Sustainable Solutions for Small Ruminants

Genome-wide association analysis of resistance to gastro-intestinal parasites in dairy sheep

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Introduction

Gastro-intestinal nematodes (GIN)

- Gastro-intestinal nematodes (GIN) are one of the main health issues in grazing ruminants
- Resistance against anthelmintics is constantly increasing in terms of prevalence, geographical repartition and severity
- Selection for resistant animals may provide a feasible long-term control strategy (Bishop and Morris, 2007)

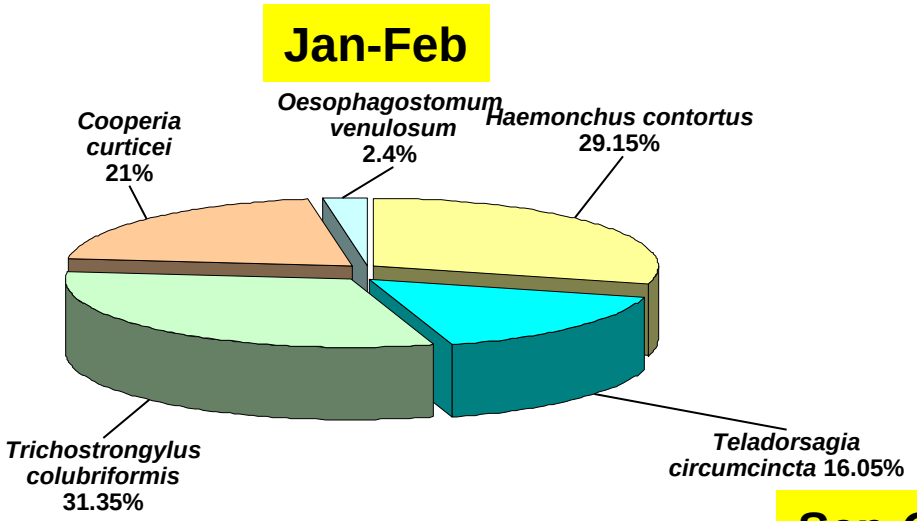
Introduction

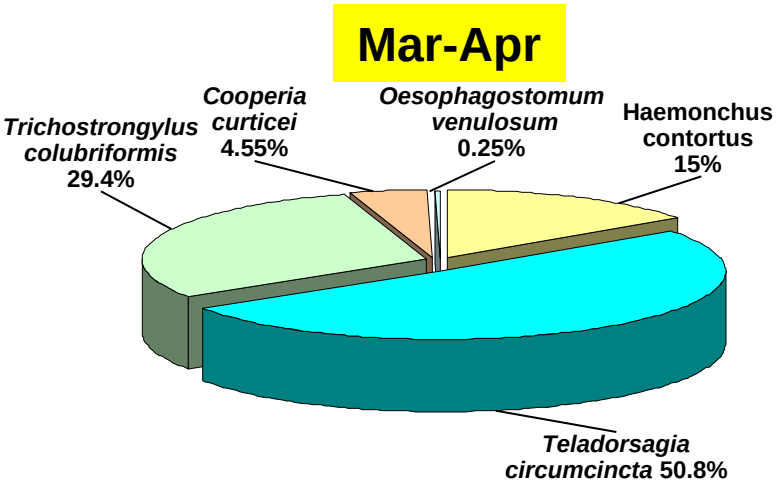
Gastro-intestinal nematodes (GIN)

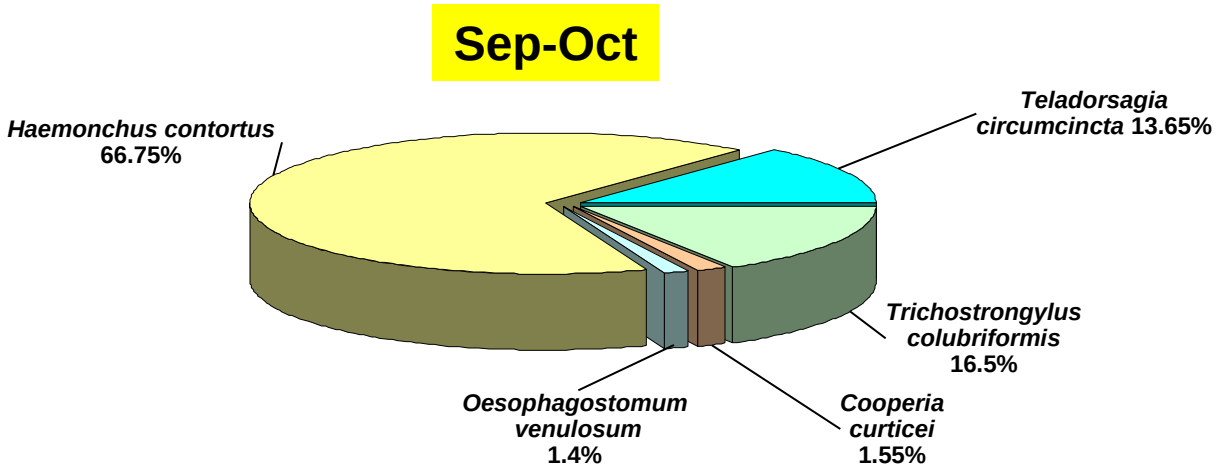
Main difficulties

- Faecal individual sampling (laborious, costly, risk of sampling not infected flocks in not representative periods)
- FEC (Faecal Eggs Count), laborious to be determined by floatation in saturated salt solution in a McMaster slide and the eggs counted (Raynaud, 1970).

Seasonal species distribution of third stage infective larvae of GI nematodes after larval culture



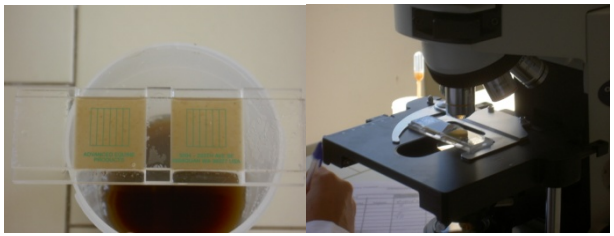




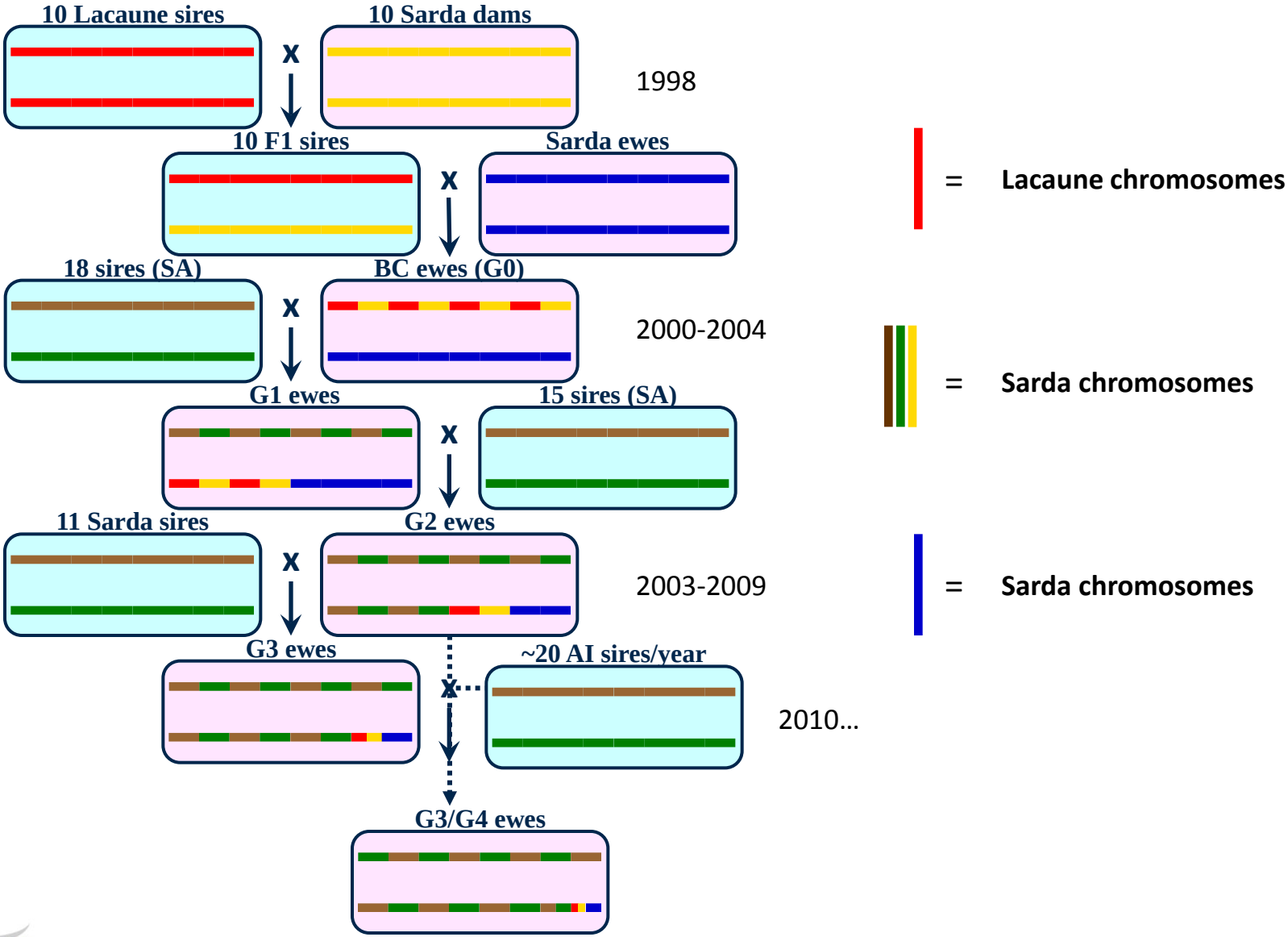
Aim

To Detect QTN affecting nematode resistance in a naturallly infected dairy sheep population by using the

Ovine SNP50 BeadChip and LD-LA approach



Experimental population



Faecal Egg Count (FEC) measurements

- Faecal Egg Count **(FEC)** under natural conditions of infection (grazing 6h/day)
- Around 50 animals were monitored in order to evaluate the percentage of infected animals and to decide whether or not to sample the whole flock
- Faeces were collected on the whole flock from **1** to **3** times per year more frequently in September and July
- Faeces were processed by floatation in saturated salt solution in a McMaster slide and the eggs counted (Raynaud, 1970)
  - lnFEC = ln(Eggs Number + 14)

Gen	# phenotyped offspring	# & genotyped offspring
G0	940	915
G1	788	765
G2	772	692
Total	2,500	2,372

Gen	# FEC (d)	# FEC (y)	Mean FEC (epg)	s.d.
G0	6,772	3,366	207.7	398.0
G1	2,513	2,093	267.2	482.0
G2	2,823	2,107	393.1	817.1
Total	12,108	7,566	263.3	545.9

Estimated additive genetic VC =0.27

Phenotypes

ln(Eggs Number + 14) adjusted for population-specific environmental effects

- **Repeatability model :** $a \sim N(0, I*\sigma^2_a)$; $e \sim N(0, I*\sigma^2_e)$
- **Environmental effects :** n. lambs born; group of management; age; physiological stage; sampling date;

Phenotypes for QTL detection = $a_i + \Sigma e_{ij}/n$

Genotypes

Ovine SNP50 BeadChip → 54,241 SNPs

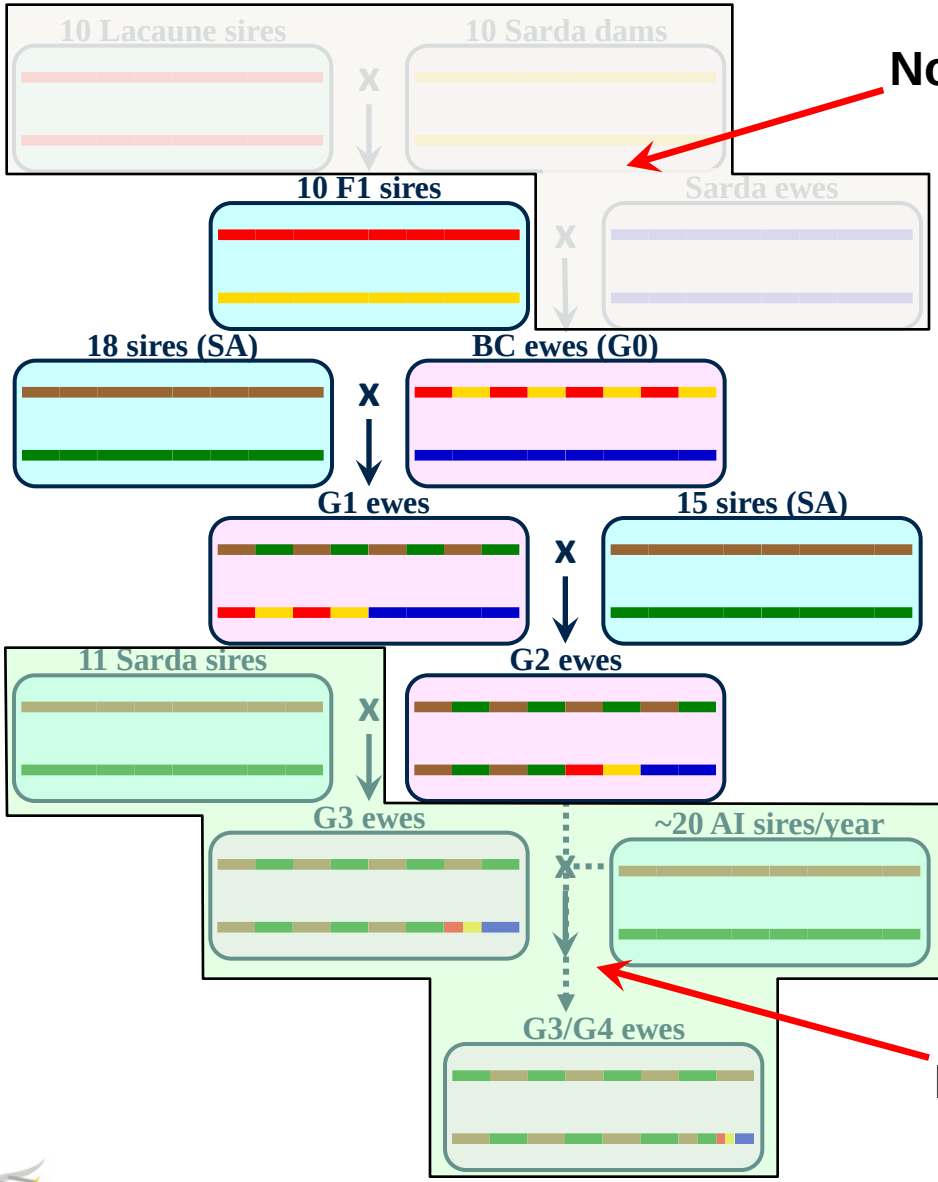
-- SNPs not located on the 26 autosome

-- SNPs with call rate < 0.95

-- SNPs with MAF < 0.05

= 44,859 SNPs

Experimental population



Not genotyped

= **Lacaune chromosomes** **(HM_L)=10**

= **Sarda chromosomes** **(HMs)=76**

= **Sarda chromosomes** **(HF)=925**

Not investigated here

SEVENTH FRAMEWORK PROGRAMME

Base Haplotypes

- **Male founder Haplotypes – MH**
  - Paternal gametes F1 (Lacaune)-**MH_L =10**
  - Maternal gametes F1 (Sarda)-**MHs = 10**
  - Both gametes SA (Sarda)-**MHs = 66**
- **Female Haplotypes –FH**
  - Maternal gametes BC (Sarda) H**925**

Offspring Haplotypes –OH

- Replicates of the base haplotypes carried by phenotyped individuals

Base haplotypes reconstruction

- **Paternal phases reconstruction (F1 & SA) (MH)**
  - *P(phase|daughter genotypes) > 1-10^{-10}*
- **Maternal gametes BC (FH)**
  - deduced from the paternal (**F1**) haplotype inherited by BC (*P>0.95*)
- **Origin of F1 chromosomes (Sarda or Lacaune)**
  - **Estimated by comparing both MHF1 with FH based on haplotypes of 6 SNPs**

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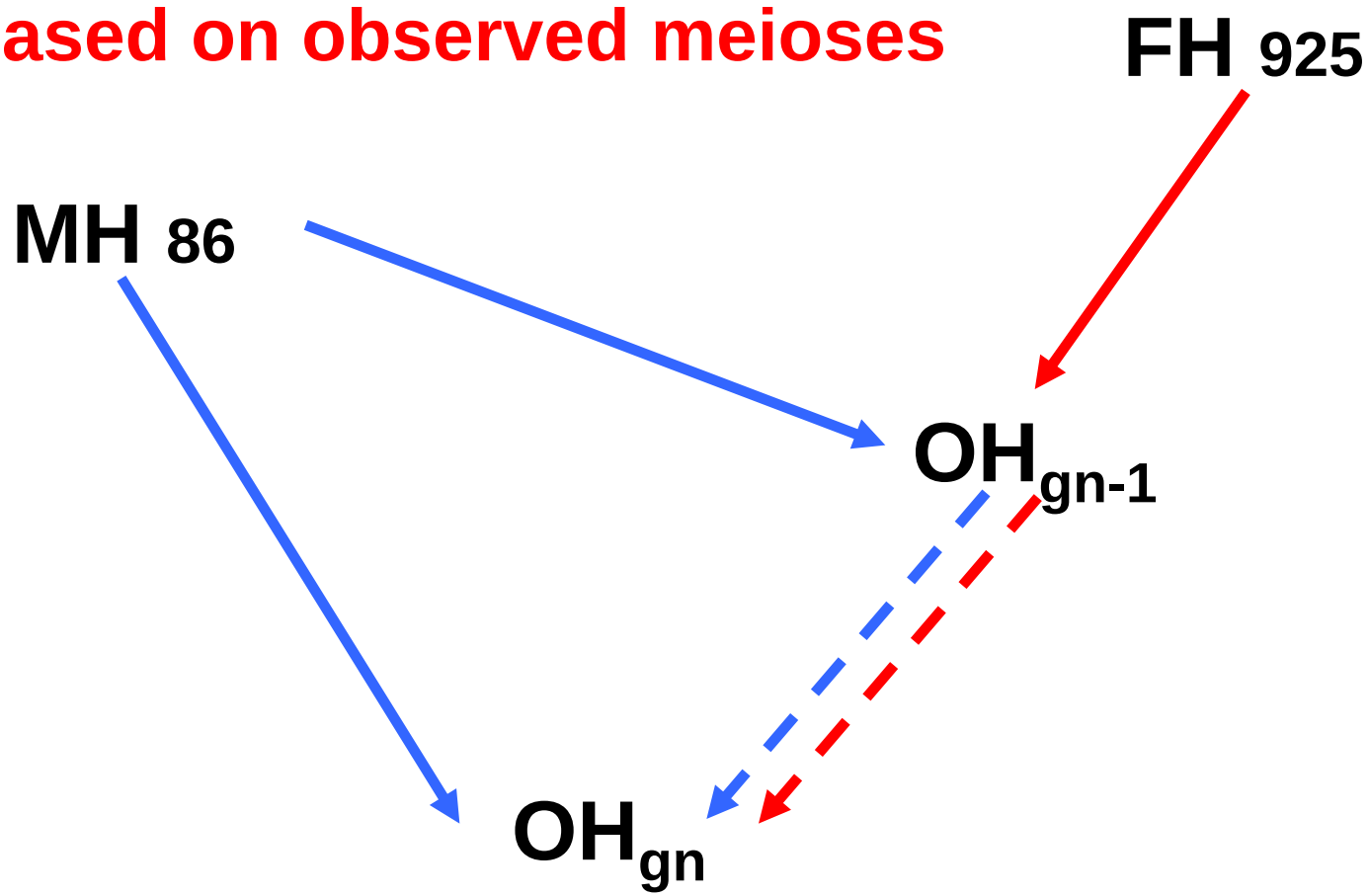
- **Transmission probability estimation**

Transmission probability of base haplotypes => OH



Each FH had few replicates in OH (1.61 on average)

V

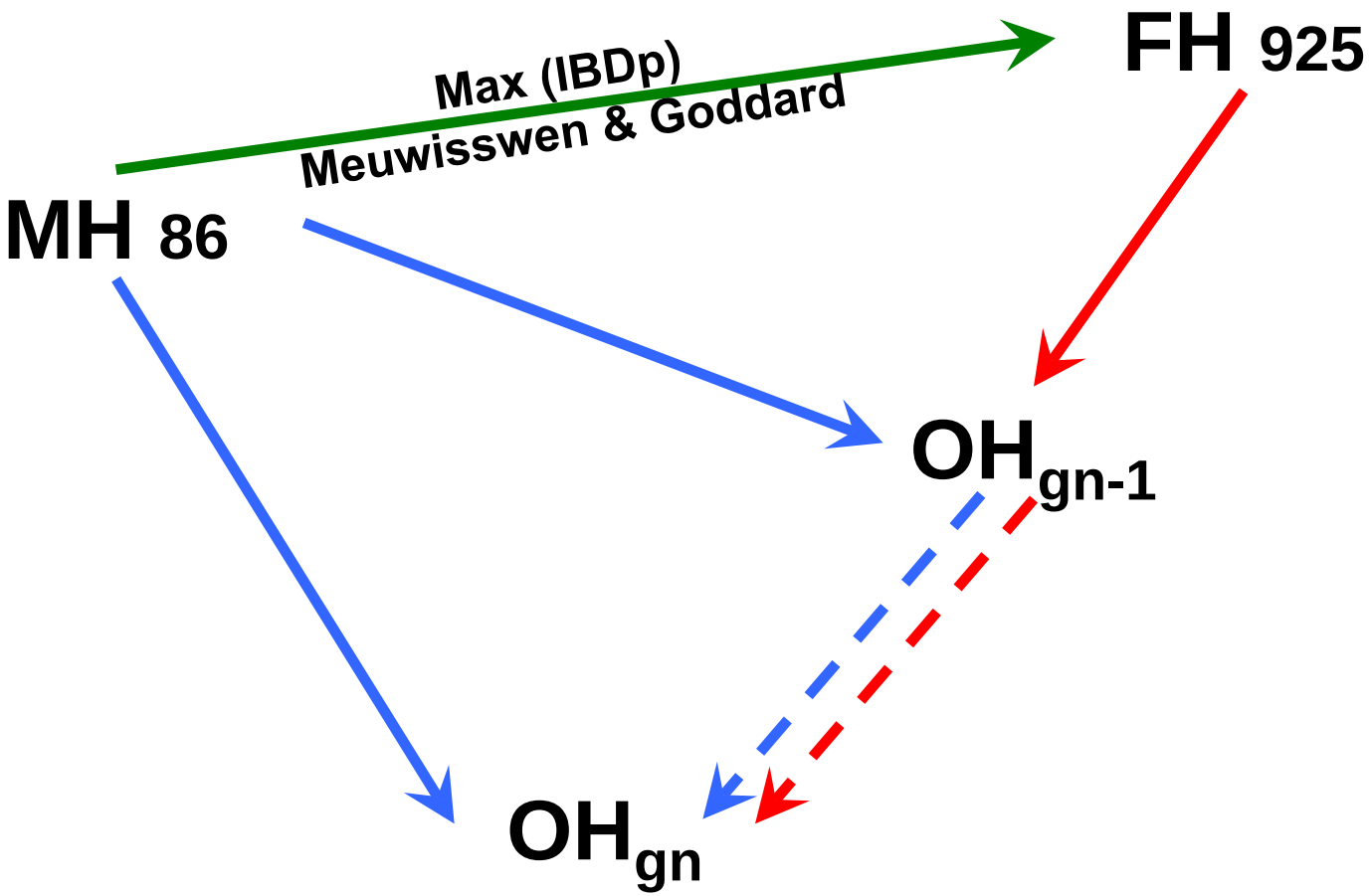
V

We focused on

MH effects estimation

To exploit the genomic information of FH they were connected to MHs through the IBDp by M&G algorithm

HIGHEST IBDp between FH and MHs



LD-LA analysis:

IBD between MH at each SNP

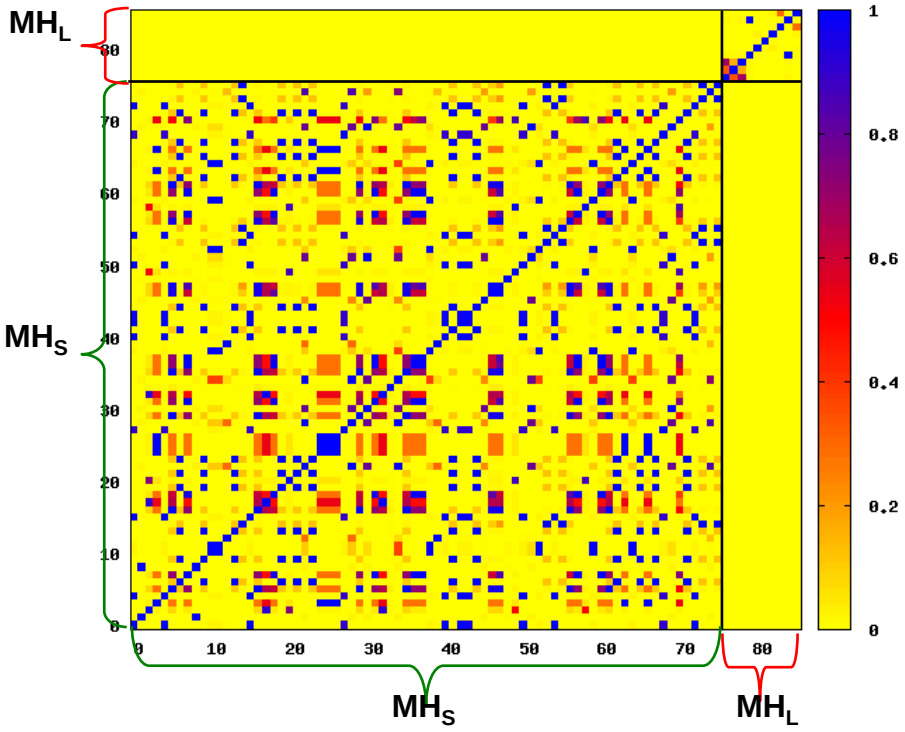
(Meuwissen & Goddard)

- **41 SNPs segment (20 left - 20 right)**
- **Expected homozygosity:**

  Sarda → p^2_S **(from FH);**

  Lacaune→ p^2_L **(from 3SR dataset)**
- **IBDp between Sarda & Lacaune hap. set to 0**

Evidence of multi-collinearity between the 76 MH_S from IBD matrix



IBD matrix → PCA → V

CP > 0.90 of variance & eigenvalue>1

LD-LA

Multiple regression of the offspring performances on the PC from MH IBD matrix

Model: $$y = 1\mu + As + XV\beta + e$$

y = vector of phenotypes; ***μ*** = overall mean; ***s*** = vector of the fixed sire effects;

² = vector of the fixed effects of selected linear combinations of MH $\beta = \begin{bmatrix} \beta_L \\ \beta_S \end{bmatrix}$

e = vector of random residuals;

A = incidence matrix relating phenotypes with sires (relationship coeff.);

X = incidence matrix allocating *"transmission"* probabilities MH -> OH ;

V = eigenvectors relating MH with selected linear combinations $V = \begin{bmatrix} V_L & 0 \\ 0 & V_S \end{bmatrix}$

SEVENTH FRAMEWORK PROGRAMME

Test and Significance threshold

F Test (at each SNP position):

Genomic $H0 \rightarrow \sigma^2 = 0$

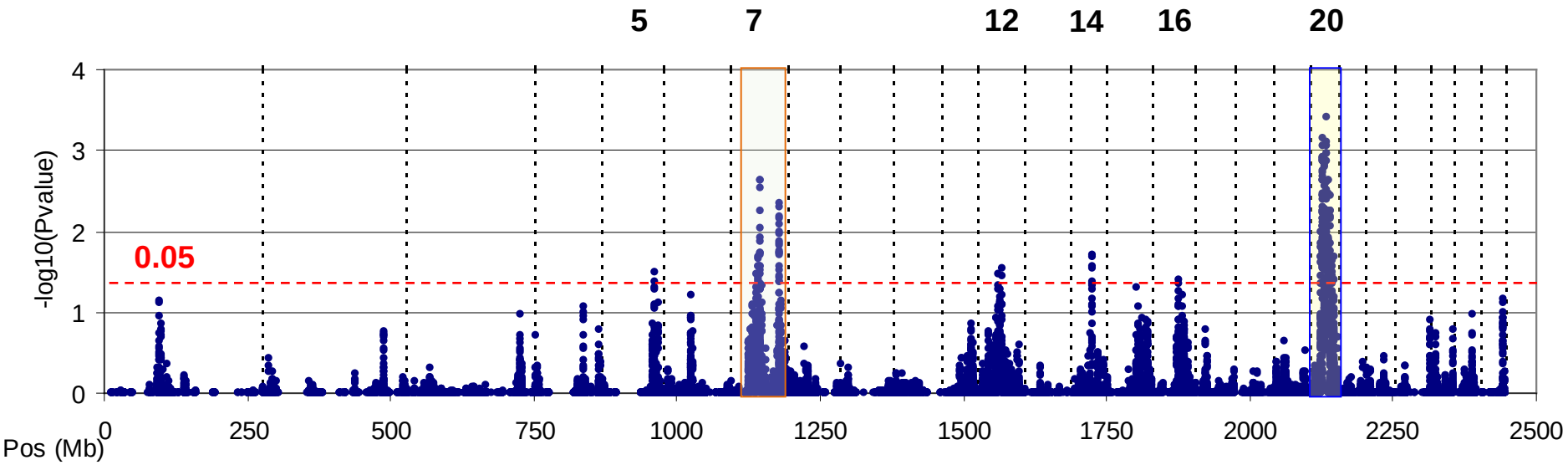
Sarda $\mathbf{H0 \rightarrow \sigma^2_S = 0}$

Lacaune $H0 \rightarrow \sigma^2_L = 0$

Significance

- **H0 chromosome wise (CW) and genome wise (GW) maximum test distribution**
- **10,000 permutations within sire family**
- **Random deviates from family effect**

Genomic regions associated with FEC

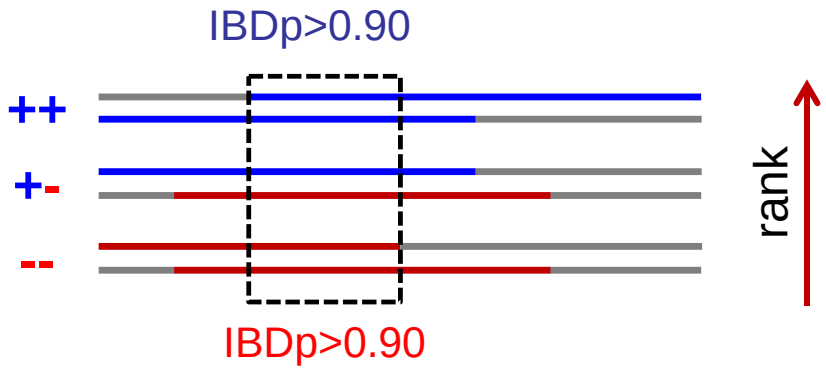


Re-sequencing

- The most significant regions were further investigated by whole genome re-sequencing of **trios of animals** in which the QTNs are expected to be segregating

$$Max\ (\alpha_{H+} - \alpha_{H-})$$

$$IBDp\ (H+, H-) = 0.00$$



Trait	OAR	Loc.(Mb)	P-value	Contrast (s.d.u)	Segment length	SNP ++; +-; --
FEC	20	25.40	0.0012	0.892	1,136,231	2,568
FEC	7	52.35	0.0023	0.583	402,400	831

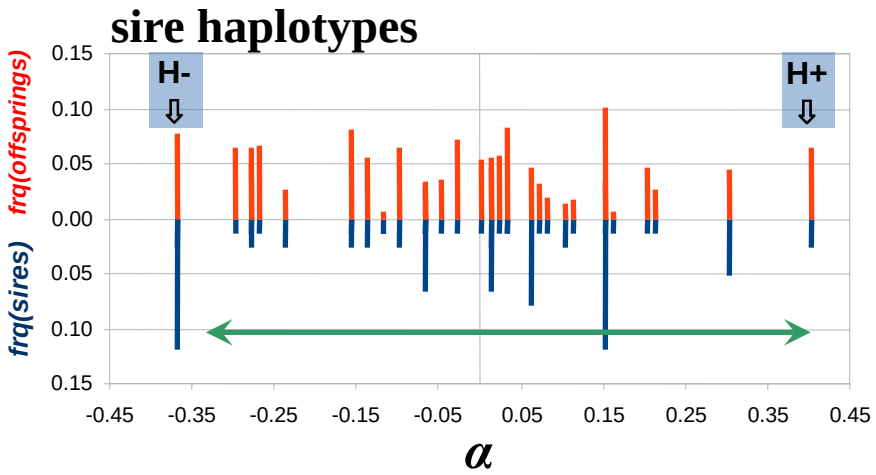
Conclusions

- The performed analysis allowed to detect:
  - 1 region 5% GW significant on OAR20
  - 2 regions 1% CW significant on OAR7
  - 4 regions 5% CW significant on OAR5; OAR12; OAR14; OAR16

- Functional role of identified SNPs is under investigation
- Estimation of haplotype frequency of the regions of interest is ongoing on the whole registered population

THANKS FOR YOUR ATTENTION!

$$\alpha_k = \sum_{l=1}^{ncp} v_{kl}\beta_l$$ LDLA (SA)



frq(sires) > 0.026 (2/76)

↓

Max (α_{H+} - α_{H-})

↓

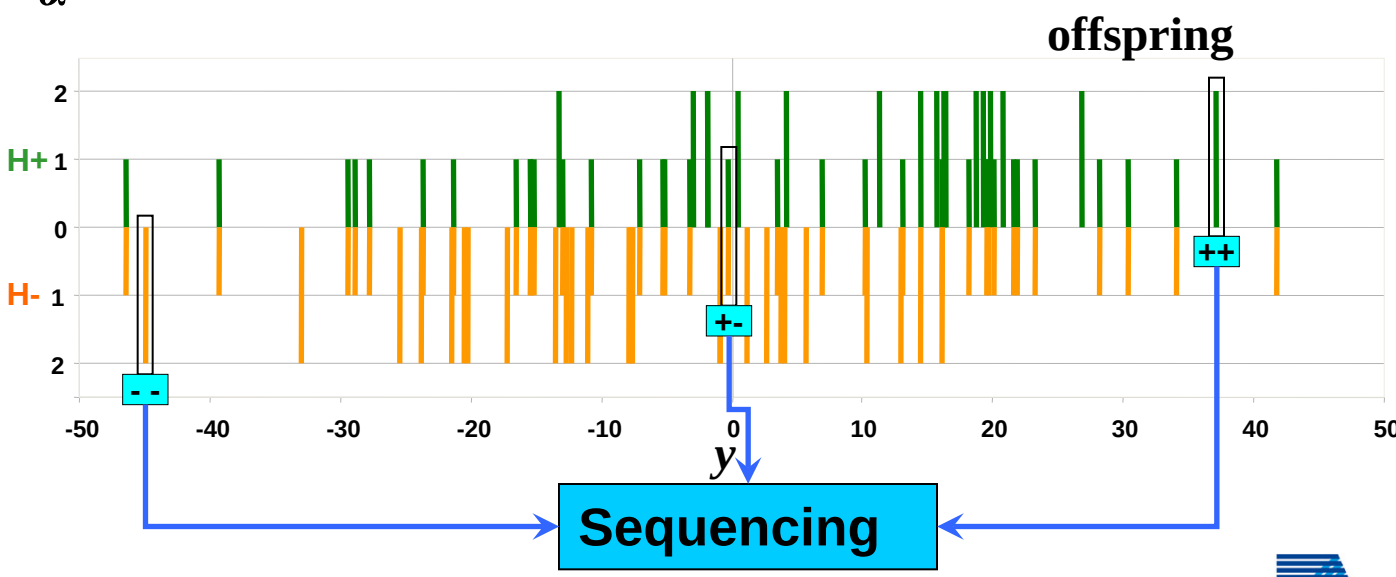
IBDp (H+ ,H-)=0.00

+ − = p(H+)>0.9; p(H-)>0.9

+ + = p(H+)=1.0; p(H-)=0.0

− − = p(H+)=0.0; p(H-)=1.0

IBD(+-)=0.0; IBD(++) & IBD(--)>0.8



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