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ORIGINAL PAPER

Detection of marker–QTL associations by studying change in marker frequencies with selection

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Abstract The value of selective genotyping for the detection of QTL has already been studied from a theoretical point of view but with the assumption of a negligible contribution (r_P^2) of the QTL to the phenotypic variance. For predicting change in gene frequency, we show that this assumption is only valid for r_P^2 less than 0.05 and for a proportion selected higher than 1%. Therefore, we develop a study of the optimization of selective genotyping without assumption on QTL effect, with selection either of both tails (bidirectional genotyping or BSG) or only one tail (unidirectional genotyping or USG). For a given population size of phenotyped plants the optimal proportion selected for selective genotyping is around 30% for each tail. For the same investment as in ANOVA, by investing more in phenotyping than in genotyping when the cost ratio of genotyping to phenotyping is higher than 1, the optimal proportion selected appears to be between 10 and 20% for each tail. It is mainly affected by the cost ratio and decreases when the cost ratio increases. At this optimum, BSG is competitive with ANOVA, or even more powerful, when the cost ratio is higher than 1. USG can also be competitive when the cost ratio is higher than 2. Using experimental data from two populations of about 300 F4 inbred families of maize, it was verified that BSG at the optimum gives the same results as ANOVA or is better whereas USG is less powerful or equivalent.

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Introduction

Since Lander and Botstein (1989), methods of QTL detection by ANOVA, regression or maximum likelihood, rest mainly upon the comparison of means at the level of marker or QTL genotypes. In order to have acceptable accuracy in the determination of QTL effect and position they need both phenotyping and genotyping of sufficiently large populations. Consequently, such methods are expensive even for a moderate population size which leads to a relatively low power of QTL detection. When, for a given individual, genotyping costs more than phenotyping, selective genotyping, i.e. genotyping only individuals from the high and low tails of the phenotypic distribution (Lander and Botstein 1989), was shown by Darvasi and Soller (1992) to lead to a marked decrease in the number of individuals genotyped for a given power, at the expense of an increase in the number of individuals phenotyped. When considering a single trait, it appears that it will almost never be useful to genotype more than the upper and lower 25% of a population. With this method, linkage between marker loci and QTL affecting the trait of interest is determined, as for classical approaches, by the comparison of marker genotype means over the pooled sample of the upper and lower tails.

An alternative approach, suggested first by Stuber et al. (1980, 1982) is to examine changes in marker

allele frequencies among selected lines originating from a cross between two parental inbred lines. It is indeed expected that, due to hitchhiking effect, selection would change frequencies of markers which are the more linked to QTLs involved in the variation of the selected trait (Lebowitz et al. 1987). This approach can be bidirectional if the two tails of the distribution are considered, or unidirectional if only one tail is considered. This last approach is particularly interesting when combined with a selection programme from an F2 or any F1-derived population, so that QTL detection becomes a co-product of selection requiring only the genotyping of selected plants or lines. From a plant breeding point of view, such an approach could be very useful to find markers associated with QTLs involved in response to selection which could be used to perform marker-assisted selection. Several applications of this method have already been reported for QTL detection or QTL validation in plants (Foolad and Jones 1993; Zhang et al. 2003; Wingbermueble et al. 2004; Coque and Gallais 2006). Furthermore, in maize, with two cycles of recurrent selection on phenotype from a population of F4 independent families, Moreau et al. (2004a) have shown that the significant changes in marker allele frequency were for marker locus located in the vicinity of detected QTLs. An additional interest of the marker frequency approach is that, it makes it possible to use DNA pooling of selected individuals to estimate the frequencies needed for the tests (Darvasi and Soller 1994). With this method, allele frequencies are evaluated from the intensities of electrophoretic bands (or other signals, depending on the technique used) rather than individual genotyping, which can strongly decrease genotyping costs.

Darvasi and Soller (1992, 1994) developed a theoretical approach for optimization of selective genotyping based upon comparisons of means and on changes in marker frequencies with DNA pooling. However, both approaches assume that the contribution of each QTL of interest to the phenotypic variance is negligible, so that phenotypic variance within marker genotype classes is approximately equal to the total phenotypic variance. In this paper, our aim is thus to revisit the theory underlying individual bidirectional (BSG) and unidirectional selective genotyping (USG) based upon change in allele frequencies, with a less restricted assumption about the size of QTL effect than Darvasi and Soller's studies, i.e. considering the possibility of the presence of one or two QTLs with large effects. Indeed, the presence of one or two QTLs with large effects will lead to departure from a normal distribution of phenotypic values and can affect the prediction of change in gene frequency. The power of BSG and USG is then compared to ANOVA for a given size of the phenotyped population and for a given investment (sum of the phenotyping and the genotyping costs). Finally, we compare USG, BSG to ANOVA with two sets of experimental data. The comparison to ANOVA is justified because, with the aim to detect markers usable in marker-assisted selection, we are more interested in the detection of markers associated to a quantitative trait than by the estimation of position and effect of QTLs, although this would also be possible.

Materials and methods

Prediction of the change in gene frequency by selection

To study the efficiency of BSG and USG for the detection of marker–QTL associations, it is first necessary to predict the change in marker allele frequency due to selection by truncation of a proportion p_S of the population in one tail. We present a general method which will be compared to the Darvasi and Soller approach and to the infinitesimal approach as formulated by Falconer (1960) and Griffing (1960).

General method

We consider as an example the case of RIL or DH population derived from an F1 between two homozygous lines, so that, at any polymorphic marker locus, there are only two alleles M and m, and two genotypes MM and mm with an initial frequency $p_0 = q_0 = 0.5$. We assume a polygenic trait with one ''major'' QTL in the vicinity of the marker locus, the other QTLs being with low effects (''minor'' QTLs), such that they do not affect normality of the distribution of phenotypes when the major QTL is fixed. Let σ^2_P be the variance of the distribution of all phenotypes. At a given marker locus close to the major QTL, this distribution is a mixture of two distributions, one for marker genotype MM and the other for marker genotype mm, with an equal variance $\sigma^2_W = \sigma^2_P - \sigma^2_M = (1 - r^2_P)\sigma^2_P$ where σ^2_M is the variance explained by the marker locus and r^2_P its contribution to the phenotypic variance. We assume that both marker distributions are normal, which is equivalent to assuming that at the level of the whole distribution there is only one QTL leading to significant departure from normality.

Let X be the difference between the selection threshold and the population mean, and $2a^*$ the difference between the means of these two subpopulations. The abscissa of the truncation point are, in standard units, $(X-a^*)/\sigma_W = \frac{x-\delta}{\sqrt{1-r_P^2}}$ for genotypes MM and $(X+a^*)/\sigma_W = \frac{x+\delta}{\sqrt{1-r_P^2}}$. for genotypes mm, where $x = X/\sigma_P$ and $\delta = a^*/\sigma_P = r_P$. Then, knowing r_P^2 and x, by using a table of selection intensities (or of normal distribution) it is possible to determine the proportions of MM and mm lines in the selected sample, f^*_{MM} and f^*_{mm}. As both MM and mm populations have the same contribution in the RIL population, in the selected sample the frequency of MM genotypes, i.e. the new frequency of M, will be $f^*_{MM}/(f^*_{MM}+f^*_{mm})$.

The approach could also be extended to the F2-derived populations with heterozygous genotypes and to populations with two unlinked major QTLs affecting normality of the distribution. In this situation with a RIL or DH population it would be necessary to consider the mixture of four sub-populations. The difference between means of these subpopulations can be affected by epistasis. Although to simplify we have not considered the presence of epistasis, the effect of digenic epistasis could be considered by the same approach. The discussion about the power of our method in presence of epistasis requires further developments which are beyond the aim of our paper.

In what follows, we call our approach the ''mixture model''. It is different from that presented by Darvasi and Soller (1992) which rests upon the comparison of means of the pooled samples of the upper and lower tails for each marker genotype. It is comparable to the study of change in gene frequency combined with DNA pooling proposed by Darvasi and Soller (1994), except that they have considered, like Darvasi and Soller (1992), that QTL effect was sufficiently small, so that its effect on variance is negligible. Indeed, they considered as marker standardized population means $(x+\delta)$ for MM and $(x-\delta)$ for mm instead of $\frac{x+\delta}{\sqrt{1-r_P^2}}$ and $\frac{x-\delta}{\sqrt{1-r_P^2}}$, because they assumed that r_P^2 is negligible in comparison to 1.

Theory for the infinitesimal model

When the contribution of any QTLs to phenotypic variance σ^2_P is sufficiently small (i.e. if the effect on phenotypic variance of each locus can be neglected) then, in a random mating population, the conditional gene frequency $p_{B/P}$ of an allele B at the QTL l, knowing the phenotype, is a linear function of the phenotype (Falconer 1960)

$$p_{B/P} = p_0 + \frac{\mathrm{cov}(f_{B/g_l}, P)}{\sigma^2_P}(P-\overline{P}),$$

with $\mathrm{cov}(f_{B/g_l}, G_l) = p_0\,\alpha_B$, where $\mathrm{cov}(f_{B/g_l}, G_l)$ is the covariance between the genetic value G_l at locus l and the conditional frequency of B knowing the genotype g at locus l ($f_{B/g_l} = 1$ for BB, 0.5 for B$\overline{\mathrm{B}}$ and 0 for $\overline{\mathrm{B}}\overline{\mathrm{B}}$, $\overline{\mathrm{B}}$ represents all other alleles, and α_B is the additive effect of allele B with frequency p_0. It results that the change in gene frequency at one locus due to selection can be written, for an allele B

$$p_1 - p_0 = \Delta p = i\,\mathrm{cov}(f_{B/g_l}, G_l)/\sigma_P, \qquad (1)$$

where i is the selection intensity in standardized units. The same approach can be applied for an inbred population derived from a random mating population, but in this situation

$$\mathrm{cov}(f_{B/g_l}, G_l) = (1+F)p_0\alpha_B,$$

where F is the coefficient of inbreeding. For a biallelic population, with alleles B and $\overline{\mathrm{B}} = b$ at a QTL, with frequency p_0 and $q_0 = (1-p_0)$, since $\alpha_B = q_0\alpha$ there results for allele B

$$\mathrm{cov}(f_{B/g_l}, G_l) = (1+F)p_0\,q_0\alpha,$$

α being the substitution effect, $\alpha = a_l - (p_0-q_0)d_l$ (Falconer 1960), where a_l is half the difference between the two homozygotes and d_l is the difference between the heterozygote Bb and the average of the two homozygotes. In the following, as we only consider F1-derived population (SSD at any generation, RIL or DH populations) where $p_0 = q_0 = 0.5$, formula (1) then becomes

$$\Delta p = 0.25(1+F)a_l/\sigma_{P_F} \quad \text{or} \quad \Delta p = 0.25(1+F)r_G\,\sigma_{G_l}/\sigma_{P_F}, \qquad (2)$$

$\sigma^2_{P_F}$ being the phenotypic variance for a population with an inbreeding coefficient F, a_l is half the difference between the two homozygotes at locus l and $r^2_G = a_l^2/\sum_l a_l^2$ the part of genetic variance explained by the QTL at the level of the homozygous population. For a population of recombinant inbred lines, there results $\Delta p = 0.5\ i\ r_P$ with $r^2_P = a_l^2/\sigma^2_P$.

Formula (2) applies for a marker strictly linked to the QTL. In case of no strict linkage between the marker and the QTL, it is possible to consider the

effect of the selection at the level of the marker loci (with allele M and m) by considering the expected marker genotype values

$$\overline{\mathrm{MM}} = \lambda a_l, \quad \overline{\mathrm{Mm}} = \lambda^2 d_l \quad \text{and} \, \overline{\mathrm{mm}} = -\lambda a_l,$$

where for a F2 population, λ = 1–2c is the linkage parameter introduced by Schnell (1963). For a DH population $\overline{\mathrm{MM}} = \lambda a_l$ and $\overline{\mathrm{mm}} = -\lambda a_l$, whereas for a RIL population λ has to be replaced by (1–2c)/(1+2c). Then, a_l can be replaced by $a_l^* = \lambda a_l$, and r_G by λr_G.

Test of marker–QTL association by change of marker allele frequency

With selection in a population of finite size, it is necessary to test whether the change in gene frequency due to selection is significant, i.e. higher than expected by random variation of frequencies. Let p_1 be the gene frequency after unidirectional selection, the expected initial frequency being 0.50. The significance of the change can be approached by a χ^2 test

$$\chi^2 = (\widehat{p}_1 - 0.5)^2 / \mathrm{var} p_1,$$

where $\widehat{p}_1$ is the observed frequency after selection and var p_1 is the expected variance of gene frequency due to sampling. To compute var p_1, we must note that there are two steps of sampling from the infinite size population with gene frequency 0.5: considering only the case of a RIL or HD population, first N lines are drawn at random and second N_S lines are selected. Taking into account both origins of genetic drift, the variance in gene frequency in the first generation of selection is

$$\mathrm{var} p_1 = p_0 q_0 \left[1 - \left(1 - \frac{1}{N} \right) \left(1 - \frac{1}{N_S} \right) \right],$$

with $p_0 = q_0 = 0.5$ the allele frequency in the population of infinite size (Waples 1989; Coque and Gallais 2006). When N is sufficiently high, var p_1 is equivalent to $p_0 q_0 / N_S$

With bidirectional selection, instead of testing the change in allele frequency in each tail separately, one can take advantage of the evaluation of allele frequencies in both tails by testing the difference between $\widehat{p}_U$, the observed gene frequency in the upper tail, and $\widehat{p}_L$, the observed gene frequency in the lower tail by the χ^2 test

$$\chi^2 = \frac{(\widehat{p}_U - \widehat{p}_L)^2}{\mathrm{var}(p_U - p_L)}, \tag{3}$$

with, since these two gene frequencies are independent

$$\mathrm{var}(p_U - p_L) = \mathrm{var} p_U + \mathrm{var} p_L = 2 \mathrm{var} p_1.$$

In what follows we use the u-test which is the square root of a χ^2, i.e. the normal distribution when change in marker frequencies are only due to sampling.

Comparison with the ANOVA test

In the case considered where at one locus there are only two marker genotypes, the ANOVA test is equivalent to a t-test for the comparison of the two marker genotype means. For a given population size N and a given within marker genotype variance σ^2_W, and assuming the same size (N/2) of each marker genotype class, the expectation of the t-test is

$$E(t) = \frac{a}{\sqrt{\sigma^2_W / N}} = r_P \sqrt{N} / \sqrt{(1 - r^2_P)}, \tag{4}$$

where a is half-difference between the means of marker genotypes.

With selective genotyping, the expectation of the u-test for the comparison of p_U and p_L is

$$E(u) = \frac{\theta \Delta p}{\sqrt{0.25 \, \theta / N_S}} = 2 \Delta p \sqrt{\theta \, p_S \, N}, \tag{5}$$

with θ = 1 for USG and 2 for BSG.

Expression (5) shows that $E(u)_{BSG} > E(u)_{USG}$, which means that, as expected, BSG will be more efficient than USG to detect marker–QTL associations. With DNA pooling, as shown by Darvasi and Soller (1994), technical error in the determination of gene frequency through intensity of the electrophoretic band could be taken into account by adding it to the sampling error 0.25 θ / N_S in (5).

Note that the ratio of $E(u)/E(t)$, which can be taken as a measure of selective genotyping relative efficiency, is independent of the population size. Furthermore, expression (5) shows that for a given size of the phenotyped population, and a given r^2_P, $E(u)$ is expected to be maximum for an optimal selection intensity because selection intensity has two antagonistic effects: it increases the expected change in gene frequency (Δp), which is favourable, but by reducing N_S it also increases the variance in gene frequency which is unfavourable. It appears also from (5) that this optimum is independent of the population size and is the same for BSG and USG (θ = 1 or 2) for a given size of the phenotyped population. In contrast, it can be predicted as being dependent on r^2_P which affects both variance

of the marker genotype distribution and abscissa of truncation point of this distribution. This optimum will be determined numerically. Both methods, ANOVA and selective genotyping will also be compared according to their power (Appendix). Obviously the optimal proportion to select is the same by using $E(u)$ or the power.

Comparison of the methods for the same investment as ANOVA

For a given experiment with N individuals, genotyping of the two tails cannot be as powerful as ANOVA test based upon genotyping of all individuals. Similarly BSG will always be more powerful than USG. Comparison of BSG or USG to ANOVA is more justified at their optimum for a given investment.

The cost C_{an} of QTL detection by ANOVA can be written

$$C_{an} = N_{an}(c_p + c_g),$$

where N_{an} is the number of phenotyped individuals and c_p (c_g) is the cost of phenotyping (genotyping) for one individual, whereas the cost C_{sg} of selective genotyping will be

$$C_{sg} = N_{sg}c_p + \theta N_S c_g = N_{sg}(c_p + \theta p_S c_g),$$

where N_{sg} is the number of phenotyped individuals with selective genotyping, N_S the number of selected and genotyped individuals in one tail and $\theta = 1$ for USG and 2 for BSG, p_S is the proportion of selected individuals in one tail.

Then, if we consider the same investment in selective genotyping as in ANOVA, there results the following relationship between the number of individuals to phenotype with selective genotyping and the number of individuals studied in ANOVA

$$N_{sg} = N_{an}\frac{c_g + c_p}{c_p + \theta p_S c_g},$$

which can be simplified by defining a cost ratio $r_{gp} = c_g/c_p$

$$N_{sg} = N_{an}\frac{1 + r_{gp}}{1 + \theta p_S r_{gp}}. \quad (6)$$

Note that when $p_S = 0.50$, then $N_{sg} = N_{an}$, but with $p_S < 0.50$, $N_{sg} > N_{an}$. According to expression (6) the expectation of the u-test is such that,

$$[E(u)]^2 = 4(\Delta p)^2 \theta p_S N_{an}\frac{1 + r_{gp}}{1 + \theta p_S r_{gp}}. \quad (7)$$

Expression (7) shows that, again, the relative efficiency of selective genotyping $E(u)/E(t)$ is independent of the population size studied for ANOVA (N_{an}). Furthermore, it also shows that there is an optimal p_S independent of population size N_{an}. In contrast this optimum is expected to be affected by r_P^2, r_{gp} and θ. It can be noted that the optimal p_S for the same investment as ANOVA is also the optimal p_S for a given investment I. Indeed, in this case, as in unit of phenotyping cost ($c_p = 1$), $I = N_{sg}(1 + \theta p_S r_{gp})$, $N_{sg} = I/(1 + \theta p_S r_{gp})$ and

$$[E(u)]^2 = 4(\Delta p)^2 \theta p_S \frac{I}{1 + \theta p_S r_{gp}},$$

which is maximum for the same p_S value as (7) for given I, r_{gp} and r_P^2.

Numerical application

To simulate the effect of selection on the frequency of a marker linked to a QTL, in a general approach, the abscissa of the truncation point can be considered as a parameter (x) which allows determination of the truncation point for each normal distribution of phenotypic values associated with a marker genotype. It is also possible to directly use the proportion selected p_S, and determine the abscissa of the truncation point by the use of the property of normal distribution, from which it results the truncation point for each normal distribution of phenotypic values associated with a marker genotype. When r_P^2 is lower than about 0.40, the results of these two approaches are nearly the same. Thus, as the results are more explicit for the breeder by directly using p_S as parameter, we have used the second approach.

After simulation of the effect of selection, expected χ^2 or u values were derived for BSG ($\theta = 2$) and USG ($\theta = 1$) according to the value of the parameters: N_{an}, r_P^2, p_S, and r_{gp}. They were compared to the expected ANOVA F values which are affected only by N_{an} and r_P^2. Furthermore, power of the tests was also computed. To determine optimal proportion selected, values of p_S were incremented modulo 0.025, from 0.025 to 0.35 and the values for r_P^2 were incremented from 0.05 to 0.35, modulo 0.05. Considered values of cost ratio r_{gp} were between 0.5 and 5, realistic values in plant breeding being presently between 1 and 3 depending on the laboratory, trait of interest and field experiment orga-

nization. As an example, in grain maize, considering that about 100 markers are required to cover the genome, on the basis of $2 for each marker, the genotyping cost of one genotype will be around $200. Phenotyping cost could be evaluated as $25 per plot, leading with 4 replications (i.e. 4 sites, one rep per site) to a total phenotyping cost around $100 and thus to a genotyping/phenotyping cost ratio around 2.

Experimental approach

Theoretical conclusions were evaluated with data from QTL detection in two populations: (1) a population (SAM) of 300 F3:4 lines of maize, with 77 codominant markers already used by Moreau et al. (2004b) and (2) a population (LHRE) derived from the F2 of the previous population with four generations of random mating and then selfed twice to produce 322 F3:4 lines (L. Moreau et al. unpublished results). For this second population the equivalent map length was 5,617 cM, and we have used 336 codominant markers. In these inbred populations, the population size being sufficiently large, the sampling variance of gene frequency is var $p_1 \sim 0.25\ (1 + F)/2N_S$ (Weir 1990), with $F = 0.5$. By varying the number of selected lines, we have tried to determine on the one hand the optimum for BSG for a given population size, and on the other hand the optimum for a given cost. u values were computed for USG and BSG and compared to F values given by marker by marker ANOVA. To represent the correspondence between the two tests we have used the correlation between $\sqrt{F}$ and u, considering only markers for which ANOVA on the entire population leads to significant test at a α-threshold 0.05 in order to avoid the high weight of non-significant markers. To take into account linkage between markers, degrees of freedom for the test of this correlation were based on the approximate number of independent chromosome regions derived by consideration of the distribution of the ANOVA tests on the chromosomes.

To compare BSG and USG to ANOVA for a given investment, as the number of phenotyped lines for selective genotyping is higher than that for ANOVA we have considered BSG or USG with 300 phenotyped lines, the size of both populations studied. Then, using expression (8), knowing the number of phenotyped lines for selective genotyping, the cost ratio and the optimal proportion selected, the corresponding population size for ANOVA was determined as $N_{an} = 300\frac{1+\theta p_S r_{gp}}{1+r_{gp}}$. To take into account random variation due to the drawing of N_{an} lines among 300, 50 independent samples were considered for a given N_{an}; then for a given N_{an} we have considered the mean of F values and the mean of the correlations between square root of F ANOVA and u-test for BSG or USG for each drawing, taking into account only markers detected in the complete ANOVA.

Results

Comparison of three models for predicting change in marker allele frequencies

The numerical comparison of ''infinitesimal'' and ''mixture'' approaches shows that the ''infinitesimal'' approach leads to an overestimation of allele frequencies at high selection intensity and high value of r_P^2, giving even predicted values higher than 1 (Fig. 1). With r_P^2 less than 0.15 and percentage of selected plants greater than 15%, both approaches give about the same results. If high selection intensities are used, for example with 5% of selected plants, the normal approximation must be excluded except if low QTLs effects are expected (r_P^2 less than 0.05). Darvasi and Soller's approach always leads to an underestimation of allele frequencies which is all the greater as r_P^2 is high. For $r_P^2 < 0.05$ and $p_S > 0.10$, the three predictions are about the same. Such differences justify the reconsideration of power and optimization of selective genotyping.

Optimization of selective genotyping for a given size of phenotyped population

The optimal proportion selected leading to the highest $E(u)$ or power for BSG and USG slightly increases with increasing r_P^2. For BSG, it is around 27.5% for $r_P^2 = 0.05$ and 32.5% for $r_P^2 = 0.25$ (Fig. 2). However, the

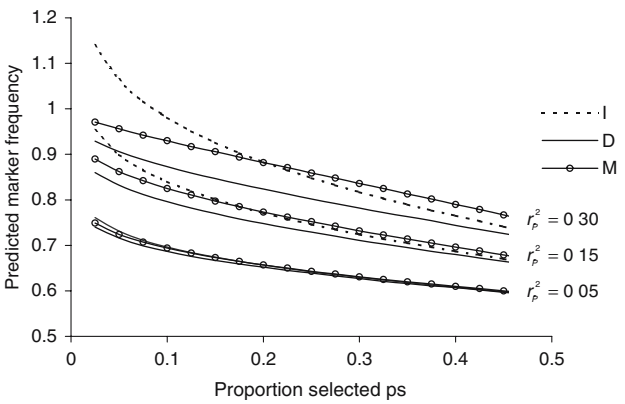

Fig. 1 Predicted change in marker frequency according to the model, by selection within a RIL population: *I* infinitesimal model, *D* Darvasi's model (1992), *M* Mixture (true) model

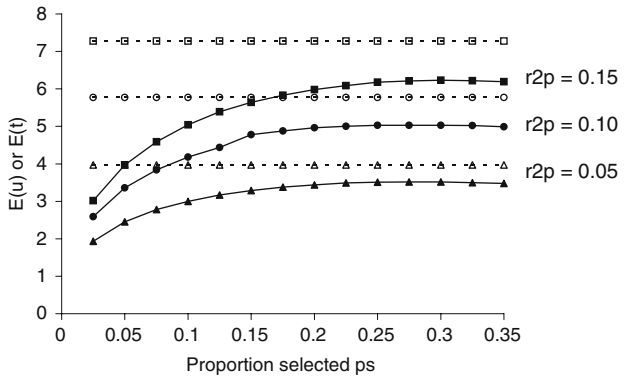

Fig. 2 Optimal proportion selected (p_S) for selective genotyping (BSG, *full symbols*) determined by the expected u value and according to r_P^2 for a given size of the phenotyped population. *Horizontal lines* with the same empty symbols corresponds to expected t for ANOVA with the same r_P^2 value

optimum is very flat for r_P^2 values between 25 and 40%. As expected, $E(u)$ is always less than $E(t)$ value for ANOVA. Considering the previous given optima, ratio $E(u)/E(t)$ is respectively, 0.88 and 0.82. For USG, as expected, the optimum p_S is the same as for BSG, but the ratio $E(u)/E(t)$ is lower: 0.66 with $r_P^2 = 0.05$ and 0.59 for $r_P^2 = 0.25$.

Optimisation of BSG for the same investment as in ANOVA method

Bidirectional selective genotyping

The two parameters which affect the optimal proportion selected p_S are the cost ratio r_{gp} and the proportion of variance explained by the marker r_P^2. Increasing the relative cost of genotyping decreases the optimum proportion p_S whereas increasing r_P^2 increases optimum p_S (Fig. 3). With a cost ratio $r_{gp} = 1$, a rather flat optimum is observed at $p_S = 0.20$ for $r_P^2 = 0.05$ with $E(u)/E(t) = 1.03$, and at $p_S = 0.25$ for $r_P^2 = 0.25$with $E(u)/E(t) = 0.93$, which means that the two methods are very close from the point of view of type I error. With $r_{gp} = 4$, the optimum is $p_S = 0.125$ for $r_P^2 = 0.05$ with $E(u)/E(t) = 1.26$, and $p_S = 0.15$ for $r_P^2 = 0.25$ with $E(u)/E(t) = 1.07$; thus, BSG leads to a more significant test for the presence of QTL than classic ANOVA. This is due to the increase in the population size of phenotyped individuals. As an example, for a cost ratio $r_{gp} = 4$, with $N_{an} = 200$, for the same investment in BSG, 500 genotypes can be phenotyped and 125 genotyped.

Figure 4 shows that at the optimal proportion selected, the power of BSG is higher than the power of ANOVA for a given cost and a given type I error when $r_{gp} > 1$. For example, for detecting a QTL with $r_P^2 = 0.05$, with an investment of 600 units of phenotyping cost, a cost ratio $r_{gp} = 2$ and a risk I threshold 0.01, the power for ANOVA is 0.73 whereas it is 0.87 for BSG. When the cost ratio is 4, with the same population size for ANOVA as previously (200) and the same risk I threshold, the power for BSG rises to 0.93. With a risk I threshold 0.001 with the same population size for ANOVA (200) and a cost ratio 4, the ANOVA power is 0.48 whereas it is 0.80 for BSG (curve not shown on Fig. 4). For the detection of a marker with $r_P^2 = 0.10$, a population size 100 for ANOVA and a risk I threshold 0.001, the power for ANOVA is 0.47 whereas it is 0.66 for BSG with a cost ratio 2, and 0.80 with a cost ratio 4.

Whatever the cost ratio r_{gp}, the conditions for the efficiency of optimal BSG corresponds to those of ANOVA. Optimal BSG can even be more efficient than ANOVA for a low population size and a high cost ratio. In these conditions, the power of BSG is quite similar to or better than that of ANOVA (Table 1). The power for BSG is higher than that of ANOVA,

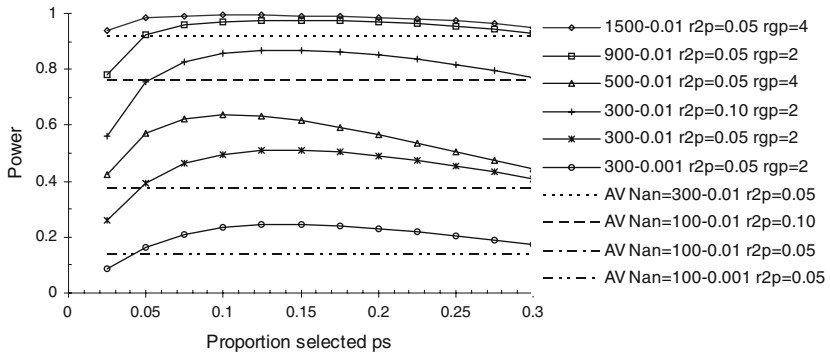

Fig. 3 Maximization of test power for BSG according to the proportion selected (p_S), total cost (first number in the legend), risk I threshold (0.01 or 0.001), r_P^2 (% of variance explained by the marker and cost ratio (r_{gp}). *Horizontal lines* represent ANOVA (AV) results. For risk I = 0.01 and $r_P^2 = 0.05$ $N_{an} = 300$ is a control for BSG with cost 900 for $r_{gp} = 2$ and 1,500 for $r_{gp} = 4$. $N_{an} = 100$ is a control for a BSG cost 300 and corresponding risk I and r_P^2

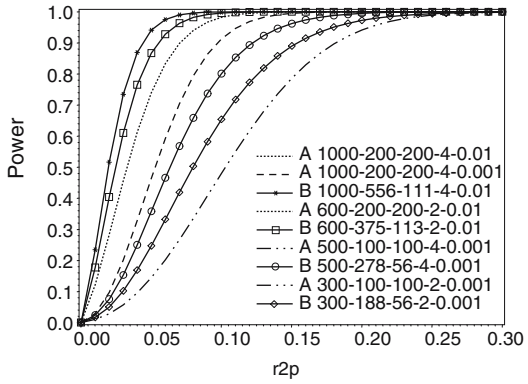

Fig. 4 Power of the bidirectional selective genotyping (*curves* B) for a given cost. The legend gives the value of the parameters: *I*–*N*1–*N*2–r_{gp}–risk I, where *I* is the total investment in units corresponding to the phenotyping cost of one genotype, *N*1 the number of phenotyped individuals, *N*2 the number of genotyped individuals, and r_{gp} the cost ratio. ANOVA corresponds to the case *N*1 = *N*2. Note that ANOVA curves (*broken lines* A) for *I* = 1,000, r_{gp} = 4 and for *I* = 600, r_{gp} = 2 are confounded as curves for *I* = 500, r_{gp} = 4 and for *I* = 300 and r_{gp} = 2, because the ANOVA power does not depend on r_{gp} for a given population size and type I error risk

when $r_{gp} > 1$ and if the efficiency of ANOVA is low, i.e. for a low population size and a low r^2_P. When r_{gp} is high, and r^2_P low, the greater efficiency of BSG is due to the fact that the sampling error on marker frequency becomes low due to the increased size of genotyped sample.

Gain in power due to BSG in comparison to ANOVA can be expressed in terms of number of genotypes which will have to be added to ANOVA in order to have the same power. With an investment of 1,000, a cost ratio 4 (population size 200 in ANOVA) and a risk I threshold 0.01, to have the same power (0.93) for the detection of markers with r^2_P = 0.10 as with the optimal BSG (556 phenotyped individuals and 111 genotyped), it would have been necessary to study about 325 lines, i.e. 125 supplementary lines. The gain is lower for situations leading to a lower power: low investment, low risk I threshold, low cost ratio.

Unidirectional selective genotyping

For USG the optimal proportion selected p_S is only slightly higher than for BSG (about + 0.03–0.05, i.e. 0.22 for r^2_P = 0.05, 0.27 for r^2_P = 0.25 with r_{gp} = 1 and 0.15 for r^2_P = 0.05, 0.20 for r^2_P = 0.25 with r_{gp} = 4). In comparison to ANOVA, if r_{gp} = 2, the power of USG is always below that for ANOVA, but not very much so when realistic population sizes are considered. With a population size 300 in ANOVA (leading to an investment of 900 in phenotyping units) at the risk I threshold 0.01, the power of unidirectional selective genotyping for the detection of a QTL with r^2_P = 0.05 is 0.83 while it is 0.92 with ANOVA; with r^2_P = 0.09 both powers are equivalent, about 99% (Fig. 5). For r_{gp} = 4, at the optimum, USG power is higher that ANOVA power, but with only a slight superiority. For example, with an investment of 500 phenotyping units (study of 100 RIL with ANOVA), with a risk I threshold 0.001, ANOVA power is 48% and USB power is 53%. USG appears then to be competitive in comparison to ANOVA with cost ratios 3 or 4, which are in a realistic range of values.

To have USG competitive with ANOVA (i.e. ratio $E(u)/E(t)$ around 1 and power 0.90), it is necessary to have a high cost ratio and a sufficiently large population (Table 2). Indeed a high cost ratio r_{gp} allows an increase in size of phenotyped and then of genotyped population, which leads to a gain in accuracy in the evaluation of marker frequency.

Application

Optimization of BSG for a given size of phenotyped population (N_{an})

For both populations, when considering all markers, whatever the trait, there is a strong correlation between square root of *F* ANOVA with all lines genotyped and *u*-test of BSG or USG with 50 to 110 lines

Table 1 Power of BSG at the optimum for the *u*-test (first number) compared to ANOVA power (second number) for the same cost

R_{gp}	r^2_P	p_S at opt for *u*-test	$E(u)/E(t)$ at opt	α = 0.01			α = 0.001		
				N_{an} = 100	N_{an} = 200	N_{an} = 300	N_{an} = 100	N_{an} = 200	N_{an} = 300
1	0.05	0.19	1.03	0.42/0.37	0.78/0.74	0.94/0.92	0.18/0.14	0.52/0.46	0.79/0.75
	0.10	0.21	1.01	0.78/0.76	0.98/0.98	1.00/0.99	0.53/0.48	0.92/0.91	0.99/0.99
	0.15	0.23	0.98	0.93/0.94	0.99/0.99	1.00/1.00	0.78/0.79	0.99/0.99	1.00/1.00
2	0.05	0.15	1.13	0.51/0.37	0.87/0.74	0.97/0.92	0.25/0.14	0.65/0.46	0.89/0.75
	0.10	0.17	1.10	0.86/0.76	0.99/0.98	1.00/0.99	0.64/0.48	0.96/0.91	0.99/0.99
	0.15	0.19	1.06	0.97/0.94	1.00/1.00	1.00/1.00	0.87/0.79	0.99/0.99	1.00/1.00
4	0.05	0.11	1.26	0.64/0.37	0.93/0.74	0.99/0.92	0.34/0.14	0.79/0.46	0.96/0.75
	0.10	0.12	1.22	0.94/0.76	1.00/0.98	1.00/0.99	0.80/0.48	0.99/0.91	1.00/0.99
	0.15	0.13	1.17	0.99/0.94	1.00/1.00	1.00/1.00	0.95/0.79	1.00/0.99	1.00/1.00

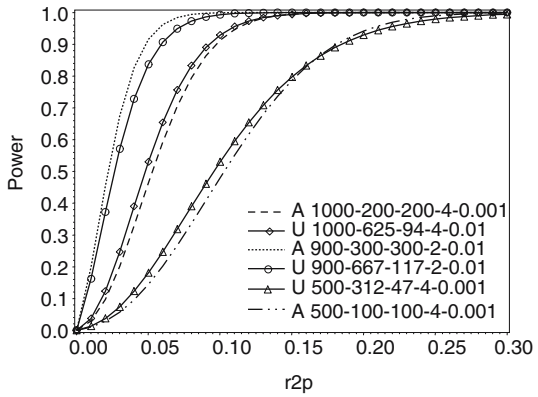

Fig. 5 Power of the unidirectional selective genotyping (*curves* U) for a given cost. The legend gives the value of the parameters *I*–*N*1–*N*2–r_{gp}–risk I, where *I* is the total investment in units corresponding to the phenotyping cost of one genotype, *N*1 the number of phenotyped individuals, *N*2 the number of genotyped individuals, and r_{gp} the cost ratio. ANOVA (*curves* A) corresponds to the case *N*1 = *N*2

genotyped (data not shown). Considering only markers significant at 0.05 in the ANOVA with all lines and the number of independent regions, for both types of populations and both traits (grain yield and grain moisture), the change in correlation according to the size of selected sample shows a rather flat optimum in the proportion selected between 23 and 36% (Table 3). This tends to confirm that p_S around 30% in each tail is the optimal proportion selected for a given size of phenotyped population, which is quite consistent with the theoretical prediction.

At the theoretical optimum there is a very good correspondence between regions detected by ANOVA on complete populations and those detected by BSG, for both traits and both populations (see Tables 4, 5 for grain yield). On average in both populations we always have *F* ANOVA > χ^2-BSG > χ^2–USG_{up} > χ^2–USG_{low}. The inequality *F* ANOVA > χ^2-BSG > χ^2-USG was expected. However, for LHRE population χ^2-BSG is closer to *F* ANOVA than to χ^2–USG_{up}. The lower statistic for USG_{low} than for USG_{up} was not expected. This is observed for both populations for grain yield, but not for kernel moisture. However χ^2–USG_{up} is closer to χ^2–USG_{low} than to χ^2-BSG. For grain yield, BSG detect 13/18 regions in LHRE and 9/10 in SAM population. The correlation between BSG and ANOVA is 0.78** for LHRE population (with 18 regions) and 0.89** for SAM population (with 10 regions). Correlations between USG and ANOVA are lower. This is due to a problem of sampling leading to lack of accuracy, mainly for USG_{low}. Indeed, in LHRE population, a selected sample of 140 individuals instead of 90, does not change the correlation for USG_{up} but increases the correlation from 0.52 to 0.61 for USG_{low} (but simultaneously, for BSG the correlation drops from 0.78 to 0.67, because a sample size of 140 is too far from the optimum). Poor correlations (not significant) were observed between *u*-USG_{up} and *u*-USG_{low} for both populations and both traits.

Optimization of BSG for the same investment as ANOVA

With the same amount of investment, BSG appears to be better than ANOVA whatever the cost ratio, the trait and the population (Table 6). USG (upper or lower) is equivalent to ANOVA with the same investment for yield in both populations. For kernel moisture, it is even better than the corresponding ANOVA for both populations and a cost ratio 3 or 4. For r_{gp} = 4, due to a decrease in the size of phenotyped and genotyped populations the correlation with com-

Table 2 Power of USG at the optimum for the *u*-test (first number) compared to ANOVA power (second number) for the same cost

R_{gp}	r_P^2	p_S at opt for *u*-test	$E(u)/E(t)$ at opt	α = 0.01			α = 0.001		
				N_{an} = 100	N_{an} = 300	N_{an} = 500	N_{an} = 100	N_{an} = 300	N_{an} = 500
1	0.05	0.22	0.79	0.22/0.37	0.71/0.92	0.93/0.99	0.07/0.14	0.44/0.75	0.77/0.99
	0.10	0.23	0.78	0.49/0.76	0.97/1.00	1.00/1.00	0.23/0.48	0.87/0.99	0.99/1.00
	0.15	0.25	0.76	0.70/0.94	1.00/1.00	1.00/1.00	0.43/0.79	0.98/1.00	1.00/1.00
	0.20	0.27	0.74	0.84/0.99	1.00/1.00	1.00/1.00	0.63/0.94	1.00/1.00	1.00/1.00
2	0.05	0.20	0.90	0.30/0.37	0.83/0.92	0.98/0.99	0.11/0.14	0.61/0.75	0.98/0.99
	0.10	0.21	0.88	0.62/0.76	0.99/1.00	1.00/1.00	0.35/0.48	0.96/0.99	1.00/1.00
	0.15	0.23	0.85	0.83/0.94	1.00/1.00	1.00/1.00	0.58/0.79	0.99/1.00	1.00/1.00
	0.20	0.25	0.83	0.94/0.99	1.00/1.00	1.00/1.00	0.79/0.94	1.00/1.00	1.00/1.00
4	0.05	0.15	1.04	0.42/0.37	0.94/0.92	0.99/0.99	0.18/0.14	0.79/0.75	0.99/0.99
	0.10	0.17	1.00	0.78/0.76	1.00/1.00	1.00/1.00	0.53/0.48	0.99/0.99	1.00/1.00
	0.15	0.18	0.97	0.93/0.94	1.00/1.00	1.00/1.00	0.77/0.79	1.00/1.00	1.00/1.00
	0.20	0.20	0.91	0.98/0.99	1.00/1.00	1.00/1.00	0.93/0.94	1.00/1.00	1.00/1.00

Table 3 Correlation between the *u*-test of BSG and USG for different number of selected lines and the square root of *F* ANOVA (for all lines and considering only significant markers) for grain yield in both populations (LHRE and SAM)

N_s (p_S)	30 (10%)	50 (16.6%)	70 (23.3%)	90 (30%)	110 (36.6%)
BSG in LHRE population	0.51	0.72	0.83	0.78	0.65
BSG in SAM population	0.79	0.93	0.83	0.91	0.90
USG_{up} in LHRE population	0.29	0.47	0.70	0.67	0.68
USG_{up} in SAM population	0.65	0.80	0.83	0.84	0.85
Average correlation	0.56	0.73	0.79	0.80	0.77

Table 4 Detection of genomic regions associated with grain yield in LHRE population according to different systems of detection

Chromo	Position in cM[a]	Number of markers	*F* ANOVA[b]	χ^2 BSG[b]	χ^2 USG^b_{up}	χ^2 USG^b_{low}
1	715–729	2	6.6*	6.4*	2.6	3.8
1	836	1	8.3**	8.8**	4.8*	4*
2	188–221	5	9.4**	6*	4.6*	1.8
2	223–269	6	10**	6.1*	7.1**	0.6
2	382–484	7	11.9***	9.8**	6.2*	4*
4	144–167	5	10.6**	8.9**	8.1**	2
4	174–206	4	10.1**	11.4***	7.7**	4.2*
4	301–329	4	12.2***	9.9**	4.2*	5.7*
4	395–397	2	8.8**	4.8*	2.9	2
4	435–527	6	10.4**	10**	3.4	6.9**
4	550	1	14.3***	14.2***	6.7**	7.5**
5	312–331	3	9.4**	8**	6.9**	1.9
6	0–48	4	15***	8.8**	5.1*	3.9*
6	116–142	5	18.3***	13.5***	8.3**	5.4*
8	122–139	4	10.8***	11.7***	9.3**	3.3
8	157–211	5	9.6**	9.4**	5.2*	4.3*
9	109	1	12.3***	8.6**	2.1	7.4**
9	364–368	2	7.2**	6.1*	3.1	3
Average statistics			10.8	9.0	5.5	4.0

ANOVA with all lines, USG and BSG at their optimum (p_S = 30%) for a given size of phenotyped population (322)

*,**,*** significant at 0.05, 0.01 and 0.001, respectively

[a] In reference to the equivalent map length

[b] Average statistics for the markers in the interval

Table 5 Detection of genomic regions associated with grain yield in SAM population according to different systems of detection

Chromo	Position cM	Number of markers	*F* ANOVA[a]	χ^2 BSG[a]	χ^2 USG^a_{up}	χ^2 USG^a_{low}
1	0	1	10.6***	9.6**	8.5**	2.1
1	168	1	14.7***	11.8***	5.8*	6*
2	40–84	4	23.3***	18.4***	8.4**	10.2**
2	104–126	3	18.7***	15.9***	6.9**	9.1**
3	13	1	12***	7.1**	3.7	3.5
4	8	1	10.9***	4.6*	5.6*	0.4
4	44–96	4	25.8***	13.3***	5.6*	8**
7	62–70	2	10.1**	4.7*	4.3*	1
8	0	1	8.3**	3.8*	2.3	1.5
8	58	1	7.2**	3.2	2.6	0.9
Average statistics			14.2	9.2	5.4	4.3

ANOVA with all lines, USG and BSG at their optimum for a given size of phenotyped population (300)

*,**,*** significant at 0.05, 0.01 and 0.001, respectively

[a] Average statistics for the markers in the interval

Table 6 Correlation coefficients between square root of the *t*-test for complete ANOVA (300 individuals) and *u*-test for optimal BSG or USG compared to ANOVA with the same investment and different cost ratios

r_{gp}	System	N^{c}_{pheno}	N^{d}_{geno}	Cost[e]	LHRE population		SAM population	
					Yield	Moisture	Yield	Moisture
1	BSG	300	120	420	0.77**	0.91**	0.92**	0.95**
	Anova/BSG[a]	210	210	420	0.69**	0.86**	0.88**	0.91**
	USG_{up}	300	68	368	0.56*	0.76**	0.85**	0.93**
	USG_{low}	300	68	368	0.67**	0.77**	0.63	0.77*
	Anova/USG[b]	184	184	368	0.61**	0.80**	0.82**	0.88**
2	BSG	300	106	512	0.76**	0.84**	0.92**	0.94**
	Anova/BSG	170	170	510	0.57*	0.78**	0.80**	0.88**
	USG_{up}	300	60	420	0.56*	0.76**	0.85**	0.93**
	USG_{low}	300	60	420	0.67**	0.77**	0.63	0.77*
	Anova/USG	140	140	420	0.48	0.74**	0.72**	0.83**
3	BSG	300	90	570	0.73**	0.88**	0.92**	0.94**
	Anova/BSG	143	143	572	0.52*	0.72**	0.76**	0.81**
	USG_{up}	300	53	459	0.51*	0.76**	0.81**	0.91**
	USG_{low}	300	53	459	0.60*	0.64**	0.61	0.81**
	Anova/USG	114	114	456	0.44	0.66**	0.68**	0.76*
4	BSG	300	75	600	0.68**	0.89**	0.86**	0.92**
	Anova/BSG	120	120	600	0.46	0.69**	0.70**	0.73*
	USG_{up}	300	45	480	0.48*	0.75**	0.78**	0.8*
	USG_{low}	300	45	480	0.50*	0.70**	0.62	0.83**
	Anova/USG	96	96	480	0.39	0.60**	0.64*	0.73*

Only markers significant at 0.05 in the complete ANOVA were considered. The significance of the correlation coefficient is approximative: it considers the number of independent regions (i.e. for yield, 18 regions for LHRE population and 10 for SAM population, and for kernel moisture 24 regions for LHRE population and 9 for SAM population)

*,** significant at 0.05 and 0.01, respectively

[a] ANOVA for the same cost as BSG

[b] ANOVA for the same cost as USG

[c] Size of the phenotyped population

[d] Size of the genotyped population

[e] Cost in phenotyping units

plete ANOVA tends to be lower than for $r_{gp} = 1$ or 2. However BSG is always the best method.

Discussion

For predicting change in gene frequency, infinitesimal model (Falconer 1960; Griffing 1960) and Darvasi and Soller's model (1992, 1994) were shown to be valid only for low QTL effects ($r^2_P < 0.05$) and low selection intensities. However, using our BSG approach without assumption on QTL effect, the optimal proportion to select, for a given investment, were close to those determined by the Darvasi and Soller (1992) approach based on the comparison of the means of pooled sample of the upper and lower tails for each marker genotype. In our approach, the optimum depends on the proportion r^2_P of variance explained by the marker, but the optima being relatively flat, the conclusions in terms of the power are not very different.

It is necessary to distinguish the optimization for a given size of the phenotyped population and for a given investment. For a given size of the phenotyped population, the optimal proportion to select is around 30%. In this situation, BSG is expected to lead to the detection of marker–QTL linkage with a lower power, but with an acceptable power when N_{an} is sufficiently large. For example for $N_{an} = 200$, $r^2_P = 0.10$ and type I error $\alpha = 0.01$, the power of BSG is 0.937 whereas that for ANOVA is 0.982. In our experiments, on both populations with N_{an} around 300, almost the same markers were detected. Furthermore, this result was obtained with a lower cost. With a cost ratio $r_{gp} = 4$, BSG allows about 33% cost reduction and USG leads to a 56% cost reduction, which could be a point to consider although USG is less competitive from the point of view of the power. With $r_{gp} = 2$, the cost reduction is still 27% for BSG and 47% for USG. In fact, it is better to compare USG and BSG to ANOVA with the same investment. For the same investment as

in ANOVA, BSG or USG allow the study of a larger population size. For example, assuming a cost ratio r_{gp} = 4, N_{an} = 200 and p_S = 0.30, the same investment in BSG leads to phenotype 294 lines and about the same power as ANOVA (0.99) would have been obtained. However, at the optimum, i.e. p_S around 0.125, BSG will be still more efficient than ANOVA (the ratio $E(u)/E(t)$ is 1.26).

For the same amount of investment, BSG appears to be quite competitive with ANOVA when the cost of genotyping of an individual is higher than the cost of phenotyping. Even USG, which was not considered by Darvasi and Soller (1992), can be competitive when the cost of genotyping of an individual is more than three times the cost of phenotyping. This is due to the fact that for the same investment it is possible to phenotype more individuals and then to select the best 15–20% allowing a selection of a sufficiently high number of individuals in order to have an acceptable accuracy of the genotype marker frequencies. Thus, the interest of BSG and USG depends on the relative cost of genotyping to phenotyping. Examination of the situation in Europe for a species like maize, leads to the conclusion that the cost ratio is around 2, i.e. a value which justified BSG.

Simulations from experimental data confirm the predicted results: at the optimum population size, for the same amount of investment, BSG is expected to be better than ANOVA, whereas USG is expected to be equivalent. The non significant correlations observed between u-USG_{up} and u-USG_{low} could have two origins. First, it could be due to a too low size of the selected sample. Indeed the correlation increases with the number of selected lines, but it remains relatively low. It could also be due to epistasis, i.e. interaction between QTL and genetic background which is not the same in both distribution tails. Furthermore, in presence of digenic epistasis there is asymmetry between USG_{up} and USG_{low} for the effect of selection on gene frequency. This asymmetry could also be due to an asymmetrical distribution. However, distributions of line values for yield and grain moisture were non significantly different from a normal distribution and thus were not asymmetrical.

The main limitation of selective genotyping in multi-trait selection is that it would be necessary to develop a specific population for each trait. However, the breeder could be mainly interested in finding QTLs of one major trait in order to develop a marker-assisted selection for this trait. Furthermore, if the breeder is interested in finding markers associated with different traits, it is possible to use DNA pooling of selected individuals as proposed by Darvasi and Soller (1994). Indeed it is possible to evaluate marker frequency on a DNA mixture [Dubreuil et al. (1999) for RFLP and Dubreuil et al. (2006) for microsatellites]. A DNA pooling of selected individuals will then be carried out for each trait. This is even the most economical method for the detection of associations between markers and QTLs. As proposed by Darvasi and Soller (1994) it will be necessary to take into account technical error in the determination of allele frequencies from the band intensities. The presence of such an error requires replications of the determination, but this will not suppress the strong economic advantage of the method even if two or three main traits are considered. For the same investment this method will allow increasing the population size and thus it will result an increase in the power.

One of the main interest of the selective genotyping is that it allows breeder to detect markers associated to QTLs by using only the selected individuals in one or two tails; then, these markers can be used in subsequent generations to perform marker-assisted selection by selecting only on markers. With this aim, as shown by Moreau et al. (1998) a relatively high type I error can be taken, mainly at low heritability, because false QTLs have a limited impact on the effectiveness of marker-assisted selection and the most important is to retain markers which are associated to QTLs with significant contribution to the trait variation. With this strategy of selective genotyping, detection of markers useful in selection can be more easily introduced in the process of conventional selection than the classical methods of QTL detection involving genotyping of all individuals. It could then favour the development of marker-assisted selection in plant breeding.

Appendix. Derivation of the power

Power of t-test for the comparison of means

The t-test for comparison of the two marker genotype means $\overline{MM}$ and $\overline{mm}$, can be written

$$t = \frac{\overline{MM} - \overline{mm}}{2\sqrt{\sigma^2_W/N}} = \frac{(\alpha_U - \alpha_L)}{2\sqrt{\sigma^2_W/N}} + \frac{a^*}{\sqrt{\sigma^2_W/N}}$$

where εU (εL) are random variation due to sampling and $a^*/\sqrt{\sigma^2_W/N} = r_P\sqrt{N}/\sqrt{(1-r^2_P)} = E(t) = c$ is the noncentrality parameter, i.e. the expected value of test knowing that the H_0 assumption of absence of difference between the two means is wrong. Then, the probability β associated with risk II, i.e. to conclude on the absence of difference knowing that they is a difference, is (Dagnélie 1975)

$\beta = \Phi(u') - \Phi(u)$ and
the power is $P = 1 - \beta = 1 - [\Phi(u') - \Phi(u)]$

where Φ is the normal distribution with variance 1, and u' and u'' define an interval centered on $-c$: $u' = u - c$, $u'' = -u - c$, u being defined for threshold $1-\alpha/2$ ($\Phi(u) = 1-\alpha/2$). This approach gives the same results as Charcosset and Gallais (1996).

Power *u*-test for the comparison of marker frequencies

The u-test for the difference in marker frequency in the situation of BSG, can be written $u = \frac{P_U - P_L}{\sqrt{0.5/N_S}} = \frac{(\varepsilon_U - \varepsilon_L)}{\sqrt{0.5/N_S}} + \frac{2\Delta p}{\sqrt{0.5/N_S}}$ where ε_U (ε_L) are random variation in gene frequency due to sampling and $2\Delta p/\sqrt{0.5/N_S} = E(u) = c$ is the noncentrality parameter, i.e. the expected value of test knowing that the H_0 assumption of absence of difference between p_U and p_L is wrong. Then, from this noncentrality parameter, the probability β associated with risk II, is derived as above. For USG, the noncentrality parameter is $\Delta p/\sqrt{0.25/N_S}$.

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