)xMo17[c]	264	8	6	14
B73xMo17($F_{2:3}$)xV78[d]	112	2	9	13
B73xMo17($F_{2:4}$)[d]	112	5	8	23
B73xMo17($F_{2:3}$)[e]	100	5	8	21

[1]Population is described by the inbred parents used to derive the progeny and the type of progeny that were evaluated:
a. Stuber and Sisco, 1991.
b. Edwards et al., 1992.
c. Stuber et al., 1992.
d. Beavis et al., 1994.
e. Beavis, Hallauer and Lee (unpublished).
[2]Magnitude of QTL effects are reported as the minimum and maximum percentages of phenotypic variability explained by significant QTL.
[3]NR = Not Reported.

and several additional QTL that explained relatively smaller amounts of the phenotypic variability (data not shown).

The estimated numbers, magnitudes and distribution of genetic effects are similar for both traits and were not related to the germplasm or types of progeny that were evaluated. Interestingly, the estimates of numbers, magnitudes and distribution of plant height and yield QTL are similar to the estimates based on QTL analyses of flowering morphology identified using progeny from crosses of maize by teosinte (Doebley et al., 1990; Doebley and Stec, 1991). Are the results of QTL studies an artifact of the experiments or are there really fewer QTL involved in the expression of yield than we previously thought?

Beavis et al. (1991) compared the genomic sites for plant height QTL from four populations (Table 1) that were evaluated with about 100—150 F_2 derived lines *per se* and found that no QTL mapped to the same site in all four populations. However, the QTL did show congruency with many of the 27 loci with genes known to have major effects on plant height in maize. They proposed that the most likely explanation for the lack of congruency among populations was that different sets of polymorphic alleles were segregating in the different genetic backgrounds.

In order to remove the confounding aspect of genetic background, a comparison to consider is one where QTL were identified in the same genetic background. Beavis et al. (1994) recently reported that yield QTL did not

map to the same genomic sites as the yield QTL identified and reported by Stuber et al. (1992). Although both studies used the same data analysis techniques on progeny from the same genetic background, there are still a number of confounding aspects with the comparison: First, different sets of genetic marker loci were used in each of the studies. Thus, relative placement of QTL within linkage groups from the two studies was tentative. However, most of the differences were among linkage groups, rather than placement within linkage groups. Second, the sources of the parental lines used to generate the populations were not the same, so there might have been different sets of QTL with segregating alleles. Third, different samples of progeny were evaluated in different genetic backgrounds. Stuber et al. (1992) identified QTL with 264 $F_{2:3}$ progeny backcrossed to the parents, referred to herein as the NCS progeny. Beavis et al. (1994) identified QTL using 112 $F_{2:4}$ progeny evaluated *per se*, referred to herein as the PHI progeny. Because the progeny had very different types of genomic background, it is possible that the different sets of identified QTL were a manifestation of espistatic and/or epigenetic factors that influenced the expression of the QTL. Fourth, the NCS and PHI progeny were evaluated in different sets of environments, although there was little evidence for QTL × environment interaction effects within either study (Stuber et al., 1992; Beavis and Keim, 1995).

In order to investigate some of the confounding aspects of the comparison, Arnold Hallauer graciously provided phenotypic data and seed from 100 $F_{2:3}$ lines derived from B73 × Mo17, referred to herein as the ISU progeny. The ISU progeny were evaluated *per se* for yield and plant height at four Central Iowa environments in 1985 and 1986, (Covarrubias-Prieto et al., 1989). Each of the ISU progeny were RFLP-typed using the same 96 RFLP markers used to genotype the PHI lines and QTL were identified using the same data analysis techniques as described in Beavis et al. (1994).

Although the parental sources used to generate the ISU progeny were different from the PHI sources, the RFLP patterns at all 96 markers were the same (data not shown). The estimated numbers, magnitudes and distribution of genetic effects based on the ISU progeny were similar to those identified with the NCS and PHI progeny (Table 1, references d and f, and Table 2, references d and e). However, the estimated genomic sites for plant height and yield QTL were not the same as those identified with the PHI progeny (Table 3), nor do they appear to be the same as those identified with the NCS progeny (Stuber et al., 1992).

The comparison of QTL from the PHI and ISU progeny are based on similar numbers and types of progeny from the same genetic background using the same set of marker loci. It is unlikely that the lack of congruency of identified QTL in the two studies was due to different sets of segregating QTL, but the comparison still has confounded factors. First, the progeny were not evaluated in the same sets of environments. Second, the progeny from the two studies were not evaluated at the same level of inbreeding: The

Table 3—Estimated genomic position and amount of phenotypic variability described by plant height and yield QTL identified in two independent sets of F_2 derived lines (denoted ISU and PHI) from the maize cross B73xMo17

Estimated Genomic Position		Estimated Amount of Phenotypic Variability (%) Explained by the QTL*	
Chromosome	Flanking Markers	ISU Progeny	PHI Progeny
Plant Height (cm) QTL:			
1	php1122/bnl7.21	17	7
1	bnl8.10/php20518	16	—
2	umc131/php20005	—	8
3	bnl8.35/umc10	—	10
3	umc60/bnl6.16	9	—
4	umc42/umc19	—	5
6	umc62/php20599	4	—
8	bnl12.30/bnl10.24	7	—
9	wx1/css1	—	10
10	php15013/php10033	—	12
Yield (Mg/ha) QTL:			
1	umc13/php1122	14	—
1	bnl8.10/php20518	—	8
2	umc34/php10012	26	—
2	umc36/php20622	—	10
3	bnl6.16/umc63	7	—
4	umc31/bnl5.46	—	7
5	bnl6.10/php60012	—	9
6	umc62/php20599	6	—
8	bnl9.11/bnl10.39	13	—
9	php10005/bz1	—	23

*Development and evaluation of plant height and yield QTL for the ISU progeny are described in Covarrubius-Prieto et al. (1989) and the PHI progeny are described in Beavis et al. (1994). The RFLP genotyping and QTL analysis techniques used for both sets of progeny are described in Beavis et al. (1994).

PHI study evaluated $F_{2:4}$ progeny whereas the ISU study evaluated $F_{2:3}$ progeny. Third the two studies used independent samples of F_2 derived progeny.

Although the environments used to evaluate the NCS, PHI and ISU progeny were not the same, multiple environments were used within each of the studies. Stuber et al. (1992) reported that there was little evidence for environmental influence on the identification of QTL using the NCS progeny despite being evaluated in six "diverse environments". Beavis and Keim (1995) reported that most QTL identified with the PHI progeny were consistent across stressful and non-stressful environments, although there was a significant QTL by environment interaction at one of the yield QTL. Finally, there was little evidence for environmental influence on QTL identified with the ISU progeny, (data not shown). Thus, given the consistency of QTL across environments

within these studies, it is unlikely that different test environments were responsible for the lack of congruent QTL among the studies.

The second confounding aspect of the ISU vs PHI comparison is that plant height and yield were evaluated in progeny with different levels of inbreeding. However, before invoking a novel model for gene expression, it is important to consider that these studies used independent samples of F_2 derived progeny from B73 × Mo17 and the lack of congruency may have been an artifact of sampling. This hypothesis was investigated by testing for plant height QTL in an independent sample of 400 $F_{2:3}$ progeny derived from a population of B73 × Mo17 that had been sib-mated for four generations (Covarrubias-Prieto et al., 1989), herein referred to as the SYN4 progeny. All 400 SYN4 progeny were genotyped with 20 RFLP marker loci that previously had been associated with plant height QTL in B73 × Mo17 (Table 3) and were evaluated for plant height at four Central US Cornbelt environments in 1991 and 1992. QTL analyses were conducted using IM with multiple QTL allowed in the model, as described by Beavis et al. (1994), on data from all 400 progeny as well as on four subsets consisting of 100 lines each.

The QTL analyses of all 400 SYN4 progeny identified plant height QTL in association with four of the ten linkage groups where QTL had previously been identified (Table 4). As with previous maize plant height QTL studies that used progeny from B73 × Mo17, no evidence for QTL × environment interactions was detected in this study (data not shown). None of the four QTL were consistently identified among subsets of 100 progeny and the estimated genetic effects of significant QTL were larger in the subsets than in the complete set.

Table 4—Estimated phenotypic variability explained by significant plant height QTL identified using 400 $F_{2:3}$ lines (complete set) and four subsets of 100 $F_{2:3}$ lines from B73 × Mo17 SYN4 population (Covarrubius-Prieto et al., 1989). Lines were evaluated for plant height at four environments in Iowa and Illinois in 1991 and 1992

Chromosome	Flanking Markers	Complete	Subset 1	Subset 2	Subset 3	Subset 4
1	php1122/bnl7.21	3	—	—	—	—
1	bnl8.10/php20518	—	—	—	—	—
2	umc131/php20005	—	—	—	—	—
3	bnl8.35/umc10	4	—	10	8	—
3	umc60/bnl6.16	—	—	—	—	—
4	umc42/umc19	—	—	—	—	—
6	umc62/php20599	—	—	—	—	17
8	bnl12.30/bnl10.24	7	- -	15	13	13
9	wx1/css1	8	17	—	16	23
10	php15013/php10033	—	—	—	—	—

The results of this study (Table 4) affirmed that sampling can have a significant impact on the number of QTL that are identified. That is, experiments that use 100 $F_{2:3}$ progeny identify fewer QTL than experiments conducted with 400 progeny. However, we still do not know how many plant height QTL are segregating in B73 × Mo17. Six previously identified QTL were not identified with these progeny. As additional markers are evaluated in this progeny, it is likely that additional QTL will be identified. How many additional QTL will be identified after the genome is saturated with marker loci? How many QTL would be identified with 1000 progeny?

The results (Table 4) also suggest that the estimated magnitudes of genetic effects are affected by the number of progeny that are evaluated, i.e., experiments that evaluated 100 $F_{2:3}$ progeny provided estimates of genetic effects that were inflated relative to the experiment that used all 400 progeny. If additional QTL are identified as markers are added to the genome, then the simultaneous estimates of genetic affects at the QTL already identified will change (Knapp et al., 1992). Would the estimated genetic effects change further if 1000 progeny were used?

MONTE CARLO SIMULATIONS

One method for gaining insight into to the limitations of experiments is through Monte Carlo simulations. In the context of QTL experiments, the idea is to simulate a set of QTL with known genetic locations and effects in a segregating population and then evaluate if the QTL can be consistently identified among independent samples from the population. The frequency with which simulated QTL are correctly identified provides an indication of the power of the experiment to identify QTL, and the difference between the estimated genetic effects and the simulated genetic effects provides an indication of the bias, or deceit, of the estimates from the experimental results.

Methods. To investigate the information that can be obtained from QTL experiments where the trait is polygenic, I simulated diploid F_2 populations with 10 or 40 QTL underlying the expression of a quantitative trait, (Table 5). All of the simulated genotypic variability in each population was due to equal additive effects at the QTL where all positive effects came from one of the parents. The sum of the additive effects accounted for 30, 65 or 95 percent of the phenotypic variability (heritability) among 100, 500 or 1000 F_2 progeny. The phenotypic values that were assigned to each F_2 were calculated by adding random error, which was normally distributed with mean 0 and a variance determined by the heritability, to the sum of the additive effects. The magnitudes of the genetic effects of each QTL can be represented as the percentage of the phenotypic variability that each contributes. For example, if there are 10 segregating QTL that are responsible for a trait that is 30 percent heritable, then each one contributes three percent to the phenotypic variability (Table 5).

Table 5—Estimates of power and amount of phenotypic variability explained by simulated QTL in a diploid genome consisting of 75 independently segregating linkage groups. Each of ten or 40 QTL with equal additive effects from one of the parents were randomly assigned to the middle of a 20 cM linkage group with marker loci placed at the ends of each linkage group. 200 simulatioins were conducted for each set of simulation conditions, which consisted of ten or 40 QTL with equal additive effects that explained 30, 63 or 95% of the phenotypic variability among 100, 500 or 1000 progeny

Number of Simulated QTL	Number of F_2 Progeny	Heritability*	Power	Average Estimated Phenotypic Variability Explained By QTL*
10	100	30	0.117	8.880
10	100	63	0.327	12.700
10	100	95	0.391	18.680
10	500	30	0.574	4.330
10	500	63	0.864	7.130
10	500	95	0.935	10.100
10	1000	30	0.845	3.020
10	1000	63	0.988	6.310
10	1000	95	0.999	9.500
40	100	30	0.025	15.780
40	100	63	0.044	16.315
40	100	95	0.059	16.525
40	500	30	0.112	3.170
40	500	63	0.294	3.555
40	500	95	0.458	3.945
40	1000	30	0.253	1.460
40	1000	63	0.594	1.975
40	1000	95	0.774	2.555

*Because all simulated QTL had equal additive effects, the amount of phenotypic variability that any one was simulated to explained is equal to the heritability devided by the number of simulated QTL. For example, each of ten QTL were simulated to explain 3% of the variability in a trait that exhibited 30% heritability. Averaged estimates that deviate from this value indicate the average amount of bias obtained from the 200 simulated experiments.

Each QTL was randomly assigned to the middle of an independent linkage group. Because linked QTL can confound the results of QTL analyses, (Haley and Knott, 1992; Martinez and Curnow, 1993), no more than one QTL was assigned to each linkage group. The genome consisted of 75 independent linkage groups, each consisting of 20 recombinants per 100 gametes produced by the F_1 between two inbred parents. Marker loci were assigned to the ends of each linkage group. Thus, the genome consisted of about 1500 cM and was completely and uniformly covered with genetic markers. For each set of experimental conditions consisting of 10 or 40 QTL that explained 30, 63 or 95 percent of the phenotypic variability in 100, 500, or 1000 F_2 progeny, 200 simulations were generated.

Each of these 3600 simulated data sets, consisting of genotypic data at 150 marker loci and phenotypic data for a quantitative trait, were analyzed for QTL using interval mapping (IM), as implemented by MAPMAKER/QTL, v.0.9, (Lincoln and Lander, 1991). The threshold for declaring the presence of a QTL in this simulated genome was determined by applying a permutation test, (Doerge and Churchill, 1994), to 200 of the simulated data sets and choosing a maximum LOD value associated with no more than 25% of the permuted data sets (LOD = 2.5). The power to identify simulated QTL was calculated as the frequency of the simulated QTL that were correctly identified on the linkage group. The magnitudes of the genetic effects of each correctly identified QTL was estimated as the percent of the phenotypic variability explained by the estimated additive and dominance effects at the point on the linkage group with the maximum LOD score.

Results. The power to identify ten simulated QTL that account for 65% of the variability among 100 F_2 progeny was 0.117 (Table 5). In other words, of the 2000 QTL that were generated in the 200 simulations with ten segregating QTL that explained 63% of the phenotypic variability among 100 F_2 progeny, 234 were correctly identified to be on the 20 cM linkage group with a simulated QTL. It was possible to consistently identify virtually all ten independently segregating QTL, but only if they were responsible for at least 65% of the phenotypic variability and 1000 F_2 progeny were used to evaluate the trait (Table 5). If 40 independent QTL were segregating in the population, then it was not possible to consistently identify all of the QTL even if the heritability among 1000 progeny was 95%. Most QTL experiments reported to date have been conducted with 100 to 200 progeny in replicated tests where the estimated heritability has been in the range of 60 to 90%. Interpolating from the results in these simulations, the expectation is to identify three to six QTL if there are 10 independently segregating QTL in 100 to 200 F_2 progeny responsible for the expression of a trait that exhibits 65 to 95 percent heritability. On the other hand, if there are 40 independently segregating QTL in 100 to 200 F_2 progeny responsible for the expression of a trait that exhibits 65 to 95 percent heritability, then the expectation is to identify two to seven of the QTL in any given experiment. Obviously, inferences about the number of independently segregating plant height and yield QTL cannot be drawn from these experiments.

The averaged estimated magnitudes of genetic effects associated with correctly identified QTL were greatly overestimated if only 100 progeny were evaluated, slightly overestimated if 500 progeny were evaluated and fairly close to the actual magnitude when 1000 progeny were evaluated (Table 5). The bias was due to overestimates of both additive and dominance effects, (data not shown). Recall there were no simulated dominance effects. The distribution of the estimated percent of the phenotypic variability described by each correctly identified QTL from the simulated data sets where the heritability was 65% among 100 progeny indicates that the estimates were

not symmetrically distributed (Fig. 1). Notice that the estimates consist of a few QTL with large estimated effects and most QTL had relatively small estimated effects. This represents the same pattern of estimated genetic effects observed in experimental QTL studies. Of the 654 correctly identified QTL in the 200 simulations with only 10 independently segregating QTL, the most frequent estimates were fairly close to the actual value of about six percent, although a few were estimated to explain as much as 35 percent of the phenotypic variability. On the other hand, when 40 independently segregating QTL were responsible for variability in the trait, then the magnitude of the estimated genetic effects were severely biased for all 352 correctly identified QTL. The distribution of estimated additive and dominance effects associated with these data followed similar patterns (data not shown).

Discussion. Previous reports of simulation studies have investigated the power of IM to identify either one, (van Ooijen, 1992) or as many as six independently segregating QTL, (Carbonell et al., 1992; Carbonell et al., 1993), but to my knowledge, the power of IM to identify polygenic traits, i.e., those with ten or more independently segregating QTL, have not been reported. van Ooijen (1992) evaluated the power of IM to identify a single simulated QTL with additive effects that accounted for one to ten percent of the phenotypic variability in 100, 200 and 400 backcross and F_2 progeny. He reported that the simulated QTL was identified in all 500 simulations if the QTL explained ten percent of the phenotypic variability among 400 progeny. If the QTL explained 1% of the phenotypic variability among 100

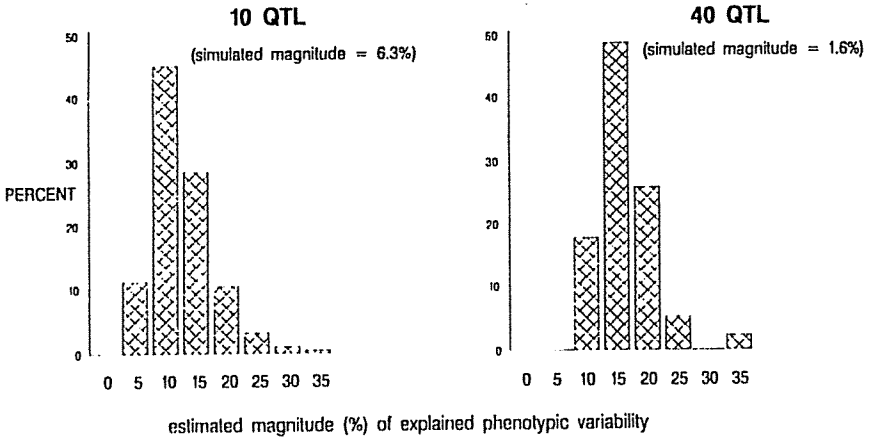

Figure 1. Frequency distribution of the estimated amount of phenotypic variability explained by correctly identified simulated QTL. Each simulated QTL contributed either 6.3 (for 10 QTL) or 1.6 (for 40 QTL) percent of the phenotypic variability in 100 F2 progeny that exhibited a heritability of 63%. Each frequency distribution was obtained from interval mapping of 200 simulated populations.

progeny, then it was correctly identified in less than 2% of 500 simulations. Carbonell et al. (1992) evaluated the power of IM to identify six independently segregating QTL with additive + dominance effects that accounted for 5 to 23% of the phenotypic variability in 250 F_2 progeny. Simulated QTL that accounted for more than 15% of the phenotypic variability were identified in all simulations, but if the simulated additive + dominance effects accounted for 5% of the phenotypic variability, then the QTL were identified in just 60 to 65% of the simulations. Carbonell et al. (1993) reported similar estimates of power for six simulated QTL segregating independently in backcross and doubled haploid progeny.

The results of simulation studies indicates that there is very little power to identify small effect QTL with small numbers (< 500) of progeny, (Table 5), (van Ooijen, 1992; Carbonell et al., 1992; Carbonell et al., 1993; this report). Since Lander and Botsetin (1989) showed that IM improved the power to identify QTL relative to marker trait analysis (Soller et al., 1976), it has been the goal and hope to improve the power through data analysis techniques. Recent focus has been on merging multiple regression and IM techniques (Jansen, 1993; Rodolphe and Lefort, 1993; Zeng, 1993). Based on theoretical considerations, Knapp and Bridges (1990) showed that the power to identify QTL can be improved by using data analyses which accommodate multiple-QTL in the model, and Beavis et al. (1994) reported that the number of identified plant height and yield QTL increased when they used multiple QTL models that were constructed using a stepwise algorithm. However, even with multiple QTL models, there was a lack of congruency in the identified QTL among the NCS, PHI and ISU studies. The challenge with multiple QTL models is that decision rules for including or excluding linkage intervals as "co-factors" in a multiple QTL model are somewhat arbitrary. Inclusion of too many "co-factors" that are not associated with QTL will reduce the power to identify QTL relative to IM (Zeng, 1994). Perhaps heuristic model building algorithms will improve the power to identify QTL for polygenic traits in small populations. I suspect that increased power through modifications in data analysis techniques will have a minor impact on analyses of polygenic traits because the most important factors are primarily a function of the experimental design, i.e., the numbers and types of progeny as well as the field plot designs that are used to evaluate the traits.

van Ooijen (1992) reported no bias in the estimated genetic effects of a single simulated QTL when it was correctly identified, whereas Carbonell et al. (1992; 1993) reported that the estimated genetic effects for all six independently segregating simulated QTL were slightly overestimated. The estimates of the small effect QTL were more biased than the large effect QTL, but none of the estimates were as biased as the estimates that were obtained in my simulations (Table 5). The large biased estimates of genetic effects associated with small numbers of progeny are due to multi-collinearit-

ies in the data that are especially prevalent when there are large numbers of independently segregating QTL responsible for the genetic variability in small numbers of progeny. One way to reduce the bias in the estimates is to use data analyses which accommodate multiple-QTL in the model to obtain simultaneous estimates of all identified QTL (Knapp et al., 1992). The extent to which such models can reduce the bias in the estimated genetic effects when there are large numbers of small effect QTL still needs to be investigated.

Perhaps further refinements in data analyses that use information from segregating "co-factors" (Zeng, 1993; Jansen, 1993) will improve the power to identify QTL and/or reduce the bias in the estimated genetic effects. Further research is needed to quantify the improvements in power and reduction of bias that can be attained with data analyses that account for segregating "co-factors", especially under conditions where there are large numbers of small effect QTL in the genome.

INFERENCES

Monte Carlo simulations of QTL can provide insight into the results of QTL experiments, but great care should be exercised to recognize the limitations of the simulated data so that the limitations of the inferences are also recognized. The QTL that were simulated had equal additive effects with all positive alleles from one of the inbred parents. They were placed in the middle of independently segregating linkage groups that were 20 cM long, and flanked with genetic marker loci. There were no missing genotypic data, nor were there any errors in the genotypic data. Obviously, the genome and QTL that I simulated were designed to optimize the conditions for identification of small effect QTL by IM. Thus, QTL analyses that use experimentally obtained data will be less powerful than the simulated data.

The results of my simulations extend the results of van Ooijen (1992) and Carbonell et al. (1992, 1993) by showing that if there are a large number of small effect QTL segregating in the genome, then the expectation is to identify a few in every experiment. Thus, inferences about the number of QTL underlying the expression of many quantitative traits cannot be drawn from QTL experiments that use small numbers of progeny. These results also provide an explanation for the lack of congruency of identified QTL among the studies that have used small numbers of progeny from B73 × Mo17, (Stuber et al., 1992; Beavis et al., 1994). If there are numerous independently segregating QTL with equal or near equal small effects, then they will all have a similar small probability of being identified in any given experiment. And, if a small proportion of such QTL are identified in one experiment there is little reason to expect the same set of QTL to be identified in other experiments that use independent sets of progeny.

Because unique sets of QTL were identified with the NCS, PHI and ISU sets of progeny, the inference is that there are a large number of QTL

responsible for the expression of both plant height and yield in B73 × Mo17. More precise estimates of the number of QTL may be possible by considering that identification of QTL in independent samples of progeny is analogous to the statistical experiment of estimating the number of balls in an urn through sampling with replacement. In order to adapt the probability distribution function, (pdf) from the balls-in-an-urn experiment, (Darroch, 1958), to the problem of estimating the number of independently segregating QTL, it will be necessary to have low type I error (false positive) rates and have accurate and precise estimates of QTL position. Type I error rates on a genome basis can be kept low through the application of permutation tests (Churchill and Doerge, 1994). Data analysis techniques such as composite interval mapping (Zeng, 1993) will improve the precision of QTL mapping, although the power to identify QTL may be reduced (Zeng, 1994). Precision also can be improved through experimental design by evaluating progeny in which linkage disequilibrium has been relaxed. The primary limitation to precision when using F_2, backcross, or doubled haploid progeny is the low frequency of informative, i.e., recombinant, lines. Furthermore, the use of these types of progeny will set an upper limit on the estimated numbers of QTL (Lande, 1981). Random mated progeny are easy to produce in maize and provide more precise estimates of QTL position (Beavis and Lee, unpublished data).

In simulated populations with a small number of progeny and QTL with equal effects, the estimates of the genetic effects were very biased (Table 5). The estimates also exhibited a skewed distribution (Fig. 1), i.e., a few QTL were estimated to have large effects while most had relatively small estimated effects. Estimates of genetic effects using experimental populations of F_2 progeny exhibit similarly skewed distributions of estimated genetic effects. Because this type of distribution of estimated effects can be obtained from effects that are simulated to be equal, inferences about the relative magnitudes of genetic effects from most experimental studies conducted to date are suspect.

Better estimates of the relative magnitude of genetic effects can be obtained with larger numbers of progeny or perhaps more efficiently through re-sampling strategies. In populations where independent samples of progeny have been used to identify QTL, some QTL have been identified in multiple sets of progeny. For example, the plant height QTL in proximity to the *wx1* locus on chromosome 9 has been identified with several independent samples of about 100 F_2 derived lines (Tables 3, 4 and 5). It seems reasonable to suggest that the magnitude of the genetic effects at this locus is fairly large in B73 × Mo17. It should be possible to adapt the probability density function that underlies the balls in an urn experiment to account for unequal effects, (Chao et al., 1992), and use information from re-sampled populations to estimate not only the numbers of segregating QTL responsible for polygenic

traits, but also obtain less biased estimates of the magnitude of the genetic effects.

In addition to providing inferences about the numbers and magnitudes of QTL the simulation results have implications for plant breeding. The application of molecular markers to plant breeding was originally viewed as augmenting existing breeding strategies, by providing a tool that would increase the realized heritability and/or decrease the resources needed to evaluate the progeny. The greatest gains in efficiency have been shown to be for those traits that exhibit low heritability (Lande and Thompson, 1991), assuming that the QTL can be identified. The paradox is that there is little power to identify QTL in a trait that exhibits low heritability (Table 5), especially if small numbers of progeny are evaluated for the quantitative trait. Most maize breeders prefer to evaluate small numbers of progeny from many breeding crosses. Because different sets of QTL will be identified in different sets of progeny from the same cross, two breeders working with independent sets of progeny from the same cross will select for different arrays of QTL. It should then be possible to combine these unique arrays for further genetic gains. Of course, maize breeders currently do this without the use of molecular markers, but the use of molecular markers may make such pyramiding strategies more efficient and effective. The results of the Monte Carlo simulations also suggest that new breeding paradigms based on evaluation of large numbers of progeny may be necessary to realize the full potential of marker aided selection (Gimelfarb and Lande, 1994).

SUMMARY

The expression of phenotypic variability for most agronomically important traits is quantitative and complex. The development of ubiquitous sets of molecular markers has made it possible to investigate the numbers and magnitudes of the underlying genetic factors, or QTL, responsible for variability in these types of traits. However, the results from several studies which have used the same genetic background, B73 × Mo17, to identify plant height and yield QTL have been inconsistent for both estimated genomic location and magnitude of genetic effects. Based on simulation experiments, the inconsistencies occured because most QTL studies conducted to date have used small numbers of progeny (<500) which are not very powerful if there are a large number of small effect QTL in the genome. Under these conditions only a small fraction of QTL are identified and the estimated genetic effects at these QTL are overestimated. The good news is that most QTL that have been identified are not false, as long as thresholds for test statistics are determined in a statistically responsible manner, and the desirable allele probably has been correctly identified. Thus, it may be possible to obtain accurate estimates of the numbers and magnitudes of QTL for complex traits through resampling strategies. The implication for breeding is that realized genetic gain from marker aided selection for traits such as grain yield will be variable and unpredictable, especially if initial QTL identification is based on small numbers of progeny. There will be some impressive results such as that reported by Stuber (1994), but there also will be disappointing results that may not be reported. Finally, to realize the full potential of molecular markers in breeding for polygenic traits, it may be necessary to develop new breeding methods, rather than augmenting existing methods with marker aided selection.

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How Much Land Can Ten Billion People Spare for Nature?

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JEFFERSON'S IMPERATIVE

After millennia of shying away from wilderness because outlaws, witches and trolls lived there, people in the Seventeenth Century began to change their minds. People—at least Rousseau, Wordsworth and Thoreau—discovered nobility, tranquility and beauty in Nature. In our times, people added justifications of ecological services, biological diversity, and greenhouse gas for preserving Nature. So legislatures proclaim and patrons purchase land for Nature. But will the ten billion people expected in the Twenty-first Century crowd out Nature, anyway?

I fear proclamations and deeds on paper won't keep hungry or unemployed off preserves. Thomas Jefferson went further (Jefferson 1785). Representing the American states in France, he followed the French court to Fontainebleau, where after talking with a poor woman along a path, he wrote James Madison,

> I asked myself what could be the reason that so many should be permitted to beg who are willing to work in a country where there is a very considerable proportion of uncultivated lands? These lands are kept idle mostly for the sake of game. . . .
>
> Whenever there is in any country, uncultivated lands and unemployed poor, it is clear that the laws of property have been so far extended as to violate natural right . . . If we do not [furnish employment after appropriating land], the fundamental right to labour the earth returns to the unemployed. . . .

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