Fine mapping of QTLs and genomic selection for production traits in an experimental population of Sarda dairy sheep.

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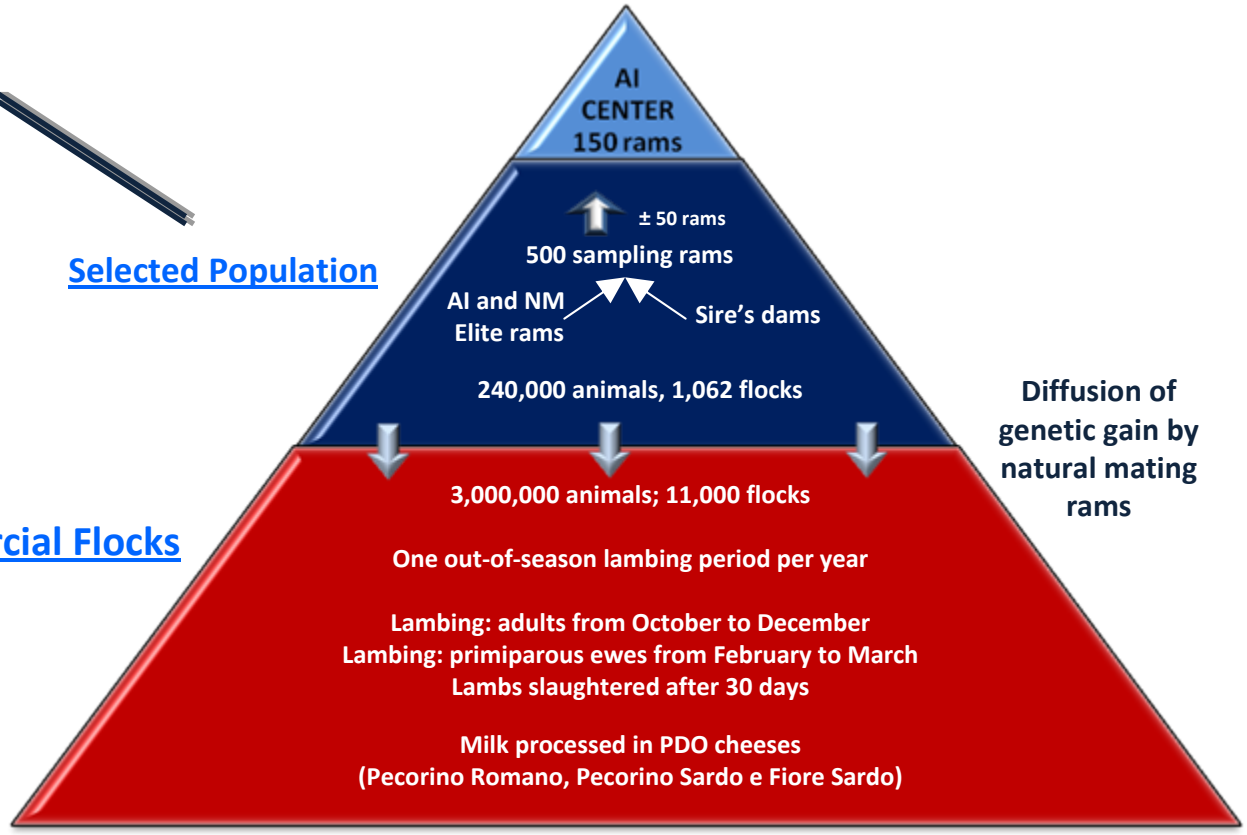
The breeding programs currently ongoing based on the traditional quantitative approach have achieved appreciable genetic gains for milk yield.

Selection scheme of Sarda dairy sheep breed

Official recording:
- Milk Yield
- Fat and Protein Content
- Udder Morphology
- Somatic Cell Count
- PrP Genotyping



Possibility of including **other traits**:

- SCC, FC, PC udder morphology (included with simplified recording)
- resistance to diseases (mastitis, internal parasites, paratuberculosis)
- milk nutritional value (fatty acid composition)
- milkability traits

limited by

high organizational effort needed to apply the traditional quantitative approach

high recording costs for traits difficult to measure

reduction of public funding

The application of selection schemes assisted by molecular information is potentially useful in dairy sheep

- advent of affordable high-throughput technology for SNP
- reduction in sequencing costs

shift to SNP markers for **QTL mapping** and **genome-wide selection studies**

Genome-wide selection seems still unachievable in most dairy sheep breeds

The main reasons:

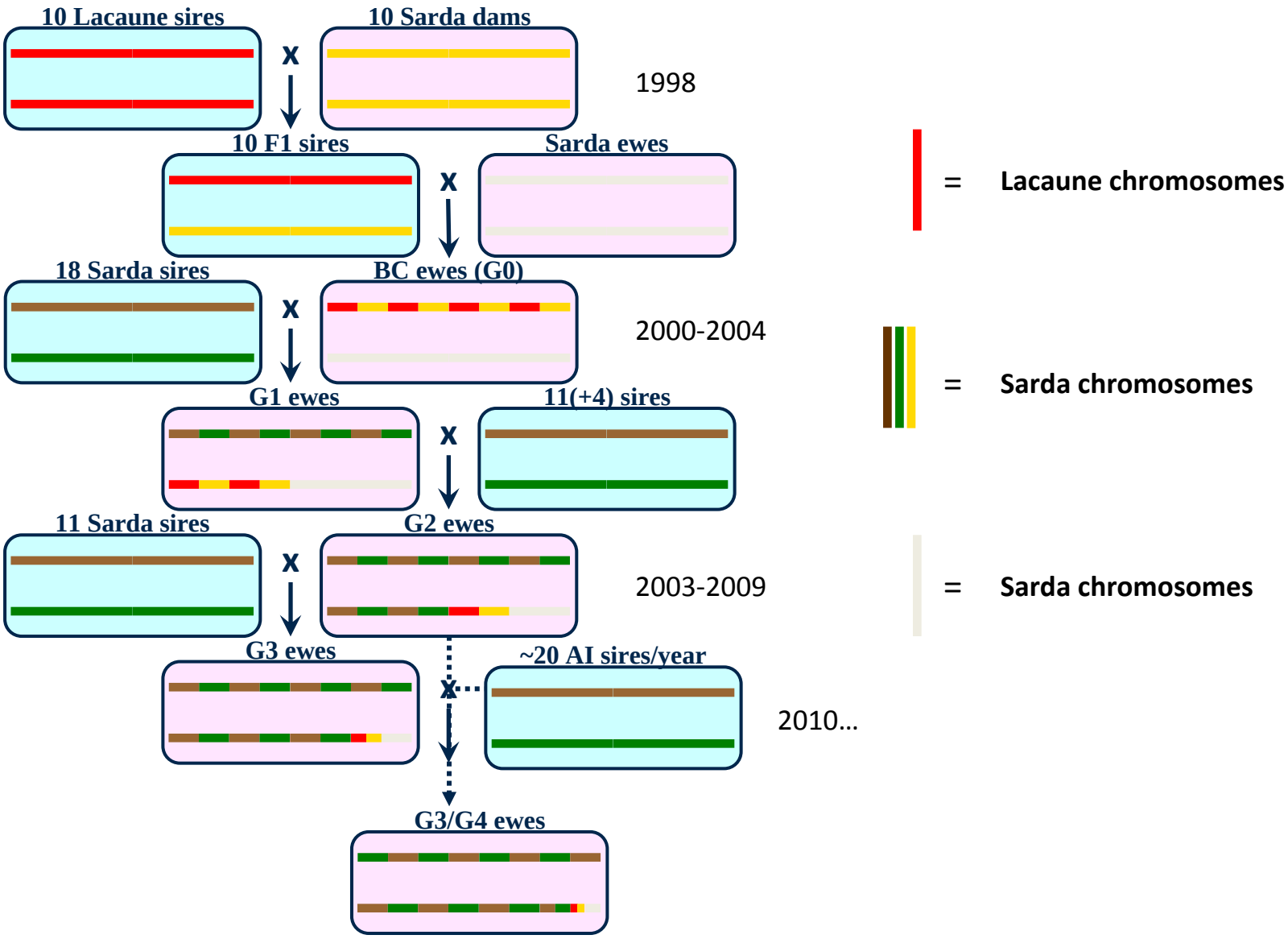
- **high cost** of HD SNP arrays mainly if related to the number of recorded traits
- difficulty in finding **well-structured training populations** to estimate SNP effects

Aim of this study

To present **preliminary elements** to evaluate **the potential evolution to nucleus flocks of pre-existing experimental populations** created for QTL detection purposes

to increase the efficiency of the selection process

Experimental population



Description of the experimental nucleus flock of the Sarda breed

Population	Generation	Number of individuals	Number of families	Mean size of the families	Procreated for
BC	G0	910	10	96.9	QTL detection
S1	G1	780	18	45.8	QTL Validation/Detection
	G2	718	15	49.0	QTL Validation/Detection
	G3,G4	508	11	45.8	QTL Validation/Detection
S2	G3,G4	344	40	8.6	EBV and DGV estimation

BC generated by mating F1 Lacaune x Sarda sires with Sarda ewes
S1 generated by mating Sarda sires with S0 ewes
S2 generated by mating AI Sarda rams with S1 ewes

S2 has been designed to be the nucleus flock (**NF**) of the Sarda breed **with the aim of estimating EBV and DGV of the most important blood lines**

Materials and methods

Phenotypes

Measured Trait	Frequency of measurement
Productive traits	
• Daily Milk yield	Fortnightly
• Fat, Protein and Lactose Content	Fortnightly
• Body Weight (adult ewe)	Monthly
• Body Condition Score	Monthly
Milkability and udder morphology	
• Kinetics of milk flow	Fortnightly
• Udder Morphology type traits	2-3 times/year linear score
• Udder's digital pictures	once in 2nd lactation
• Vacuometer	once in 2nd lactation
Health Traits	
• Somatic Cell Count	Fortnightly
• Clinical Mastitis	visual detection + microbiological essay
• Faecal Egg Count	2 times/year
• ELISA test for paratuberculosis	2 times/year
• Histo-pathological examination for paratuberculosis	once at slaughter
Milk quality traits	
• Fatty acid content	once in 2nd lactation
Reproductive traits	
• Prolificacy	once/year
• Fertility	once/year

Genotypes

Genotyping: *Porto Conte Ricerche* - iScan Platform of Illumina.

Individuals with a portion of missing genotypes higher than 10%

X chromosome and SNPs which could not be mapped

SNPs with call rate lower than 95%

MAF lower than 1%

(final data set: 44,859 SNP)

Genotyped animals (Illumina Inc. OvineSNP50 Beadchip):

- **Around 2,000 G0**, **G1** and **G2** ewes
- 10 **F1 Lacaune x Sarda sires**,
- 62 out of the 88 **Sarda sires** used in nucleus flock (**SA**)
- 94 **AI old Sarda rams** with the highest genetic impact on the selected population (**HI**)

Fine mapping of QTLs

- LA with "*extended*" families: including phenotypes and genotypes of grand- and great-grand-daughters
- At each SNP position a within-family linear regression of phenotypes on the probability of inheriting one of the QTL alleles of the family founder
- The model also included a founder effect and was tested by likelihood ratio test (**LRT**).
- Separate analyses were performed for **F1** and **SA** families in order to detect QTL segregating between breeds and within the Sarda breed respectively.
- *Chromosome-wise and genome-wise significance thresholds* were defined by 10,000 within family permutations.

Genetic link between the selected population and the nucleus flock

- Evaluated by relationship and genomic relationship matrices
- Relationship matrix included 2,894 **selected population (SP) sires** of ewes with lactation records in 2011 and **SA rams**.
- The genomic relationship matrix included 94 **HI** and 62 **SA** rams.

DGV and EBV calculation

DGV were **estimated** by LASSO-LARS (**LL**) procedure **using** 1,464 **ewes** of G1 and G2.

LL procedure was run until 500 SNPs were fitted in the model.

At each step the correlation between DGVs obtained by the current set of active SNP effects and the corresponding EBVs was calculated.

The DGV for MY of HI and SA rams was calculated as the sum of the genotype effects derived from the estimated SNP allele substitution effects.

DGV and EBV correlations

1. **SA rams: within NF and** official **EBV s**
2. **SA rams: EBV** estimated on NF daughters and **DGV** : to estimate the SNP effects' predictive ability
3. **HI rams: EBV** estimated in the official genetic evaluation and **DGV :** to estimate the SNP effects' predictive ability of EBV estimated for SP rams

Results and discussion

QTL fine mapping

* chromosome-wise significant (p<0.05); § genome-wise significant (p<0.05)

	OAR	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	TOT
MY	F1			§				*						*	*	*	*	*	*	*	*				§	*	*	13
	SA									*	*		*							*	*				*			6
FC	F1	§		§	*	*		§	*	§		*				*	*		*		§	*			*			14
	SA	*			§	*	§							*			*	*	*		*		*					10
PC	F1	§	*	*		*	*	*		*		§	*	§	*	*	§	*		*	*	*		*			*	19
	SA	*	*				§			*			*		*		*											7

Number of QTL detected in F1 was larger than in SA sires

Milk Yield

- **2 QTL** exceeding the genome-wise significance threshold in F1 families
- **No SA QTL** was genome-wise significant.

Fat Content

- **5 QTL** exceeded the 0.05 genome-wise significance threshold in F1 families.
- **2 QTL** exceeded the 0.05 genome-wise significance in SA sires

Protein Content

- **4 QTL** exceeding the genome-wise significance threshold in F1 families
- **1 QTL** exceeding the genome-wise significant threshold in SA.

Results and discussion

QTL fine mapping

* chromosome-wise significant (p<0.05); § genome-wise significant (p<0.05)

	OAR	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	TOT
MY	F1			§				*						*	*	*	*	*	*	*	*				§	*	*	13
	SA									*	*		*							*	*				*			6
FC	F1	§		§	*	*		§	*	§		*				*	*		*		§	*			*			14
	SA	*			§	*	§							*			*	*	*		*		*					10
PC	F1	§	*	*		*	*	*		*		§	*	§	*	*	§	*		*	*	*		*			*	19
	SA	*	*				§			*			*		*		*											7

- **Among the 16 QTLs** in F1 and SA on the same chromosomes, only **OAR20 for MY, OAR 6 and 16 for PC** showed close peaks (<5 Mb)
- Results **do not allow** to clearly **define causative mutation or LD markers**
- **LD and LDLA analyses** are expected to **improve the power of fine mapping**
- Further improvements are expected by **the increasing of the size of the genotyped and phenotyped portion of NF** and by the **sequencing of a set of target animals**

Results and discussion

Genetic link between selected population and nucleus flock

Frequencies of maximum relationship between pairs of SP and HI sires with SA sires extracted from the relationship and genomic relationship matrix respectively.

Mmaximum relationship	Pairs SP with SA (pedigree)	Pairs HI with SA (genomic)
0.00	0.01	0.00
0.00-0.063	0.07	0.00
0.064-0.125	0.32	0.10
0.126-0.250	0.44	0.66
0.251-0.500	0.14	0.21
> 0.500	0.02	0.03

- 90% of HI have a **genomic relationship higher than 0.125** with at least one SA sire
- 80% of SP sires had a **relationship coefficient higher than 0.0625** with at least one SA.
- The **percentage of HI and SP genome represented in SA seems to be sufficient t**o allow either a **correct estimation of SNP effects** or a sufficiently **accurate evaluation** of SP blood lines.

DGV and EBV estimation

- Correlation between SA within NF EBV and official **EBV** was **0.51**
- Correlation between SA within NF **EBV and DGV** was **0.91**
- Correlation between official **EBV** and **DGV** of HI sires was **0.43**.

These results confirm that SNP effects estimated by genotypes of NF ewe have a promising predictive ability of SA and HI sires EBVs.

The genomic tools originally used to detect QTLs can be also used also for genome-wide selection

Further improvements are expected by **increasing the number** of genotyped and phenotyped NF ewes.

QTL fine mapping

•The addition of the following generations of genotyped and phenotyped ewes combined with more sophisticated statistical methods will lead to a more precise definition of the QTL regions.

High density sequencing techniques on target animals should allow to identify causal mutations or LD SNPs to be used for selection.

DGV and EBV estimation

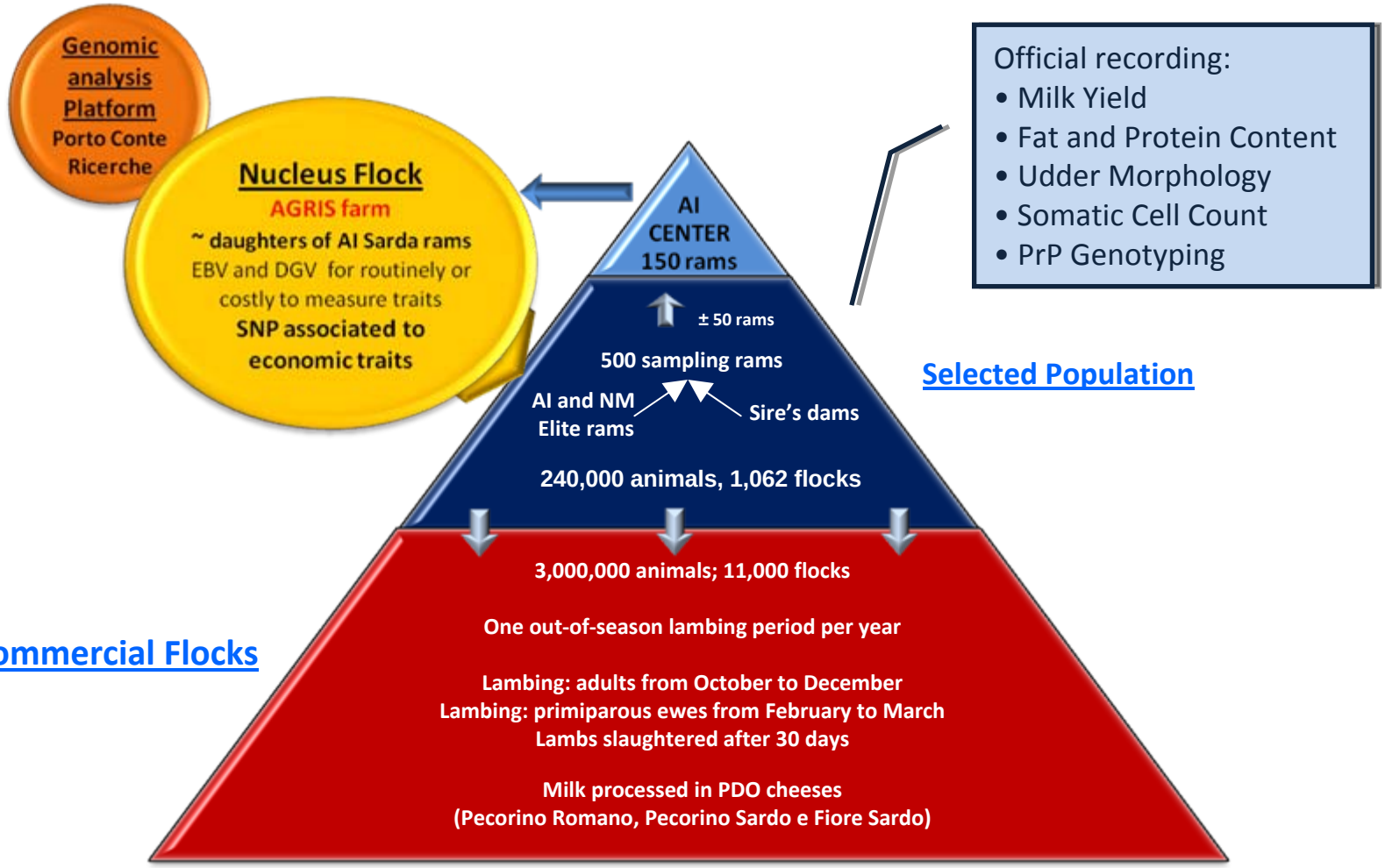
•The correlation between the DGV based on SNP effects from the nucleus flock and EBV estimated by progeny test in the selected population confirms that the nucleus flock can be useful to predict genetic merit of rams used in the selected population.

General Conclusions

- Results seem promising for including the pre-existing experimental flock created for QTL detection purposes in the breeding program of the Sarda breed with the aim of increasing the efficiency of the selection process.

- In the next future, simulation studies will be carried out to optimize the size of the nucleus flock and to find objective methods for choosing the blood lines to include in the nucleus flock and defining the size of the sire families.

Potential Evolution of selection scheme of Sarda dairy sheep breed



Thank you for your attention

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