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Quantitative trait loci underlying milk production traits in sheep

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Summary

Improvement of milk production traits in dairy sheep is required to increase the competitiveness of the industry and to maintain the production of high quality cheese in regions of Mediterranean countries with less favourable conditions. Additional improvement over classical selection could be reached if genes with significant effects on the relevant traits were specifically targeted by selection. However, so far, few studies have been undertaken to detect quantitative trait loci (QTL) in dairy sheep. In this study, we present a complete genome scan performed in a commercial population of Spanish Churra sheep to identify chromosomal regions associated with phenotypic variation observed in milk production traits. Eleven half-sib families, including a total of 1213 ewes, were analysed following a daughter design. Genome-wise multi-marker regression analysis revealed a genome-wise significant QTL for milk protein percentage on chromosome 3. Eight other regions, localized on chromosomes 1, 2, 20, 23 and 25, showed suggestive significant linkage associations with some of the analysed traits. To our knowledge, this study represents the first complete genome scan for milk production traits reported in dairy sheep. The experiment described here shows that analysis of commercial dairy sheep populations has the potential to increase our understanding of the genetic determinants of complex production-related traits.

Keywords daughter design, milk production, quantitative trait locus, sheep.

Introduction

The world's commercial dairy sheep industry is primarily concentrated in Mediterranean countries, and is linked to the exploitation of local breeds for the production of high quality cheese. This regional diversity, together with obvious economic factors, has limited the number of genomic studies undertaken in dairy sheep when compared with the numerous quantitative trait locus (QTL) discovery experiments carried out in dairy cattle (reviewed in Khatkar *et al.* 2004; Smaragdov *et al.* 2006). Apart from the historical and cultural significance, as well as the unique richness of locally produced cheese (Boyazoglu & Morand-Fehr 2001), traditional dairy sheep production systems are relevant for the maintenance of sheep populations in less favoured areas and exhibit valuable ecological features. Therefore, major efforts should be made to strengthen and maintain these genuine production systems.

Milk production traits in dairy sheep are moderately to highly heritable (Barillet 1997; Sanna *et al.* 1997; Othmane *et al.* 2002; Legarra & Ugarte 2005), and classical breeding programmes have been designed for the main dairy sheep populations in Europe (Barillet 2007). Farmers are paid according to the milk yield (MY) as well as the total solids produced, which are the major determinants of cheese yield (Othmane *et al.* 2002). Hence, in order to address the needs of the sector, the selection objectives include milk composition-related traits in addition to MY. Classical genetic breeding has led to substantial improvement in milk production traits, although major selection achievements could be realized if genes with significant effects on the considered traits were specifically targeted by selection. Currently, sheep genetic maps and other genomic resources (Maddox & Cockett 2007) provide the dairy sheep scientific research community with the tools required to identify and characterize genetic factors underlying production traits of interest. This will facilitate a better understanding of the

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underlying biology of a specific trait, paving the way towards implementation of molecular information in breeding programmes.

The first reported search for QTL underlying milk production traits in dairy sheep was performed by our research group in Churra sheep, an indigenous, highly specialized milk production breed farmed in the region of Castilla y León, Spain (Díez-Tascón *et al.* 2001). We studied ovine chromosome 6 because of its conserved synteny with bovine chromosome 6, for which a large number of milk-related QTL have been previously reported in dairy cows (Spelman *et al.* 1996; Lipkin *et al.* 1998; Velmala *et al.* 1999). Díez-Tascón *et al.* (2001) identified a putative QTL for milk production traits on sheep chromosome 6 and illustrated the suitability of the so-called daughter design (Soller & Genizi 1978) as an experimental approach for studying the genomic structure of the Churra sheep population.

In the framework of the European-funded project *gene-sheepsafety*, an extended population of Churra sheep was later subjected to whole-genome scan analysis to detect QTL for traits of economic interest in dairy sheep. In this study, we present the QTL identified for milk production, whereas the findings related to other traits analysed in this population, e.g. somatic cell score and udder morphology, have been reported elsewhere (Gutiérrez-Gil *et al.* 2007, 2008b). Whole-genome searches for QTL affecting milk production traits in dairy sheep have also been undertaken in other sheep populations, but only preliminary results have thus far been reported (Barillet *et al.* 2005, 2006).

The current work describes a whole-genome linkage analysis for the production traits currently considered as selection targets in the official Churra breeding scheme, which are MY, milk fat percentage (FP) and milk protein percentage (PP), as well as two other traits classically considered in dairy cattle QTL studies: protein yield (PY) and fat yield (FY). The regression analysis carried out in this study involved half-sib pedigrees that originated via artificial insemination by 11 sires that were members of the breed selection nucleus. To our knowledge, this study represents the first full genome scan for milk production traits reported in dairy sheep.

Materials and methods

Resource population and phenotypic measurements

A daughter design comprising 11 half-sib families of Churra sheep, with a total of 1213 ewes distributed in 17 different flocks, was analysed in this study. All animals belonged to the selection nucleus of the National Association of Churra Breeders (ANCHE) and were bred by artificial insemination. The average family size was around 110, ranging from 47 to 223 daughters per sire. The phenotypic data considered in this research were the records collected routinely by ANCHE in the official milk recording process: MY, PP and FP. The average lactation length in Churra sheep is approximately 140 days. The first test-day record was obtained at least 3 days after lamb weaning (between days 31 and 75 post-partum). Subsequent records were obtained at approximately monthly intervals thereafter (El-Saied *et al.* 1999). The phenotypic database included a total of 10 203 test-day records distributed among 3779 lactations, with an average of 3.11 lactations per ewe and 2.7 test-day records per ewe and lactation. PY and FY were calculated by multiplying PP and FP with the corresponding test-day MY. The basic statistics for MY, PP, FP, PY and FY are provided in Table 1.

Table 1 Basic statistics for milk production traits measured on test-day records from 1213 Churra ewes.

Trait	*n*	Mean	SD	Minimum	Maximum
MY (ml)	10 203	1092.7	562.4	151.5	3969.0
PP	10 203	5.6	0.8	3.6	10.0
FP	10 203	6.9	1.9	3.1	13.1
PY (g)	10 203	59.4	2.7	6.7	211.5
FY (g)	10 203	71.5	3.5	6.4	358.8

SD, standard deviation.

Genotyping and linkage map

DNA was extracted from the blood of ewes and the semen of sires following standard procedures (Sambrook *et al.* 1989). Microsatellite markers were selected based on their location and information content (IC) from available ovine linkage maps (de Gortari *et al.* 1998; Maddox *et al.* 2001) to obtain a predicted uniform coverage of approximately one marker every 20 cM across the ovine autosomes. During the first stage, each marker was individually assayed to evaluate the locus-specific amplification and to determine optimal PCR conditions and allele ranges in the Churra population. PCRs were performed in a total volume of 10 μl, including 25 ng of DNA, 1× $MgCl_2$-free PCR buffer (Applied Biosystems), 1.5–3 mM $MgCl_2$ (depending on the primer pair), 200 μM of each dNTP, 0.25 μM of each primer and 0.5 U of *Taq*-polymerase or *Taq*-Gold (depending on the primer pair) (Applied Biosystems). After a 95 °C incubation step for 5 min, PCR amplifications were performed for a total of 30 cycles using the following conditions: denaturation at 94 °C for 30 s, annealing at *X*°C for 40 s (*X*°C ranged from 45 to 62 °C depending on the primer pair) and extension at 72 °C for 40 s, with a final extension step at 72 °C for 10 min. Microsatellite markers were grouped according to their PCR conditions and forward primer fluorescent-labelling with the aim of using a multiplex PCR strategy for their amplification. Multi-loading combinations were later designed and analysed with an ABI 377 automatic sequencer using the GENESCAN and GENOTYPER software packages (Applied Biosystems). An SNP marker located on chromosome 13 was genotyped as described by Alvarez *et al.* (2006).

Marker maps, which were based on the genotypes of 1421 ewes, were constructed using the CRI-MAP 2.4 software (Green *et al.* 1990). The Morton test for heterogeneity (Morton 1956) was applied to the two-point LOD scores to detect residual genotyping anomalies. Multi-point marker maps were constructed using the *build*, *flips* and *chrompic* options in CRI-MAP. The IC of the maps was measured as described by Coppieters *et al.* (1998).

QTL analysis

Quantitative trait locus mapping was performed using the multi-marker regression method described by Knott *et al.* (1996) for half-sib designs implemented with HSQM software (Coppieters *et al.* 1998). The response variables used in the QTL analysis were the yield deviations (YD), which are unregressed weighted averages of ewes' performances adjusted for environmental effects (Vanraden & Wiggans 1991). Hence, YD were calculated from the phenotypic records of MY, PP, FP, PY and FY as deviations from the population mean that were corrected for the environmental effects considered in the statistical model described in detail by Othmane *et al.* (2002). Briefly, test-day record measurements were corrected for the following systematic environmental effects: flock test-day (1294 levels), week of lactation (13 levels) and lambing age (six levels).

The HSQM program (version 6) (Harmegnies *et al.* 2006) was used to perform 10 000 random permutations of the phenotypic data against all analysed marker genotypes (Churchill & Doerge 1994) to obtain genome-wise significance thresholds for the five considered traits. QTL effects were considered significant if they exceeded the 5% genome-wise significance threshold (P_g-value < 0.05). We also considered the suggestive linkage level, for which one false positive is expected in a genome scan (Lander & Kruglyak 1995). Therefore, QTL were considered suggestive if the test statistic exceeded a threshold reached, on average, once per phenotype permutation. Assuming that the 'threshold exceeding events' are distributed according to a Poisson distribution, the *P*-value corresponding to the suggestive threshold also corresponds to the proportion of permutations for which the suggestive threshold is exceeded at least once across the genome, which is $1 - 0.37 = 0.63$ (Harmegnies *et al.* 2006). To graphically present our results, the statistical significance of a QTL was expressed as the $\log(1/P_g)$, where P_g is the proportion of the phenotype permutations for which the QTL test statistic was exceeded anywhere across the genome (Harmegnies *et al.* 2006). Therefore, the significance thresholds considered here (the 5% genome-wise significance level and the suggestive significance level) corresponded to $\log(1/P_g) = 1.3$ and $\log(1/P_g) = 0.2$ respectively. The 95% confidence intervals (95% CI) for the QTL locations were estimated by bootstrapping (Visscher *et al.* 1996).

For each QTL identified at the whole-population level, the within-family analyses revealed the families' segregation for the corresponding QTL (chromosome-wise *P*-value, $P_c < 0.05$) and the estimate for the allelic substitution effect.

Following the method of Weller *et al.* (1990), the statistical power of our experiment to detect a QTL with a substitution effect of 0.3 phenotypic SD units and two alleles at equal frequency, and affecting a trait with a heritability of 0.3 (considering the last estimates for MY and PP in Churra sheep; L.F. de la Fuente, unpublished data) was estimated to be 56.4%. This was assuming a type I error rate of 0.05, a 10% recombination frequency between the QTL and marker, and that half of the analysed sires were heterozygous at the QTL.

Results

Linkage map

The linkage map constructed for the Churra sheep population studied in this experiment has been reported elsewhere (Gutiérrez-Gil *et al.* 2008a). Briefly, the total map length was 3269 cM Kosambi (4058 cM Haldane) and included 182 genetic markers (181 microsatellites and one SNP) evenly distributed over the 26 ovine autosomes. Despite certain length discrepancies, the marker order and genetic distances were in agreement with previously published sheep genetic maps (de Gortari *et al.* 1998; Maddox *et al.* 2001).

QTL analysis results

The IC of the Churra sheep linkage map across the entire genome is shown in Fig. 1. On average, 57% of the potential information was extracted using the linkage map based on male information. The highest IC value was found on chromosome 23 (92%), coincident with the position of marker *CSSM31*, whereas the lowest value for this parameter was found on chromosome 3 (21%). The average IC per chromosome ranged between 41.5% (chromosome 8) and 75% (chromosome 6). The IC exceeded 50% for 67% of the genome analysed.

Results from the genome-wise across-family regression analysis for the five milk production traits analysed here are depicted in Fig. 2, where the statistical significance obtained for each cM (Haldane) across the genome is represented as the $\log(1/P_g)$.

One significant QTL exceeding the 5% genome-wise significance threshold, [$\log(1/P_g) > 1.3$], was identified on chromosome 3 for PP (indicated by the star symbol in Fig. 2). This genome-wise significant association is characterized according to the results of the across-family analysis in Table 2a. The magnitude of this QTL effect is provided for each of the segregating families identified by the within-family analyses.

In addition, there were eight other chromosome-trait combinations (indicated by the arrow symbol in Fig. 2) that exceeded the suggestive significance threshold (Lander &

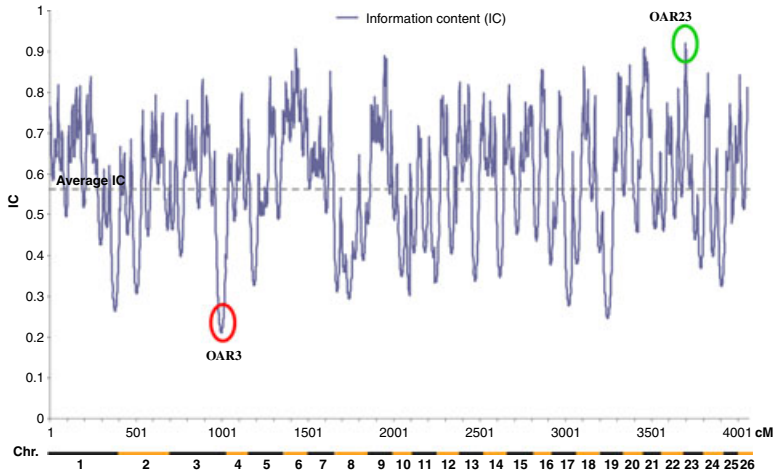

Figure 1 Information content (IC) across the marker linkage map constructed for the Churra sheep population considered in the present study (*y*-axis). The linkage map positions (cM Haldane) are presented in the *x*-axis, where the alternate orange and black horizontal lines mark the limits between the 26 sheep autosomes. The average IC across the total map length (IC = 57%) is represented with a horizontal dashed line. The minimum and maximum IC values, ranging from 21% (chromosome 3) to 92% (chromosome 23), are also indicated.

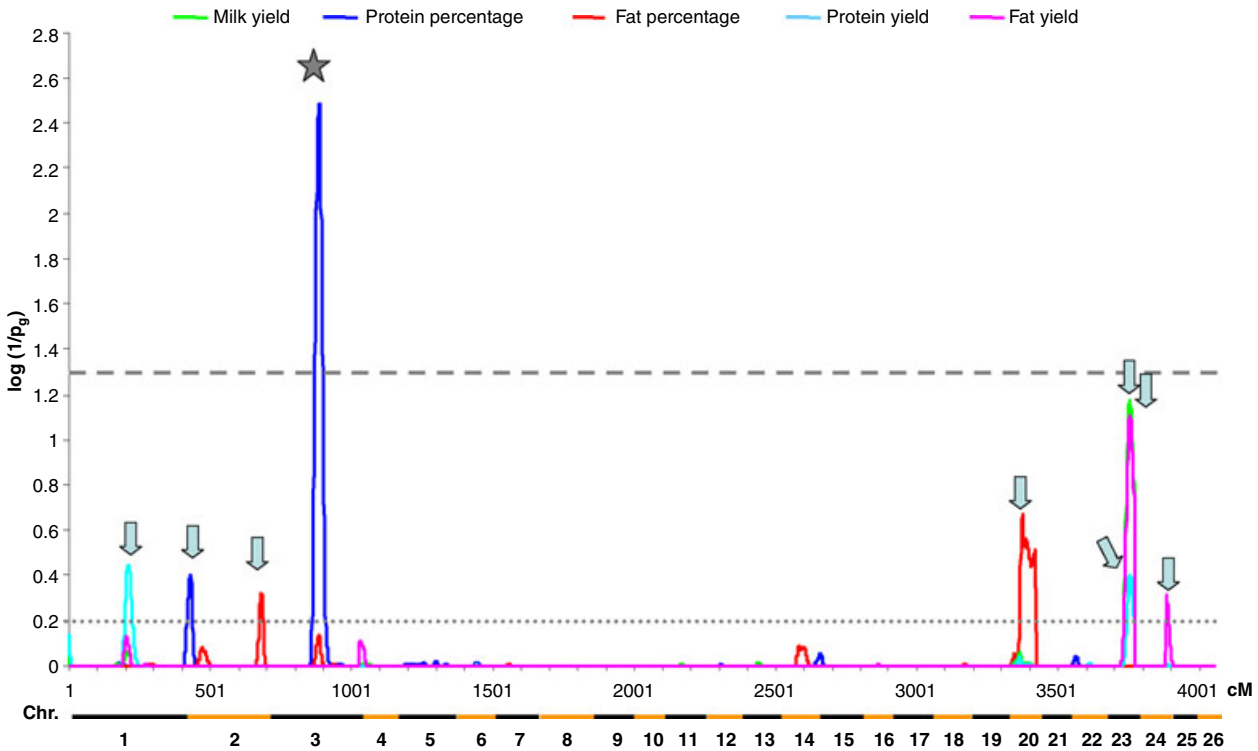

Figure 2 Genome-wise statistical significance of the regression analysis carried out for milk production traits in Churra sheep. The statistical significance for the five traits analysed is expressed as the log(1/P_g), where P_g is the proportion of the phenotype permutations for which the QTL test statistic was exceeded anywhere across the genome. The length of the linkage map is indicated in cM (Haldane) on the *x*-axis. The alternate orange and black horizontal lines mark the limits between the 26 sheep autosomes. The two horizontal dashed lines indicate the suggestive [log(1/P_g) < 0.2] and 5% genome-wise [log(1/P_g) < 1.3] significance thresholds.

Kruglyak 1995), which corresponds to a log(1/P_g) > 0.2, as explained in Materials and methods. These suggestive associations were found to influence PY on chromosome 1, PP and FP on chromosome 2, FP on chromosome 20, MY, PY and FY on chromosome 23, and FY on chromosome 25. Details of the results obtained from the across- and within-

Table 2 Genome-wise significant associations, at the 5% risk and suggestive significance levels, detected for milk production traits in Churra sheep. Across-family analysis results for the chromosome-trait combinations identified as exceeding the 5% genome-wise (a), and suggestive (b) significance thresholds. Details of the within-family analysis are also shown for those families displaying segregation of the QTL (chromosome-wise P_c-value < 0.05).

	Across-family analysis					Within-family analysis		
Significance level	Chr.	Trait	Position cM[1,2]	Flanking interval[3]	Genome-wise P_g-value[4]	Segregating families	Position cM[5]	Size effect, trait units[6] (SD units)
(a)								
Genome-wise significance (P_g-value < 0.05)	OAR3	PP	186 [165–205]	***KD103***–OARVH34	0.0033	5	186	0.187 (0.227)
						6	255	0.188 (0.229)
						7	179	0.246 (0.299)
						11	186	0.280 (0.340)
(b)								
Suggestive significance (P_g-value < 0.63)	OAR1	PY	210 [1–274]	*ILSTS004–CSSM4*	0.3589	1	200	1.353 (0.493)
						6	220	1.019 (0.371)
	OAR2	PP	25 [2–256]	***MCM147***	0.3954	5	26	0.196 (0.238)
						11	187	0.209 (0.254)
	OAR2	FP	274 [48–292]	*BMS356–OARFCB11*	0.4794	1	39	0.588 (0.304)
						5	67	0.429 (0.222)
						7	261	0.594 (0.307)
	OAR20	FP	69 [21–116]	***OLADRBPS***	0.2142	1	59	0.432 (0.525)
						4	39	0.8 (0.972)
						6	70–116	1.196 (1.453)
	OAR23	MY	99 [76–115]	*MCM136–URB031*	0.0670	7	94	166.671 (0.296)
						11	80–115	234.728 (0.417)
	OAR23	FY	100 [7–115]	*MCM136–URB031*	0.0776	7	95	1.088 (0.309)
						10	97	2.349 (0.667)
	OAR23	PY	100 [20–115]	*MCM136–URB031*	0.3922	–	–	–
	OAR25	FY	1–2 [1–75]	***MCM200***–*ILSTS060*	0.4868	7	2	0.731 (0.208)
						8	2	1.005 (0.285)

Chr., chromosome number; MY, milk yield; PP, protein percentage; FP, fat percentage; PY, protein yield; FY, fat yield.

[1,5]Position (cM Haldane) of the chromosome where the maximum *F*-statistic value was obtained in the across- and within-family analyses respectively.

[2]The 95% confidence interval obtained by bootstrapping analysis (Visscher *et al.* 1996) is shown in square brackets (cM Haldane).

[3]Markers flanking the position of the maximum *F*-statistic in the across-family analysis. Markers in bold caps are <2 cM from the maximum *F*-statistic.

[4]Genome-wise P_g-value associated to the maximum *F*-statistic value of the across-family analysis, calculated through a genome-wise permutation test (Churchill & Doerge 1994) implemented through the HSQM software (version 6) (Harmegnies *et al.* 2006).

[6]Magnitude of the allelic substitution effect calculated for each segregating family, expressed in units of the trait (kg for yield traits, and percentage points for composition traits) and in phenotypic SD units (value in brackets).

family analyses for these suggested QTL are provided in Table 2b.

The most significant QTL was localized in the second third of chromosome 3 (186 cM) for PP (P_g-value = 0.0033), with the peak QTL position located 1 cM from marker *KD103* (see Fig. 3). This QTL was accompanied by a parallel behaviour of the statistical profile for FP, although this effect did not reach the suggestive significance threshold. For the genome-wise significant QTL found in chromosome 3 for PP, the within-family analysis revealed four segregating families (Families 5, 6, 7 and 11).

The QTL peaks for these segregating families were consistent with the position of the statistical peak in the across-family analysis. Estimates of the allele substitution effect on PP for these four informative families ranged from 0.227 to 0.340 phenotypic SD units. The 95% CI for this QTL involved a 40-cM-long interval spanning positions 165–205 cM.

Eight other regions showed suggestive significant effects on the traits studied. The central region of chromosome 1 was associated with PY. The average allelic substitution effect of this QTL for the two segregating families identified was 0.432 phenotypic SD units. In the proximal region of chromosome 2, a suggestive QTL influencing PP was identified. Two families (Families 5 and 11) showed evidence of segregation, although the peak location of their statistical

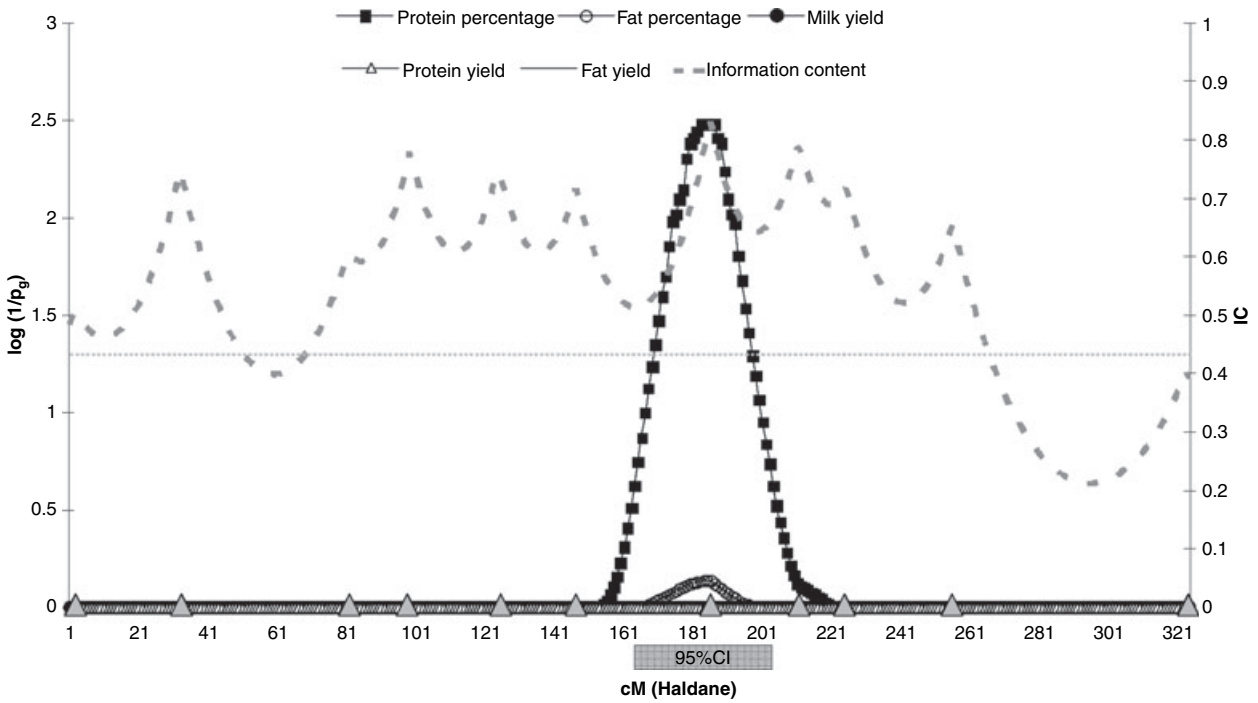

Figure 3 Statistical profiles obtained on chromosome 3 for five milk production traits analysed in the present study. The *x*-axis indicates the relative position on the linkage map (cM Haldane) The *y*-axis represents the log(1/P_g-value). The horizontal line indicates the 5% genome-wise significance threshold. Information content (IC) obtained along the linkage groups is represented at the right, *y*-axis. Beginning at the centromeric end, the triangles on the *x*-axis indicate the relative position of the markers, which were *BMS1350*, *OARCP34*, *BM1831*, *INRA131*, *BM304*, *ILSTS042*, KD103, *OARVH34*, *CSRD2111*, *BMS1248* and *MCMA13*. The confidence interval (95% CI) calculated by bootstrapping analysis for the protein percentage QTL is shown as a grey box at the bottom of the figure.

profiles differed substantially (26 and 187 cM). The allelic substitution effect for this suggestive QTL showed an average magnitude of 0.246 phenotypic SD units. Another suggestive QTL for FP was localized to the most distal marker interval analysed on chromosome 2. Three families showed evidence of segregation for this genetic effect (Families 1, 5 and 7), although for two of them (Families 1 and 5), the QTL peak mapped to the first third of the chromosome (see Table 2b). The allelic substitution effect for this QTL ranged from 0.222 to 0.307 phenotypic SD units.

There was another QTL for FP on chromosome 20 that demonstrated significance at the suggestive level. In the across-family analysis, the peak of the FP statistical profile was coincident with the location of marker OLADRBPS. Three out of the 11 analysed families showed statistical evidence of segregation for this QTL (Families 1, 4 and 6), although there were certain discrepancies regarding the QTL map location identified for each individual family. The peaks for Families 1 and 4 were found in the second third of the chromosome (39 and 59 cM respectively), whereas significant values for Family 6 were observed as a plateau involving the three distal marker intervals of the chromosome (70–116 cM). For this QTL, the estimates for the allelic substitution effects of FP ranged from 0.525 to 1.453 phenotypic SD units. Three overlapping QTL affecting MY, FY and PY were detected at the suggestive level in the last third of chromosome 23. The effects for MY and FY reached the 10% genome-wise significance threshold, and were found to segregate in two families, one of which showed segregation for the two trait effects (Family 7). The average allelic substitution effects estimated for the MY and FY QTL detected on chromosome 23 were 0.357 and 0.488 phenotypic SD units respectively. For the suggestive PY QTL also detected on chromosome 23, none of the analysed families showed significant allelic substitution effects ($P_c < 0.05$). However, the suggestive effect detected in the across-family analysis could be explained by the three families exhibiting significant values at the 10% chromosome-wise level (Families 6, 7, 11). For the single family that appeared to be segregating for the three overlapping QTL detected on chromosome 23 (Family 7), the sign of the allelic substitution effects varied in the same direction (data not shown), indicating that the increase in MY is accompanied by a concomitant effect on FY and PY. However, a slight dilution effect (more remarkable for PP) was observed when the QTL effects for this family were compared with the average milk composition of the population. Another suggestive significant QTL for FY was identified at the proximal end of chromosome 25, close to marker *MCM200*. For the

two segregating families identified for this QTL (Families 7 and 8), the average magnitude of the allelic substitution effect was about 0.247 phenotypic SD deviation units. The 95% CI length calculated for all of the suggestive associations identified in this study comprised most of the chromosomal length (Table 2b).

Discussion

In the present study, QTL exhibiting considerable effects on milk production traits were identified in a commercial population of Churra sheep. The male linkage map constructed for this population comprised 182 genetic markers and spanned 4058 cM (Haldane) across the entire ovine genome (Gutiérrez-Gil *et al.* 2008a). Using this linkage map, we determined that approximately 60% of the theoretically available information was extracted from the pedigree material for QTL detection, which validates this map as a practical tool for the genetic dissection of complex traits in this population.

The genome-wise regression analysis detected significant associations for the five dairy traits analysed herein. One QTL was significant at the 5% genome-wise level, whereas eight additional linkage associations were detected at the suggestive significance level (Table 2). A principal component analysis performed using SAS software (1998) showed that three principal components account for 99% of the phenotypic variance observed for milk traits in this population, similar to estimations made in dairy cattle (Spelman *et al.* 1996). Hence, considering that three independent traits were analysed in this work, substantially more QTL were detected than the 0.15 and 1.89 significant associations that would be expected by chance alone at the 5% genome-wise and suggestive significance levels respectively.

Segregating QTL with large effects have also been detected after several decades of intensive selection for milk traits in elite populations of dairy. Therefore, the number of genuine QTL segregating in this population of Churra sheep, whose selection scheme was recently initiated (de la Fuente *et al.* 1995), is very likely to be higher than the numbers reported herein.

The daughter design has been utilized in few dairy cattle studies (Ron *et al.* 2004) because of the higher power offered by the granddaughter design. However, the daughter design better fits the structure of most commercial populations of dairy sheep including Churra sheep (Gutiérrez-Gil *et al.* 2008b). The statistical power of the experiment might have been increased by including more individuals in the study and, in particular, by increasing the number of daughters per sire (Weller *et al.* 1990). However, this study had to be adapted to fit the inherent limitations of the resource population studied. At present, samples from additional families are being collected to be included in future analyses that will provide confirmation and fine-mapping of the most significant effects reported herein.

We considered the fact that a substantial proportion of genuine QTL may not have been detected by our analysis; therefore, we consider it important to report the regions detected at the suggestive significance level. This significance threshold was suggested by Lander & Kruglyak (1995) for initial genetic analyses, such as those presented here, with the aim of striking a fair balance between using very conservative corrections and using significance thresholds that are too lenient.

QTL mapping

We present a discussion of the significant QTL results on a trait basis, and also relate the present study to the findings in the literature. For this purpose, we used the latest version of the sheep linkage map (version 4.7; http://rubens.its.unimelb.edu.au/~jillm/jill.htm), the sheep–cow comparative map available through the CMAP tool (Nicholas *et al.* 2004), and published information on dairy cattle QTL obtained from recent reviews (Khatkar *et al.* 2004; Smaragdov *et al.* 2006) and publicly accessible databases such as CATTLEQTLDB (http://www.animalgenome.org/QTLdb/cattle.html) and QTLMAP (http://www.vetsci.usyd.edu.au/reprogen/QTL_Map/).

Preliminary QTL results for milk traits obtained from two other populations present in the European funded project g*enesheepsafety*, a Sarda × Lacaune backcross population and a commercial population of Lacaune sheep (Barillet *et al.* 2006), were utilized for the comparative study presented herein.

Protein percentage

This trait is of great interest for the cheese industry because of its positive effect on cheese yield and the high genetic correlation between these two traits (Othmane *et al.* 2002). For PP, our analysis revealed a genome-wise significant QTL on chromosome 3, and a suggestively significant QTL on chromosome 2 (Table 2). The QTL on chromosome 3 was the most significant association detected in this study, and also the effect for which more segregating families were detected, including the two largest half-sib groups involved in the experiment (Families 5 and 7). The shadow effect observed for FP in the same region of chromosome 3 is probably due to the high genetic correlation that exists between FP and PP (Barillet 1997; Othmane *et al.* 2002).

Although results from daughter design analyses can only provide limited information on QTL allele frequencies in the general population (Weller *et al.* 2002), the large proportion of segregating families containing a coincident within-family position for the QTL affecting PP on chromosome 3 may indicate that the alternative alleles at the QTL have frequencies of about 0.25 and 0.75 in the Churra population. If this is confirmed, and the favourable allele

demonstrates the lower frequency, the development of a marker-assisted selection protocol for this genetic effect in this population might be feasible. However, continued efforts towards a saturated genetic map in this QTL confidence interval, and the study of positional candidate genes, are required before this can be achieved.

A search for correspondence of the PP QTL on chromosome 3 with previously reported QTL revealed similar results to those observed in studies carried out in both dairy sheep and milk cows. Barillet *et al.* (2006) identified significant effects for MY, FY and PY linked to the *BMC1009* marker, which is 4 cM away from *KD103*. The orthologous bovine region maps to the first half of chromosome 5 and has been associated with PP in independent studies (Mosig *et al.* 2001; Plante *et al.* 2001; Bennewitz *et al.* 2003). The most striking similarity is represented by a QTL for protein content on bovine chromosome 5 (Bennewitz *et al.* 2003), which maps to the orthologous region of marker *KD103*. Further research is required to assess the possibility that the same locus underlying these effects influences milk PP in both ruminant species. It is worth noting that marker *KD103* maps within the sequence of the *HDAC7A* gene, which encodes histone deacetylase 7A. Nuclear histone acetylation has been associated with casein mRNA induction in the mammary gland (Hirose *et al.* 1985). According to the VIRTUAL SHEEP GENOME BROWSER v1.2.1 (http://www.livestockgenomics.csiro.au/perl/gbrowse.cgi/vsheep1.2), other genes mapping close to marker *KD103* in sheep include *VDR* (vitamin D Receptor) and *PP11* (placental protein 11 precursor). Further research is needed to assess the possible association of these genes with the QTL effect on PP reported herein.

For the suggestive QTL affecting PP on chromosome 2, the different peak position observed for the two segregating families may indicate that two different loci contribute to the effect detected at the across-family level. The orthologous region to the across-family position for this QTL corresponds to the second third of cattle chromosome 8, for which a QTL for PP was reported in dairy cows following a selective DNA pooling approach (Mosig *et al.* 2001). The within-family QTL location suggested for Family 11, close to marker *ILSTS030*, is coincident with significant effects on PP identified in a granddaughter design study in French dairy sheep breeds (Barillet *et al.* 2006), and with orthologous QTL identified in the first half of bovine chromosome 2 for PP and FP in dairy cattle (Zhang *et al.* 1998; Mosig *et al.* 2001; Ashwell *et al.* 2004).

Fat percentage

Milk fat represents about 6–7% of total sheep milk and is one of the main components responsible for the characteristic taste and flavour of sheep cheese (Harper 1959). There is an increasing trend among health-conscious consumers to avoid animal fat because of the known association between dietary saturated fatty acids and the risk of heart disease (Hayes & Khosla 1992). Despite its high fat content, sheep milk is also a source of high-quality nutrients, such as essential fatty acids, e.g. linoleic and linolenic acids, and other fatty acids with positive effects on human health, e.g. conjugated linoleic acid (Khanal & Olson 2004).

Because of the interest in modifying sheep milk fat content and fatty acid composition through genetics, the QTL identified for milk FP in this study may be good candidates for further research, although further analyses are required to confirm and redefine these suggestive QTL.

The QTL for FP identified on chromosome 20 showed the highest allelic substitution effect detected in this study, with an average magnitude across the segregating families of about 1 phenotypic SD unit. For this QTL, the discrepancies regarding QTL position suggested by the within-family analyses may be explained by the differences in the informativeness of the markers across segregating families (e.g. Family 6 sire was not informative for the last three markers analysed in this linkage map, as previously reported in Gutiérrez-Gil *et al.* 2007). An alternative explanation for such discrepancies is the segregation of different QTL influencing FP in the different families. Interestingly, this QTL is coincident with a previously reported QTL for somatic cell score in the same population of Churra sheep (Gutiérrez-Gil *et al.* 2007). As Family 6 showed overlapping significant effects for the two traits, further studies should assess the possibility that these two QTL may be either due to a single locus with pleiotropic effects or, in contrast, due to the action of two different loci that are linked. For this purpose, additional genetic markers should be analysed in order to increase the informative value of the markers on the last third of the chromosome.

In a Sarda × Lacaune population, Barillet *et al.* (2006) identified QTL for MY, FY and PY within the Major Histocompatibility Complex region, which are coincident with the location of the chromosome 20 QTL detected by our across-family analysis. The same authors also reported a QTL for FP linked to marker *OARHH56* (GenBank accession L13871), which maps within the plateau of the statistical profile for Family 6 in our study. These findings highlight the distal third of ovine chromosome 20 as a candidate chromosomal region for the presence of one or more QTL influencing milk production traits. In relation to the published data for dairy cattle, the second half of the orthologous bovine chromosome 23 has been described by different groups as carrying QTL for milk fat-related traits (Ashwell *et al.* 1997; Zhang *et al.* 1998; Plante *et al.* 2001; Bennewitz *et al.* 2004). It is worth mentioning that the *prolactin* gene (*PRL*) maps between markers *OLADRPBS* and *BP34/OARHH56*, as do genes coding for other placental prolactin-related proteins (*PLRP1*, *PLRP3*, *PLRP4*). Prolactin has proven essential for mammary gland development, lactogenesis and the expression of milk protein genes (Horseman *et al.* 1997), and polymorphisms in this gene have been

associated with variations in MY and FP (Cowan *et al.* 1990; Brym *et al.* 2005; Alipanah *et al.* 2007). A recent study showed that the PLRP proteins do not possess lactogenic activity in sheep (Ushizawa *et al.* 2007). Based on this, the ovine *PRL* gene appears to be a positional and functional candidate gene for the effects detected on sheep chromosome 20 in relation to FP.

Another suggestive significant QTL for FP was found in the distal region of chromosome 2, with three families segregating for this genetic effect. One of the flanking markers for this QTL, *BMS356*, is close to the *ALPI* gene, which encodes intestinal alkaline phosphatase. In mouse, this enzyme has been shown to be involved in lipid absorption (Nakano *et al.* 2007), although there is no previous evidence that this gene influences milk composition in ruminants. In the bovine orthologous region of this QTL, which corresponds to the distal end of bovine chromosome 2, Harder *et al.* (2006) reported a QTL for FY persistence.

Milk yield, protein yield and fat yield

When selection is orientated towards cheese production, the negative correlation between MY and milk composition makes it necessary to find a compromise between MY and milk composition. Hence, when assessing the implementation of molecular markers associated with milk production traits, it is important to understand the mode of action of the underlying locus, with the aim of avoiding any undesirable dilution effects on milk components. A single QTL was found to influence MY at the suggestive level on chromosome 23, and was accompanied by significant suggestive effects on FY and PY. These effects are very likely to result from the highly positive genetic correlations between the affected traits (Barillet 1997). The orthologous region to this QTL region maps to the distal end of bovine chromosome 24, which contains the *ACAA2* gene. This gene encodes acetyl-coA acyltransferase 2, which catalyses the last step in the mitochondrial fatty acid beta-oxidation spiral. No previous studies in cattle or sheep have described a milk-related QTL in this region.

The bovine region orthologous to the QTL for PY on chromosome 1 identified in our study corresponds to the first third of bovine chromosome 1, where QTL for MY (Nadesalingam *et al.* 2001; Khatkar *et al.* 2004), PY (Rodriguez-Zas *et al.* 2002; Liu *et al.* 2004) and FY (Liu *et al.* 2004) have been previously reported. In dairy sheep, milk-related QTL have been found on chromosome 1, although at different marker intervals than those detected in our study (Calvo *et al.* 2004; Barillet *et al.* 2006). In the region orthologous to the chromosome 25 QTL detected for FY, which corresponds to the proximal end of bovine chromosome 28, QTL influencing MY (Rodriguez-Zas *et al.* 2002; Ashwell *et al.* 2004), PP and FP (Zhang *et al.* 1998) have also been found.

Surprisingly, no QTL were found in chromosomes 6 or 9. These chromosomes correspond to the bovine chromosomes on which causal mutations in dairy cattle QTL have been detected, in the *ABCG2* (Olsen *et al.* 2007) and *DGAT1* (Grisart *et al.* 2002) genes respectively. These genes appear not to segregate in the Churra population analysed herein because, even at the within-family analysis level, no evidence for significant effects on any of these chromosomes was found. In contrast, *DGAT1* is an obvious candidate for an FP QTL identified by Barillet *et al.* (2005). This finding, which was obtained in a granddaughter design study performed in French dairy sheep breeds, suggests that an assessment of the role of specific allelic variants of this gene in relation to variations in milk fat content and milk fat composition will be of interest.

In the present study, we did not confirm the PP QTL identified by Díez-Tascón *et al.* (2001) in Churra sheep close to the κ-casein locus on sheep chromosome 6. Only three families were common to both studies, and that QTL segregated in only one of these families (Family 5 in our study). These observations may suggest a low frequency of this putative QTL in the Churra population.

Conclusion

For the first time in a commercial population of dairy sheep, a whole genome scan using microsatellite markers revealed a genome-wise significant QTL, as well as other suggestive genetic effects that influence milk production-related traits. This study highlights the potential of a commercial dairy sheep population to increase our understanding of the genetic determinants of complex production-related traits. Additional analyses are underway with the aim of confirming and refining the genome-wise significant QTL detected on chromosome 3 for PP, as well as the suggestive significant QTL affecting FP on chromosome 20. For these two QTL regions, the agreement between our results and those obtained in the Sarda × Lacaune population (Barillet *et al.* 2006) suggests that a meta-analysis combining the information obtained in these two independent works would be a plausible option for overcoming the statistical limitations of studying a commercial population such as that analysed in the present study.

Many of the genetic effects detected in our study, even those at the suggestive significance level, are coincident with milk production QTL mapped in dairy cattle. This supports our results and indicates the possibility that some of the mechanisms underlying genetic variation in dairy-related traits are conserved across different ruminant species. Further studies are needed to determine if the coincident effects mapped in sheep and cow can be explained by the same biological mechanism.

The results summarized herein represent a first step towards a better understanding of the genetic control of

production traits in dairy sheep. With the advent of genomic progress and the commercial nature of the population used in this experiment, this molecular information may guide Churra dairy sheep breeders to enhance the yield and quality of their products in the near future.

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References

Alipanah M., Kalashnikova L. & Rodionov G. (2007) Association of *prolactin* gene variants with milk production traits in Russian Red Pied cattle. *Iranian Journal of Biotechnology* **5,** 158–61.

Alvarez L., Arranz J.J. & San Primitivo F. (2006) Identification of a new leucine haplotype (ALQ) at codon 154 in the ovine *prion protein* gene in Spanish sheep. *Journal of Animal Science* **84,** 259–65.

Ashwell M.S., Rexroad C.E.R. Jr, Miller R.H., VanRaden P.M. & Da Y. (1997) Detection of loci affecting milk production and health traits in an elite US Holstein population using microsatellite markers. *Animal Genetics* **28,** 216–22.

Ashwell M.S., Heyen D.W., Sonstegard T.S., Van Tassell C.P., Da Y., VanRaden P.M., Ron M., Weller J.I. & Lewin H.A. (2004) Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. *Journal of Dairy Science* **87,** 468–75.

Barillet F. (1997) Chapter 20: genetics of milk production. In: *The Genetics of Sheep* (Ed. by I. Piper & A. Ruvinsky), pp. 539–64. CAB International, Oxon.

Barillet F. (2007) Genetic improvement for dairy production in sheep and goats. *Small Ruminant Research* **70,** 60–75.

Barillet F., Arranz J.J. & Carta A. (2005) Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep. *Genetics Selection Evolution* **37**(Suppl. 1), S109–23.

Barillet F., Arranz J.J., Carta A., Jacquiet P., Stear M. & Bishop S. (2006) *Final Consolidated Report of the European Union Contract of Acronym genesheepsafety, QTLK5-CT-2000-00656*, p. 145.

Bennewitz J., Reinsch N., Grohs C. *et al.* (2003) Combined analysis of data from two granddaughter designs: a simple strategy for QTL confirmation and increasing experimental power in dairy cattle. *Genetics Selection Evolution* **35,** 319–38.

Bennewitz J., Reinsch N., Guiard V. *et al.* (2004) Multiple quantitative trait loci mapping with cofactors and application of alternative variants of the false discovery rate in an enlarged granddaughter design. *Genetics* **168,** 1019–27.

Boyazoglu J. & Morand-Fehr P. (2001) Mediterranean dairy sheep and goat products and their quality. A critical review. *Small Ruminant Research* **40,** 1–11.

Brym P., Kamiński S. & Wójcik E. (2005) Nucleotide sequence polymorphism within exon 4 of the bovine *prolactin* gene and its associations with milk performance traits. *Journal of Applied Genetics* **46,** 179–85.

Calvo J.H., Marcos S., Beattie A.E., Gonzalez C., Jurado J.J. & Serrano M. (2004) Ovine alpha-amylase genes: isolation, linkage mapping and association analysis with milk traits. *Animal Genetics* **35,** 329–32.

Churchill G. & Doerge R. (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138,** 963–71.

Coppieters W., Kvasz A., Farnir F., Arranz J.J., Grisart B., Mackinnon M. & Georges M. (1998) A rank-based nonparametric method for mapping quantitative trait loci in outbred half-sib pedigrees: application to milk production in a granddaughter design. *Genetics* **149,** 1547–55.

Cowan C.M., Dentine M.R., Ax R.L. & Scchuler L.A. (1990) Structural variation around *prolactin* gene linked to quantitative traits in an elite Holstein sire family. *Theoretical and Applied Genetics* **79,** 577–82.

Díez-Tascón C., Bayón Y., Arranz J.J., De La Fuente F. & San Primitivo F. (2001) Mapping quantitative trait loci for milk production traits on ovine chromosome 6. *Journal of Dairy Research* **68,** 389–97.

El-Saied U.M., Carriedo J.A., De La Fuente L.F. & San Primitivo F. (1999) Genetic parameters of lactation cell counts and milk and protein yields in dairy ewes. *Journal of Dairy Science* **82,** 639–44.

de la Fuente L.F., Fernández G. & San Primitivo F. (1995) Breeding programme for the Spanish Churra sheep breed. *Cahiers Options Méditerranéennes* **11,** 165–72.

de Gortari M.J., Freking B.A., Cuthbertson R.P., Kappes S.M., Keele J.W., Stone R.T., Leymaster K.A., Dodds K.G., Crawford A.M. & Beattie C.W. (1998) A second-generation linkage map of the sheep genome. *Mammalian Genome* **9,** 204–9.

Green P., Falls K. & Crooks S. (1990) *Documentation for* CRI-MAP, *Version 2.4*. Washington University school of Medicine, St Louis.

Grisart B., Coppieters W., Farnir F. *et al.* (2002) Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. *Genome Research* **12,** 222–31.

Gutiérrez-Gil B., El-Zarei M.F., Bayón Y., de la Fuente L.F., San Primitivo F. & Arranz J.J. (2007) Genome Scan analysis for detection of QTL influencing somatic cell score in dairy sheep. *Journal of Dairy Science* **90,** 422–6.

Gutiérrez-Gil B., Arranz J.J., El-Zarei M.F., Álvarez L., Pedrosa S., San Primitivo F. & Bayón Y. (2008a) A male linkage map constructed for QTL mapping in Spanish Churra sheep. *Journal of Animal Breeding and Genetics* **125,** 201–4.

Gutiérrez-Gil B., El-Zarei M.F., Alvarez L., Bayón Y., de la Fuente L.F., San Primitivo F. & Arranz J.J. (2008b) Quantitative trait loci underlying udder morphology traits in dairy sheep. *Journal of Dairy Science* **91,** 3672–81.

Harder B., Bennewitz J., Reinsch N. *et al.* (2006) Mapping of quantitative trait loci for lactation persistency traits in German Holstein dairy cattle. *Journal of Animal Breeding and Genetics* **123,** 89–96.

Harmegnies N., Davin F., De Smet S., Buys N., Georges M. & Coppieters W. (2006) Results of a whole-genome quantitative trait

locus scan for growth, carcass composition and meat quality in a porcine four-way cross. *Animal Genetics* **37,** 543–53.

Harper W.J. (1959) Chemistry of cheese flavors. *Journal of Dairy Science* **42,** 207–13.

Hayes K.C. & Khosla P. (1992) Dietary fatty acid thresholds and cholesterolemia. *FASEB Journal* **6,** 2600–7.

Hirose M., Sarui K. & Sunagawa A. (1985) Correlation between nuclear histone acetylation and casein messenger RNA induction in the mammary gland. *Journal of Biochemistry* **97,** 781–9.

Horseman N., Zhao W., MOntecino-Rodriguez E., Tanaka M., Nakashima K., Engle S.J., Smith F., Markoff E. & Dorshkind K. (1997) Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the *prolactin* gene. *EMBO Journal* **16,** 6926–35.

Khanal R.C. & Olson K.C. (2004) Factors affecting CLA content in milk, meat, and egg: a review. *Pakistan Journal of Nutrition* **3,** 82–98.

Khatkar M.S., Thomson P.C., Tammen I. & Raadsma H.W. (2004) Quantitative trait loci mapping in dairy cattle: review and meta-analysis. *Genetics Selection Evolution* **36,** 163–90.

Knott S.A., Elsen J.M. & Haley C.S. (1996) Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *Theoretical and Applied Genetics* **93,** 71–80.

Lander E. & Kruglyak L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics* **11,** 241–7.

Legarra A. & Ugarte E. (2005) Genetic parameters of udder traits, somatic cell score, and MY in Latxa sheep. *Journal of Dairy Science* **88,** 2238–45.

Lipkin E., Mosig M.O., Darvasi A., Ezra E., Shalom A., Friedmann A. & Soller M. (1998) Quantitative trait locus mapping in dairy cattle by means of selective milk DNA pooling using dinucleotide microsatellite markers: analysis of milk protein percentage. *Genetics* **149,** 1557–67.

Liu Y., Jansen G.B. & Lin C.Y. (2004) Quantitative trait loci mapping for dairy cattle production traits using a maximum likelihood method. *Journal of Dairy Science* **87,** 491–500.

Maddox J. & Cockett N. (2007) An update on sheep and goat linkage maps and other genomic resources. *Small Ruminant Research* **70,** 4–20.

Maddox J.F., Davies K.P., Crawford A.M. *et al.* (2001) An enhanced linkage map of the sheep genome comprising more than 1000 loci. *Genome Research* **11,** 1275–89.

Morton N.E. (1956) The detection and estimation of linkage between genes for elliptocytosis and the Rh blood type. *American Journal of Human Genetics* **8,** 80–96.

Mosig M.O., Lipkin E., Khutoreskaya G., Tchourzyna E., Soller M. & Friedmann A. (2001) A whole genome scan for quantitative trait loci affecting milk protein percentage in Israeli-Holstein cattle, by means of selective milk DNA pooling in a daughter design, using an adjusted false discovery rate criterion. *Genetics* **157,** 1683–98.

Nadesalingam J., Plante Y. & Gibson J.P. (2001) Detection of QTL for milk production on chromosomes 1 and 6 of Holstein cattle. *Mammalian Genome* **12,** 27–31.

Nakano T., Inoue I., Koyama I. *et al.* (2007) Disruption of the murine intestinal alkaline phosphatase gene *Akp3* impairs lipid transcytosis and induces visceral fat accumulation and hepatic steatosis. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **292,** G1439–49.

Nicholas F.W., Barendse W., Collins A. *et al.* (2004) Integrated maps and Oxford grids: maximising the power of comparative mapping. In: *Proceedings of the 29th International Conference on Animal Genetics*: 11–16 September 2004; Tokyo, Japan (ISBN-4-9902072-1-1)

Olsen H.G., Nilsen H., Hayes B., Berg P.R., Svendsen M., Lien S. & Meuwissen T. (2007) Genetic support for a quantitative trait nucleotide in the *ABCG2* gene affecting milk composition of dairy cattle. *BMC Genetics* **21,** 32.

Othmane M.H., De La Fuente L.F., Carriedo J.A. & San Primitivo F. (2002) Heritability and genetic correlations of test day milk yield and composition, individual laboratory cheese yield, and somatic cell count for dairy ewes. *Journal of Dairy Science* **85,** 2692–8.

Plante Y., Gibson J.P., Nadesalingam J., Mehrabani-Yeganeh H., Lefebvre S., Vandervoort G. & Jansen G.B. (2001) Detection of quantitative trait loci affecting milk production traits on 10 chromosomes in Holstein cattle. *Journal of Dairy Science* **84,** 1516–24.

Rodriguez-Zas S.L., Southey B.R., Heyen D.W. & Lewin H.A. (2002) Interval and composite interval mapping of somatic cell score, yield, and components of milk in dairy cattle. *Journal of Dairy Science* **85,** 3081–91.

Ron M., Feldmesser E., Golik M. *et al.* (2004) A complete genome scan of the Israeli Holstein population for quantitative trait loci by a daughter design. *Journal of Dairy Science* **87,** 476–90.

Sambrook J., Fritsch E.F. & Maniatis T. (1989) *Molecular Cloning. A Laboratory Manual*, 2nd edn, Volumes 1, 2, and 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanna S.R., Carta A. & Casu S. (1997) (Co)variance component estimates for milk composition traits in Sarda dairy sheep using a bivariate animal model. *Small Ruminant Research* **25,** 77–82.

SAS Institute (1998) *SAS User's Guide: Statistics, Release 6.12*. SAS Inst., Inc., Cary, NC.

Smaragdov M.G., Prinzenberg E.M. & Zwierzchowski L. (2006) QTL mapping in cattle: theoretical and empirical approach. *Animal Science Papers and Reports* **24,** 69–110.

Soller M.A. & Genizi A. (1978) The efficiency of experimental designs for the detection of linkage between a marker locus and a locus affecting a quantitative trait in segregating populations. *Biometrics* **34,** 47–55.

Spelman R.J., Coppieters W., Karim L., van Arendonk J.A. & Bovenhuis H. (1996) Quantitative trait loci analysis for five milk production traits on chromosome six in the Dutch Holstein-Friesian population. *Genetics* **144,** 1799–808.

Ushizawa K., Takahashi T., Hosoe M., Ohkoshi K. & Hashizume K. (2007) Expression and characterization of novel ovine orthologs of bovine placental prolactin-related proteins. *BMC Molecular Biology* **25,** 95.

Vanraden P.M. & Wiggans G.R. (1991) Derivation, calculation, and use of national animal model information. *Journal of Dairy Science* **74,** 2737–46.

Velmala R.J., Vilkki H.J., Elo K.T., de Koning D.J. & Mäki-Tanila A.V. (1999) A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle. *Animal Genetics* **30,** 136–43.

Visscher P.M., Thompson R. & Haley C.S. (1996) Confidence intervals in QTL mapping by bootstrapping. *Genetics* **143,** 1013–20.

Weller J.I., Kashi Y. & Soller M. (1990) Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *Journal of Dairy Science* **73,** 2525–37.

Weller J.I., Weller H., Kliger D. & Ron M. (2002) Estimation of quantitative trait locus allele frequency via a modified granddaughter design. *Genetics* **162,** 841–9.

Zhang Q., Boichard D., Hoeschele I. *et al.* (1998) Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. *Genetics* **149,** 1959–73 (Erratum in: *Genetics* 1999, **153**, 513).