



# The impact of using old germplasm on genetic merit and diversity—A cattle breed case study

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## Abstract

Artificial selection and high genetic gains in livestock breeds led to a loss of genetic diversity. Current genetic diversity conservation actions focus on long-term maintenance of breeds under selection. Gene banks play a role in such actions by storing genetic materials for future use and the recent development of genomic information is facilitating characterization of gene bank material for better use. Using the Meuse-Rhine-Issel Dutch cattle breed as a case study, we inferred the potential role of germplasm of old individuals for genetic diversity conservation of the current population. First, we described the evolution of genetic merit and diversity over time and then we applied the optimal contribution (OC) strategy to select individuals for maximizing genetic diversity, or maximizing genetic merit while constraining loss of genetic diversity. In the past decades, genetic merit increased while genetic diversity decreased. Genetic merit and diversity were both higher in an OC scenario restricting the rate of inbreeding when old individuals were considered for selection, compared to considering only animals from the current population. Thus, our study shows that gene bank material, in the form of old individuals, has the potential to support long-term maintenance and selection of breeds.

## KEYWORDS

ex-situ conservation, gene bank, genetic diversity, genetic merit

## 1 | INTRODUCTION

Decades of artificial selection, targeting the improvement of economically important traits, has drastically impacted livestock breeds. Substantial increases in genetic gain have been observed for traits linked to production (i.e., milk and meat yield or growth rate; Thornton, 2010) yet indubitably associated with a loss of genetic diversity (Notter, 1999). Genetic diversity, however, is essential to enable future selection and ultimately breed conservation. Considering

that livestock production and its environment are likely to change in the future, it is important to keep variability available for adaptation to changes in breeding goals in the future, possibly following changes in environment (e.g., due to climate change). In addition, it is necessary to maintain diversity for fitness related traits of the breeds.

Conservation actions can be performed on the living population (in-situ) and focus on the selection of breeding individuals, the management of mating design as well as the control over the individuals' contributions to the next

generation (Ballou & Lacy, 1995; Caballero & Toro, 2000; Fernández & Toro, 1999; Meuwissen, 1997). As a result, it primarily limits the increase in inbreeding. The Food and Agriculture Organization recommends to limit such increase to 0.5%–1% per generation (FAO, 1998). Alongside in-situ conservation actions, ex-situ actions also exist in the form of gene bank collections. Gene banks allow to conserve the overall population genetic diversity in the form of reproductive material (sperm, ova and embryos) for an indefinite time. By its temporal fixation, the stored material is thus free from the impact of evolution or drift on genetic variability. Ex-situ material enables to access or recover old and specific variation and introduce it in the current population but also to restore extinct breeds or support breeds at risk of extinction or control breed design in the case of reorientation of the breeding goal (Hiemstra, van der Lende, & Woelders, 2006; Oldenbroek, 2017). Issues related to conservation of genetic diversity in livestock are especially important for small local breeds. Such breeds are likely to be neglected as their competitiveness for economically important traits is expected to be lower than of mainstream breeds. In small local breeds, the percentage of artificial insemination might be considerably lower compared to the mainstream breeds. Small local breeds may therefore suffer from sporadic pedigree recording and lack of thorough characterization of the breed. Moreover, they are likely to be more at risk of extinction due to their limited number of individuals that are alive and able to reproduce. Ex-situ conservation, therefore, is especially important for small local breeds as it supports the preservation of additional breed material.

The increasing availability of genomic information enables its use for both selection and conservation of genetic diversity. For instance, single nucleotide polymorphism (SNP) chips give power to better genetically characterize breeds, to identify individual uniqueness, to identify genome regions or even specific markers of importance (i.e., deleterious variants, signals of selection) and to accurately estimate relationships between breeds and individuals (Toro, Fernández, & Caballero, 2009). As a result, using genomic information has the potential to improve decisions for conservation of genetic diversity compared to the traditional information brought by pedigree records (Hanotte & Jianlin, 2006).

The main objective of this study was to test how old individuals, present in the gene bank, are of potential use to maintain and improve the level of genetic diversity in the current population and enable long-term maintenance of small local breeds under selection. To answer this question, we used the Meuse-Rhine-Issel (MRY) cattle breed (The cattle site, 2017) as an example of a small local breed subject to ex-situ conservation measures. To our knowledge,

our study is the first one to do this on empirical data, while this has been done previously using simulated data (Leroy, Danchin-Burge, & Verrier, 2011). In addition, we described the evolution of livestock genetic merit and genetic diversity through time in this breed.

## 2 | MATERIALS AND METHODS

### 2.1 | Selection decision and evaluation

To infer the potential use of old individuals for genetic diversity conservation of the current population of the breed, we compared selection decisions based on the current population only versus on the whole population, including the old individuals.

Selection of animals as parents of the next generation was performed using the optimal contribution (OC) strategy (Meuwissen, 1997) implemented in the Gencont programme allowing to simultaneously optimize conservation of genetic diversity, while maximizing genetic merit. In this study, we focused on two selection strategies. The first strategy only targeted conservation of genetic diversity (*cons*) by minimizing the average relatedness between selected individuals and thus managing the rate of inbreeding between the current and next generation. The other strategy (*impcons*) simultaneously maximizes genetic merit (i.e., the average BV of the selected individuals) while restricting the loss of genetic diversity. In this strategy, the generational rate of inbreeding ( $\Delta F$ ) is restricted to a value of 1%, following FAO (1998) recommendation. Rate of inbreeding was computed from changes in average population relatedness. Using the genetic information available in the form of genotypes, we measured relatedness between individuals by computing a similarity-based relationship matrix. We expect that using such matrix allows better reduction in loss of overall genetic diversity than other relationship matrices when combined with OC strategy (Eynard, Windig, Hiemstra, & Calus, 2016). Similarities are based on the count of identical alleles averaged across loci between two individuals (Eding & Meuwissen, 2001; Nejati-Javaremi, Smith, & Gibson, 1997):

$$G_{jk} = \frac{2}{N} \sum_i (x_{ij} - 1)(x_{ik} - 1),$$

where  $N$  is the number of markers and  $G_{jk}$  is the estimated relationship between individual  $j$  and  $k$  across all markers,  $x_{ij}$  and  $x_{ik}$  are the genotype (0, 1 or 2 with 0 and 2 being the homozygous and 1 the heterozygous) of individual  $j$  and  $k$  for marker  $i$ . Computing relationships using the similarity-based method is equivalent to using the methods described by VanRaden (2008) and Yang et al. (2010) assuming allele frequencies of 0.5 for all loci.

The whole population was split into two groups based on year of birth: (a) individuals born from 2000 onwards, hereafter referred to as the “current population,” and (b) individuals born before 2000, hereafter referred to as the “old population.” This split was chosen such that the former group indeed represents a pool of currently and recently used bulls, while both groups had sufficient numbers of bulls to perform selection. Two different constraints were applied to the *impcons* scenario. Following the basic formula for rate of inbreeding (Falconer & Mackay, 1996):

$$\Delta F = \frac{F_{t+1} - F_t}{1 - F_t},$$

where  $\Delta F$  is fixed to 1% and  $F_t$  is the initial average inbreeding coefficient for the whole population (*impCONS*) or the current population (*IMPcons*), we computed the expected average inbreeding coefficient in the year  $t+1$  ( $F_{t+1}$ ) as half the relatedness in this year. Thus, in the *IMPcons* scenario, a constraint is placed on the inbreeding rate between the current generation and the next generation, while the scenario *impCONS* controls inbreeding further back in time by placing the constraint on the whole population's inbreeding coefficient. Moreover, because average relatedness was initially lower in the whole population compared to the current population *impCONS* is a stricter constraint than *IMPcons* and thus places more emphasis on the conservation of genetic diversity compared to *IMPcons* that places more emphasis on the improvement of the response to selection.

The three approaches *cons*, *impCONS* and *IMPcons* were tested in two scenarios, considering selection from (a) the current population (*current\_cons*, *current\_impCONS* and *current\_IMPcons*), or (b) the whole population (*tot\_cons*, *tot\_impCONS* and *tot\_IMPcons*) in which all individuals regardless their birth date were considered for selection. In these scenarios, the number of animals selected was (a) the optimal number of individuals with their associated contributions to the next generation, as defined by Gencont, denoted hereafter by “ $x_{\text{weight}}$ ” (b) the optimal number of individuals with equal contributions to the next generation, denoted hereafter “ $x$ ” or (c) 100, 50, 20 or 10 individuals with equal contributions. A summary of all the tested scenarios is given in Table 1.

For each selection decision, the selected groups were compared based on (a) the average genetic merit and (b) average observed and expected heterozygosity. The observed heterozygosity is the average heterozygosity status of the selected individuals, while the expected heterozygosity is the heterozygosity predicted for a population under Hardy–Weinberg equilibrium with the same allele frequencies.

**TABLE 1** Description of the optimal contribution selection scenarios

Variables	Values taken
Selection criteria	<i>cons</i> : Minimize relatedness <i>impCONS</i> : Maximize genetic merit + Restrict inbreeding rate from whole population 1% (strict) <i>IMPcons</i> : Maximize genetic merit + Restrict inbreeding rate from current population 1% (relaxed)
Selection from	<i>current</i> : Current population <sup>a</sup> ( $N = 119$ ) <i>tot</i> : Whole population <sup>b</sup> ( $N = 413$ )
Scenarios names	<i>current_cons</i> , <i>current_impCONS</i> , <i>current_IMPcons</i> <i>tot_cons</i> , <i>tot_impCONS</i> , <i>tot_IMPcons</i>
No. of selected individuals	$x$ , $x_{\text{weight}}$ , 100, 50, 20, 10

Note.  $x$  is the selection decision scenario where the optimal number of individuals were selected by Gencont and equal contributions were given to them,  $x_{\text{weight}}$  is the selection decision scenario where the optimal number of individuals were selected by Gencont and unequal optimal contributions were given to them.

<sup>a</sup>Stands for the current population of individuals born from 2000 onward.

<sup>b</sup>stands for the complete population of all individuals regardless their date of birth.

## 2.2 | Description of the MRY breed

Distributed in the east and south of the Netherlands, along the three rivers, Meuse, Rhine and Issel, where it takes its name from, the herd book of this breed was created in the early 1900s'. Used until the 70s as one of many dual purpose breeds, thereafter the number of purebred breeding individuals from the Dutch MRY cattle breed has drastically reduced mostly because of crossbreeding and replacement by Holstein Friesian cattle. The population size went from more than 500,000 in the 70s to 15,000 in 2008 (Hiemstra & de Haas, 2004). A pure-breed breeding programme is managed by the Cooperative Cattle Improvement Organisation CRV BV (Arnhem, the Netherlands) and supported by two regional breeders associations (MRY-East and South) to maintain the breed standard of a calm, robust and strong cow that combines high milk production with good health, fertility and meat value (<http://www.thecattlesite.com/breeds/dairy/111/meuse-rhine-issel/>). In an effort to characterize the genetic material present in their gene bank, the Centre for Genetic Resources of the Netherlands (CGN) of Wageningen University and Research carried out genotyping of the stored individuals.

Our objective was to investigate the potential to maintain or even improve the level of genetic diversity in a small local breed under selection considering the use of gene bank material, and therefore, only purebred animals were considered in our study. For this study, a total of 413

purebred MRY bulls (87.5%–100% MRY) were available. Of these 413, 192 bulls have semen straws stored in the gene bank at the CGN. The other 221 bulls were not included in the gene bank collection, but used by farmers through artificial insemination and included in the breeding programme of CRV. A pedigree containing 5,226 records was available. The number of generation equivalents (sum over all ancestors of  $[1/2]^n$ , with  $n$  being the number of generations between the individual and ancestor of interest (Maignel, Boichard, & Verrier, 1996)) was calculated. In total, 403 bulls had known contribution to the population and contributed on average 1,675 female offspring in the population. The old bulls contributed on average for 2,270 female offspring, while the current bulls contributed on average 206 female offspring. The individual Breeding Values (BV) used in the analysis were those for the NVI, the Dutch Flemish total merit index estimated through traditional genetic evaluations based on pedigree information, of sires for bull ranking in the Netherlands and Flanders (Genetische Evaluatie Stieren, 2017), as calculated in April 2017.

### 2.3 | Genomic information

Genotypes of 436 individuals from the Meuse-Rhine-Issel (MRY) cattle breed were available before any quality control. Based on the BovineSNP50 BeadChip (Illumina Inc., San Diego, CA, USA), a set of 49,438 markers remained when combining the different genotyping batches, keeping only markers that have been called for all the individuals. This initial genotype data set was put through the following quality control steps: (a) individual call rate >85% (22 individuals removed), (b) marker call rate >95% (5,004 markers removed), (c) each marker allele should be present at least three times in the data set (equivalent to using a minor allele frequency threshold of 0.0036; 4,539 markers removed), (d) if only the two homozygous or only the heterozygous are present then the marker is discarded (five markers removed), (e) opposing homozygous markers <2% between genotyped parent offspring pairs (from the 200 pairs tested, one individual was removed because of having a high percentage of opposing homozygous markers with both his offspring), (f) marker Mendelian inconsistency <5% (97 markers removed). The final genotype data included 39,793 markers for 413 individuals. Missing genotypes on the remaining markers were imputed using Fimpute (Sargolzaei, Chesnais, & Schenkel, 2014).

### 2.4 | MRY bulls population characterization and changes through time

To gain more insight into the structure of the data and its changes over time, we analysed genetic merit and genetic

diversity. Therefore the population was described on the one hand by measuring its average genetic merit  $G = \overline{NVI}$  and how it changed through time with the rate of genetic merit ( $\Delta G$ ) per year, as the slope of the linear regression of average NVI per year of birth.

On the other hand, Principal Component Analysis (PCA) based on genomic relationships between individuals was used to allow visual characterization of the population genetic diversity. The inertia of the PCA cloud representing each population was calculated as the sum of the eigenvalues of the individuals included in the population of interest. Moreover, the individual observed heterozygosity was measured as the proportion of heterozygous markers per individual and populational observed heterozygosity was measured as the average heterozygosity of the population. At last, individual inbreeding coefficients were measured in three different ways: (a) based on pedigree information ( $F_A$ ), (b) from the similarity-based genomic relationship matrix ( $F_G$ ), as the diagonal  $-1$  or (c) based on Runs Of Homozygosity (ROH) larger than 1 Mb (i.e., 1,000,000 bp) and represented by a minimum of 50 successive SNPs ( $F_{ROH}$ ). The formula used to compute the inbreeding from ROH is:  $F_{ROH_i} = (\sum L_{ROH_i}) / (L_{\text{autosome}})$ , where  $\sum L_{ROH_i}$  is total length of ROH in the genome of individual  $i$ , and  $L_{\text{autosome}}$  is the length of autosomal genome covered by the SNP chip used (Purfield, Berry, McParland, & Bradley, 2012), in this case  $L_{\text{autosome}} = 2,500,604,901$  bp or 2,500.6 Mb. The “age” of the segment can be inferred roughly from the length of the ROH using  $ROH (l \text{ in megabases, Mb}) = 100/2g$ , where  $g$  is the number of generation as this ROH exists (Purfield, McParland, Wall, & Berry, 2017; Thompson, 2013). Thus, in this study, it was possible to infer inbreeding coming from approximately up to 50 generations in the past with the ROH length of 1 Mb. In addition to informing on the old inbreeding carried by the population, such ROH length of 1 Mb is close to the minimal size that can be analysed using SNP of limited density like the 50 K SNP chip. To describe changes in genetic diversity through time, we measured the rate of inbreeding ( $\Delta F$ ) based on the proposed estimators.  $\Delta F$  per year being calculated as follows (de Roos, Schrooten, Veerkamp, & van Arendonk, 2011),

$$\Delta F = 1 - \left( \frac{1 - F_{\text{year}_t}}{1 - F_{\text{year}_i}} \right)^{\frac{1}{\text{year}_t - \text{year}_i}},$$

with  $\text{year}_t$  and  $\text{year}_i$  being the final and initial years for which average inbreeding coefficient  $F_{\text{year}_t}$  and  $F_{\text{year}_i}$  of the population have been estimated.

To allow meaningful averages of genetic merit, heterozygosity and inbreeding, when a year group did not contain at least four individuals it was combined with the next year until reaching a minimum of four individuals.

This was applied to avoid large deviation from the mean and bias in trend due to a small number of really influential individuals present in the early years when plotting the estimates against time.

### 3 | RESULTS

#### 3.1 | Population characterization and evolution through time

The 413 bulls had 0 to 6 full generations (i.e., all parents present) in the pedigree, and their number of generation equivalents ranged from 0.5 to 8.42. These individuals available for this study were born between 1962 and 2014 and had genetic merit ranging from  $-386$  to  $140$ . The old population, born between 1962 and 1999, had an average genetic merit of  $-183$ . The current population, born between 2000 and 2014, had an average genetic merit of  $5$ .

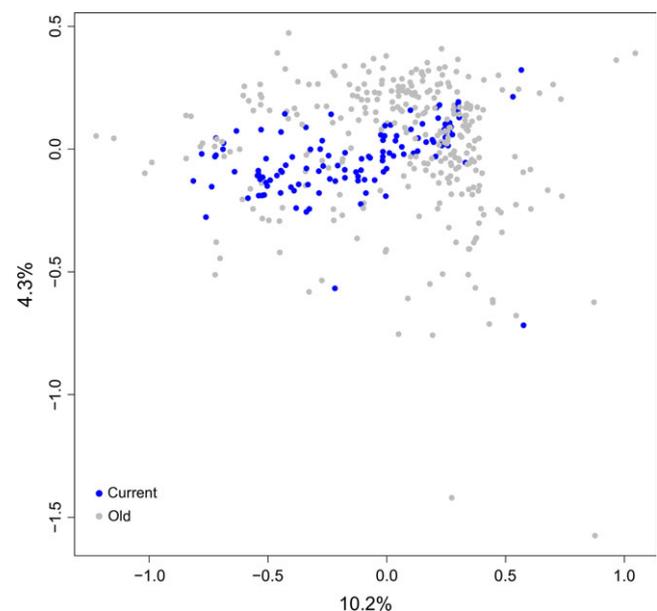
The PCA was used to visually infer if there were subgroups in the population. The whole population clustered in one group and 17.24% of the total population diversity, measured as the inertia, was explained by the current population while the remaining 82.76% was explained by the old population (Figure 1). This indicates that the old population carries most of the genetic diversity observed in the whole MRY population. It should be noted that this was expected because the old population represents more birth years than the current population and thus more of the breed history. Moreover, genetic diversity, measured as individual observed heterozygosity, ranged from 0.27 to 0.37 with an average of 0.34. Observed heterozygosity for the old and current population were very similar, on average 0.34 and 0.33, while the expected heterozygosity was 0.30 for both old and current population. Equal expected heterozygosity for the old and current population suggests an active management of genetic diversity at the population level, which is also supported by a slightly higher observed heterozygosity compared to expected heterozygosity. We can presume that the breeding programme managed to slow down the increase in inbreeding, for example by avoiding mating between relatives in the population. Inbreeding measured based on pedigree ( $F_A$ ), similarity-based relationship matrix ( $F_G$ ) and ROH ( $F_{ROH}$ ) were on average 0.02, 0.32 and 0.07. In the old population, the average inbreeding coefficients were 0.02, 0.32 and 0.06 for  $F_A$ ,  $F_G$  and  $F_{ROH}$ , respectively while in the current population they were slightly higher, being 0.04, 0.33 and 0.08 (Table 2). Spearman's rank correlations between  $F_A$  and  $F_G$ ,  $F_A$  and  $F_{ROH}$ , and  $F_G$  and  $F_{ROH}$  were 0.71, 0.70 and 0.96, respectively. Therefore, we expect little ranking differences between inbreeding coefficient computed from the two estimators based on genomic information ( $F_G$  and  $F_{ROH}$ ). Measures of average inbreeding were assumed to be proxy

of inbreeding in the next generation under the assumption of random mating in our current population. For consistency, using the same estimator for individual and population inbreeding and OC selection decision is necessary. However, the choice of which inbreeding estimator to use over the others might slightly affect the breeding decisions taken by promoting the selection of different individuals. The genetic merit increased by more than 400 points NVI throughout the complete period, equivalent to a rate of change in genetic merit ( $\Delta G$ ) of +8 points NVI per year (results not shown). Rate of inbreeding ranged from 0.05% to 0.09% per year (Figure 2). Overall, genetic merit increased when going from old to current population at the expense of a small decrease in genetic diversity.

#### 3.2 | Genetic merit and diversity after selection

##### 3.2.1 | Selection decision on current population

The optimal contribution scenarios *current\_cons\_x\_weight*, *current\_impCONS\_x\_weight*, and *current\_IMPcons\_x\_weight*, resulted in 53, 47 and 27 selected individuals respectively with contributions to the next generation ranging from 0.14% to 6.49%, 0.04% to 6.65% and 0.13% to 9.52%. All selection decisions at least achieved a genetic

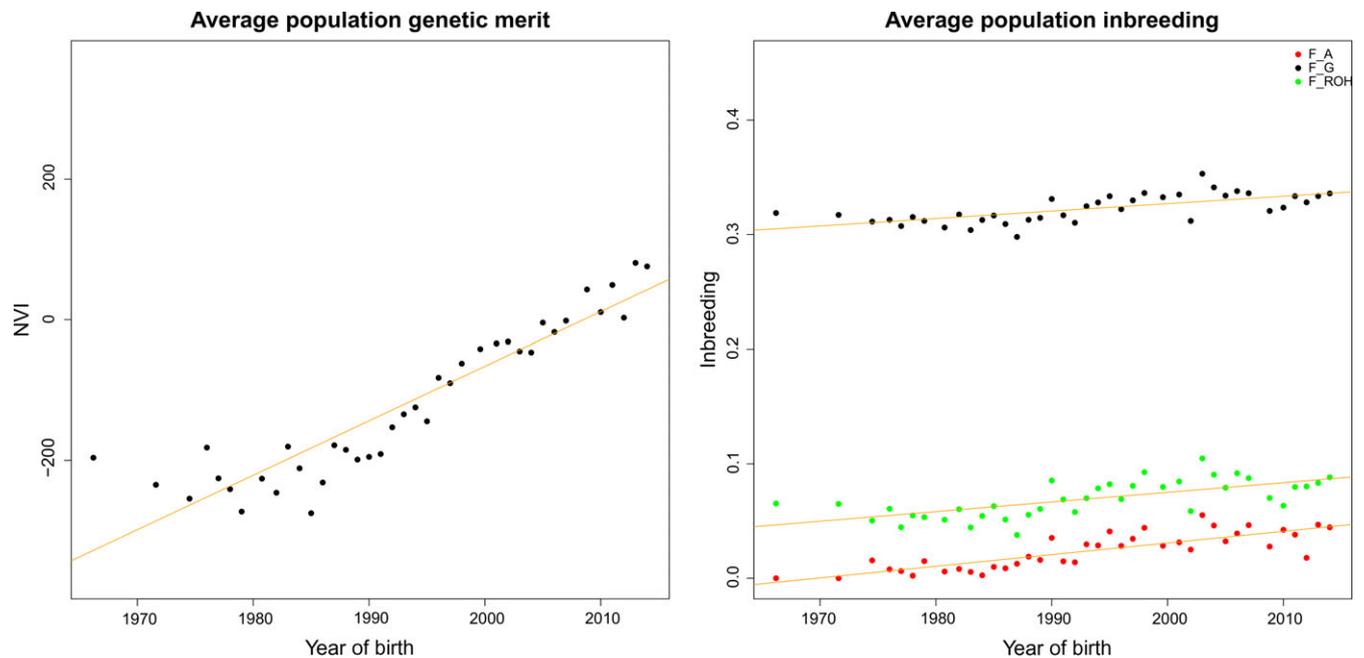


**FIGURE 1** Principal Component Analysis of the relationships between individuals. The X-axis is the first component and the Y-axis the second. First and second components explain 14.5% of the population variance. The grey dots are the old individuals (born before 2000) and the blue dots are the young individuals (born from 2000 onwards). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 2** Whole, current and old populations characteristics

	Whole population ( $N = 413$ )			Current population ( $N = 119$ )			Old population ( $N = 294$ )		
	Min	Average <sup>a</sup>	Max	Min	Average <sup>a</sup>	Max	Min	Average <sup>a</sup>	Max
Year of birth	1962	1992	2014	2000	2007	2014	1962	1988	1999
NVI	-368	-129	140	-201	5	140	-368	-183	121
Observed heterozygosity	0.271	0.338	0.370	0.299	0.332	0.360	0.271	0.341	0.370
$F_A$	0	0.024	0.144	0	0.039	0.144	0	0.018	0.142
$F_G$	0.261	0.321	0.451	0.280	0.333	0.397	0.261	0.316	0.451
$F_{ROH}$	0.004	0.068	0.246	0.014	0.082	0.165	0.004	0.062	0.246

<sup>a</sup>For Year of birth the median is given instead of the average.

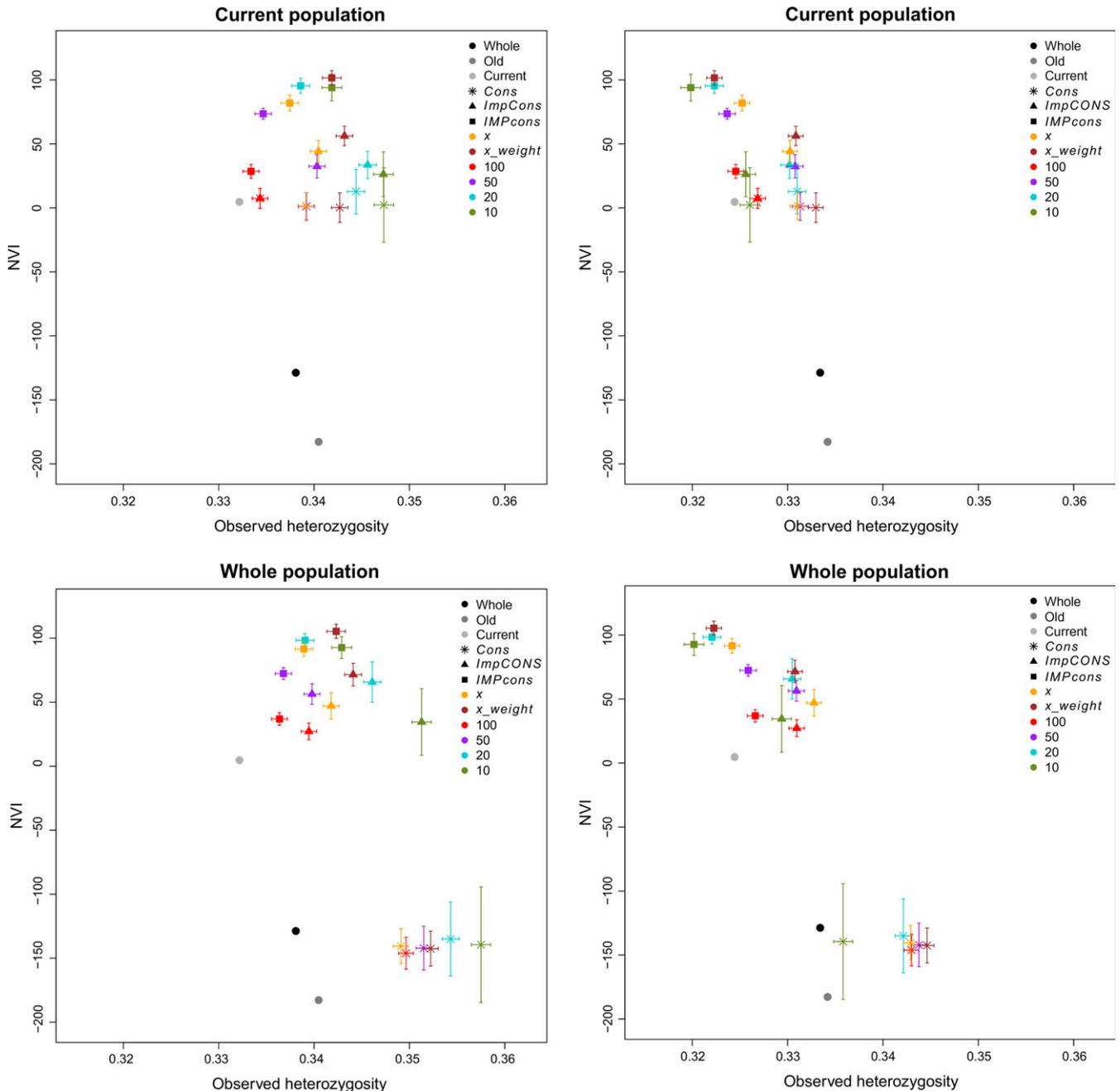


**FIGURE 2** Evolution through time of genetic merit and inbreeding. The figure on the left represents the change in average genetic merit of the whole population through time. The figure on the right represents the change in average inbreeding coefficients through time with in red the inbreeding coefficient based on pedigree, the black based on genomic relationship matrix (similarity-based) and the green based on Runs Of Homozygosity (ROH). The X-axis is the year of birth and the Y-axis is the yearly average genetic merit and average inbreeding coefficients. The orange lines are the linear regression lines. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

merit of the selected group similar to that observed in the complete current population, while using the *impcons* strategies even increased the genetic merit (Figure 3). This increase was even larger for *IMPcons* as its constraint for inbreeding rate from the current population was less stringent. Average observed heterozygosity of the selected groups was always higher than of the complete current population and increased further when less individuals were selected as these decisions forced the selection of only the most diverse individuals. Expected heterozygosity of the selected groups was higher than in the complete current population for the *cons* and *impCONS* strategies and lower for the *IMPcons*. The strategy *impCONS* allowed for more genetic diversity as expected (Figure 3).

### 3.2.2 | Selection decision on whole population

The optimal contribution scenarios *tot\_cons\_x\_weight*, *tot\_impCONS\_x\_weight*, and *tot\_IMPcons\_x\_weight* resulted in 81, 43 and 24 selected individuals with contributions to the next generation ranging from 0.08% to 4.47%, 0.02% to 7.14% and 0.23% to 9.49%, respectively. Genetic merit was lower for the *cons* selection decisions compared to the current population, while it was slightly improved for the *impcons* selection decisions and highest for *IMPcons* (Figure 3). On the one hand, average observed heterozygosity of the selected groups were always higher than in the current population. As expected, the *cons* strategy was the best to reach higher observed



**FIGURE 3** Comparison of genetic merit and diversity between selection decisions for the current and whole population. The X-axis is the average observed heterozygosity and expected heterozygosity and the Y-axis is the average genetic merit of the selected group. The black, dark grey and light grey circles represent the whole population, the current population (born after 2000) and the old population (born before 2000) respectively. The orange, blue, red, purple, light blue and green circles represent the number of individuals selected: optimal number, 100, 50, 20 or 10 selected individuals with equal contribution to the next generation. The brown circles represent the optimal number of selected individuals with their respective contributions to the next generation. The stars stand for the *cons* selection strategy, the triangles for the *impCONS* strategy (constraint on inbreeding based on whole individuals) and the squares for the *IMPcons* strategy (constraint on inbreeding based on the current individuals). Each value is plotted with standard errors. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

heterozygosity. On the other hand, expected heterozygosity of the selected groups was only significantly higher than in the current population for the *cons* and *impCONS* strategies. The selected group of the optimal number of

individuals,  $x_{weight}$ , in the *cons* strategy gave the highest expected heterozygosity, while for the *impCONS* strategy it provided the best compromise between genetic merit and genetic diversity (Figure 3).

### 3.2.3 | Comparison of selection decisions on current versus whole population

Between the 81 selected individuals of *tot\_cons\_x\_weight* and the 53 of *current\_cons\_x\_weight*, 19 were the same, with, however, different contributions (Supporting information: Figure S1). As expected the selected groups from the current population had higher genetic merit whereas the selected groups from the whole population had higher genetic diversity (Figure 4).

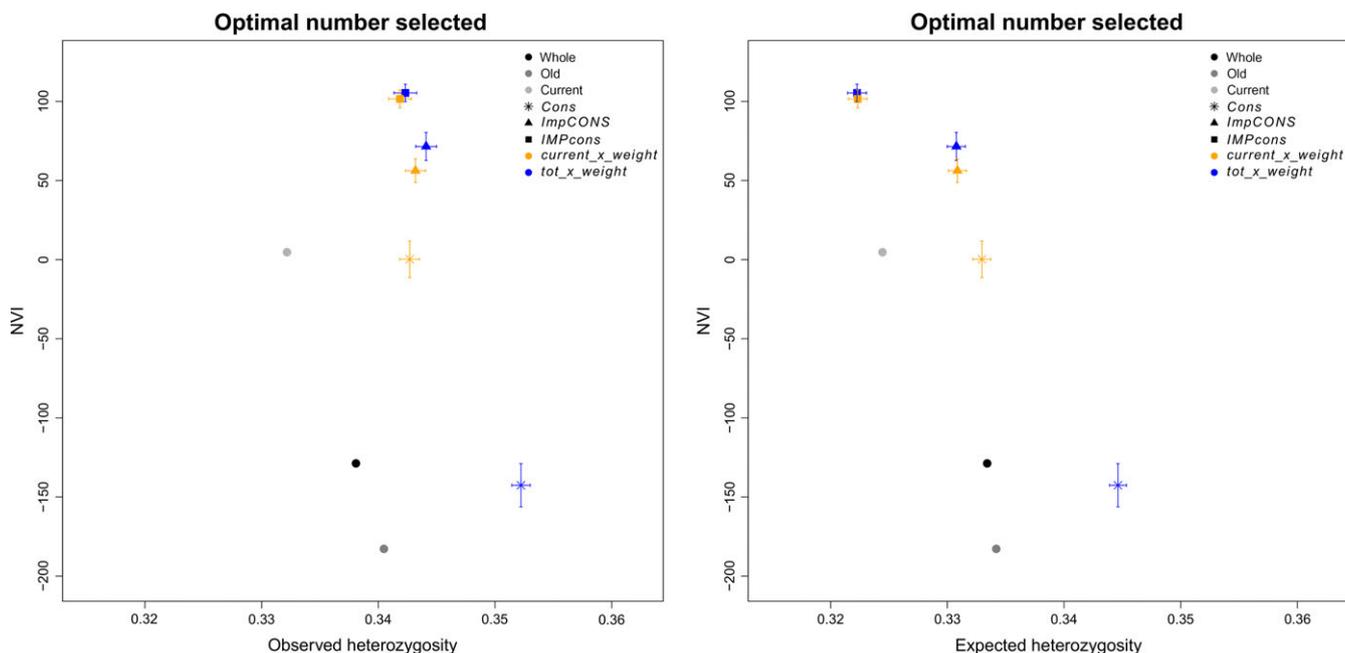
Between the 24 selected individuals of *tot\_IMPcons\_x\_weight* and the 27 of *current\_IMPcons\_x\_weight*, 22 of the selected individuals were identical. When selected from the whole population only two extra individuals were added whereas when selected from the current five additional individuals were selected, however, they add small contribution to the next generation. These scenarios showed the highest genetic merit and both selected groups from the current or whole population give really similar genetic merit and observed and expected heterozygosity (Figure 4).

Between the 43 selected individuals of *tot\_impCONS\_x\_weight* and the 47 of *current\_impCONS\_x\_weight*, 33 of the selected individuals were identical. When selected from the whole population 10 extra individuals were added whereas when selected from the current 14 additional individuals

were selected, however, they add small contribution to the next generation. Unexpectedly, the genetic merit of the selected group from the current population was slightly lower than from the whole population. Also, the genetic diversity in the selected group was smaller for the current population when considering the observed heterozygosity and was the same when considering the expected heterozygosity (Figure 4).

## 4 | DISCUSSION

Most conservation efforts focus on small local breeds as their survival in current livestock production may be threatened by their limited economic potential. Gene bank collections store unique genetic diversity from these particular breeds over time. An important question is whether use of gene bank material can make a positive contribution to current populations. Our data set provided the opportunity to measure the impact of selecting both current and old individuals to produce the next generation on genetic merit and genetic diversity. This study is to our knowledge the first one use empirical germplasm data instead of simulated data (Leroy et al., 2011). In addition, to better understand the dynamics of this population over time, we reviewed the



**FIGURE 4** Comparison between optimum selection decisions for the current and whole population. The X-axis is the average observed heterozygosity and expected heterozygosity and the Y-axis is the average genetic merit of the selected group taking into consideration the optimum contribution affected to each selected individual. The black, dark grey and light grey circles represent the whole population, the current population (born after 2000) and the old population (born before 2000) respectively. The orange and blue circles represent the selection decisions for current and whole population respectively. The stars stand for the *cons* selection strategy, the triangles for the *impCONS* strategy (constraint on inbreeding based on whole individuals) and the squares for the *IMPcons* strategy (constraint on inbreeding based on the current individuals). Each value is plotted with standard errors. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

changes in genetic merit and diversity that happened in the past 50 years of selection.

#### 4.1 | Changes in genetic merit and diversity through time

The evolution through time showed that selection was successful in increasing genetic merit at the cost of a loss of genetic diversity. In fact the average genetic merit, measured by the total merit index NVI, increased by more than 400 points over the 52 years period from which the data came from, which is equivalent to an increase in on average 8 points per year. The observed change in trend in the 80s, with the average NVI starting to follow a linear increase (Figure 2), is likely due to the implementation of breeding value estimation in combination with a breeding goal more closely resembling the current NVI. Between 1980 and 2000, the average genetic merit of Holstein Friesian cattle in the Netherlands increased by about 450 points NVI (de Jong & Stoop, 2014), while in the same period this was only 220 for the MRY. This difference can be explained by the larger scale and probably higher selection intensity of the Holstein breeding programme. For genetic diversity management in ongoing breeding programmes, rates of inbreeding are commonly expressed per generation. Assuming a generation interval of 5.5 years (Hiemstra & de Haas, 2004), our results for the MRY cattle translate into 0.3% and 0.5% increase per generation, which is below the FAO recommended threshold of 0.5%–1% (FAO, 1998) for all three inbreeding measures. As hypothesized from our results, management of the breeding decisions might have avoided large losses of genetic diversity. However, we observed that the old individuals explained a larger proportion of complete MRY population genetic diversity. At last, allele frequencies are likely to have changed through time, leading to changes in heterozygosity status at specific marker sites, between old and current population. Observed and expected heterozygosity measured on sliding windows (Engelsma, Veerkamp, Calus, Bijma, & Windig, 2012) across the complete genome are overall diminished in the current population compared to the old population (Supporting information: Figure S2 and Supporting information: Figure S3) making old individuals more diverse than current ones.

#### 4.2 | Potential of using old individuals for successful selection

The MRY breeding programme has changed through time from being purebred dual purpose to now targeting both purebred and crossbred performance for milk production (CRV catalogue, 2010; Hiemstra & de Haas, 2004). Thus old individuals might not harbour the best genetic merit for

the current breeding goal as they might have been used for breeding in previous times when the breeding goal was different from today. Nevertheless, in our study adding old individuals to the current population raised genetic merit by a few NVI points. As shown by Leroy et al. (2011), using old individuals is of interest to recover genetic diversity due to drift or selection, or when major shifts in breeding goal occur as they are likely to carry interesting variation that might have been erased from the current population due to ongoing selection. Our study indeed confirmed the potential to recover genetic diversity. Supporting information: Figure S4 we looked at the trends of changes through time for the different traits underlying the current breeding goal, as measured through the NVI. Important traits included in the current breeding goal for MRY are the production index (Inet), longevity, fertility, meat value and conformation. Inet, longevity and conformation score increased through time whilst, as expected, fertility and meat value decreased. Old individuals selected in the different proposed scenarios often appeared in the highest part of the distribution for all these traits. It is particularly interesting to see that old individuals selected in our best scenario, *tot\_impCONS\_x*, appear to have high values for fertility and meat. The increased use of such individuals for breeding might also allow conservation of valuable genetic variants for such traits. In fact, the gene bank strategy to select bulls to be stored could partly be based on genetic merit for traits that did not improve much since the origin of the breed, such as meat and fertility in MRY, in contrast to milk production. In general, having a larger sample of individuals from the past would have the advantage to give more alternatives to increase genetic diversity and would presumably have a larger impact on genetic merit. The amount of genetic merit and the level of genetic diversity conserved after selection, in optimal contribution, depend on the number of individuals to select from and the constraint applied. The wanted average genetic diversity might not be reached because there is no possible combination of individuals for such constraint. Moreover, if the number of selected individuals is too low it might be impossible to select a group harbouring enough diversity, if the number of selected individuals is forced too high related individuals will be selected. Larger group of individuals to select from should help to pin-point the best set of constraints to optimize both genetic merit and genetic diversity conservation. One of our optimal selection strategies considered the use of a combination of individuals from the current population, having the best genetic merit for a specific trait of interest, and old individuals, to recover or support the maintenance of genetic diversity. Such findings could help support selection decisions balancing genetic merit and diversity conservation in small local breeds. Studies have shown the benefit of using

optimal contribution strategies to conserve genetic diversity in the long term in the context of genomic selection (Clark, Kinghorn, Hickey, & Van der Werf, 2013; Eynard et al., 2018; Sonesson, Wooliams, & Meuwissen, 2012). The implementation of genomic selection in small local breeds has been recently under consideration in order to allow such breeds to compete in a market mostly dominated by mainstream breeds. However, special care should be taken to efficiently conserve genetic diversity in this fast evolving context. Therefore, further studies focusing on combining the use of optimal contribution for the reference population design as well as for the breeding decisions in small local breeds, taking into consideration current and old available individuals, are needed.

### 4.3 | Gene banking for future use in selection

Practices describe above in breeding decision should be supported by a constant gene banking effort. In the past, the Dutch gene bank sampled most of the available individuals (Danchin-Burge, Hiemstra, & Blackburn, 2011). Nowadays, sampling for gene bank collections should focus on collecting old individuals as representative as it can be of the former population, as well as individuals carrying unique diversity and to collect current individuals of potential interest for the future. The ongoing effort made by gene banks (IMAGE, 2017) to characterize the available material should be supported by studies reporting and inferring the potential of old samples, mostly present in gene bank, to successful long-term animal breeding. Gene banks could benefit from such studies to design optimal sampling strategies for the future, moving from sampling any available individual to targeted sampling of the most relevant individuals. In addition, more emphasis could be put on storing female material and in general to the contribution of female population for genetic diversity conservation. The results of this study show that it appears to be possible to design sustainable breeding populations for small population in need of conservation with a restricted sample of best individuals for both genetic merit and diversity coming from the current and old population. Leroy et al. (2011) performed a long-term analysis, using simulation, and concluded that using gene bank samples to recover genetic diversity was of interest, especially for small local breeds. Their assessment was more reserved for mainstream breeds for which the use of gene bank samples had a substantial negative impact on genetic merit in the long term. Our study, even though based on a short-term analysis, draws similar conclusions from empirical data, in the context of small local breed under selection. However, it should be noted that these results hold only if the contributions drawn from the OC strategy are used for the selection decisions taken in practice. The implementation of such contributions

might be the limiting factor for long-term benefit in practice. A gene bank, by containing most of the old genetic resource, has the potential to contribute to long-term selection and genetic merit and diversity conservation, especially in the case of small local breeds.

## 5 | CONCLUSIONS

The recent interest in characterization of the available genetic material in gene bank collections is going along with questioning how to use gene bank collections for long-term selection targeting simultaneously genetic merit and diversity. We studied the Dutch Meuse-Rhine-Issel cattle population whose genetic diversity is both under threat due to ongoing selection and its small population size. Even though the breed of interest is considered to be a small local breed, it evolved through time as expected for any breeds under selection, by gaining genetic merit but also losing genetic diversity. Thus, we hypothesized that using old bulls in addition to the current breeding population could benefit long-term genetic diversity. Combining the use of optimal contribution (OC) strategy with the utilization of individuals coming both from the current and the old population it was possible to improve genetic merit and genetic diversity simultaneously. Our conclusions show possible benefits of using gene bank genetic material to support long-term selection decisions, especially in small populations under selection.

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## AUTHOR'S CONTRIBUTIONS

MPL, JJW, SJH and SEE designed the study. IH provided the data. SEE performed the analysis and drafted the manuscript. MPL, JJW, SJH and SEE contributed to the interpretation of results, the discussion and commented on the manuscript. All authors read and approved the manuscript.

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## REFERENCES

- Ballou, J. D., & Lacy, R. C. (1995). *Identifying genetically important individuals for management of genetic variation in pedigreed populations*.
- Caballero, A., & Toro, M. A. (2000). Interrelations between effective population size and other pedigree tools for the management of conserved populations. *Genetics Research*, 75(3), 331–343. <https://doi.org/10.1017/S0016672399004449>
- Clark, A. S., Kinghorn, B. P., Hickey, J. M., & Van der Werf, J. H. J. (2013). The effect of genomic information on optimal contribution selection in livestock breeding programs. *Genetics Selection Evolution*, 44, 45.
- CRV catalogue. (2010). *MRY – The most modern breed for cross-breeding*. Retrieved from [http://www.reproduccionanimal.com.mx/AIC\\_RAGLyRF\\_MRY%20a%20la%20vanguardia%20de%20su%20Establo%202009%202010.pdf](http://www.reproduccionanimal.com.mx/AIC_RAGLyRF_MRY%20a%20la%20vanguardia%20de%20su%20Establo%202009%202010.pdf). 20 April 2017.
- Danchin-Burge, C., Hiemstra, S. J., & Blackburn, H. (2011). Ex situ conservation of Holstein-Friesian cattle: Comparing the Dutch, French, and US germplasm collections. *Journal of Dairy Science*, 94(8), 4100–4108. <https://doi.org/10.3168/jds.2010-3957>
- Eding, H., & Meuwissen, T. H. E. (2001). Marker-based estimates of between and within population kinships for the conservation of genetic diversity. *Journal of Animal Breeding and Genetics*, 118(3), 141–159. <https://doi.org/10.1046/j.1439-0388.2001.00290.x>
- Engelsma, K. A., Veerkamp, R. F., Calus, M. P. L., Bijma, P., & Windig, J. J. (2012). Pedigree- and marker-based methods in the estimation of genetic diversity in small groups of Holstein cattle. *Journal of Animal Breeding and Genetics*, 129(3), 195–205. <https://doi.org/10.1111/j.1439-0388.2012.00987.x>
- Eynard, S. E., Croiseau, P., Laloë, D., Fritz, S., Calus, M. P. L., & Restoux, G. (2018). Which individuals to choose to update the reference population? Minimizing the loss of genetic diversity in animal genomic selection programs. *G3: Genes, Genomes Genetics*, 8(1), 113–121.
- Eynard, S. E., Windig, J. J., Hiemstra, S. J., & Calus, M. P. L. (2016). Whole-genome sequence data uncover loss of genetic diversity due to selection. *Genetics Selection Evolution*, 48, 33. <https://doi.org/10.1186/s12711-016-0210-4>
- Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics*, 4th ed. Harlow, UK: Longman Scientific & Technical.
- FAO (1998). *Inbreeding and brood stock management*. Rome, Italy: Electronic Publishing Policy and Support Branch, Communication Division FAO.
- Fernández, B. J., & Toro, M. A. (1999). The use of mathematical programming to control inbreeding in selection schemes. *Journal of Animal Breeding and Genetics*, 116(6), 447–466. <https://doi.org/10.1046/j.1439-0388.1999.00196.x>
- Genetische Evaluatie Stieren. (2017). Retrieved from <http://www.gesfokwaarden.eu/>. 7 April 2017.
- Hanotte, O., & Jianlin, H. (2006). *Genetic characterization of livestock populations and its use in conservation decision-making*. Hiemstra, S. J., & de Haas, Y. (2004). *MRY*. Retrieved from [http://www.regionalcattlebreeds.eu/publications/documents/5384\\_mrij%20koeien\\_engels.pdf](http://www.regionalcattlebreeds.eu/publications/documents/5384_mrij%20koeien_engels.pdf). 8 March 2017.
- Hiemstra, S. J., van der Lende, T., & Woelders, H. (2006). The potential of cryopreservation and reproductive technologies for animal genetic resources conservation strategies. In J. Ruane, & A. Sonnino (Eds.), *The role of biotechnology in exploring and protecting agricultural genetic resources* (pp. 45–59). Rome, Italy: FAO.
- IMAGE (2017). *IMAGE - Innovative management of animal genetic resources*. Retrieved from <http://www.imageh2020.eu/>. 8 October 2017.
- deJong, G., & Stoop, M. (2014). *Genomic bulls in The Netherlands and their impact on the population – Genetic trend B&W bulls*.
- Leroy, G., Danchin-Burge, C., & Verrier, E. (2011). Impact of the use of cryobank samples in a selected cattle breed: A simulation study. *Genetics Selection Evolution*, 43, 36. <https://doi.org/10.1186/1297-9686-43-36>
- Maignel, L., Boichard, D., & Verrier, E. (1996). *Genetic variability of French dairy breeds estimated from pedigree information*. Paper presented at the Interbull meeting, Veldhoven, The Netherlands.
- Meuwissen, T. H. E. (1997). Maximizing the response of selection with a predefined rate of inbreeding. *Journal of Animal Science*, 75(4), 934–940. <https://doi.org/10.2527/1997.754934x>
- Nejati-Javaremi, A., Smith, C., & Gibson, J. P. (1997). Effect of total allelic relationship on accuracy of evaluation and response to selection. *Journal of Animal Science*, 75(7), 1738–1745. <https://doi.org/10.2527/1997.7571738x>
- Notter, D. R. (1999). The importance of genetic populations diversity in livestock populations of the future. *Journal of Animal Science*, 77(1), 61–69. <https://doi.org/10.2527/1999.77161x>
- Oldenbroek, K. (2017). *Genomic management of animal genetic diversity*. Wageningen, The Netherlands: Wageningen Academic Publishers. <https://doi.org/10.3920/978-90-8686-297-9>
- Purfield, D. C., Berry, D. P., McParland, S., & Bradley, D. G. (2012). Runs of homozygosity and population history in cattle. *BMC Genetics*, 13, 70. <https://doi.org/10.1186/1471-2156-13-70>
- Purfield, D. C., McParland, S., Wall, E., & Berry, D. P. (2017). The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. *PLoS ONE*, 12(5), e0176780. <https://doi.org/10.1371/journal.pone.0176780>
- de Roos, A. P. W., Schrooten, C., Veerkamp, R. F., & van Arendonk, J. A. M. (2011). Effects of genomic selection on genetic improvement, inbreeding, and merit of young versus proven bulls. *Journal of Dairy Science*, 94(3), 1559–1567. <https://doi.org/10.3168/jds.2010-3354>
- Sargolzaei, M., Chesnais, J. P., & Schenkel, F. S. (2014). A new approach for efficient genotype imputation using information from relatives. *BMC Genomics*, 15, 478. <https://doi.org/10.1186/1471-2164-15-478>
- Sonesson, A. K., Wooliams, J. A., & Meuwissen, T. H. E. (2012). Genomic selection requires genomic control of inbreeding. *Genetics Selection Evolution*, 44, 27. <https://doi.org/10.1186/1297-9686-44-27>
- The cattle site. (2017). *Meuse rhine issel*. Retrieved from <http://www.thecattlesite.com/breeds/dairy/111/meuse-rhine-issel/>. 9 October 2017.
- Thompson, E. A. (2013). Identity by Descent: Variation in meiosis, across genomes, and in populations. *Genetics*, 194(2), 301. <https://doi.org/10.1534/genetics.112.148825>

- Thornton, P. K. (2010). Livestock production: Recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1554), 2853. <https://doi.org/10.1098/rstb.2010.0134>
- Toro, M. A., Fernández, J., & Caballero, A. (2009). Molecular characterization of breeds and its use in conservation. *Livestock Science*, 120(3), 174–195. <https://doi.org/10.1016/j.livsci.2008.07.003>
- VanRaden, P. M. (2008). Efficient methods to compute genomic predictions. *Journal of Dairy Science*, 91(11), 4414–4423. <https://doi.org/10.3168/jds.2007-0980>
- Yang, J. A., Benyamin, B., McEvoy, B. P., Gordon, S., Henders, A. K., Nyholt, D. R., ... Visscher, P. M. (2010). Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics*, 42(7), 565–569. <https://doi.org/10.1038/ng.608>

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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