Introgression of wool-shedding genes into the Romane breed sheep

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ABSTRACT: Wool shedding data were measured on 2762 Romane females and after an experimental design aiming to introgress wool-shedding genes from the Martinik Hair into the Romane breed through 4 successive backcrossing generations. Ability to shed varied from 42.7 % to 52.8% according to age but only 1.2% of adult Romane ewes showed total wool shedding. Estimated heritability of wool shedding was high (0.69) and wool shedding in lambs was strongly genetically correlated (0.86) to wool shedding in adults. After the introgression process, a large increase of the ability to shed (+75.3%) and shedding extent (+145.7%) was observed. This introgressed breeding stock (n=120 ewes) was then selected using EBV. A high genetic gain (+1 σ_g) was observed after 2 generations of selection indicating that a rapid progress can be achieved through introgression of wool-shedding genes and then selection in the Romane breed.
Keywords: Sheep; Romane breed; Wool shedding introgression

Introduction

Wool production in Europe is now generally unprofitable and may indeed be undesirable compared to meat or milk production. The income from wool is less than the costs associated with shearing and the removal of soiled wool (‘dagging’) from lambs prior to slaughter or to prevent fly strike (Vipond, 2006). Thus with increasing shearing costs, there is an interest in Europe for the use of breeds that have no wool or shed their wool. Some farmers in the UK or Germany are experimenting with crossing their sheep with other breeds that either shed their wool annually, or are recognised ‘hair’ sheep breeds, such as the Wiltshire Horn, the Dorper, Kaahdin and Barbados Blackbelly, with varying degrees of success (Conington, 2010). No similar attempt has been made in France up to now. In France, the Romane breed, a 50:50 composite breed (Ricordeau et al., 1992) between the “Berrichon du Cher” (meat breed) and the Romanov (prolific breed with high maternal ability) shows a large variability in its fleece type. The highly variable birthcoat type was found to be related to lamb survival (Allain et al, 2014) and the kempy fleece in the adult shows some ability to shed annually (Allain et al., 2010). As only a few animals shed their fleece completely, it has been decided to evaluate a strategy based on gene introgression of wool shedding from the Martinik Hair breed, a hairy sheep known to shed annually (Leimbacher et al., 2010) in the Romane breed. The present paper i) investigates genetic variability of wool shedding in the Romane breed and ii) describes the experimental design with first results aiming to evaluate introgression of shedding genes pool from the Martinik Hair into the Romane breed through 4 successive backcrosses with selection of breeding stock on shedding ability at each generation.

Materials and Methods

Experiment 1: Genetic variability of fleece shedding in the adult Romane ewe. A flock of 350 ewes of the Romane (RM) breed was used as experimental support since 2002 at the INRA farm of La Fage on the Causses-du-Larzac, a calcareous plateau at an altitude of 800m in the South of France. This flock was kept outside all year round on a dry rangeland as previously described (Allain et al, 2014). For reproduction, AI was used on all ewes in November from the age of 8 months. All ewes were measured for fleece shedding from 2002 once a year at the end of June, just before annual shearing from the age of 15 months.

Experiment 2: Introgression of fleece shedding genes into the Romane breed.

Animals and experimental conditions. A flock of 50 Martinik Hair (MH) ewes and a flock of 600 RM ewes were used as experimental support at the INRA farm of Bourges. Animals are raised in a barn with open sides. Depending on grazing availability, season, and experimental requirements, animals were fed indoors or on pasture during the day from spring to autumn. RM ewes were mated for the first time at the age of 9 months in July, at the age of 18 months in April for the second time and then once a year in April. MH ewes were mated once a year in July. All females of the RM flock were measured for fleece shedding at 9 months of age as lambs and one year later at 19 months of age as adults. All females were measured for fleece shedding at mid-June before annual shearing from at both 10 and 19 months of age from 2009 to 2013.

Introgression design. The experimental introgression design was implemented in two phases. In a first step, 4 F1 (MH*RM) rams were mated to 600 RM ewes to create a population of backcrossed animals (BC1: F1*RM) born from December 2003 to December 2008 and used for QTL detection for nematode resistance in sheep (Sallé et al, 2012). A total of 33 BC1 males were measured for fleece shedding at mid-June at 7 and 19 months of age in 2008 and 2009 respectively. In a second step, 6 BC1 males which had shed their fleece were mated in 2009 to 120 RM females of 10 months of age that had also shed their fleece to create a BC2 (BC1*RM) generation. BC2 males were measured for fleece shedding at 7 months of age. Then 10 BC2 males that had shed their fleece were mated to 180 RM females of 10 months of age that had also shed their fleece to create a BC3 (BC2*RM) generation. The first BC3 generation was born in December 2010 and considered as a new population which was then selected on wool shedding.

Selection of the introgressed BC3 population. BC3 males of the first generation were selected in two steps. At 3 months of age, about a quarter of the males within each sire family were selected on staple length (shorter staples). These pre-selected males and all females were measured for fleece shedding at 7 months of age. In October 2011, all BC3 females (n=136) of 10 months of age were mated to 7 selected males of different sire families having the most wool shedding. From 2012, animals were measured for fleece shedding at both 4 and 7 months of age. About a quarter of males were preselected phenotypically on wool shedding in June at 4 months of age. All females and preselected males were also measured for wool shedding at 7 months of age. Breeding values were estimated within BC3 population in order to select breeding stock of the next generation. The BC3 ewes were mated for the first time at the age of 9 months in October, at the age of 18 months in July for the second time then once a year.

Fleece shedding measurement. Fleece shedding was measured in two steps once a year in June before shearing for animals older than 7 months and twice a year in June and September for younger lambs. On farm, the part of animal body area that had shed was drawn on a standard sheep profile. This drawing was then analysed using image software (Visilog) to estimate the extent of fleece shedding. Two traits were defined: the ability to shed at least a part of the fleece or not, as a binary trait and the extent of shedding as the ratio of wool shed area to total body area. Distribution of the extent of shedding being non normal, this trait was categorized in 8 classes (1: <5%; 2: 5 to <20%; 3: 20 to <35%; 4: 35 to <50%; 5: 50 to <65%; 6: 65 to <80%; 7: 80 to <95% and 8: >=95% wool shedding extent).

Data Analysis. Data were analysed in 3 separate datasets with the TM (Threshold Model) program (Legarra et al, 2008) using a Bayesian analysis and performing numerical integration through the Gibbs sampler to estimate genetic parameters in the adult RM ewe (dataset 1), both RM lambs and hogget (dataset 2) and BC3 or introgressed population (dataset 3). A chain of 210,000 iterations was used, with a burn-in of 10,000 rounds saving a sample every 100 iterations. The mean and standard error of the estimated marginal posterior density were used as estimates and standard errors of genetic parameters. ***Dataset 1*** included a total of 2984 measurements from 1259 RM ewes from 1 up to 5 years of age made between 2002 and 2013 (experiment 1). The pedigree data included relationships between 2485 animals over up to 15 generations. Classes of wool shedding extent were analysed according a mixed model including age of ewe at measurement (from 1 to 5 yr), year of birth, age of dam, year of production with the numbers of lambs produced and suckled respectively as fixed effects, and additive genetic, permanent environment and residual error as random effects. ***Dataset 2*** included a total of 1503 records from RM ewes measured for fleece shedding in mid-June at both 9 and 21 months of age from 2009 to 2013 (experiment 2). Classes of wool shedding extent in lambs and in adult ewes were analysed as separate traits through a bivariate mixed model including year of birth, age of dam, type of birth and type of rearing as fixed effects, and additive genetic and residual error as random effects. For 19-month old ewes, type of birth and rearing type effects were replaced by the numbers of lambs produced and suckled. Pedigree data included 3385 animals. ***Dataset 3*** included a total of 416 BC3 animals measured for fleece shedding at 4 (n=299) and/or 7 (n=416) months of age from 2011. Thereafter, 180 BC3 animals (n= 251 records) were also measured for fleece shedding as adult ewes in mid-June before shearing and after the first and second reproductive cycle. Classes of wool shedding extent were analysed according to a mixed model including sex, age at measurement (4, 7, 19 or 31 months), year of birth (from 2011 to 2013), type of birth, rearing type and age of dam as fixed effects. For ewe records, effects of type of birth and number of lambs suckling their dam were replaced by the numbers of lambs produced and suckled by the ewe respectively at each reproductive cycle. Three random effects were included in the model: additive genetic, permanent environment and the residual error. Solutions for additive genetic effects of the mixed models were used as EBV.

Results and Discussion

Variability and heritability of wool shedding in the adult RM breed. Table 1 shows basic statistics of wool shedding. Ability to shed at least a part of their fleece once a year during spring was observed in 42.7% of female lambs, 52.8% of 19 month ewes and 41.3% of adult ewes in Bourges and La Fage flocks respectively. Mean fleece shedding extent was 6.0%, 13.7% and 14.4% in female lambs and 19 month ewes of Bourges flocks, and adult ewes of La Fage flock respectively. Total wool shedding was only observed on 0.9% and 1.2 % of adult ewes in Bourges and La Fage flocks. A general pattern was observed over body regions, with wool shedding first from the neck and belly, then moving progressively in a dorso-posterior direction to end on the rump. The RM breed being a 50:50 composite line between Romanov and Berrichon du Cher, genes involved in shedding ability probably originated from the Romanov breed where fibre shedding has been reported (Bykova, 1973) while it never occurs in "Berrichon du Cher". Table 2 shows wool shedding according to age in the adult RM ewes of the La Fage flock. Age of the ewe, reproductive activity and numbers of lambs produced and suckled have highly significant effects on wool shedding. Ewes 1 year of age shed less ($p<0.001$) than older ewes. Increase of wool shedding with age has been also reported in Wiltshire-Merino crosses (Rathie et al., 1994). Such a difference of wool shedding in young ewes can be due either to gestation and reproductive activity or to body growth development at the age of 7 months as the decision on the age of first mating (7 or 19 months) was conditioned by a minimum live weight of 35kg at 7 months of age. Thereafter, no age effect on wool shedding was observed. However breeding ewes producing and suckling lambs shed more fleece than others and wool shedding increased with the number of lambs produced and suckled.

Table 1. Basic statistics of the ability to shed (%) and shedding extent (%) at different ages in the Romane breed and the first BC3 unselected population.

	RM			BC3	
	7 mo[3]	19 mo[3]	Adult[4]	7 mo[3]	19 mo[3]
N =	1303	1004	2984	159	114
Ability to shed[1]	42.7	52.8	41.3	74.8	94.7
Shedding extent[2]	6.0	13.7	14.4	14.8	34.4
	(12.3)	(20.3)	(22.4)	(18.3)	(30.9)

[1] % of animals that partly shed their fleece
[2] mean (± SD) of the ratio of shed area to total body area
[3] lambs (7 months) and yearling (19 months) ewes from Bourges flock
[4] adult ewes (1 to 5 years old) from La Fage flock

Table 2. Wool shedding according to age in the adult Romane ewe.

Age	1 year	2 years	3 years	4 years
Ability to shed [1]	29.0	48.1	52.4	45.5
Shedding extent [2]	9.7	17.8	17.2	15.6
	(17.9)	(24.4)	(24.7)	(24.1)

[1 2] see table 1

High heritability estimates of wool shedding in the RM breed were observed: 0.73 (± 0.11), 0.42 (± 0.09) and 0.69 (± 0.05) in lamb females, yearling and adult ewes respectively. These results are in agreement with preliminary observations in this breed (Allain et al., 2010), the New Zealand Wiltshire (O'Connell et al, 2012) and another study observed on a flock composed of a range of shedding breeds and composite animals (F1 and BC1) between non shedding and shedding breeds in order to introduce wool-shedding genes in the flock (Pollott, 2011). A high genetic correlation (0.86 ± 0.09) was observed when wool shedding in lambs and yearling ewes were considered as separate traits indicating that it is genetically almost the same trait, as also observed by Pollott (2011).

Introgression of wool-shedding genes into the RM breed. Table 1 shows ability to shed and shedding extent observed in the first cohort of BC3 animals born in 2011 compared to RM breed**.** In BC3 females, ability to shed and shedding extent increased by 75.3% and 145.7%, and 79.4% and 150.1% at 7 and 19 months of age respectively in the first BC3 cohort compared to the RM breed. A phenotypic selection on wool shedding at lamb age on both RM female and BC males was undertaken through back-crossing generations up to the first BC3 cohort. Such a selection process during the introgression scheme was efficient. Thereafter BC3 animals were measured for wool shedding at the age of 4 and 7 months and selected on EBV. A phenotypic gain was observed: ability to shed and the extension of shedding increase by 19.4% and 81.6% respectively. In the BC3 generation born in 2013, 89.4% of animals shed at least a part of their fleece at 7 months of age with a mean fleece shedding of 29.3%. A high heritability estimate (0.50 ± 0.09) was observed in this BC3 population. A gain of 1.0 genetic standard deviation was observed in BC3 animals born in 2013 compared to the first BC3 generation.

Pollott (2011) proposed that the genetic control of wool shedding can be separated in two parts: i) the ability to shed for which the mode of inheritance would be autosomal dominant with a low level of incomplete penetrance and ii) the extent of shedding which would be a polygenic trait. Our results cannot demonstrate the existence of a dominant autosomal gene. However, it is clear that introgression of wool-shedding genes through 4 successive backcrossing generations from the shedding MH breed to the RM breed followed by selection using EBV is an efficient way to increase wool shedding.

Conclusion

Wool shedding with a high heritability estimate was observed in the pure Romane sheep breed. However as only a few animals completely shed their fleece annually it could take a long time to obtain shedding sheep by selection. Our first results about introgression of shedding genes from the Martinik Hair breed through 4 successive backcrossing generations seems to be an interesting selection strategy to lead more quickly to a shedding sheep and thus to create genetic progress by improving adaptive traits in the Romane breed which has a high production potential (Francois et al., 2010).

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