

USE OF MOLECULAR MARKERS AND OTHER INFORMATION FOR SAMPLING GERMLASM TO CREATE AN ANIMAL GENE BANK

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Introduction

The need to conserve farm animal biodiversity is accepted by many countries through the ratification of the Convention of Biological Diversity (CBD, 1992). Although farm animal breeds are not well defined in general terms, they are the typical unit for conservation. Based on an inventory of the actual breeds (on the global scale this is done by FAO through the world watch list, Scherf 2000), the specific breed characteristics and their genetic diversity, decisions need to be made on what should be conserved.

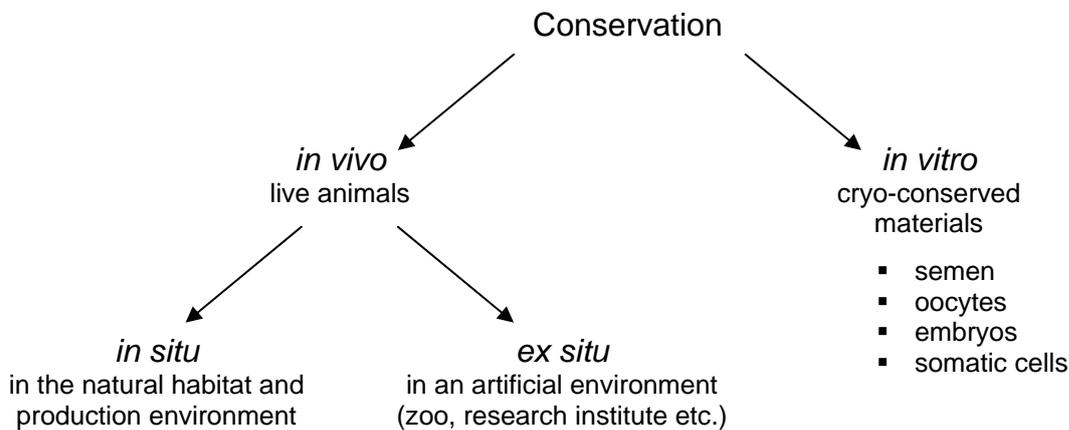


Figure 1: Systematic overview of basic conservation schemes in farm animals.

Figure 1 gives an overview of the different conservation schemes available in farm animals. It is generally accepted, that whenever possible preference should be given to in vivo conservation schemes. In vitro schemes often are started as an additional safeguard when the population size of the endangered breeds is very small or the breed is at high risk of extinction. For cryo-conservation, semen and embryos are the first choice. It should be noted, though, that while long-term storage of deep frozen semen is feasible in all major farm animal species, the use of cryo-conserved embryos is only established in cattle and small ruminants and under development in a number of other species (Hall, 2004). Storing somatic cells to produce clones in the future may be seen as a simple and inexpensive option since Dolly (Wilmut et al. 1997), but up to now practical experience with this technique is rather limited in most species.

Table 1 shows some of the main features of in vivo and in vitro conservation schemes. It should be noted that the apparent advantage that a live population genetically adapts to changing conditions often is overemphasized, since genetic change due to natural selection is not expected to be very large in only a few generations. The costs of in vitro schemes often are considered to be very low. If, however, costs of reactivating the cryo-serve at the end of the planning horizon are included, this may not be true. Reist-Marti (2004) pointed out that even the collecting and storing phase of a cryo-conservation scheme will be expensive if the

technical infrastructure and the expertise is not available, which is the case in most developing countries.

Table 1: Main features of *in vivo* and *in vitro* conservation schemes for farm animal biodiversity.

| | Conservation scheme | |
|---|---------------------|-----------------|
| | <i>in vivo</i> | <i>in vitro</i> |
| Genetic drift and inbreeding | operating | not operating |
| Genetic adaptation to changing conditions | happening | not happening |
| Cultural and socio-economic role of breed | maintained | eroding |
| Cost | moderate to high | low to moderate |

Creating an animal genebank may refer not only to *in vitro*, but also to *in vivo* repositories, in the latter case it might be understood as identifying a sub-population which is actively managed in a conservation breeding program, e.g. through a sire rotation system (KIMURA AND CROW, 1963).

Use of molecular markers

Molecular markers are a tool to study the diversity on the genetic level. The most widespread use of molecular markers in this context is the assessment of diversity within and between breeds. Although in principle all types of markers would be suitable for this purpose, microsatellites are used in 90 per cent of all diversity studies (BAUMUNG ET AL. 2004). A joint committee of FAO and ISAG has recommended a standard set of microsatellite markers for the major farm animal species, this recommendation was recently reviewed and extended to a larger number of species (HOFFMANN ET AL. 2004). These markers are chosen to represent neutral genetic variability in the genome.

In addition, one might also consider markers associated with so-called quantitative trait loci (QTL), i.e. markers that reflect the genetic potential of an animal for a given quantitative or qualitative trait. Farm animal research focuses very strongly on mapping QTLs and single genes, so that such markers increasingly will be available in the future. Of special interest will be markers that are linked to disease resistance QTL, like trypanotolerance in cattle (HANOTTE ET AL. 2003), nematode resistance in sheep (COLTMAN ET AL. 2001), or E.coli-resistance in pigs (MEIJERINK ET AL. 2000).

Creating a genebank

Creating a genebank can be considered as a multi-stage decision making process with the following stepwise decisions:

1. Which breeds to conserve?

WEITZMAN (1992, 1993) has presented a formal framework for this decision making process. While the diversity metric suggested by Weitzman has been criticised as not accounting for within breed diversity (see e.g. CABALLERO AND TORO, 2002), the general framework to make decisions based on the expected conserved diversity has a strong appeal. The original proposition, which was first adopted by THAON D'ARNOLDI ET AL. (1998) for farm animals, is based on a diversity metric which is derived from a genetic distance matrix. Genetic distances can be estimated from allele frequencies at marker loci between populations: the more different these frequencies are, the more distant are the breeds. Diversity is a measure for the total variability of a distance matrix for a set of breeds: the more distant the breeds in the set are, the larger will be the diversity.

Using actual allele frequencies results in the present diversity. Combining the information with extinction probabilities, i.e. the probability that a breed will go extinct over a given time

horizon, 50 years, say, results in the expected diversity at the end of the time horizon. The expected diversity always will be less than the actual diversity. The objective is to design conservation programs in such a way that the expected diversity is maximised.

WEITZMAN (1993) suggests the conservation potential as the single most informative criterion to rank breeds w.r.t. to conservation priority. The conservation potential of a breed basically reflects the amount of expected diversity that can be conserved if a breed is made completely safe. SIMIANER ET AL. (2003) found that this criterion correctly identified the optimum sub-set of 6 breeds to be conserved in a set of 23 African cattle breeds. PINENT ET AL. (2005) show that the derivation of the conservation potential for a set of breeds always should take into account information on related breeds outside the set of candidate breeds for conservation (e.g. foreign breeds or commercial breeding populations) to avoid ‘false positives’.

SIMIANER (2002, 2005) suggests to combine expected diversity with other criteria resulting in the expected total utility as a maximisation criterion. This criterion may encompass presence of special genetic traits (like disease tolerance), production, cultural, or environment values of breeds etc. A similar, but less formal argument was made by PIYASATIAN AND KINGHORN (2003).

2. Optimal allocation of resources

Once the decision is made which breeds should be sampled, it is necessary to assign appropriate shares of the conservation budget to the different breeds. In the EU, about 40 mio Euro are spent per year for the conservation of farm animal genetic resources (SIGNORELLO AND PAPPALARDO, 2003). Although this is not a centralised budget which is distributed in a uniform and rational process, one could in principle compare the theoretically optimal allocation with the real situation, revealing the relative efficiency of the implemented conservation programs.

SIMIANER ET AL. (2003) have suggested a formal approach to find the optimal allocation of a given budget. This basic approach was further refined by REIST-MARTI (2004). In a first application, SIMIANER (2002) showed that the optimal allocation of a hypothetical conservation budget to a set of 26 African cattle breeds resulted in a 60 per cent increase of efficiency (in terms of conserved diversity per conservation Euro) compared to uniform distribution or allocation of the total budget to the three most endangered breeds only.

3. Which conservation scheme for a given breed?

This decision is not independent from the choice of breeds to conserve and the optimal allocation of resources. To do these first steps, costs and effects (in terms of reduced extinction probability) need to be known for the different conservation schemes. The costs typically can be subdivided in fixed cost, necessary to establish the conservation scheme in this breed, and variable cost, which depend on the number of animals, herds, cryo-conserved samples etc. included. With known cost functions for different conservation schemes in the same breed, it is always possible to identify the optimum conservation scheme for a given investment level within breed.

This is demonstrated in REIST-MARTI (2004), where three out of four different conservation schemes were found to be preferable in at least one out of eight African cattle breeds chosen for conservation. If such a planning process is considered on an international level, factors like the exchange rate of currencies, relative labor costs in different countries etc. play an important role. Labor-intensive *ex situ* conservation schemes may be cheaper than cryo-conservation in some countries, where wages are low and the infrastructure for cryo-conservation is not available (REIST-MARTI, 2004).

4. Which germplasm should be stored?

Once the aforementioned decisions are made, individual genotypes need to be identified to become part of the conservation scheme. Some general criteria can be defined concerning the desirable genetic properties of the sample:

- it should represent the genetic portfolio of the breed;
- it should have a maximum effective population size;
- special genetic traits should be conserved.

Fulfilling these criteria may lead to a conflict of goals, since maximizing the effective population size suggests to collect extreme genotypes, which may not be representative for the population.

Using parameters from population genetics, the group of animals chosen should have minimum inbreeding and minimum relationship to each other. Note that the level of inbreeding is less critical than the average relationship, because the actual inbreeding is removable, while the level of relationship inevitably determines the long term level of inbreeding.

If reliable pedigree data are available, these parameters can be calculated for any possible sample and used to identify the optimum group of animals to be stored. If pedigree information is not available, genetic markers can be used to approximate these criteria. EDING AND MEUWISSEN (2001) suggested to estimate MALÉCOT'S (1948) kinship coefficient based on molecular marker information and to derive what they call a 'core set' to conserve.

Conclusions

Molecular markers are an indispensable tool to understand the genetic structures of populations. For the sampling of germplasm to create an animal genebank, they are necessary, but in no way sufficient to make adequate decisions. Besides diversity information derived from molecular data, a good knowledge of breed characteristics and values, the risk status of breeds, availability and cost efficiency of possible conservation programs, among others, need to be understood and specified. Considering the present situation in livestock, diversity information often is easier accessible than information on many of the other factors listed (RUANE, 2000). Therefore it is strongly recommended to concentrate co-ordinated genotyping efforts to fill the still existing 'white spots' on the global maps of farm animal diversity, and to re-allocate funds to develop a better understanding of the other components of a rational decision making process.

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