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## Experimental designs for Quantitative trait loci detection in the New Zealand dairy industry.

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### ABSTRACT

Detection of loci that affect quantitative traits such as milk production has been undertaken with daughter and granddaughter designs in the N.Z. dairy industry. Two other experimental designs, selective DNA pooling of trait extremes and the use of first cross Holstein-Friesian-Jersey bulls, offer the opportunity to detect more loci than the current two designs. Selective DNA pooling involves the collective pooling of DNA from daughters at each extreme (high and low) of the trait distribution. A sire family of 100,000 daughters with DNA pools created from the top and bottom 1% of progeny pooled has the same power (probability of detection) as approximately 5,000 progeny being individually genotyped with less than 1% genotyping effort. Crossbreeding in the New Zealand dairy industry opens up the opportunity to identify QTL alleles that contribute to the genetic differences between the two breeds. Identification of breed specific QTL alleles can be achieved at a power of 90% based on some 600-1500 progeny from matings of F1 Holstein-Friesian x Jersey bulls to F1 cows.

**Keywords:** Quantitative trait loci; experimental design; dairy industry.

### INTRODUCTION

Quantitative trait loci (QTL) studies in dairy cattle are being undertaken in many countries (Georges *et al.*, 1995; Ron *et al.*, 1996; Vilkki *et al.*, 1997). Dairy industries in New Zealand and the Netherlands are collaborating through Livestock Improvement (LIC) and Holland Genetics (HG) to detect QTL for milk production traits using granddaughter and daughter designs (Weller *et al.*, 1990). Thirty-two sires with an average of 35 progeny tested sons make up the granddaughter design and 8 sires with an average of 800 daughters comprise the daughter design. Selective genotyping (Darvasi and Soller, 1992) has been applied in the daughter design to increase power of detection per unit of genotyping. This work has led to QTL being successfully identified in the granddaughter design (Spelman *et al.*, 1996; Arranz *et al.*, 1998; Huisman *et al.*, 1998) and in the daughter design (Singireddy, 1998).

Darvasi and Soller (1994) outlined an extension of selective genotyping where the DNA from the selected animals from each extreme of the distribution are pooled. These authors showed the genotyping cost is minimal yet this design has high statistical power for sires that have many daughters. Selective DNA pooling has been successfully applied in the Israeli dairy industry (Lipkin *et al.*, 1998). Large half-sib families exist in the New Zealand dairy industry and thus make this experimental design attractive.

In livestock species such as pig and poultry, experimental populations have been bred using inbred or divergent lines in F<sub>2</sub> or backcross designs for QTL detection. These designs are more powerful than the granddaughter and daughter designs due to greater marker informity and larger allelic contrasts than occurs within outbred

populations. In dairy cattle, experimental populations have not been formed due to long generation intervals, low female fecundity and the inability to maintain reproductive rate in inbred lines. The New Zealand dairy industry has two predominant breeds, Holstein-Friesian and Jersey that are farmed as straightbreds and crossbreds. The two breeds differ in their yield and milk composition and thus a cross between the two could be viewed as a divergent cross. QTL alleles that contribute to the genetic differences between the two breeds could be exploited in a breeding programme that uses marker-assisted selection and crossbreeding.

This paper outlines the statistical power of QTL detection for the current experimental designs: granddaughter and daughter design, and for prospective QTL experimental designs: selective DNA pooling and Holstein-Friesian and Jersey cross, that are available to the New Zealand dairy industry.

### METHOD AND RESULTS

**Granddaughter design:** In the granddaughter design a sire and his progeny-tested sons are genotyped and phenotypic records are collected on the granddaughters of the sire to calculate estimated breeding values (EBV) for the sons. Segregation of heterozygous marker loci is followed for the two sire alleles from the sire to the sons. Significant differences in mean EBV between the two groups of sons inheriting alternate sire alleles at a marker locus indicates the presence of a linked QTL.

Statistical power, where power is 1 minus the type II error rate or equivalently the probability of detecting the QTL, was calculated using the chi-square approach of Weller *et al.* (1990). The parameters used in the power calculations reflect the size of the current LIC/HG design;

thirty-two sires with an average of 35 half-sib sons each progeny tested from 85 daughters. A nominal type I error of 0.001 is used which accounts for the multiple testing that is undertaken at each of some 200 marker loci and for each trait investigated. Other assumptions are that the heritability of the trait is 25%, the frequency of the QTL allele in the population is 0.5 and the recombination rate between marker and QTL is 0.05 (equivalent to a separation of 5 cM or some 500,000 base pairs). Allelic frequencies deviating from 0.5 have lower probability of being detected as the expected heterozygosity decreases.

**TABLE 1:** Power of granddaughter daughter and selective DNA pooling design for different QTL effects, nominal type I error of 0.001, trait heritability of 25%, QTL allele frequency is 0.5 and 0.05 recombination rate between marker and QTL.

| QTL effect |            | Experimental design |      |         |
|------------|------------|---------------------|------|---------|
| $\sigma_p$ | $\sigma_G$ | Gddtr               | Dtr  | Pooling |
| 0.10       | 0.20       | 0.01                | 0.06 | 0.40    |
| 0.15       | 0.30       | 0.05                | 0.33 | 0.92    |
| 0.20       | 0.40       | 0.19                | 0.68 | 1.00    |
| 0.25       | 0.50       | 0.48                | 0.88 | 1.00    |
| 0.30       | 0.60       | 0.78                | 0.95 | 1.00    |
| 0.35       | 0.70       | 0.94                | 0.98 | 1.00    |
| 0.40       | 0.80       | 0.99                | 0.99 | 1.00    |

Gddtr= granddaughter; thirty-two sires with an average of 35 progeny tested sons each.

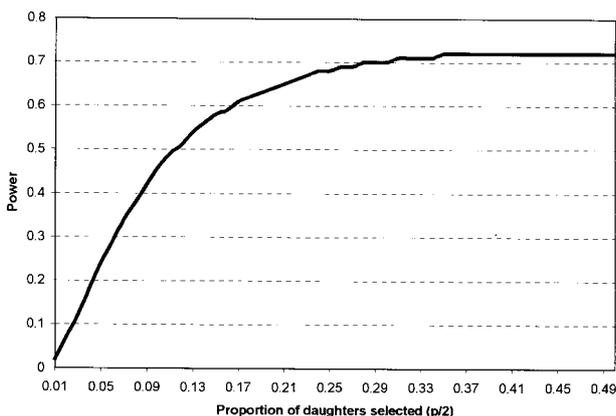
Dtr = daughter; eight sires with an average of 800 daughters each with the top and bottom 25% of daughter selectively genotyped.

Pooling = Selective DNA pooling; 1 heterozygous sire with 75,000 daughters with the top and bottom 1% separately pooled.

The granddaughter design has near full power for the largest simulated effect of 0.4  $\sigma_p$  (Table 1). A QTL of this size would explain 8% of the phenotypic variance or equivalently 32% of the genetic variance assuming two QTL alleles at equal frequency. For QTL 0.20  $\sigma_p$  the power of the granddaughter design is low (Table 1). Only large QTL ( $\geq 0.25 \sigma_p$ ) will be detected using this design.

**Daughter design:** The daughter design is similar to the granddaughter design but daughters of the sire are

**FIGURE 1:** Power of the daughter design for different proportion of daughters selected for eight sires with an average of 800 daughters each, nominal type I error of 0.001, trait heritability of 25%, QTL allele frequency is 0.5 and 0.05 recombination rate between marker and QTL.



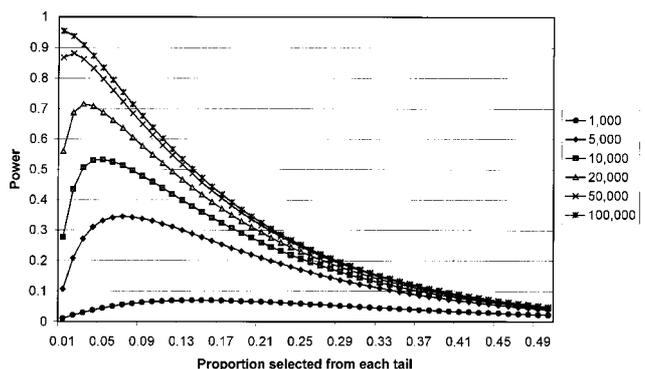
genotyped and phenotyped. The granddaughter has 3-4 times more power than the daughter design for the same number of genotype assessments due to greater accuracy in EBV evaluation of progeny tested bulls than lactating cows. Selective genotyping (Darvasi and Soller, 1992), which is individual genotyping of the daughters at the extremes of the trait distribution, is a tool that can increase the power per genotype. QTL effects from an experiment using selective genotyping are inflated due to a positive correlation between residual effects and genotype value. This bias can easily be adjusted for (Darvasi and Soller 1992). Figure 1 outlines the power of the daughter design for 8 sires and 800 daughters each for different proportion of the daughters being genotyped for a QTL of 0.2  $\sigma_p$  (all other parameters as before). The power of the daughter design does not decrease dramatically until the proportion selected is less than 20-25% from both tails of the distribution. The power of this design for different sized QTL is outlined in Table 1 with a selected proportion of 25% from each tail and with the fore-mentioned assumptions.

The power of the daughter design with 8 sires and 800 daughters selectively genotyped is greater than the granddaughter design with 32 sires and 35 progeny tested sons (Table 1). However, it must be noted that the daughter design requires nearly 3 times more genotyping for the same power. Selective genotyping in this example reduces the number of genotypes by half with minimal reduction in power. Therefore the granddaughter design is still 1.5-2 times more powerful per genotype than the daughter design with selective genotyping. The daughter design is likely to detect QTL that are  $\geq 0.20 \sigma_p$ .

**Selective DNA pooling:** Selective DNA pooling is an extension of selective genotyping with the DNA from the daughters from each tail of the distribution pooled (Darvasi and Soller, 1994). The two pools are genotyped and the marker allele frequencies are estimated for each pool. When there is a significant difference in sire marker allele frequency this indicates the presence of a linked QTL.

The extensive use of elite bulls in the New Zealand dairy cattle population results in sires having up to 100,000 lactating daughters. The large family size has an impact on the power of detection as the larger the half-sib family the

**FIGURE 2:** Power of selective genotyping for a heterozygous sire with a QTL effect of 0.15  $\sigma_p$  for different proportion of daughters selected and for different sized half-sib families.



greater the power (Figure 2). The optimum percentage of daughters to select decreases as the size of the half-sib family increases. However more daughters have to be sampled for the larger half-sib families. For example, for a family of 5,000 or 10,000 the optimum number of daughters to be selected from each tail is 400-500 whereas for half-sib families of 50,000 or 100,000 the optimum number of daughters is 1,000 from each tail.

The power for a half-sib family of 100,000 for a  $0.15\sigma_p$  QTL is some 95% (Figure 2). To attain 95% power with total genotyping some 5,000 daughters would be required and some 3,000 daughters with selective genotyping. For a genome scan with 200 markers this would require 400 (2 pools 200 markers) genotypes for selective DNA pooling, 600,000 for selective genotyping and 1,000,000 for total genotyping.

Power of detection with selective DNA pooling is far superior to that of the granddaughter and daughter design (Table 1). This experimental design is able to detect QTL of small size ( $0.1\sigma_p$ ).

#### Holstein-Friesian and Jersey QTL experiment:

The primary objective of a QTL experiment with the two breeds would be to identify chromosomal regions that contribute to the genetic differences between the Holstein-Friesian and Jersey breeds. The design would first involve purebred Jersey and Holstein-Friesian animals being interbred to form  $F_1$  bulls and cows. The  $F_1$  bulls will be heterozygous at all loci that are fixed with different allelic forms in the two breeds. Gametes produced by the  $F_1$  animals will segregate the alternative forms of alleles. The options for the experiment are either a backcross ( $F_1$  bull mated to HF and J cows) and/or  $F_2$  experiment ( $F_1$  bulls interbred with  $F_1$  cows). Approximately twice as many animals are required for the backcross design to have equivalent power to the  $F_2$  design and therefore the  $F_2$  design is the preferred option.

Three possible scenarios that may occur when detecting differences between the two breeds need to be considered when calculating the power of a  $F_2$  design;

- i) The two breeds are homozygous for different allelic forms at the QTL loci and marker haplotypes can be identified at breed level.
- ii) The two breeds are not homozygous at the QTL and share QTL alleles in common, but at different frequencies, and marker haplotypes can be identified at breed level.
- iii) The two breeds are not homozygous at the QTL and share QTL alleles in common but at different frequencies, and they have some marker allele sharing which reduces the ability to identify marker haplotypes at breed level.

Microsatellite markers are usually highly informative and therefore breed origin should be able to be ascertained for the markers. Furthermore, the development of new markers in the next five years will enable the selection of breed specific markers. Therefore the first two scenarios are used for power calculations.

The number of  $F_2$  offspring required to attain 90% power (assumptions as before for power calculations)

increases as the degree of allele sharing increases between the two breeds (Table 2). For example when the breeds are fixed for different alleles 958  $F_2$  offspring are required to attain 90% power for a  $0.2\sigma_p$  QTL. A gene of  $0.2\sigma_p$  is some 4.5 kg for fat, 3.3 kg for protein, 105 l for milk and 6.9 kg for liveweight. The breed differences between Holstein-Friesian and Jersey are some 10 kg for fat, 17 kg for protein, 850 l for milk and 89 kg for liveweight. When the allele frequency in one breed is 90% and 10% in the other breed 1,497  $F_2$  offspring are required and when the allele frequency in one breed is 80% and 20% in the other breed 2,662  $F_2$  offspring are required (Table 2).

**TABLE 2:** Required number of  $F_2$  offspring to have 90% power for different QTL sizes and different degrees of QTL allele sharing between the two breeds.

| QTL effect ( $\sigma_p$ ) | Degree of QTL allele sharing |         |         |
|---------------------------|------------------------------|---------|---------|
|                           | 1:0                          | 0.9:0.1 | 0.8:0.2 |
| 0.10                      | 4,732                        | 7,394   | 13,145  |
| 0.15                      | 1,704                        | 2,662   | 4,732   |
| 0.20                      | 958                          | 1,497   | 2,662   |
| 0.25                      | 613                          | 958     | 1,703   |
| 0.30                      | 426                          | 665     | 1,183   |
| 0.35                      | 313                          | 489     | 869     |
| 0.40                      | 240                          | 374     | 665     |

Note: the allele frequencies in the two breeds do not have to equal 1, but have arbitrarily been chosen in this way.

## DISCUSSION

Genetic responses from computer simulated marker-assisted selection in dairy cattle breeding schemes have been significant when the proportion of genetic variance explained by markers is reasonably large (e.g. 20%). The granddaughter and daughter designs both have medium to high power for the QTL with large effects. Unless the genetic variance is predominantly comprised of large sized QTL these designs will not explain a large proportion of the genetic variance. Utilisation of the QTL in marker-assisted selection will increase the rate of genetic gain and increase dairy industry returns but the impact will not be large (Spelman and Garrick, 1997, 1998).

Selective DNA pooling is a design that utilises the large daughter numbers from the elite sires due to their extensive use enabled by liquid semen technology. The power of selective DNA pooling with half sib families of 50,000-100,000 is very high even for small sized QTL ( $0.15\sigma_p$ ). In addition, the required number of genotypes to identify the QTL is less than 1% of that required if the equivalent power was achieved in a daughter design with selective genotyping. However, individual pools have to be formed for each trait analysed with selective DNA pooling. So for the three milk production traits (milk volume, milk fat and milk protein) and the two percentage traits (milk fat %, milk protein %) the required number of genotypes would increase 5-fold but would still only be some 3% of that required with selective genotyping. In the future it is likely that there will be statistical development that will enable QTL effects to be estimated for traits other

than the trait that selection was undertaken as has occurred with selective genotyping (Bovenhuis and Spelman, 1998).

The use of milk as the source of the DNA (Lipkin *et al.* 1998) removes the cost of collecting blood from the identified daughters that could be spread through many hundreds of herds throughout New Zealand. Over 90% of the New Zealand dairy cattle are milk recorded / herd tested and all of this milk is analysed at the one site.

Marker-assisted selection has the potential to be utilised with crossbreeding in the New Zealand dairy industry. To identify the QTL that constitute the genetic differences between the two breeds the F<sub>2</sub> design with approximately 1,000 female progeny would be very powerful. This design would enable other traits such as milk characteristics, fertility and disease resistance to be analysed for marker-QTL linkages. Currently this can not be done as the traits phenotyped in the dairy industry are milk yield and conformation traits.

Once the chromosomal regions have been identified in the two breeds the potential is there to generate crossbred individuals that have a combination of the best QTL alleles from the two breeds. These crossbred individuals would be probably be bulls to be progeny tested and then the genes disseminated through semen. Genes from crossbred females could be disseminated through cloning if it is operational in the dairy industry in the future.

## CONCLUSIONS

Selective DNA pooling and a F<sub>2</sub> design involving Holstein-Friesian and Jersey are QTL experimental designs that could utilise unique aspects of the New Zealand dairy industry; large half-sib families and crossbreeding. These two QTL experimental designs have the potential to identify large proportions of the genetic variance that will enable marker-assisted selection to have a large impact on the New Zealand dairy breeding scheme.

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