POWER OF ASSOCIATION AND TRANSMISSION DISEQUILIBRIUM TESTS

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INTRODUCTION

Livestock genetic improvement has been largely achieved without knowledge of individual genes underlying traits under selection. Nevertheless, higher rates of genetic gain are possible when molecular and quantitative information of genes is used in selection schemes (Meuwissen *et al.,* 2001). Gene mapping relies on linkage disequilibrium (LD) between markers and trait loci. Association tests use LD at the population level, whereas linkage uses LD within families. Because of this, association tests are powerful statistical techniques through which high resolution mapping can be achieved, given sufficient marker coverage (Terwilliger and Weiss, 1998). However, population stratification, caused by recent migrations and/or non-random mating, can lead to detection of spurious disequilibrium (disequilibrium without linkage). Spurious disequilibrium is a major confounding factor for some association tests (e.g. Neale *et al.,* 1999). Family-based association tests, such as the transmission-disequilibrium test, or TDT (Spielman *et al.,* 1993), are robust to population stratification, albeit they may be less powerful than non-robust tests (Risch and Teng, 1998).

The power and robustness of association tests have been thoroughly studied regarding discrete traits (e.g. Xiong and Guo, 1998). However, fewer studies have focused on contrasting power between family-based and non family-based association tests for quantitative traits (e.g. Page and Amos, 1999 ; Sham *et al.* 2000). This paper studies power and robustness across six tests (five association tests, four of which are family-based tests, and a linkage test). Based upon our results, we recommend the use of non family-based tests when spurious disequilibrium is unlikely, and otherwise the use of the family-based test described in Rabinowitz (1997).

MATERIAL AND METHODS

Power of the tests was empirically estimated as the ratio of significant analyses over the total number of analyses in computer simulations and also was deterministically predicted through the calculation of the non-centrality parameters (λ). For each test, the distribution of statistics under the alternative hypothesis of association is determined by λ. The area under the non-central distribution exceeding a predetermined threshold is the power of the test.

Tests. The tests used in this study were : 1) one-way ANOVA, with genotype as factor 2) two-way ANOVA, with genotype and mating-type as factors 3) nested ANOVA, with genotype nested within mating-type 4) TDT1 (Allison, 1997), 5) TDT2 (Szyda *et al.,* 1998), and 6) TDT3 (Rabinowitz, 1997). The first test is a non family-based association test. Tests 2, 4, 5, and 6 are family-based association tests. Test 3 is a linkage test. Statistical thresholds were set for each test so that a 0.05 rate of false positive results was expected under the null hypothesis of no association.

Simulations. All tests were compared across 9 scenarios defined by different combinations of parameters (i.e. N, P and σ^2_q ; see table 1), and 1000 replicates per scenario. Each sample consisted of independent family trios (two parents and one offspring). Genotypes were available for all family members, and phenotypes were available in the offspring. The recombination rate between two biallelic loci, the marker and the trait loci, is denoted with C. The error terms were independently drawn from a normal distribution with mean zero and variance $\sigma^2_e = 1$. There were neither polygenic effects nor dominance at the trait locus. Simulations were repeated with and without population stratification. The population was divided into two independent groups of equal size to simulate stratification. Each group differed in trait and marker allele frequencies, and only within-group matings were allowed. Sires were all heterozygous for the marker (dams were in Hardy-Weinberg equilibrium), as TDTs use only families with at least one heterozygous parent.

Predictions. For each test, λ was calculated using the sample size, allele frequencies and proportion of variance explained by the trait gene.

RESULTS AND DISCUSSION

Table 1 shows deterministic (De) and empirical (Em) power calculations when analysing a trait locus that explains a fraction σ^2_q of the total phenotypic variance, the positive allele has frequency P and the sample size (number of independent trios) is N. There was no population stratification in this set of simulations.

Table 1. Empirical and deterministic power calculations at the trait locus

Parameters			One-way [F]		Nested [G]		TDT1 [H]		TDT2 [I]		TDT3 [J]	
σ^2_q [A]	P [B]	N [C]	De[D]	Em[E]	De	Em	De	Em	De	Em	De	Em
	0.1	114	0.85	0.86	0.69	0.71	0.80	0.79	0.81	0.81	0.84	0.86
0.05	0.3	219	0.90	0.92	0.69	0.77	0.80	0.85	0.74	0.81	0.86	0.86
	0.5	247	0.90	0.90	0.69	0.68	0.80	0.80	0.71	0.70	0.86	0.85
	0.1	56	0.86	0.86	0.68	0.67	0.80	0.75	0.81	0.79	0.81	0.84
0.1	0.3	105	0.89	0.88	0.69	0.64	0.80	0.77	0.73	0.72	0.84	0.85
	0.5	118	0.90	0.90	0.69	0.68	0.80	0.77	0.71	0.71	0.84	0.87
	0.1	36	0.85	0.85	0.66	0.69	0.80	0.76	0.81	0.76	0.77	0.80
0.15	0.3	67	0.89	0.89	0.68	0.66	0.80	0.77	0.73	0.71	0.82	0.83
	0.5	75	0.90	0.90	0.68	0.68	0.80	0.79	0.70	0.70	0.82	0.86

[A] Ratio of trait locus variance over phenotypic variance ; [B] Allele frequency of positive allele ; [C] Sample size ; [D] Deterministic power ; [E] Empirical power from 10^3 replicates ; [F] One-way ANOVA ; [G] Nested ANOVA ; [H] Allison (1997) ; [I] Szyda *et al.* (1998) ; [J] Rabinowitz (1997).

The one-way ANOVA is the test showing maximum power consistently across all scenarios. Tests 2 and 4 (see Material and Methods) were alternative parameterisations of equivalent statistical models, thus only TDT1 is shown.

The rates of false positive results corresponding to table 1 were around 0.05 (results not shown) for all tests. However, when population stratification was simulated (N = 36, σ^2_q = 0.15, LD = 0 within population, 10^3 replicates), the one-way ANOVA showed a positive correlation between the rate of false positive results and the strength of stratification (table 2).

Table 2. Simulation results : Rate of false positive results with stratification [A]

Stratification	1	0.8	0.6	0.4	0.2	0
One-way	0.215	0.098	0.083	0.045	0.06	0.045
TDT1	0.046	0.023	0.053	0.042	0.073	0.038
TDT2	0.056	0.054	0.069	0.054	0.064	0.075
TDT3	0.041	0.041	0.034	0.039	0.047	0.045
Nested	0.046	0.041	0.048	0.063	0.067	0.037

[A] Allele frequency (P) difference between populations, averaging 0.5

Table 1 shows the maximum power the tests can achieve under the parameter combinations considered, because analyses were performed at the trait locus. Power will decay as C increases between marker and trait locus (figure 1). The parameters used to obtained results shown in figure 1 were σ^2_q = 0.15, N = 100, and LD = 0.125 (e.g. 50 % of maximum LD when P = 0.5), and 10^3 replicates. The ranking of the tests is consistent across different C values : the most powerful is always one-way ANOVA, followed by TDT3, TDT2, TDT1, and nested ANOVA.

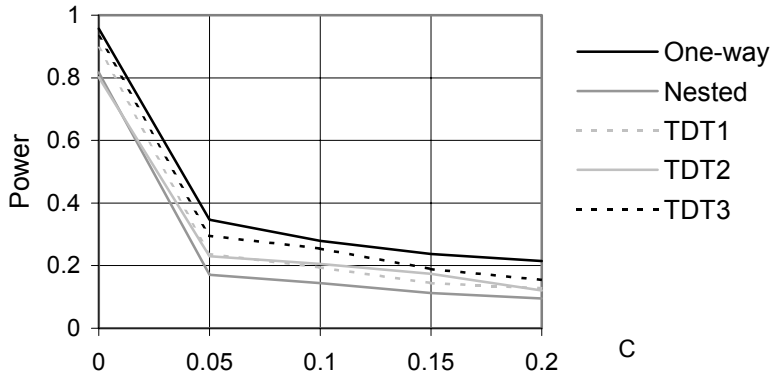

Figure 1. Power decay due to recombination rate (C)

CONCLUSION

Non family-based association tests, e.g. one-way ANOVA, are generally more powerful than family-based association tests, e.g. TDT, at detecting genetic effects when analysing single markers because they use all available information, i.e. information between and within families. However, an advantage of family-based tests, compared to non family-based tests, is

their robustness to false positive results caused by spurious disequilibrium. The level of spurious disequilibrium may be high in livestock populations when a recent hybridisation event has occurred, the effective population size (N_e) is low, or mating is not at random. Hybridisation among pure lines is commonly used for endowing commercial products with hybrid vigour, or for creating new synthetic breeds. Furthermore, low N_e and non-random mating are the norm in all selected breeds. Hence, spurious disequilibrium ought to be considered when mapping genes in livestock.

The power of detection of a genetic effect at a marker locus depends on N, C, LD and P. Power decreases with decreasing values of N and LD, and increasing values of C. Maximum genetic variation at a marker, i.e. maximum information, is found at intermediate allele frequencies. In addition, deterministic power analysis, through calculation of λ, provides a quick and efficient way of comparing different tests.

Unless samples are drawn from populations known to be genetically homogeneous, we encourage the use of family-based tests in order to avoid misleading results. From among the family-based tests compared in this work, the TDT of Rabinowitz (1997) showed the greatest power across all scenarios.

Association tests work best when markers are closely linked to trait loci, with power decreasing quickly as C increases. Therefore dense marker maps should be used for scanning entire genomes with these tests. Then, it will be necessary to correct for multiple tests.

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