A CROSSBREEDING EXPERIMENT TO DETECT QUANTITATIVE TRAIT LOCI IN DAIRY CATTLE

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INTRODUCTION

In the last decade, advances in molecular genetics have made it possible to dissect the genetic variability of complex traits into quantitative trait loci (QTL). In France, two large programmes were carried out for QTL detection. The first one, in collaboration with the French artificial insemination industry, was a typical granddaughter design and used national records for milk production and health traits. This programme, now completed, intended to detect genes responsible for within breed variability, *i.e.* the variability available for selection (Boichard *et al*, 2002). The second one, carried out at the INRA experimental farm of Le Pin-Au-Haras (Normandy, France), and still running, has intended to detect genes responsible for the variability between the Holstein and Normande breeds. The purpose of the present paper was to describe its design, *i.e*. the current family structure obtained from specific reproduction and mating methods and the traits recorded. Finally, the expected QTL detection power, based on the current design, was estimated by simulation.

MATERIAL AND METHODS

In 1993, a QTL experiment was designed at INRA by crossing Holstein and Normande breeds. These two breeds had been present on the experimental farm for twenty years. The Normande breed is a dual-purpose breed, less productive than the Holstein but with a higher milk concentration and better-cheese making properties. More beefy, it also exhibits a better fertility level and a lower fat mobilization after calving. Although these differences were much smaller than differences commonly observed between dairy and beef breeds, they were large enough for these two breeds to be good candidates to this experiment.

After preliminary simulation studies for assessing the expected QTL detection power, the initial design was crossing the two breeds in order to obtain at least 600 F2 females recorded in first lactation. Because these two breeds could not be considered as inbred lines, F2 females were generated in order to get as large full-sister families as possible, with embryo transfer and partly with embryo sexing, in order to save recipient requirements. F2 families were further extended using groups of full-sisters as F1 dams. Although more complex to analyse, this option provided additional detection power by increasing the effective family size.

The initial scheme (figure 1) was hierarchical and considered 10 F0 males (5 Holstein and 5 Normand), 30 F0 females (3 per F0 male), 3 or 4 F1 females per F0 female, and 5 F2 females per F1 female. Mating and selection rules were the following : 1) Least related F0 parents were selected to maximize initial within-breed variability ; 2) F0 and F1 females were selected on their embryo production performances only, in order to maximize family size ; 3) one F1 son was retained for each F0 sire; 4) matings between F1 avoided producing inbred F2 progeny ;

5) F1 full-sisters were mated to the same F1 sire; 6) finally, the objective was to obtain 60 granddaughters per F0 male and 60 daughters per F1male.

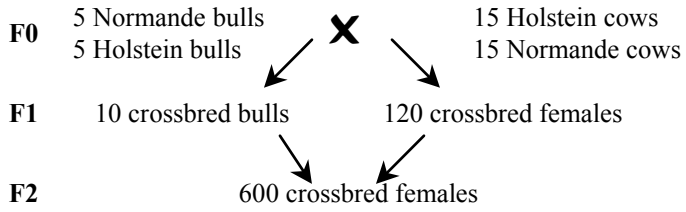


Figure 1. Initial design

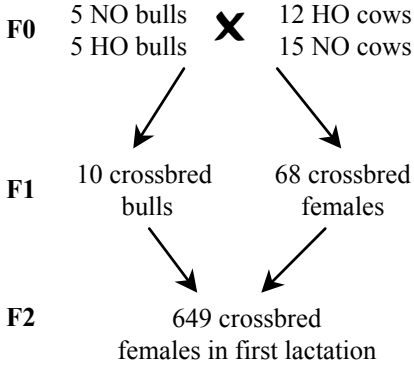
Reproduction management. The experimental farm capacity was 200 calvings and 130 first lactations per year. Embryo transfer as well as partial sexing were systematically used. On average, 40 donors were collected 3 times each year. One third of the embryos were fresh-transferred, the second third was sexed and female embryos (40%) were fresh-transferred, and the rest was frozen. F0 females were preserved from reproduction treatments during their first lactation. They were collected later than 90 days after calving during the second lactation, and every second month. Most F1 females were collected as heifer at 14 months. Then, they were inseminated after 1 to 3 collections and calved at 3 years of age. Embryo collections started again during lactation. The first lactation of the F2 females was treatment-free and these females were used as recipients for their first calving. In order to give room to first lactations, many cows were culled or sold in second or third lactation in the first two months after calving, resulting in a rather atypical age structure in the herd.

Current design. The experiment started in 1993 with the choice of F0 parents. F1 males were born in 1995-1996 (40 obtained, 10 retained), whereas F1 females were born in 1995-1998 (160 obtained, 68 retained). F2 female birth started in 1997 and will continue up to 2003. A total of 649 first lactations of F2 will be available by 2005. Figure 2 summarizes the design obtained. In comparison with the initial design, numbers of F0 and F1 females were reduced and F2 full-sister family size was increased. Table 1 shows the situation for each family. On average, each F0 male was mated to 2-4 F0 females to obtain 5-8 F1 females (by groups of 1 to 4 full sisters) and 55 to 78 granddaughters (by groups of 3 to 21 full sisters).

Recorded traits. During this experiment, many traits were measured. In addition to the classical measures, many traits difficult to record in commercial herds were considered. Most traits were recorded before or during the first lactation of the F2 females. Milk was recorded at each milking, whereas fat, protein, lactose, and cell counts were measured once a week. To measure cheese making ability, Camembert cheese were produced from 25 litres milk of each individual cow. Cheese yield and 30 physico-chemical factors were recorded during the process, as well as plasmine and plasminogene milk content. At each clinical mastitis event, a teat description and a bacteriological analysis of milk were carried out and repeated one month after. Teat thickness before and after milking twice in lactation, vacuum needed to open teat sphincter also twice during lactation, and milk emission kinetic parameters once in lactation were indicators of milkability. Both Normande and Holstein breed associations independently scored all cows two months after calving for 23 traits. Furthermore, all animals were photographed and a comprehensive description of their coat color was made twice, as a calf

and as a cow. To appreciate the ability to fat mobilization, body condition was scored every month, 6 metabolic components were sampled three times during the lactation and an active adrenaline test was also practiced. Age at puberty and post partum ovarian activity were monitored by progesterone assay every tenth day.

Figure 2. Current design*



* HO= Holstein, NO= Normande

Table 1. Survey of family situation

F0 Bulls *	F0 Females mated to F0 bulls	F1 Females	F2 Females / F1 female
HO1	4	8	9.75
HO2	3	8	9.75
HO3	3	5	11.80
HO4	3	8	8.13
HO5	2	7	8.57
NO1	2	7	8.57
NO2	3	5	12.40
NO3	2	6	9.17
NO4	3	7	8.71
NO5	2	7	10.14
Total	27	68	9.54

* HO = Holstein bull, NO = Normande bull

QTL Detection power. Detection power was estimated by simulation. The family structure of both initial and current designs was simulated. Polygenic values of F0 animals were drawn from normal distributions allowing for a difference between breeds, and polygenic values of F1 and F2 animals were generated from parental values and a Mendelian sampling effect, without non-additive effects. A single biallelic additive QTL was simulated with different breed frequencies. This QTL was closely linked to a completely informative marker with 2n alleles, where n was the number of F0 founders. This assumption may be considered too optimistic but is nearly equivalent in practice to a set of linked microsatellite markers. It provides an upper bound of the detection power that can be achieved with such a design.
The model of analysis was an animal model and included a mean, the polygenic effect, groups of unknown parents to account for the difference between breeds, and the fixed effect of the F0 founder markers received by the F2 progeny. Heritability was defined by the ratio polygenic variance/ (polygenic variance + environmental variance) and the true values were used in the analysis. The statistical test for the marker effect was a F test. Because some founder genes could be lost in the segregation, the number of degrees of freedom was not constant and the empirical test distribution under H0 was obtained with 5000 replicates assuming a null QTL effect. For each H1 hypothesis, 1000 replicates were computed. The value of increasing family size was measured by comparing the detection power with the initial and the current designs. Results are presented in table 2 and are in accordance with those of Gomez-Raya and Sehested (1999) for F2 families of 10 piglets.

Table 2. Detection power (%) of the original and the current designs with Type-I error =0.05, as a function of QTL effect, QTL within breed frequencies and heritability

Gene effect[1]	heritability	Initial design		Current design	
		QTL frequencies [2]			
		0.0 / 1.0	0.3 / 0.7	0.0 / 1.0	0.3 / 0.7
0.2	0.1	24	18	30	22
	0.5	30	22	37	24
0.3	0.1	59	46	71	53
	0.5	72	49	82	55
0.4	0.1	93	80	97	84
	0.5	97	84	99	88

[1] Gene effect is expressed in phenotypic standard deviation within breed x QTL genotype
[2] Frequencies of the favourable allele in the first and in the second populations, respectively

When the QTL was not fixed within breed, results showed that detection power was high for QTL with substitution effects of at least 0.4 standard deviation. When the QTL was fixed, detection power was still high for moderate QTL effects (0.3 standard deviation). The range of heritability values was rather high (0.1-0.5) : however, power corresponding to the lowest values was only slightly reduced. Finally, the current design was more powerful than the initial design, thus illustrating the effect of increasing family size. The largest difference was found for fixed QTL within breed and moderate effects (0.3), where power increased from 59 to 71%.

CONCLUSION

Simulation demonstrated that this crossbreeding experiment is likely to detect genes with substitution effect larger than 0.3 – 0.4 within breed x QTL genotype phenotypic standard deviation. This was obtained due to the use of enhanced reproduction methods for cattle (approximately 10 full-sisters per F2 family). Extending the size of maternal family would have still increased detection power. However, this would imply the use of even more intensive techniques such as IVEP for donors : this technique was not available at the beginning of the experiment.

A preliminary analysis is planned to be carried out in 2003, based on about 380 F2 females recorded in first lactation. The last data will be obtained in 2005 and will be processed for the final analysis. Then, the QTLs detected for between-breed variation will be compared to the QTLs currently detected within each breed and effectively used for marker assisted-selection.

REFERENCES

Boichard, D. et al (2002) *Genet. Sel. Evol.* (submitted)
Gomez-Raya, L. and Sehested, E. (1999). *Genet. Sel. Evol* **31** : 351-374