

## QTL DETECTION WITH GENETIC MARKERS IN A DAIRY SHEEP BACKCROSS SARDA X LACAUNE RESOURCE POPULATION

A. Carta<sup>1</sup>, F. Barillet<sup>2</sup>, D. Allain<sup>2</sup>, Y. Amigues<sup>3</sup>, B. Bibé<sup>2</sup>, L. Bodin<sup>2</sup>, Sara Casu<sup>1</sup>,  
E. Cribiu<sup>4</sup>, J.M. Elsen<sup>2</sup>, A. Fraghi<sup>1</sup>, L. Gruner<sup>5</sup>, P. Jacquiet<sup>6</sup>, S. Ligios<sup>1</sup>, C. Marie-  
Etancelin<sup>2</sup>, L. Mura<sup>1</sup>, G. Piredda<sup>1</sup>, R. Rupp<sup>2</sup>, S.R. Sanna<sup>1</sup>, A. Scala<sup>7</sup>, L. Schibler<sup>4</sup>  
and S. Casu<sup>1</sup>

<sup>1</sup>Istituto Zootechnico e Caseario per la Sardegna, 07040 Olmedo, Italy

<sup>2</sup>INRA-SAGA, France; <sup>3</sup>LABOGENA, France; <sup>4</sup>INRA-LGBC, France; <sup>5</sup>INRA-PII, France

<sup>6</sup>UMR INRA-ENVY, France; <sup>7</sup>Sassari University, Italy

### INTRODUCTION

The dairy sheep industry in Western European countries is mainly based on the production of high quality PDO cheeses. Thus dairy sheep selection has been oriented towards milk yield and milk composition (protein and fat content) to maintain competitiveness of this production. Due to the evolution of the EU agricultural policy and consumers demand, more attention has been now given to traits related to the reduction of production costs (milkability, functional traits, longevity), health (resistance to mastitis or parasitic diseases) and safety food (milk content in fatty acids related to human health). In most situations, only a part of these new traits is extensively recorded in the nucleus flocks of the purebred breeding schemes (Barillet, 1997; Rupp *et al.*, 2002 ; Sanna *et al.*, 2002). Therefore research combining classical quantitative approach and QTL detection is needed, either on-farm by implementing experimental recording schemes, or in experimental flock especially for traits difficult and costly to record. Crosses between breeds to produce F2 or backcross experimental population allow to exploit linkage disequilibrium for genes differing between breeds and to detect genome regions controlling the traits of interest. In the light of this, an experimental Sarda x Lacaune backcross resource population was produced in 1999 by INRA (France) and IZCS (Italy) in order to detect QTL both on milk production and on traits difficult to measure through a whole genome scan. The Sarda and the Lacaune are the two most numerous French and Italian dairy sheep breeds, and they differ for a lot of traits as body size, growth rate, wool characteristics, prolificacy, milk yield level and milkability. Their dairy selective history is also very different (Barillet *et al.*, 2001 ; Carta *et al.*, 2002). The purpose of this paper is to give an overview of the Sarda x Lacaune project and to illustrate it by summarizing the results of first QTL analysis for milk production traits in first lactation on sheep chromosome three (OAR 3).

### OVERVIEW OF THE BACKCROSS SARDA x LACAUNE PROJECT

In 1998, 14 elite Lacaune rams from France were mated in Italy by AI to 100 Sarda ewes to produce F1 rams. Among those, 10 sons of different Lacaune sires were mated to 3,000 Sarda ewes to procreate 967 backcross females born in 1999. These ewes have been reared in an experimental farm in Sardinia and in 2002 they were in third lactation. The farm is located in the South of Sardinia under a dry Mediterranean climate. The ewes were fed with the same nutritional regime based on 4-5 hours of grazing irrigated mixed swards of ryegrass and

berseem clover with important supplementations of alfalfa hay, maize silage and concentrates particularly in winter and late spring. The ewes are milked by machine twice a day. A description of the traits recorded in the experiment and the way of measuring related phenotypes are reported in table 1.

**Table 1. Description of the main phenotypic measures**

Traits	Periodicity/Tools
<b>Production traits:</b>	
Daily milk yield	Three times per month/INRA electronic jar
Protein and fat content	Bi-monthly/INRA electronic jar
Growth (as ewe lamb)	Bi-monthly body weight/electronic balance
Body Weight (adult ewe)	Monthly body weight/electronic balance
Body condition score (adult ewe)	Monthly subjective score
Wool weight	Once a year/electronic balance
Wool quality	As ewe lamb/objective measurements & score
<b>Milkability and udder morphology</b>	
Kinetics of milk flow	Three times per month/INRA electronic jar
Udder morphology type traits	Monthly/subjective score
Udder measurements	Once a year/numeric picture analysis
<b>Health traits</b>	
Udder health : Somatic cell count	Bi-monthly/INRA electronic jar
Udder health : clinical mastitis detection	daily/visual detection +microbiological assay
Nematode parasite resistance	2- 3 times per year /fecal eggs count
Oestrus ovis resistance	Once a year/antigen ELISA test
<b>Reproductive traits</b>	
Ovulation rate	Once a year/laparoscopy
Fertility (after synchronization)	Date of lambing on the controlled cycle
Prolificacy	Litter size at lambing
Ability of out-of season mating	Once a year before mating/progesterone assay
Anti PMSG antibody	3 ELISA tests around AI
Embryo mortality	Radioimmunoassay of PSPB
<b>Milk quality traits</b>	
Milk fatty acids composition	Once a year /gas chromatography

**Genome scan.** INRA has developed a panel of 155 microsatellite markers to enable a complete scan of the autosomal sheep genome. Most genotypings have been carried out by IZCS in multiplex using an ABI377-96 automatic sequencer. In January 2002, 117,318 genotypings were available. Over the 120 genotyped markers, the percentage of informative meioses was 62 % on average.

### QTL DETECTION FOR MILK TRAITS IN 1st LACTATION ON OAR 3

**Phenotypic data.** A total of 887 first lactation milk yield (MY), protein yield (PY), fat yield (FY), protein (P %) and fat (F %) contents were available. Milk recording was performed using INRA electronic jars allowing to record also kinetics of milk flow (Ricard *et al.*, 1995).

Lactation yields were calculated by the Fleischmann method for the milking period only, considering an average suckling period of 21 days. Descriptive statistics of these traits are summarized in table 2.

**Table 2. Family means and residual standard deviation (rsd) for milk traits (1st lactation)**

Sire	N of daughters	MY (liters)		PY (liters)		FY (liters)		P %		F %	
		Mean	rsd	Mean	rsd	Mean	rsd	Mean	rsd	Mean	rsd
1	96	195	32.00	9.53	1.43	12.84	1.95	4.84	0.26	6.53	0.45
2	91	199	34.75	9.77	1.63	12.78	2.24	4.87	0.23	6.35	0.38
3	112	192	26.76	9.51	1.22	12.93	6.94	4.93	0.24	6.70	0.42
4	101	224	29.41	10.95	1.48	14.31	1.75	4.83	0.26	6.35	0.38
5	76	159	41.46	8.03	2.04	10.58	2.65	5.01	0.26	6.62	0.46
6	102	207	31.02	10.69	1.42	14.25	2.03	5.12	0.31	6.81	0.47
7	78	231	37.39	11.69	1.89	16.56	2.72	5.03	0.23	7.14	0.43
8	80	231	27.04	10.73	1.10	14.67	1.55	4.60	0.22	6.29	0.34
9	83	162	31.70	8.22	1.52	10.96	2.12	5.06	0.22	6.75	0.40
10	68	180	38.38	9.09	1.88	12.44	2.61	4.99	0.24	6.83	0.41

**OAR 3 genotyping.** Ten microsatellite markers were available on OAR 3 covering a segment from 37 to 284 cM with an average map distance of 27 cM. The number of heterozygous sires ranged from 5 to 9 and the percentage of informative meioses from 40 to 80 %.

**Statistical analysis.** Prior to the QTL analysis, phenotypes were adjusted for different combinations of fixed effects specific for each milk trait. The main factors were the length of the milking period, the lambing season and the number of lambs. Single trait QTL analysis was carried out according to the methodology proposed by Knott *et al* (1996) and Elsen *et al* (1999) by within-sire linear regression using the following model :

$$Y_{ij} = s_i + (2p_{ij}-1)a_i + e_{ij}$$

where  $Y_{ij}$  was the individual phenotype adjusted as described above,  $s_i$  is the sire,  $p_{ij}$  was the probability of inheriting one defined QTL allele from sire  $i$  for the daughter  $j$  given the marker information,  $a_i$  was half the substitution effect of the putative QTL carried by the sire  $i$ , and  $e_{ij}$  was the residual assumed to be normally distributed with a zero expectation and a heterogeneous variance  $\sigma_{ei}^2$ . The probability for each possible phase of the sires was estimated from progeny marker information. The most likely phase was retained and the probability that each progeny received one or the other chromosomal segment was estimated at every position, given this phase, using a 1 cM step. The rejection thresholds were estimated for each trait by within-family permutations as proposed by Churchill and Doerge (1994), using 10,000 permutations.

**Results and discussion.** Across family analysis was performed to test for evidence of different location effects in different families. All locations close to significance at  $\alpha_c$  chromosome-wise level of 0.05 [*i.e.* with  $(26 \times 5 \times 0.05) = 7$  type I errors expected by chance under the null hypothesis] are reported in table 3. There is evidence for an important QTL affecting PY, MY and FY with the most probable location near the marker BMC1009 (a region homologous to bovine chromosome 5). The most important effects were detected in families 4 and 6 (about 0.9 and 1 within family residual standard deviation units for PY). Another close to the  $\alpha_c$

significance level QTL was detected for F% near the marker ILSTS45 (bovine chromosome 11). A particularly important effect of 1 within family residual standard deviation unit was detected in family 5.

**Table 3. LRT peak, chromosome-wise P value, and most probable location**

Trait	Families	LRT at the peak	P value	Most probable location (cM) - closest marker
MY	All	39.10	0.0013	195 - BMC1009
PY	All	46.30	0.0001	197 - BMC1009
FY	All	29.20	0.0214	195 - BMC1009
F %	All	25.90	0.0558	37 - ILSTS45

### CONCLUSION

Results obtained in this analysis of OAR 3 for milk traits showed that the Sarda x Lacaune backcross design allows to detect with sufficient power QTL showing substitution effects of 1 within family residual standard deviation. Since all the results of the genome scan will be available in 2002 before the end of the third lactation, QTL detection for a number of recorded traits will be performed this year.

This experiment is part of an European research project for the detection of QTL for traits related to the quality and safety of sheep products (Barillet, 1999) and for their comparison in different environments x populations. It will be done for mastitis resistance and milkability in three populations of dairy sheep (backcross Sarda x Lacaune in Italy, Churra families in Spain, Lacaune and Manech families in France) and for nematode parasite resistance in three populations of dairy and meat sheep (backcross Sarda x Lacaune in Italy, Churra families in Spain, and backcross between a lean and a fat line of Blackface in United Kingdom).

### ACKNOWLEDGEMENTS

This program was supported by an European contract (QLK5-2000-00656).

### REFERENCES

- Barillet, F. (1997) In "The Genetics of Sheep", p. 539-540, CAB International, Oxon, UK
- Barillet, F. (1999) Proposal QLRT-1999-30656, 5<sup>th</sup> European Framework Programme.
- Barillet, F., Marie, C., Jacquin, M., *et al.* (2001) *Livest. Prod. Sci.* **71**: 17-29.
- Carta, A., De Candia, M., Fois, N., Ledda, A., Ligios, C., Ligios, S., Molle, G., Sanna S. R., Scala, A., Casu, S. (2002) In "Datasheet on Sardinian breed - Animal health and production compendium". CAB International, Oxon, UK (in press)
- Churchill, G.A., Doerge, R.W. (1994) *Genetics* **138** : 963-971.
- Elsen, J.M., Mangin, B., Goffinet, B., *et al.* (1999) *Genet. Sel. Evol.* **31** : 213-224.
- Knott S.A., Elsen, J.M., Haley, C.S. (1996) *Theor Appl Genet.* **93** : 71-80.
- Ricard E., Arhainx J., Guillouet, P., *et al.* (1995) *EAAP Publication* **75** : 47-51.
- Rupp ,R., Boichard, D. *et al.* (2002) *These proceedings* .
- Sanna S.R., Casu Sara, Carta, A. (2002) *These proceedings*.