Use of old cryopreserved bull semen for genetic variability management in the dairy cattle breed Abondance

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Use of old cryopreserved bull semen for genetic variability management in the dairy cattle breed Abondance

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ABSTRACT

Background: In domestic animal populations, artificial selection and genetic drift are leading to an erosion of genetic diversity. Cryopreserved genetic resources could allow an efficient management of genetic diversity of farm animals by reintroducing lost variants. This study focused on a concrete case of cryopreserved semen use of an ancient bull from the 80's into the breeding scheme of the 2000's in the Abondance breed.

Methods: We aimed at describing the genetic contribution of the ancient bull on both genetic diversity and genetic gain using pedigrees and SNP data. We characterized the subsequent impact of this bull on genetic structure of the population.

Results: We found that cryopreserved semen restored genetic diversity lost over time. A shift in milk production performance could be absorbed in a few years by reasoned mating choices. The re-use of this old bull had positive impacts integrated into the breeding scheme, such as the contribution of proven genetic originality but also qualities on traits that are less subject to strong selection pressure in the past such as reproductive abilities.

Conclusions: The use of cryopreserved semen to manage the genetic variability of animal populations is a possible solution. However, certain aspects need to be carefully monitored to avoid undue disadvantages associated with the provision of genetic originality, notably a discrepancy in the genetic values of selected traits. Therefore, recommendations for the use of the genetic resources available in cryobanks should be put in place in order to enhance the value of all the collections already established and to ensure the sustainability of the selected breeds.

Key Words: genetic diversity, ex-situ conservation, cryobank, genetic resources, livestock

INTRODUCTION

Genetic diversity is one of the Essential Biodiversity Variables (EBVs) necessary for the study and management of biodiversity, as well as the basis for monitoring programs around the world (Pareira, 2013). Moreover, the genetic diversity enables living beings to adapt to new challenges. Indeed, in recent decades, environmental fluctuations have been increasingly felt, particularly with global warming. These environmental disturbances may lead to a shortage of food or water resources, but also to a change in the distribution of diseases, forcing animal populations to adapt to new, potentially hostile environments. Consequently, genetic diversity is becoming an essential aspect of the maintenance and survival of animal populations. Indeed, the absence of genetic diversity makes a population much more vulnerable to the various hazards it might encounter, such as the arrival of new pathogens. All these new issues, associated with changes in agronomic practices, require species to have significant evolutionary potential, which is characterized by a large gene pool, i.e. genetic diversity (Barrett, 2008).

In the case of domesticated animal species, the management of populations has a significant impact on the level of diversity, notably because of the selection pressure that has been taking place for many years. The improvement of traits of high economic values for animal production environment has been possible through years of artificial selection (Thornton, 2010). It is known that this selection process inevitably leads to a more or less drastic reduction in genetic diversity depending on the breeds and the various selection goals (Notter, 1999). Strong selection pressure aimed at the rapid improvement of production traits leads to an even more drastic drop in diversity. Moreover, the emergence of genomic selection may have an impact on genetic diversity. As genomic evaluations are implemented in regional breeds, genetic diversity should be carefully monitored to ensure the sustainability of there breeding system (Doublet, 2019 and Senesson, 2012). However, as mentioned above, it is necessary for farm animal populations to be able to adapt to the new challenges. Consumer expectations may change rapidly and therefore involve the definition of new breeding goals. For example, today there is an increasing demand for products from free-range or organic farming systems and animals selected for housing conditions could be unadapted to these potentially harsh environments. In this way, various studies reveal the importance of genetic diversity for the adaptation and evolutionary potential of animal populations (Carvalho, 1993 and Lai, 2019). Successful adaptation of domestic animal populations to the reorientation of breeding goals will only be possible if there is a sufficient level of genetic variability. Moreover, maintaining genetic diversity within the populations subject to selection ensures a response to selection. The absence of diversity for a trait inevitably leads to a lack of response to selection for that trait (Carey, 1983). On the other hand, some animal populations are not subject to the pressure of artificial selection but still suffer from an erosion of their genetic diversity due to genetic drift. Breeds with low effective size are the most susceptible to this phenomenon, which makes them highly vulnerable. Specifically, the effects of genetic drift are all the stronger in breeds with low effective population size, Ne (Wright, 1931).

Genetic diversity of domestic animal species is characterized by among breed diversity but also within breed and conservation measures must be applied at both levels to ensure the maintenance of an overall level of diversity of a species. It then becomes essential to know how to characterize and measure the level of genetic diversity in order to manage it. Genetic diversity is generally assessed through inbreeding or kinship levels estimated from pedigree data (Caballero, 2000 and Meuwissen, 1992). The main biases associated with these methods result from the quality of the genealogies. Indeed, the more complete the pedigree is with sufficient depth, the more the estimates will reflect the real diversity. However, in general, pedigree data leads to an underestimation of inbreeding and kinship levels and therefore to an overestimation of the genetic diversity of a breed. Thus, the use of molecular data allows more efficient measurements of diversity in animal populations. Real inbreeding can then be assessed by identical-by-descent (IBD) segments, named runs of homozygosity (ROH) and considering that this long segments of consecutive homozygous SNPs originate from common ancestors (McQuillan, 2008 and de Cara, 2013). Heterozygosity is also a measure of diversity to be taken into account, especially since the loss of heterozygosity has been shown to have a deleterious effect on population fitness (Reed, 2003).

The conservation of animal genetic resources is carried out at different levels, which should complement and corroborate each other in order to represent reservoirs of genetic diversity to be exploited in the future. It is within the framework of the accompaniment of the green revolution by the FAO and the international agricultural research centres that Otto Frankel, first in the field of plant biology, conceptualized the notion of genetic resources as early as 1967. It was in 1992 that the use of genetic resources was politically highlighted and defined as "material of plant, animal, microbial or other origin containing functional units of heredity" having actual or potential value at the Convention on Biological Diversity (CDB, 1992). Genetic resources can be conserved *in situ*, within their natural environment. At the same time, the conservation of genetic resources is also carried out *ex situ*, outside their natural environment, either *in vivo* or *ex vivo*. The management of genetic resources *in vivo* mainly involves a limitation of inbreeding by reasoned choice of breeders as well as the definition of mating plans and the management of the contributions of individuals over successive generations (Ballou, 1995). According to FAO recommendations, the increase in inbreeding should be limited to 0.5%-1% per generation (FAO, 1998). The fairly recent development of new biotechnologies, in particular cryopreservation but also the development of gene banks, makes it possible to promote *ex vivo* conservation measures for genetic resources. Currently, the organization of French gene banks concerns a wide range of species allowing the sustainable conservation of genetic resources and a diversity of their possible future uses (Danchin-Burge, 2006 and Verrier, 2003). The French National Cryobank was created at the end of 1999 for the conservation of semen and embryos of domestic animal breeds with the aim of hosting samples representative of the genetic diversity of all French breeds. During the 2000s, all the genetic collections saw a boom in France, with the main actor being the bovine species at the end of 2001. Genetic material deposits have become more regular for all three major dairy breeds, but also for two local breeds, including the Abondance (Idele, 2003). The use of such *ex situ* genetic resource conservation programs, which should be combined with *in situ* breed conservation, has been recommended by FAO in its Global Plan of Action (FAO, 2007). Some approaches have been proposed to use genetic resources to improve genetic diversity of threatened breeds (Sonesson, 2002). The real progress in setting up and running gene banks makes it possible to use former individuals in order to reintroduce genetic diversity (Danchin-Burge, 2011),

however the overall use of these resources is quite low.

The indeterminate conservation of genetic material (semen, ova, embryos) raises general issues about the use of cryopreserved ancient resources. Indeed, as some studies reveal, the use of ancient individuals, whose genetic material has been cryopreserved, as breeders in a contemporary population could be considered as an interesting method for managing genetic variability (Leroy, 2011). These issues are obviously of interest for local breeds that may represent small populations subject to selection (Eynard, 2018). However, the use of ancient genetic resources can hinder genetic progress for traits that are currently being selected. Indeed, a gap in terms of genetic values is expected (Leroy, 2011). More specifically, the more a population will have been subjected to a strong selection and will have had a high genetic gain over successive generations, the more the conserved genetic resource may present a delay for the selected traits. In addition, there is a risk of increasing the level of inbreeding in the population. Effectively, the reuse of an ancient individual is associated with a probability that he will mate with his own descendants, which would lead to an acceleration of the increase in inbreeding. It then becomes clear that the use of cryopreserved old seed can have a significant harmful impact on a population undergoing breeding. A real compromise must be made between providing genetic diversity and slowing down genetic progress. Thus, even if the evaluation and conservation of genetic diversity have been developed in recent years, in particular through the establishment of cryobanks, the mobilization of the diversity present in the collections is today shy. This is largely explained by the lack of recommendations for the use of those genetic resources, which are already available currently.

In this study, we looked at a concrete case of reusing the semen of an old bull. The objective was to analyze the impact of using a former cryopreserved bull to restore genetic diversity within the dairy cattle breed Abondance. Indeed the emergence of genomic selection within dairy cattle selection schemes may have an impact on genetic diversity. Abondance is therefore a good study model for estimating the consequences of reintroducing genetic diversity through the use of former breeding stock. NAIF is a bull born in 1977, whose semen has been cryopreserved. This bull has been used in two distinct periods, with a first use in the years 1980-1990 and a second use in the years 2000-2010. We used genealogical and genotyping data to determine whether or not the reuse of NAIF was successful under the current Abondance breeding scheme. We will thus study both the performances and the genetic diversity to detail this case study.

MATERIALS AND METHODS

1 | Description of breed and animals

Abondance is a French dairy cattle breed. This regional breed is the 4th place among French dairy breeds in terms of milk production. It produces quality milk with strong cheese-making abilities, allowing it to be used for the production of cheese with designation of origin labels (AOP). Abondance breed originates from the French Alps (Massif du Chablais) and is a hardy breed whose morphology and functional aptitudes allow it to thrive in alpine territories. This breed has a population of 55,000 cows that nearly represents 1.3% of the French dairy herd.

We studied a particular bull named NAIF (identifier: FR3877011640) born in 1977 which semen was cryopreserved. This bull was first used from 1980 to 1993 and then its cryopreserved semen was reused between 2005 and 2009.

We defined two males cohorts corresponding to genotyped breeding sires contemporary to both uses of NAIF. Cohort 1 corresponds to 62 males, born between 1970 and 1991, which produced offspring between 1980 and 1993 along with NAIF. Cohort 2 corresponds to 124 males, born between 1982 and 2005, that produced offspring between 2004 and 2007, corresponding to the period when NAIF was mainly reused.

Finally we also defined a population consisting of all individuals born in 2017 to study the longer term effect of NAIF reuse.

2 | Pedigree data

We used a data set that included all individuals in the Abundance selection scheme extracted from the national database. The pedigree included a total of 25010 individuals born from 1944 to 2018. The quality of the pedigree was evaluated by the NGEN module of the PEDIG software (Boichard, 2002). The pedigree quality was correct with 3.19 (sd=0.41) and 5.55 (sd=0.17) equivalent number of known generations for cohort 1 and cohort 2 respectively. The equivalent number of known generations was 7.57 (sd=0.08) for the cohort of 2017. The direct descendants of NAIF were identified and associated to cohort 1 or 2 depending on their birth year. NAIF contributions were calculated from pedigree data using the PEDIG software (contribution function). Total NAIF contributions were calculated for each year from 1980 to 2017. Then two types of contributions were defined, an old contribution from the first use of NAIF (1980-1993) and a recent contribution from the contemporary use of the cryopreserved NAIF genetic resource (2004-2009). These contributions were calculated using the same method as for the total contributions. The contemporary use of NAIF was computed by affecting a new identifier to NAIF when used during the second period with both parents unknown.

3 | Molecular data

We had the genotypes of 6958 individuals with the 50K SNPs chip (Illumina Infinium® BovineSNP50 BeadChip).

Quality control was performed by removing SNPs with a call rate inferior to 99% or a minor allele frequency (MAF) less than 1%. No individual had less than 99% genotyped SNPs. After quality control, no SNPs were deleted and 43,801 markers remained in the data sets. The density of markers averaged to one SNP every 57.2 ± 60.0 kb. 26 pairs of markers were found to have identical positions in the genome. These 52 SNPs were removed from the analyses.

Measurements of heterozygosity

The heterozygosity of NAIF, and of the 2 sire cohorts were computed for each chromosome and across the entire genome using the following formula:

$$Het_{i,j} = \frac{\Sigma \, SNP\_het_{i,j}}{\Sigma \, SNP\_tot_j}$$

$Het_{i,j}$ the heterozygosity rate of individual i for the part of genome j considered (chromosomes or whole genome)

$\Sigma \, SNP\_het_{i,j}$ the number of heterozygous markers on the j portion of the genome

$\Sigma \, SNP\_tot_j$ the total number of markers covering portion j of the genome

The means of the two breeding groups (with or without NAIF) were compared using a two-factor ANOVA test (group effect and chromosome effect).

Measurement of inbreeding

Inbreeding was assessed from molecular data using the Run of Homozygosity (ROH hereafter). ROHs represent long autozygous segments of the genome. A ROH is considered to be a homozygous segment of at least 15 SNPs and 1000 kb in length, with at least one SNP per 70 kb. Two consecutive SNPs could not be included in the same ROH if they were separated by more than 140 kb. ROHs were detected using the "homozyg" PLINK 1.9 function (Purcell & Chang, Year).

The size of the sliding window has been set to 15 SNPs. The number of heterozygous calls in the sliding window was limited to 1, and the limit for missing data was 5. For an SNP to be included in an ROH, the success rate of all scan windows containing it had to be at least 0.05.

Inbreeding estimates based on the ROH, $FROH_i$, were calculated as the proportion of the genome included in the ROH as follows:

$$FROH_i = \frac{\Sigma \, \text{LROH}_i}{Lgen}$$

$\Sigma \, LROH_i$ is the total length of ROH for individual i

Lgen is the size between the first and last marker covering the considered genome

The 1,148 individuals born in 2017 were grouped according to their genealogical link to NAIF, forming two groups, one with a recent link to NAIF where he appears to be a father on his second use and the other with no link to recent use of NAIF. These same individuals were also grouped according to the number of genealogical links to NAIF.

The inbreeding of these individuals was also studied at the chromosome level by calculating an inbreeding coefficient per chromosome:

$$FROH_{i,j} = \frac{\Sigma \, \text{LROH}_{i,j}}{Lgen_j}$$

$\Sigma \, LROH_{i,j}$ is the total length of ROH for individual i on chromosome j

$Lgen_j$ is the size between the first and last marker on chromosome j

The effects of chromosome and link to a recent use of NAIF in the pedigree on inbreeding were tested by an ANOVA test.
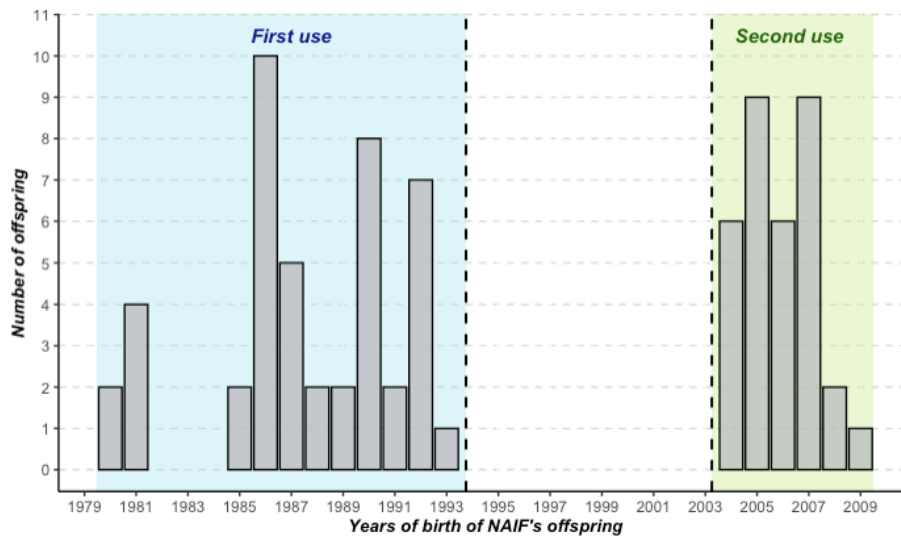
Figure 1*: Production of direct offspring by NAIF during its breeding career between 1980 and 2009
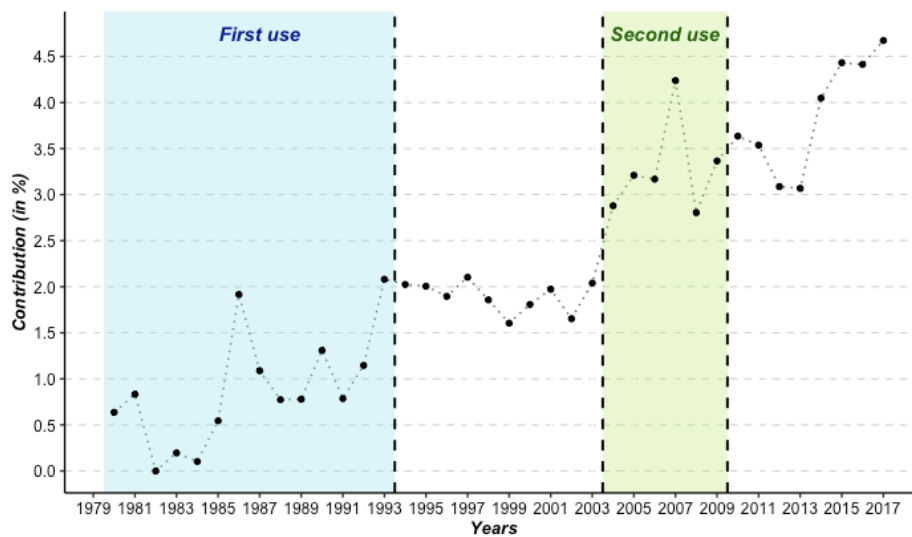(Blue: first period of use - Green: second period of use)*

Figure 2*: Total annual contribution of NAIF based on pedigree from 1980 to 2017
(Blue: first period of use - Green: second period of use)*

Figure 3*: Old and recent contribution of NAIF evaluated from pedigree from 2004 to 2017
(In blue: old contribution from the first period of NAIF use - In green: recent contribution from the second period of NAIF use)*

Genetic Structure by Multivariate Analysis

Principal Component Analyses were conducted with cohort 1 and 2 using the ade4 package (Dray S, Dufour A). NAIF was added as a supplementary individual to the PCAs (i.e. it is not contributing to the construction of the principal components).

A Between-Class Analysis was then carried out on the 2017 cohort specifying the links of the individuals with NAIF. Individuals were separated into 6 classes representing the possible combinations of the different uses of NAIF, either the absence of a link, or the presence of one or two old or recent links (consequence of its first or second use through a single parent or both of them). This analysis aimed to maximize the between groups variance while minimizing within groups variance.

4 | Genomic performance data evaluated in 2017

Genomic estimated breeding values were assessed in 2017 for all genotyped individuals. We extracted these values for the cohort 1, cohort 2 and NAIF.

We focused on three different integrative indices, the total merit index (ISU, *Indice de Synthèse Unique*), the dairy merit index (INEL, *Indice National Economique Laitier*) and the reproductive merit index (REPRO). These three integrative indices well represent the value of individuals.

5 | Statistical analysis

All statistical analysis and graphical representations were made using R (R Core team, 2019) and the ggplot2 package (Wickham H). Statistical tests were made using the lm function and post-hoc and comparisons were made using the emmeans package (Searle SR, Speed FM, Milliken GA), type II ANOVA were performed using the car package (Fox J, Weisberg S).

RESULTS

1 | Pedigree data

Production of NAIF

The NAIF bull was first used to produce 45 direct progeny born between 1980 and 1993 (*Figure 1*). Following a 10-year period of inactivity, his semen was used again to produce 33 progeny born from 2004 to 2009. The years from 2004 to 2007 were marked by a strong production of individuals with NAIF as a sire with a total of 30 descendants in those 4 years alone. He was not used anymore for artificial insemination after 2009.

Contribution of NAIF

In its first period of use, the overall contribution of NAIF increased from 1980 to 1993. From 1994 to 2003, its contribution remained fairly constant. During its contemporary use, NAIF's contribution has increased again. From 2009 onwards, a decline appears over the next four years, followed by a marked increase from 2014 to 2017 due to the use of NAIF's descendants (*Figure 2*). Distinguishing between past and recent NAIF contributions reveals the impacts of the two periods of NAIF use. Older contributions are stronger than recent ones, with the exception of the year 2017. For the year 2007, the recent contribution is greater than for the other years (*Figure 3*). The first offspring of NAIF born in 2004 are reaching sexual maturity and have also been able to reproduce for the first time. From 2014 onwards, the recent contribution increases year by year due to the use of descendants from the contemporary use of NAIF. Thus, the evolution of the recent contribution of NAIF shows that descendants of contemporary use of NAIF have not been excluded from the Abondance selection scheme.
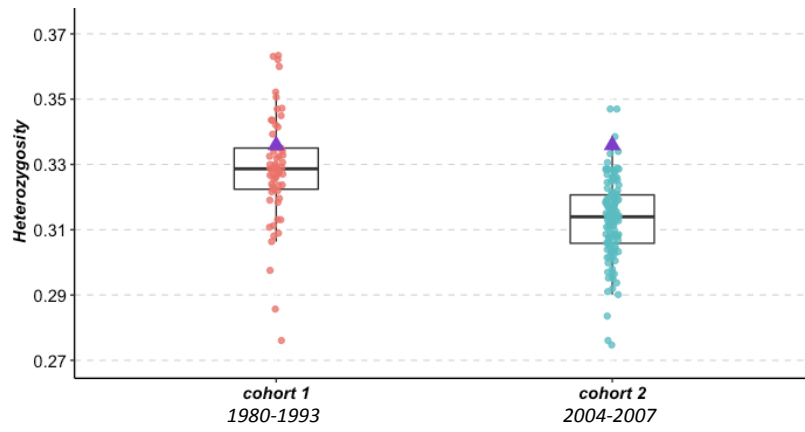
Figure 4: Average heterozygosity of contemporary cohorts at both uses of the NAIF bull
(The 62 bulls in Cohort 1 are shown in pink, the 124 bulls in Cohort 2 are shown in blue, NAIF is represented by the purple triangle)
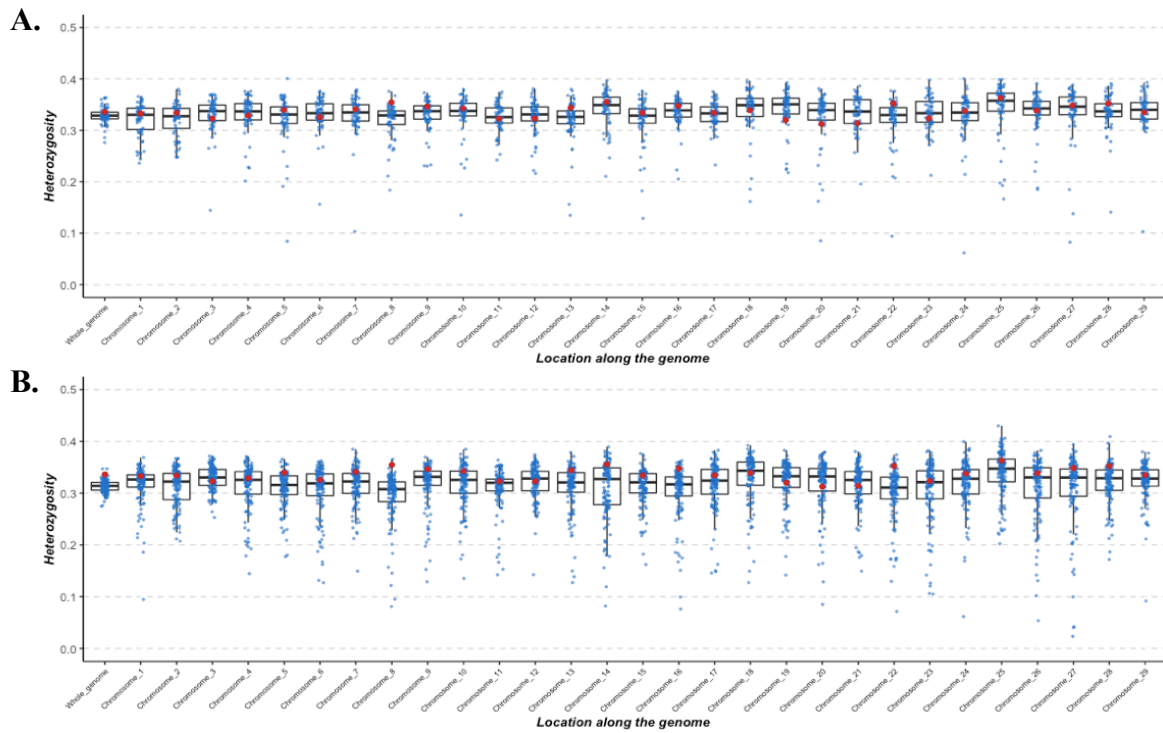

Figure 5: Average heterozygosity by chromosome for bulls of Cohort 1 (**A**) and Cohort 2 (**B**)
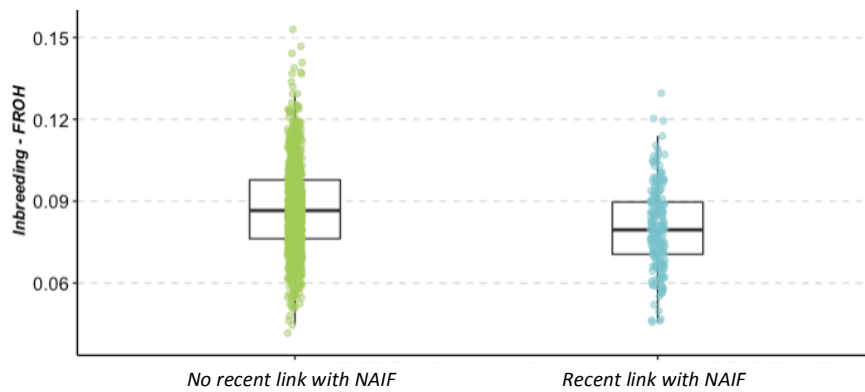*(NAIF is represented by the red dot)*

Figure 6: Inbreeding of individuals of cohort 2017 depending on their link with the recent use of NAIF
(Green: individuals not from recent use of NAIF (noRECENT_LWN) - Blue: individuals from recent use of NAIF (RECENT_LWN))

2 | Molecular data

Measurements of heterozygosity

The average heterozygosity along the genome of the 62 sires from cohort 1 was 0.329 (sd=0.016) while that of the 124 sires from cohort 2 was 0.313 (sd=0.012) (*Figure 4*). Over the whole genome, the mean heterozygosity decreased between the two cohorts (one-factor ANOVA test, $p<10^{-4}$). NAIF has a heterozygosity rate of 0.336. Compared to cohort 1, he had a good heterozygosity rate and belongs to the third quartile of the distribution while he was one of the most heterozygous individuals of the cohort 2 (figure 4).

The levels of heterozygosity varied according to the chromosome studied for the two populations (*Figure 5*). For each chromosome, the average heterozygosity of cohort 1 was higher than that of cohort 2 (two-factor ANOVA test, $p<0.001$ for all chromosomes). NAIF also had different heterozygosity per chromosome. Although it remained heterozygous throughout the genome, for some chromosomes it was not among the most heterozygous individuals of the two populations (chromosome 18 to 21).

Inbreeding measurements

For cohort 2017, 85 animals were unrelated to NAIF (0_LWN), 436 animals had a single link with NAIF through either the maternal or paternal way (1_LWN) and 627 animals had two NAIF relationships through both parents (2_LWN), with average inbreeding of 0.0867 ± 0.0181, 0.0864 ± 0.0158 and 0.0858 ± 0.0162 respectively. Inbreeding was not significantly different according to the kinship with NAIF (ANOVA test, p=0.76). Thus, individuals who were inbred as a result of NAIF did not have a higher level of inbreeding across the genome than other individuals. Inbreeding was significantly different between chromosomes (ANOVA test, $p<10^{-4}$). In addition, 197 individuals had a link to NAIF from its second period of use while 951 individuals independent to the recent use of NAIF. Mean inbreeding was 0.0874 ± 0.0162 and 0.0801 ± 0.0151, respectively for individuals with no link to the recent use of NAIF and those related to recent NAIF use (*Figure 6*). A significant decrease in inbreeding was observed following the use of the cryopreserved bull (ANOVA test, $p<10^{-4}$).

Figure 7: Principal Component Analysis of genotyping data for Cohort 1 (**A**) and Cohort 2 (**B** and **C**)
(NAIF is represented by the blue dot)
*(For **C**, Green: individuals with no link to NAIF (noLWN) - Red: individuals with a link to NAIF (LWN))*
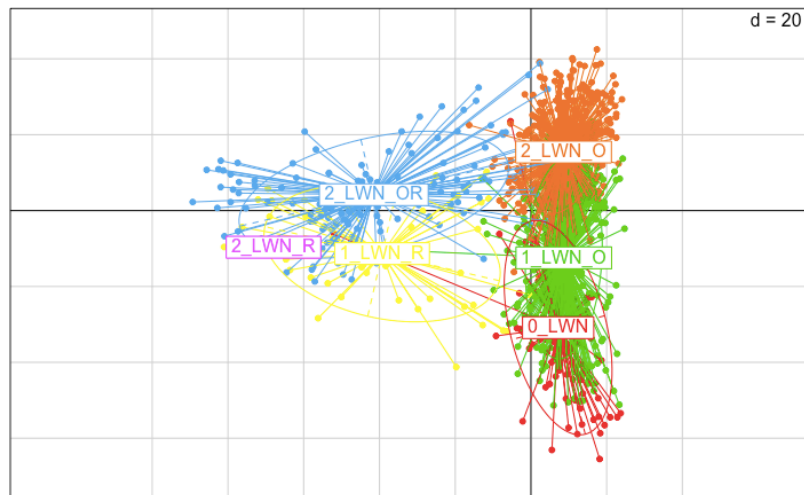

Figure 8: Between-Class Analysis of genotyping data of the cohort 2017
(Red: individuals not related to NAIF - Green: individuals with 1 old link with NAIF - Orange: individuals with 2 old links with NAIF - Blue: individuals with 1 old and 1 recent link with NAIF - Yellow: individuals with 1 recent link with NAIF - Purple: individuals with 2 recent links with NAIF)

Genetic Structure by Multivariate Analysis

Principal Component Analyses allowed NAIF to be genetically placed within the two cohorts of breeding males of its time of use. As a first step, NAIF was added to cohort 1. The first two components account for 11.6% of the variability of this reproductive population, a percentage explained by the large number of markers used. NAIF appears to be an average individual of the cohort 1 since it did not differ from other bulls that bred during its first use (*Figure 7 A*). Reversely when looking at the position of NAIF within cohort 2, it appeared more extreme and different from the population mean. The PCA was built with the 124 bulls that bred during the second use of NAIF and the first two axes accounted for 7.5% of the total variability. Although he was not the individual with the greatest distance from the barycentre of the point cloud, he remained the bull with the most negative coordinate on the second component. (*Figure 7 B*). In addition, individuals with a link to NAIF (LWN), i.e. where NAIF appears in their pedigree traceback, have been identified. Of the 124 bulls in cohort 2, 61 had this link to NAIF and 63 were totally independent of this lineage. The PCA showed a distinction between these two groups of individuals (*Figure 7 C*).

For the Between-Class Analysis of the 2017 cohort, the 1148 individuals were divided into 6 groups. 85 individuals had no link to NAIF (0_LWN), 49 individuals had a recent link to NAIF through one of their parents (1_LWN_R), 387 individuals had a past link to NAIF through one of their parents (1_LWN_O), 479 individuals had two past ties to NAIF by each parent (2_LWN_O), 1 individual had two recent ties to NAIF by each parent (2_LWN_R) and 147 individuals had one past and one recent tie by both parents (2_LWN_OR). The 3 groups with recent use of NAIF (1_LWN_R, 2_LWN_R, 2_LWN_OR) were grouped together compared to the other 3 groups with no link to NAIF (0_LWN) or only links due to its first use (1_LWN_O, 2_LWN_O) (*Figure 8*). The recent use of NAIF has led to a certain originality that differentiated the three groups from the rest of the population.

3 | Genomic performance data evaluated in 2017

The ISU, INEL and REPRO values of NAIF were 70, -24 and 0.8 respectively. The values were 75.46 (sd=15.72), -19.70 (sd=15.85) and 0.26 (sd=0.36) for cohort 1 and 91.29 (sd=15.44), -6.44 (sd=15.04) and 0.14 (sd=0.58) for cohort 2 for ISU, INEL and REPRO respectively. The distribution of these values for cohort 1 and cohort 2 and the relative position of NAIF were represented on figure 9.
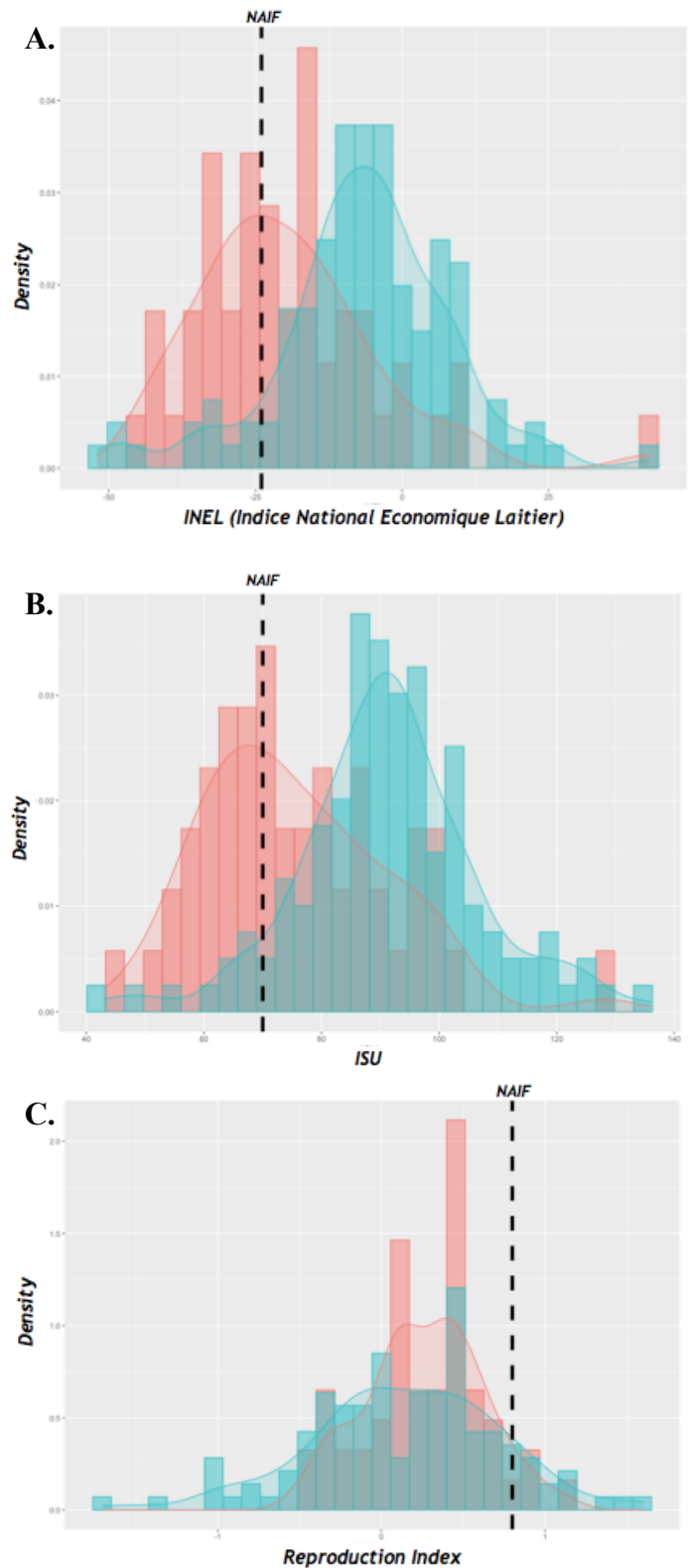

Figure 9: Distribution of INEL (**A**), ISU (**B**) and a reproduction index (**C**) for Cohort 1 and Cohort 2
(The 62 bulls in Cohort 1 are shown in pink, the 124 bulls in Cohort 2 are shown in blue, NAIF is represented by the black dotted line)

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DISCUSSION

NAIF was the bull chosen to bring genetic diversity back into the Abundance selection scheme. Indeed the 1980s, Red Holsteins individuals were used into the Abondance breed selection scheme to improve milk performance. This practice was abandoned in the late 1990s, when the goal was to return to a pure breed. In addition, it was known that some pure Abundance lines no longer produced approved bulls for testing and were therefore doomed to disappear, as was the case for the Amiens bull family. The NAIF bull, born in 1977, belonged to the Amiens family. NAIF was an average bull that was used to purify the breed. In addition, this individual had sufficient cryopreserved doses to expect a high enough progeny production to impact the Abundance selection scheme. He was therefore a good candidate to successfully bring back genetic diversity, especially blood from the Amiens lineage.

NAIF produced 45 individuals in the first use and 33 in the second but in a shorter time period. The second use was thus more intense than its first use. These two successive uses had a strong genetic contribution of NAIF to the population. Its overall contribution increased in both periods of use with stagnation when not used. However, his contribution continued to increase after 2009 when he was no longer used as a sire. This increase in contribution was achieved by using NAIF's progeny into the Abondance breeding scheme. Moreover, while the old contribution remained almost stable from 2004 to 2017, recent contributions increased over the end of this period. Indeed, the recent contribution of NAIF became almost equivalent to the old contribution from 2016 onwards. Previous years did not necessarily follow the same pattern. This difference is explained by the time needed for the population to bridge the performance gap between NAIF and its contemporary sires during its second use.

The performance study revealed that NAIF was a pretty decent individual when first used. However, a discrepancy was observed when placed against a more recent cohort. Thus, when reintroduced, NAIF was found to lag behind pure performances such as the INEL or the ISU. Indeed, considering a gap of nearly 2 generations between cohorts 1 and 2 resulted in an increase of 7.90 points per generation and 6.63 points per generation, that is to say an increase of about 1.58 points and 1.33 points per year for the ISU and INEL respectively. This aspect was expected and has already been highlighted in other studies (Leroy, 2011 and Eynard, 2018). A bull with semen stored for several generations will have poorer genetic values as evidenced by NAIF compared to cohort 1. This difference will be likely to be even greater if genetic gain is strong, which is often the case for large dairy cattle breeds. Thus, it appears that the largest is ΔG, the more older individuals will lead to a strong discrepancy in the selected traits. For dairy breeds, this discrepancy is strongly reflected in dairy production indices. However, although scenarios developed by Leroy et al (2011) showed that the use of conserved bulls with a lower genetic value would not be so advantageous, the case of NAIF reveals that this gap can be absorbed by mating choices with good females. A latency period is indeed necessary in order to close the performance gap, yet the qualities brought back by NAIF could be integrated into the breeding scheme especially by its progeny. Thus, as suggested by Eynard et al (2018), adding older bulls to a current breeding population provides long-term genetic diversity in breeds undergoing selection without too much impact on the genetic merit showing the advantage of using animals in the gene bank.

In spite of the negative impact on pure performance, the use of NAIF has increased qualities in other traits. NAIF had a good reproductive index. The use of NAIF brought back past quality for some traits whose improvement was not necessarily within the past breeding objectives, as for reproductive index for instance. This underlines the fact that traits such as fertility, calving ease or vitality at birth, which are strongly affected in many dairy breeds by the selection toward production traits only focus, can be improved using cryoconserved resources. NAIF's contribution has been retained in the breeding scheme through a judicious and reasoned choice of females with which it has been mated. Indeed, NAIF was used on females with high production performances. These matings made it possible to minimize the gap in milk performance, while bringing back the reproductive qualities and genetic originality of the Amiens family. In plant breeding, Allier et al. (2019) showed that collaborative diversity panels (i.e. genetic resources and elite lines) coupled with genomic prediction seemed relevant to identify and exploit genetic resources to enrich elite germplasm in maize. Thus, the use of a former bull with elite females would maximize the reduction of the gap in genetic values to benefit from diversity without drastically impacting genetic gain on future generations. In addition, the high heterozygosity of NAIF gives it a wide gametic variance (i.e. a large genetic variability of its gametes). Associated with a large number of offspring when it was reused, the gametic variance allowed it to offer a rather large panel of diversity among its descendants that were hereafter selected in the breeding scheme. Other studies have revealed that the use of parents who produce more variable gametes may provide a response to selection by increasing the probability of reproducing a high level genotype (Bijma, 2020). This prospect is all the more interesting since genomic evaluation via GEBV of a straw from a former individual present in the cryobank is feasible and would make it possible to determine the right candidates to use to bring back genetic diversity.

The study of the Abundance population for a few generations after the reuse of NAIF allowed to ensure that the originality brought back by NAIF was not removed by selection. The study of the 2017 population, nearly two generations after its second use highlighted the success of this reintroduction of diversity within the breeding scheme. Inbreeding did not increased achieved did not increase due to the reuse of NAIF, a decrease in inbreeding between individuals from recent NAIF matings and the standard population was even observed. This significant difference in inbreeding can be explained by the choice of females that were mated with NAIF. Indeed, the action of bringing out an old bull can favour inbreeding matings if the individual has been heavily used in the past and if vigilance is not carried out on mating plans. Conversely, in the case of NAIF use, we observed a slowing down of the increase in inbreeding rate, ΔF. It emerges that the use of an old bull in a contemporary population to which he has already contributed nearly 40 years in the past, requires vigilance on the level of inbreeding. Indeed, Doekes et al (2019) showed that inbreeding in recent generations was more detrimental than inbreeding in distant generations, leading to inbreeding depression in many traits. Finally, multivariate analyses revealed genetic uniqueness of NAIF. When first used, NAIF was representative of its contemporary sires. However, when used for the second time, NAIF appeared to be an original individual relative to the other active sires. This genetic originality was passed on to NAIF's progeny when it was reused. The BCA reveals that all individuals with a recent link to NAIF are distinguishable from other individuals. NAIF was thus able to bring part of the lost genetic specificity of the Amiens family back into the 2017 population. It is this genetic originality that is gleaned by the players in the sector to promote the use of NAIF's sons within selection schemes and reintroduce diversity.

CONCLUSION

This study showed that the use of a cryopreserved ancient bull can allow for reintroducing genetic diversity within a current breeding scheme. Nevertheless, points of vigilance are highlighted for the use of ancient genetic resources. Due to selection over time for certain traits, the introduction of older individuals may impact the expected genetic gain. A shift in the level of traits being selected can lead to a decline in genetic merit for these production traits. However, the use of older individuals also affects traits not subject to selection. Indeed, while selection allows progress on production traits, morphological or functional traits can often be counter-selected when negative genetic correlations exists (i.e. allocation of resources) or can drift and decrease due to a loss of genetic variability. Thus, the reintroduction of a former bull allows for selection on these declining traits by reintroducing genetic variance at these traits and valuable candidates. The rise of genomic selection allows an opening on these compromises. Evaluations of former individuals are possible at present for traits that were not selected at the time by assessing them relative to the contemporary population. Molecular tools provide access to accurate genomic data that discriminate the best breeding stock to use to successfully introduce diversity. The rate of heterozygosity and gametic variance (using haplotypes for instance) are indicators to be taken into account. It makes it possible to characterize the diversity of populations but also to detect the genetic originality of older bulls. It is therefore possible to increase the diversity of an animal population under selection by using ancient genetic resources. However, nowadays there are no recommendations to facilitate the consideration of the different aspects that will ensure the success of the reintroduction. Although genetic resources are well conserved, they are still too little used. There is a need for further studies to provide recommendations for the use of genetic resources that respond to the objectives and expectations of each species, in order to fulfill the expectation of the stakeholders to ensure the sustainability of our breeds.

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