Ex Situ Conservation of Endangered Farm Animal Genetic Resources in Austria

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Introduction

In Austria from the beginning of gene conservation in rare and endangered breeds in the early eighties of the last century the emphasis lay on in situ conservation measures. The core of the programs has always been a gene conservation program planned and supervised by the ÖNGENE and executed by the responsible acknowledged breeding associations (Organisations in charge; OC). The OCs keep the herdbooks, are responsible for the selection and the identification of breeding animals and establish breeding goals and breeding standards. Last not least the OCs confirm the breeding animals as purebred and eligible for subsidies within the programs (FISCHERLEITNER 2002). In breeds with very small populations mating plans try to minimize inbreeding (SIMON, 1991).

A new program was launched in 2007 including compulsory recording for all participating rare breeds. In this program characterization and sustainable use of traditional and endangered Farm Animal Genetic Resources (FAnGR) play a major role linking wherever possible AnGR to the ecologically sound production of regional specialities. For example the Original Pinzgau cattle is the official breed of the Austrian National Park “Hohe Tauern” (Salzburger Bauer 2006). A picture of these very characteristic dual purpose cattle is on every package of a brand of organic fresh milk in Austria.

All in situ programs should be backed by ex situ conservation for safety measures in case of diseases, catastrophes or unwanted loss of genetic diversity caused by selection.

Ex situ conservation – material, methods and discussion

Ex situ – in vivo conservation

In FAnGR mammals ex situ - in vivo conservation plays no big role. However it is important for awareness raising in the public with no agricultural background. Therefore many zoos in Austria display farm animals of endangered breeds and some participate in conservation breeding programs (e.g. Tiergarten Schönbrunn, Alpenzoo Innsbruck and Tierpark Herberstein in Styria). If the animals are part of the regular breeding programs there can be a continuing exchange between the on farm population and the ex situ population.

The situation differs in poultry. Here the conservation of rare breeds is in the hands of fancy breeders. The majority of them are not farmers. They keep the birds as a hobby and not for production. The drawback in this situation is that no recording is possible, as breeding usually is targeted on show birds.

Ex situ – cryoconservation:

The Austrian Gene Bank

The Austrian Gene Bank was founded in 1997. The basic idea was to back up the in situ conservation programs and to provide material for planned mating in highly endangered breeds. For mainly financial reasons the collection concentrated on semen although a small number of cattle embryos from previous programs is stored as well. When the Institute was still a commercial AI centre research in embryo transfer, in-vitro fertilisation and embryo splitting led to a small collection of embryos from Original Austrian Brown and Tux-Zillertal cattle. Some of these embryos (Original Austrian Brown) were used to produce AI bulls for the in situ breeding program. From another successful splitting experiment identical Tux-Zillertal females were born.

The collection was established to produce semen from cattle, sheep and goats according to EU regulations for using the material in situ. As a starting point the concept of SMITH (1984) was tested: For conserving 98% of the genetic diversity of a breed it is necessary to store at least 25 not related males. It soon became clear it that was impossible to find 25 not related sires of an highly endangered breed when sufficient pedigree information of at least 3 generations does exist. Therefore the limit was raised to 50 sires per breed from as many different parents as possible, even including father/son

relations if the mothers are not related, but excluding full siblings (GANDINI and OLDENBROEK, 1999). We are aware that in highly endangered breeds this may mean to collect every existing male. Potential donor animals are selected by the OCs considering phenotype and if possible desirable production traits as well as pedigree information. They are tested according to EU regulations for semen production. The semen is collected, processed and stored at the AI centre of the Institute for Organic Farming and Farm Animal Biodiversity. The owner of the donor animal gets a fee to compensate for the time the animal is not available for natural mating. The collection is owned by the Austrian government. From the active collection the OCs may order semen at moderate prices for their breeding programs. According to international guidelines the base collection and the duplicate collection for safety reasons are not accessible except in case of catastrophes (HIEMSTRA and MÄKI-TANILA, 2004).

Today the Austrian Gene Bank for rare breeds contains 29 breeds with 391 sires (Fig 1). If we consider the goal of 50 sires per breed only 31,8% of the gene bank were filled in the last 10 years. A major drawback is the use of males accustomed to free range breeding. About 20% of the selected sires do not accept to mount on teaser animals or dummies and ejaculate into an artificial vagina. This behaviour seems to be most common in pigs and horses.
The number of donors of a breed does not always correlate with the population size. The OCs strongly influence the number of the selected donors. For example the populations of the two goat breeds Pinzgau and Chamois Alpine differ widely: Pinzgau numbered 330 breeding animals in 2006 and Chamois Alpine 850 but Pinzgau has more donors than Chamois Alpine (37 vs. 32). The third ranking breed Styrian Pied goat has only 130 registered breeding animals but is rather well represented in the collection (19 donors).
To complete the base collection quickly BREMOND (2003) recommends the use of epididymal semen from slaughter animals. This semen must be stored separately as it does not fulfil the EU Regulations. As the routine use of such semen in breeding plans is not possible except by special permission this option has not been used yet.

Table 1 shows the relatedness (R) of donors in the Austrian Gene Bank in 5 selected rare breeds. Only breeds with more than 10 sires in the collection and a pedigree completeness index (VI; SCHMIDT et al., o.A.) of at least 25% over 5 generations in the pedigrees are shown. The biggest amount of R is due to the pairs of fathers and sons. Although no full siblings are taken into the collection the R_{max} in all breeds is equal or above 50%. This shows clearly the close relations in purebred small populations. A specific selection of donors for maximal genetic variation by marker information like recommended by TORO and MÄKI-TANILA (1999) has not been done yet. Currently the rise in inbreeding in the highly endangered breeds can be checked effectively by strict mating plans and the use of gene bank material (BERGER and FISCHERLEITNER, 2004).

The Austrian Gene Bank includes not only farm animals. A semen bank for endangered species of freshwater fish was added to the Austrian Gene Bank in 1998 to build a genetic archive and to use valuable genetics from free living populations more efficiently. It contains now semen from Brook Trout, Charr, Great Lake Trout, Danube Salmon, Grayling, Common White Fish, Bleak, Sneep and Burbot.

The Austrian Cattle Archive (ACA)
The Convention for Biological Diversity (CBD, 1992) and the Global Plan of Action (FAO, 1999) demand to include all FAnGR into national gene banking not only rare breeds. Therefore the ACA was founded in 1999 as an agreement between the Austrian cattle AI centres and the ÖNGENE.

From every test bull entering an Austrian AI centre 50 doses of semen are stored in the ACA together with complete pedigree and DNA information for scientific purposes. Remaining doses may be used for breeding on special demand but not before 15 years of storage. At least 5 doses must stay in the archive. The ACA consists now of 20 breeds with 1485 sires (Table 2). If an AI centre culls semen from long time storage and the bull is not yet registered in the ACA, 50 doses go to the collection not to lose the genetic information forever. The eldest bull in the collection is an Austrian Brown Cattle bull born in 1959.
Pinzgau Cattle and Austrian Grey cattle have always maintained their own collections at the cattle AI centres Kleßheim (Salzburg) and Birkenberg (Tyrol). This explains the high number of bulls of these breeds in the ACA in relation to the living herdbook populations.

This close collaboration between commercial AI centres and the Institute ensures a continuing genetic documentation of Austrian cattle breeding.

The Austrian DNA Bank (FADNAB)
The goal of the FADNAB project which started in 2006 is to provide a comprehensive DNA archive for all Austrian FAnGR.

The concept is to store 50 to 100 samples per breed and generation and to repeat the collection after 5 to 6 generations to build a genetic inventory (BREMOND, 2003). The collection can help to analyse many problems like measuring genetic distances between breeds or keeping track of rare alleles during a strict selection scheme. Isolated genomic DNA can be acquired and stored easily and cheaply. As DNA is currently regarded as not revivable there are few restrictions regarding property rights and access.

Every sire in the Gene Bank or ACA is added to the FADNAB collection. Additionally females are collected in the field by tissue or blood samples to document as broad a genetic base as possible.

For autochthonous and endangered poultry breeds the FADNAB will be the first documentation and characterisation instrument in Austria. The analysis results can help to establish sustainable in situ conservation schemes.

All three collections will be entered into the upcoming “Cryo-net”-module of the EFABIS-net project in which the Institute for Organic Farming and Farm Animal Biodiversity acts as partner organisation.

Conclusions
The in situ programs for endangered breeds of FAnGR in Austria are backed by cryo-collections according to international guidelines. Unfortunately this applies only to mammals as currently there are no in situ programs for poultry breeds.

The three existing cryo-collections contain semen, cattle embryos and genomic DNA. The Austrian Gene Bank for endangered breeds of FAnGR was founded in 1997 and consists of a working collection for planned mating as well as a base and duplicate collection. One major problem beside the use of males accustomed to natural mating is the sometimes very small number of donors of rare breeds and the high relations between the donors in the collection.
As only 31,8% of the Gene Bank were completed during the last 10 years the use of epididymal semen for documentation purposes seems feasible.

The other two collections the Austrian Cattle Archive and the Farm Animal DNA Bank have scientific purposes.

- The ACA represents a complete collection of Austrian cattle breeding since 1999 the eldest bull reaching back to 1959. For the Austrian Simmental cattle which is still the main breed the ACA may well be the best genetic documentation of the change from a classic dual purpose breed into dairy lines and suckler cow lines.
- In the FADNAB not only cattle but all species and breeds of Austrian FAnGR will be stored. This is the chance to analyse the genetic diversity in the remaining strains of Austrian poultry breeds. Although these are show animals a description of their performance on farm should be done. Sustainable breeding programs could be developed based on the results.

Although the genetic diversity of FAnGR in Austria seems to be well documented much work remains to be done. The priorities for ex situ projects the next years will be the completion of the Austrian Gene Bank and the conservation and characterisation of poultry breeds.

Summary
The main method of Austrian gene conservation programs is the in situ – on farm conservation of endangered Farm Animal Genetic Resources (FAnGR). Ex situ – in vivo plays only a small role in conservation but is important to build and maintain public awareness. Accordingly most of these collections are displayed in zoos or animal parks.

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The Austrian Gene Bank for endangered breeds of FAnGR was founded in 1997 and consists of a working collection of semen for planned mating and some cattle embryos as well as a base and duplicate collection. A semen bank for endangered species of freshwater fish was added to the Austrian Gene Bank in 1998 to build a genetic archive and to use valuable genetics from free living populations more efficiently.

The Austrian Cattle Archive (ACA) was founded in 1999 as an agreement between the Austrian cattle AI centres and the ÖNGENE. The ACA represents a complete collection of Austrian cattle breeding since 1999. The eldest bull in the collection was born in 1959. For the current change in the Austrian Simmental breed from dual purpose to either dairy or suckler cow lines the ACA is the best genetic document.

In the FADNAB founded in 2006 DNA of all species and breeds of Austrian FAnGR will be stored.

Although the genetic diversity of FAnGR in Austria seems to be well documented much work remains to be done. The priorities for ex situ projects the next years will be the completion of the Austrian Gene Bank and the conservation and characterisation of poultry breeds.

Zusammenfassung
Die wichtigste Methode in der Generhaltungszucht bei österreichischen gefährdeten Nutztierrassen ist die in situ – on farm Erhaltung. Die Erhaltung ex situ – in vivo spielt nur eine kleine Rolle in der eigentlichen Generhaltung, ist aber für die Öffentlichkeitsarbeit sehr wichtig. Aus diesem Grund werden die meisten dieser Sammlungen in Zoos und Tierparks der Öffentlichkeit präsentiert.

Nach internationalen Richtlinien werden alle in situ Programme durch die ex situ Kryokonservierung in der Österreichischen Genbank für gefährdete Nutztierrassen ergänzt. Das trifft allerdings nur auf die Säugetiere zu, weil derzeit keine in situ Programme für Geflügel existieren. Die drei bestehenden Kryo-Sammlungen beinhalten Samen, Rinderembryonen und genomische DNA.

Die österreichische Genbank für gefährdete landwirtschaftliche Nutztierrassen wurde 1997 gegründet und besteht aus einem Kurzzeitlager für Samen für gezielte Paarungen, einigen Rinderembryonen und aus einem Langzeit- und einem Sicherungslager. 1998 wurde eine Samenbank für gefährdete Arten von Süßwasserfischen als genetisches Archiv und um wertvolle Genetik aus Wildpopulationen effizienter zu nutzen hinzugefügt.

Das österreichische Dokumentationslager Rind wurde 1999 in Form eines Übereinkommens zwischen den österreichischen Rinderbesamungsstationen und der ÖNGENE gegründet. Das Dokumentationslager stellt eine vollständige Sammlung der österreichischen Rinderzucht seit 1999 dar. Der älteste Stier in der Sammlung wurde 1959 geboren. Für das österreichische Fleckvieh ist das Archiv zugleich ein Dokument der Umzüchtung von einer Zweinutzungsrasse in spezialisierte Milch- bzw. Mutterkuhlinien.

In der im Jahr 2006 gegründeten FADNAB wird DNA von allen österreichischen genetischen Ressourcen der Nutztiere gelagert werden.

Obwohl die genetische Vielfalt der Nutztiere in Österreich gut dokumentiert wird bleibt noch viel zu tun. In den nächsten Jahren wird bei ex situ Projekten das Hauptaugenmerk auf der Vervollständigung der Genbank und auf der Erhaltung und Charakterisierung von Geflügelrassen liegen.

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Table 1 Relations (R) between sires in the Gene Bank

Breed	Donors	R_{mean}	R_{min}	R_{max}	VI (%)
Cattle					
Tux-Zillertal	17	11,756	0	65,6	33,9
Pustertal spotted	13	6,55	0	50	40,7
Murboden	33	5,61	0	53,1	27,8
Sheep					
Carinthian	28	9,27	0	59,37	50,7
Goat					
Tauern Pied	13	19,44	0	54,39	62

VI (%) = Pedigree completeness over 5 generations

Table 2 **Austrian Cattle Archive**

Breed	**Donors**
Austrian Simmental	795
Brown Swiss	213
Holstein Friesian	35
Limousin	10
Charolais	4
Angus	2
Blonde d´Aquitaine	3
Belgian Blue-White	4
Galloway	1
Jersey	1
Pinzgau (incl. Original Pinzgau)	221
Austrian Grey	77
Murboden	35
Tux-Zillertal	19
Pustertal Spotted	10
Ennstal Pied	22
Carinthian Blond	16
Waldviertel Blond	14
Original Austrian Brown	3
Sum	1485

Fig. 1 **Content of the Austrian Gene Bank**

(Donors per breed)