

Inbreeding Becomes A Serious Issue

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Abstract

Analyses of single nucleotide polymorphism (SNP) data of Holstein genotypes present at Anafibj has been done to verify trends in genetic variation over time. Results show a linear decline of SNP heterozygosity in the pre-genomics era from 1990-2010, and a 5-fold larger linear decline in the genomics era from 2010-2023. This is a clear signal that the increased annual genetic progress from genomic selection goes together with a strong decrease in genetic variation. Pedigree based inbreeding rates show Italy, USA and Canada having the highest annual inbreeding rates for Holsteins among countries. Strong market competition for artificial insemination (AI) centers results in the intense selection of a smaller number of elite animals. Emphasis on short-term genetic gain might be harmful for maintaining the long-term health and diversity of the breed. In Italy, research is underway to attempt to counter the harmful effects of decrease in genetic variation as well as genetic disorders.

Key words: Inbreeding, Holstein, effective population size, genetic diversity, SNP, Runs-Of-Homozygosity, genomic selection

Introduction

A closed population is a population in which there is no gene flow from other populations. This means that the only source of new genetic variation in the population are mutations. Breeding in any closed population will therefore gradually increase inbreeding and hence reduce genetic variation, unless sufficient mutations occur. Selecting a small number of sires and selecting candidates that are genetically very similar will strongly increase the rate of inbreeding. Inbreeding can lead to a number of problems, including reduced fertility and fitness, increased susceptibility to disease, and decreased genetic diversity. Since the advent of genomic selection, genetic variation is declining at a strongly increased rate, due to a stronger selection intensity and shorter generation intervals. When genetic variation is reduced, populations are less likely to be able to adapt

to change and may become more vulnerable to extinction.

The Holstein breed worldwide is the leading dairy breed, but the genetic base is not as large as might seem from the number of animals. Less than 10.000 animals were imported from Europe in North-America before 1890. And few lines became the leading sire lines in the <1900 period (Neptune H and Hulleman), 1920-1940 (Rag Apple and Burke), 1960-1970 (Chief and Elevation). The latter 4 foundation sires all descent from the first 2. So, in reality there are just two male lines remaining (Yue et al., 2015). And effectively there have been multiple genetic bottlenecks.

Materials and Methods

The Anafibj genomic databank was used for analysing annual trends in genetic variation of Holstein SNP genotypes. After imputation, annual average SNP heterozygosity of 88068

SNPs from animals born from 1990-2023 was computed. Animals without pedigree were excluded as well as non-genotyped animals. The year 2010 was considered as the transition point between pre-genomic and genomic selection.

The average inbreeding coefficient within a year was computed as (homozygosity this year-homozygosity first year) / (1-homozygosity first year). Average generation intervals were computed per year for males and females separately and then averaged between sexes. The relative year since 1990 was divided by the annual generation interval to estimate how many generations would be passed since 1990 at the current generation interval. Using a linear fit on SNP heterozygosity, the inbreeding coefficient **F** was computed per year. The effective population size (**Ne**) was estimated as $Ne = 1/(2 \cdot 2 \cdot ((1-F)^{(1/(\text{number of generations}))}))$ (Frankham, Bradshaw and Brook, 2014).

Inbreeding depression was calculated for 305d milk yield including in a linear mixed model with milk yield as response variable and the inbreeding calculated based either on Pedigree data or on Runs-Of-Homozygosity (ROH) as regressor.

Results & Discussion

Results (Table 1) from the pedigree-based annual inbreeding rates, from word Holstein Frisian federation (WHFF) show that Italy (0.26), USA (0.26) and Canada (0.23) have the highest inbreeding rates, since genomic selection. This probably results from the strong competition between AI centers within the Intercontinental Consortium.

Results of the analysis of SNP heterozygosity by birth year are shown in Figure 1. Both the pre-genomic as well as the genomic era showed a decrease in annual average SNP heterozygosity. The 1990 SNP heterozygosity was 0.3561 after which the annual decline was -0.0004. The linear regression in the pre-genomics era shown in Figure 1 has an R² of 0.82. From 2010

onwards SNP heterozygosity was 0.3480 and the annual decline was -0.0020. The R² of the linear regression in this period was 0.97. So, results show a 5-fold increase in the decline of SNP heterozygosity. If the SNP heterozygosity is 0.32 and the (linear) annual decline is 0.0020, then after $0.32 / 0.0020 = 160$ years we will be at a heterozygosity of 0.00.

Table 1. Countries with highest pedigree-based inbreeding rates per year as shown by WHFF

Country	2010-2019
ITA	0.26
USA	0.26
CAN	0.23
FIN	0.20
POL	0.20
ESP	0.20
CHE	0.19
SLO	0.18
NLD	0.16
FRA	0.15
DEU	0.15
IRL	0.15

In Figure 2, the average inbreeding coefficient, annual inbreeding rate and inbreeding rate per generation are shown per birth year. The change in 2010 is evident. Genomic selection had an enormous impact on all 3 variables.

Figure 3 illustrates the decline in the effective population size of the Holstein breed. Since genomic selection started the decline has been from nearly 90 to 60. Note that FAO considers an Ne value of 50 to be a critical threshold for the long-term survival of a breed. (FAO, 1988).

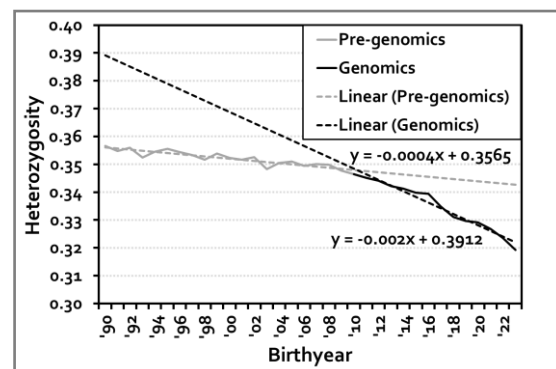


Figure 1. Pre- and post-genomic trends of SNP heterozygosity by birth year

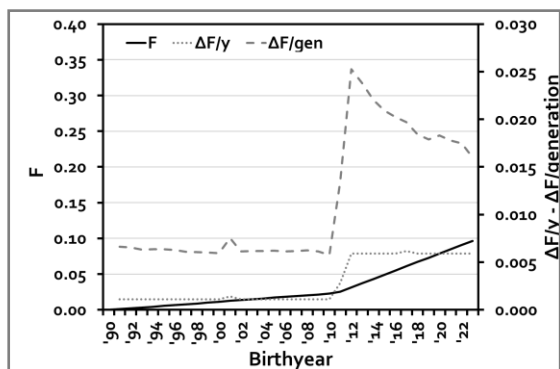


Figure 2. Inbreeding coefficient (F), annual inbreeding rate ($\Delta F/y$) and inbreeding rate per generation ($\Delta F/gen$) by birth year

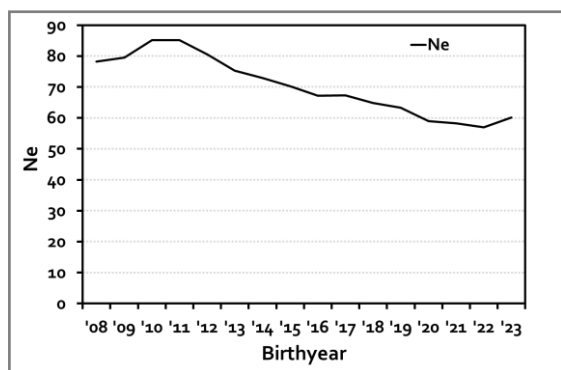


Figure 3. Decline of effective population size (N_e) during the most recent 16 birth years

In our ongoing study in Italian Holsteins, inbreeding depression resulted in declines of 44 kg and 61 kg of milk yield per % increase in pedigree and ROH inbreeding coefficients, respectively. Over the last 5 years, with a +2.35% change in inbreeding coefficient (ΔF) (Ablondi et al., 2021), this results in an inbreeding depression of 103 kg based on pedigree and 143 kg based on ROH. Considering realized genetic progress of 415 kg during this period, including the impact of inbreeding, progress without inbreeding might have been 518 kg (+103 kg based on pedigree) and 558 kg (+143 kg based on ROH). Hence 103 kg which means 20% was lost if estimated with pedigree, whereas 143 kg meaning 26% was lost based on ROH. In conclusion, the impact of inbreeding is substantial.

The decline of within breed genetic diversity, should trigger adjustments in the breeding programs to mediate harmful results. Breeders can take steps to maintain genetic diversity, such as using a wider range of bulls from different sires and dams and countries, and breeding for a variety of traits. This can help to prevent the over-use of a few elite bulls. However, this will require a change in mindsets, as breeders may need to sacrifice some short-term genetic gain in order to maintain the long-term health and diversity of the breed.

At Anafibj, research is underway to estimate genomic expected future inbreeding. Aim is to provide a premium to animals which have a lower relatedness to the expected future population, and a penalty to more related animals. Current focus is on ROH, which enables to focus on more recent inbreeding, which is considered more harmful. We aim to compute genomic expected future inbreeding coefficients, which is the probability in an autosomal segment that the haplotype transmitted from a random mate (of a reference population reflecting the future population) is identical to the transmitted haplotype of this individual, i.e. a ROH. In practice identical by state is used as if identical by descent. The importance is to lower the future inbreeding caused by the bulls rather than the own inbreeding of the bulls themselves, because own inbreeding does not pass to the descendants, given only half the chromosomes transmit to the offspring.

Conclusions

Analyses of SNP heterozygosity over time show a clear linear decline in the pre-genomics and genomics periods. A 5-fold increase in the decline of SNP-heterozygosity was found in the genomics era. It shows that the increased annual genetic progress comes at a cost of more rapid decline of genetic variation. Care has to be taken to avoid damage from the loss of genetic variation for future generations or

due to homozygosity for recessive genetic disorders.

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