ISSN 0370-2731/2000 Proceedings of the New Zealand Society of Animal Production **60:** 281-283

Experimental design for detection of quantitative trait loci for susceptibility to Facial Eczema in dairy cattle.

R. J. SPELMAN, M. D. SELLARS AND K. SANDERS

Livestock Improvement Corporation, Private Bag 3016 Hamilton

ABSTRACT

Three hundred and fifty farmers involved in the Livestock Improvement Corporation Sire Proving scheme (SPS) were surveyed, in May 1999, on the incidence of Facial Eczema (FE) in their herds. The SPS farmers were asked to score their 2- and 3-year-old SPS daughters on a five point scale; 1=not affected, 2=slightly affected, 3=severely affected (still alive), 4=culled due to FE and 5=died due to FE, and to comment on any calves or yearlings that were in categories 3 to 5. In the herds with at least one case of FE (50% of respondees), 89% of the animals were not affected, 5% were slighty affected, 5% severely affected and 1% were either culled or died primarily from FE. Heritabilities of 3-17% verified that FE in this dataset was controlled in part genetically and with the DNA bank for all SPS daughters, enables a QTL study to be undertaken. The proposed experimental design is an association study with the pooling of DNA for the 516 daughters that were scored 3 or higher. The control for the experiment would be paternal half sibs for each of the 'affected' daughters from the same herd, if possible. To detect linkage disequilibrium (LD) a panel of some 1000 microsatellites would be genotyped.

Keywords: Facial Eczema; genetics, QTL; linkage disequilibrium.

INTRODUCTION

The late summer/autumn period in 1999 was reported in the general farming press as being one of the worst occurrences of Facial Eczema (FE) in the North Island. One way of reducing the incidence of FE may be through genetics. Resistance to FE in dairy cattle has been shown to have a genetic component with heritability estimates up to 0.77 (Morris *et al.*, 1990, Morris *et al.*, 1998).

Livestock Improvement Corporation (LIC) progeny tests approximately 235 bulls per year, which is termed the Sire Proving Scheme (SPS). The daughters of the progeny test bulls are farmed in approximately 420 herds. Paternity testing is undertaken in the LIC SPS on all progeny test daughters. An important by-product of the parentage testing is that DNA samples are collected and stored for some 15-20,000 daughters on an annual basis. This offers the opportunity of undertaking quantitative trait loci (QTL) experiments now, and in the future, without having to resample animals for DNA.

LIC has identified QTL for milk production (Spelman *et al.*, 1996, Arranz *et al.*, 1998) and has started to utilise the QTL through marker-assisted selection (MAS) by pre-selecting bulls before progeny testing. Candidate genes for FE have been identified and tested in sheep (Phua *et al.*, 1999). Regions of interest identified in the sheep models, have to date been unsuccessfully tested in bovine QTL searches.

This paper outlines survey data on the incidence of FE in the North Island during 1999, presents heritabilities for the trait as scored by farmers and discusses possible QTL experiments for FE and the implications for the New Zealand dairy industry.

SURVEY

Three hundred and fifty North Island SPS farmers were surveyed, in May 1999, on the incidence of FE in their herds. The SPS farmers were asked to score their 2 and 3 year old SPS daughters (approximately 20,000 cows, older daughters do not have DNA stored) on a five-point scale; 1- not affected, 2- slightly affected, 3- severely affected (still alive), 4- culled due to FE and 5- died due to FE, and to comment on any calves or yearlings that were in categories 3 to 5. All reported calves and yearlings were used in the estimation of heritability.

Approximately two-thirds of the surveyed farmers responded, with 50% of the respondees having at least one case of FE (score of 3, 4 or 5) in the designated animals. In the herds with at least one case of FE, 89% of the animals were not affected, 5% were slighty affected, 5% severely affected and 0.5% were culled due to FE and 0.5% died primarily from FE. The majority of farmers undertook preventative measures for FE.

Heritability: The model fitted to the data was:

$$y = Xb + Zu + e$$

where; y is the array of FE scores, X is the design matrix for the fixed effects (b), Z is the incidence matrix for the random effects (u), and e is the random residual. The fixed effects used in the model were overall mean, herd, age (years) and breed. Breed was separated into 3 classes; Holstein-Friesian (HF) (≥ 12/16ths HF), Jersey (J) ((≥ 12/16ths J) and the remaining animals in the other class. Jersey animals were more susceptible than the other two classes. Country of origin for the sire was not included in the final model because it was not significant.

The current dataset has 8592 records with 745 sires represented, thus, an average of 11.5 daughters per sire. The number of daughters per sire ranged from 1 to 244 with 11 sires having more than 50 daughters each. Holstein-Friesian cows made up the majority of the dataset with 5022 observations, Jersey 1868 observations and the balance in the other category. There were 516 affected animals (scored

as 3-5) in the dataset.

Heritabilities were estimated using AIREML (Johnson and Thompson 1995) primarily in an animal model setting. Heritability estimates were slightly higher for the non-transformed 1 to 5 scale compared to the Log_e and Snell-transformed variables when all of the data were analysed together (Table 1).

TABLE 1: Heritability estimates for FE susceptibility for the non-transformed scale of 1 to 5 and the Log_e and Snell-transformed variables using an animal model.

	Non-transformed	Log_e	Snell
All data	0.067 ± 0.024	0.053 ± 0.020	0.050 ± 0.018
Jersey	0.137 ± 0.063	0.099 ± 0.057	0.104 ±0.058
Holstein-Friesian	0.034 ± 0.021	0.035 ± 0.020	0.037 ± 0.021
Other	0.027 ± 0.071	0.010 ± 0.067	0.000 ± 0.069

The data were split into three groups based on the breed classification, and heritabilities estimated for each dataset using the same model as above, but without breed as a fixed effect. The heritability for the Jersey breed was higher than that for Holstein-Friesian for all 3 of the FE variables.

The heritabilities for FE estimated in this study are substantially lower than those of Morris *et al.* (1998) who reported values of up to 0.77 ± 0.13. The trait studied by Morris *et al.* (1998) was log_e gamma-glutamyltransferase, which is a more defined measurement of liver damage than the discrete 1 to 5 scale of visual FE used in this study. Morris *et al.* (1998) also found significantly higher estimates for Jersey than Holstein-Friesian, for the heritability of FE.

QTL EXPERIMENTAL DESIGN

Undertaking a family-based linkage QTL study, such as affected sib-pair sharing, on the described dataset will have low power due to the small number of families (110) that have at least two affected sib pairs (score of 3-5). Risch and Merikangas (1996) showed that linkage analysis is limited to identifying genes that have large effects for disease traits, whereas population-based association studies have greater power.

Risch and Teng (1998) and Teng and Risch (1999), presented papers on linkage disequilibrium tests for disease traits with individual genotyping and pooling of sampling. Linkage disequilibrium is when a marker allele always, or nearly always, segregates with a specific allele at the gene of interest (two loci on the same chromosome) for all families in the population. In contrast, linkage is an association between a marker locus and the gene locus, and in different families, different marker alleles may be associated with a specific allele at the gene of interest. The basis of association and linkage disequilibrium tests is the comparison of the marker allele frequency in the affected group in comparison to the control group. The control group can be parents, unaffected sibs or unrelated individuals.

Risch and Teng (1998) and Teng and Risch (1999), showed that there was higher statistical power when unrelated individuals were used as the control group, especially when there was more than one affected sib per family. The drawback of using unrelated individuals as the control group is that it is susceptible to population stratification, which is occurs in the current dataset. As described, the dataset can be split into at least 3 "breeds", and there is also the admixture of overseas and New Zealand genes and mating is non-random in the population, which all contribute to population stratification.

Individual genotyping provides more information than pooling as it has more statistical power and is more robust to population stratification. Teng and Risch (1999) reported that 20-30% fewer families were required with individual genotyping compared to pooling to achieve the same statistical power.

The power of a QTL experiment, assuming that the 516 affected animals in the dataset are singletons (one affected animal per family or sire in this study), is shown in Table 2 for different modes of genetic action and allele frequencies. It is assumed that the biallelic marker is the gene itself in the following power calculations (for full details see Risch and Teng (1998)).

TABLE 2: Statistical power (%) to detect linkage disequilibrium in the Facial Eczema dataset with 516 singleton affected families and varying number of unaffected half sibs, parents not collected, using pooling (sig. level of 0.00005).

	Unaffected animals		
	1	**2**	**5**
Dominant			
p=0.05	90	99	100
p=0.20	99	100	100
p=0.70	2	3	6
Recessive			
p=0.05	0	0	0
p=0.20	22	40	57
p=0.70	100	100	100
Multiplicative			
p=0.05	9	20	35
p=0.20	78	94	99
p=0.70	82	94	98
Additive			
p=0.05	28	53	76
p=0.20	91	99	100
p=0.70	50	71	85

The values of statistical power in table 2 are based on the formula of Risch and Teng (1998) for unaffected full sibs being used as the control group rather than half sibs, which will be the case in the FE study. In effect, the power in the FE study using half sibs as the control will be slightly higher than that presented in Table 2 because the degree of allele sharing between half sibs is less than between full sibs. In addition, the presence of multiple affected animals for individual sires in the current dataset will also increase the statistical power above that presented in Table 2. There is reasonable power to identify a QTL in most of the scenarios presented in Table 2 (i.e., in most scenarios greater than a 50% chance of identifying the QTL), except for a recessive allele at low frequencies or a dominant allele at high frequency. It is unlikely that the true situation will be the latter due to the observed low frequency of affected animals in the population.

In human studies, there are estimates that one needs some 500,000 single nucleotide polymorphic markers for a genome wide linkage disequilibrium study (Kruglyak, 1999) based on linkage disequilibrium stretching some 3kilobases. Farnir *et al.* (2000) reported linkage disequilibrium stretching over tens of centimorgans in dairy

cattle. The genetic size of the genome for dairy cattle is some 3000 cM and, therefore, if linkage disequilibrium is over tens of centimorgans, then the number of microsatellite markers that would be needed is far less than that needed in human studies and is in the order of 1000-2000. Currently, some 1500 microsatellite markers exist in dairy cattle. Farnir *et al.* (2000) also reported disequilibrium between non-syntenic loci (i.e., loci on different chromosomes). This could result in false positives (i.e. associations between alleles at loci on different chromosomes) in experiments such as the one outlined in this paper. Linkage disequilibrium should then be tested using the transmission disequilibrium test (Spielman *et al.*, 1993). This will ensure that the marker is both in linkage and disequilibrium and negate the false positives derived in the association study due to the non-syntenic disequilibrium identified in dairy cattle.

USE OF FE QTL IN THE BREEDING SCHEME

If a QTL for FE resistance was identified in the described study it could be directly used in the breeding scheme due to the identified marker allele being in linkage disequilibrium with the QTL. In contrast, if a linkage study was undertaken, the applicability of the results to marker assisted selection would be limited. This is due to the linkage results only holding on a within-family level and not population-wide. Therefore, for each new family in the breeding scheme the linkage between marker and QTL would have to be re-evaluated, which would not be possible as FE phenotypes are not collected on a wide-scale and routine basis.

The degree of linkage disequilibrium between the marker and the QTL would affect the usefulness of any results, with the best case scenario being the causative mutation being identified. In addition, the application of the QTL will depend on the proportion of genetic variation that the QTL control for FE resistance or conversely susceptibility. It is unlikely that FE susceptibility is controlled by one locus, so therefore the QTL that are identified will only control a proportion of the genetic variation for FE susceptibility.

CONCLUSIONS

The dataset of phenotypes for FE that was collected in 1999 are controlled in part genetically, which enables it to be used for QTL studies. The most appropriate QTL design is an association study that utilises pooling. With the different heritability estimates in the Jersey and Holstein-Friesian populations for FE, it would be preferable to have two samples; one of all breeds and another of Jersey only, for the QTL study. The control for the study would be half-sibs preferably from the same herd. If a QTL is identified from the association study, individual genotyping should be undertaken in the genetic region of interest.

ACKNOWLEDGEMENTS

The help of Anne Winkelman and Dave Johnson in the calculation of the heritabilities is appreciated.

REFERENCES

Arranz, J-J., Coppieters, W., Berzi, P., Cambisano, N., Grisart, B., Karim, L., Marcq F., Moreau L., Nezer C., Riquet, J., Simon P., Vanmanshoven P., Wagenaar D., and Georges M. 1998 A QTL affecting milk yield and composition maps to bovine chromosome 20: a confirmation. *Animal Genetics* **29:** 107-115.

Farnir, F.; Coppieters, W.; Arranz, J-J.; Berzi, P.; Cambisano, N.; Grisart, B., Karim, L., Marcq F., Moreau L., Mni M., Nezer C., Simon P., Vanmanshoven P., Wagenaar D., and Georges M. 2000. Extensive genome wide linkage disequilibrium in cattle. *Genome Research* **10:** 220-227.

Johnson, D.L.; Thompson, R.; 1995. Restricted maximum likelihood estimation of variance components for univariate animal models using sparse matrix techniques and average information. *Journal of Dairy Science* **78:** 449-456.

Kruglyak, L. 1999. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. *Nature Genetics* **22:** 139-144.

Morris, C.A.; Burton, L.J.; Towers, N.R.; Cullen, N.G.; Rendel J.M.; Johnson, D.L. 1998. Genetics of susceptibility to facial eczema in Friesian and Jersey cattle. *New Zealand Journal of Agricultural Research* **41:** 347-357.

Morris, C.A.; Towers, N.R.; Tempero, H.J.; Cox, N.R.; Henderson, H.V. 1990. Facial eczema in Jersey cattle: heritability and correlation with production. *Proceedings of the New Zealand Society of Animal Production* **50:** 255-259.

Phua, S.H.; Dodds, K.G.; Morris, C.A.; Paterson, K.A.; McEwan, J.C.; Garmonsway, H.G.; Towers, N.R.; Crawford, A.M. 1999. Catalase gene is associated with facial eczema disease resistance in sheep. *Animal Genetics* **4:** 286-95.

Risch, N.; Merikangas, K. 1996. The future of genetic diseases of complex human diseases. *Science* **273:** 1516-1517.

Risch, N.; Teng, J. 1998. The relative power of family-based and case-control designs for linkage disequilibrium studies of complex human diseases. I DNA pooling. *Genome Research* **8:** 1273-1278.

Spelman, R.J., W. Coppieters, L. Karim, J.A.M. van Arendonk, and H. Bovenhuis, 1996 Quantitative trait loci analysis for five milk production traits on chromosome six in the Dutch Holstein-Friesian population. *Genetics* **144:** 1799-1808.

Spielman, R.S.; McGinnis, R.E.; Ewens, W.J. 1993. Transmission test for linkage disequilibrium: The insulin region and insulin-dependent diabetes mellitus (IDDM). *American Journal of Human Genetics* **52:** 506-516.

Teng, J.; Risch, N. 1999. The relative power of family-based and case-control designs for linkage disequilibrium studies of complex human diseases. II Individual genotyping. *Genome Research* **9:** 234-241.