Association mapping in plant populations

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Introduction

The objective of genetic mapping is to identify simply inherited markers in close proximity to genetic factors affecting quantitative traits (Quantitative trait loci, or QTL). This localization relies on processes that create a statistical association between marker and QTL alleles and processes that selectively reduce that association as a function of the marker distance from the QTL. When using crosses between inbred parents to map QTL, we create in the F_1 hybrid complete association between all marker and QTL alleles that derive from the same parent. Recombination in the meioses that lead to doubled haploid, F_2, or recombinant inbred lines reduces the association between a given QTL and markers distant from it. Unfortunately, arriving at these generations of progeny requires relatively few meioses such that even markers that are far from the QTL (e.g., 10 cM) remain strongly associated with it. Such long-distance associations hamper precise localization of the QTL. One approach for fine mapping is to expand the genetic map, for example through the use of advanced intercross lines, such as F_6 or higher generational lines derived by continual generations of outcrossing the F_2 (Darvasi and Soller, 1995). In such lines, sufficient meioses have occurred to reduce disequilibrium between moderately linked markers. When these advance generation lines are created by selfing, the reduction is disequilibrium is not nearly as great as that under random mating.

The central problem with any of the above approaches for fine mapping is the limited number of meioses that have occurred and (in the case of advanced intercross lines) the cost of propagating lines to allow for a sufficient number of meioses. An alternative approach is "association mapping", taking advantage of events that created association in the relatively distant past. Assuming many generations, and therefore meioses, have elapsed since these events, recombination will have removed association between a QTL and any marker not tightly linked to it. Association mapping thus allows for much finer mapping than standard bi-parental cross approaches. In our review of this topic, we first define association quantitatively and describe mechanisms that generate it. To motivate our discussion of rigorous methods to test for marker association with a quantitative trait allele, we then discuss in some detail an example from the plant breeding literature.

Next, we review an analysis frequently used in human genetics to find marker associations with disease susceptibility alleles, the transmission / disequilibrium test (TDT). We touch upon work to extend the TDT to quantitative traits and to identify QTL by environment interactions (QTL x E). We describe recent developments making use of multiple-marker haplotypes to locate QTL and conclude with some points concerning the power of association mapping.

Association between a neutral Mendelian marker and the phenotype

A statistical association between a neutral marker allele and the phenotype occurs when marker alleles are in gametic phase disequilibrium (GPD) with alleles at a QTL. Two alleles at distinct loci are in positive GPD if they occur together more often than predicted on the basis of their individual frequencies. This definition of association says nothing concerning the physical position of the loci or of the alleles' joint effects on the phenotype. The term "gametic phase disequilibrium" is used synonymously with the term "linkage disequilibrium," but we use the former term since it avoids reference to linkage (as unlinked markers can still be in GPD) and emphasizes that associated alleles must co-occur in gametes.

In the example of Table 1, the combination of alleles (or haplotype) QM is observed with frequency $p_{QM} = 0.4$, while its predicted frequency is only $p_Q p_M = 0.3$. The alleles Q and M are in GPD with disequilibrium coefficient $D = p_{QM} - p_Q p_M = \text{cov}(Q,M) = 0.1$. Note that since D can be expressed as a covariance, we can bound its possible values by considering the case when the correlation is +/- 1, giving

$$|D| \leq \sigma_Q \sigma_M = [\, p_Q(1-p_Q)\, p_M(1-p_M)\,]^{1/2} \tag{1}$$

For a pair of diallelic loci, the expected value of the estimate of D is equal in magnitude irrespective of the haplotype frequencies used and can be calculated as $D = p_{QM} p_{qm} - p_{Qm} p_{qM}$. For each generation of random mating, D decays by a factor of $(1-r)$, where r is the recombination rate between the two loci considered. Thus, after t generations, only $(1-r)^t$ of the initial disequilibrium remains.

A variety of mechanisms generate linkage disequilibrium, and several of these can operate simultaneously. Some of the more common mechanisms are:

1. Populations expanding from a small number of founders. The haplotypes present in the founders will be more frequent than expected under equilibrium. Three special cases are noteworthy. First, genetic drift affects GPD by this mechanism in that a population experiencing drift derives from fewer individuals than its present size. Second, by considering an individual with a new mutation as a founder, we see that its descendants will predominantly receive the mutation and loci linked to it in the same phase. Linked marker alleles will therefore be in GPD with the mutant allele. Finally, an extreme case arises in the F_2 population derived from the cross of two inbred lines. Here, all individuals derive from a single F_1 founder genotype and association between loci can be predicted based on there mapping distance (e.g., Lynch and Walsh 1998).

2. Gametic phase disequilibrium arises in structured populations when allelic frequencies differ at two loci across subpopulations, irrespective of the linkage status of the loci. Admixed populations, formed by the union of previously separate populations into a single panmictic one, can be considered a case of a structured population where substructuring has recently ceased.

3. Negative GPD will occur between loci affecting a character in populations under stabilizing or directional selection as a result of the Bulmer effect.

4. Positive GPD will occur between loci affecting a character under disruptive selection.

5. When loci interact epistatically, haplotypes carrying the allelic combination favored by selection will also be at higher-than-expected frequencies.

Effects of population admixture and selection on association: an illustration

Studies to determine association between a marker allele and the phenotype can take two forms. In one form, groups are distinguished on the basis of their divergent phenotypes (diseased vs. healthy; low vs. high trait value) and allele frequencies are compared across groups. Such studies are often referred to as case-control studies in the human genetics literature since they contrast disease-affected individuals (cases) with unaffected (control)

individuals. The second type of study uses groups distinguished on the basis of their marker genotypes, and phenotypic means are compared across groups. An example of this is Beer *et al.* (1997), who analysed 13 quantitative traits on 64 North American oat varieties and landraces grouped according to RFLP genotype at 48 loci. Significant associations between RFLP fragments and group means occurred for 11.2% of fragments when testing at a 1% type I error rate, indicating many more associations than expected by chance alone. Some caution is in order, because (as the authors point out), the observed the marker-trait association does not necessarily imply that markers showing a significant effect on the phenotype are linked to QTL. Rather, the marker-trait disequilibrium may exist in the absence of linkage, and instead may have arisen simply as a consequence of population structure.

A classic example from humans of this population stratification effect is Knowler *et al.* (1988), who examined candidate haplotypes for Type 2 diabetes in members of the Pima and Tohono O'odham tribes of southern Arizona. Individuals with one particular haplotype had only an 8% rate of diabetes, while those lacking this haplotype had a 30% rate of diabetes. However, this particular haplotype is much more common in Caucasian populations than in full-heritage Native American populations. When correcting for this population difference by only considering individuals of full-heritage, 59% of individuals with the haplotype had diabetes, while 60% of the individuals lacking the haplotype had diabetes. In a similar fashion, the marker alleles associated with significantly different trait values observed by Beer *et al.* may have become associated with the phenotype through admixture of genetically divergent populations (for both markers and QTL), or through the effects of selection on both marker frequency and phenotype. In the former case, we can conceptualize the association between marker allele and phenotype as arising from the allele's association with the polygenic effect. If two populations diverge in phenotypic mean and in frequency of a marker allele, then admixture of these populations will create such an association. Under random mating, an unlinked marker allele's association with the phenotypic variance will be divided by four in each generation. Unfortunately, this rule only applies to outbreds that may conceivably random-mate. It will be more difficult to predict the decay of marker association with

phenotype in a germplasm pool of self-pollinators, such as Beer *et al.*'s oat data. One obvious population structure in the Beer *et al.* data is the distinction between spring and winter oat varieties, which differ in both phenotype and in marker frequencies (Souza and Sorrells, 1991). Beer *et al.* did not take these two divergent subpopulations into account in their analysis.

Another potential level of population structuring is a temporal one: Beer *et al.* analysed germplasm spanning about four decades of genetic improvement. Varieties grouped by year of release are expected to differ in mean for traits such as grain yield and harvest index. Under selection, the frequency of favorable QTL alleles at all loci increases and covariances among marker alleles across generations arise. These covariances hamper the estimation of the phenotypic effect associated with any single marker (Kennedy et al., 1992). In effect, we may consider the germplasm pool analysed by Beer *et al.* as an admixture of old and modern subpopulations, the one having undergone less selection than the other. We then would expect to find fewer associations between marker alleles and phenotypes within each subpopulation than in the combined pool. Beer *et al.* performed this analysis and found only 6.5% and 4.9% of allele-trait associations were significant in the subpopulations of old and modern varieties, respectively. Some of the decline in the frequency of significant results would be due to the difference in power between tests on the combined pool versus within each subpopulation. It seems likely, however, that the difference in the results also indicates that the partition of the combined pool into old and modern varieties successfully separates subpopulations that are divergent in both phenotypic mean and in allele frequencies at certain markers.

The obvious weakness of group-comparison studies is that the grouping method may result in groups that contain predominantly individuals from different subpopulations. To eliminate this weakness, family-based control methods seek case and control individuals or marker alleles within the same family.

The transmission / disequilibrium test

The problem of population admixture is ubiquitous in human disease mapping, promoting considerable work to develop unbiased association estimators. Perhaps the most successful is the TDT of Spielman *et al.* (1993) to identify loci contributing to disease susceptibility in humans in the presence of population structure. For outbred species, the test employs family trios consisting of both parents and a progeny that is affected by disease (or, in general, that belongs to one category of a dichotomous trait). One of the parents must be heterozygous and carry one copy of the focal marker allele putatively linked to the disease susceptibility allele. The test consists of determining the frequency of transmission of the focal allele to affected progeny. A chi-square or binomial test can determine whether that frequency deviates from the expectation of 0.5. Two conditions are necessary for a significant deviation: the marker allele must be both in GDP with and also linked to a disease susceptibility allele. In the TDT, both case and control marker alleles are in effect within the same heterozygote parent. Random Mendelian segregation therefore ensures that the distribution of the TDT statistic under the null hypothesis is unaffected by population structure or selection within the pedigree (Spielman and Ewens, 1996).

No TDT tests have been developed for predominantly selfing species. The extension, however, should be straightforward. A selfing TDT could employ marker information on F_1 hybrid / selfed progeny pairs, where the F_1 is heterozygous at a putatively linked marker locus and the progeny is affected. In this situation, transmission frequencies have the same expectations as for the TDT test, even if several generations of selfing occur between F_1 and inbred progeny. If the F_1 itself was not genotyped, its genotype may be inferred from either the known genotypes of its inbred parents or by pooling DNA from a number of its progeny derived by selfing. A potential complication (especially in hybrids) is gametic selection, which can bias transmission ratios. Hence, when using a TDT, one should always also perform a test of equal allelic transmission when phenotypic value is ignored.

While the TDT is always a valid test of linkage, researchers have devoted substantial effort to inferring in what cases the TDT is a valid test of population-wide association (Spielman and Ewens, 1996). In particular, when the family trios used are related, the test may detect association that exists solely in the pedigree from which those families derive but not in the general population (Martin et al., 2000). We view the problem as one of determining the correct inference space for the test result. When the test uses multiple related families the correct inference space for association is the pedigree from which they derive, not the general population. Asserting broader inference would be an example of pseudoreplication (Hurlbert, 1984). Further, while the TDT remains a valid test for linkage, the critical interest in using association mapping is in finding tightly linked markers. A TDT based on multiple related families may detect association based on fairly distant marker-QTL pairs simply because recombination within the confines of the single pedigree evaluated will fail to reduce their association.

Extensions of the TDT to quantitative traits

As developed, the TDT only applies to traits that can be scored as dichotomously in the progeny, though these traits may be influenced by more than one underlying genetic factor. For populations undergoing artificial selection on a quantitative trait, Bink *et al.* (2000) take advantage of the insight that "selected" versus "not selected" constitutes a dichotomous trait. All families with selected progeny are therefore genotyped and the standard TDT is applied to those data. In the case of recurrent selection, the observed families will generally not be independent of each other, related as they are through cycles of intermating. As discussed, care must be taken in determining the inference space for positive association results. Data sets containing genotype information on current and previously released varieties of inbred crops could be analysed using the TDT in this way. Indeed, variety pedigrees are generally known (though some pedigrees may contain errors, e.g., Lorenzen et al., 1995). We can assume that a derived variety was selected from its parental varieties because of its agronomically favorable traits. Thus, a preferentially transmitted marker allele could be inferred to be in GPD with an agronomically favorable QTL allele.

Allison (1997) proposed five extensions of the TDT for quantitative traits. These extensions either compare the means of progeny conditional on whether they received the putatively associated allele, or examine the frequency of inheritance of the allele among progeny whose trait values are above or below specified thresholds. In this latter case, we see that the use of thresholds reduces quantitative traits to dichotomous traits, bringing us back to the standard TDT. Unfortunately, these tests impose restrictive conditions on usable family trios: one heterozygous and one homozygous parent, and only one offspring. In practice, one family may have multiple progeny and / or the parents may lack genotypic data. To gain power from such data, Monks and Kaplan (2000) present a parametric procedure that relaxes family restrictions, allowing families of different types and several progeny per family to be used. The test defines a statistic, T_{MK}, based on the mean crossproduct between the deviation of the progeny phenotype from the population mean and the transmission of the focal marker allele from heterozygous parents. For large sample sizes, T_{MK} is approximately distributed as a unit normal [$T_{MK} \sim N(0,1)$]. To apply the test to small sample sizes or when multiple markers or marker alleles are used, Monks and Kaplan (2000) describe permutation procedures to obtain empirical distributions for T_{MK}. Finally, to account for environmental covariates that affect the quantitative trait of interest, the population mean can be adjusted by regression of the trait on the environmental covariates (Rabinowitz, 1997). A cross product is then calculated using the progeny deviation from this adjusted mean.

While plant geneticists have long been interested in genotype by environment interaction, efforts to account for it within human genetics and in association tests in particular are more recent (Guo, 2000a; Guo, 2000b; Schaid, 1999a). In the standard TDT, QTL x E would lead to environmental influences on the transmission frequency of the focal marker allele from a heterozygotic parent to affected progeny. Such an effect could be detected by grouping family trios according to their environment or level of exposure to a risk factor. Heterogeneity of transmission frequency across groups would provide evidence in favor of QTL x E (Schaid, 1999a). Similarly, for the Monks and Kaplan test, environments would affect the magnitude of T_{MK} in the presence of QTL x E. Existence

of QTL x E could then be inferred if the variance of T_{MK} across environments is significantly greater than zero. In observational studies where environments cannot be randomized across family trios, interpretation of such a result would need to be treated carefully: an association between environments and different subpopulations could also lead to heterogeneity of transmission or of T_{MK} in the absence of QTL x E.

Association mapping with multiple markers

Given data on multiple linked markers, each particular combination, or haplotype, can be considered an allele at a "supralocus." Extensions to the TDT for multiple marker alleles can then be applied to this supralocus (McIntyre et al., 2000; Spielman and Ewens, 1996). A drawback to these methods is that they fail to make full use of all the haplotype information, as some haplotypes are more closely related (i.e., fewer mutational / recombinational steps away) than others. This potentially induces a correlation structure among haplotypes that needs to be considered. Several approaches have been developed to use the full haplotype information to pinpoint more precisely the location of mutations affecting disease status or the value of a quantitative trait. These methods are like typical linkage methods of QTL mapping in that, for specified map locations, they relate identity by descent (IBD) probabilities with phenotypic resemblance among individuals. For this task, however, linkage methods can calculate exact IBD probabilities based on meiotic events recorded in a pedigree. Association methods cannot rely on a recorded pedigree and so use haplotype similarities either to infer IBD probabilities directly or to create cladograms, which can be considered as approximate pedigrees. We describe three approaches.

Templeton and coauthors (Templeton and Sing, 1993; Templeton et al., 1987) use the haplotype marker profiles to construct a cladogram that estimates the evolutionary history and relationships among haplotypes. Assume that a mutation occurred at some point in this history on one branch of the cladogram. Haplotypes along that branch will be IBD for the mutation and distinct from haplotypes along other branches. The branches of the cladogram therefore define nested sets of haplotypes that should have related associations

with the phenotype. Templeton *et al.* (1987) present a nesting algorithm to group haplotypes hierarchically enabling a nested analysis of variance. We note that the cladogram could also be used to define a covariance matrix among haplotype effects that would enable a mixed model analysis of variance to detect QTL on the basis of significant among-haplotype variance. While this approach does not localize the mutation within the set of markers used to define haplotypes, it will increase the power to detect QTL in linkage disequilibrium with those markers.

Meuwissen and Goddard (2000) use an approach to estimate the covariance matrix among haplotype effects that does help predict QTL position within the set of markers. Starting from assumptions concerning the population history since mutation caused polymorphism at the QTL (i.e., effective population size and number of generations since mutation) the algorithm repeatedly simulates haplotype evolution and samples the probability of IBD status across specified categories of identity by state (IBS) among markers within haplotypes. The covariance among haplotype effects is then based on their IBS and its inferred relation to IBD. Since the probability function P(IBD | IBS) depends on QTL location within the set of markers, the assumed QTL location affects the haplotype covariance matrix. A maximum likelihood QTL position is inferred from the covariance matrix most consistent with the observed phenotypes. Note that Meuwissen and Goddard's approach assumes a single (monophyletic) polymorphism at the QTL while Templeton *et al.*'s cladogram approach does not. Finally, the simulation approach may require information about the population history that is unavailable in practice: the population size and possible substructure over time, the age of the mutation, admixture through migration, and selection on the mutation. While Meuwissen and Goddard show that the approach is, within limits, robust to population size and mutation age assumptions, applying the analysis to real (rather than simulated) data would be of interest in testing it.

The two methods discussed above apply to a random sample of individuals chosen independent of their phenotypic value. In mapping human disease, individuals are not sampled randomly, but rather chosen precisely because they are affected. Assume now

that all affected individuals carry a disease susceptibility allele from a mutation that occurred only once in the population. For a hypothesized QTL position, the likelihood problem is now turned on its head: rather than seeking the likelihood of the phenotype measurements given marker haplotypes, we seek the likelihood of observing this sample of marker haplotypes given that all individuals share a phenotype. The likelihood of the haplotypes depends on their genealogy, which cannot be known. In this situation, a marginal likelihood is calculated by averaging likelihoods over different possible genealogies, weighted by the genealogy probabilities (Graham and Thompson, 1998; Rannala and Slatkin, 1998; Rannala and Slatkin, 2000). This high-dimensional integration over genealogies can be performed using random samples of genealogies. The algorithms to obtain samples use recent developments in the field of coalescent theory (Donnelly and Tavaré, 1995; Hudson, 1993). In essence, coalescent theory considers the haplotypes in a sample to be the tips of the genealogical tree then defines probability distributions for the time in the past when branches were joined through common ancestor haplotypes. These steps are iterated until the whole genealogy coalesces into a single common ancestor. Having defined the coalescent genealogy, different events can be placed along its branches, such as recombination events (Graham and Thompson, 1998) or marker or QTL locus mutations (Zöllner and von Haeseler, 2000). The resultant coalescent represents one possible path generating the currently observed sample. Again, the hypothesized QTL position will affect the distribution of samples obtained through this process and will produce different Monte Carlo estimates of the observed sample likelihood. Differences in likelihood resulting from QTL position then allow for fine-scale disequilibrium mapping using multiple markers (Graham and Thompson, 1998).

Power of association mapping

Risch and Merikangas (1996) discussed power in the context of a complete human genome scan to detect disease susceptibility alleles of relatively small effect. Given their assumptions about the disease genetics, they showed substantial benefits of association mapping over linkage methods available in humans. We here briefly discuss factors that

affect the power of association mapping to detect QTL and reproduce selected guidelines for sample sizes (Monks and Kaplan, 2000; Schaid, 1999b).

The first determinant of power is the magnitude of GDP itself, which depends on mechanisms previously discussed. The different mechanisms also lead to different relationships between GDP and genetic distance (Jorde et al., 1994; Laan and Pääbo, 1997). Ideally, a marker based on polymorphism in the causal locus itself is used, ensuring maximum marker-QTL GDP (Risch and Merikangas, 1996). Either candidate gene approaches or, with improving genotyping capability, exhaustive genome scan approaches, makes this ideal feasible. Even with complete disequilibrium, maximum GDP depends on the frequencies of the marker and QTL alleles, as shown above (Equation 1). Consequently, alleles with a frequency of 0.5 are most easily detected and detection power decreases for more extreme allele frequencies of either marker or QTL. These considerations further indicate that when multiple alleles exist that affect the trait (a condition called genetic heterogeneity), association mapping loses power (Ott, 1999, p. 290-291). A more subtle consequence of Equation (1) is that a marker more distant from a QTL may actually display a higher level of disequilibrium than a more closely linked marker.

A second determinant of power is the effect size of the QTL allele. In human disease mapping, a joint measure of recombination fraction between marker and QTL, and QTL effect size is given by the genotype relative risk (GRR) of disease for different marker genotype classes (Schaid and Sommer, 1993). Considering marker classes mm, mM, and MM, $GRR_1 = P(disease \mid mM) / P(disease \mid mm)$, and $GRR_2 = P(disease \mid MM) / P(disease \mid mm)$. Higher GRR indicate larger QTL effect size. Under a multiplicative model of gene mode of action where $GRR_2 = [GRR_1]^2$, disease susceptibility loci where $GRR_1 = 2$ are considered to have relatively small effects (Risch and Merikangas, 1996). For comparison to quantitative traits, these GRR are similar to the relative "risk" for individuals carrying a favorable allele of being selected under an intensity of 20% when the marker is in complete disequilibrium with an additive QTL that explains 10% of the phenotypic variance and $P_q = P_Q = 0.5$.

Finally, the mode of gene action (dominant, recessive, additive, or multiplicative) greatly influences the power of QTL detection (Schaid, 1999b). Mode of gene action determines the relationship between GRR_1 and GRR_2 as follows: dominant $GRR_1 = GRR_2$; recessive $GRR_1 = 1$; additive $GRR_2 = 2GRR_1-1$; multiplicative $GRR_2 =[GRR_1]^2$. The TDT makes no assumptions concerning this mode of action when it looks at the transmission of marker alleles to affected offspring. But because only affected offspring are sampled, the distribution of marker genotypes that they carry depends on the GRR. Consequently, the power of the TDT will depend on those GRR (Schaid, 1999b). Likelihood methods to analyse TDT data that are general across modes of gene action or specific to an assumed mode and that are more powerful than the TDT were presented by Schaid and Sommer (1993; 1994). Tables 2 and 3 reproduce results from Schaid (1999b) and Monks and Kaplan (2000) to provide a general idea of the sample sizes required to detect with 80% power QTL that affect disease susceptibility or a quantitative trait measured on a continuous scale.

Final remarks

The reader will no doubt notice the heavy influence of human genetics in much of the above discussion of association mapping. Plant breeders will do well in the future to continue to follow the human genetics literature for continued developments and refinements. As stressed by Walsh (this volume), it behooves all practitioners of quantitative geneticists to follow developments in other sub-fields outside of their own.

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Table 1. Haplotype, and marginal
marker and QTL frequencies

Marker allele	QTL allele Q	q	
M	0.4	0.2	0.6
m	0.1	0.3	0.4
	0.5	0.5	

Table 2. Number of family trios (last two columns) required to obtain 80% power of detecting association with a type I error rate of 5×10^{-8}. This error rate allows for a genome wide scan over the whole human genome. Results from Schaid (1999b).

Mutant allele frequency	Disequilibrium coefficient D	GRR_1	GRR_2	Statistical test	
				TDT	General likelihood
0.01	0.01	2	4	5730	6480
		1	2	3.86×10^7	7.72×10^5
0.10	0.09	2	4	687	776
		1	2	44800	8516
0.50	0.25	2	4	337	381
		1	2	946	710

Table 3. Number of families required to obtain 80% power of detecting association with a type I error rate of 0.01. Multiple offspring may be used per family. The segregating QTL causes 10% of the phenotypic variance and has an additive mode of action. Results from Monks and Kaplan (2000).

Mutant allele frequency	Disequilibrium coefficient D	Number of progeny per family	
		1	5
0.10	0.02	7130	1600
	0.05	1810	404
0.50	0.10	808	182
	0.25	202	45