



# World Holstein Cattle Biodiversity Study

Promoted by WHFF

Coordinated by ANAFIBJ  
11<sup>th</sup> October 2023



The Agenda of the meeting is as follow:

- 1) Welcome and Apologies for absence
- 2) Biodiversity study presentation (Martino)
- 3) Materials & Methods need for the project
- 4) General discussion
- 5) Timetable of the project
- 6) Close the meeting

○

+



### **SAB Scientific Advisory Board**

- ITALY - Cassandro, Finocchiaro, Van Kaam and Pilla and Persichilli,
- NLD - Gerben de Jong
- HUN - Laszlo Bognar
- FRA - Tiphaine MACE,
- .... other countries and names are welcome





The Agenda of the meeting is as follow:

- 1) Welcome and Apologies for absence
- 2) Biodiversity study presentation (Martino)
- 3) Materials & Methods need for the project
- 4) General discussion
- 5) Timetable of the project
- 6) Close the meeting

- **History of the proposal**

- 44° WHFF council of 15th April 2021: The idea was proposed the first time by Italy
- 45° WHFF council of 29th June 2021: The idea was discussed and Martino prepared a survey's draft
- 46° WHFF council of 20st October 2021: A draft's agreement was discussed and proposed
- 47° WHFF council of 16th Febraury 2022: The draft's agreement and appendix on M&M were discussed
- 48° WHFF council of 12 May 2022: Discusson on final paper go out to members from WHFF.

- **Answer received at the present:**

- HUN and ITA are agree to start
- DEU are not interested at the moment
- From other countries are awaiting reply
- Following 14 countries signed the agreement: FRA, LAT, DFS, POR, BEL, SPA, BRA, ITA, CAN, POL, HUN, NLD, UK, IRL





## SUMMARY

**Participants:** All Countries are invited with Holstein Friesian cattle genotyped and interested on the study.

**Costs:** No money for participants, bio-statistical analyses costs are provided by ANAFIBJ and UniMOLISE

**Benefit:** a deep analyses on the genetic relationships/structure among World Holstein pops.

**Practical outcome for farmers:** The genetic distinctiveness of the different Holstein pops and an overview on the combination of breeding objectives and use of Holstein semen among the d countries, what have generated in terms of can in term of similarities among Holstein pops

**Time:** 12 months beginning after the received of the complete data set



The Agenda of the meeting is as follow:

- 1) Welcome and Apologies for absence
- 2) Biodiversity study presentation (Martino)
- 3) Materials & Methods need for the project
- 4) General discussion
- 5) Timetable of the project
- 6) Close the meeting

## Methods and Sampling

An optimum number 250/300 random samples of cows population, or at least 25/30 samples per Holstein population, using of genotyping already available with at least 54k chip SNP minimum set born in the country not more than 10 years ago, and possibly chosen in a random way within the country, but representative of the selection applied population in the country.

The date of birth for each cow with a profile of chip SNP of 54k will be required.

---



Genotyping and quality control, genetic diversity and runs of homozygosity, population structure and admixture analyses, will be conducted.

---





## Methods

The study involves Holstein herdbooks from **14 different countries (France, Latvia, DFS, Portugal, Belgium, Spain, Brazil, Italy, Canada, Poland, Hungary, the Netherlands, United Kingdom, and Ireland)**. We aim to obtain an ideal sample size of 250 to 300 random cow samples from each country's population, or at least 25 to 30 samples from each specific Holstein population. These samples will be drawn from existing genotypes, ensuring a minimum set of 54,000 chip SNPs.

The selection of samples will be conducted in a randomized manner, with the following criteria:

- 1. Only the female population will be taken into account.**
- 2. The selected animals must have been born in the respective country within the last 10 years.**
- 3. They should have a lineage traceable to at least three generations on the maternal side born in the same country, and a total of five generations with documented registration. This ensures that the sampled animals are representative of the selective breeding applied within their country's population.**
- 4. We will require the birth date for each cow with a 54,000 chip SNP profile.**
- 5. To verify the representativeness of the sampled animals within their respective countries, we will collect some phenotypic measurements, specifically milk yield-305, protein yield-305, and fat yield-305, all measured during the first lactation.**

Subsequently, we will carry out genotypes quality control, evaluate genetic diversity, examine runs of homozygosity, and analyze population structure and admixture following our previous study on Italian Holstein (Persichilli et al., 2023).



The Agenda of the meeting is as follow:

- 1) Welcome and Apologies for absence
- 2) Biodiversity study presentation (Martino)
- 3) Materials & Methods need for the project
- 4) General discussion
- 5) Timetable of the project
- 6) Close the meeting



## • GENERAL DISCUSSION

- Avoid imputation
- Work on genotyped SNPs only, ignore SNPs which are not on all DNA chips.
- Format of the data (e.g. Plink format)
- Other HF population-countries might be download by scientific website
- Outlier population, as control
- ...
- ...



The Agenda of the meeting is as follow:

- 1) Welcome and Apologies for absence
- 2) Biodiversity study presentation (Martino)
- 3) Materials & Methods need for the project
- 4) General discussion
- 5) Timetable of the project
- 6) Close the meeting

# TIMETABLE of the Projects



Items	15 <sup>th</sup> apr- 21	last sem. '21	first sem. '22	last sem. '22	first sem. '23	last sem. '23	first sem. '24	last sem. '24
Proposed the Idea								
Discussion & Agreement definition								
Recording agreements with signatures								
Kick-off technical meeting								
Presentation at WHFF conference								
Deadline to receive data by countries						31 <sup>th</sup> dec '23		
Analyse of data								
Discusion at SAB-Scientific Advisory Board								
Presentation final results at WHFF council								dec '24



The Agenda of the meeting is as follow:

- 1) Welcome and Apologies for absence
- 2) Biodiversity study presentation (Martino)
- 3) Materials & Methods need for the project
- 4) General discussion
- 5) Timetable of the project
- 6) **Close the meeting**

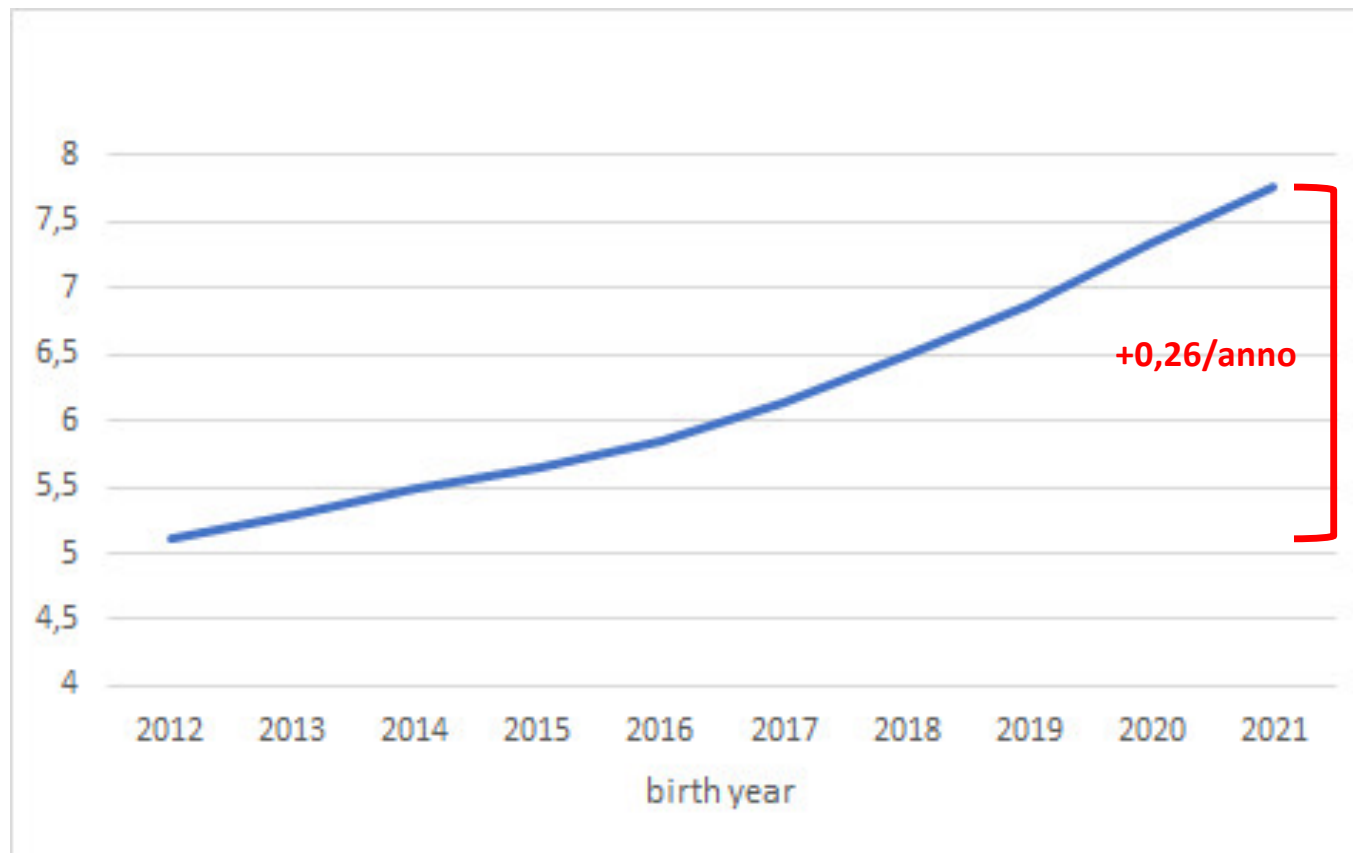


Thank you

World Holstein Cattle Biodiversity Study

Promoted by WHFF  
Coordinated by ANAFIBJ

## Trend of Inbreeding in Italian HF population





## Average increase in Inbreeding per country pe HF popolations

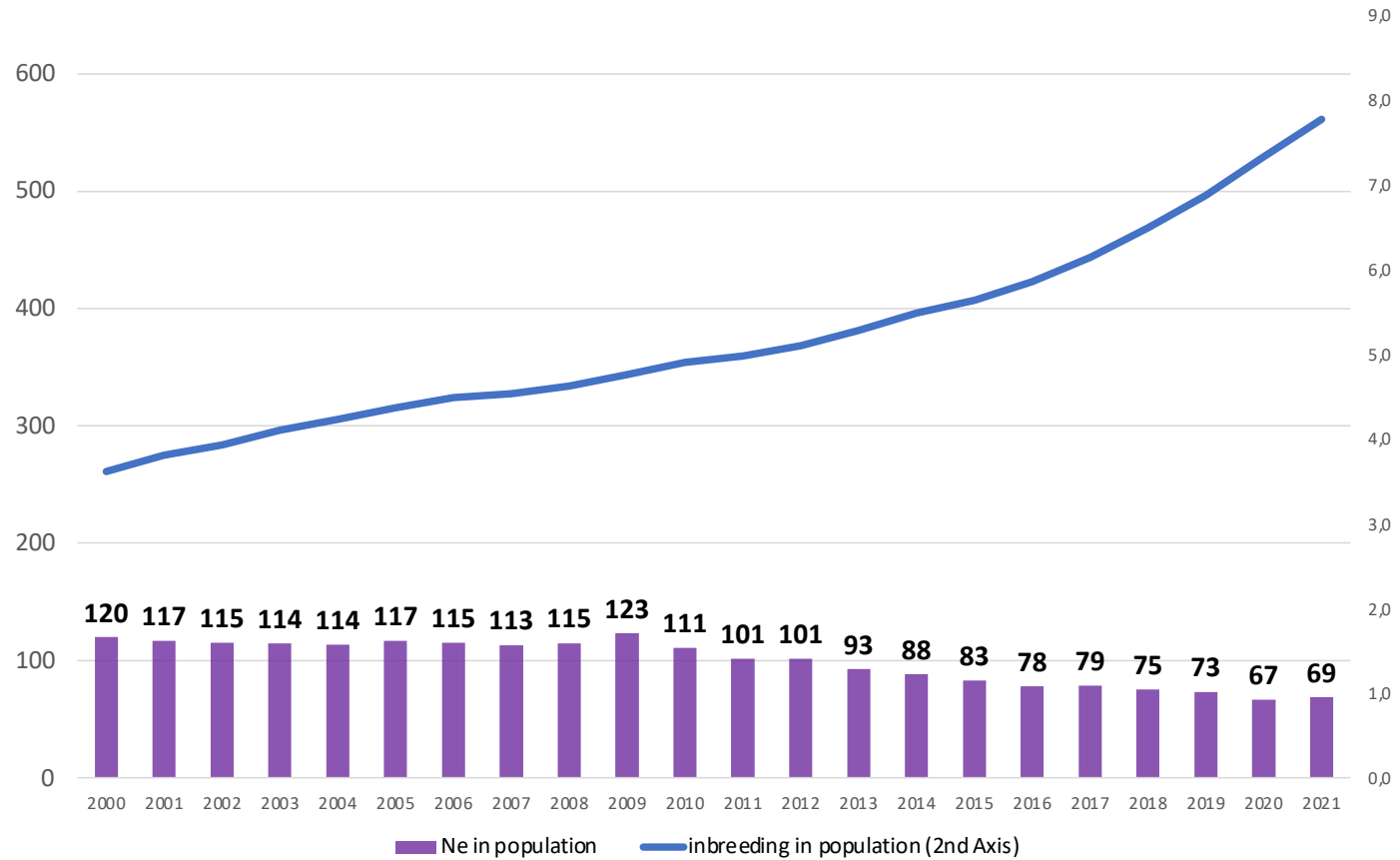
Birth year of females	1990-2000	2000-2010	2010-2020
Italy	0.18	0.14	0.26
US	0.19	0.11	0.26
Canada	0.26	0.08	0.25
Netherlands	0.17	0.03	0.16
France	0.20	0.10	0.16
Germany	0.16	0.08	0.15
Sweden	0.19	0.13	0.12
Denmark	0.18	0.12	0.10

The inbreeding level in the majority of HF cow populations has increased when genomic selection was introduced (2010-2020) compared to the situation when the traditional progeny test scheme was the key element in the breeding program (2000-2010).

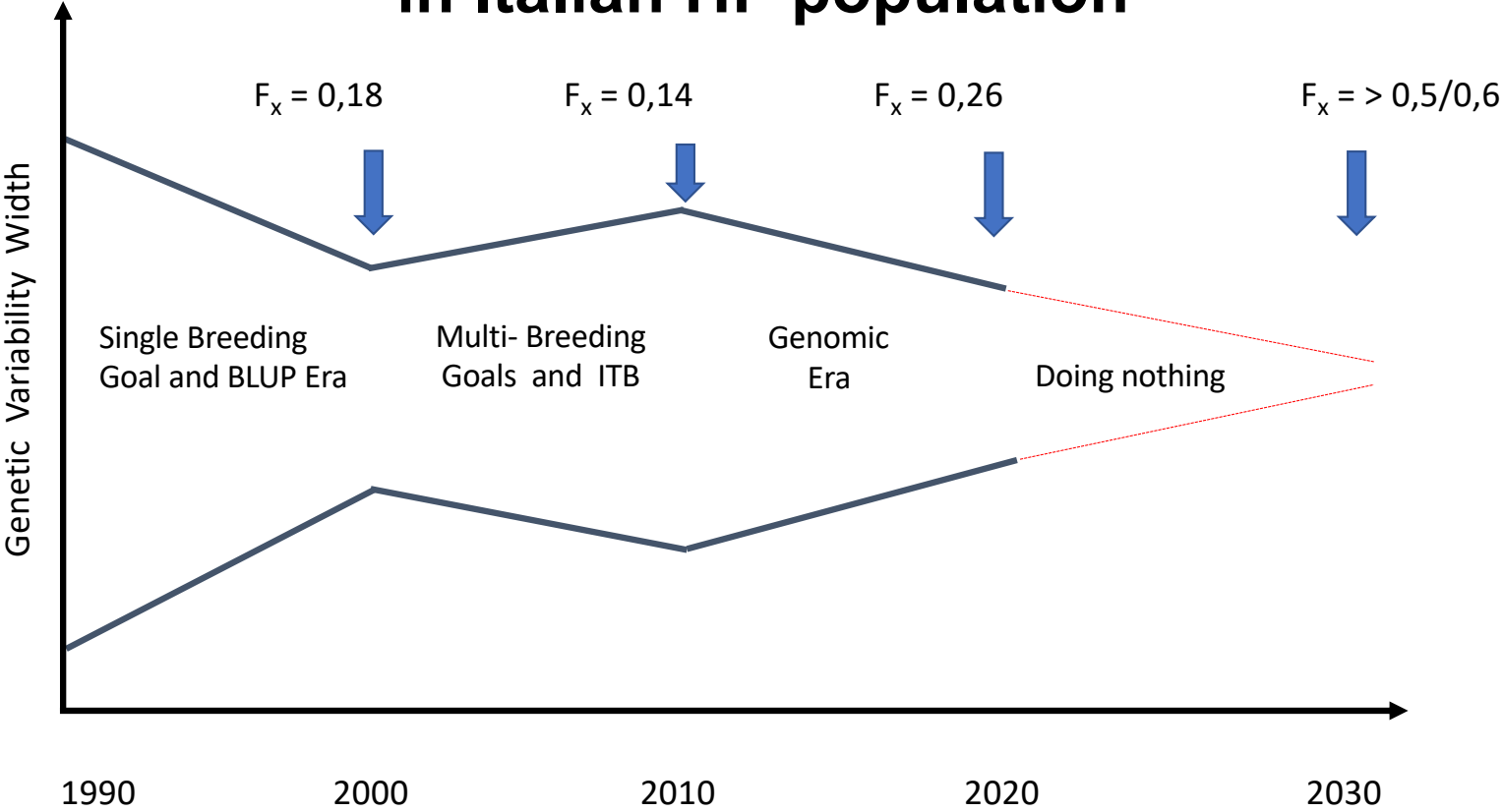
**For the Nordic Europe countries the increase is kept at the same level in the period from 2000-2020.**

Source World Inbreeding Trend in Holsteins –  
Presentation by Egbert Feddersen- view here <http://www.whff.info/>.

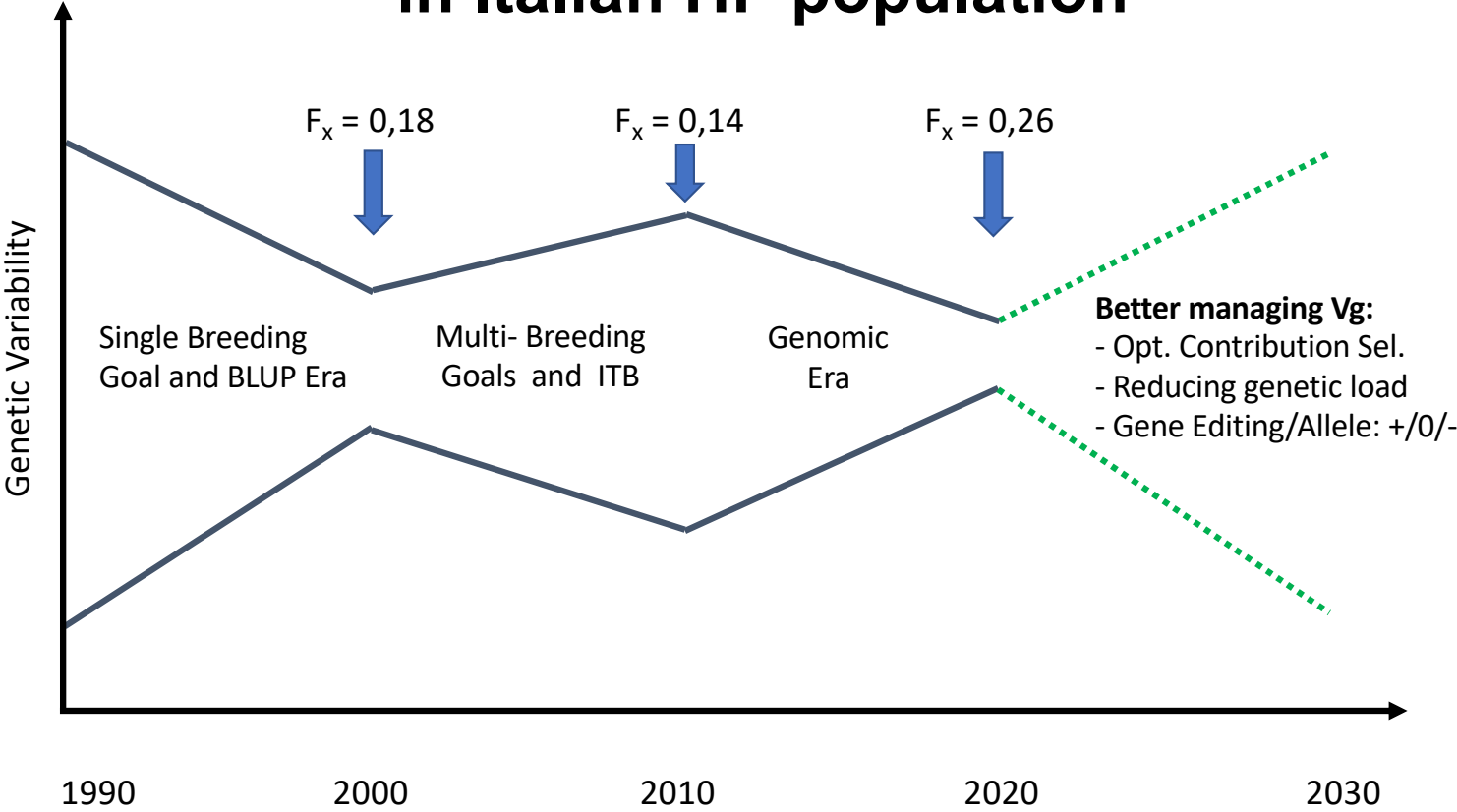
## Evolution of Inbreeding and Ne (effective Population size) for Italian Holstein (Population level)



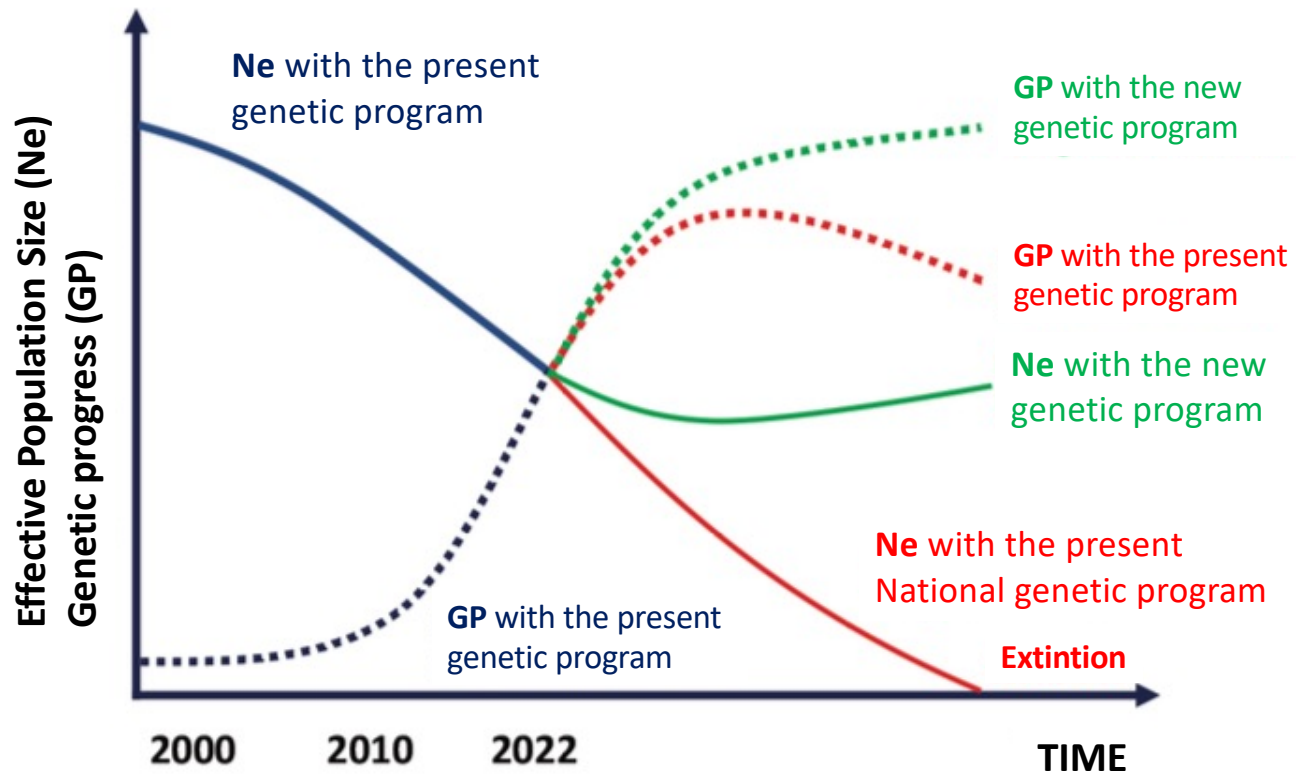
# Evolution of Genetic Variability in Italian HF population



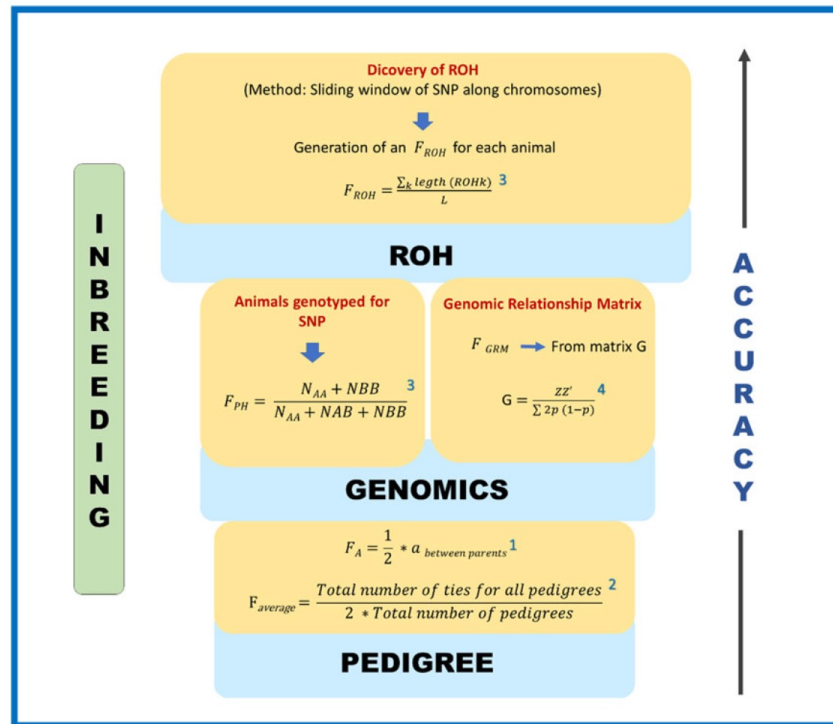
# Evolution of Genetic Variability in Italian HF population



# Expected Genetic Progress (GP) and Effective population Size (Ne)



# Main methods for the determination of Inbreeding



Gutiérrez-Reinoso MA, Aponte PM and García-Herreros M (2022). A review of inbreeding depression in dairy cattle: current status, emerging control strategies, and future prospects. Journal of Dairy Research 89, 3–12. <https://doi.org/10.1017/S0022029922000188>



**DATA TRANSFER AGREEMENT FOR A BIO-DIVERSITY STUDY OF THE  
HOLSTEIN FRIESIAN POPULATION**

BETWEEN:

National Association of Breeders of Holstein Friesian cattle of (country), a corporation/society of animal breeders, hereinafter called “**Participant**”,

Name \_\_\_\_\_ and \_\_\_\_\_ location \_\_\_\_\_, represented by

\_\_\_\_\_, CEO of the Association,

AND

ANAFIBJ-Italy, on behalf of the WHFF (World Holstein Friesian Federation), hereinafter called “**Recipient**” whose registered offices are located at via Bergamo, 292 – Loc. Migliaro, CREMONA, Italy – 26100, represented herein by Prof. Martino Cassandro,

Individually called “the party” or collectively “the parties”.

BEING UNDERSTOOD THAT

Participant agrees to participate in a WHFF-research project executed by ANAFIBJ in Italy, under supervision of the Council of the WHFF for the aims as described in step 1 to 3

The Participant (all members of WHFF) has in its possession DATA comprised of:

- SNP polymorphisms molecular data of Holstein Friesian animals official registered at herd book of Participant
- Any information oral or written provided by Participant to the Recipient for the implementation of this Agreement and which is confidential,

This DATA has been protected by a deed of industrial property, and can only be used for this agreement and for scientific publications, approved by the WHFF Council.



The Recipient (ANAFIBJ on behalf of the WHFF) is interested in the DATA (optimum number 250/300 random samples of cows population of the last 10 years or at least 25/30 samples per Holstein population, using of genotyping already available with at least 54k chip SNP) held by Participant to conduct research on genomic analyses research to provide three studies as follow and in the attachment described:

1. Collect and survey DATA provide by the Participant
2. Investigate the genetic relationships among WHFF Member Country populations.
3. Veritication of whether or not there is a genetic structure in the global Holstein Friesian population that can highlight the genetic distinctiveness of the global Holstein Friesian population and/or a participating countires/organisations.

IN CONSEQUENCE WHEREOF THE PARTIES AGREE AS FOLLOWS:

- 1 The Participant undertakes to supply the DATA to the Recipient after execution of this agreement by both parties. The DATA is supplied to the Recipient on a non-exclusive basis and for the sole purpose of the research and experiment described above. Consequently, the Recipient undertakes to use the DATA only to this end, to the exclusion of any other use.
  
- 2 The Recipient acknowledges the Participant as holder of rights to the DATA and of industrial and intellectual property rights relative hereto.  
Consequently, the Recipient will not include the DATA or any part of it in any patent application or other deed of industrial property without the preliminary written agreement of Participant.
  
- 3 The Recipient will not proceed to manipulations or alterations, which could affect the rights of Participant on the DATA, without the written and preliminary agreement of Participant.  
The Recipient is not authorised to combine, to mix or to incorporate the DATA with another material except for the needs of the research defined above. The Recipient undertakes to use the DATA according to the national and international laws and regulations and will make it its business to obtain all authorisations needed to the conduct of its research and experiment.

4 Save further express and written agreement, the Participant does not grant the Recipient any right, title deed, right of license or exploitation right to the DATA.

5 The Recipient acknowledges the confidential nature of the DATA and agrees:

- to supply this DATA only to members of his permanent staff and the WHFF Council who agree with the provisions of this agreement;
- to take all reasonable measures to avoid that his staff reveals to third parties, even for free, without written and preliminary agreement of Participant, all or any of the DATA.

The Recipient assumes the responsibility for implementing the obligations of this agreement towards every person having access to the DATA.

6 The obligations of confidentiality of the parties in this agreement do not apply to

DATA:

- which is in the public domain at the time of its disclosure by one of the parties;
- which falls in the public domain without any breach of this agreement;
- which was legally supplied by a third party not being subject to obligations of confidentiality;
- which is already known by the Participant and/or the Recipient before coming into force under this agreement without having been communicated, directly or indirectly, by one of the parties.

- 7 Regarding results obtained by the Recipient from the DATA, the Participant and the Recipient will determine together if results can be the subject of an oral or written communication and which authors of every party, will be joint authors. In all publications concerning the DATA, the Recipient should refer to the participants as the source of the DATA; as well as to WHFF.
  
- 8 The DATA supplied here is of experimental nature. The Participant declines any liability or responsibility concerning any and all damages caused by the DATA, and by the use which could be made of it. The Participant makes no representation or warranty that the use of the DATA will infringe any patent or other proprietary right.
  
- 9 This agreement will come into effect from the date it is signed, for a duration of 18 months (assuming 6 months to collect samples and 12 months for analyses and writing final report), in case of need of prolonged period the WHFF Council will decide if to continue or not the agreement.  
In any case, the obligations of confidentiality contained in this agreement will be maintained as long as the DATA is not released in the public domain.
  
- 10 This agreement is submitted to the Italian law. The parties will do their best to resolve amicably any dispute as for the interpretation or the performance of this agreement. In case of persistent disagreement, the parties will submit this one to the Italy courts.

In agreement with all participants, the data upon completion of the study will be destroy.

In witness whereof, this agreement has been drawn up in two original copies.

Done in \_\_\_\_\_, on \_\_\_\_\_

For THE Recipient

Done in \_\_\_\_\_, on \_\_\_\_\_

For the Participant

## APPENDIX: description of the DATA (including CONFIDENTIAL INFORMATION)

### **Introduction**

Assessment of genetic diversity and population structure is a fundamental task, not only to understand the evolutionary history of the origin of breeds, but also to provide important information for the conservation and management of local biodiversity [1, 2]. Indeed, evaluating the genetic diversity within and between populations provides an important control metric to avoid genetic erosion, inbreeding depression and cross-breeding between exotic and locally-adapted cattle breeds that can have deleterious consequences and eventually lead to extinctions [3–4].

The availability of genome-wide single nucleotide polymorphism (SNP) data has made it possible to conduct detailed characterizations of the genetic diversity and population structure in cattle. To date, the genetic variability, and the relationships between breeds at the genomic level have been investigated on a worldwide scale (e.g. [5, 6,]), a regional scale (e.g. [7, 8, 9]), and at the country level (e.g. [10,11, 12]), but a comprehensive study of the several Holstein Friesian worldwide populations is still lacking. Moreover several studies have conducted to explore the genetic variability and structure in cattle breeds both at local (13) and international (14) level, but Holstein Friesian was not investigated yet. By using genotype data from medium-density SNP arrays, we suggest using genome-wide comparative study of several official HF populations.

## REFERENCES

1. Boettcher PJ, Tixier-Boichard M, Toro MA, Simianer H, Eding H, Gandini G, et al. Objectives, criteria, and methods for using molecular genetic data in priority setting for conservation of animal genetic resources. *Anim Genet.* 2010;41:64–77.
2. Williams JL, Hall SJ, Del Corvo M, Ballingall KT, Colli L, Ajmone Marsan P, et al. Inbreeding and purging at the genomic level: the Chillingham cattle reveal extensive, non-random SNP heterozygosity. *Anim Genet.* 2016;47:19–27.
3. Pariset L, Mariotti M, Nardone A, Soysal MI, Ozkan E, Williams JL, et al. Relationships between Podolic cattle breeds assessed by single nucleotide polymorphisms (SNPs) genotyping. *J Anim Breed Genet.* 2010;127:481–8.
4. Ben Jemaa S, Boussaha M, Ben Mehdi M, Lee JH, Lee SH. Genomewide insights into population structure and genetic history of tunisian local cattle using the Illumina bovinesnp50 beadchip. *BMC Genomics.* 2015;16:677.
5. Gautier M, Laloë D, Moazami-Goudarzi K. Insights into the genetic history of French cattle from dense SNP data on 47 worldwide breeds. *PLoS One.* 2010;5:e13038.
6. Decker JE, McKay SD, Rolf MM, Kim J, Alcalá AM, Sonstegard TS, et al. Worldwide patterns of ancestry, divergence, and admixture in domesticated cattle. *PLoS Genet.* 2014;10:e1004254.
7. Medugorac I, Medugorac A, Russ I, Veit-Kensch CE, Taberlet P, Luntz B, et al. Genetic diversity of European cattle breeds highlights the conservation value of traditional unselected breeds with high effective population size. *Mol Ecol.* 2009;18:3394–410.
8. Mastrangelo S, Saura M, Tolone M, Salces-Ortiz J, Di Gerlando R, Bertolini F, et al. The genome-wide structure of two economically important indigenous Sicilian cattle breeds. *J Anim Sci.* 2014;92:4833–42.
9. Senczuk, G., Mastrangelo, S., Ciani, E., Battaglini, L., Cendron, F., Ciampolini, R., Crepaldi, P., Mantovani, R., Bongioni, G., Pagnacco, G., Portolano, B., Rossoni, A., Pilla, F. & Cassandro, M. 2020, "The genetic heritage of Alpine local cattle breeds using genomic SNP data", *Genetics Selection Evolution*, vol. 52, no. 1.
10. Makina SO, Muchadeyi FC, van Marle-Köster E, MacNeil MD, Maiwashe A. Genetic diversity, and population structure among six cattle breeds in South Africa using a whole genome SNP panel. *Front Genet.* 2014;5:333.
11. Yurchenko A, Yudin N, Aitnazarov R, Plyusnina A, Brukhin V, Soloshenko V, et al. Genome-wide genotyping uncovers genetic profiles and history of the Russian cattle breeds. *Heredity.* 2018;120:125–37.
12. Mastrangelo, S., Ciani, E., Ajmone Marsan, P., Bagnato, A., Battaglini, L., Bozzi, R., Carta, A., Catillo, G., Cassandro, M., Casu, S., Ciampolini, R., Crepaldi, P., D'Andrea, M., Di Gerlando, R., Fontanesi, L., Longeri, M., Macciotta, N.P., Mantovani, R., Marletta, D., Matassino, D., Mele, M., Pagnacco, G., Pieramati, C., Portolano, B., Sarti, F.M., Tolone, M. & Pilla, F. 2018, "Conservation status and historical relatedness of Italian cattle breeds", *Genetics Selection Evolution*, vol. 50, no. 1.
13. Mastrangelo, S., Ben Jemaa, S., Ciani, E., Sottile, G., Moscarelli, A., Boussaha, M., Montedoro, M., Pilla, F. & Cassandro, M. 2020, "Genome-wide detection of signatures of selection in three Valdostana cattle populations", *Journal of Animal Breeding and Genetics*, vol. 137, no. 6, pp. 609-621.
14. Moscarelli, A., Sardina, M.T., Cassandro, M., Ciani, E., Pilla, F., Senczuk, G., Portolano, B. & Mastrangelo, S. 2021, "Genome-wide assessment of diversity and differentiation between original and modern Brown cattle populations", *Animal Genetics*, vol. 52, no. 1, pp. 21-31.

An Example of output of the analyses

