File S1

Supplemental Material: A Simulation Study of Permutation, Bootstrap and Gene Dropping for Assessing Statistical Significance in the Case of Unequal Relatedness

This supplement contains a number of sections that are meant as reference material that extends on the level of detail provided in the main text. It is not designed to be read from beginning to end and does not conform to a narrative format in the way a journal article might.

Statistical Model

A typical genetic model for mapping a diploid population with alleles A and a at a locus is as follows

$$y_i = \boldsymbol{x}_i{}'\boldsymbol{\beta} + x_i^* a^* + z_i^* d^* + u_i + \epsilon_i, \quad i = 1, 2, \cdots, n \tag{1}$$

where y_i is the trait value for the i-th individual, $\boldsymbol{x}_i$ represents covariates (e.g. sex) and $\boldsymbol{\beta}$ are the corresponding effects, x_i^* is $1, 0$ or -1 if the genotype at the putative QTL is AA, Aa or aa and a^* is the additive effect of the putative QTL, z_i^* is 1 if the genotype at the putative QTL is heterozygous or 0 if the genotype is homozygous and d^* is the dominance effect, u_i represents polygenic variation, and ϵ_i denotes the residual effect. Assume that $\epsilon_i \sim N(0, \sigma^2), i = 1, 2, \cdots, n$ are independent, and $\boldsymbol{u} = (u_1, u_2, \cdots, u_n)' \sim N_n(\boldsymbol{0}, \boldsymbol{G})$ with $\boldsymbol{G} = (g_{ij})$ and is independent of $\boldsymbol{\epsilon} = (\epsilon_1, \epsilon_2, \cdots, \epsilon_n)'$. It is known (Jackquard, 1974; Abney et al., 2000) that in general

$$\begin{aligned} g_{ij} &= 2\Phi_{ij}\sigma_a^2 + \Delta_{ij,7}\sigma_d^2 + (4\Delta_{ij,1} + \Delta_{ij,3} + \Delta_{ij,5})Cov(a,d) \\ &\quad + \Delta_{ij,1}\sigma_h^2 + (\Delta_{ij,1} + \Delta_{ij,2} - f_i f_j)\mu_h^2 \\ &\stackrel{def}{=} g_{ij}^{(a)}\sigma_a^2 + g_{ij}^{(d)}\sigma_d^2 + g_{ij}^{(ad)}Cov(a,d) \\ &\quad + g_{ij}^{(h)}\sigma_h^2 + g_{ij}^{(m)}\mu_h^2 \end{aligned} \tag{2}$$

where Φ_{ij} is the kinship coefficient between the i-th and j-th individuals, f_i is the inbreeding coefficient for the i-th individual, Δ_{ij}'s are identity coefficients as defined in Lynch and Walsh (1998, pp.133) and can be calculated from the pedigree data, and $g_{ij}^{(a)}$ denotes $2\Phi_{ij}$ etc. Abney et al. (2000) suggested that the last three polygenic variance components, σ_h^2, $Cov(a,d)$ and μ_h^2, in g_{ij} are negligible, and we ignored these three variance components for ease of computation. Though it is common to only consider the additive polygenic variance component (e.g. Yu et al., 2006; Kang et al., 2008), we prefer to keep both the additive and dominance polygenic variance components.

Permutation, Bootstrap, Gene Dropping and Genome Reshuffling for Advanced Intercross Permutation

The following four simulation-based methods for estimating significance thresholds were used:

Permutation tests A permutation test is a randomization test. It is a re-sampling procedure. Typically, the data points are randomly reassigned to subjects and then the permuted data is reanalyzed to obtain the test statistic. The process is repeated many times. The values of the test statistic obtained from the permuted data are treated as a sample from the distribution of the test statistic of the original data under the null hypothesis, and the threshold at significance level α is then estimated by the $100(1-\alpha)$th percentile of this set of values.

A fundamental requirement for valid permutation is exchangeability, which should be ensured by the design of an experiment or be assumed under the null hypothesis (Anderson, 2001; Nichols and Holmes, 2001). A permutation test is exact when permutation is performed within exchangeable units. Exact permutation tests do not exist when data points are not exchangeable, for instance, in a linkage analysis where a continuous variable is used as a covariate. In this case, one may consider approximate permutation tests. Different strategies have been proposed to perform approximate permutation tests, including permutation of the raw data or residuals under null hypothesis (see e.g. Anderson, 2001), restricted permutation (Zou et al., 2005), and permutation of transformed residuals (Abney et al., 2002). The performance of approximate permutation tests varies in different experimental designs (Anderson and Braak, 2003).

Permuting the phenotypic data and permuting the genotypic data are two different ways to perform permutation in QTL mapping. We permuted genotypic data, which would retain the relationship between the trait and other predictors (e.g. sex) and could result in better estimation (O'Gorman, 2005).

Bootstrap tests Bootstrap is another popular re-sampling procedure. Bootstrap has a wide range of statistical applications including hypothesis testing (e.g. Efron and Tibishirani, 1993). There are two versions of bootstrap: non-parametric bootstrap and parametric bootstrap. While non-parametric bootstrap draws samples from the original data with replacement, parametric bootstrap generates data from a fitted model. We now briefly discuss how to use parametric bootstrap in our situation. Under the hypothesis of no QTL, model (1) reduces to $y_i = \boldsymbol{x}_i{}'\boldsymbol{\beta} + u_i + \epsilon_i, \quad i = 1, 2, \cdots, n$ and $\boldsymbol{y} = (y_1, y_2, \cdots, y_n)' \sim N_n(\boldsymbol{x\beta}, \boldsymbol{G} + \boldsymbol{I}\sigma^2)$ with $\boldsymbol{x} = (\boldsymbol{x}_1, \boldsymbol{x}_2, \cdots, \boldsymbol{x}_n)'$ and $\boldsymbol{G} = (g_{ij}^{(a)}\sigma_a^2 + g_{ij}^{(d)}\sigma_d^2)$. We can fit the model and obtain parameter estimates $\hat{\boldsymbol{\beta}}$, $\hat{\sigma}_a^2$, $\hat{\sigma}_d^2$ and $\hat{\sigma}^2$, and then generate a sample $\boldsymbol{y}^{(b)} = (y_1^{(b)}, y_2^{(b)}, \cdots, y_n^{(b)})'$ from $N_n(\boldsymbol{x}\hat{\boldsymbol{\beta}}, \hat{\boldsymbol{G}} + \boldsymbol{I}\hat{\sigma}^2)$ with $\hat{\boldsymbol{G}} = (g_{ij}^{(a)}\hat{\sigma}_a^2 + g_{ij}^{(d)}\hat{\sigma}_d^2)$. When polygenic variation is ignored, $\boldsymbol{y}^{(b)}$ is generated from $N_n(\boldsymbol{x}\hat{\boldsymbol{\beta}}, \boldsymbol{I}\hat{\sigma}^2)$ instead. We then analyze $\boldsymbol{y}^{(b)}$ the same way as we analyze the original data $\boldsymbol{y}$. The values of the test statistic calculated from a number (say 1000) of bootstrap samples are pooled to estimate significance thresholds in the same way we described for permutation tests. Our approach should be similar to what is described in ``Alternative mapping methods 2'' in Valdar et al. (2009).

Gene dropping tests Gene dropping is yet another re-sampling procedure. Instead of re-sampling phenotypes, it uses pedigree information and Mendelian segregation principles to generate genotypic data. The idea is straightforward. If we know the haplotypes in a pair of parents and recombination rates between loci, we can simulate haplotypes (and thus genotypes) in an offspring by simulating meiosis. If we know the haplotypes in the founders, a full pedigree and a genetic map, we can simulate genotypes

for any individuals in the pedigree (see Cheng et al., 2010, for more details). Gene dropping has been used to assess significance in a wide range of applications such as genetic variability (MacCluer et al., 1986; Pardo et al., 2005; Thomas, 1990), inbreeding and allele sharing (Suwanlee et al., 2007; Jung et al., 2006), and genome-wide association studies (Cheng et al., 2010). A limitation of gene dropping is the need for a pedigree.

GRAIP Genome reshuffling for advanced intercross permutation, or GRAIP, was proposed by Peirce et al. (2008) for situations where relatedness is a concern but a complete pedigree is not available. The haplotype pairs in the parents of the last generation are permuted across the parents within each sex and then genotypic data for the individuals in the last generation are generated from the permuted haplotypes by gene dropping, using the pedigree information about nuclear families only. As the haplotypes in the parents are unknown in practice, one needs to derive phase data for the parents. This was not an issue in our studies because the haplotype (and thus genotype) data were generated using the gene dropping procedure so phase was known.

Simulation Details

Additional details of our simulation studies are provided here:

Generate a pedigree We used advanced intercross lines (AIL) as our mapping population. We created a pedigree of twenty-six generations from two inbred founder strains. In F_n $(2 \leq n < 25)$, there were 144 breeding pairs and each pair produced one female and one male progeny. The 144 female progeny randomly paired with the 144 male progeny to breed the next generation. Each breeding pair in F_{25} had four progeny, which created our sample of size 576. This pedigree resulted in varying relatedness among F_{26} individuals (supplemental table S1).

Simulate genotypic and phenotypic data It was assumed that there were twenty chromosomes and 101 markers were evenly distributed every 1 cM on each chromosome. One of every five markers on the second ten chromosomes were chosen as polygenic QTL to generate polygenic variation. The additive and dominance effects of the polygenic QTL were randomly uniformly distributed in $(-0.2, 0.2)$ and $(-0.04, 0.04)$ respectively.

Phenotypic data were generated from equation (1), with an overall mean 0 and polygenic effects as stated above. The relatedness measurements were calculated from the pedigree as described in Cheng et al. (2010). The standard deviation σ of the residual ϵ_i was 0.7, 1 or 1.5, and the corresponding polygenic effects on average approximately accounted for 56%, 46%, or 32% of the total variation in the phenotype. Genotypic data were generated by gene dropping using the pedigree.

To investigate robustness of a test to misspecification of the residual's distribution, we generated data from exponential and uniform distributions in addition to normal distributions.

Obtaining significance thresholds We used four methods to test for QTL: permutation, parametric bootstrap (e.g. Efron and Tibishirani, 1993), gene dropping and genome reshuffling for advanced intercross permutation (GRAIP) Peirce et al. (2008). In the permutation test, we permuted genotypic data without restriction unless specified otherwise. We were especially interested to investigate the performance of the permutation test in the context of statistical modeling. In applications, one may choose restricted permutation if appropriate.

Type I error The genome scan for QTL under the null hypothesis of no QTL was performed on the first ten chromosomes, where there were no QTL. For each set of parameter values, 1200 datasets were generated and each dataset was analyzed using the

likelihood ratio test (LRT). The type I error rate was estimated by the proportion of the 1200 datasets for which one or more of the scanned markers were identified as QTL, meaning that the test statistic exceeded the genome-wide significance threshold at a given significance level. We generated 6000 datasets to estimate significance thresholds for each of the four methods and each set of parameter values.

The data were analyzed with polygenic variation either being ignored or being accounted for. If polygenic variation was ignored, the model to analyze the data was $y_i = \mu + x_i^* a^* + z_i^* d^* + \epsilon_i, i = 1, 2, \cdots, n$; this was model (1) without the random polygenic effect.

Statistical power In new sets of simulations, a QTL was placed in the middle of the first chromosome. The QTL had an additive effect 0.4 and a dominance effect 0.1. The QTL accounted for approximately 2.8%, 2.3%, or 1.6% of the total variance, corresponding to $\sigma = 0.7$, 1 or 1.5. Again, the genome scan for QTL under the null hypothesis of no QTL was performed on the first ten chromosomes. A QTL was identified if the test statistic at any of the scanning loci exceeded the genome-wide threshold at a given significance level. For each of the four methods and each set of parameter values, the power was estimated by the proportion of 1200 simulations where a QTL was identified. The threshold was estimated in the same way as for type I error rates.

Pooling Procedure

In practice when we have one dataset, we can permute the data N times to estimate a threshold for the test statistic. When we replicate a simulation K times, the test statistic in all the replicates follows the same distribution. Therefore, we only need one threshold for all the replicates. Suppose we permute the data N_i times in the i-th replicate simulation and get $S_i = \{x_{ij}, j = 1, 2, \cdots, N_i\}$, $i = 1, 2, \cdots, K$. Then

$$E\{\frac{\sum_{i=1}^{K}\sum_{j=1}^{N_i} I_{x_{ij}>x}}{\sum_{i=1}^{K} N_i}\} = \frac{\sum_{i=1}^{K} \alpha N_i}{\sum_{i=1}^{K} N_i} = \alpha$$

where x is the $100(1-\alpha)$th percentile of S_i and $I_{x_{ij}>x} = 1$ if $x_{ij} > x$ or 0 otherwise. This means that we can pool S_i $(i = 1, 2, \cdots, K)$ to estimate the threshold for the test statistic in all the replicate simulations.

Computational Approximation

In general there is no analytical solution to maximum likelihood estimates (MLE) for model (1). Genome scans are extremely computationally intensive and sometimes impractical without computational simplification. Note that the random effect u in model (1) is only used to control background genetic variation. A reasonable approximation will be good enough. Assume in equation (2) $g_{ij}^{(a)}\sigma_a^2 + g_{ij}^{(d)}\sigma_d^2 + g_{ij}^{(ad)}Cov(a,d) + g_{ij}^{(h)}\sigma_h^2 + g_{ij}^{(m)}\mu_h^2 = (g_{ij}^{(a)}c_1 + g_{ij}^{(d)}c_2 + g_{ij}^{(ad)}c_3 + g_{ij}^{(h)}c_4 + g_{ij}^{(m)}c_5)\sigma^2$. Then the variance-covariance matrix of $\boldsymbol{y}$ is $\boldsymbol{\Sigma} = (\boldsymbol{G}^{(a)}c_1 + \boldsymbol{G}^{(d)}c_2 + \boldsymbol{G}^{(ad)}c_3 + \boldsymbol{G}^{(h)}c_4 + \boldsymbol{G}^{(m)}c_5 + \boldsymbol{I})\sigma^2$ where $\boldsymbol{G}^{(a)} = (g_{ij}^{(a)})$ etc. If c's are known, then $\frac{1}{\sigma^2}\boldsymbol{\Sigma}$ is a known matrix and an analytical MLE solution exists. In applications, c's are unknown; however, we can estimate them under the null hypothesis and use the estimates as known values. Approximating random effects by their estimates is a known strategy in mixed-effect model models (Pinheiro and Bates, 2000) and works well in our situation.

Computational Efficiency

The permutation test as well as the other three methods is computationally intensive, which is a trade-off between reliability and computation. However, the computation is still manageable with the previous computational approximation even if there are thousands of markers. In our simulations, there were 1010 SNP markers and the sample size was 576; one genome scan took only a few seconds on a conventional desktop computer. Parallel computing can make it realistic to perform permutation tests even when there are hundreds of thousands of SNP markers.

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Table S1 Summary of Relatedness[a]

	Min.	1st Qu.	Median	3rd Qu.	Max.
$g_{ij}^{(a)}$	0.76810	0.77070	0.77180	0.77480	1.42500
$g_{ij}^{(d)}$	0.13590	0.15480	0.15540	0.15590	0.61600
$g_{ij}^{(ad)}$	0.66420	0.66930	0.67160	0.67640	1.69800
$g_{ij}^{(h)}$	0.07814	0.07917	0.07964	0.08059	0.42460
$g_{ij}^{(m)}$	0.00714	0.00816	0.00821	0.00830	0.24430

[a] Defined in equation (2) among the simulated F_{26} individuals. The different levels of relatedness means that the assumption of exchangeability is incorrect.

Table S2 Type I Error Rates[a]

Distr[b]	Method[c]	$\sigma = 0.7$			$\sigma = 1$			$\sigma = 1.5$		
		$\alpha = 0.1$	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.1$	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.1$	$\alpha = 0.05$	$\alpha = 0.01$
Exp	Permut	0.0933	0.0375**	0.0050**	0.0925	0.0475	0.0075	0.1108	0.0583	0.0133
	Bootstr	0.0925	0.0408	0.0050**	0.0817**	0.0375**	0.0075	0.1092	0.0508	0.0117
	GeneDr	0.1017	0.0442	0.0050**	0.0883	0.0475	0.0075	0.1083	0.0558	0.0125
	GRAIP	0.0925	0.0442	0.0075	0.0875	0.0425	0.0075	0.1125	0.0583	0.0158*
Norm	Permut	0.1100	0.0525	0.0108	0.1058	0.0475	0.0100	0.0958	0.0475	0.0083
	Bootstr	0.1067	0.0517	0.0108	0.0958	0.0408	0.0100	0.0958	0.0442	0.0058*
	GeneDr	0.1000	0.0525	0.0133	0.0958	0.0417	0.0100	0.0958	0.0467	0.0075
	GRAIP	0.0967	0.0525	0.0117	0.0958	0.0450	0.0100	0.1058	0.0450	0.0058
Unif	Permut	0.0908	0.0408	0.0092	0.0942	0.0483	0.0125	0.0917	0.0517	0.0108
	Bootstr	0.0892	0.0400*	0.0083	0.0950	0.0467	0.0117	0.0992	0.0558	0.0125
	GeneDr	0.0908	0.0400*	0.0083	0.0925	0.0467	0.0117	0.0883	0.0517	0.0125
	GRAIP	0.0908	0.0467	0.0092	0.0950	0.0467	0.0125	0.0958	0.0525	0.0125

[a] Estimated from 1200 simulations at genome-wide significance levels $\alpha = 0.10$, 0.05 and 0.01. Symbol *, ** or *** indicates the estimated type I error rate is significantly different from the expected level at significance level 0.10, 0.05 or 0.01.
[b] Permuting genotypic data (Permut), bootstrapping phenotypic data (Bootstr), gene dropping (GeneDr) or GRAIP.
[c] The distribution of the residual was exponential (Exp), normal (Norm) or uniform (Unif), each with a standard deviation 0.7, 1 or 1.5.

Table S3 Estimated Genome-wide Thresholds for the Body Weight Data

	Relatedness Ignored			Relatedness Not Ignored		
α level	0.1	0.05	0.01	0.1	0.05	0.01
Permut	19.45	21.09	24.52	18.70	20.23	23.56
Bootstr	19.49	21.01	24.25	19.49	21.00	24.20
GeneDr	65.17	70.46	84.48	19.53	21.08	24.45
GRAIP	57.69	62.20	72.67	19.72	21.26	24.50

Estimated from 5000 simulations at genome-wide significance levels $\alpha = 0.1$, 0.05 and 0.01 by the following methods: permuting genotypic data (Permut), bootstrapping phenotypic data (Bootstr), gene dropping (GeneDr) and GRAIP, using the likelihood ratio test (LRT).

Table S4 P-values by the Kolmogorov-Smirnov Test

	Permut	Bootstr	GeneDr	GRAIP
$\sigma = 0.7$	0.60200	0.32428	0.00000	0.00000
$\sigma = 1$	0.44558	0.44988	0.00000	0.00000
$\sigma = 1.5$	0.43282	0.10871	0.00000	0.00000

Based on 6000 simulations under the null hypothesis that when no QTL effects existed, the distribution estimated by a testing method when relatedness was ignored was identical to the distribution estimated by the same method when relatedness was taken into account. Data was generated by each of the testing methods: permuting genotypic data (Permut), bootstrapping phenotypic data (Bootstr), gene dropping (GeneDr) and GRAIP. The distribution of the residual was normal with a standard deviation 0.7, 1 or 1.5.

Supporting Data and R Scripts

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.146332/-/DC1.

File S2 R Scripts
File S3 Raw Data