Complementarity of on farm conservation and cryo-conservation of animal genetic resources

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Plan

- AnGR and conservation methods
- Changing views on importance of conservation approaches
- In-situ conservation
- Role of ex-situ genebanks: Why to freeze and what to freeze?
- State of AnGR genebanks in Europe and new initiatives
- Conclusions
Farm animal genetic resources

Those animal species that are used, or that may be used for the production of food and agriculture, and the populations within each of them

Populations within each species can be classified as:

- wild and feral populations
- landraces, and primary populations
- standardised breeds, selected lines
- and any conserved genetic material

In vivo

In vitro

FAO, 1998
Conservation methods

- "In-situ conservation" means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties.

- "Ex-situ conservation" means the conservation of components of biological diversity outside their natural habitats.
Conservation methods: AnGR

**In-situ conservation**
conservation of a *breed* through continued use by livestock keepers in the production system in which the livestock evolved or are now normally found and bred.

**Ex-situ in vivo**
conservation of a *breed* through maintenance of live populations not kept under normal management conditions (e.g. zoological parks, governmental farms) and/or outside of the area in which they evolved or are now normally found.

**Cryoconservation (in vitro)**
conservation by cryopreservation of breed’s genetic material (usually semen, embryos or somatic cells) *in vitro*, in a non-living state, so that live animals can, if necessary, be reconstituted in the future.

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FAO, 2012 & 2013
From *in-situ* to *ex-situ* in vivo

**In - situ**
- The region of origin of the breed
- The typical production system
- Conservation farms
- Research / experimental stations
- Educational facilities: schools, museums

**Ex - situ in vivo**
- Protected areas
- Farm parks, zoos

*no clear boundary between in situ and ex situ in vivo*

Adapted from Gandini, 2007
## Comparison of conservation methods

### Conservation methods and their potential to contribute to various objectives

<table>
<thead>
<tr>
<th>Objective</th>
<th>Type of conservation (if implemented as a stand-alone measure)</th>
<th>In situ</th>
<th>Ex situ in vivo</th>
<th>Cryoconservation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maintaining flexibility for the future</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insuring against changes in production conditions</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Safeguarding against diseases, disasters, etc.*</td>
<td>No</td>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Providing opportunities for research</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Genetic factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allowing continued evolution/genetic adaptation</td>
<td>Yes</td>
<td></td>
<td>Limited</td>
<td>No</td>
</tr>
<tr>
<td>Increasing knowledge of breed characteristics</td>
<td>Yes</td>
<td></td>
<td>Limited</td>
<td>Limited</td>
</tr>
<tr>
<td>Limiting exposure to genetic drift**</td>
<td>Yes</td>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Sustainable management of rural areas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Providing opportunities for rural development</td>
<td>Yes</td>
<td></td>
<td>Limited</td>
<td>No</td>
</tr>
<tr>
<td>Maintaining agro-ecosystem diversity</td>
<td>Yes</td>
<td></td>
<td>Limited</td>
<td>No</td>
</tr>
<tr>
<td>Maintaining rural cultural diversity</td>
<td>Yes</td>
<td></td>
<td>Limited</td>
<td>No</td>
</tr>
</tbody>
</table>

*Note: *Risk from disease in *in vivo* programmes can be decreased by maintaining animals in geographically dispersed locations.

**The extent of genetic drift will depend on the population size *in situ* and the number of animals sampled for cryoconservation.

Genetic drift cannot be eliminated in *in vivo* populations, but proper management can limit drift to an acceptable level.

*Source: FAO, 2013*
Changing views on conservation methods

- Since the mid XX century – focus on *in-situ* conservation of rare breeds
- Improvement of freezing methods → interest in cryoconservation mainly endangered breeds

*In that period: ex situ and in situ: alternative approaches, even mutually exclusive*

- Adoption of CBD, 1992: introduction of the concept that *in-situ* is a primary conservation method and *ex-situ* complementary method; possibility to support conservation

(Article 11: Incentive measures
Each Contracting Party shall .... adopt economically and socially sound measures to act as incentives for the conservation and sustainable use of components of biological diversity)
Changing views on conservation methods

- Since early XXI century: enhanced interest in establishment of genebanks

- At present: efforts to achieve synergy and integrate ex-situ and in-situ

FAO, 2015
Stakeholders maintaining AnGR

<table>
<thead>
<tr>
<th>In-situ conservation</th>
<th>Ex-situ in vivo</th>
<th>Cryoconservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmers</td>
<td>Farmers</td>
<td>Ministry of Agriculture</td>
</tr>
<tr>
<td>Farmers’ association</td>
<td>Research institutions</td>
<td>Public national genebanks</td>
</tr>
<tr>
<td>Food sector</td>
<td>Educational institutions</td>
<td>Private genebanks/biological banks</td>
</tr>
<tr>
<td>Tourism sector</td>
<td>Farm parks</td>
<td>Research institutions</td>
</tr>
<tr>
<td>Nature conservation authorities</td>
<td>Nature conservation authorities</td>
<td></td>
</tr>
<tr>
<td>Regional / local authorities</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Coordination and overseeing

<table>
<thead>
<tr>
<th>Conservation in vivo</th>
<th>Cryoconservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ministry of Agriculture</td>
<td></td>
</tr>
<tr>
<td>National Focal Point AnGR</td>
<td></td>
</tr>
<tr>
<td>Regional / local governmental authorities</td>
<td>Providers of genetic resources</td>
</tr>
</tbody>
</table>
In situ conservation

Key issue: How to maintain interest of farmers to keep the breed?

- Demonstrate a role for the breed:
  - Utilisation in commercial production system
  - Niche products
  - Landscape management
  - Biological significance
  - Socio-cultural role (historical witnesses, custodians of local traditions)

- Create/enhance a role for the breed - if possible

- Subsidize continued use of the breed
In situ conservation: significant role for the breed

Utilisation in commercial production system

Dorota Chmielowiec & i Maria Centkiewicz, DFE
In situ conservation significant role for the breed

Niche products

"Parmigiano Reggiano da latte di razza reggiana"

Christoph Zimmer, 2016

http://eng.agraria.org/cattle/reggiana.htm
In situ conservation significant role for the breed

Landscape management

1997: GAP Grazing Animal Project

English Nature

Natural England

RBST
In situ conservation significant role for the breed

Biological significance

Genome-Wide Association Studies Identify Two Novel BMP15 Mutations Responsible for an Atypical Hyperprolificacy Phenotype in Sheep

Julie Demars1,2, Stéphane Fabre1,2,3,4,5,6, Julien Sarry1,2, Raffaella Rosetti7, Hélène Gilbert1,2, Luca Persani2, Gwenola Tosser-Klopp1,2, Philippe Mulansant1,2, Zuzanna Nowak1,2, Wioleta Drobotik6, Elżbieta Martyniuk6, Loys Bodin5

1 INRA, UMR1444 Laboratoire de Génétique Cellulaire, Castanet-Tolosan, France; 2 INRA, UMR1444 Laboratoire de Génétique Cellulaire, Castanet-Tolosan, France; 3 IFREMER, UMR893 Physiologie de la reproduction et des comportements, Noisy, France; 4 CNRS, UMR7317 Physiologie de la reproduction et des comportements, Noisy, France; 5 Université François-Rabelais Tours, UMR Physiologie de la reproduction et des comportements, Noisy, France; 6 RCSI, UMR Physiologie de la reproduction et des comportements, Noisy, France; 7 Laboratorio di Ricerche Endocrino-Metaboliche, Istituto Auxologico Italiano, University of Milan, Milano, Italia; 8 Wydział Nauk o Zdrowiu, Akademia Główne, Poznan, Polska; 9 INRA, UMR611 Station d'Amélioration Génétique des Animaux, Castanet-Tolosan, France
In situ conservation significant role for the breed

Socio-cultural role (historical witnesses, custodians of local traditions)
Role of *ex-situ* conservation

- Complementary to *in-situ* conservation
  - Supports implementation of *in-vivo conservation programmes* and within-breed selection
  - Introduces variation into existing populations (enhances genetic diversity)
  - Allows capturing specific alleles

- Insurance for unknown future needs of animal breeding: long term storage

- Research material

- Source of genetic material for breed reconstitution

- Emergency response in case of disasters

- Provides repository of genetic resources in public domain
Support for *in-situ* conservation programmes

The Longhorn Cattle Society is a registered Charity in the UK and was founded in 1878 to promote and improve the breeding of the ancient and magnificent English Longhorn Cattle, and to maintain their purity.

**SEMEN FOR SALE**

The following bulls are available from the Society:


http://www.longhorncattlesociety.com/buy-and-sell/semen-for-sale/
Valuable alleles: Low-fat milk, straight from the cow

- Mutation resulting low-fat milk found by biotechnology company ViaLactia in New Zealand,
- Carrier cow Marge and her daughters have a fat content of 1% and high in omega-3 oils
- Because of the lower levels of saturated fat, butter from these cows is spreadable straight from the fridge, like margarine
- Lack of profitability at present but valuable mutation to keep for future use

http://www.abc.net.au/science/articles/2007/05/29/1936619.htm
Direct evidence for the feasibility of long-term conservation by cryopreservation and rescue of genetic materials when combined with animal cloning

- Restituted from somatic cells of bull 86
  - three years after his death in 1996
  - 15 years after somatic cells were frozen

- In vitro assays indicate this bull is naturally resistant to
  - *B. abortus*,
  - Mycobacterium bovis,
  - Salmonella typhimurium,
  as was the original genetic donor
Emergency response

Heritage GeneBank

- was established during the height of the Foot & Mouth epidemic in 2001 in UK
- emergency action to help sheep breeders to save their breeds and valued bloodlines at threat of extinction
- Germplasm from the Herdwick, Rough Fell, Lonk, Dalesbred, Portland, Lincoln Longwool, and Whitefaced Woodland was collected and frozen

The first stage of a national genebank for sheep in the UK

- Lessons learnt:
  - geographically concentrated breeds might be at risk

The Sheep Trust

www.york.ac.uk/org/cnap/tst/heritagegenebank.html
Restitution of a breed

Objective:

- Estimation of the amount of genetic material to be cryopreserved for reconstructing a population of **25 females and 25 males of reproductive age**
- Stochastic and deterministic simulations
- Three alternative strategies were considered:
  - embryos (embryos-only)
  - embryos
  - semen (embryos + semen)
  - semen (semen-only)
- Reconstruction was simulated for horse, cattle, swine, sheep and rabbit species
Time for restitution: depending on species and strategy

(Gandini et al., 2006. 8th World Congress on Genetics Applied to Livestock Production)
Costs of restitution: depending on species and strategy

- E Embryos only
- E + S% of share
- S only
- n MAXP

(Gandini et al., 2006. 8th World Congress on Genetics Applied to Livestock Production)
State of AnGR genebanks in Europe

### Proportion of countries reporting conservation activities

<table>
<thead>
<tr>
<th>Regions and subregions</th>
<th>Number of countries</th>
<th>In situ conservation programmes</th>
<th>Ex situ in vivo conservation programmes</th>
<th>Ex situ in vitro conservation programmes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>40</td>
<td>70</td>
<td>48</td>
<td>30</td>
</tr>
<tr>
<td>East Africa</td>
<td>8</td>
<td>75</td>
<td>63</td>
<td>50</td>
</tr>
<tr>
<td>North and West Africa</td>
<td>20</td>
<td>65</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>12</td>
<td>75</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>Asia</td>
<td>20</td>
<td>90</td>
<td>80</td>
<td>65</td>
</tr>
<tr>
<td>Central Asia</td>
<td>4</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>East Asia</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>South Asia</td>
<td>6</td>
<td>83</td>
<td>83</td>
<td>33</td>
</tr>
<tr>
<td>Southeast Asia</td>
<td>6</td>
<td>83</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Southwest Pacific</td>
<td>7</td>
<td>71</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td><strong>Europe and the Caucasus</strong></td>
<td><strong>35</strong></td>
<td><strong>100</strong></td>
<td><strong>69</strong></td>
<td><strong>86</strong></td>
</tr>
<tr>
<td>Latin America and the Caribbean</td>
<td>18</td>
<td>83</td>
<td>72</td>
<td>61</td>
</tr>
<tr>
<td>Caribbean</td>
<td>5</td>
<td>100</td>
<td>80</td>
<td>60</td>
</tr>
<tr>
<td>Central America</td>
<td>5</td>
<td>60</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>South America</td>
<td>8</td>
<td>88</td>
<td>88</td>
<td>63</td>
</tr>
<tr>
<td>North America</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Near and Middle East</td>
<td>7</td>
<td>71</td>
<td>71</td>
<td>29</td>
</tr>
<tr>
<td>World</td>
<td>128</td>
<td>84</td>
<td>63</td>
<td>55</td>
</tr>
</tbody>
</table>

*Note: Figures refer to the proportion of countries reporting conservation activities for at least one species.*

*Source: Country reports, 2014.*

FAO 2015
What to freeze?

- **Type I**: Rare breeds
- **Type II**: Exceptional animals not used as breeders
- **Type III**: Improved breeds
- **Type IV**: Selected breeding stock

(C. DANCHIN-BURGE, 2008; Institut de l'Elevage, Dépt Génétique, Paris FRANCE)
New initiatives: EUGENA

- The European Regional Focal Point on AnGR (ERFP), established a Working Group on *Ex situ* Conservation of AnGR:
  - to exchange experiences and knowledge
  - to support the establishment, further development, efficiency and effectiveness of the European national gene banks
  - to jointly develop a European strategy for gene banking & documentation

- In 2015 the European Genebank Network for AnGR (EUGENA) was established

- EUGENA is the network of gene banks in the European countries with the objective to support the *ex situ* conservation and sustainable use of AnGR

- EUGENA operates under the umbrella of the ERFP
New initiatives: IMAGE

The aims of IMAGE:

- to enhance the use of genetic collections
- to upgrade animal gene bank management

- to demonstrate the benefits brought by gene banks by:
  - Enhancing usefulness of genetic collections (livestock sector to respond to new environmental constraints and market needs)
  - minimising genetic accidents such as abnormalities or genetic variability tipping points
  - optimising complementarity between *ex-situ* and *in-situ* conservation
  - making use of the latest developments in DNA technology and reproductive physiology in collecting, storing and using biological resources
General guidance

- Noting further that the .....fundamental requirement for the conservation of biological diversity is the *in-situ* conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings.

- Noting further that...... *ex-situ* measures, preferably in the country of origin, also have an important role to play.

In AnGR conservation: Need to increase integration and synergy between *in-situ* and *ex-situ*
Increased integration of *in-situ* and *ex-situ*

- **At national level**
  - Integration at breed’s conservation programme level
  - Enhanced use of cryomaterial in *in-vivo* population
  - Identifying donors and providing valuable material for cryopreservation

- **At regional level**
  - Transboundary breeds: concerted action
  - Sharing information on status of genebanks’ collections and *in-situ* populations
  - Joined collection/storage activities?

- **At global level**
  - Research to improve efficiency and synergy of conservation methods
  - Simulation models/case studies to present opportunities and benefits of integration
## Ensuring complementarity: SWOT analysis

**S**
- NFP for AnGR: overseeing and coordination function over implementation of national conservation programmes
- Growing awareness of stakeholders
- Strong trends towards development of national genebanks

**W**
- Insufficient coordination among national stakeholders (many players, some with specific agenda)
- Different level of development of national genebanks

**O**
- EUGENA: concerted action at regional level
- IMAGE: technical and procedural development; generating knowledge on status and operation of European genebanks

**T**
- High costs of *ex-situ* conservation (example: ‘LABank’)
- Need for a long-term commitment to maintain genebank
Thank you for your attention