



HAL
open science

EIF2S2 Retroposition into IRF2BP2 Underlies Fleece Variation in Sheep through the Formation of a Long Endogenous Double-Stranded RNA

Julie Demars, Margarita Cano, Laurence Drouilhet, Stéphane Fabre, Bertrand Servin, Philippe Mulsant, Gwenola Tosser-Klopp, Daniel Allain

► **To cite this version:**

Julie Demars, Margarita Cano, Laurence Drouilhet, Stéphane Fabre, Bertrand Servin, et al.. EIF2S2 Retroposition into IRF2BP2 Underlies Fleece Variation in Sheep through the Formation of a Long Endogenous Double-Stranded RNA. Plant and Animal Genomes Conference, Jan 2017, San Diego, United States. hal-02785197

HAL Id: hal-02785197

<https://hal.inrae.fr/hal-02785197>

Submitted on 4 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

EIF2S2 retroposition into IRF2BP2 underlies fleece variation in sheep through the formation of a long endogenous double-stranded RNA

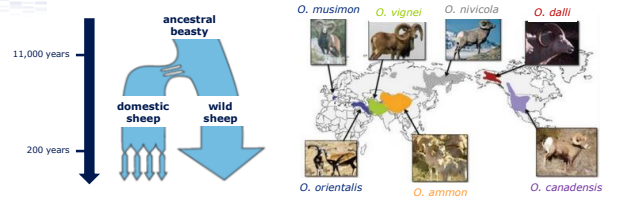


J. Demars, M. Cano, L. Drouilhet, S. Fabre, B. Servin, P. Mulsant, G. Tosser and D. Allain

Abstract W132
PAGXXV - Cattle/Sheep/Goat Workshop
Sunday, January 15, 2017

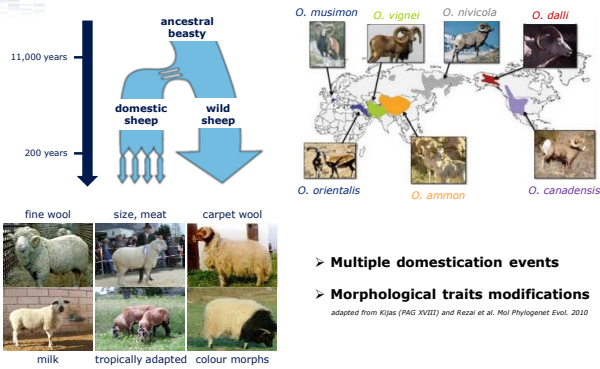


The sheep population history



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The sheep population history



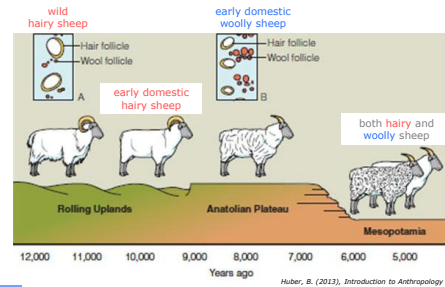
- Multiple domestication events
 - Morphological traits modifications
- adapted from Kijas (PAG XVII) and Rezaei et al. Mol Phylogenet Evol. 2010



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

Fleece variation under the sheep domestication

- Variation in composition and structure of the fleece
- Hairy sheep vs. woolly sheep



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

Hairy and woolly sheep in modern breeds

- Hairy sheep
Sheep Production Handbook : « sheeps whose coats consist of hair, more like that of cattle and goats, rather than wool »

- Phenotypes (hairy vs. woolly) easily detectable on lambs
 - Hairy long coat ≈ 23.1 mm



- Woolly short coat ≈ 8.3 mm



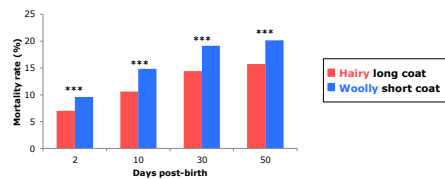
adapted from Allain et al. JAS 2013



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The fleece variation, a major agronomic trait

- Important survival component
 - Less total mortality rate in long hairy bearing coats lambs



From Allain et al. JAS 2013



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The fleece variation, a major agronomic trait

- **Important survival component**
 - Less total mortality rate in long hairy bearing coats lambs
 - More heat losses in woolly bearing coat lambs
 - Long hairy coat lambs are more resistant to weather changes
- **Heritability of lamb birthcoat type**
 - Binary trait, heritability = 0.85
 - Continuous trait, heritability = 0.56
- **Sheep breeding permanent outdoor**
 - Economical and agronomical trait = Importance to use in selection
 - French Romane breed, high productive potential breed

from Allain et al. JAS 2012

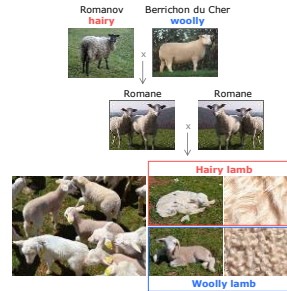


Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

Description of the study design

A familial strategy

The French Romane breed

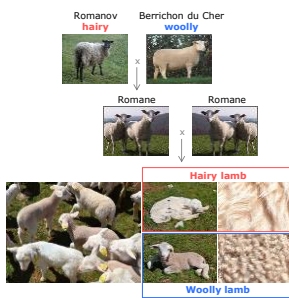


Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

Description of the study design

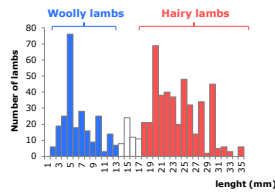
A familial strategy

The French Romane breed



- **Animals**
Half-sib QTL design in Romane
8 sires Romane * dams Romane

- **Phenotype**
Length of lamb's fleece (quantitative)
Type of lamb's fleece (binary)

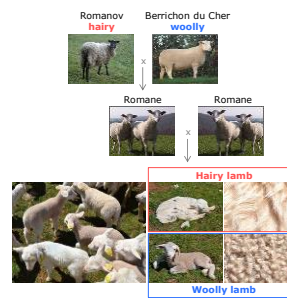


Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

Description of the study design

A familial strategy

The French Romane breed



- **Animals**
Half-sib QTL design in Romane
8 sires Romane * dams Romane

- **Phenotype**
Length of lamb's fleece (quantitative)
Type of lamb's fleece (binary)

- **Genotype**
OvineSNP50 Genotyping BeadChip (n = 759)
Ovine Infinium® HD SNP BeadChip (n = 135)

- **Methodology**
Imputation
Genome-Wide Association Analysis (GWAS)
Linkage Analysis

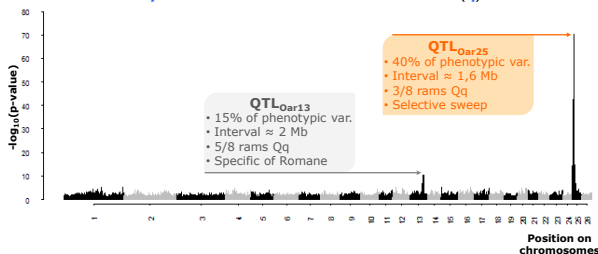


Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

Two loci underlie fleece variation in Romane

➤ Genetic determinism

- 66% of hairy lambs vs. 33% of woolly lambs
- **Hairy dominant** allele from **Romanov (Q)**
- **Woolly recessive** allele from **Berrichon du Cher (q)**



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

Fine-mapping of the QTL_{Oar25}

A familial strategy

The French Romane breed

- **Animals**
Half-sib QTL design in Romane
8 sires Romane * dams Romane

- **Phenotype**
Length of lamb's fleece (quantitative)
Type of lamb's fleece (binary)

- **Genotype**
OvineSNP50 Genotyping BeadChip (n = 759)
Ovine Infinium® HD SNP BeadChip (n = 135)

- **Methodology**
Identity By State Haplotype Identification

A population strategy

Ancestral species and French breeds

- **Hairy ancestral species**
Ovis orientalis (n = 15)
Ovis musimon (n = 18)
Ovis capadensis (n = 3)
Ovis dalli (n = 2)

- **Hairy domestic breeds**
(6 breeds and 116 individuals)
Causse du Lot, Corse, Limousine, Manech tête rousse, Rava, **Romanov**

- **Woolly modern breeds**
(18 breeds and 402 individuals)
Berrichon du Cher, Blanche du Massif Central, Charmoise, Charollais, Ile-de-France, Lacaune (lait), Lacaune (viande), **Mérino d'Arles**, Mérino de Rambouillet, Mourerous, Ouessant, Préalpes du Sud, Rouge de l'Ouest, Roussin de la Hague, Suffolk, Tarasconnaise, Texel, Vendéen



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

Fine-mapping of the QTL_{Oar25}

A familial strategy The French Romane breed

- Animals**
Half-sib QTL design in Romane
8 sires Romane * dams Romane
- Phenotype**
Length of lamb's fleece (quantitative)
Type of lamb's fleece (binary)
- Genotype**
OvineSNP50 Genotyping BeadChip (n = 759)
Ovine Infinium® HD SNP BeadChip (n = 135)
- Methodology**
Identity By State Haplotype Identification

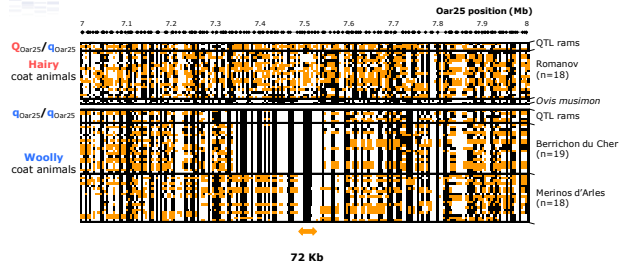
A population strategy Ancestral species and French breeds

- Animals**
Hairy ancestral species (n = 38)
(*Ovis musimon*...)
Hairy domestic breeds (n = 116)
(Romanov...)
Woolly modern breeds (n = 402)
(Berrichon du Cher, Merino d'Arles...)
- Phenotype**
None (sheep breed standards)
- Genotype**
Ovine Infinium® HD SNP BeadChip (n = 554)
- Methodology**
Haplotypes in the QTL_{Oar25} interval



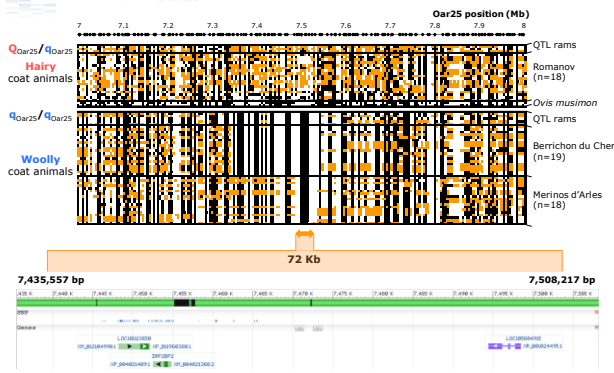
Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The QTL_{Oar25} encompasses the *IRF2BP2* gene



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

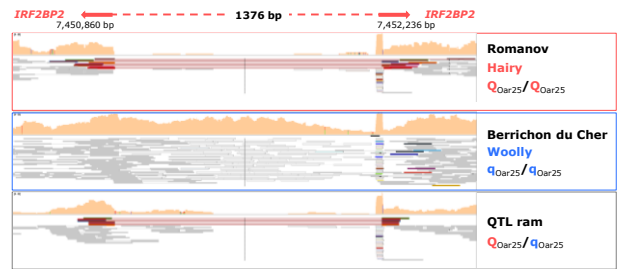
The QTL_{Oar25} encompasses the *IRF2BP2* gene



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The mutation is an insertion within *IRF2BP2*

- Whole Genome Sequencing
 - 2 Romanov animals (**hairy** and Q_{Oar25}/Q_{Oar25})
 - 3 Berrichon du Cher animals (**woolly** and q_{Oar25}/q_{Oar25})
 - 2 QTL rams heterozygous (Q_{Oar25}/q_{Oar25})



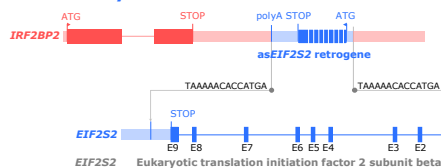
Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The insertion is an antisense *EIF2S2* retrogene

The ancestral hairy *IRF2BP2*^{wt} allele

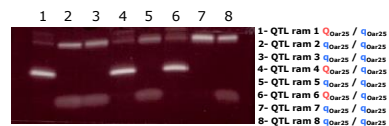


The modern woolly *IRF2BP2*^{asEIF2S2} allele



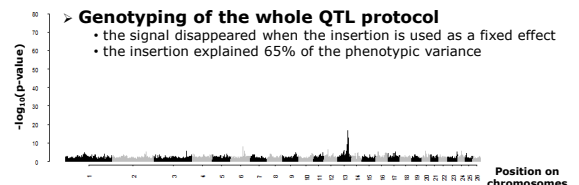
Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The insertion segregates in the Romane breed



Genotyping of the whole QTL protocol

- the signal disappeared when the insertion is used as a fixed effect
- the insertion explained 65% of the phenotypic variance



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The insertion discriminates hairy/woolly sheep

Breed	Genotyped animals (n)	Phenotype	Frequency (<i>IRF2BP2^{wt}</i>)	Frequency (<i>IRF2BP2^{asEIF2S2}</i>)
<i>Ovis orientalis</i>	15	Hairy	1.00	
<i>Ovis musimon</i>	18	Hairy	1.00	
<i>Ovis canadensis</i>	3	Hairy	1.00	
<i>Ovis dalli</i>	2	Hairy	1.00	
Causses du Lot	20	Hairy	0.85	0.15
Corse	16	Hairy	1.00	
Limousine	18	Hairy	0.94	0.06
Manech tête rousse	25	Hairy	1.00	
Rava	19	Hairy	0.92	0.08
Romanov	18	Hairy	0.94	0.06
	154		0.96	0.04
Berrichon du Cher	35	Woolly		1.00
Blanche du Massif Central	20	Woolly		1.00
Chamoise	22	Woolly		1.00
Charollais	22	Woolly		1.00
Île-de-France	23	Woolly		1.00
Lacune (lait)	35	Woolly		1.00
Lacune (viande)	34	Woolly		1.00
Mérino d'Aries	18	Woolly		1.00
Mérino de Rambouillet	27	Woolly		1.00
Nourerous	16	Woolly	0.06	0.94
Ouessant	18	Woolly		1.00
Préalpes du Sud	17	Woolly	0.03	0.97
Rouge de l'Ouest	16	Woolly		1.00
Roussin de la Hague	21	Woolly		1.00
Suffolk	18	Woolly		1.00
Tarascennaise	15	Woolly		1.00
Texel	24	Woolly	0.04	0.96
Vendéen	21	Woolly		1.00
	402		0.01	0.99



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The IRF2BP2/asEIF2S2 mRNA is transcribed

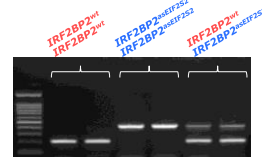
The IRF2BP2 transcript



The chimeric IRF2BP2/asEIF2S2 transcript

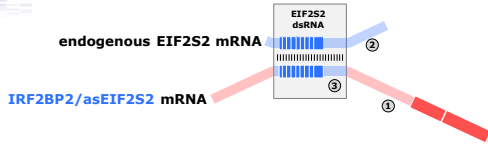


Genotyping of RNA extracted from skin



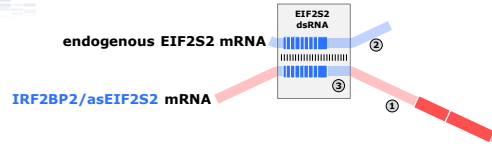
Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

A double-stranded EIF2S2 RNA is created



Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

A double-stranded EIF2S2 RNA is created



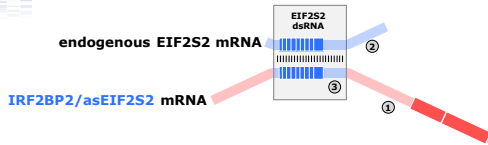
RNAseA protection assay

- ribonuclease that clives only ssRNA (EIF2S2 dsRNA is protected)
- IRF2BP2^{wt}/IRF2BP2^{wt}* versus *IRF2BP2^{asEIF2S2}/IRF2BP2^{asEIF2S2}*



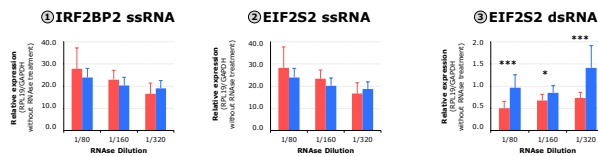
Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

A double-stranded EIF2S2 RNA is created



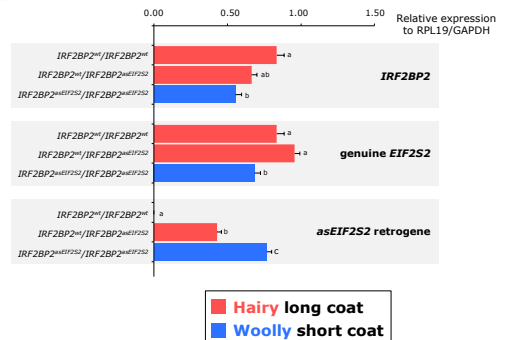
RNAseA protection assay

- ribonuclease that clives only ssRNA (EIF2S2 dsRNA is protected)
- IRF2BP2^{wt}/IRF2BP2^{wt}* versus *IRF2BP2^{asEIF2S2}/IRF2BP2^{asEIF2S2}*



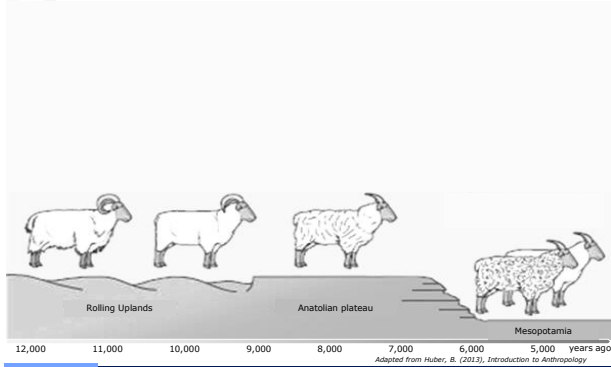
Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

The dsRNA alters EIF2S2 and IRF2BP2 stability

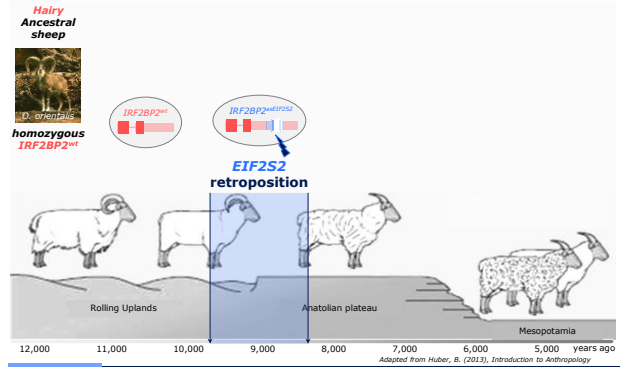


Julie Demars - Abstract W132 - PAGXXV - Cattle/Sheep/Goat Workshop

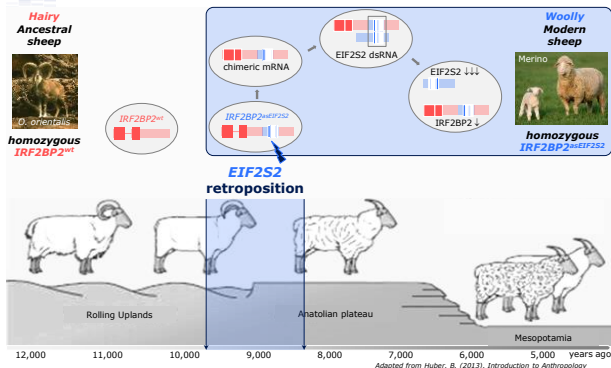
To conclude ...



To conclude ...



To conclude ...



INTA
M. Cano

INRA (GenPhySE lab)

- GENROC team
L. Drouilhet, S. Fabre, F. Plisson-Petit, J. Sarry, G. Tosser-Klopp, F. Woloszyn
- GESPER team
D. Allain, C. Moreno
- DYNAGEN team
B. Servin
- Experimental farms
D. Foulquier, F. Carrière, M. Aletru
- Bioinformatic and Genomic platforms
P. Bardou, O. Bouchez, H. Berges

A future **permanent position** is still under evaluation in the **Animal Genetic Division** of the **French National Institute for Agricultural Research**

How epigenetic mechanisms contribute to the determinism of agronomic importance traits ?

- GenPhySE lab, INRA, Toulouse, France
<http://genphyse.toulouse.inra.fr/groups/genepi>
- GenEpi group, Juliette Riquet
juliette.riquet@inra.fr
- INRA job opportunities, news at the end of january
<http://jobs.inra.fr/en/Career-opportunities/Researchers/Research-scientists>