Optimizing selection decisions in the Danish Dairy Shorthorn conservation program

Advisory report from DCA – Danish Centre for Food and Agriculture, Aarhus University

Alban Bouquet, Charlotte Æ. Meinert, Morten Kargo

Center for Quantitative Genetics and Genomics

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Data sheet

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Authors:	Assistant Professor Alban Bouquet, Research Assistant Charlotte Æ. Meinert and Senior Researcher Morten Kargo, Center for Quantitative Genetics and Genomics, Aarhus University
Reviewer:	Senior Researcher Hanne Marie Nielsen, Center for Quantitative Genetics and Genomics, Aarhus University
Quality assurance, DCA:	Senior Consultant Johanna Höglund & Senior Consultant Louise Dybdahl Pedersen, DCA Centre Unit, Aarhus University
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Content

Summary

The Danish Dairy Shorthorn is a small cattle population experiencing a rapid loss of genetic diversity, as demonstrated by both pedigree and genomic analyses. There is therefore an urgent need to manage inbreeding more effectively to safeguard the long-term viability of this endangered breed. The last initiative to address this critical situation was the use of two Northern Dairy Shorthorn bulls—a related Shorthorn line from the UK.

In this research-based advisory report, we developed a data-analysis pipeline that uses available pedigree information to support breeders in making informed decisions about which animals to retain and how to design matings that minimize inbreeding increase in the next generation(s). The pipeline is built around Optimal Contribution Selection (OCS), a state-of-the-art method for controlling inbreeding in small populations. OCS analyses highlighted the need to broaden the set of bulls used for breeding, including gene bank bulls, and to distribute their contributions more evenly. We also integrated genomic data from the entire live population and from bulls with cryopreserved semen to evaluate the added value of genomics within the OCS framework.

Overall, genomic and pedigree-based OCS yielded consistent recommendations. Genomic information revealed several deviations between expected and realized inbreeding and relationships. In such cases, genomic OCS placed greater emphasis on individuals that were less related to the population than expected from pedigree records. This is especially useful when selecting among closely related breeding candidates. Our analyses showed that strategic use of two gene bank bulls, Thy Texas and U. Ralf, could produce offspring little related to the population that would be valuable for the conservation program. Using this pipeline, the current inbreeding level of Danish Dairy Shorthorn could be reduced from 0.20 to 0.16 by implementing the pedigree-based mating plan, and even to 0.13 with genomic information. The OCS recommendations incorporating genomic data were not sensitive to the method used to build the genomic relationship matrix.

Genomic data also helped identify Northern Dairy Shorthorn–sired bull calves with potential to enrich the gene bank. Among the five calves genotyped from the 2025 cohort, one exhibited low kinship both with living females and existing gene bank bulls, making it a strong candidate for inclusion. The remaining calves shared the same maternal grandsire—an individual with a very high contribution to the current population—reducing their usefulness for improving genetic diversity. Additional biological samples from recently born bull calves have been collected and are currently undergoing genotyping.

Overall, these results demonstrate the substantial benefits of integrating genomic information into the management of genetic diversity in endangered livestock populations. The tools and workflow developed in this project can readily be adapted for other species within the national conservation program.

Dansk sammendrag

Den danske bestand af Malkekorthorn er lille, og der sker et hurtigt tab af genetisk diversitet, hvilket analyser baseret på både afstamning og genomiske data viser. Der er derfor et stort behov for at håndtere indavl mere effektivt for at sikre denne truede races langsigtede overlevelse. Det seneste initiativ til afhjælpning af den kritiske situation er brug af to Northern Dairy Shorthorn-tyre – en beslægtet Korthornslinje fra Storbritannien.

I denne forskningsbaserede rådgivningsrapport har vi udviklet et redskab, der bruger den tilgængelige stamtavleinformation til at støtte avlere i at træffe informerede beslutninger om, hvilke dyr der bør bevares, og hvordan parringer bør planlægges for at minimere indavlsstigningen i næste og kommende generation(er). Redskabet bygger på Optimal bidrags Selektion (OCS), en avanceret metode, som kan anvendes til styring af indavl i små populationer. OCS-analyserne viste, at der er et behov for at udvide udvalget af tyre, der anvendes i avlen – herunder tyre fra genbanken – og for at udligne deres genetiske bidrag ved at bruge dem mere ligeligt. Vi analyserede også genomiske data fra hele den levende bestand og fra tyre med nedfrosset sæd for at vise, hvad anvendelse af genomisk information kan bidrage med til OCS-metoden.

Overordnet gav genom- og stamtavlebaseret OCS enslydende anbefalinger; dog afslørede de genomiske data flere afvigelser mellem forventet og faktisk indavl og slægtskab. I sådanne tilfælde lagde genomisk OCS større vægt på individer, der viste sig at være mindre beslægtede med den samlede population end forudsagt på basis af stamtavlen. Det er især nyttigt ved udvælgelse blandt nært beslægtede avlskandidater. Vores analyser viste også, at strategisk brug af to genbanktyre, Thy Texas og U. Ralf, vil betyde, at der vil fødes kalve, som er væsentlig mindre beslægtede med populationen end "gennemsnitlige" korthornskalve. Disse kalve vil være meget værdifulde for bevaringsprogrammet for Dansk Malkekorthorn. Ved brug af programmet vil det aktuelle indavlsniveau i Dansk Malkekorthorn kunne reduceres fra 0,20 til 0,16, hvis programmet anvender afstamningsinformation – og helt ned til 0,13 hvis der anvendes genomisk information. Anbefalingerne baseret på genomisk information var ikke følsomme over for den metode, der blev anvendt til at konstruere slægtskabsmatricen.

Genomiske data bidrog også til at identificere de tyrekalve efter de to Northern Dairy Shorthorn tyre som havde potentiale for at styrke avlsmaterialet i genbanken. Blandt de fem tyrekalve fra 2025-årgangen, der blev genotypebestemt, var der én kalv med meget lavt slægtskab til både de levende køer og til genbanktyrene. Det gør denne kalv til en stærk kandidat til optagelse i genbanken. De øvrige kalve delte samme morfar – en tyr med meget stort genetisk bidrag til den nuværende population – hvilket reducerer deres værdi i forhold til at øge den genetiske diversitet. Der er desuden indsamlet biologiske prøver fra nyligt fødte tyrekalve til genotypning, og analyserne af disse prøver er i gang.

Samlet viser resultaterne en betydelig fordel ved at anvende genomisk information i forvaltningen af genetisk diversitet i truede husdyrracer – her vist for Dansk malkekorthorn. De værktøjer og arbejdsgange, der er udviklet i dette projekt, kan dermed forholdsvis let tilpasses andre husdyrracer i det nationale bevaringsprogram.

Preface

Through the Interlaken Declaration, Denmark has committed to working toward the sustainable use, development, and conservation of animal genetic resources for food and agriculture.

Shorthorn dairy cattle of the original dual purpose type is one of the Danish breeds considered worthy of conservation and efforts have been made to preserve and restore genetic diversity in this population. The last initiative was the use of semen from two Northern Dairy Shorthorn bulls, a related Shorthorn line from the UK. However, there is a need for a formal breeding plan that breeders can follow to make the best possible use of the imported semen and, more generally, to optimize breeding work within the Danish Shorthorn population.

In an order submitted on 17 February 2025, the Danish Agricultural and Fisheries Agency has asked DCA – National Centre for Food and Agriculture at Aarhus University (AU) to develop such a breeding plan.

1 Background

As part of Denmark's commitment to conserving animal genetic resources, a national conservation program has been established to preserve Danish heritage livestock breeds. The Danish Dairy Shorthorn (Dansk Malkekorthorn) is one of five cattle breeds included in this initiative. Originally native to Great Britain, the Shorthorn breed has expanded worldwide into several subpopulations selected for different breeding objectives such as dairy, dual-purpose or beef production. The breed was introduced to Denmark in the 1800s, and by 1920s-1930s, about 30% of cattle in Jutland were Shorthorns (Sørensen and Nielsen, 2012). Adapted to the cold and humid climate and moorlands of Northern Europe, the breed thrived in Denmark (Landbrugsstyrelsen, 2025; Sørensen & Nielsen, 2012).

In the second half of the 20th century, Danish Shorthorns have been progressively replaced or crossed with more productive breeds better suited for intensive production systems. In the 1980s, the last remaining individuals from pure Danish Dairy Shorthorn belonged to the Lynge herd (Trinderup et al., 1999). Animals descending from these lines form the basis of today's conservation program. The breed has declined to critically low population size; in 2007, only 28 breeding cows and 4 bulls were part of the *in situ* conservation program (Sørensen and Nielsen, 2012). Over the last decade these numbers have stabilized and even slightly increased to reach around 50 cows. Additionally, semen of 13 bulls born between 1961 and 2015 was cryo-conserved to constitute an *in vitro* gene bank.

Previous genetic diversity studies revealed high inbreeding level within the Danish Dairy Shorthorn population (Szekeres et al., 2016). The breed is classified as endangered according to guidelines for genetic resource preservation (FAO, 2013). These results suggested that a substantial portion of genetic diversity may already have been lost and that the high inbreeding rate could expose the breed to potential negative effects of inbreeding depression on fitness traits. Therefore, there is a pressing need for strategies and tools to help breeders manage inbreeding more effectively.

The present research addresses that need. First, we developed a data analysis pipeline to assist breeders in making informed decisions about which animals to keep on their farms and how to mate them optimally to retain as much genetic diversity as possible. Indeed, while decisions occur at the farm level, it is crucial for such a small population that individual decisions align with the broader goal of preserving the breed's genetic diversity. The pipeline was initially developed to analyze pedigree data and was enhanced to incorporate genomic information.

Moreover, as a transboundary breed, other Shorthorn subpopulations might serve as reservoirs of genetic diversity to rejuvenate the Danish gene pool. Genomic analysis of global Shorthorn subpopulations revealed the unique genetic makeup of the Danish Shorthorn (Ravn, 2020), underscoring its conservation value while highlighting the challenge of introducing individuals from other subpopulations without compromising its distinctiveness. In 2023 it was decided to import semen of two bulls from the Northern Dairy Shorthorn population through a collaboration with the British Shorthorn breed association (Bevaringsudvalget, 2023). Inseminations were carried out in 2024, and the first calves were born in 2025. We are now at a turning point where it needs to be decided which bull calves could be considered to store semen in the gene bank.

Thus, this report will tackle the three following objectives mentioned in the order received from the Danish Agricultural and Fisheries Agency:

1. Evaluate the current status of genetic diversity in the Danish Dairy Shorthorn population considering all alive individuals and bulls with cryo-preserved semen.
2. Design a pipeline based on Optimal Contribution Selection to produce an optimized mating that can be used by breeders and evaluate the benefits of incorporating genomic information to improve genetic diversity preservation
3. Identify which bull calves (sired by Northern Dairy Shorthorn bulls) would be interesting to complement the gene bank

This report has been structured around these three objectives. First, we assessed the genetic diversity of the Danish Dairy Shorthorn breed using indicators derived from both pedigree and genomic data. This step was important to get a better insight into the population structure and obtain individual measures of diversity such as the inbreeding coefficient of animals and the kinship coefficients between animals.

Next, we used this information in an algorithm designed to optimize the selection and mating of breeding animals, with the aim of maximizing genetic diversity in the next generation. This algorithm, known as Optimal contribution Selection (OCS), is a well-established methodology for managing inbreeding in small populations. We also examined the potential benefits of integrating genomic information into this approach.

Finally, we used genomic data to identify bull calves sired by Northern Dairy Shorthorn bulls with potential to enrich the gene bank, specifically animals that are weakly related to both males and females in the population. Genomic information was crucial for this purpose, as it enabled decisions to be based on realized genomic relationships that could not be obtained from pedigree data alone.

2 Analysis of genetic diversity in the Danish Dairy Shorthorn breed

2.1. Presentation of data

At the start of the project, data were obtained for Danish Shorthorn animals included in the *in situ* conservation program (data received 14/03/2025). The initial list comprised 191 purebred Shorthorn animals born until 31/12/2024 across 13 herds, including 159 females and 32 males, as well as 13 bulls with semen stored in the gene bank. An overview of gene bank bulls, born between 1961 and 2015, can be found online on the website of the Danish Agency for Food, Agriculture and Fisheries (https://lfst.dk/landbrug-og-planter/husdyrgenetiske-ressourcer/bestil-genmateriale). It is worth noticing that two of them have no viable semen or very little semen available, so only 11 bulls can be used in practice. Information on ongoing pregnancies were also obtained for females in the list. Information on calves born in 2025 was updated using the central livestock register website (CHR, https://chr.fvst.dk/chri/faces/frontpage). In total, the list comprised 265 animals. All pedigree information was extracted from the Danish Cattle database by SEGES Innovation, resulting in a pedigree file containing 2951 animals.

In April-May 2025, Shorthorn breeders shipped ear biopsies from 180 live animals included in the conservation program, including 34 calves born in 2025. All samples were sent to Eurofins genotyping lab (Galten, Denmark) and genotyped using a custom version of the Illumina 80K SNP beadchip (version EuroG_MDv5; Illumina, San Diego, California). Genomic data were obtained for 174 animals (received on 10/07/2025). DNA of poor quality was obtained for six animals which were therefore not genotyped. A second batch comprising cryo-preserved semen from the 11 bulls available in the gene bank was sent for genotyping in November 2025. In total, genomic information was available for 185 animals.

2.2. Description of the population structure

In 2024, 46 purebred Shorthorn calves were born and registered in the conservation program. In 2025, 70 pregnancies were registered (via insemination or natural service) resulting in 54 calves born by 30/10/2025. The distribution of cows across herds is highly uneven. In 2024, one herd accounted for 28 calves (60%), while the remaining 18 calves were spread across nine different herds. Calves born in 2024 were sired by 12 bulls, primarily through natural service. Sire usage was also uneven: one natural service bull produced 27 offspring, representing 57% of all calves born that year. The use of gene bank bulls was limited with only four calves born from three different bulls. Finally, eight calves had no declared sire.

In 2025, pregnancies involved 13 different bulls. The two Northern Dairy Shorthorn bulls contributed to 21 pregnancies in total, followed by the natural service bull (18 pregnancies) that sired 27 calves born in 2024. Gene bank bulls were used more extensively in 2025, with 15 inseminations performed with six different bulls. Overall, paternal origins of calves born in 2024 and 2025 remained dominated by a few sires, restricting the genetic diversity of candidates available for population renewal.

The generation interval of calves born in 2024-2025, i.e. the average age of parents at the birth of their offspring, was relatively high (6.55 years). This is due to the use of bulls from the gene bank born in the 1960s-1980s but also to the long productive life of some cows (up to 14 years).

2.3. Genetic diversity analysis through the lens of pedigree information

In this paragraph, we evaluated the rate at which genetic diversity is eroded in the Danish Shorthorn population using pedigree data. In this context, pedigree data is relevant because it is available for all animals and over many generations, yet with different degree of completeness. The pedigree data was used to compute inbreeding coefficients (Fped) of animals and kinships between them. The inbreeding coefficient measures the probability that two copies of a gene inherited by an individual from both parents come from the same ancestor. The closer the common ancestor(s) shared by parents in the pedigree, the higher the inbreeding. The kinship (or coancestry) coefficient evaluates the probability that two individuals have inherited the same alleles at a locus from a common ancestor. Therefore, the inbreeding coefficient of an individual is equal to the kinship coefficient between its parents. Inbred individuals are expectedly more homozygous than non-inbreds. A higher loss of genetic diversity is likely to occur when inbreeding rapidly increases in a population. Therefore, the increase in inbreeding over time – or equivalently the increase in coancestry coefficients between parents - is a relevant indicator of genetic diversity erosion.

Analyses were carried out for 221 reference individuals having sufficient pedigree information with the OptiSel R package (Wellmann, 2021). In the present study, we considered sufficient pedigree information as an animal having a pedigree completeness index (PCI) of 0.60 calculated by tracing genealogies over 6 generations back in time. Indeed, in case of no or very shallow pedigree information, inbreeding coefficients are set to zero and genetic diversity indicators are uninformative.

Using pedigree information, we also characterized breed composition as the proportion of genes inherited from founders of different breeds detected in the pedigree. The proportion of other breeds is very low in live Danish Dairy Shorthorn animals (<1%) indicating a low degree of admixture (Figure 1). Looking at the gene flow from other Shorthorn subpopulations, 10-15% of founders stem from four main foreign subpopulations (namely from Great Britain, USA, Canada and Germany) with about 3-7% of genes consistently coming from US Shorthorn founders (Figure 1). In 2025, we can notice the much higher contribution of Shorthorn founders from Great Britain due to the use of semen of Northern Dairy Shorthorn bulls.

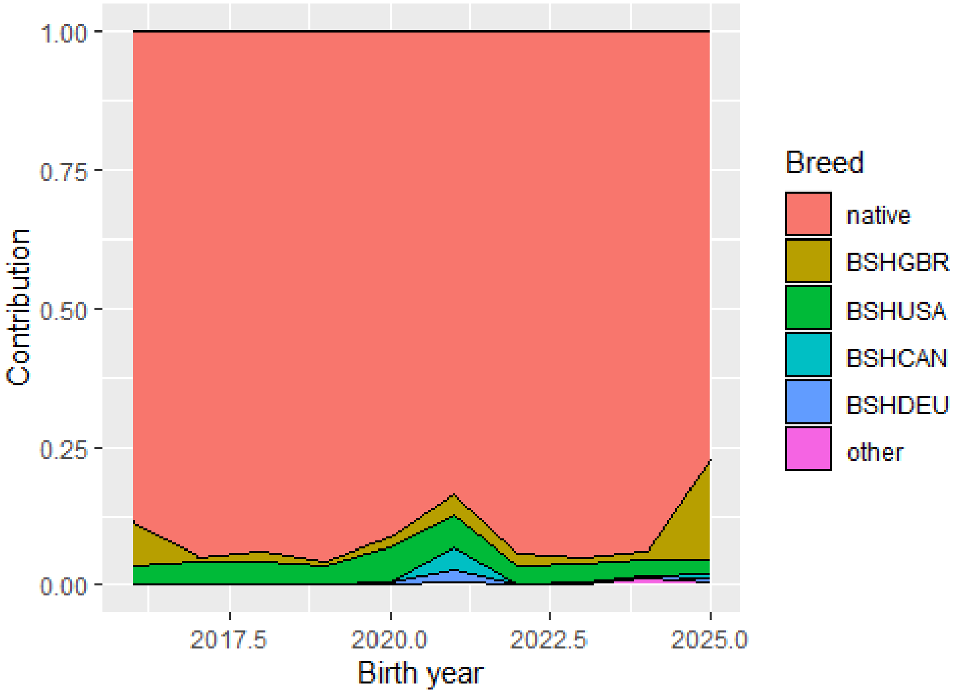

Figure 1. Founder genetic contributions of Danish Shorthorn animals born between 2015 and 2025 represented by Shorthorn country of origin (native: Denmark, BSHGBR: Great Britain, USA, BSHCAN: Canada, BSHDEU: Germany).

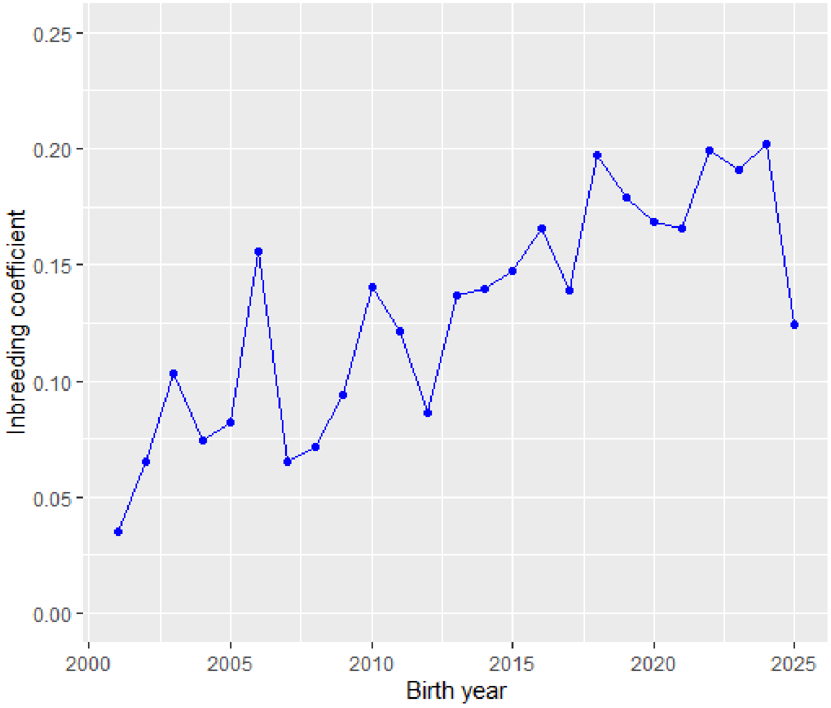

Figure 2. Inbreeding trend estimated in the Danish Dairy Shorthorn population.

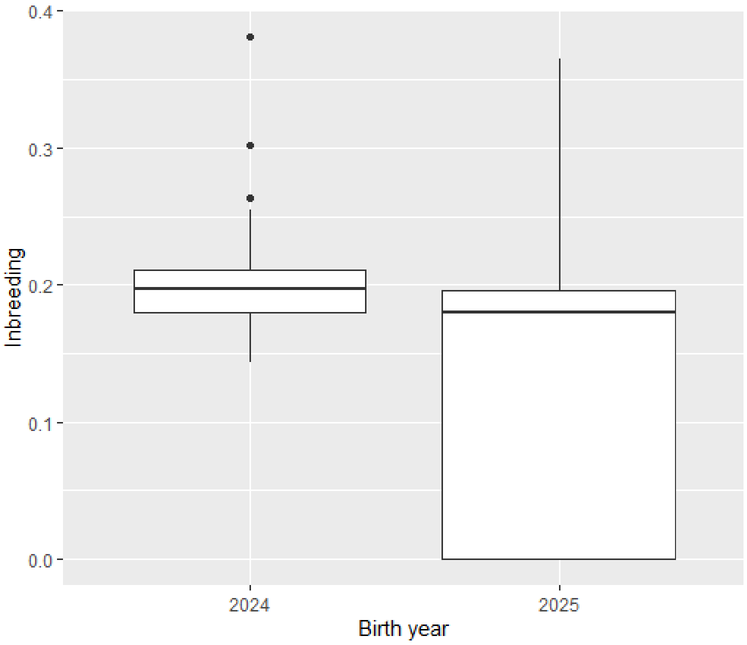

Figure 3. Distribution of inbreeding coefficients for Danish Shorthorn calves born in 2024 and 25, including calves with Northern Dairy Shorthorn sires born in 2025.

Inbreeding coefficients were estimated for the 221 animals in the reference group and their pedigree and were averaged per year of birth (Figure 2). A clear trend was observed in this population with an absolute increase in inbreeding of around 0.15 between 2001 and 2018. Inbreeding levels have stabilized around 0.17-0.20 over the last 6 years. In 2025, accounting for calves sired by Northern Dairy Shorthorn bulls, the average inbreeding coefficient was reduced from about 0.20 to 0.125. This is due to the absence of known ancestors shared among Danish and Northern Dairy Shorthorn animals: all offspring of Northern Dairy Shorthorn bulls had an inbreeding coefficient of 0 even though their pedigree was known for several generations (Figure 3).

Effective population size is a key metric for monitoring the evolution of within-population genetic diversity. It represents the size of an idealized randomly mating population that would experience the same decrease in genetic diversity as the population under study. The higher the inbreeding rate (or increase in coancestry) in the population, the lower the effective size and the higher the risk of loss in genetic diversity. The effective population size was estimated using the methodology presented by Cervantes et al. (2011) and implemented in OptiSel (Wellmann, 2021). This method is based on the rate of increase in coancestry estimated between breeding animals and accounts for their pedigree completeness to limit estimation bias (Cervantes et al., 2011).

The effective population size (N_e) of Danish Dairy Shorthorn was estimated at 16.2 individuals. According to guidelines on genetic resource preservation of the Food and Agriculture Organization of the United Nations (FAO, 2013), a population with $N_e < 50$ is considered at risk. These results indicate a rapid erosion of genetic diversity in Danish Shorthorn, as already reported by Szekeres et al (2016), and highlight the importance to provide breeders with tools designed to curb inbreeding rates.

2.4. Genetic diversity evaluated through the lens of genomic data

A description of genomic diversity has previously been carried out to map the Danish Dairy Shorthorn population among other Dairy Shorthorn subpopulations (Ravn, 2020). The use of genomic data also improves the evaluation of within-population genetic diversity as this information reflects realized genome sharing between animals, not just expected proportions. The results of this paragraph are important for the interpretation of the mating plan in the next chapter.

Genomic-based inbreeding and kinship estimators use information at SNP genetic markers to finely trace chromosome segments that are transmitted from parents to offspring across generations. In this sense, these estimators provide a more accurate picture than pedigree-based estimators, which rely solely on expected proportion of genome sharing (50% for parent-offspring, 25% for grandparent-offspring, etc). Genomic information can capture deviations from the expected genome sharing that arise due to recombination between chromosomes or the random sampling of homologous chromosomes during meiosis. Moreover, when pedigree information is missing for a given animal, pedigree-based estimators assume the individual is assumed to be unrelated to the rest of the population. In contrast, genomic data can reveal shared ancestry between individuals even when pedigree information is unavailable or incomplete. For these reasons, genomic data was essential in the current work to improve the selection of breeding animals and optimize the mating plan to preserve as much genetic diversity as possible regardless of the completeness of pedigree records.

2.4.1. Quality control of genomic data

Genomic data were obtained for 174 animals belonging to the *in situ* conservation program and 11 gene bank bulls. Two calves had parents that were not declared as purebred Shorthorn in the database and three animals born before 2025 had foreign Shorthorn sires (two animals had a Shorthorn sire from Great Britain and the third animal had a sire from the US). These animals were kept in exploratory analyses but were discarded for the constitution of the mating plan in the next chapter.

Quality control filtering was carried out with PLINK 1.9 software (Chang et al., 2015). Two animals had low call rates (77% informative SNPs). As these two individuals had several genotyped progenies, we decided to keep their genomic data, and uninformative SNPs were imputed as described below. SNP markers with low call rates (<0.90) were discarded. Finally, for further analysis, we retained SNP markers located on autosomes with minor allele frequencies higher than 0.05. Thus, genomic analyses included 185 animals having information for 34 847 SNP markers.

Parentage was checked for pairs of parent-offspring that were genotyped prior to imputation with FImpute v3 (Sargolzaei at al., 2014). One conflict was detected between genomic data of a heifer born in 2024 and her presumed dam. The heifer was compatible with her sire, that had other genotyped offspring. Her presumed dam had another genotyped offspring with which it was compatible. No other genotyped cow was compatible with this heifer. In the following, the dam of the heifer was thus listed as missing in the pedigree file. Finally, six genotyped animals had no sire declared in the pedigree file. We screened genomic data with FImpute v3 to find bulls that could be compatible among the genotyped bulls but did not find any. As for animals with foreign ancestry, we kept genomic data on these six animals in exploratory analyses but not for the constitution of the mating plan.

2.4.2. Population stratification

First, we carried out a principal component analysis (PCA) on genomic data to investigate whether clear stratifications could be detected in the Danish Shorthorn population that would indicate recent admixture events. It is particularly relevant to map animals with British ancestry to assess whether they would cluster together and be very distinct from purebred Danish Shorthorns. The PCA was carried out using PLINK 1.9 software (Chang et al., 2015) and plotted using the ggplot2 R package (Wickham 2016).

The first two principal components of the PCA explained 19% (PC1) and 13% (PC2) of the dataset variability (Figure 4). The first component clearly separated one over-represented family from the rest of the population (natural service bull on the far right of Figure 4). There was no obvious clustering of individuals sired by Northern Dairy Shorthorn bulls in any of the first PCA components. It should be noticed though that there were only six calves from the Northern Dairy shorthorn bulls, making it potentially difficult to detect subtle stratifications based on genomic data.

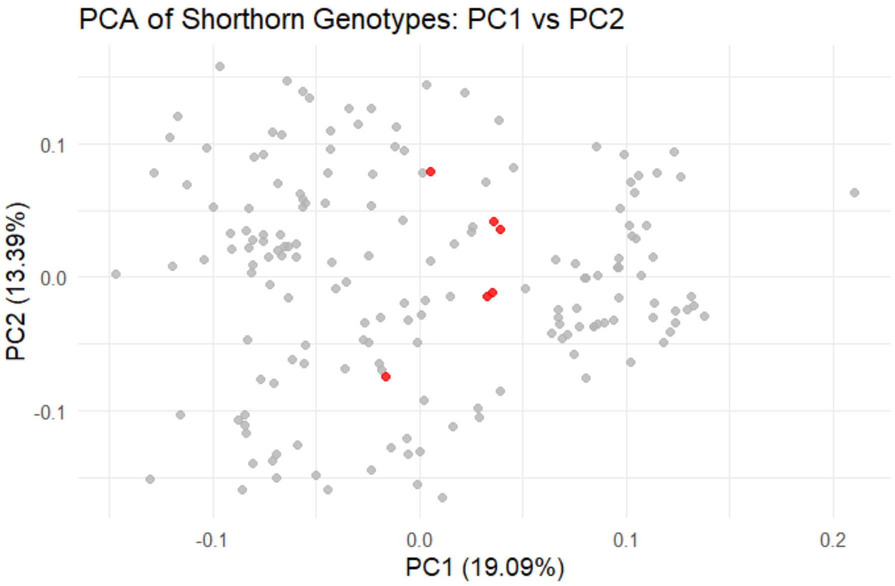

Figure 4. Representation of individuals based on the first two components (PC1, PC2) of the principal component analysis using genomic data. Animals sired by Northern Dairy Shorthorn sires are highlighted in red.

2.4.3. Estimation of inbreeding and relatedness between individuals

The estimation of inbreeding and relationship coefficients was performed using two homozygosity-based measures explained below that are relevant for inbreeding management in small populations (Meuwissen et al., 2020). Other estimators were investigated but not included in this report as they produced misleading inbreeding trends using this dataset and were shown to be less efficient in controlling the rise in homozygosity through Optimal Contribution Selection (Meuwissen et al., 2020).

The first estimator is based on excess-of-homozygosity (termed Hom afterwards) and was computed using method 1 in Van Raden (2008) considering allele frequencies of 0.5 for all SNP markers. This estimator interprets any excess of homozygosity compared to a fully outbred base population as inbreeding. Computations were carried out with the BLUPF90 program (Misztal et al., 2014). This program builds a full genomic relationship matrix that contains both inbreeding coefficients of each animal (1+Fhom on diagonal elements) and relationship coefficients between genotyped animals (off-diagonal elements). It also automatically rescales genomic inbreeding coefficients so that their average matches mean pedigree inbreeding coefficient of genotyped animals.

The second estimator is based on the detection of runs-of-homozygosity (ROH). Runs of homozygosity are long chunks of genomes that are completely homozygous. It is expected that inbred animals carry more ROHs due to inheritance of identical by descent (IBD) segments from parents having a common ancestor. The closer the inbreeding event in the pedigree, the longer the ROH because of lower probability of breakage of this segment by recombination. In contrast, short ROHs have gone through multiple recombination events over generations and are indicative of ancient inbreeding events (Kirin et al., 2010).

Compared to the previous method, the use of ROHs allows the separation of recent from ancient inbreeding events, the former being more problematic than the latter due to a higher probability to carry deleterious mutations not purged yet by natural selection. Runs of homozygosity were detected using --homozyg option in PLINK 1.9 (Chang et al., 2015). Following Meyermans et al. (2020) and Tenhunen et al. (2024), we used the following parameters for ROH detection:
a ROH has 1) a minimal length of 1 Mb, 2) contains at least 35 SNP markers without any gap of more than 500kb between 2 consecutive SNPs, 3) at least 1 SNP per 100kb on average, and 4) the genome was scanned considering a sliding window of 20 SNPs. Inbreeding coefficients (Froh) were then estimated as the sum of all detected ROH segments divided by the genome length. A similar approach was used for estimating relationship coefficients between animals using pairwise shared segments (IBD). An IBD segment analysis determines all IBD segments shared between relatives from which relatedness can be inferred. Analyses were performed with the KING 2.3.1 software (Manichaikul et al., 2010) using a segment length of 1Mb for the detection to be consistent with the ROH analysis. Relationship coefficients based on shared segments will hereafter be mentioned as “KING” estimates.

The average pedigree-based inbreeding coefficient of genotyped animals was 0.17±0.07. Inbreeding coefficients estimated based on ROHs was higher (0.23±0.06) than Fped and Fhom (0.17±0.07). The correlation between pedigree and genomic inbreeding coefficients was moderate to high: 0.60 between Fped and Fhom and 0.71 between Fped and Froh (Figures 1 and 2 in the Annex). These correlations indicate that both pedigree and genomic inbreeding estimators can adequately identify animals with high and low inbreeding coefficients and are consistent with already published studies. However, many re-rankings were observed between estimators for intermediate inbreeding values. As expected, we can see great variation in genomic inbreeding coefficients among animals having null pedigree inbreeding coefficients due to no or limiting pedigree information showing the importance of genomic data (Figures 1 and 2 in the Annex).

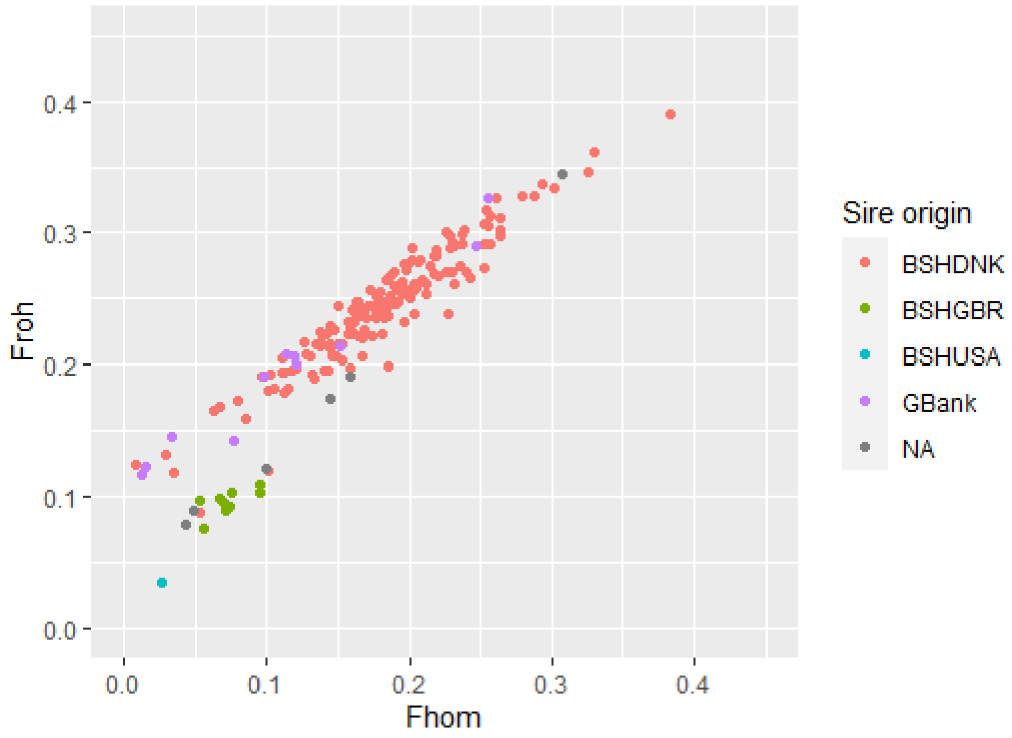

Figure 5. Comparison of genomic inbreeding estimators based on homozygosity (Fhom) or runs of homozygosity (Froh) depending on the sire origin (BSHDNK: Danish Shorthorn, BSHGBR: British Shorthorn, BSHUSA: US Shorthorn, NA: unknown sire). Gene bank bulls (GBank) were represented as purple dots.

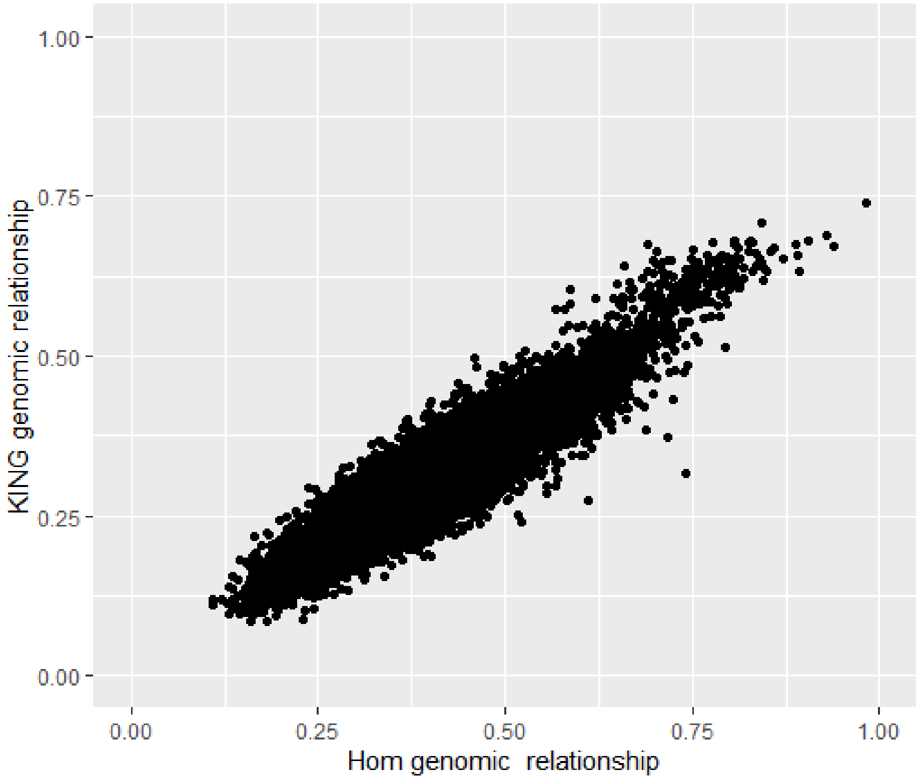

Figure 6. Comparison of genomic relationship estimates based on excess of homozygosity (Fhom) or IBD segment sharing (KING).

The correlation between genomic inbreeding coefficients Fhom and Froh was high (0.94). Both genomic inbreeding estimators ranked purebred Danish Shorthorn animals in a similar way (Figure 5). Interestingly, animals with a foreign sire constituted a separate group with lower Froh values for similar Fhom. Indeed, these animals systematically carry much shorter ROHs which suggests that common ancestors to Danish and foreign Shorthorn subpopulations trace far back in time. So, even with similar Fhom estimates, the distribution of homozygous SNPs is different along the genome in purebred Danish Shorthorns and animals with a foreign sire. This slightly nuances the PCA results that did not point out a clear stratification between purebred Danish Shorthorns and calves sired by Northern Dairy bulls. Finally, most gene bank bulls displayed lower genomic inbreeding than live animals. Only two of these bulls (born in 2000 and 2007) had rather high inbreeding coefficients (Figure 5).

Genomic relationship coefficients were also very highly correlated with each other (0.93, Figure 6) and highly correlated with pedigree estimates (0.77-0.78). However, KING relationship estimates were lower and less dispersed than homozygosity-based measures. Furthermore, there was still substantial variation in KING relationships for a given level of Hom relationship. This is likely because only shared homozygous segments (>1Mb) are accounted for in the estimation of KING relationships, whereas all SNPs are accounted for in the relationship calculation based on excess of homozygosity.

2.5. Added value of gene bank bulls to the population diversity

Semen cryo-preserved in the gene bank is crucial to the conservation program because it makes it possible to restore genetic diversity that has been lost over time. Therefore, we performed a more detailed analysis of genetic diversity within gene bank bulls to refine the choice of candidate bull calves that would best complement them. We calculated the genetic contributions and kinship coefficients of gene bank bulls to live females to determine whether some of these bulls should be used in priority to bring in genetic diversity. In the following figures, we present genomic kinship coefficients obtained with the Hom estimator as these coefficients were scaled to match pedigree-based coefficients, making the comparison easier. However, results obtained with the KING estimator led to similar patterns.

The representation of genetic contributions from gene bank bulls to the live population offers a contrasted picture (Figure 7). Interestingly, the representation of pedigree and genomic kinship coefficients of the same bulls with live animals is much more homogeneous (Figure 8 and 9). Four gene bank bulls have pervasive genetic contributions to most live animals in the conservation program and are amongst the five major ancestors in the breed (Figure 7), with genetic contributions higher than 20% (Nordt Håls, Thy Kløv, Nordt Finn, Thy Krœn). These gene bank bulls are closely related to each other; for example, Thy Kløv is the sire of Nordt Finn and grand sire to Thy Krœn. So, these contributions are partly redundant – the genetic contribution of Nordt Finn is embedded in the one of Thy Kløv - and do not sum up to 1. However, because they are distant ancestors, bulls like Nordt Håls and Thy Kløv are less related with live females than more recent bulls.

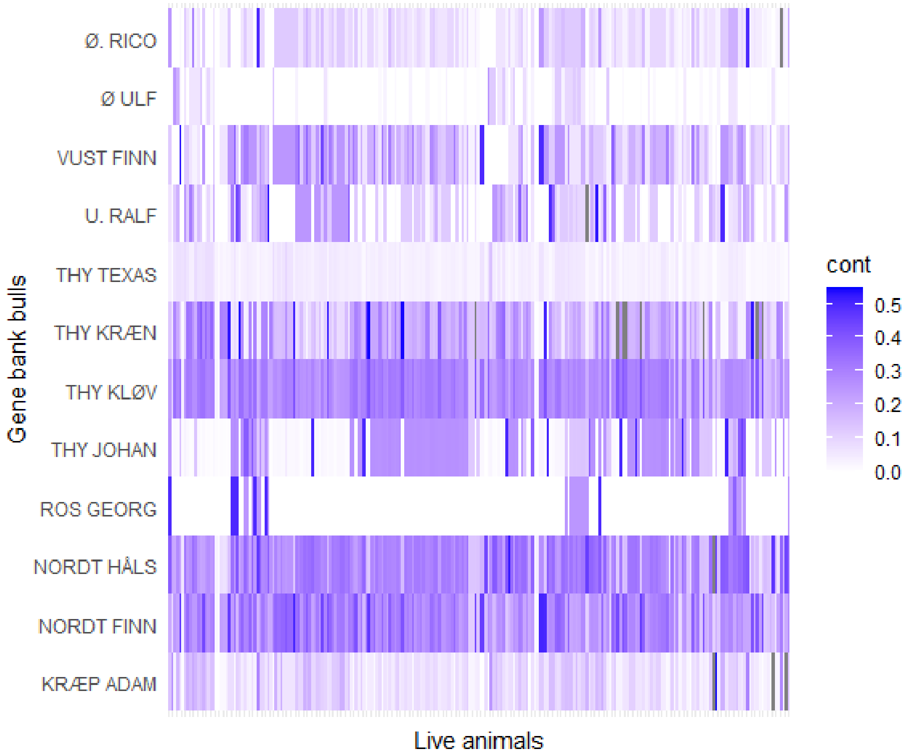

Figure 7. Genetic contributions (cont) of bulls with semen stored in the gene bank to the current population

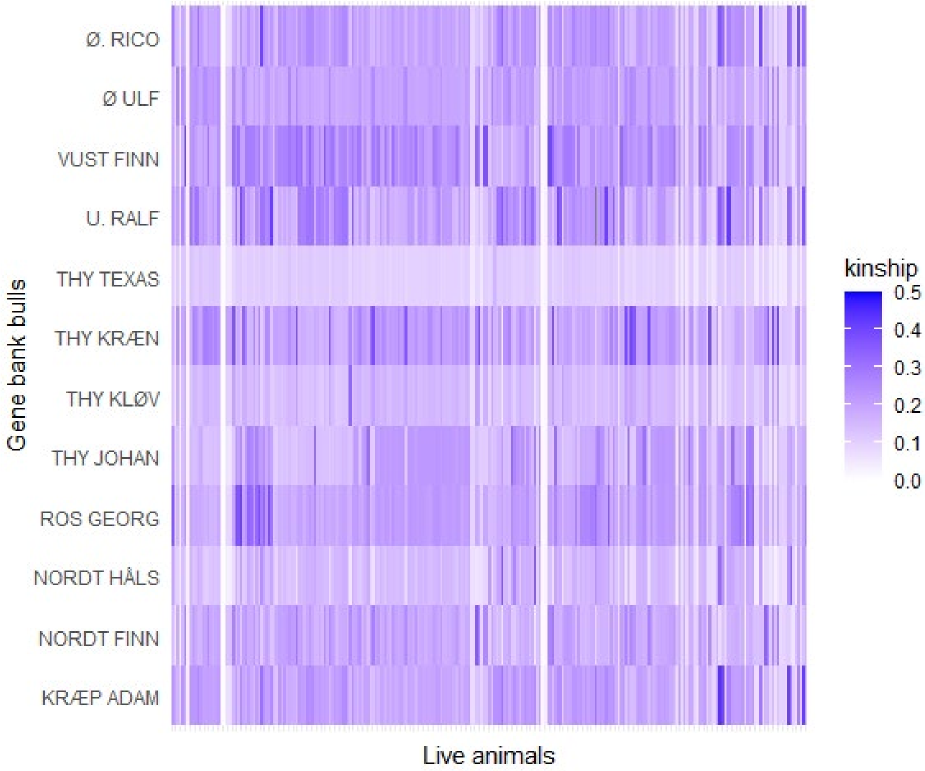

Figure 8. Pedigree kinship coefficients estimated between the bulls with semen available in the gene bank and live animals in the conservation program

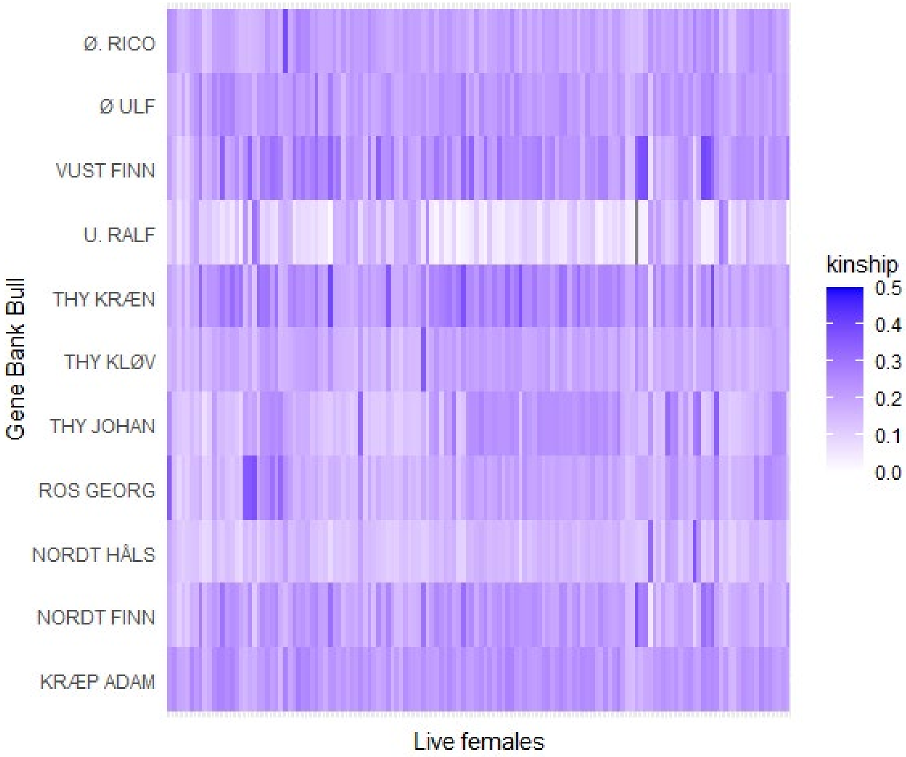

Figure 9. Genomic kinship coefficients (Homozygosity-based) estimated between the bulls with semen available in the gene bank and live females in the conservation program

The youngest bull in the gene bank (Ros Georg) has a low direct genetic contribution to the current population. Despite this, it is moderately to highly related to most females in the population. Indeed, this bull has two half-sibs that were used as natural service bulls and cumulated many offspring born in 2023-25.

A few bulls stand out:

- The bull Thy Texas has low genetic contribution and low pedigree kinship to the current population. This bull was not genotyped due to the low number of semen straws stored in the gene bank. However, we expect that its genomic kinship to other Danish animals will be low as his sire was US Shorthorn. A targeted use of the remaining straws could be of high interest to restore genetic diversity in the Danish Shorthorn population. To do so, it is critical to coordinate with breeders to find out which females would be optimal mates to Thy Texas to maximize diversity relative to the current population.
- The bull U. Ralf has moderate genetic contributions to the live population. Genomic data revealed lower genomic kinship with most females, suggesting that his offspring will be more heterozygous than expected based on pedigree information.

Similar conclusions can be drawn for natural service bulls. Most of them are on average moderately to highly related to many females. However, genomic kinship estimators tend to reveal slightly more contrasted results than pedigree-based estimates (Figure 5 in the Annex).

In conclusion,

- The Danish Dairy Shorthorn breed is a small population with a fast decline in genetic diversity.
- The unbalanced bull usage and limited genetic origins of bulls in the gene bank created bottlenecks.
- Overuse of some natural service bulls highly related to the gene bank bulls reduces the interest of the latter to restore genetic diversity.
- The genomic inbreeding coefficients explored here (Fhom and Froh) agree well with each other in ranking purebred Danish Shorthorn cattle. The ROH estimator tends to give lower levels of inbreeding for animals with foreign ancestry due to shorter ROHs.
- Genomic data is valuable to estimate realized inbreeding and kinship (relationship) coefficients of gene bank bulls with females. A few animals were more diverse than expected according to pedigree information.

3 Mating plan using optimal contribution selection and genomic information

3.1. Description of the analysis pipeline

Optimal contribution selection (OCS) is a methodology that aims to optimize the selection of breeding individuals in a population and their use (or genetic contribution) to get the best compromise between selection response and diversity maintenance. In a conservation program, this approach can be used to minimize the increase in inbreeding in the next generation as there is no aim for genetic improvement. Since its development, OCS has become a gold standard approach to preserve genetic diversity in small populations by ensuring that coancestry between selected parents is minimized both between and within sexes. In standard OCS programs, the optimization process is carried out assuming random mating between males and females. The inbreeding level achieved in the next generation can be further reduced by optimizing matings between males and females using their optimal contributions as guidelines.

Using genomic information in OCS can be a gamechanger for inbreeding management through more accurate breeding decisions and mating plans (Meuwissen et al., 2020). Indeed, genomic data offers several benefits compared to pedigrees:

- It can be used to validate parentage
- It gives a fine picture of realized inbreeding and kinship coefficients which is particularly interesting for animals with shallow pedigree information or foreign ancestry.

While genomic data are routinely used in mainstream breeding programs, this is not the case for most conservation programs. In this section we will describe our analysis pipeline and present the results with respect to the optimized mating plan. In the next paragraph, we will describe how we incorporated genomic information in OCS along with parameters and constraints considered in the optimization

The pipeline is made up of two main elements:

1. A shell script to manipulate genomic data and build genomic relationship matrices. This workflow was presented in paragraph 2.4. Genomic data are read and filtered according to quality control criteria (detailed in 2.4.1). Parentage relationships are verified, and genomic matrices are built. In the present study, we ran OCS with two genomic estimators presented in 2.4 to evaluate their impact on the mating recommendations
2. An R script that reads the genomic relationship matrix, combines this information with pedigree data and prepares input data for the OCS step carried out with the OptiSel R package (Wellmann, 2021). This R script then uses outcomes from the OCS step to produce an optimized mating plan and check for interesting bull calves to retain in the conservation program.

We focused on available software that can perform OCS analyses. As a preliminary step, we benchmarked EVA (Henryon et al., 2015) and the OptiSel R package (Wellmann, 2021). Programs differ in their structure and execution mode, but recommendations were similar. So, we decided to use OptiSel in this pipeline to facilitate its integration in the R script.

3.2. Data and parameters used in the optimization

The program OptiSel needs different elements to perform the OCS analyses:

- A list of candidates that are available for breeding to produce the next generation. For this case study, we considered all active breeding males and females born until 2024 that belonged to the conservation program. We also included the 12 bulls with semen available in the gene bank. We excluded candidates declared as crossbreds in the database and with unknown or foreign parent to prevent the OCS algorithm from picking these individuals that are theoretically more diverse but not of direct interest for the conservation program.
    - 129 females were candidates and allocated up to 1 mating each. We aimed to select 75 of these females for establishing the mating plan to be close the number of matings recorded in 2025.
    - 22 natural service bulls were allowed up to 10 matings each.
    - For gene bank bulls we allocated up to 10 matings to bulls with no semen restrictions, 5 matings for the bull Kræp Adam due to limited number of doses, and 3 matings to older bulls subjected to potential restrictions (sanitary or limited number of straws).
    - There was no constraint on the total number of bulls to select or on the distribution between natural service and insemination to produce the 75 matings.
- A pedigree file including parents of all candidates and tracing all ancestors back in time. The program uses birth year to evaluate generation intervals. We also included all offspring of candidates, that are not candidates themselves, to consider that some candidates might already have had some contributions to the population (calves born in 2025).
- A relationship matrix that was built
    - Using pedigree information and the pedIBD() function in OptiSel
    - When incorporating genomic information in the OCS, we replaced elements of the pedigree-based relationship matrix with genomic inbreeding and relationship estimates for the genotyped individuals

First, we performed OCS using only pedigree information. Then, we performed OCS with the same list of candidates but an updated relationship matrix including genomic-based inbreeding and relationship estimates for genotyped candidates as explained above. Optimal contribution contributions were calculated assuming that matings were carried out at random. Based on these recommendations, we established an optimized mating plan that will further minimize expected inbreeding in the next generation using the matings() function in OptiSel (Wellmann, 2021).

3.3. Results

The selection of bulls and their optimal number of matings were relatively consistent irrespective of the relationship matrix used in the OCS. Eighteen bulls were selected in each scenario with a relatively balanced number of matings (Table 1).

Table 1. Number of matings recommended by OCS for bull candidates calculated using pedigree-based and genomic relationship matrices (bulls with names are gene bank bulls).

			Genomic relationship	
		Pedigree relationship	**Homozygosity based**	**ROH-KING**
Bull ID	**Bull name**	**N matings**	**N matings**	**N mating**
1277800593		6	5	6
5291001694	ROS GEORG	6	6	6
5743500975		6	1	5
5743501042		6	6	0
8048700128	Ø ULF	6	6	5
8048701400		6	5	6
8048700073	Ø RICO	6	6	6
9207400511		6	6	1
4587406397		5	5	6
5743501023		5	6	6
6878500024	KRÆP ADAM	3	1	3
8887300018	U RALF	3	6	5
198604057	NORDT HÅLS	2	2	2
6293300074	THY KLØV	2	2	2
6293300076	NORDT FINN	2	2	2
6293300230	THY KRÆN	2	0	0
6293300249	THY TEXAS	2	2	2
6293300228	THY JOHAN	1	2	2
9207400380		0	6	6
8887300054	VUST FINN	0	0	4

The OCS framework tended to equalize bull contributions over several bulls, with at most six matings allocated to bulls while the upper limit was set to 10. Interestingly, most gene bank bulls were allocated matings suggesting that they are still of value to restore genetic diversity in this population. Older bulls were constrained in the optimization process to three matings due to potential restrictions for usage. Their optimal contributions might have been higher if the number of allowed matings was increased to 10.

It is worth noticing that the gene bank bull U Ralf and his son (92074-00380) are strongly promoted in genomic OCS calculations as these animals were shown to be more diverse with genomic data than expected according to pedigree.

Selection of the 75 dams was also largely consistent between pedigree and genomic selection with few differences observed. While two sisters may be selected based on pedigree, genomic OCS will in most cases select one sister over another based on their realized relationship to the sires. This shows how pedigree OCS captures the main population structure while genomic OCS can refine selection decisions.

When looking at the optimized mating plan using only purebred Danish animals, the average inbreeding expected in prospective offspring was 0.16 when estimated with pedigree and 0.13 when estimated with Fhom estimator. This is much lower compared to the current inbreeding level of 0.20 for animals born in 2024 (Figure 2). The mean kinship between suggested parents in the optimized mating plan was 0.11 using the IBD sharing approach to be compared to genomic inbreeding estimated from ROH of 0.23. By following the optimized mating plan, we expect that future offspring will harbor fewer long ROHs (>1Mb).

Overall, the results of the two methods agree well on the selection of breeding animals, and differences could be explained by results presented in paragraph 2.5. Through the observed differences, the pedigree-based optimization was shown to put more focus on the differences between “families”. The pedigree OCS would view full-siblings as interchangeable. While genomic optimization showed greater focus on the differences within families and between individuals. These results are likely due to the use of realized kinship coefficients between closely related individuals that the pedigree cannot describe. Slight differences between the two genomic OCS can be explained by the fact that the OCS based on KING genomic relationships aims to minimize the length of ROHs while the OCS based on excess-of-homozygosity estimator aims to minimize genome-wide homozygosity. Meuwissen et al. (2020) observed in their simulations that KING-based OCS led to more breeding animals being selected. This was not observed here, but the number of bulls to choose from was rather limited.

In conclusion, the final choice of the genomic estimator to be used in practice depends on the primary focus of the conservation program: maximizing heterozygosity or minimizing the risk of inbreeding depression. In our case study, both investigated genomic estimators led to consistent selection decisions and number of matings.

4 Choice of bull calves to complement the gene bank

When importing semen from two bulls from Northern Dairy Shorthorn, it was decided to store semen from one or two of their offspring to expand the genetic diversity of the Danish Dairy Shorthorn gene bank. To find out which bull calves would complement the current gene bank best, we used genomic data to estimate kinship coefficients with live females and bulls in the gene bank. Genomic data were more relevant than pedigree information because Northern Dairy bulls do not share any known ancestors with Danish animals. Genomic data were prepared using the pipeline described in chapters 2 and 3.

Five bull calves with a Northern Dairy sire were genotyped. The bull calf 57435-1077 was consistently less related to both females and gene bank bulls (Table 2). Looking at detailed kinship coefficients, this bull calf would generate offspring with very low inbreeding with most cows (Figure 10). Other bull calves were more related to the female population because they all shared the same maternal grandsire that has a high genetic contribution to the current female population. As expected, running the OCS pipeline by allowing these bull calves a high number of matings (50 each) promoted a large use of the bull calf 57435-1077 and a minor use of all others.

Table 2. Average genomic kinship coefficients (KING based on IBD segment sharing and Hom based on excess of homozygosity) of genotyped bull calves with live females and gene bank bulls.

		Kinship with females		**Kinship with gene bank bulls**	
Bull calf	**Sire**	**KING**	**Hom**	**KING**	**Hom**
5743501077	BSHGBRM000206896300003	0.091	0.120	0.096	0.118
5743501070	BSHGBRM000206896300003	0.099	0.136	0.097	0.122
5743501072	BSHGBRM000372121600021	0.101	0.141	0.117	0.145
5743501076	BSHGBRM000372121600021	0.102	0.152	0.101	0.139
5743501068	BSHGBRM000206896300003	0.11	0.159	0.109	0.143

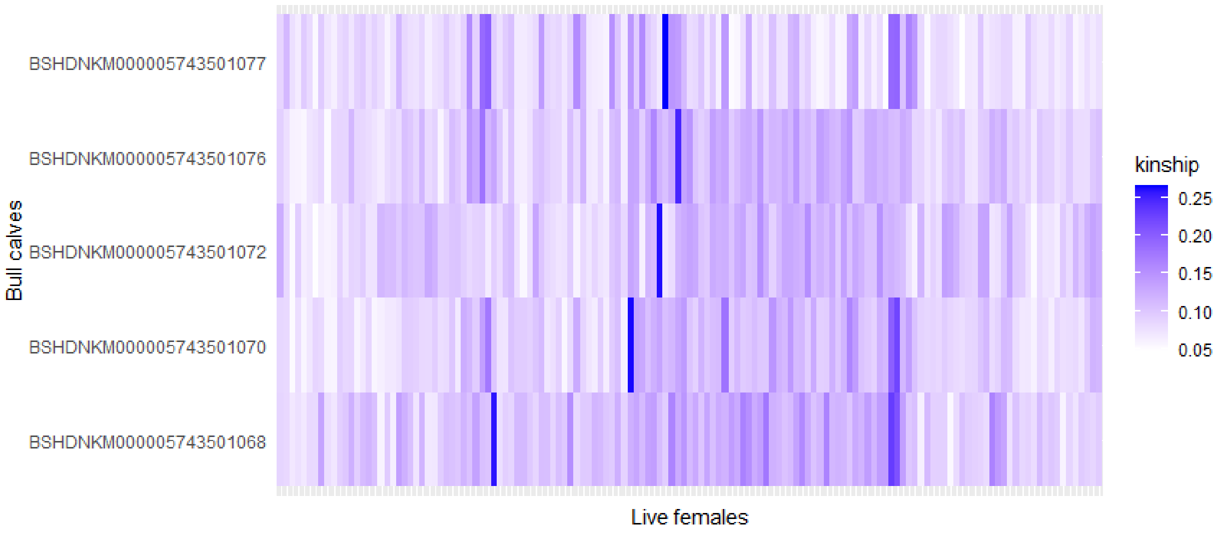

Figure 10. Genomic kinship coefficients between bull calves sired with Northern Dairy Shorthorn bulls and live females.

Discussion and final recommendations

The Danish Dairy Shorthorn is a small livestock population characterized by a rapid decline in genetic diversity. Most available natural service bulls are closely related to the gene bank lineages, which themselves form a narrow genetic base, highlighting the need for mating strategies that introduce novel genetic variation.

The last initiative was to use semen of two bulls from the Northern Dairy Shorthorn to rejuvenate the Danish gene pool by increasing heterozygosity and breaking down long runs of homozygosity, thereby reducing the risk of inbreeding depression. Calves sired by these two bulls are already born. Using genomic data, we were able to identify one bull calf with low kinship to both the male and female populations, making it a strong candidate for inclusion in the gene bank. We should underscore here that, for such strategic initiatives for the Danish Shorthorn conservation program, it is crucial to guide breeders in finding a panel of cows with sufficiently diverse origins to produce offspring that will be of interest for conservation. Indeed, in the first batch of genotypes, four out of five bull calves sired by a Northern Dairy Shorthorn bull had the same maternal grandsire that already had a high contribution to the current population. Additional bull calves sired by Northern Dairy Shorthorn sires were born over the last 6 months. Samples were collected and are presently being genotyped to identify another candidate for the gene bank.

In addition to introducing new bulls to the gene bank, the strategic use of the current gene bank bulls still exists. For example, the bull Thy Texas has hardly been used due to low number of straws (8). This bull was not genotyped but we expect his progeny to be little related to the rest of the population and, hence, to be very valuable for the conservation program. Likewise, genomic data revealed that the bull U. Ralf and his (grand)sons were less related to live females than expected based on pedigree information. Putting more emphasis on this family will help limit the accumulation of inbreeding in this breed.

Effective management of inbreeding in small populations is challenging, especially when herd sizes are unbalanced and breeding decisions are made independently at the herd level, which can be suboptimal for maintaining genetic diversity at the population scale. For example, the overuse of a natural service bull in a large herd has reduced the value of related gene bank bulls for the whole population. In this context, Optimal Contribution Selection (OCS) offers a powerful framework to regulate the use of breeding animals by retaining animals that minimize genetic relationships both within and between sexes. The pipeline developed in this project can be routinely implemented in the Danish Dairy Shorthorn conservation program and adapted for other breeds, provided dedicated personnel is available to perform analyses, validate results, coordinate with breeders, and centralize the decision-making process.

In this study, a maximum of 10 matings per bull was imposed, with lower limits set for older gene bank bulls due to sanitary constraints or limited semen availability. While we expected to select 10–12 bulls as in current practice, the OCS pipeline recommended a broader set of bulls, particularly older gene bank animals that reached their allowable usage. Accordingly, it is important that these results and the current practices are discussed within the conservation committee and with breeders to adjust constraints, so they reflect current practices.

Overall, selection decisions based on pedigree and genomic OCS were largely consistent, although genomic information revealed deviations from expected pedigree relationships in a few cases. Genomic data are especially valuable in situations where pedigree data are incomplete to ensure correct parentage, but also for putting more emphasis on within-family selection. We compared two estimators of genomic inbreeding and kinship. While they slightly differed in mean level due to different modes of calculation, they were very highly correlated with each other. Two important goals of conservation programs are to maintain diversity (heterozygosity) and to limit the risk of inbreeding depression. Meuwissen et al. (2020) showed by simulation that both investigated estimators should adequately reach these goals when incorporated in genomic OCS. They mentioned that the genomic matrix built with ROH and KING estimators might better reflect identical-by-descent sharing than the homozygosity-based approach and, thus, be more effective for inbreeding management. Based on these simulations, we could advise the use of ROH and IBD segment sharing (KING) methodology. However, in our case study, the choice of the genomic estimator only led to minor differences in the OCS outcomes.

Existing license-free OCS tools such as OptiSel (Wellmann, 2021) and EVA (Henryon et al., 2015) estimate genetic contributions assuming random mating and then produce an optimized mating plan considering all possible matings. In practice, some of these matings are not possible due to external constraints (e.g., a natural service bull is only used in one herd). Therefore, a ranked list of alternative matings can be edited for each cow by the data analysis pipeline. This list can be used in case the optimal mating recommendations cannot be followed. If deviations to the optimized mating plan occur due to on-farm constraints, genetic contributions estimated will be updated at the next time step because OCS dynamically adapts to the data structure. However, it would be desirable to get new features implemented in OCS tools to better account for practical constraints and facilitate implementation.

Finally, this report underscores the critical importance of effective cross-herd coordination in conservation programs to ensure that all actions and breeding decisions align with the overarching objective of optimally preserving genetic diversity In this context, genomic data are valuable as they help to reduce the risk of suboptimal or misguided decisions. However, their effective use requires robust data management and careful handling of parentage inconsistencies. Moreover, genotyping the entire population represents a substantial initial investment, which may be a limiting factor in small populations unless being financially supported. Once this initial effort has been completed, the annual cost of genotyping newborn calves becomes considerably more manageable. It would therefore be worthwhile to explore different funding schemes to support breeders in adopting genotyping, given the expected long-term benefits for genetic diversity conservation.

Conclusion

This work confirmed that the Danish Shorthorn population has a narrow genetic base and is experiencing a rapid accumulation of inbreeding. Genotyping the entire population, including bulls stored in the gene bank, proved highly valuable as it enabled a more accurate assessment of each animal's contribution to the genetic diversity of the population. In particular, several gene bank bulls showed greater conservation value than previously expected based on pedigree data alone and should therefore be used more extensively. This work also highlighted the need for coordinated breeding decisions at the population level to ensure that all decisions align with the goal of optimally preserving genetic diversity.

In this project, a framework combining genotyping and Optimal Contribution Selection has been developed to help breeders manage inbreeding and thereby enhance genetic diversity preservation. Incorporating genomic data further improved the accuracy of mating recommendations by considering realized, and not just expected genomic sharing. Mating recommendations have been discussed for bulls in this report and will be presented to Shorthorn breeders. While the full mating plan was not included in the report, it can be provided upon request. Given the clear benefits expected for managing inbreeding in the Shorthorn population, we recommend to use this framework in other populations included in the conservation program. The pipeline developed in this project can be readily adapted to their specific needs and characteristics.

Finally, genomic data were instrumental in identifying bull calves sired by Northern Dairy Shorthorn bulls whose semen should be stored in the national gene bank. We identified one strong candidate that will significantly contribute to enriching genetic diversity. Additional recently born bull calves are currently being genotyped to identify further promising candidates.

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Annex

Comparison of estimators of inbreeding and kinship coefficients

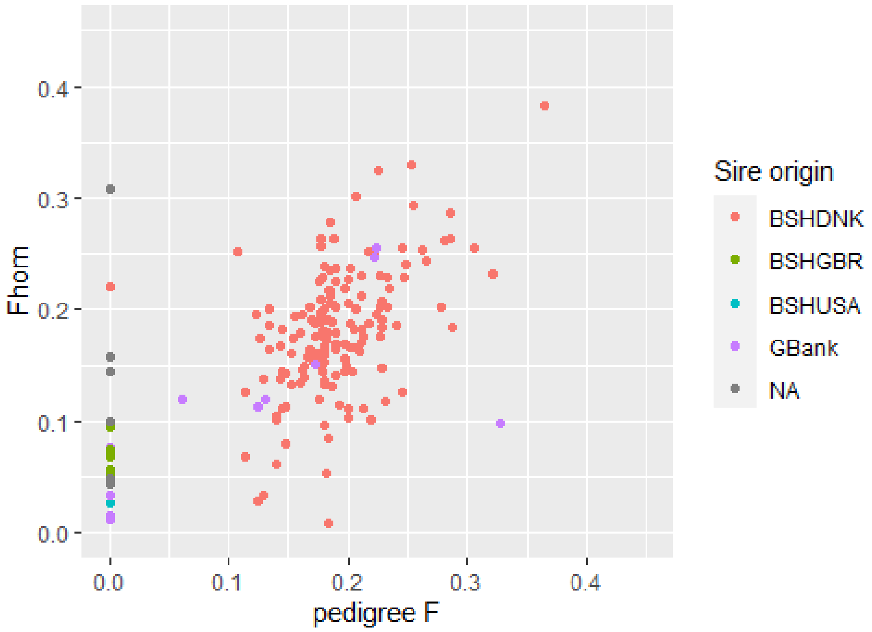

Figure 1. Comparison of genomic inbreeding estimators based on homozygosity (Fhom) and pedigree inbreeding coefficients depending on the sire origin (BSHDNK: Danish Shorthorn, BSHGBR: British Shorthorn, BSHUSA: US Shorthorn, NA: unknown sire). Gene bank bulls (GBank) were represented as purple dots.

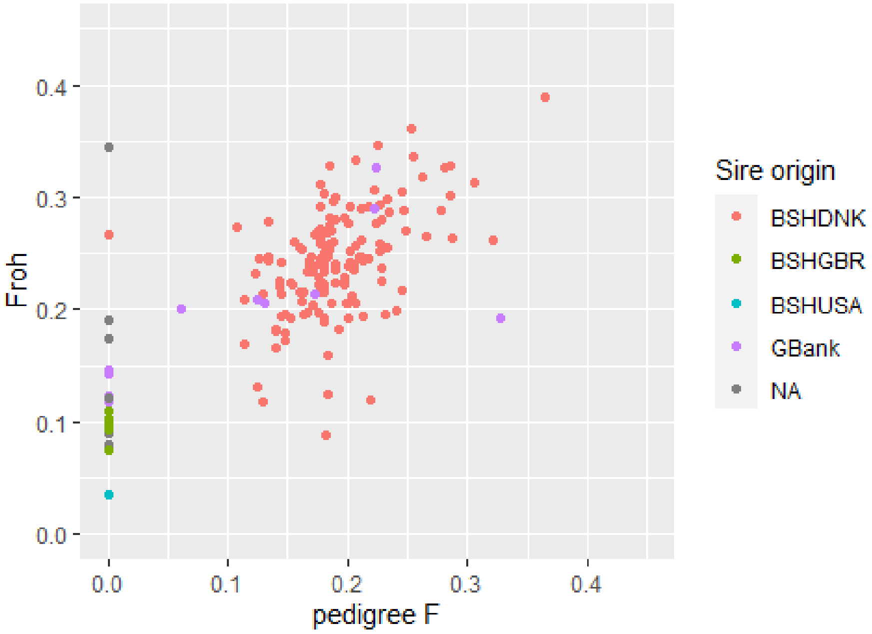

Figure 2. Comparison of genomic inbreeding estimators based on runs-of-homozygosity (Froh) and pedigree inbreeding coefficients depending on the sire origin (BSHDNK: Danish Shorthorn, BSHGBR: British Shorthorn, BSHUSA: US Shorthorn, NA: unknown sire). Gene bank bulls (GBank) were represented as purple dots.

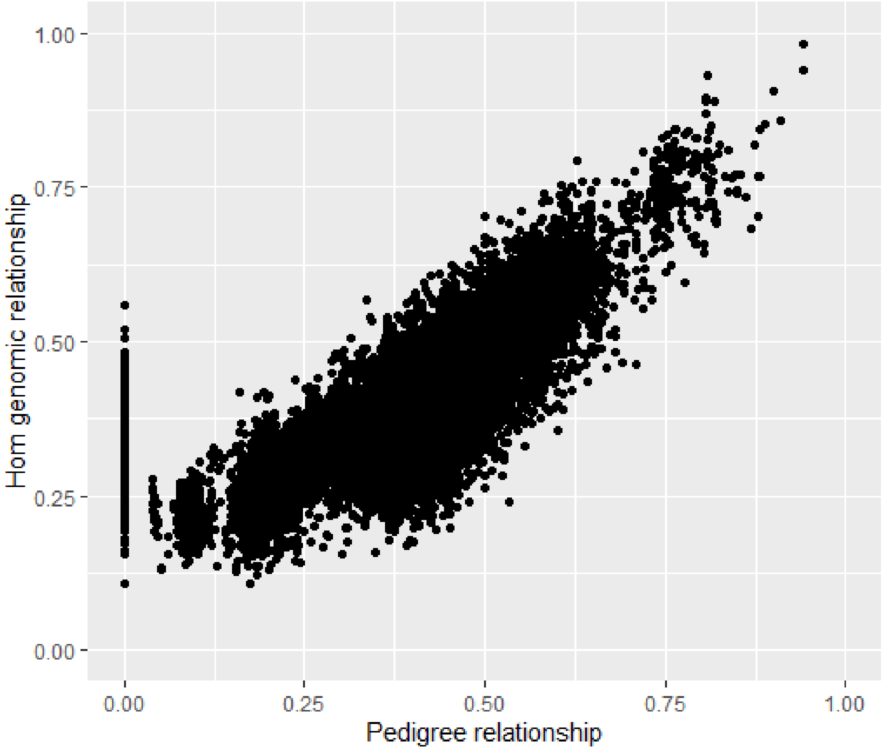

Figure 3. Comparison of genomic relationship estimator (homozygosity-based) and pedigree relationship coefficients.

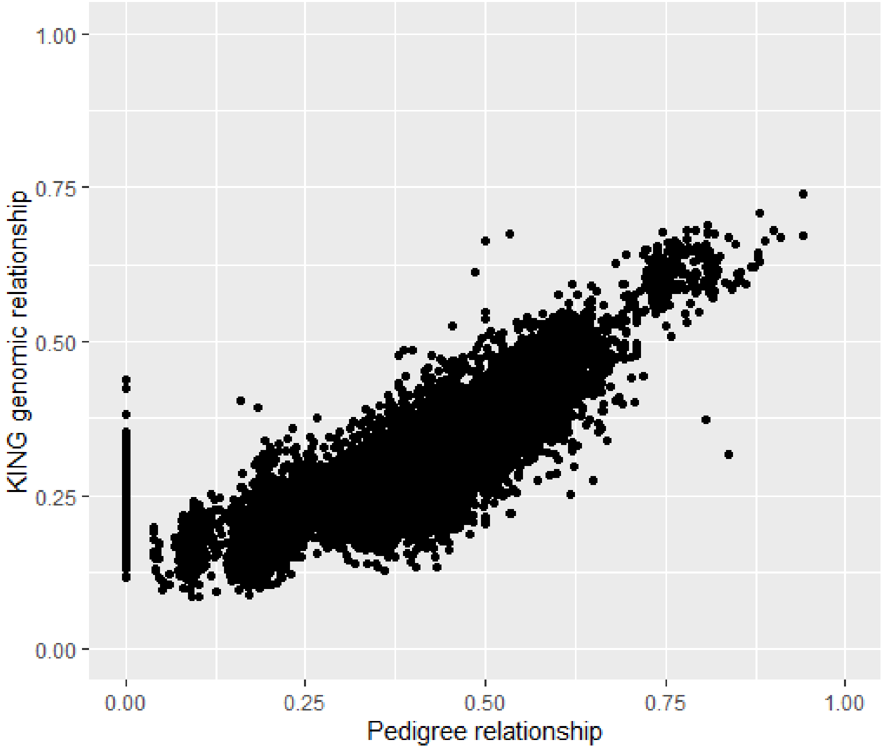

Figure 4. Comparison of genomic relationship estimator (KING IBD segment sharing) and pedigree relationship coefficients.

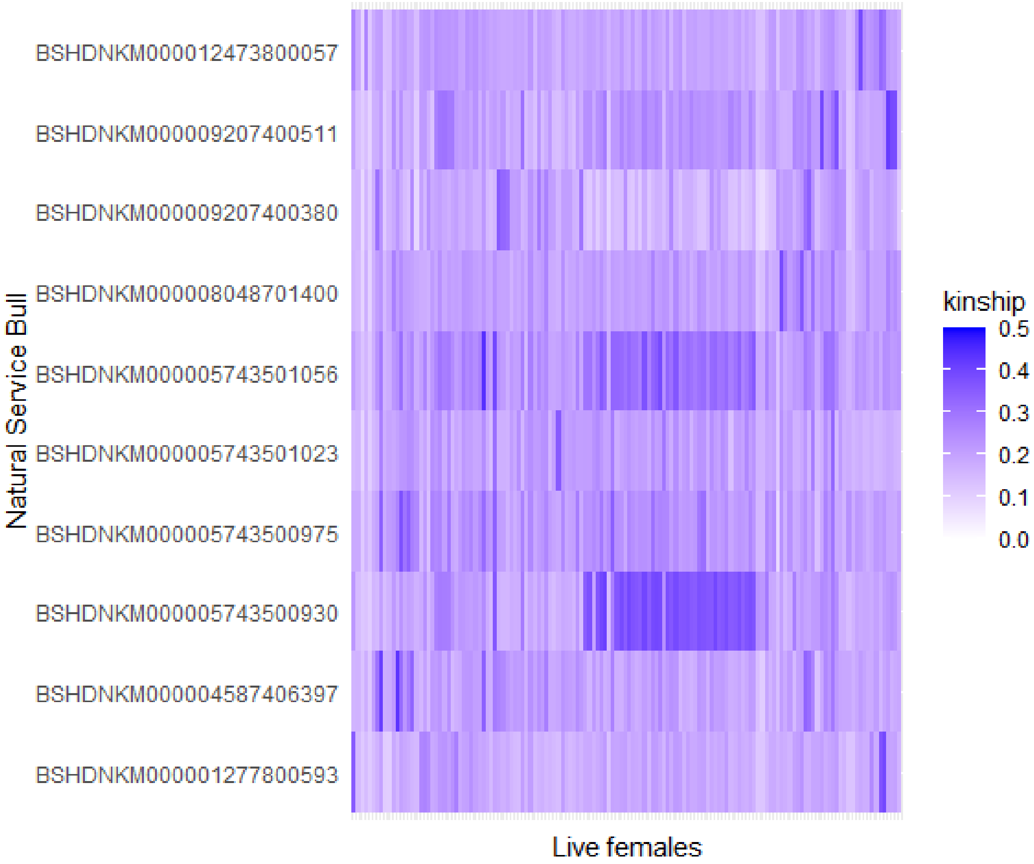

Figure 5. Genomic kinship coefficients (Homozygosity based) estimated between natural service bulls and live females in the conservation program.