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Optimising the Use of Cryopreserved Genetic Resources for the Selection and Conservation of Animal Populations

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ABSTRACT

Genetic diversity is essential for the sustainability and adaptability of populations, and is thus a central pillar of the agro-ecological transition. However, within a population, it is inevitable that some amount of genetic variability is lost, and efforts must be made to limit this as much as possible. A valuable tool in this endeavour could be the use of cryopreserved genetic resources in cryobanks, which could assist in the management of various animal populations in the contexts of both selection and conservation. We performed simulations that revealed that the most appropriate use of *ex situ* genetic resources depends on characteristics of the target population and its management objectives. For populations under conservation, the aim is to maintain genetic diversity, which was best achieved by the regular use of cryopreserved genetic resources at each generation. For populations under selection, instead, the concern is the addition of additive genetic variability, which benefited from the use of cryopreserved collections over only a few generations based primarily on the genetic values of donors. The use of cryopreserved semen had a beneficial effect when breeding objectives were changed. In both cases, the use of cryopreserved individuals in animal populations requires a large amount of reproductive material: for breeds under selection because the number of offspring is high, and for breeds under conservation because the frozen semen is used repeatedly over a long period. The use of cryopreserved material appears to be an effective means of managing the genetic variability of an animal population, either by slowing down the erosion of variability or by helping to redirect a selection objective. However, care must be taken with populations under selection to limit the disadvantages associated with the reintroduction of old genetic material, in particular the gap in breeding values for traits of interest. Finally, our study highlights the need for a sufficiently large stock of cryopreserved material in collections (e.g., number of doses, straws) to ensure the most efficient use.

1 | Background

As part of the agroecological transition aimed at improving the sustainability of animal production, efforts are currently underway to diversify farming systems at different scales (Dumont et al. 2013). Diversification has also been recommended in support of locally adapted and complementary food systems that preserve biodiversity (IPES-Food 2016). This idea is especially relevant in the field of livestock breeding, where diversity is needed to redefine new, more sustainable breeding systems (Dumont et al. 2020; Ducos et al. 2021) that can overcome the many challenges—both environmental and societal—that have arisen in recent years (Hoffmann 2010). For example, climate change has the potential to affect populations, thus increasing the importance of good adaptive capacities (Gaughan et al. 2019; Pasqui and Di

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Giuseppe 2019). Similarly, the demand for products that are more respectful of the environment and animal welfare (i.e., local farming or free-range farming) has increased considerably in Europe, requiring a conversion of livestock production systems (Thornton 2010; Escribano 2016). In this context, genetic diversity and genetic resources serve as a fundamental pillar for the implementation of new breeding systems and for the maintenance of the adaptive capacities of livestock breeds (Notter 1999). However, it is well known that genetic diversity tends to decrease in animal populations over time (Hagger 2005; Eynard et al. 2016; Doekes et al. 2019; Doublet et al. 2019; Hulsegge et al. 2022). In addition to reducing the adaptive capacity of populations, a low level of genetic diversity yields slower genetic gain (Meuwissen 1997; Sonesson et al. 2012) and an increased level of inbreeding, which the Food and Agriculture Organisation recommends limiting to a 0.5%–1% increase per generation (FAO 1998).

In order to conserve animal genetic diversity in the long term, many countries have set up gene banks to cryoconserve their genetic resources. For example, the French National Cryobank collects all of the available reproductive resources of livestock species (Danchin Burge et al. 2006). Thus far, though, existing cryopreserved resources have not been widely used in breeding programs. This may be due to a lack of knowledge, as the benefits of using cryopreserved resources in animal breeding programs have been investigated in only a few studies. Early work on this topic presented simulations showing that the use of bulls from a cryobank can help to limit the loss of genetic diversity in a population under selection, and can be used to change breeding goals (Leroy et al. 2011). Indeed, it may even be possible to use cryopreserved genetic resources to bring back alleles for adaptation to climate change, as was shown for Nordic populations (Kantanen et al. 2015). Using real data, some studies have shown the potential of using cryopreserved material to improve the short-term management of local and international breeds in the Netherlands (Doekes et al. 2018; Eynard et al. 2018). Recently, the effectiveness of using frozen semen of an ancient bull from a French germplasm collection was demonstrated in practice (Jacques et al. 2023).

We hypothesize that the limited use of cryopreserved germplasm is mainly due to a lack of available recommendations. To address this, in the present study we simulated various scenarios for the use of cryopreserved resources in different breeding schemes with different goals. In particular, we investigated the potential impact of using these resources in a local breed under conservation or for a breed under selection, with or without a change in selection objective; in each case, we identified the best strategy. This information can be used to promote the use of *ex situ* genetic resources for the management of genetic diversity.

2 | Methods

We performed stochastic simulations of breeding programs using the R package MoBPS, version 1.6.64 (Pook et al. 2020; Pook 2021). All additional calculations and graphical representations were performed using R, version 4.2.1 (R Core team 2020), and the ggplot2 R package (Wickham 2011).

2.1 | Definition of Scenarios: Type of Breeding Program, Prolificacy and Uses of Germplasm Collections

We defined four types of scenarios that differed in the selection process used, and considered two traits of interest, hereafter named Trait 1 and Trait 2. Both traits have a heritability of 0.4 and exhibit a negative genetic correlation of −0.3.

The scenario rm. corresponds to a random choice of parents among the candidates; the scenario max_BV corresponds to a choice of parents that maximises genetic gain on a synthetic index combining both traits; the scenario max_GD corresponds to a choice of parents that minimises the loss of genetic diversity; and the scenario OCS corresponds to a choice of parents that maximises genetic gain under the constraint of a maximal increase in kinship of 0.5% per generation, as recommended by the FAO (FAO 1998). The max_BV, max_GD, and OCS scenarios were modelled using the optimal contribution strategy (OCS) (Meuwissen 1997) implemented in MoBPS via the optiSel package, version 2.0.5 (Wellmann 2019, 2021). The relationship matrix in the OCS was calculated using the vanRaden method (VanRaden 2008). For the scenarios involving the use of OCS, we set a uniform constraint for the female kernel, allowing us to consider their contributions to be equal. The maximum contribution of each male candidate was set at 5% for the max_BV scenario, and 1% for the max_GD and OCS scenarios, in order to obtain a realistic minimum number of fathers at each generation. For the OCS scenario, an upper bound to restrict the average kinship of the progeny was calculated for each generation, based on an estimate of average kinship within the male and female kernels proposed as candidates and prohibiting an increase beyond 0.5%.

The effect of prolificacy was studied by simulating three levels: 1, 2 or 10 offspring per mating. These values were chosen to cover the majority of livestock species (horses, ruminants and pigs).

Finally, these scenarios were implemented either with the contemporary kernels only, or by adding 40 cryopreserved sires to the pool of candidates. In the latter case, cryopreserved individuals were systematically re-evaluated at the same time as contemporary individuals. All scenarios were replicated 20 times.

2.2 | Definition of Scenarios: Uses of Germplasm Collections During a Change in Breeding Goal

This case applied only to populations under selection. Three scenarios considering changes in the breeding goals were considered. They all occured after the pre-burn-in and selection burn-in periods, (see Figure 1) and were simulated during 15 generations. The breeding goal changes were defined according to the weights

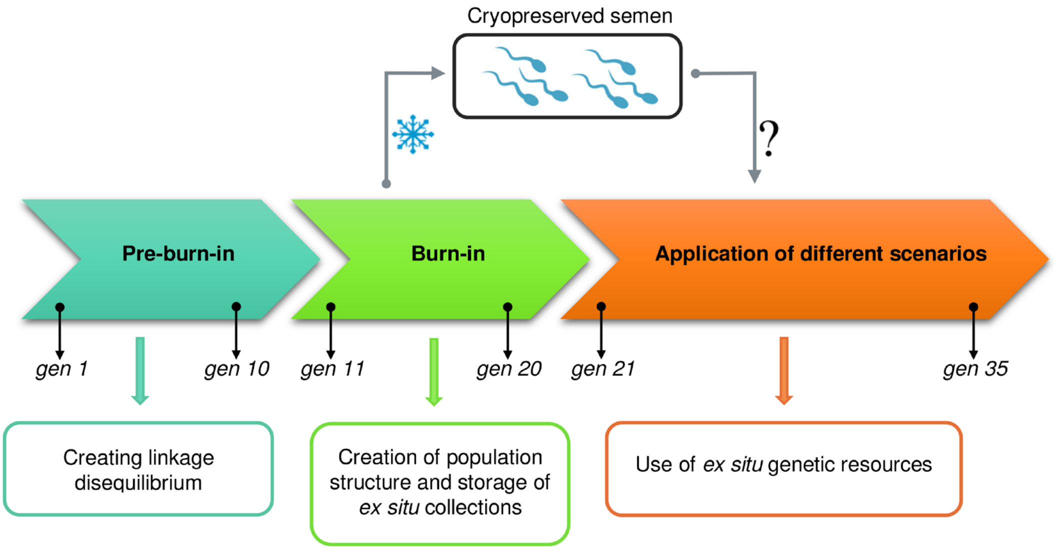

FIGURE 1 | Breeding steps of simulations. [Colour figure can be viewed at wileyonlinelibrary.com]

given to Trait 1 and Trait 2 in the synthetic index. The first scenario corresponded to 'No' change of selection objective in which Trait 1 had the highest weight (0.8); the second scenario corresponded to a 'moderate' change in selection objective, in which each trait had an equal weight (0.5); and the last scenario corresponded to a 'strong' change in objective, with a complete reversal of the weights of Trait 1 and Trait 2 in the synthetic index (i.e., $\text{weight}_{\text{Trait1}} = 0.2$ and $\text{weight}_{\text{Trait2}} = 0.8$). Each category of breeding goal change was tested for the three levels of prolificacy.

2.3 | Initialization of Simulated Populations and Pre-Burn-In

2.3.1 | Genetic Architecture and Definition of a Synthetic Index

First, we simulated a founder population of 500 individuals with a sex ratio of 0.5, with 5000 genetic markers (SNPs) distributed over 4 chromosomes. SNPs were regularly distributed along the genome, that is, the average distance in centimorgans between neighbouring SNPs was 4.105 cM.

Each trait was simulated with 500 additive quantitative trait loci (QTLs), which were randomly sampled from the SNP data with effects sizes drawn from a Gaussian distribution. The parameters for the two traits were initialized with a genetic mean of 0 and a variance of 1. The true breeding values (TBVs) were generated by the genotype simulation and used to standardise the EBV for each trait. Then, EBVs were combined in a synthetic index using a weight of 0.8 for Trait 1 and a weight of 0.2 for Trait 2. Thus, for each animal, the index value was obtained as follows:

$$\text{Index}_i = \text{weight}_{\text{Trait1}} \frac{\text{EBV}_{\text{Trait1}_i} - \overline{\text{EBV}_{\text{Trait1}_{\text{cand}}}}}{\text{sd}\left(\text{TBV}_{\text{Trait1}_{\text{cand}}}\right)} + \text{weight}_{\text{Trait2}} \frac{\text{EBV}_{\text{Trait2}_i} - \overline{\text{EBV}_{\text{Trait2}_{\text{cand}}}}}{\text{sd}\left(\text{TBV}_{\text{Trait2}_{\text{cand}}}\right)}$$

with Index_i the synthetic index value of animal i; $\text{EBV}_{\text{Trait1}_i}$ and $\text{TBV}_{\text{Trait1}_i}$ the estimated breeding value and the true breeding value, respectively, of animal i for Trait 1 (idem for Trait 2); and $\text{weight}_{\text{Trait1}}$ and $\text{weight}_{\text{Trait2}}$ the weights of Trait 1 and Trait 2 in the selection objective, respectively (here, 0.8 and 0.2).

The candidate sires were ranked according to this index. The estimated genetic values of individuals (EBVs) were obtained using GBLUP evaluation models incremented in MoBPS software. The SNPs involved in the QTLs of the two traits were not taken into account in the evaluations, which is generally the case with the SNP chips used routinely in breeding schemes as the causal SNPs are not available. The reference population for the estimates consisted of all phenotyped individuals available at the time of evaluation.

2.3.2 | Pre-Burn-In and Initial Population Creation

We performed a pre-burn-in process to generate linkage disequilibrium and genetic structure for 20 simulated populations. To do this, we simulated 20 founder kernels of 250 males and 250 females, then random breeding cycles for 10 generations. Each new cohort included 250 males and 250 females (candidates for selection) resulting from the mating of the 20 best males and the 250 best females of the previous cohort, with prolificacy set at 2, half-sib matings prohibited, and the selection of sires based on the synthetic index defined above (see Figure S1). At the end of these 10 generations, the aim was to obtain a population with an average kinship of 10% in generation 10.

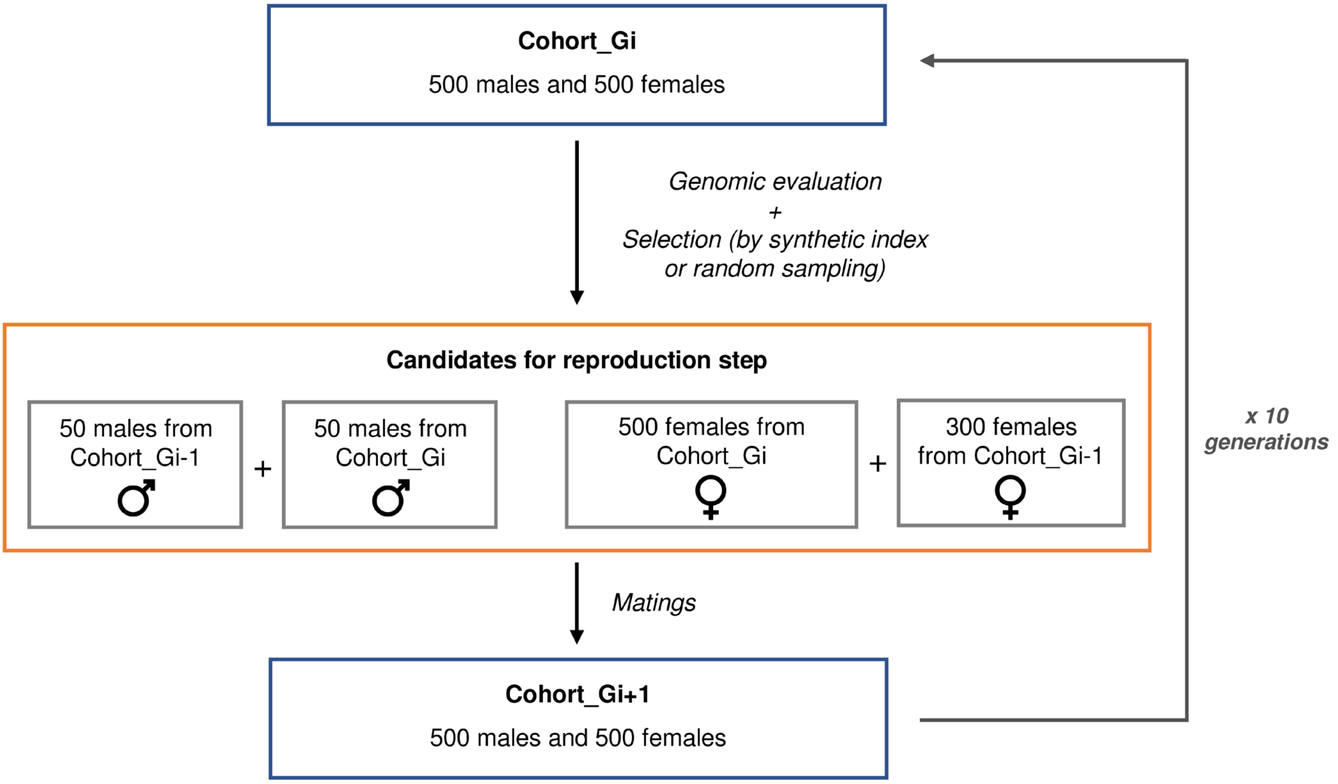

FIGURE 2 | Breeding steps for the burn-in phase for populations under selection or conservation. [Colour figure can be viewed at wileyonlinelibrary.com]

2.4 | Burn-In Phases, Breeding Scheme and Set Up of Germplasm Collection

2.4.1 | Burn-In and Simulated Population Size

Following the pre-burn-in phase, we performed two types of burn-in: one to mimic a population under selection and the other to mimic a population under conservation.

We simulated a breeding program for livestock with overlapping generations (see Figure 2). Each new cohort was composed of 500 males and 500 females, obtained from matings between a kernel of 100 males and 800 females from the last two generations, with prolificacy always set at 2. Individuals were selected for reproduction (i) on the basis of the synthetic index defined above for selection burn-in or (ii) by random sampling in the case of conservation burn-in (in which case the only influence on genetic diversity was genetic drift). These burn-in processes, replicated 20 times, were performed over 10 generations to obtain an average kinship in the 20th generation of (i) 14%–15% in the case of the selection scheme or (ii) 10%–11% in the case of the conservation scheme.

2.4.2 | Simulation of Cryopreserved Individuals: Creation of Germplasm Collection

From the 50 individuals available as potential sires for each generation, 10 were chosen from cryopreserved samples. Thus, cryopreserved individuals may have already been used as sires before, which is generally the case for the sires present in the French National Cryobank. The simulated cryobank contained 10 cryopreserved sires randomly selected from generations 11, 13, 15 and 17; representing a constitution of collections spread out over time. These 40 individuals represented a rough approximation of the current heterogeneity of the French National Cryobank (Jacques et al. 2024). The cryobank had a fixed composition over the generations, and there was no replacement of cryopreserved sires over the generations. Cryopreserved individuals were included among the potential sire candidates at each generation for the different scenarios.

2.5 | Criteria for Comparing Scenarios

2.5.1 | Use of Cryopreserved Individuals

For each scenario involving the use of *ex situ* genetic resources, we quantified the effective use of cryopreserved individuals by counting their average number of direct descendants (i.e., sons or daughters), as well as indirect descendants (e.g., grandchildren, great-grandchildren). Thus, for each rank (the number of generations separating a cryopreserved father from his descendants, e.g., rank 1 for children, rank 2 for grandchildren, rank 3 for great-grandchildren, etc.), the number of descendants resulting from the use of cryopreserved individuals was recorded.

2.5.2 | Breeding Values

For each generation, the 'true' genetic values (TBVs) of the two traits were calculated, together with the empirical genetic

TABLE 1 | Number of cryopreserved individuals used and direct offspring produced in populations under conservation (rm and max_GD).

	rm		**max_GD**	
Scenario type	**Nb. Cryopreserved sires used**	**Nb. Offspring per cryopreserved sire**	**Nb. Cryopreserved sires used**	**Nb. Offspring per cryopreserved sire**
Prolificacy 1	40 [sd = 0]	107.5 [sd = 2.24]	40 [sd = 0]	162.5 [sd = 4.25]
Prolificacy 2	40 [sd = 0]	107.8 [sd = 2.41]	40 [sd = 0]	160 [sd = 5.19]
Prolificacy 10	40 [sd = 0]	109.9 [sd = 4.23]	39.90 [sd = 0.31]	152.6 [sd = 9.81]

Note: The random mating scenario is abbreviated with 'rm' and the scenario which maximises the genetic diversity is abbreviated with 'max_GD'.

variances for these two traits (i.e., variance of the genetic values of the simulated individuals). The EBVs for traits 1 and 2, as well as the value of the sires' standardised synthetic index, were evaluated at each generation. We also calculated a value for the 'true' selection index, that is, based on EBVs and without standardisation, for each generation by applying their respective weights in the selection objective. In this way, we were able to compare the simultaneous evolution of the two traits.

2.5.3 | Genetic Diversity

The evolution of genetic diversity was studied by comparing its value at the start of the program (generation 20) with that obtained at the end of the program (generation 35). To do this, we extracted the genotypes of 1000 individuals from generations 20 and 35 for each simulation and estimated various measures of diversity using PLINK 1.9 software (Purcell et al. 2007; Chang et al. 2015).

First, we measured the mean heterozygosity values at these two time steps (HeG20 and HeG35) to obtain the following differential:

$$\Delta \mathrm{Het} = \overline{\mathrm{He}_{\mathrm{G35}}} - \overline{\mathrm{He}_{\mathrm{G20}}}$$

In a similar way, we investigated variations in allele frequencies over time between generation 20 and generation 35 of each simulation using PLINK's 'freq' function. We then calculated the mean difference between the allelic frequencies in generation 35 and generation 20 for all SNPs according to the following equation:

$$\overline{\Delta \mathrm{Freq}} = \sum_{N_{\mathrm{SNP}}} \left(\mathrm{freq\_G35}_{\mathrm{SNP}_i} - \mathrm{freq\_G20}_{\mathrm{SNP}_i} \right) / N_{\mathrm{SNP}}$$

with $\mathrm{freq\_G35}_{\mathrm{SNP}_i}$ the frequency of marker i in the 35th generation, $\mathrm{freq\_G20}_{\mathrm{SNP}_i}$ the frequency of marker i in the 20th generation, and N_{SNP} the total number of markers considered.

We also examined the number of enriched rare SNPs, defined as those with a minor allele frequency (MAF) below 5% in the G20 cohort and above 5% in the G35 cohort.

Average kinship was calculated for each generation using the 'kinship.emp.fast' function of the MoBPS package, which estimates the real kinship based on the recombination points of genotypes by sampling the population. This estimator is based on the notion of shared identical segments per progeny. From this, the increase in average kinship (ΔK) was computed.

Finally, the genetic distance between generation 20 and generation 35 was estimated for each scenario using Nei's method (Nei 1972) with MoBPS's 'get.distance' function. The Nei distance takes into account the effects of mutation and genetic drift, and its estimated value is proportional to evolutionary time. It is calculated using the following expression:

$$D = -\ln\left(\sum_l \sum_u X_u Y_u \Big/ \sqrt{\left(\sum_l \sum_u X_u^2 \right)\left(\sum_l \sum_u Y_u^2 \right)} \right)$$

with X and Y the two population at the 20th and 35th generations and X_u (or respectively Y_u) the frequencies for the allele u of the locus l in the population X (or respectively in the population Y).

2.6 | Statistical Analyses

Statistical tests were performed using the 'lm' function, post hoc tests were conducted using multcomp and emmeans packages (Hothorn et al. 2002; Lenth et al. 2021), and type II ANOVAs were performed using the car package (Fox et al. 2019). All the analyses of variance models used in this study have been written in the Table S1.

3 | Results

3.1 | Population Under Conservation

Two scenarios were considered for this type of population: random mating (rm) and maximisation of genetic diversity (max_GD).

3.1.1 | Quantifying the Use of Ex Situ Resources

Almost all sires from *ex situ* collections were used in rm. and max_GD (Table 1). There was no significant difference in the number of cryobanked sires used (two-factor ANOVA, $F = 2.11$, df = 114, $p = 0.07$ see Table S1).

Whatever the scenario, collections of cryopreserved genetic resources were used throughout the 15 simulated generations (Figure S2) and the offspring of cryopreserved sires were used as fathers or mothers in the next generations, allowing the preservation of these lineages in populations (Figure 3). The average number of offspring per cryopreserved sire was higher in max_GD (two-factor ANOVA test, $F = 537.8$, $\mathrm{df} = 114$, $p < 0.05$; see Table S1) with a higher heterogeneity between sires as compared to rm., as shown by the higher standard deviation. A higher prolificacy also increased the standard deviation of the number of offspring, due to the constrained population size.

3.1.2 | Impact on Genetic Diversity

The average kinship in a population increased less rapidly when cryopreserved collections were used (Figure 4), particularly for max_GD (Table S1). The same pattern was observed for the average observed heterozygosity of the populations (Figure 5), which, although inevitably decreasing over time, was less degraded in the scenarios using *ex situ* collections (Table S1). Similar findings were noted in the evolution of allelic frequencies and MAF (Figure 5), which declined less when using cryopreserved collections (Table S1), resulting in a smaller decrease in expected heterozygosity. The use of cryopreserved genetic resources led to

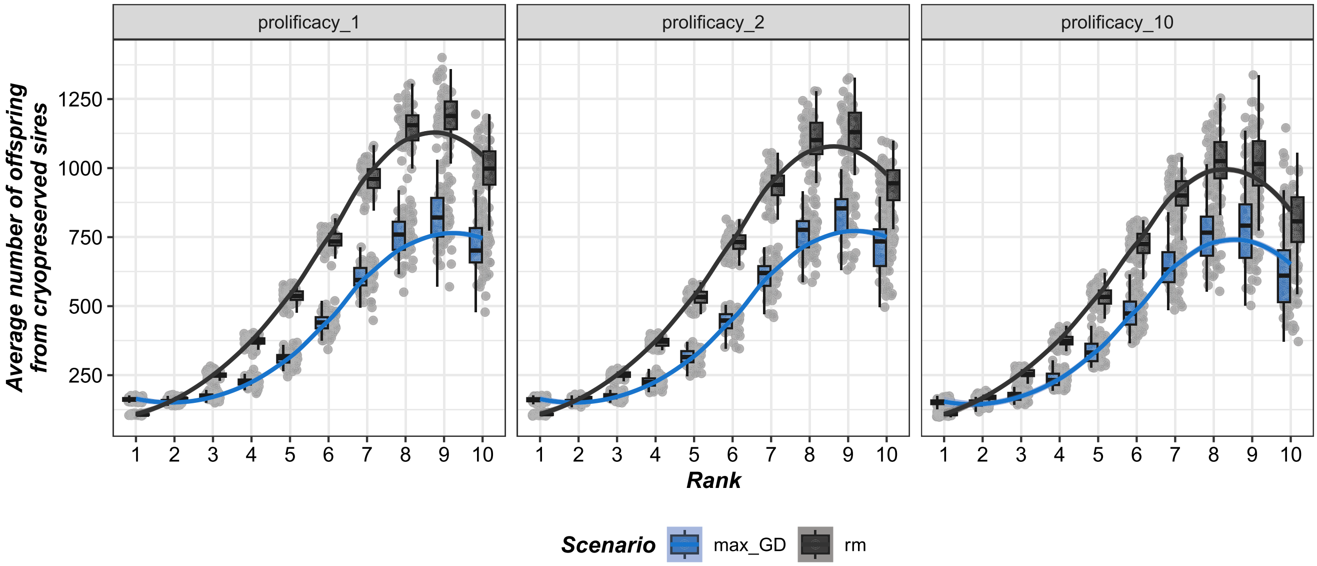

FIGURE 3 | Number of offspring of cryopreserved individuals across generations in the conservation programs. The *x*-axis corresponds to the genealogical rank of individuals in relation to their ancestors from the *ex situ* collections (i.e., rank 1 for children, rank 2 for grandchildren, rank 3 for great-grandchildren, etc.). The random mating scenario (rm) is represented in black and the scenario which maximises the genetic diversity (max_GD) is represented in blue. [Colour figure can be viewed at wileyonlinelibrary.com]

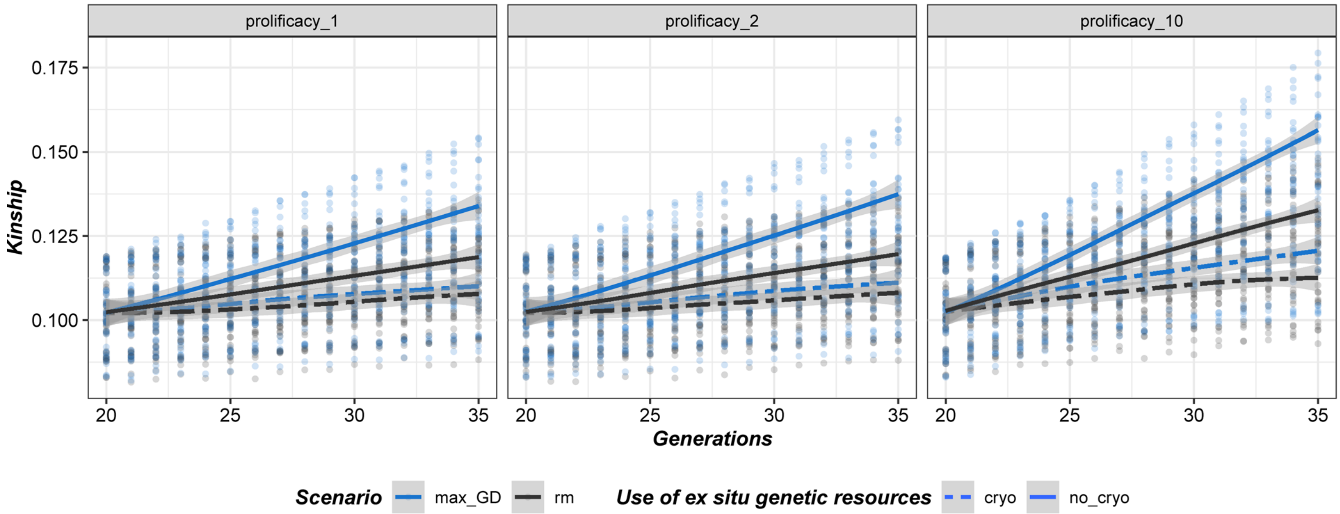

FIGURE 4 | Evolution of average kinship across generations within populations in the conservation scenarios. The solid and dashed lines represent results without and with the use of *ex situ* collections, respectively. The random mating scenario (rm) is represented in black and the scenario which maximises the genetic diversity (max_GD) is represented in blue. [Colour figure can be viewed at wileyonlinelibrary.com]

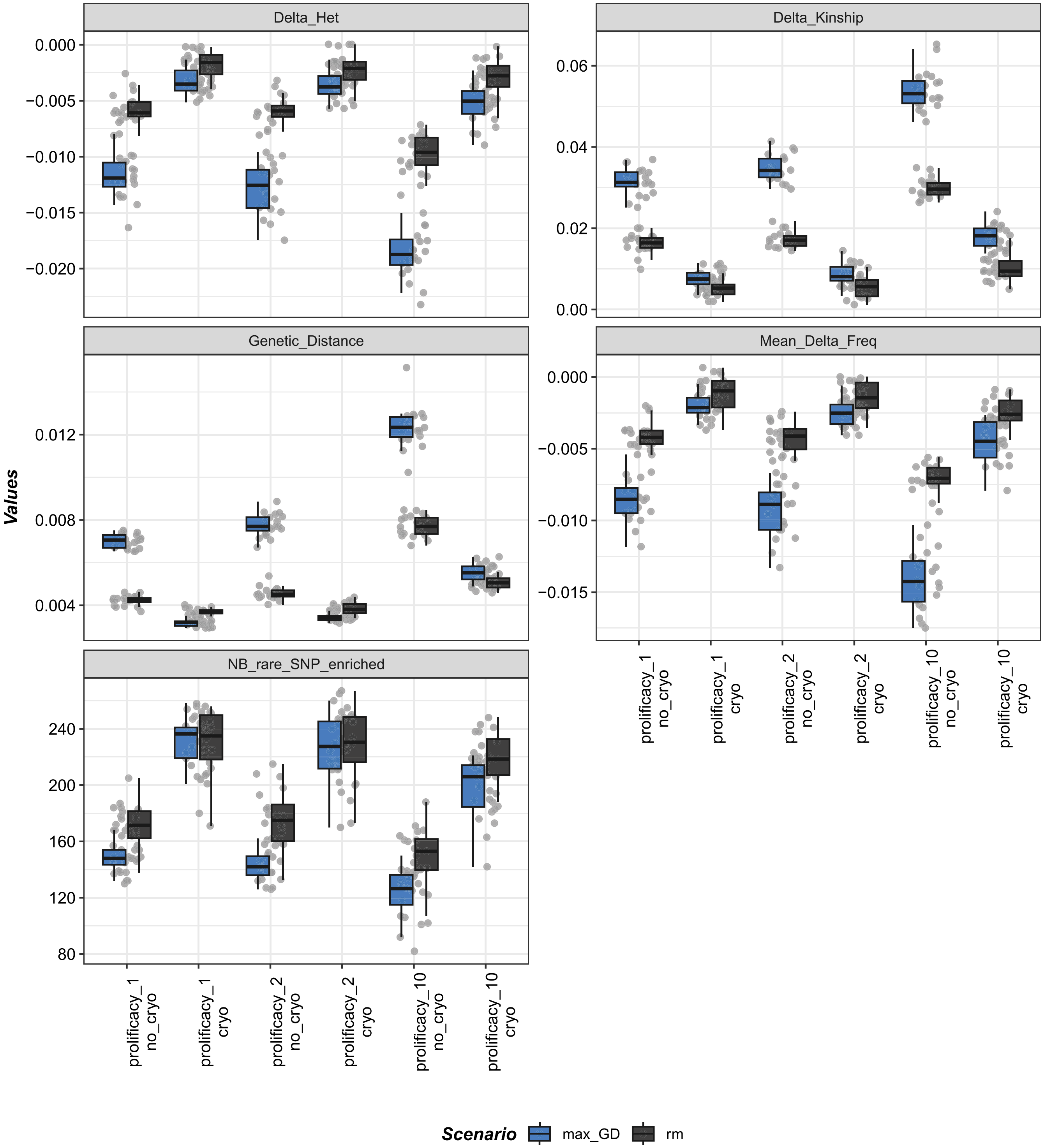

FIGURE 5 | Measurements of genetic diversity for scenarios of conservation populations. In black, scenario with random mating (rm). In blue, scenario maximising genetic diversity (max_GD). Each dot represents one replicate. Delta_Het: The difference in heterozygosity between generations 20 and 35; Delta_Kinship: The difference in kinship between generations 20 and 35; Genetic_Distance: Nei's genetic distance between generations 20 and 35; Mean_Delta_Freq: The difference in allelic frequencies between generations 20 and 35; NB_rare_SNP_enriched: The number of SNPs with a MAF lower than 0.05 in generation 20 whose frequency increased in generation 35. [Colour figure can be viewed at wileyonlinelibrary.com]

a significant increase in the number of rare SNPs conserved in populations (Table S1). Details of the measurements of genetic diversity for the different scenarios are presented in Table S2.

Populations differentiated less over time when individuals from *ex situ* collections were used, with lower Nei distances between generations 20 and 35 in all scenarios (Table S1).

TABLE 2 | Number of cryopreserved individuals used and direct offspring produced in populations under selection (max_BV and OCS).

	OCS		max_BV	
Scenario type	**Nb. Cryopreserved sires used**	**Nb. Offspring per cryopreserved sire**	**Nb. Cryopreserved sires used**	**Nb. Offspring per cryopreserved sire**
Prolificacy 1				
None	0.30 [sd = 0.47]	23 [sd = 10.84]	0 [sd = 0]	NA
Moderate	1 [sd = 0.79]	25.86 [sd = 14.90]	0.20 [sd = 0.52]	95.5 [sd = 2.29]
Strong	2.70 [sd = 2.13]	31.1 [sd = 10.42]	0.75 [sd = 0.78]	138.7 [sd = 57.96]
Prolificacy 2				
None	0.25 [sd = 0.44]	27.2 [sd = 10.83]	0.05 [sd = 0.22]	108 [sd = NA]
Moderate	1 [sd = 0.97]	28.5 [sd = 18.73]	0.20 [sd = 0.52]	113 [sd = 29.44]
Strong	2.25 [sd = 1.48]	27.2 [sd = 10.94]	0.55 [sd = 0.83]	136.7 [sd = 49.91]
Prolificacy 10				
None	0.35 [sd = 0.59]	25 [sd = 18.70]	0 [sd = 0]	NA
Moderate	0.80 [sd = 0.89]	31.4 [sd = 13.43]	0 [sd = 0]	NA
Strong	2.30 [sd = 1.78]	41.1 [sd = 13.98]	0.55 [sd = 0.69]	132.8 [sd = 60.47]

Note: The scenario which maximise genetic progress is abbreviated with 'max_BV' and the scenario which maximises genetic progress under a constraint of genetic diverisy is abbreviated with 'OCS'. None: scenario with no change in the weighting of the two traits; Moderate: weighting of both traits increased to 0.5; Strong: complete inversion of weights between the two traits.

3.2 | Population Under Selection

Scenarios considered were those maximising breeding values: the maximisation of breeding values (max_BV) and the optimal contribution selection (OCS).

3.2.1 | No Change in Selection Objective

3.2.1.1 | Quantifying the Use of Ex Situ Resources. Very few cryopreserved sires were used in the max_BV scenario (Table 2). Actually, the use of cryopreserved sires occurred only once, in a case when prolificacy was set to two. A slightly higher number of cryopreserved sires was used in the OCS scenario (Table 2 and Figure 6) with the preferential use of sires from the most recent generations (Figure S3).

3.2.1.2 | Impact on Genetic Values. Overall, for each scenario, the use of *ex situ* collections had no impact on the value of the synthetic index (see Figure S4). The use of cryopreserved collections enabled Trait 2 to be maintained or even improved (in contrast to the deterioration that occurred without *ex situ* contributions), whereas Trait 1 deteriorated slightly (Figure 7). These observations were similar for both scenarios (i.e., max_BV and OCS), although the effect was more moderate for the OCS scenario. Prolificacy had no significant impact on the genetic values of Trait 1 and Trait 2 (Table S1). The decrease in additive genetic variance along generations observed in max_BV was slowed down by the inclusion of cryopreserved sires as candidates (Figure 8), whereas no impact of the use of cryopreserved sires was observed on genetic variance for OCS.

3.2.1.3 | Impact on Genetic Diversity. The average kinship in the population increased less rapidly when cryopreserved sires were available as candidates; this change was more moderate in OCS than in max_BV (Figure 9). Similar results were obtained for the other measurements of genetic diversity in both scenarios (see Figure 10 for max_BV and Figure 11 for OCS). The average observed heterozygosity and the allele frequencies were less degraded when proposing cryopreserved sires, while a significant increase in the number of rare SNPs was also observed (four-ways ANOVA results in Table S1). Populations differentiated less over time when individuals from *ex situ* collections were proposed, with lower Nei distances between generations 20 and 35 for both selection scenarios. Details of the measurements of genetic diversity for the different scenarios are presented in Additional_file7 (Table S3 for max_BV and Table S4 for OCS).

3.2.2 | Change in Selection Objective

3.2.2.1 | Quantifying the Use of Ex Situ Resources. In both scenarios (i.e., max_BV and OCS), more cryopreserved individuals were used when a 'strong' change in selection objective was applied (Table 2). This increase was not influenced by prolificacy (three-ways ANOVA, $F = 21.87$, df = 346, $p < 0.05$ see Table S1). The average number of offspring per cryopreserved sire increased with the magnitude of the change in selection objective (from 'none' to 'strong') (three-ways ANOVA, $F = 8.71$, df = 346, $p < 0.05$ see Table S1).

In the long term, the overall contribution of individuals from cryopreserved collections was significant, with family origins persisting over generations, especially with OCS, or when a strong change in selection objective took place (Figure 6).

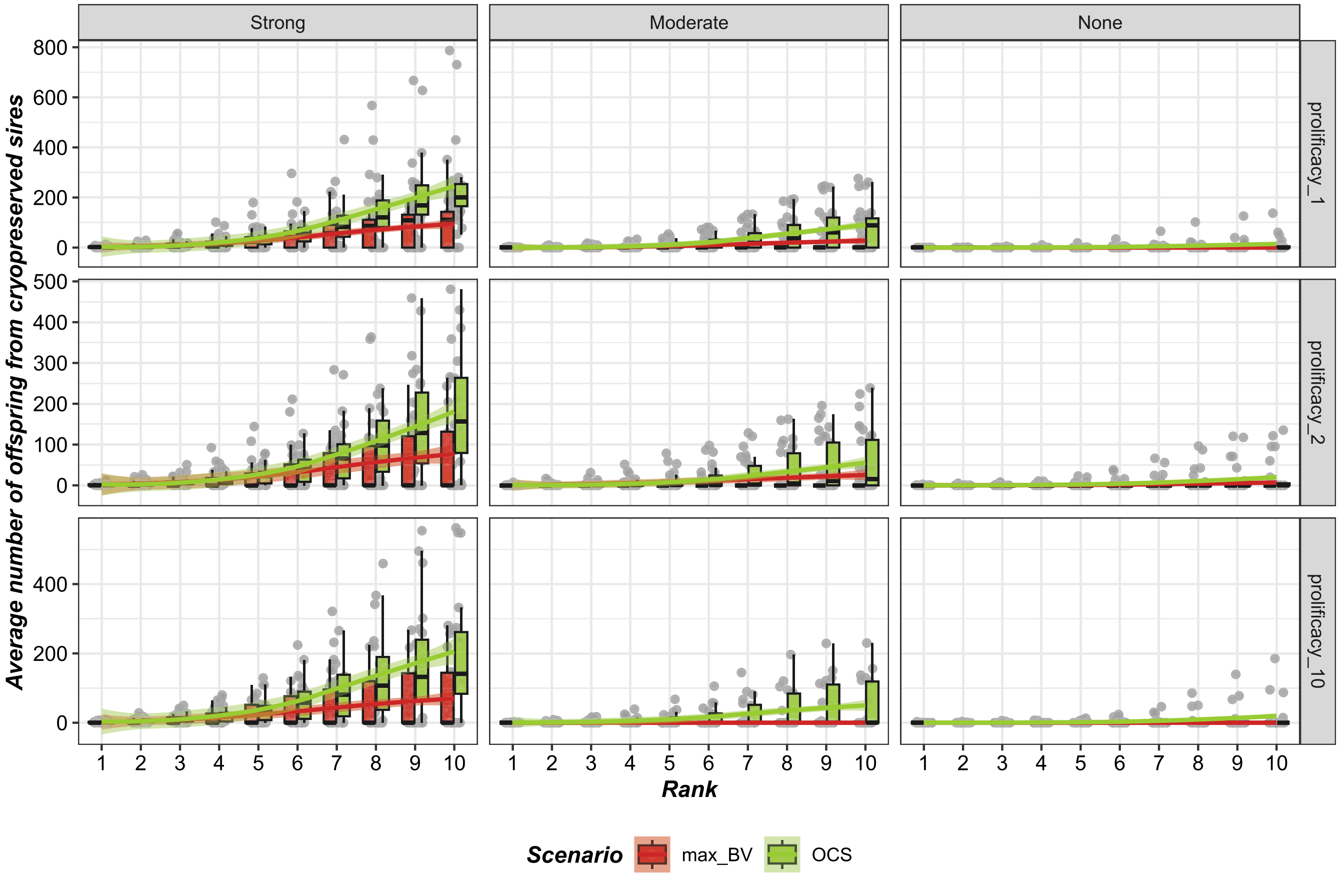

FIGURE 6 | Number of offspring of cryopreserved individuals across generations in the different management programmes of selected population. The *x*-axis corresponds to the genealogical rank of individuals in relation to their ancestors from the *ex situ* collections (i.e., rank 1 for children, rank 2 for grandchildren, rank 3 for great-grandchildren, etc.). The scenario which maximise genetic gain (max_BV) is represented in red and the scenario which maximises genetic gain under a constraint of genetic diverisy (OCS) is represented in green. [Colour figure can be viewed at wileyonlinelibrary.com]

For scenarios with a strong change in selection objective, cryopreserved individuals were used over several generations (from two to five consecutive generations) and more ancient cryopreserved individuals were used as sires (see Figure S3).

3.2.2.2 | **Impact on Genetic Values.** For scenarios where the change of objective was moderate, the use of cryopreserved sires had no impact on the genetic progress of Trait 1 and Trait 2. In cases where the change in objective was strong, we observed the opposite pattern observed in scenarios with no change in selection objective: a positive impact of *ex situ* collections on Trait 1, together with a slight degradation of Trait 2, especially for the max_BV scenarios (Figure 7). These results were similar for both max_BV and OCS scenarios, but with less marked effects for OCS. In the case of a change in selection objective, the responses of the two traits were not affected by prolificacy (see Table S1).

Figure 8 shows the evolution of the genetic variances of Trait 1 and Trait 2 according to the different scenarios. For max_BV, the use of *ex situ* resources could weakly slow down the degradation of additive genetic variance. For OCS, the use of cryopreserved genetic resources had no significant impact on the additive genetic variance of either trait (Table S1).

3.2.2.3 | **Impact on Genetic Diversity.** The average increase in kinship was limited by the use of cryopreserved collections for max_BV (Figure 9). Other measures of diversity showed that the use of cryopreserved sires helped to maintain genetic variability when selection objectives changed (Figure 10). For OCS, which already took into account a constraint on diversity, no difference was noted in average kinship evolution when selection objectives changed (Figures 9 and 11). There was no strong effect of prolificacy in the different scenarios; however, the exception was OCS, for which the impact on average kinship was only visible when prolificacy was set to 10 (Figures 9 and 11).

4 | Discussion

Our study quantified how the use of contemporary and cryopreserved sires over several generations could affect the genetic diversity and genetic gain of populations being managed either for selection or conservation purposes. To our knowledge, this work is the first to use stochastic simulations to model and optimise the use of cryopreserved genetic material over multiple generations; previous research has focused on either deterministic simulations or real data analysis addressing the case of a single generation (Leroy et al. 2011; Doekes et al. 2018; Eynard et al. 2018).

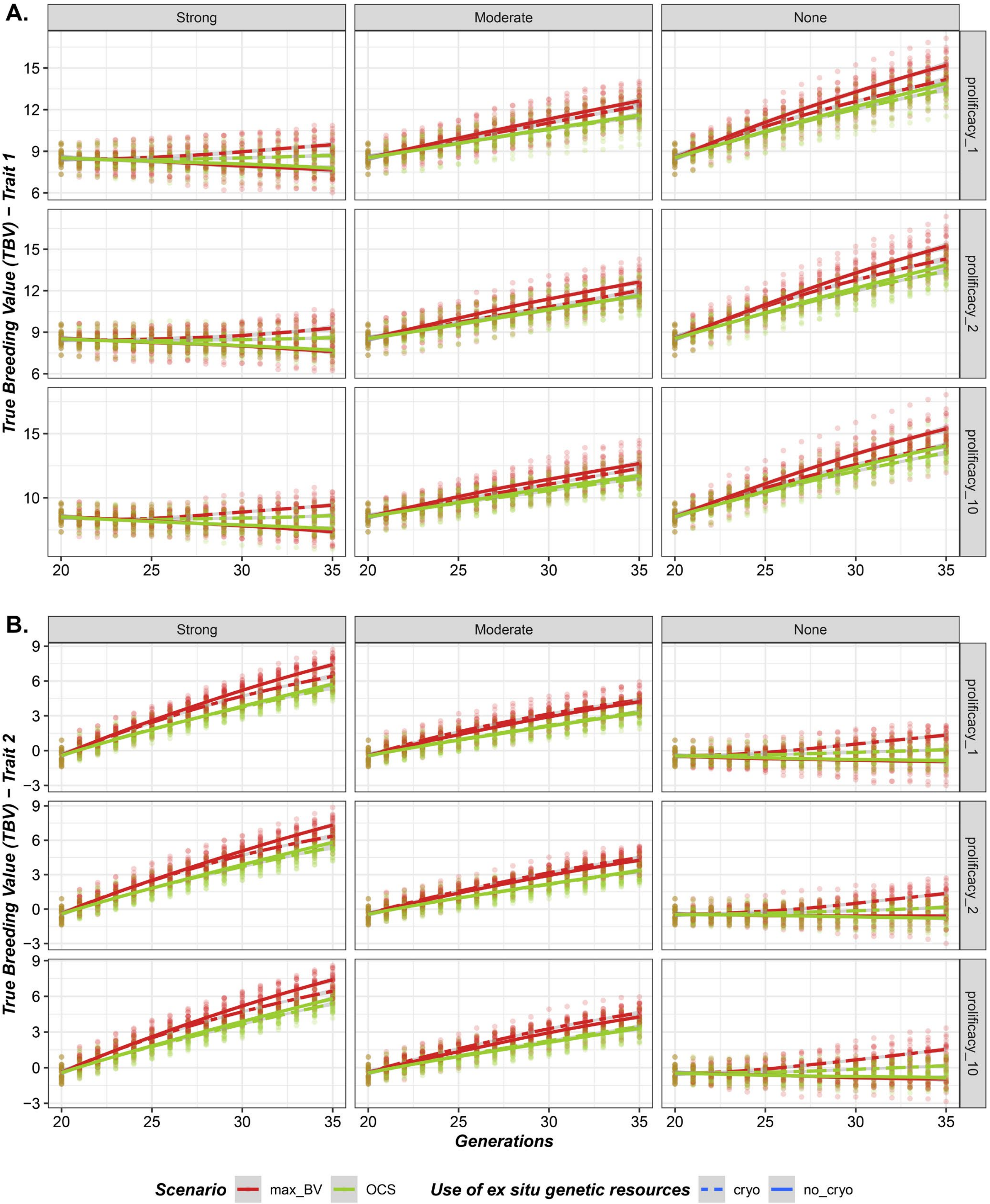

FIGURE 7 | Evolution over time of the average genetic value of populations for the two traits of interest in the different scenarios, with and without the use of cryopreserved collections. The solid and dashed lines represent results without and with the use of *ex situ* collections, respectively. The scenario which maximises genetic gain (max_BV) is represented in red and the scenario which maximises genetic gain under a constraint of genetic diversity (OCS) is represented in green. [Colour figure can be viewed at wileyonlinelibrary.com]

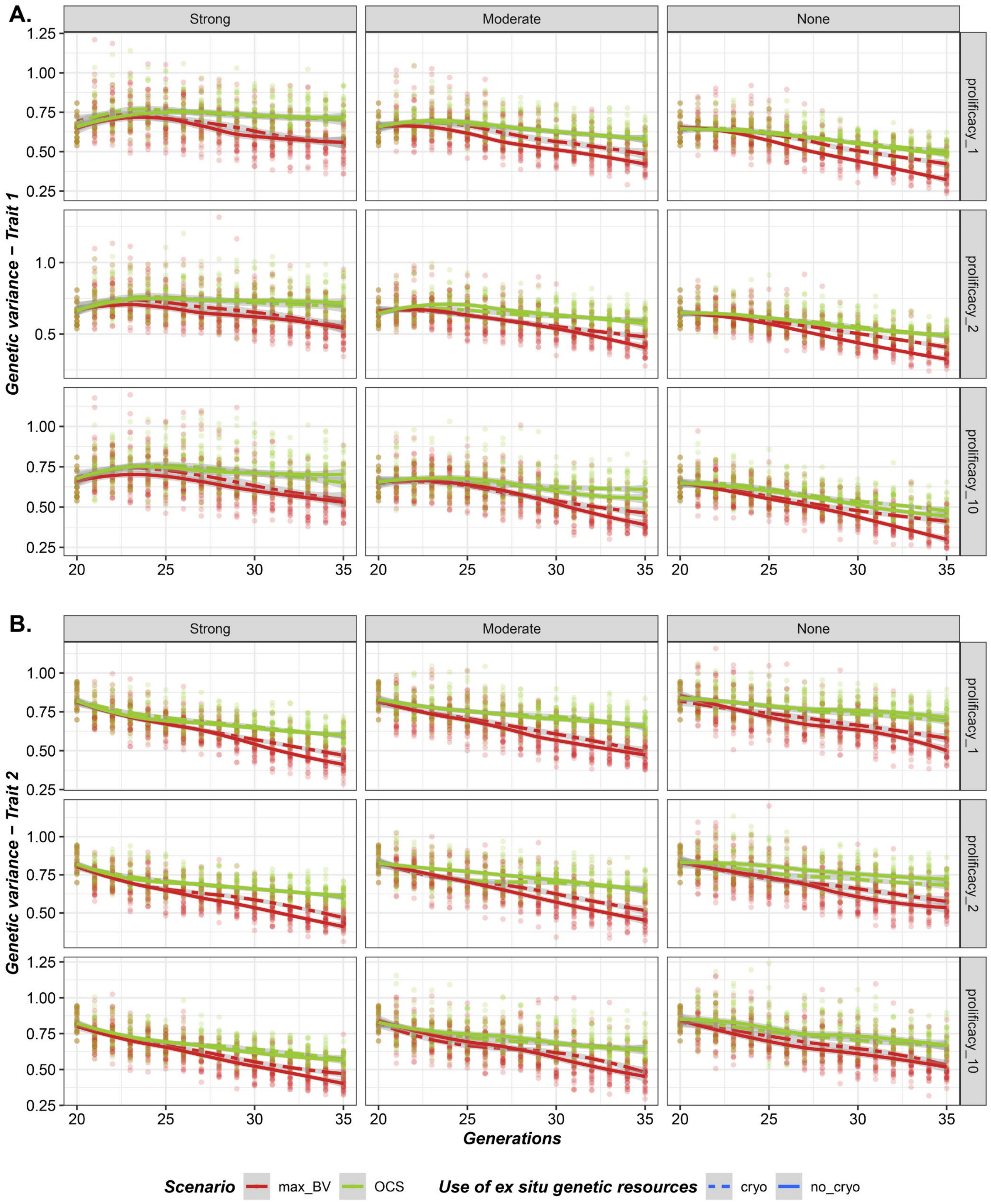

FIGURE 8 | Evolution over time of the average additive genetic variance of populations for the two traits in the different scenarios, with and without the use of cryopreserved collections. The solid and dashed lines represent results without and with the use of *ex situ* collections, respectively. The scenario which maximises genetic gain (max_BV) is represented in red and the scenario which maximises genetic gain under a constraint of genetic diversity (OCS) is represented in green. [Colour figure can be viewed at wileyonlinelibrary.com]

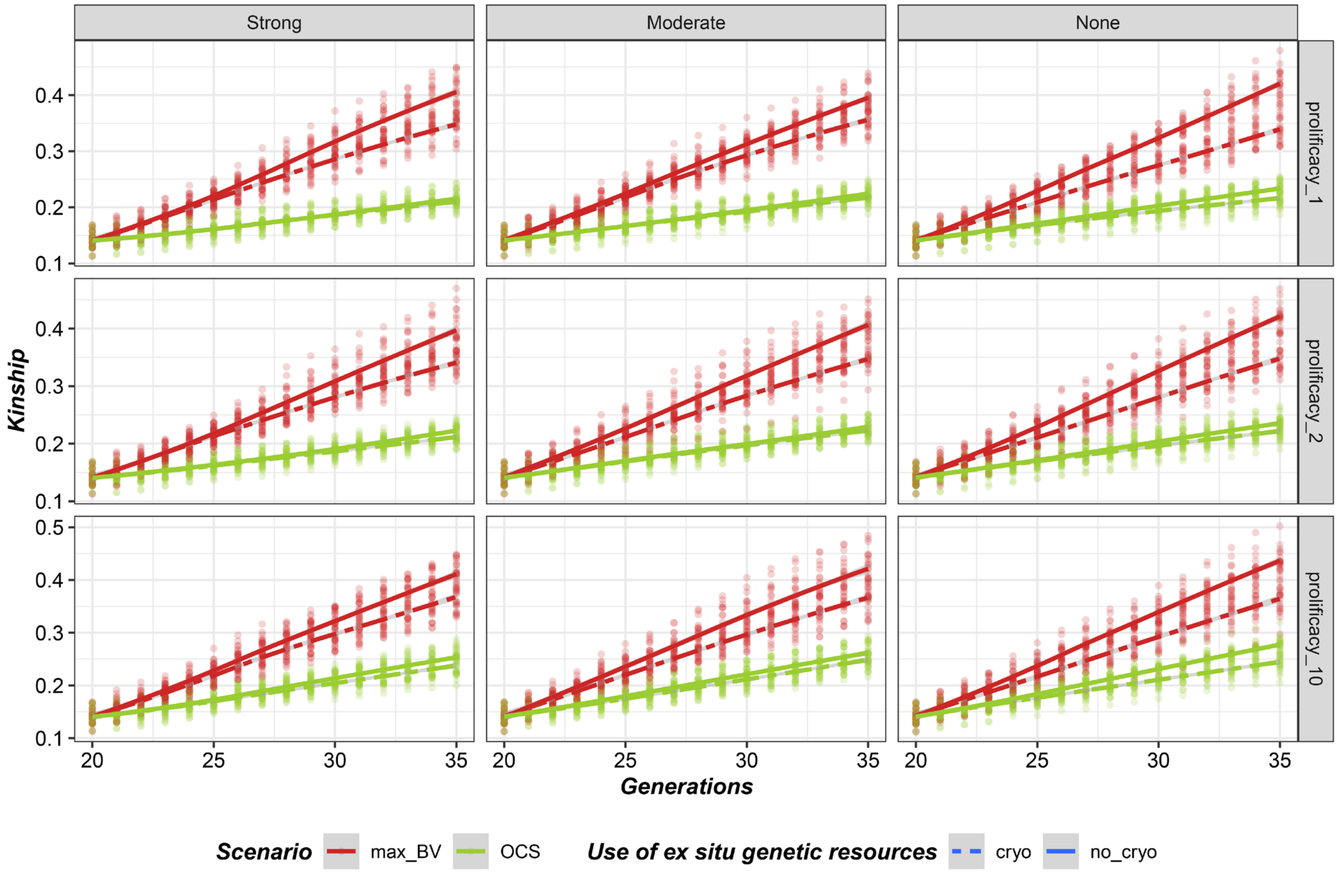

FIGURE 9 | Evolution of average kinship across generations within populations in the different scenarios of selected population. The solid and dashed lines represent results without and with the use of *ex situ* collections, respectively. The scenario which maximise genetic gain (max_BV) is represented in red and the scenario which maximises genetic gain under a constraint of genetic diverisy (OCS) is represented in green. [Colour figure can be viewed at wileyonlinelibrary.com]

The number of males used from the cryobank varied among the scenarios. Both the OCS and random strategies yielded generally similar results, although the random strategy, strictly from the viewpoint of conservation of genetic diversity, would have a higher impact and might therefore be preferred (e.g., the random strategy avoided matings between closely related individuals). For highly selected populations, only a few individuals from collections were used, but they had large numbers of offsprings in the subsequent generations, that is, higher values than those obtained with OCS. This strategy ensures that a sufficiently large panel of offspring is available to be used as parents, preserving the re-introduced lineage over time. This utilisation approach was reported in the reuse of an old sire within a cattle population under selection: frozen semen was used to produce several offspring, of which a few became confirmed sires in the breed (Jacques et al. 2023). Indeed, the larger the number of progeny produced by a single male, the more variability resulting from meiosis is obtained (i.e., gametic variance), thus increasing the chance of obtaining more favourable allelic combinations. This is especially likely when the donor exhibits a high level of heterozygosity.

The impact of using cryoconserved males on genetic values differed according to the scenarios as summarised in Table 3. The results of our simulations confirm the effectiveness of using cryopreserved collections to maintain or reintroduce genetic diversity in animal populations. Even in the case of highly selected populations, the use of cryoconserved sires had a positive impact on genetic variance (Table 3). Depending on the type of breeding programme considered, the integration of cryopreserved materials makes it possible to maintain a high level of diversity or at least prevent its rapid deterioration. This is especially obvious in conserved populations, where the use of cryoconserved sires limited the loss of genetic diversity over time (i.e., reduced genetic drift). These results are consistent with those obtained by Sonesson et al. (2002), who showed that the use of cryopreserved sires enables one to maintain the genetic diversity of a population in conservation, and that better results were obtained when using cryopreserved sires from two different generations as compared to sampling these sires from a single generation. In addition, levels of observed heterozygosity also benefited from the use of cryopreserved resources. Combined with reduced levels of kinship, this can have a positive impact on the fitness of conserved populations by limiting inbreeding depression (Chapman et al. 2009; Szulkin et al. 2010). This underlines the necessary complementarity between in situ and *ex situ* resources to optimise the management of conserved populations. The collections should not only serve as a safe for the future, but also as a resource available for populations at present. An indicator to guide the choice of cryopreserved sires for the management of diversity was recently proposed with that aim (Jacques et al. 2022).

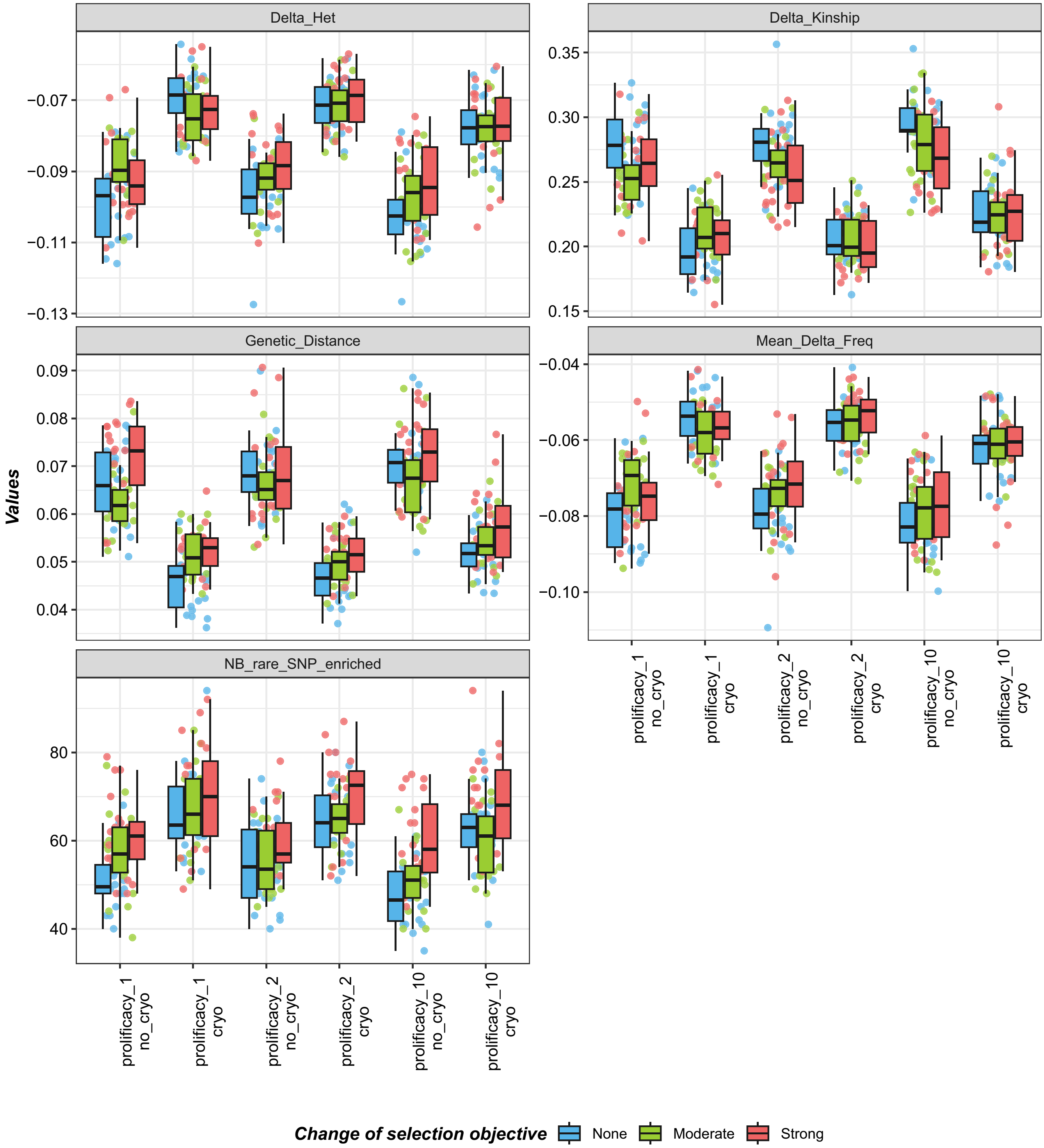

FIGURE 10 | Measurements of genetic diversity for scenarios which maximise genetic gain (max_BV). In blue, none: Scenario with no change in the weighting of the two traits; in green, moderate: Weighting of both traits increased to 0.5; in red, strong: Complete inversion of weights between the two traits. Delta_Het: The difference in heterozygosity between generations 20 and 35; Delta_Kinship: The difference in kinship between generations 20 and 35; Genetic_Distance: Nei's genetic distance between generations 20 and 35; Mean_Delta_Freq: The difference in allelic frequencies between generations 20 and 35; NB_rare_SNP_enriched: The number of SNPs with a MAF lower than 0.05 in generation 20 whose frequency increased in generation 35. [Colour figure can be viewed at wileyonlinelibrary.com]

For populations under selection, where the only aim is to improve breeding values, the use of cryopreserved individuals still had a positive impact on genetic diversity (Table 3). The advantage of using cryoconserved collections is to increase the number of selection candidates in the breeding stock, and thus limit genetic drift. Even if the cryopreserved sires are not retained, the optimisation of contributions (OCS) implemented in max_BV led to a lower kinship at the population level. This

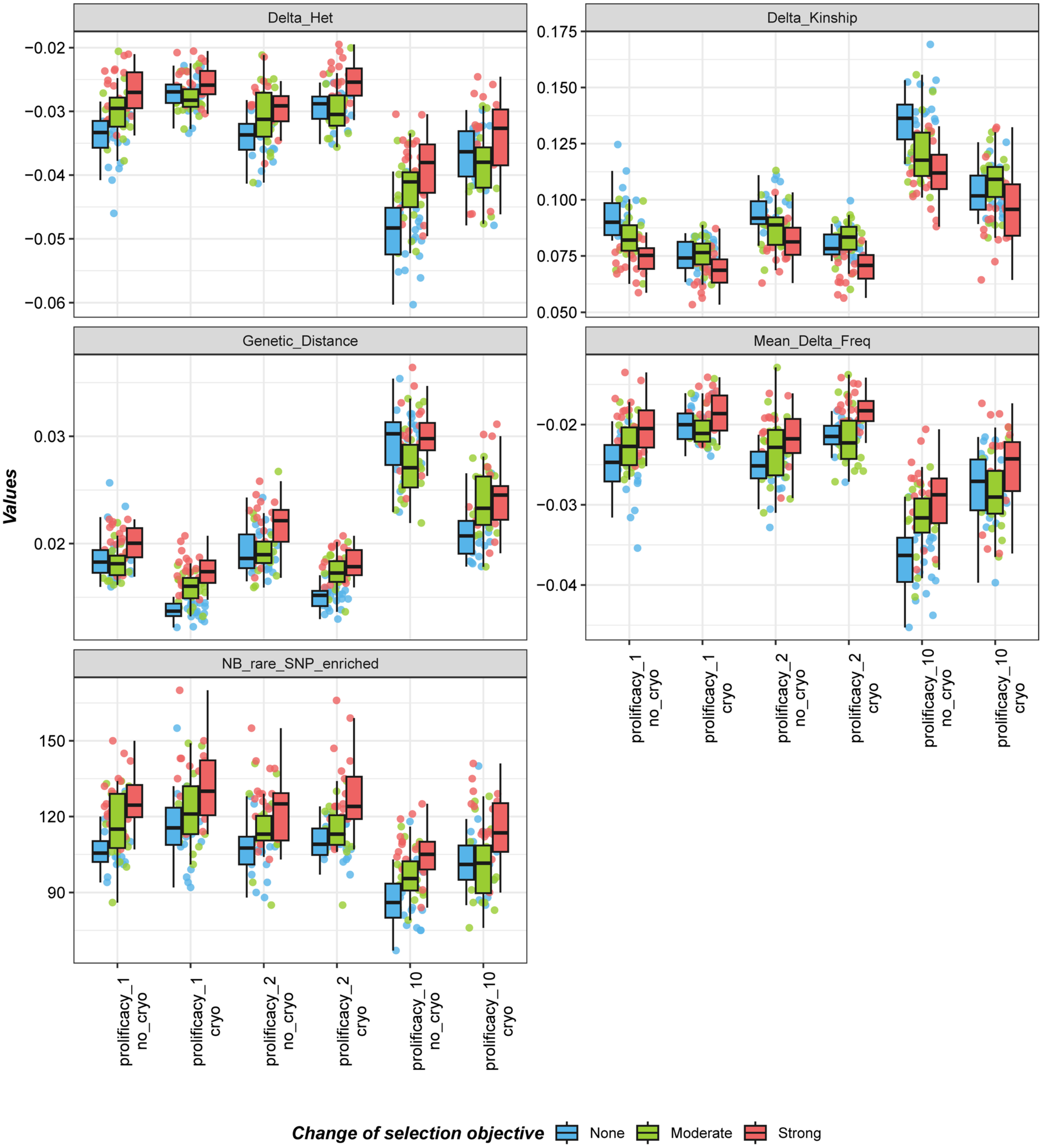

FIGURE 11 | Measurements of genetic diversity for scenarios which maximises genetic gain under a constraint of genetic diverisy (OCS). In blue, none: Scenario with no change in the weighting of the two traits; in green, moderate: Weighting of both traits increased to 0.5; in red, strong: Complete inversion of weights between the two traits. Delta_Het: The difference in heterozygosity between generations 20 and 35; Delta_Kinship: The difference in kinship between generations 20 and 35; Genetic_Distance: Nei's genetic distance between generations 20 and 35; Mean_Delta_Freq: The difference in allelic frequencies between generations 20 and 35; NB_rare_SNP_enriched: The number of SNPs with a MAF lower than 0.05 in generation 20 whose frequency increased in generation 35. [Colour figure can be viewed at wileyonlinelibrary.com]

counter-intuitive result may be due to the estimation of contributions in OCS methods that is often close to zero but not null for individuals with lower breeding values (i.e., cryopreserved individuals in our case) that consequently limits the maximal contribution of the best individuals (i.e., it sums to one), thus indirectly leading to more balanced use of retained sires.

TABLE 3 | Summary of the impact of the use of cryopreserved collections on breeding values and genetic variance for different breeding programmes.

Programs	Change in selection objective	Diversity	Breeding values			Genetic variance	
			Synthetic index	Trait 1	Trait 2	Trait 1	Trait 2
Random mating	NA	+++	NA	NA	NA	NA	NA
Maximised genetic diversity	NA	+++	NA	NA	NA	NA	NA
Optimal contribution	None	++	=	-	++	=	=
	Moderate	++	=	=	=	=	=
	Strong	+++	=	++	=	=	=
Maximised breeding value	None	+	=	--	+++	+++	++
	Moderate	=	=	-	=	++	++
	Strong	=	=	+++	-	+	++

Note: --, moderate deterioration; -, small deterioration; +, small improvement; ++, moderate improvement; +++, large improvement; =, no change.
Abbreviation: NA, not applicable.

The increase in genetic diversity through the use of *ex situ* collections also had a direct impact on selection: introducing this 'neutral' genetic diversity resulted in increased genetic variance (Table 3), demonstrating that even without the intention of reintroducing genetic diversity, cryopreserved resources are useful for enabling a selection response in the long term. While the synthetic index did not change based on the introduction of old material, the impact on a given trait strongly differed depending on the change in breeding weights for that trait. These results are in line with those of Doekes et al. (2018), who showed that the impact of cryopreserved bulls depended on the trait or index observed, particularly with a strong interest in indices more recently considered in selection objectives.

In the case of a strong change in selection objective, we can conclude, as did Leroy et al. (2011), that the use of cryopreserved sires is particularly beneficial. At the difference of the approach of Leroy et al. (2011) who put a constraint on the maintenance of sire lineages, in our approach the use of cryopreserved sires was driven by their ability to fulfil the breeding goals, considering or not genetic diversity. Furthermore, we assessed population diversity by a range of parameters, beyond kinship. In case of the reversal in the weight of a trait (negatively correlated to the other trait) in the selection index, the use of old material prevented a strong degradation of this trait (Table 3). Conversely, if there was no change in objective, the benefit of cryopreserved resources lay in improving or maintaining the lower priority trait which, without the introduction of diversity, would deteriorate due to the negative correlation with the higher priority trait. This suggests that the real-world benefits of cryopreserved materials to breeding populations may be all the more important, as numerous traits are under selection and their importance varies with changes in the environment. In the context of the agro-ecological transition—aimed at adaptation to climate change and concomitant changes in breeding practices—it is highly likely that the number of traits considered will increase, and that their relative importance in selection objectives will vary (Dumont et al. 2013; Wezel et al. 2020; Ducos et al. 2021). For example, over the last few decades, numerous studies have focused on the inclusion of new traits such as environmental footprint or animal robustness (Nielsen et al. 2006; Merks et al. 2012; Herrero et al. 2013). Thus, cryopreserved genetic resources will be particularly useful for optimising the transition of highly selected populations into agro-ecological systems. In addition, some breeding programs have already had to reorient their selection objectives in order to consider new traits that were not necessarily under selection or have been degraded by selection. For instance, many breeding programs are now taking care of eliminating genetic defects, such as halothane in pigs or scrapie in sheep, which may totally change the breeding value of some sires; weights of health traits are also getting a growing importance in cattle or poultry (Rupp and Boichard 2003; van der Most et al. 2011). One study also looked at the reintroduction of genetic diversity on the Y chromosome of Holstein in the United States, which was only possible through the remobilization of ancient *ex situ* genetic resources (Dechow et al. 2020).

Populations managed with OCS appeared to benefit less from the contribution of cryopreserved collections (Table 3). There was no impact of their use on genetic variance, regardless of a change in selection objective. This result can be explained by the efficiency of the optimal contribution strategy in combining genetic improvement while maintaining genetic diversity. Regarding breeding values, though, the situation was different: the use of cryoconserved collections had a positive impact on the lower priority trait in the case of a constant selection objective, as well as on the higher priority trait in the case of a strong change in selection objective. Indeed, the use of OCS based on genomic data makes it possible to select the cryopreserved individuals that are most relevant for managing changes in breeding objectives, both in terms of maximising genetic gain and by taking into account a constraint on increasing relatedness.

In the present study, prolificacy did not change the impact of using cryoconserved collections on either genetic diversity or breeding values. Because of this, our conclusions regarding the use of cryoconserved resources should remain valid across a range of livestock species with different litter sizes. However, prolificacy did have an impact on the number of cryopreserved sires used. This can be explained by the constant size of the simulated population, which sets a fixed number of offspring. For a given number of offspring, the number of artificial inseminations will differ based on litter size. This imposes a strong constraint on the female pathway when prolificacy is high, leading to a reduction in the number of dams and matings and thus the possibility of adjusting genetic contributions by OCS.

Some parameters that were not included in our simulations deserve further study. First, in the present study, both simulated traits had the same heritability. It would be interesting to analyse a scenario in which heritability differs between traits, which is generally the case in different livestock species (Oliveira Junior et al. 2021; Mucha et al. 2022) Second, as mentioned above, it would be informative to specifically examine the impact of prolificacy on the use of cryopreserved collections. For this, it would be necessary to compare breeding schemes with a fixed number of sires instead of a fixed number of offspring, as we chose to do here. Moreover, for our simulations, we chose to use OCS, but other optimization methods are available. For example, another possibility for optimising a breeding program is to use the minimum genomic parentage selection method (i.e., SPMG), which relies on a reverse rationale by setting the desired genetic gain and then minimising inbreeding in the next generation (Colleau et al. 2004). This method is routinely used in France in Alpine and Saanen goats breeding schemes (Palhière et al. 2022) but also in some French poultry breeds (Chapuis et al. 2016). In this case, if the set of the genetic gain is reasonable (i.e., not too high), we can expect that the cryopreserved individuals could have a stronger beneficial effect since the management of genetic diversity is the main target of the optimization procedure. In the present study, we did not investigate the impact of using older material on the accuracy of genomic evaluations and therefore on selection efficiency. Indeed, for an accurate evaluation, the training (or reference) population must be similar to the population to be evaluated, as the estimation of marker effects relies on the link with causal alleles (Rincent et al. 2012); if the structure of the linkage disequilibrium changes, the effect of the markers may no longer be correctly predicted. As this structure evolves over time, it is likely that older individuals will be more disconnected from the current population, as well as from the reference population, which should remain contemporary. It would therefore be important to understand (i) the extent to which this could lead to a loss of accuracy in the assessments of older individuals and (ii) what kind of impact mixing individuals with more- or less-accurate predictions would have on population evolution. Another point to consider is that here we simulated a trait that can be measured directly on individuals, but a number of traits are measured indirectly. For phenotypes that are not directly measurable from breeding stock (e.g., a sire's milk value estimated from the performance of his daughters, egg production in roosters), the accuracy of the estimation of genetic value depends mainly on measurements made on related individuals, and therefore on the number of offspring produced. In this case, the animals with the best-characterised phenotypes would be the oldest individuals, who would be expected to have the most accurate evaluations. These questions should be explored in greater detail with future simulations, so that new recommendations may be provided for the evaluation of cryopreserved genetic resources going forward.

Overall, our simulations demonstrate that, given the same cryobank architecture, the best use of cryopreserved individuals can be drastically different depending on the type of breeding scheme in place. Moreover, we did not consider in our simulations any constraints or limits on the number of available insemination doses, which may not be the case for all breeds or males (as demonstrated by an analysis of the French National Cryobank; (Jacques et al. 2024)). Conserved populations require management that optimises genetic diversity, including the use of cryopreserved genetic resources at each generation, while populations under selection would be more likely to require periodic inputs from germplasm collections, in which the choice of individuals would focus predominantly on performance for a combination of traits that may change over time. In addition, here the use of cryopreserved sires in the various selection schemes was quite high across generations, which would require the availability of a sufficient number of doses (e.g., straws) in collections. If cryopreserved collections are used to meet different objectives, the ideal constitution of the collection, if it exists, would have to be defined according to the management programme of each population. Further studies are needed to investigate the suitability of different strategies for developing germplasm collections. Indeed, although cryopreserved genetic resources can be stored indefinitely, the collection and storage of such materials generate costs that must be controlled (De Oliveira Silva et al. 2019). It is therefore necessary to develop strategies for managing the content of cryobanks so that (i) they remain relevant for a range of populations over time and (ii) managers of cryopreserved *ex situ* genetic resources retain some degree of freedom regarding the sampling and distribution of collections.

5 | Conclusions

In conclusion, the use of cryoconserved collections was shown here to be beneficial for the management of genetic diversity in the long term in all of the scenarios investigated. For populations under selection, the use of cryobank resources is particularly useful in the case of a radical change in selection objectives. For this, the occasional use of a few sires, judiciously chosen so as not to affect genetic gain too much, should be sufficient. The use of the Optimal Contribution Strategy (OCS) promotes the use of *ex situ* collections and the long-term maintenance of their progeny in breeding schemes, even if the impact on diversity and genetic gain is more moderate (because the application of OCS optimises the trade-off between diversity and genetic gain before using cryopreserved collections). We also highlight that OCS remains an effective tool for maintaining genetic diversity, regardless of whether cryopreserved material is used. For populations under conservation, cryobanks bring major benefits, as the regular use of *ex situ* genetic resources could slow down the erosion of genetic variability. However, when germplasm collections are employed in these situations, it is important that the number of offspring generated is appropriate given the size of the population, which implies having a sufficient stock of material. Future studies should examine the value of cryobanks in a larger set of breeding schemes, particularly from the viewpoint of the agro-ecological transition, which could create new prospects for the better exploitation of cryopreserved genetic resources.

Author Contributions

G.R. and M.T.B. conceived the project. A.J. performed the analysis and drafted the manuscript. G.R. and M.T.B. supervised the analyses. A.J., M.T.B., and G.R. interpreted the results. A.J., G.R., and M.T.B. contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.