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## Genetic determinism of quality of lamb leather in Lacaune dairy sheep breed

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### Abstract

Lacaune lamb skins are well known for their high quality and their use in the leather industry and luxury ready-to-wear clothing. In France, for several decades, the quality of this leather has deteriorated, mainly due to two skin defects: pinhole and straw-like bushiness. To explore the genetic determinism of these defects, around 1400 Lacaune lambs, from 72 rams, were phenotyped for the 2 skin defects and 4 wool traits. The pinhole defect showed a high heritability ( $0.59 \pm 0.07$ ) and was highly genetically correlated to the wool predicted core bulk ( $r_g = 0.54 \pm 0.08$ ). The straw-like bushiness defect had a lower heritability ( $0.25 \pm 0.06$ ), and was correlated to the mean fiber diameter ( $r_g = 0.41 \pm 0.15$ ). A genome wide association study revealed for the pinhole defect one major signal on chromosome 3 close to a cluster of keratin genes, known to play an important role in skin development.

### Introduction

Lamb skins are generally considered to be a by-product of sheep production and genetic studies of traits directly related to sheep skin are infrequent in the literature. The Lacaune sheep milk breed is bred in south of France, in the Roquefort cheese production area. Lacaune lamb skins are well known for their high quality and their use in the leather industry and luxury ready-to-wear clothing. Therefore, historically, a large majority of French tanneries was located in the same geographical region. However, for several decades, a degradation of this leather quality was observed, mainly due to the presence of two skin defects: pinhole and straw-like bushiness. Pinhole consists of small holes in the grain surface of the dewooled pelt, which decreases the leather quality. The straw-like bushiness defect is defined as perforations due to the presence of forage debris planted in the thickness of the dermis. In the present study, we explored the genetic determinism for these two main skin defects and their possible relationship with wool traits.

### Materials & Methods

#### *Animals and phenotypes.*

Around 1400 Lacaune lambs (from 72 rams) were born around February 2020 and 2021 (in 16 different farms) and identified at birth with electronic ear tags. At around 35 days-old lambs were brought together in a unique fattening farm. Around 60 days-old, a blood and a wool samples of each animal were collected (they were carried out in the fattening herd within the framework of the usual practices of breeding by authorized persons from OVI-test AI organization). After an average fattening period of 79 days, animals were slaughtered and their skins were sent to the ALRIC tannery to proceed to the tanning. Each skin was identified in order to be assigned to one lamb. The two defects were scored at the end of the tanning procedure: skin pinhole score (from 0=absent to 4=abundant corresponding to several heavily marked regions), and straw-like bushiness score (from 0=absent to 2=abundant). Wool

samples were analysed by Optical Fibre Diameter Analyser (OFDA) for determining mean fibre diameter (MFD,  $\mu$ ), coefficient of variation of fibre diameter (CVD, %) and mean fibre curvature (MFC,  $^{\circ}/\text{mm}$ ). Predicted core bulk (PCB), expressed in  $\text{cm}^3/\text{g}$ , represents the occupied volume by 1 gram of wool under a pressure of  $10^{-3}$  N (Sumner et al., 2005). At the end, 1379 lambs were phenotyped for wool traits, 1166 for leather defects, and 1158 for both (Table 1).

### ***SNP genotypes.***

DNA of a subset of 1248 animals was extracted from blood sample and genotyped using the Illumina OvineSNP50 BeadChip. Genomic analyses were performed using 1248 phenotyped and genotyped lambs, plus their 72 rams' genotypes (available from a previous project). SNP quality was checked (i.e. SNP call rates  $>95\%$ , minor allele frequency  $>5\%$ , inconsistent for Hardy-Weinberg disequilibrium). Finally, 38,439 autosomal SNPs were retained and 14 lambs failed QC and were removed.

### ***Statistical analysis.***

Variance-covariance components were estimated with the airemlf90 software (Misztal et al., 2002) using pedigree information (8575 animals over 6 generations) combined with SNPs information. Heritabilities were estimated with linear single trait animal models, and genetic correlations with linear bivariate animal models. For leather defects, the models included environmental fixed effects of birth herd (16 levels), year\*housing condition during the fattening period (3 levels), litter size (2 levels: 1, 2 or more), duration of fattening period (as co-variable,  $79\text{d} \pm 15.71$ ) and a random additive genetic animal effect. For wool traits, the models were the same excepting for duration of fattening period replaced by the age at sampling ( $61\text{d} \pm 12.07$ ). Genome wide association analysis (GWAS) was performed using GEMMA software (Zhou and Stephens, 2012) using phenotypes adjusted for environmental effects defined above with the procedure GLM of SAS software, the threshold of significance was at the genome level (probability of 0.05 divided by the number of SNP used).

## **Results**

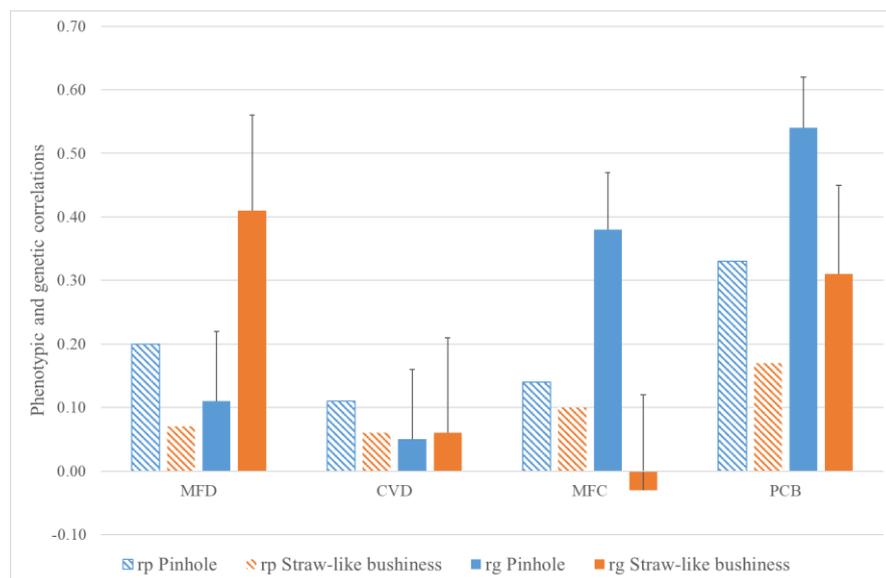
Phenotypes characteristics of leather and wool traits are presented in Table 1. The mean of the pinhole score was 1.47, and 25% of skins did not express the defect whereas the percentages of skins classified of 1 to 4 were of 28%, 27%, 17% and 4% respectively. For Straw-like bushiness, the mean was 0.86, and 45% of skins showing no defect, whereas 27% presented this defect with a low density (score 1) and 28% with a high density (score 2). Straw-like bushiness presented a moderate estimated heritability of 0.25, whereas the other traits had a high estimated heritability ranging from 0.48 for MFD to 0.61 for MFC (Table1).

**Table 1. Summary of phenotypes used for estimation of genetic parameters and heritability estimations.**

	Records	Mean	Std	Heritability	SE <sup>1</sup>
Pinhole	1166	1.47	1.14	0.59	0.07
Straw-like bushiness	1166	0.86	0.84	0.25	0.06
MFD	1379	19.31	2.02	0.48	0.07
CVD	1379	25.75	5.85	0.51	0.06
MFC	1379	93.45	10.38	0.61	0.06
PCB	1379	28.02	1.43	0.57	0.06

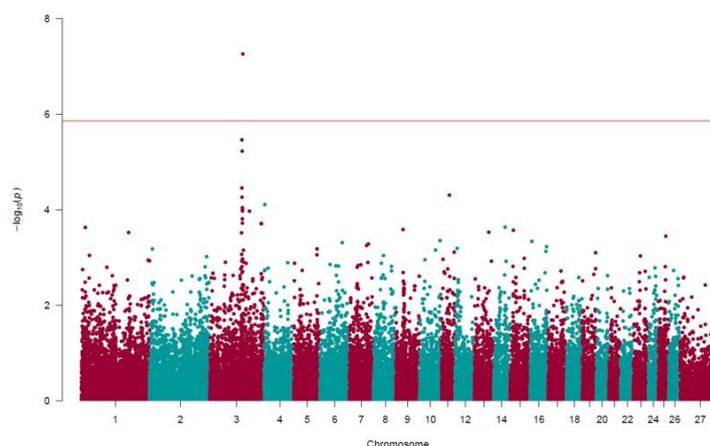
<sup>1</sup>: approximated standard error of heritability

Phenotypic and genetic correlations estimated between each leather defect and wool traits are shown in Figure 2. The two leather defects showed low to moderate phenotypic correlations with wool traits and reach 0.33 and 0.17 with PCB (for pinhole and straw-like bushiness defects respectively). Although the two defects were genetically highly correlated ( $0.66 \pm 0.11$ ), their correlations with wool traits were somewhat different. Pinhole defect presented a strong positive genetic correlation with MFC ( $0.38 \pm 0.09$ ) and PCB ( $0.54 \pm 0.08$ ). Lambs with high MFC or high PCB wool had genetically a skin with more pinhole defects. Straw-like bushiness defect showed a moderate positive genetic correlation with PCB ( $0.31 \pm 0.14$ ) and a higher genetic correlation with MFD ( $0.41 \pm 0.15$ ). The two defects were related to PCB.



**Figure 1. Phenotypic and genetic correlations between leather defects and wool traits** (rp: phenotypic correlation, rg: genetic correlation, vertical line: approximated standard error).

Genome wide association studies were performed on 1147 animals for the pinhole defect and the straw-like bushiness defect. A significant signal was detected at the genome-wide threshold for the pinhole defect, localized on OAR3 (Figure 2).



**Figure 2. Manhattan plot for GWAS of pinhole defect.** This plot is based on  $-\log_{10}(P)$  from GWAS against chromosome position, each colour represents different chromosome. Red line indicates genome-wide significant threshold.

We identified the most significant SNP at a position of 146.2 Mb on the ovine reference genome (Oar\_rambouillet\_v1.0). Considering the significant region at the chromosome-wide threshold, it encompasses 4Mb and the cluster of Keratin genes localised between 143-144 Mb. In contrast, no significant signal was found for the straw-like bushiness defect.

## **Discussion**

A defect similar to the pinhole defect was studied in New-Zealand and Australia. We found an estimation of pinhole heritability of 0.59, which was close to the estimation of 0.6 found by Campbell et al. (1997) on fewer animals. We have found a high genetic correlation (0.54) between pinhole defect and PCB, a higher level of wool PCB being related to a higher presence of pinhole defect. This can be related to previous studies carried out in New-Zealand, where a phenotypic positive relationship was found between the presence of the pinhole defect and PCB (Scobie et al., 1997; Cooper, 2002). In these previous studies, high PCB was related to a more pronounced curvature of fibers. These authors proposed that when removing the fleece, fibers that have a high curvature remove part of the collagen matrix at the same time, affecting the skin (Scobie et al., 1997).

No data on genetic parameters for the straw-like bushiness defect are available in literature. Its heritability estimation was 0.25, suggesting a large influence of the environment on this skin defect. Length of the fattening period (highly correlated with age at slaughter) seemed to influence the presence of this defect. Indeed, lambs with a longer fattening period had more straw-like bushiness defects, which could be linked to a longer exposition of their skin to the plant debris.

Our genetic study led to the identification of a region nearby the cluster of Keratin genes localized on OAR3. Keratin genes is a family of structural fibrous proteins also known as scleroproteins. It is the key structural material making up scales, hair, nails, feathers, horns, claws, hooves, and the outer layer of skin among vertebrates. Keratin also protects epithelial cells from damage or stress. Sequencing of finely chosen animals is currently on-going to identify one or several candidate mutations in our region of interest.

For pinhole defect, its high heritability, its strong genetic correlation with a wool trait measurable on live animals and the detection of a QTL affecting it, indicate that a genetic selection could be considered in order to decrease its frequency in Lacaune breed. However, additional studies must be conducted in order to better understand its genetic determinism and to evaluate its relationship with traits currently selected in this dairy sheep breed.

## **Acknowledgements**

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