Functional investigation of a QTL region affecting resistance to *Haemonchus contortus* in sheep

Guillaume Sallé[2], Carole Moreno[1], Julien Ruesche[1], Frédéric Bouvier[1], Mathias Aletru[1], Jean-Louis Weisbecker[1], Françoise Prévot[2], Jean-Paul Bergeaud[2], Cathy Trumel[3], Christelle Grisez[2], Dominique François[1], Andres Legarra[2], Emmanuel Liénard[2,3], Philippe Jacquiet[2,3]

[1] INRA Animal Genetics Department (UR631, UE632, UE)
[2] INRA Animal Health Department (UMR 1225)
[3] Toulouse University, INP - Ecole Nationale Vétérinaire de Toulouse

EAAP 2013, Nantes, France

INTRODUCTION

- Gastro-intestinal nematodes : a curse for sheep breeding
  - ✓ Impact sheep health and welfare
  - ✓ Strong economical burden for breeders (~85 M£ in the UK *Nieuwhof & Bishop 2005*)
  - ✓ Anthelminthic resistant populations have been selected for worldwide
- Breeding for more resistant animals could help controlling nematodes in a more sustainable fashion
  - ~30 % of the observed variation in resistance due to the genetic background
  - Strong between breeds differences
  - Need to know the genes : good understanding of mechanisms + improve breeding schemes

Our working basis *(Aumont et al. 2003, Terefe et al., 2007)*

Martinik Black-Belly

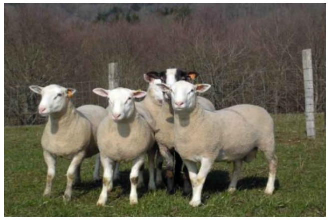
Subtropical breed

Resistant

Romane

French meat breed

Susceptible



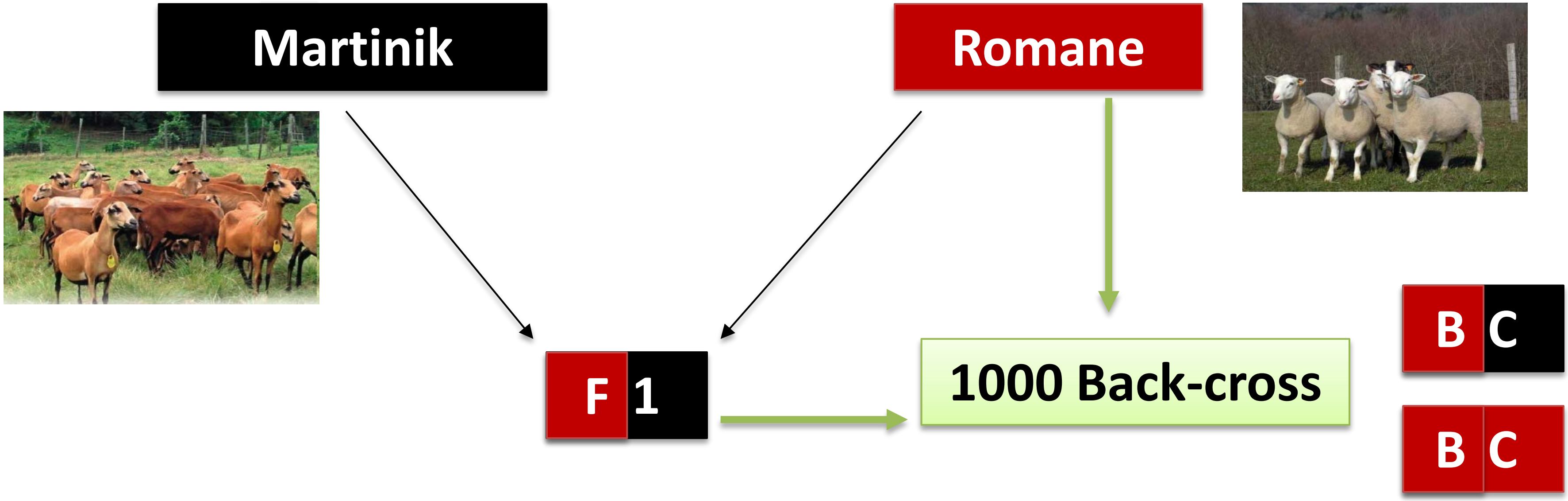
- Martinik sheep are far more resistant than the Romane sheep to *H. contortus*
  - 3 to 10 fold less excreted *H. contortus* eggs
- Phenotypic differences also observed on the immunological background
  - Higher and quicker eosinophilic infiltration of the abomasal mucosa
  - IL5 and IL13 over-expressed in Martinik abomasal mucosa
  - Key differences observed at 1st infection

Next question: what are the genetic mechanisms controlling these differences ?

SUMMARY

- **OAR12: an interesting candidate**
- **Investigating the functional properties of this region**
- **Conclusion & perspectives**

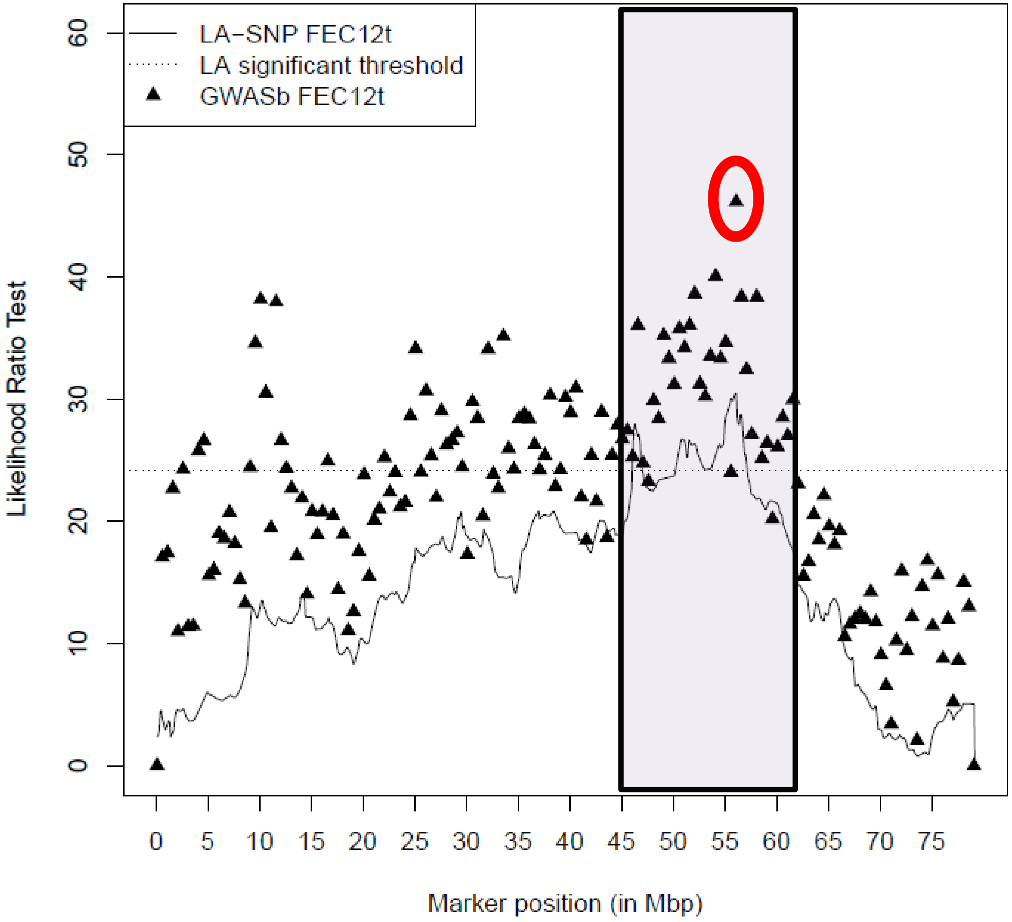
Looking for regions associated to *H. contortus* resistance



- **Identify regions of the genome affecting resistance to *H. contortus***
  - ***1,000 lambs experimentally challenged twice***
  - ***Measured for Fecal Egg Counts and Haematocrit***
  - ***Genotyped for 50K SNP***

- **Choose one interesting region**

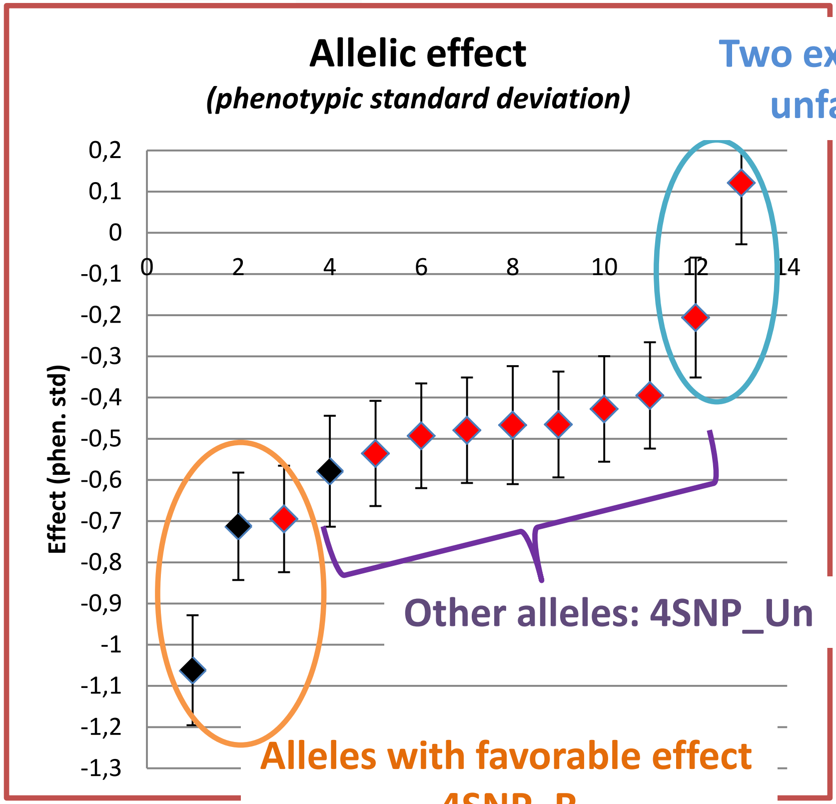
Association of 4 SNPs with resistance to *Haemonchus contortus*



- **One region affecting FEC each time the sheep faced *H. contortus***
- **Second highest effect (0.19 σ_p)**
- **Found in other sheep populations:**
  - ✓ **Sarda*Lacaune** ***(Moreno et al., 2006)***
  - ✓ **Merino** ***(Beh et al., 2002)***
  - ✓ **Soay sheep** ***(Beraldi et al., 2007)***

G. Sallé et al., J. Anim. Science, 2012

The 4-SNP allelic effects



Two extreme alleles with unfavorable effects

Other alleles: 4SNP_Un

Alleles with favorable effect 4SNP_R

- **13 alleles have been identified**
- **3 favorable alleles *versus* 2 very unfavorable alleles**
- **One allele from the Romane breed within the favorable alleles (!)**

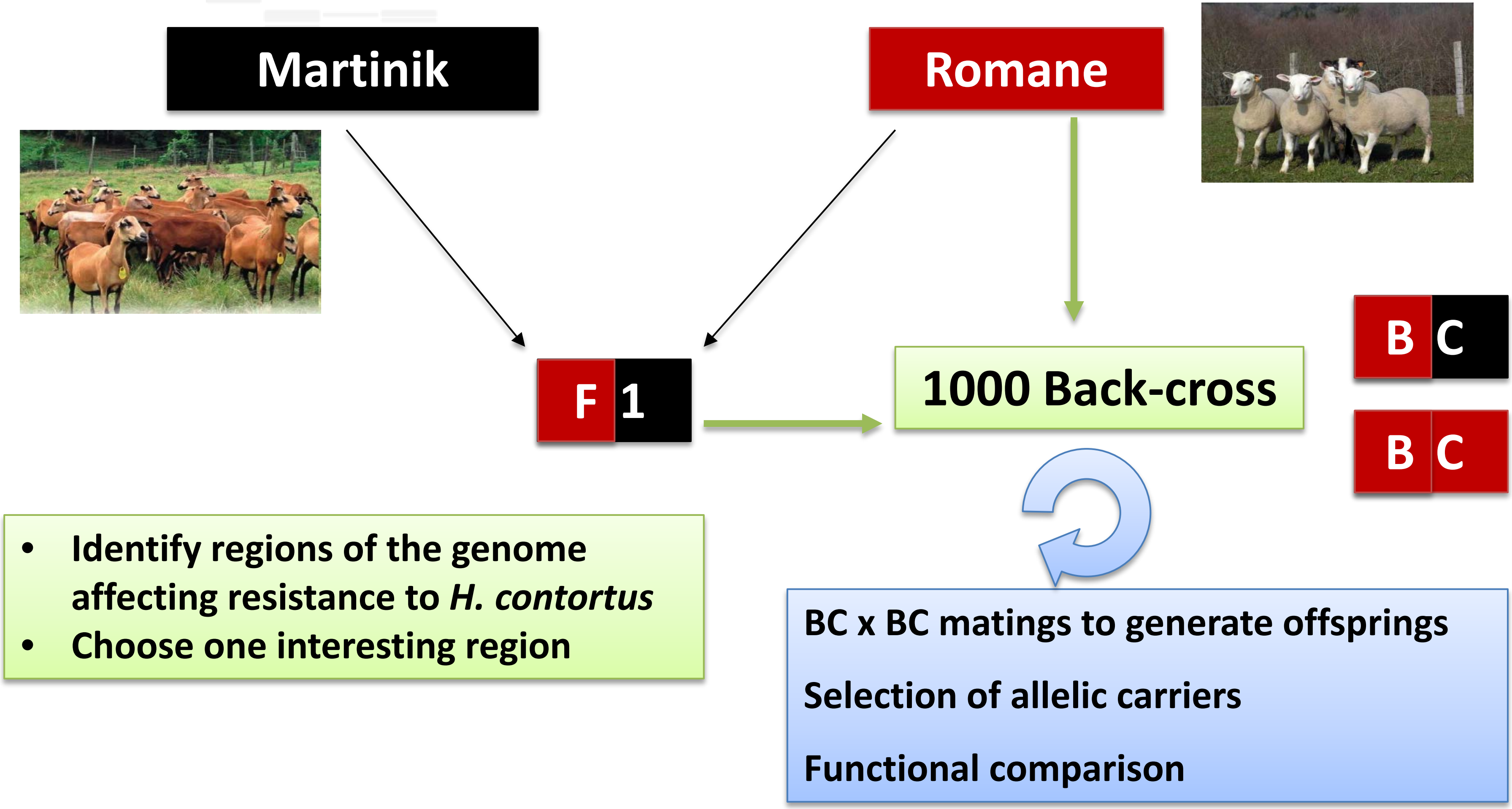
◆ **Martinik (resistant) origin**

◆ **Romane (susceptible) origin**

SUMMARY

- OAR12: an interesting candidate
- Investigating the functional properties of this region
- Conclusion & perspectives

The experimental design



Martinik

Romane

F 1

1000 Back-cross

B C

B C

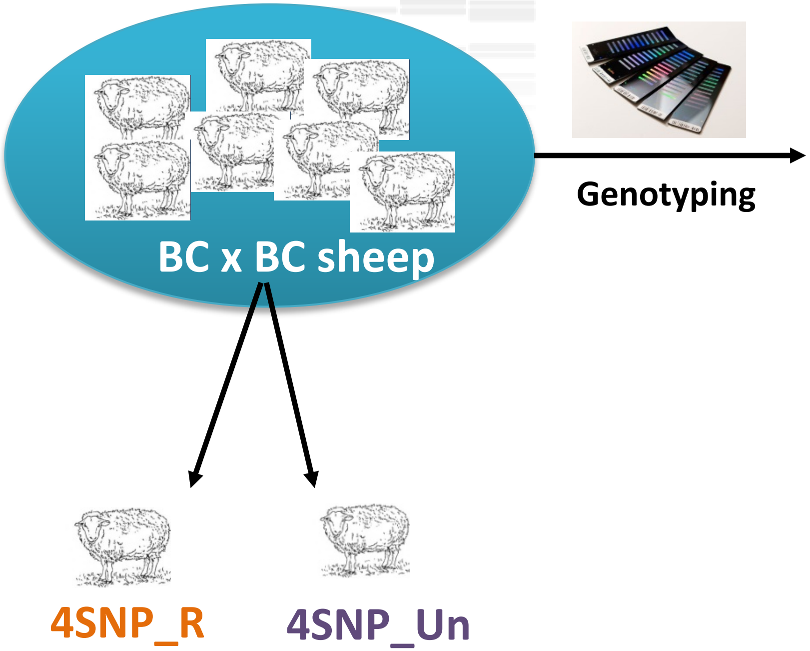
- **Identify regions of the genome affecting resistance to *H. contortus***
- **Choose one interesting region**

BC x BC matings to generate offsprings

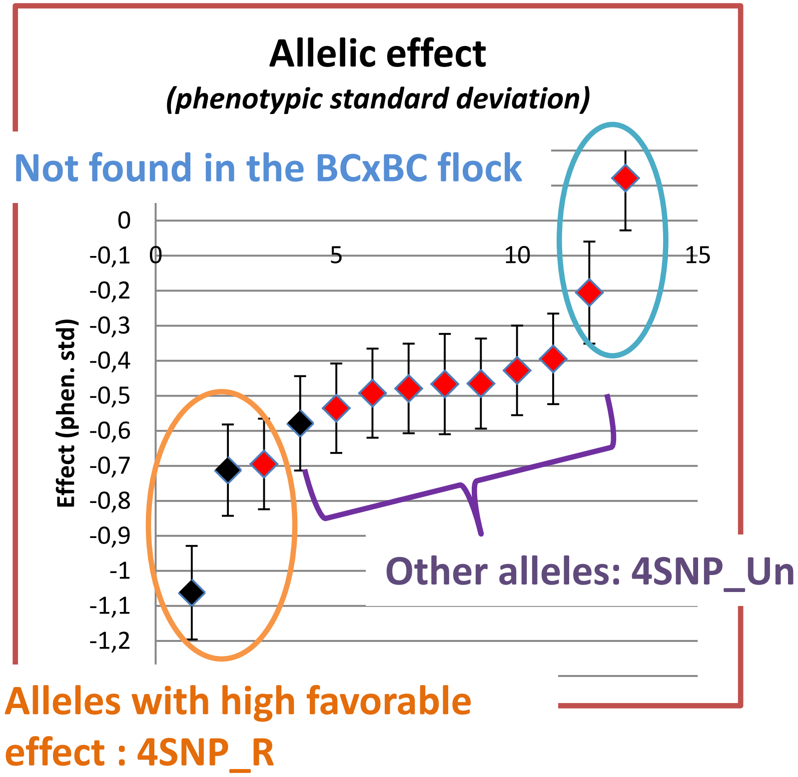
Selection of allelic carriers

Functional comparison

Functional validation



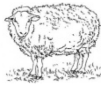
- Determination of the inherited alleles
- Estimation of the genomic background effect



- **Two groups of BCxBC sheep selected on the 4SNP alleles they inherited :**
  - **4SNP_R animals carried the favorable alleles**
  - **4SNP_Un animals inherited “neutral” alleles**

Functional validation

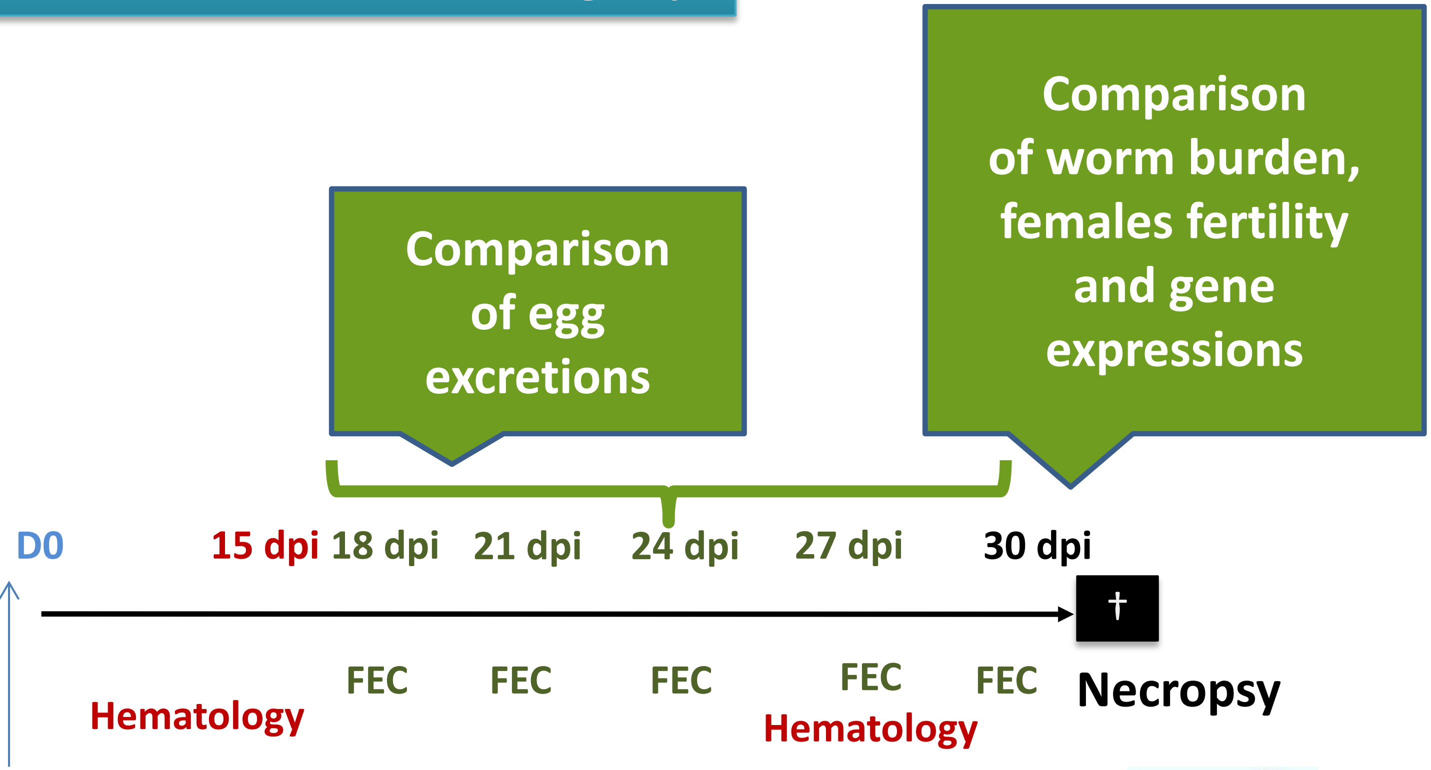
- 2 BC*BC groups based on their 4SNP alleles
- Equivalent genomic value between the two groups



4SNP_R

44 challenged
(16 vs 28)

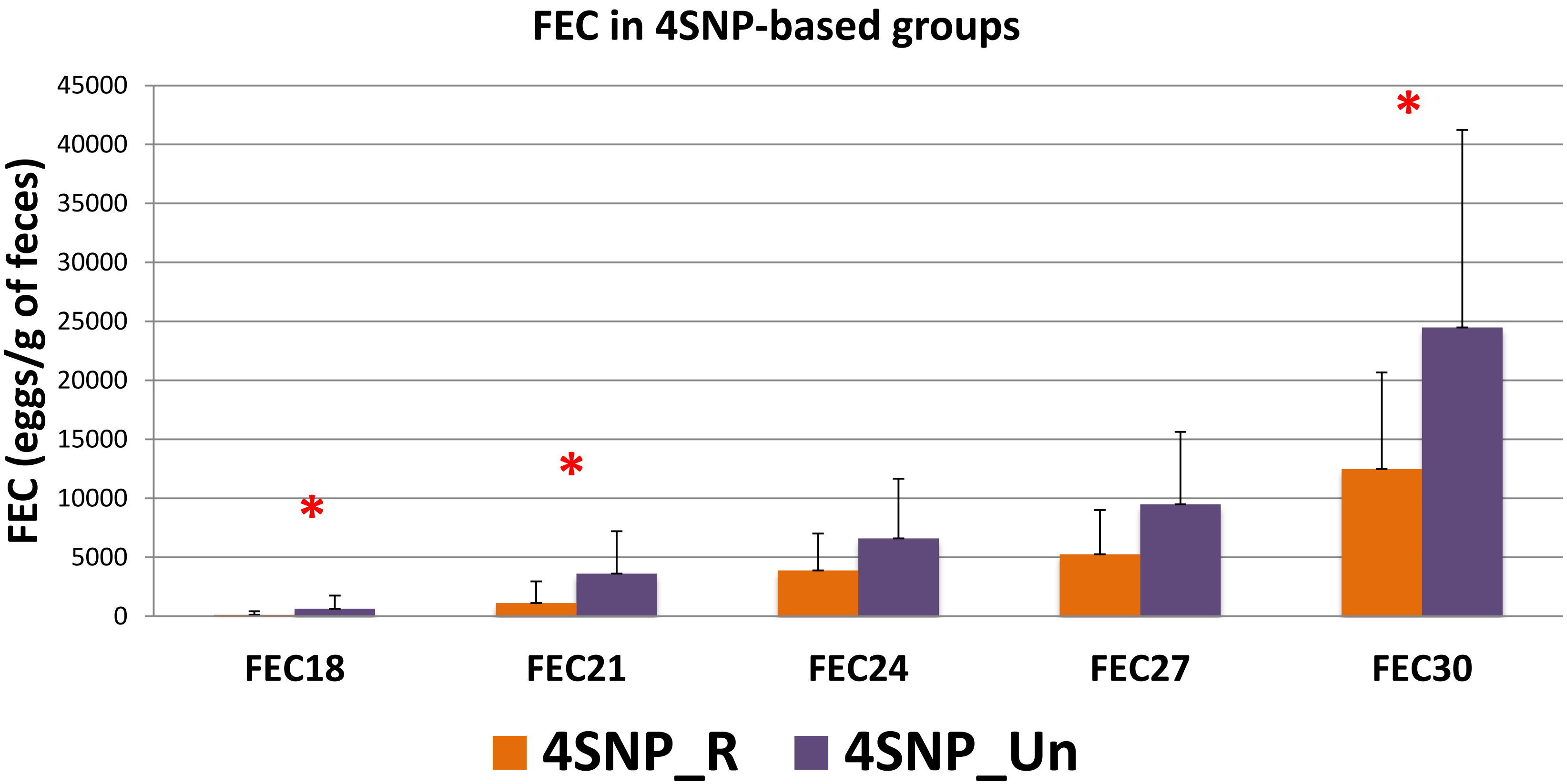
4SNP_Un



Comparison of egg excretions

Comparison of worm burden, females fertility and gene expressions

An effect on fecal egg counts…

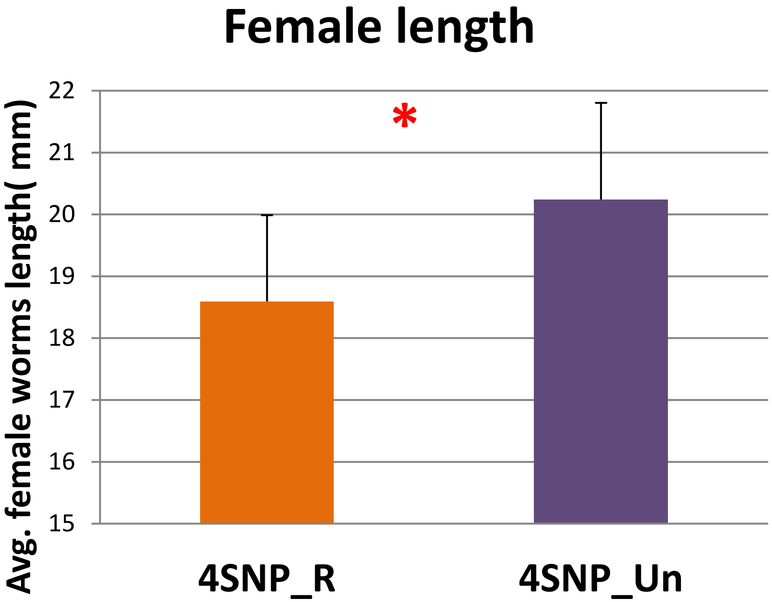


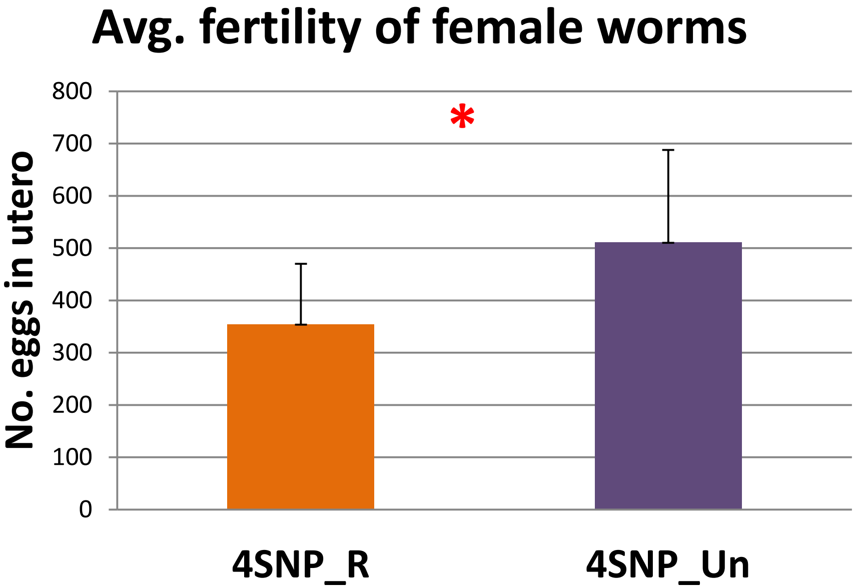
4SNP_R sheep excreted less eggs than 4SNP_Un

The effect on FEC could be explained by a difference in female worms fecundity

For each BC*BC infected sheep:

- **The length of 35 *H. contortus* females was measured**
- **The number of *in utero* eggs of 20 *H. contortus* females counted**





No differences for **worm burden**…

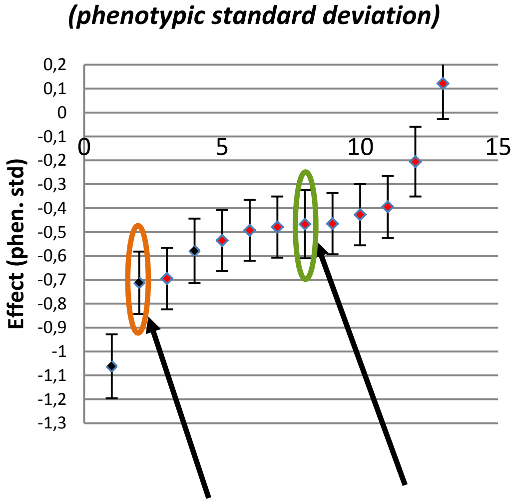
4SNP_R lambs also exhibited **significantly less blood loss** at 30 dpi

Any candidate genes ?

Selection of the most frequent alleles in each group:

GAAG$_{MBB}$ *vs* GACA$_{RMN}$

(N = 9) **(N =8)**



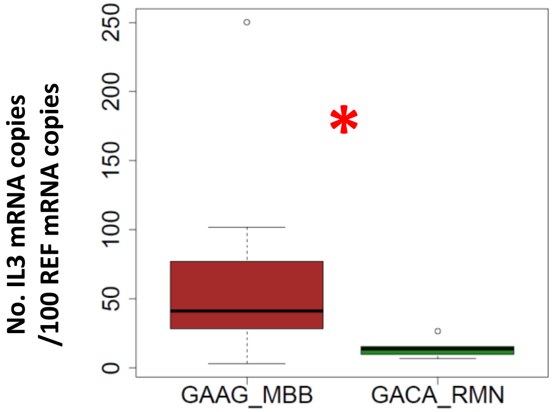
Gene expression compared between carriers

- Set of differentially expressed genes from micro-array experiment between pure breeds MBB and RMN sheep *(Liénard et al., 2011)*

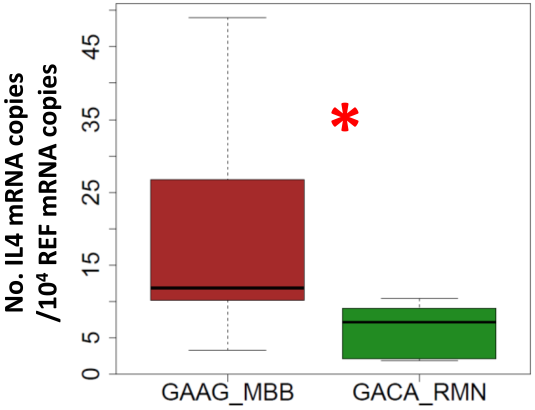
- *PAPP-A2* (pappalysin) gene underlying the 4SNP region (regulation of Insulin-like Growth Factor (IGF) activity)

Any candidate genes ?

IL-13



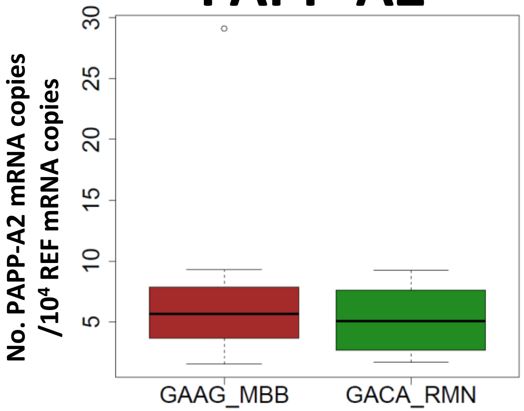
IL-4



Abomasal mucosa, 30 days post-infection

Stronger Th2 response

PAPP-A2



***PAPP-A2* was not differentially expressed between the two groups**

SUMMARY

- OAR12: an interesting candidate

- Investigating the functional properties of this region

- **Conclusion & perspectives**

CONCLUSION & PERSPECTIVES

Take home message

- ✓ A 4SNP region in OAR12 was associated to sheep resistance
- ✓ This functional study seems to confirm its role
- ✓ This region seems to impact worms fertility (1 σp)
- ✓ This region was associated to a Th-2 biased over-expression

Perspectives

- ✓ Refine… : association analysis in Martinik and Romane sheep
- ✓ Investigating the host-nematode system as a whole

ACKNOWLEDGEMENTS

Supervisors
Pr. P. Jacquiet
Dr. C. Moreno

Quantitative genetics
S.C. Bishop
J.M. Elsen
A. Legarra
O. Filangi

Parasitology
J.-P. Bergeaud
C. Grisez
E. Liénard
F. Prévot

Lab – Farm interface
D. François
J. Ruesche

Experimental farms staff
M. Aletru
Y. Bourdillon
F. Bouvier
T. Fassier
D. Marcon
J-C. Thouly
J. L. Weisbecker

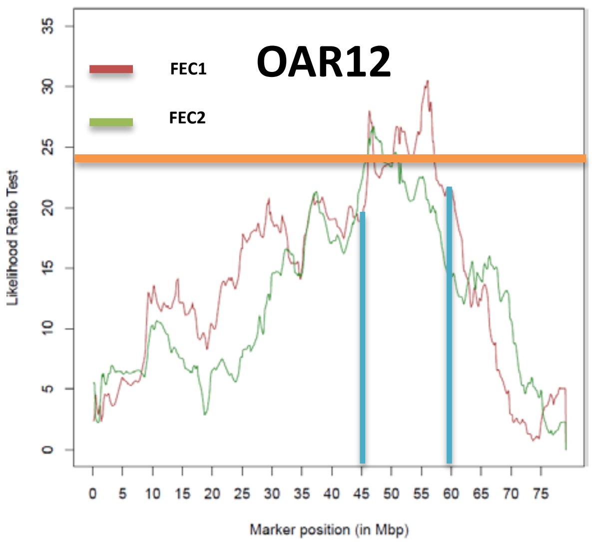
Financial support

Thank you for your attention

Merging knowledge together

Positional knowledge

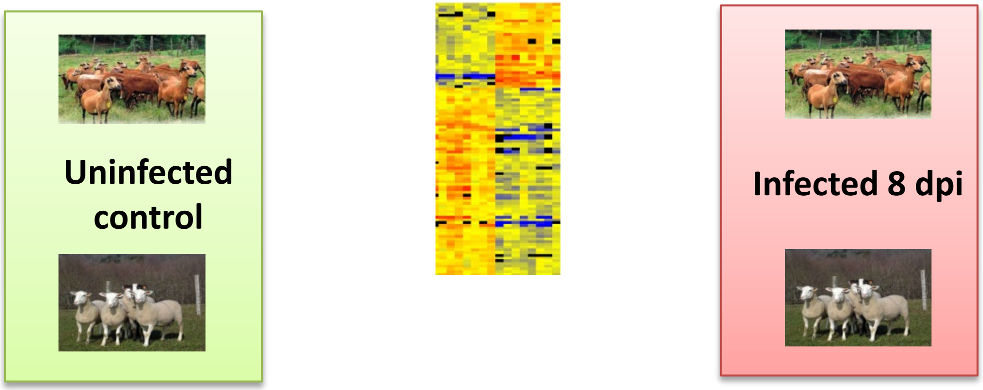
- Regions involved
- No gene



Differential gene expression

(Liénard et al., WAAVP 2011)

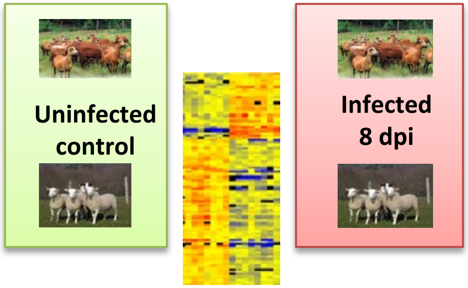
- Breed comparison
- Control and 8 dpi
- Tissue samples from abomasum and lymph nodes
- Comparison of the gene expression of ~15,000 genes (microarray)



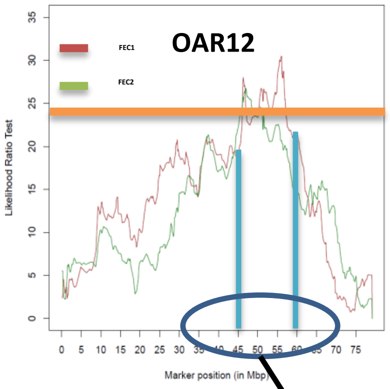
Uninfected control

Infected 8 dpi

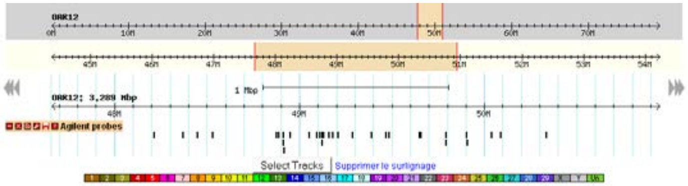
Merging knowledge together



157 genes with fold change > 1.5

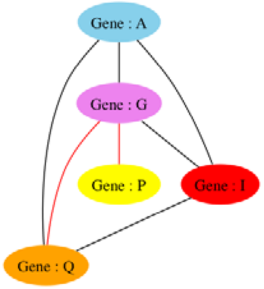


- **Significant QTL**
- **Annotated genes contained within LRTmax position +/- 4 Mbp**



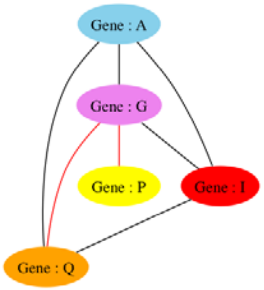
Gene set

Gene network analysis

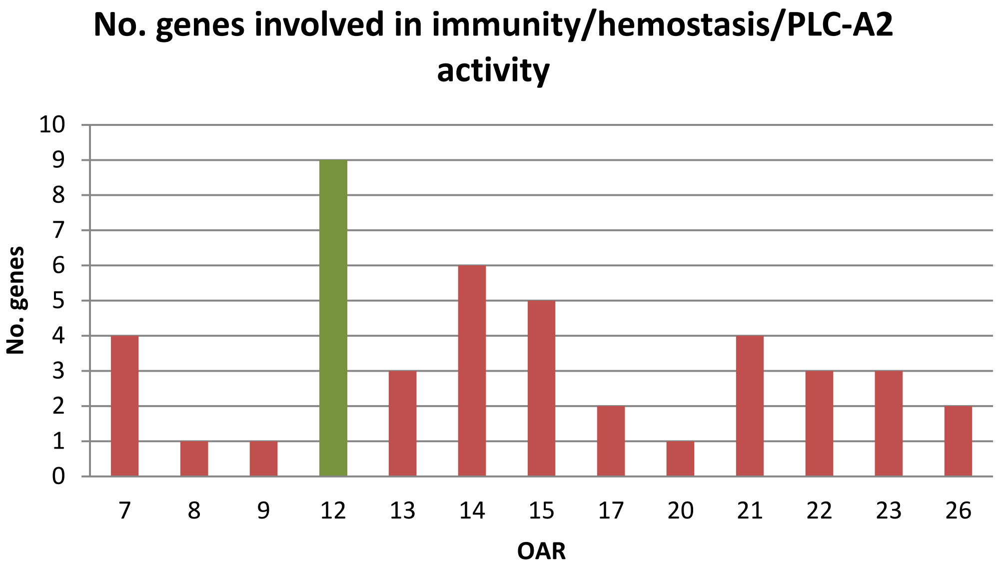


Networks

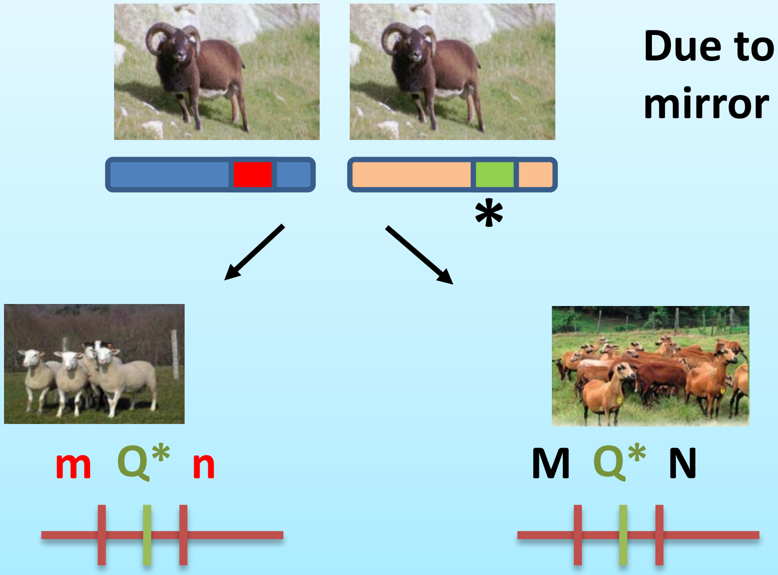
Results of the bioinformatic approach



Within the networks related to haemonchosis: a high number of genes mapped on OAR12

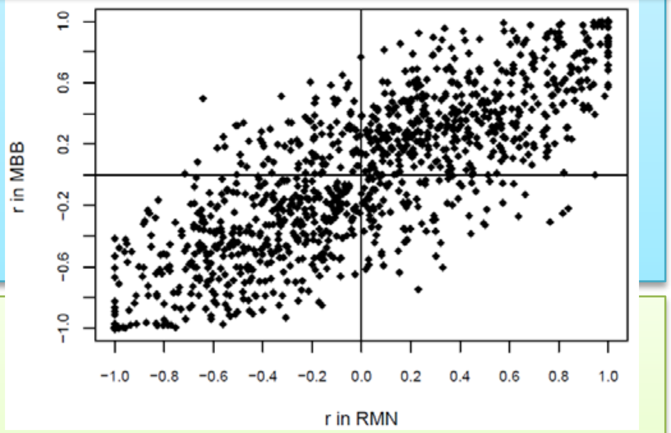


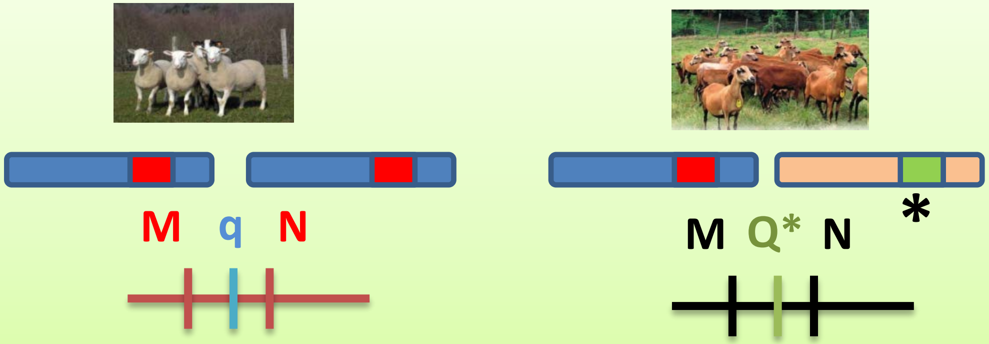
Association analysis & breeds



Due to the two pure breeds histories, SNP alleles may not mirror the same QTL alleles

Relationship between *r* of the Martinik breed and the Romane breed (0-10 Kb)





The mutation can have appeared after breeds diverged

Population genetics input

- Different environments ⇔different selection pressure
- Stronger selection pressure due to GIN in MBB sheep

Is there any gene that has been selected in the MBB sheep and not in the RMN breed?

Sweep detection

- *In collaboration with S. Boitard*
- **Additional genotyping of MBB and RMN individuals**
- **One suggestive sweep at 42 M**
- **Fitting the sweep genotype in the QTL detection model erodes the QTL profile**