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Gene banks for conserving and managing genetic diversity in the era of genomics and new reproductive technologies

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History and purpose of gene banks

The first animal gene bank collections started over 40 years ago. The Dutch gene bank, for example, started rather informally in the 1980s when a tank was placed in a research institute with cattle and horse semen discarded by breeding companies to avoid property taxes. It was followed by an initiative of a private foundation safeguarding cattle semen and resulted in the 1990s in the current national gene bank. Since then, gene banks have further institutionalised and professionalised, with guidelines for managing gene banks (Boes *et al.* 2023) and international networks (e.g. Eugena in Europe). A main purpose of gene banks was to serve as a backup in case of disasters, allowing the possibility to restore a breed or a breeding line after extinction. However, gene banks serve more purposes (Fig. 1). In particular, gene banks can support in situ populations in various ways and they can function as a resource for research.

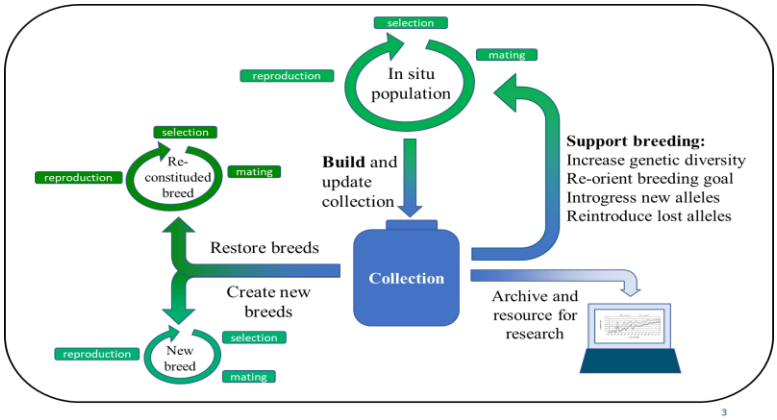

Figure 1. Uses of gene bank collection. Adapted from Boes *et al.* 2023.

Recent developments in genomics and reproductive technology. Gene banks can take advantage of recent innovations in genomics and reproductive technology. Currently individuals can be genotyped in detail at a relatively low cost and whole genome sequencing (WGS) is possible. DNA information from cryo-conserved sires and animals in the living populations has become available. This can be used to realize three opportunities: 1) to make the gene bank a more complete archive of genetic diversity, 2) to determine the history of the genetic diversity, and 3) to improve the performance and genetic diversity of living populations (Oldenbroek and Windig 2022). Reproductive technologies have evolved as well. The maternally inherited genome (i.e. store embryos and ovaries) can be conserved, cloning techniques can be applied to generate offspring from cryopreserved somatic cells, and for poultry, Primordial Germ Cells (PGC's) can be conserved next to semen. When implemented, these developments will influence all the different purposes of gene banks.

Collection building and updating

From a genetic viewpoint the Optimal Contributions method (OC) that minimises genetic relatedness of candidates is the best method to build a gene bank collection (Meuwissen 1997). Constraints can be built in to adapt the vector of contributions for specific gene bank purposes. For example, the number of animals that can be stored is fixed, or each animal selected has to contribute equally. In case the collection is extended, the contribution of animals already represented in the gene bank may be fixed, so that the animals added to the gene bank will minimise the relatedness of the collection, given the current collection.

Traditionally, kinships needed for OC are estimated using pedigrees, but when animals are genotyped they can be estimated from DNA. For the latter several methods exist, with the main difference being whether a correction for allele frequencies is used (e.g. as done for excess of heterozygosity implemented in Plink) or not (e.g. based on similarities). Using the former, the resulting gene bank collection will more closely mimic the allele frequencies of the in situ population, with the latter rare alleles will be represented more in the collection. Eynard *et al.* (2016) showed that more rare alleles are saved when using sequence information and kinships are estimated by using similarities. DNA can also be used to estimate relatedness between breeds, which is generally not possible with pedigrees. To prioritise breeds for conservation, the method of Eding *et al.* (2002), based on OC, can be used.

Nowadays, semen, embryos and more can be conserved. However, reproductive techniques do not function equally well in all species. Embryo transfer in pigs requires large numbers of embryos in order to have one successful pregnancy, while in poultry it is not possible at all. In poultry, insemination success can be quite low. The use of PCGs has great promise (Bernal Juarez 2025), but due to its complexity and costs, further developments are needed.

Restoring breeds

The required number of embryos needed to restore a population with a certain effective population size and probability of successful recovery can be calculated. If the success rate per embryo transplant is known, the total number of recreated individuals follows a binomial distribution. In case of semen, only the number of doses and donors can be calculated similarly, although one has to take into account that after insemination of females of a surrogate breed several generations of backcrossing are needed until the required purity is reached. The advantage of PGCs with respect to the numbers stored is that they can be cultured so that per animal huge numbers can be stored. Because the technique has not been used yet at a large scale its success is yet unknown and the number of animals from which PCGs need to be stored for a successful recovery cannot yet be determined. In general, storing embryos or other material such as ovaries and PCGs is more complex and costly than storing semen. However, a good strategy may be to store large quantities of semen combined with a relatively low number of embryos or PCGs because a recovery program where both are used can be successful even with a low number of embryos or PCGs.

The Dutch gene bank has been evaluated to determine whether enough material is stored for a full recovery. To this end, three scenarios were evaluated: 1) a safe scenario in which a population with an effective population size of 200 can be reached without selection (or 150 with some selection), 2) a moderate scenario with Ne of 100 (75), 3) a minimum scenario with Ne of 50 (34). For each species a plot was made with the scenario possible for each combination of the number of doses and donors (e.g. for cattle Fig. 2). This depended on the insemination and pregnancy success rate in the different species.

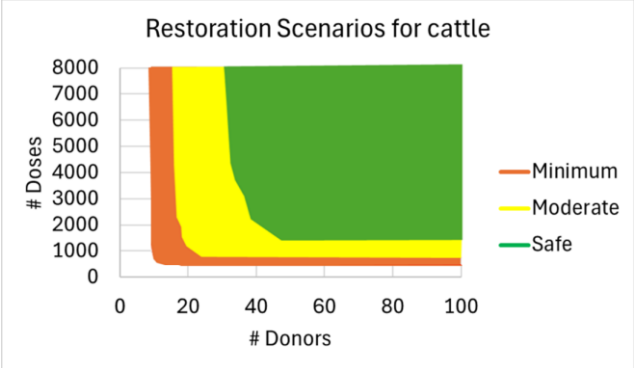

Figure 2. Number of donors and doses of semen needed for restoration of cattle breeds: 95% probability of succes and an effective population size of 50 (orange), 100 (yellow) 200 (green).

Supporting in situ populations

Breeds can profit from using gene bank material. One possibility is to introgress beneficial alleles that have been lost or are rare in the in situ population. An example is the prion protein in goat breeds. There are several alleles that differ in their sensitivity for the lethal scrapie disease, with the fully resistant allele being rare or absent in most breeds. Semen of local breeds stored in the gene bank was genotyped for the alleles (Windig *et al.* 2012). In the rare Dutch Toggenburger breed, an unexpectedly high frequency of this resistant allele was found. This breed can now serve as a resource for other breeds to increase the frequency of the resistant allele. In general, introgression of alleles in an in situ population can become more precise and faster by using new techniques (such as the use of PCGs, Ballentyne *et al.* 2021).

Even without genotyping, breeds may profit from diversity present in the gene bank. In Abondance cattle, a lost lineage was reintroduced using semen from the gene bank (Jacques *et al.* 2023). Although initially there was a slight decrease in milk production, it did lead to lower inbreeding levels and better reproductive performance. When breeding goals change, selection decisions made in the past may not have been optimal. In such cases, using gene bank material may correct these decisions. Doekes et al. (2018b) investigated whether the use of gene bank material in Holstein cattle could lead to higher breeding values by using OC. With the current breeding goal this was only the case when kinship was restricted to a low value (i.e. high diversity). In case the breeding goal changed, for example to high fertility levels, use of gene bank material always resulted in higher breeding values, with or without restrictions on kinship. Gene banks may also help to broaden reference populations needed for genomic selection.

Resource for research

Gene bank collections can be a valuable resource to investigate genetic differences between and within breeds and changes over time, especially because they have been set up to conserve all the genetic diversity present in all breeds at the time of sampling as best as possible. This limits the need for sampling in the field. In this way relationships between cattle breeds in the Netherlands have been investigated (van Breukelen *et al.* 2019) and based on that data, a test was developed to determine the breed of individuals not yet registered in the herd book.

If material is stored in the gene bank over a long time period, changes in genetic diversity over time can be investigated. Using WGS, Hulsegge *et al.* (2022) investigated changes in genetic diversity in Dutch Friesian cattle (DF), one of the ancestral breeds of Holstein cattle. DF is largely replaced by Holstein since the 1980s and is now a rare breed. Genetic diversity before 1990 was compared to genetic diversity since 2003 using semen stored in the gene bank. They showed that the Dutch Friesian diverged in that period more from the Holstein breed, lost some

genetic diversity, but that inbreeding levels did not increase, probably due to the breeding management system employed. The advantage of using WGS was that even with a relatively low number of animals, changes in genetic diversity can be uncovered.

Changes in genetic diversity over time in the Holstein breed were investigated by Doekes *et al.* (2018b) by analysing data of genotyped bulls born between 1980 and 2015. Two major points of change were detected. Around 2000, when OC was introduced and multiple traits were added to the breeding goal, inbreeding and kinship rates decreased. After 2010 they increased, which coincided with the introduction of genomic selection. Similar research on local cattle breeds from Norway, the Netherlands and France showed that introduction of genomic selection does not lead automatically to a loss of genetic diversity, but that the policy for genetic management is all the more important.

Strategy for sampling and genotyping animals

The following strategy may be followed to optimise gene bank management. Although rare breeds at the brink of extinction may be prioritised for storage in the gene bank, one should strive to store genetic material of all breeds, from local rare breeds up to the large global commercial breeds. Sampling should be repeated in time and the more intensive selection is, the more often sampling should be repeated. In this way gene banks can function as a backup not only for extinction but also for unwanted changes, or when changes in breeding goals are required. The value of the gene bank will increase further when genotypes are publicly available. A limited number of animals per breed selected with OC can be sequenced, while the remainder can be genotyped with a SNP-chip.

Conclusion

Developments in genomics and reproduction techniques have a high potential to make gene banks more effective in the storage and use of genetic diversity. Moreover, these developments will increase opportunities to use material from gene banks to better support in situ populations and animal breeding in general.

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