Section D

Conservation

1 Introduction

A substantial proportion of the world's livestock breeds are at risk of extinction (see Part 1 Section B). The need for action to protect them is recognized in the Global Plan of Action for Animal Genetic Resources (FAO, 2007a), whose Strategic Priority Area 3 is devoted to conservation. The state of implementation of conservation programmes (comprehensiveness of coverage, extent of use of different conservation methods, extent of involvement of different stakeholder groups, etc.) is described in Part 3 Section D. The present section describes the "state of the art" in the field, i.e. the methods, tools and approaches that can be drawn upon in order to design and implement effective conservation programmes and strategies. It serves as an update of the equivalent section of the first report on *The State of the World's Animal Genetic Resources for Food and Agriculture* (first SoW-AnGR) (FAO, 2007b). It draws heavily on two guideline publications on conservation prepared by FAO since 2007 – *Cryoconservation of animal genetic resources* (FAO, 2012) and In vivo *conservation of animal genetic resources* (FAO, 2013) – and focuses in particular on recent developments.

Various methods can be used to conserve animal genetic resources (AnGR). Conservation activities can be categorized according to whether they involve the maintenance of genetic material *in vivo* or *in vitro* (see Box 4D1). *In vivo* conservation can, in turn, be classified according to whether it takes place *in situ* or *ex situ*. *In situ* conservation is undertaken in the traditional production system of the conserved AnGR. *Ex situ* conservation is undertaken elsewhere (clearly, all *in vitro* conservation is *ex situ*). *In situ* and *ex situ* conservation are usually regarded as complementary (FAO, 2012; 2013)[1] and in combination they can form the basis of a powerful conservation strategy.

The first part of the section focuses on themes common to all conservation methods: planning tools; methods for identifying breeds at risk of extinction (including a description of the updated risk classification system developed by FAO since the first SoW-AnGR was published); and methodologies for determining the conservation value

[1] See also the "rationale" of Strategic Priority 9 of the Global Plan of Action for Animal Genetic Resources (FAO, 2007a).

Box 4D1
Glossary: *in vivo* and *in vitro* conservation

***In vivo* conservation** is conservation through the maintenance of live animal populations. It encompasses both *in situ* conservation and *ex situ in vivo* conservation.
***In situ* conservation** is conservation through continued use of live animal populations by livestock keepers in the production system in which the respective populations evolved or are now normally found and bred.
***Ex situ in vivo* conservation** is conservation through the maintenance of live animal populations not kept under normal management conditions (e.g. in a zoological park or a governmental farm) and/or outside the area where they evolved or are now normally found and bred.
***Ex situ in vitro* conservation** is conservation through the maintenance, under cryogenic conditions, of cells or tissues that have the potential to be used to reconstitute live animals and populations at a later date.

of a breed as a basis for priority setting. This is followed by in-depth discussions of the two major categories of conservation: first *in vivo* conservation methods and then *in vitro* methods (otherwise referred to as cryoconservation). The subsection on *in vivo* conservation includes a look at institutional arrangements, methods for maintaining genetic variability in small populations, and strategies and methods for increasing demand for at-risk breeds. The subsection on *in vitro* conservation discusses the infrastructure and institutional frameworks for the operation of a gene bank, strategies for the development and assessment of gene bank collections, developments in cryobiology and reproductive physiology, developments in information systems and documentation of gene banked material, and legal aspects of gene banking.

A number of different arguments have been put forward as to why efforts should be made to conserve AnGR (see the first SoW-AnGR[2] for more detailed discussion). Conservation programmes for AnGR usually address one or more of the following objectives:

- economic – maintaining the livestock sector's capacity to respond to ecological changes (e.g. those caused by climate change), changing market demands, changing regulatory frameworks, changes in the availability of inputs, and so on;
- social and cultural – maintaining the roles of livestock in the cultural and historical identities of the communities that developed them (and for the social and cultural benefit of society more broadly);
- environmental – AnGR make an intrinsic contribution to biodiversity and they also contribute to maintaining capacity to utilize livestock in the provision of ecosystem services and to reduce the negative environmental effects of livestock production; and
- research and training – maintaining resources that are valuable for research or educational purposes (e.g. in the fields of immunology, nutrition, reproduction, genetics, genomics and adaptation to climatic and other environmental changes).

As well as considering arguments for conservation, the discussion presented in the first SoW-AnGR also addressed differences between genetic resources conservation in the plant and animal sectors.[3] A number of biological (e.g. reproductive rates, generation intervals and level of diversity within breeds/varieties), operational (e.g. feasibility and costs of activities such as in vitro conservation, germplasm collection and clonal propagation) and institutional (e.g. patterns of ownership and use of genetic resources and the state of development of gene banks) differences between the two sectors were identified. The combined effect of these differences is that AnGR conservation programmes are generally more complicated to organize than those for plant genetic resources. A particular difference is the primary role of the private sector in managing AnGR. Individual animals are usually owned by individuals or groups of individuals, which can make implementation of organized conservation programmes more complex. Owner prerogative as to the direction of selection and mating strategies adds a unique and dynamic nature to conservation actions in this sector.

The various types of conservation programme each have advantages and disadvantages with respect to addressing particular conservation objectives. These advantages and disadvantages are summarized in Table 4D1. This summary refers to situations in which only one of the types of conservation is used. For example, if only *in vitro* conservation is used and no *in vivo* population is present, the conserved AnGR will be making no ongoing contribution to rural development.

In situ conservation is considered to have a number of advantages, including:

- allowing the conserved breed to continue adapting to its production environment as it changes over time;

[2] FAO, 2007b, pages 444–488.

[3] FAO, 2007b, pages 449–451.

- facilitating the maintenance of local knowledge regarding the breed and its management; and
- providing opportunities for the development of strategies that enable the breed to become self-supporting (i.e. that remove the need for external support).

However, *in situ* conservation is not without risks. For example, a population maintained *in situ* may be struck by a disease outbreak or other disaster or may be affected by inbreeding, genetic drift or introgression from another breed. *Ex situ* conservation decreases these risks by providing a backup that can be drawn upon if required. *Ex situ* conservation as a stand-alone strategy does not allow for adaptation. However, if the population is also maintained *in situ*, regularly collecting and conserving new samples *in vitro* can help to maintain the potential for future adaptation.

As described above, *ex situ* conservation can be undertaken either *in vivo* or *in vitro*. While in many circumstances maintaining a live *ex situ* population adds little to a conservation strategy that already includes *in situ* and *in vitro* components, it can have some advantages. For example, *ex situ in vivo* programmes are usually under centralized control, which can facilitate management actions such as the control of mating. In cases where the population size is very small and no facilities are available for cryopreservation, *ex situ in vivo* conservation may be the only viable option. One weakness of *ex situ in vivo* conservation is that, because the populations are usually small (and thus highly subject to genetic drift) and animals are often kept in a single location that may not replicate their original production environments, the conserved population will usually not maintain the complete genetic diversity of the original founder population.

Table 4D1 helps demonstrate the benefits of using complementary approaches to conservation. If an *in vivo* population is maintained along with an *in vitro* collection, then the living population can be periodically sampled to enrich the *in vitro* collection and account for changes in gene frequency that occur via the adaptive process. Likewise, although in the absence of an *in vivo* population an *in vitro* collection cannot contribute to the ongoing development of rural areas, if both types of programme are in place then material from the *in vitro* collection can be actively used in the management of genetic variation in the *in vivo* population.

TABLE 4D1

Conservation methods and their potential to contribute to various objectives

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

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.

Box 4D2

Analysis of strengths, weaknesses, opportunities and threats (SWOT analysis) of Groningen White Headed cattle in the Netherlands

The Groningen White Headed is a native Dutch cattle breed. The first description of the breed dates from the fourteenth century. Pictures of red and of black White Headed cows were painted during the Middle Ages. A herdbook was founded at the end of the nineteenth century. Around that time, 90 percent of all cattle in the Province of Groningen (in the northern part of the Netherlands) were White Headed cattle. They were dual-purpose animals used for milk and beef production. Animals belonging to the breed were also found near the cities of Utrecht and Leiden (in the southwest), where their milk was used for cheese production. Around 1970, the breed had 20 000 milk-recorded females, but due to cross-breeding with Holstein-Friesians, the number of milk-recorded pure-bred females had fallen to approximately 600 in 2014.

A number of national and regional groups of farmers and breeders are interested in the breed. One of them, the "Blaarkop Stichting", is very active in promoting it.

A SWOT analysis undertaken for this breed produced the following results:

Strengths: good performance in terms of functional traits and milk quality; distinctive appearance.

Weaknesses: relatively low milk yield; risk of genetic drift and loss of genetic variation.

Opportunities: renewed interest in functional traits is increasing the use of pure-bred Groningen White Headed sires for cross-breeding with Holstein-Friesians; increasing use of the breed for beef production and as suckler cows.

Threats: the abolition of milk quotas in the European Union will increase the emphasis given to the efficiency of milk production.

Based on the results of the SWOT analysis, the breed interest groups decided to initiate three strategic actions:

1. stimulating farmers to keep the breed or to use pure-bred sires for cross-breeding with Holstein Friesians (some 20 sires are marketed by artificial insemination studs), thus taking advantage of the breed's strength of having good functional traits;
2. making Groningen White Headed semen from the National Gene bank (CGN) available to breeders when its use will increase the genetic variability in the population of pure-bred females (CGN has collected semen from 70 sires since 1973), thus addressing the weakness related to genetic variation; and
3. producing cheese and beef for niche markets and using the breed in the provision of ecological services, thus addressing the threat posed by the abolition of milk quotas by providing alternative sources of income.

Source: Adapted from Hiemstra *et al.*, 2010.

Photo credit: Veeteelt.

Photo credit: Zwanet Faber.

2 Planning a conservation strategy

The planning process for a conservation strategy for a region or a country should start with a review of the status of each breed or breeding population potentially targeted for conservation activities. If inventories of breeds and populations are incomplete, effort should be made to improve them (see Part 4 Section A), as unrecorded breeds will clearly not be included in the planning process and not accounted for in the conservation strategy (although they may benefit indirectly from measures that support the sustainability of the production systems in which they are kept).

The characteristics of each breed should be described, along with its production environment and its uses, roles and values. It is also important to evaluate drivers of change and how they are affecting production systems and the breed's roles within them. Data on the size and structure of the breed population and how these are changing over time are also essential. See Part 4 Section A for a discussion of data collection methods. The estimation of risk status is discussed in greater detail below in Subsection 3. Specific threats – whether associated with production system trends, weaknesses in management or exposure to risks such as disease outbreaks or climatic disasters – should, as far as possible, be identified and evaluated (see Part 1 Section F). The overall objectives of the conservation strategy also need to be considered, i.e. which of the objectives described above in Subsection 1 are to be prioritized?

Once the relevant information has been assembled, priorities can be set (see Subsections 3 and 4) and management strategies for individual breeds can be developed. One approach to planning a conservation strategy for an individual breed is to undertake a SWOT (strengths, weaknesses, opportunities and threats) analysis of the breed and its production system (Martin-Collado *et al.*, 2013) (see Box 4D2 for an example). Threats or opportunities can be identified by analysing trends and drivers of change in the production system. Strengths and weaknesses can be determined by considering the characteristics of the breed in relation to the requirements of production systems and national objectives for conservation and livestock development. Also relevant are population-level factors that affect risk of extinction (e.g. the size, structure and distribution of the breed population, the demographics of the livestock-keeping population) or affect capacity to implement conservation and other management activities (e.g. the presence or absence of breeders' organizations).

3 Identifying breeds at risk

Population size and rate of change in population size are the most important criteria for determining a breed's risk of extinction and should be recorded regularly. The two aspects of breed extinction – loss of animals and loss of gene variants – are deeply interconnected. The loss of breeding animals and consequently a low number of parents available to breed the next generation increases the average relationship between parents and may lead to a higher occurrence of genetic defects and inbreeding depression.

Species differ greatly in terms of their reproductive capacity, and this influences the ability of populations to recover after a decline. For example, a small population size creates a higher risk of extinction in horses than in pigs. In order to account for differences of this kind, FAO's amended risk categorization system (FAO, 2013) distinguishes between species with low and high reproductive capacities and includes different risk-status thresholds for each group (see Tables 4D2 and 4D3; note also that a new category – "vulnerable" – has been added to the classification system).

Once a breed's risk category has been assessed, different objectives for the management of its population can be considered. Four (non-mutually exclusive) means of strengthening the position of the breed can be distinguished:

- enlarging the population;
- managing diversity;

- selecting for improved productivity; and
- establishing a store of cryoconserved genetic material.

The relevance of each of these objectives for breeds in the various risk-status categories is indicated in Table 4D4.

In addition to population size and trends, other demographic factors can influence risk status. Concentration of the population in a restricted area or in a limited number of herds may place it at greater risk of extinction (Carson *et al.*, 2009). Another factor to consider is the possible presence

TABLE 4D2

Risk categories for species with high reproductive capacity

Population trend and pure-breeding proportion[1]	Population size[2] (n)							
	Males (n)	≤80	81 – 120	121 – 800	801 – 1 200	1 201 – 1 600	1 601 – 2 400	>2 400
Increasing trend and >80% pure-breeding	≤5							
	6 – 20							
	21 – 35							
	>35							
Stable or decreasing trend or ≤80% pure-breeding	≤5							
	6 – 20							
	21 – 35							
	>35							

= Critical = Endangered = Vulnerable = Not at risk

Note: High reproductive capacity species = pigs, rabbits, guinea pigs, dogs and all avian species.
[1] Many countries do not have historical data with which to determine population trends or do not regularly monitor the proportion of pure-breeding. When this information is not available, the lower part of the table should be used.
[2] Some combinations with large numbers of females relative to males are not realistic, especially in the absences of artificial insemination. However, they illustrate that increasing numbers of one gender may not compensate for small numbers of the other.
Source: FAO, 2013.

TABLE 4D3

Risk categories for species with low reproductive capacity

Population trend and pure-breeding proportion[1]	Population size[2] (n)							
	Males (n)	≤240	241 – 360	361 – 2 400	2 401 – 3 600	3 601 – 4 800	4 801 – 7 200	>7 200
Increasing trend and >80% pure-breeding	≤5							
	6 – 20							
	21 – 35							
	>35							
Stable or decreasing trend or ≤80% pure-breeding	≤5							
	6 – 20							
	21 – 35							
	>35							

= Critical = Endangered = Vulnerable = Not at risk

Note: Low reproductive capacity species = horses, donkeys, cattle, yaks, buffaloes, deer, sheep, goats and camelids.
[1] Many countries do not have historical data with which to determine population trends or do not regularly monitor the proportion of pure-breeding. When this information is not available, the lower part of the table should be used.
[2] Some combinations with large numbers of females relative to males are not realistic, especially in the absences of artificial insemination. However, they illustrate that increasing numbers of one gender may not compensate for small numbers of the other.
Source: FAO, 2013.

TABLE 4D4
Relative importance of population management objectives according to risk status

Risk category	Enlarging the population	Managing diversity	Selection for productivity	Cryoconservation
Critical	+++	+++	-	+++
Endangered	++	+++		++
Vulnerable	+	+	+++	+
Not at risk		+	+++	

Note: The larger the number of plus (+) signs, the more important the objective. Minus (-) signs indicate that the objective should not be pursued. Absence of a sign means that the objective can or should be pursued, but the decision as to whether to do so should take other factors (e.g. the cost) into account.
Source: FAO, 2013.

of controlled or uncontrolled cross-breeding. The average age of breeders, their plans to continue livestock-keeping activities and their "exit strategies" and "legacy plans", if any, can also be significant. In many developed countries, significant proportions of livestock keepers are quite advanced in years and sufficiently financially secure to keep relatively unprofitable breeds because of tradition or as a hobby. When these breeders retire from active livestock keeping, the breeds they raise may be lost if younger breeders are not willing to take their place.

4 Determining the conservation value of a breed

All breeds or breeding populations categorized as being at risk of extinction can be considered candidates for inclusion in a conservation programme. However, it may be necessary to set priorities among these candidates. Risk status is often considered the most important criterion in setting conservation priorities. However, the value of conserving a given breed will be affected by a range of factors. Potentially relevant criteria include genetic uniqueness, within-breed genetic variation, traits of economic importance, unique traits and traits related to adaptation to a specific environment. The sociocultural value of the breed or its role in maintaining a unique ecosystem may also be reasons for assigning it a high priority.

When multiple factors need to be taken into account in establishing conservation priorities, one approach is to develop a "conservation priority index" that assigns different weights to the various factors (FAO, 2013). Once breeds have been prioritized, the costs of potential conservation programmes, along with their probability of success, need to be taken into account. Breed-ranking methods that include non-market values along with genetic variation and market values continue to be developed (e.g. Martin-Collado *et al.*, 2014; Zander *et al.*, 2013). However, to date such methods have mainly been limited to research. They are not widely used by countries when prioritizing breeds for conservation. Developments in this field are discussed in greater detail in Part 4 Section E.

In the case of transboundary breeds (see Part 1 Section B), prioritization may be complicated by the need to consider risk status not just at national level, but also across several countries. Collaboration at regional or global levels in the prioritization and planning of conservation activities should help ensure that transboundary breeds are not neglected because stakeholders at national level assume that they will be conserved elsewhere.

Molecular genetic data can contribute to the setting of conservation priorities (e.g. Tadano *et al.*, 2013). The panel of 30 species-specific microsatellite markers recommended by ISAG-FAO Advisory Group (FAO, 2011) still has some utility, especially for minor species, but is quickly being superseded

by more advanced approaches. Genomic techniques, such as detecting large numbers of single nucleotide polymorphisms (SNPs) or whole genome sequencing, allow the variety of alleles, haplotypes and genotypes within the genome to be established and the presence of rare alleles and unique genome sequences to be verified. The state of the art in the use of molecular tools is discussed in Part 4 Section B.

5 *In vivo* conservation

In vivo conservation programmes can involve a range of different types of action. In the case of *in situ* conservation, the general objectives are to support livestock keepers that raise at-risk breeds, to promote the sustainability of production systems in which at-risk breeds are kept and to promote developments that enable at-risk breeds to become more self-sustaining. More specifically, *in situ* programmes can involve (*inter alia*):

- breeding programmes that focus on increasing the productivity of at-risk breeds while managing their genetic diversity;
- efforts to promote the marketing of products from at-risk breeds;
- efforts to promote alternative uses for at-risk breeds;
- efforts to promote community-level initiatives to improve the management of at-risk breeds;
- the provision of advice on the management of at-risk breeds; and
- the provision of support payments to the keepers of at-risk breeds.

The range of activities that can be undertaken at an *ex situ in vivo* conservation site is more limited. Direct support payments are generally considered to be feasible only on a short-term basis.

The success of an *in vivo* conservation programme is likely to depend on the presence of an appropriate institutional framework. The tasks involved in organizing such a framework are discussed below in Subsection 5.1. Specific tools and approaches are discussed in Subsections 5.2 and 5.3.

5.1 Institutional arrangements

The context for *in vivo* conservation programmes will vary greatly between countries and between species. However, sustainable and realistic plans and appropriate mechanisms for involvement of livestock keepers and other stakeholders will always be required. An *in vivo* conservation programme, particularly an *in situ* programme, is likely to involve a wide array of stakeholders. Depending on the circumstances, these may include livestock keepers and breeders, government institutions, breeders' associations, breeding companies, research and education institutes, NGOs, consumers and marketers. Livestock keepers and breeders are the cornerstones of any *in situ* conservation programme and ensuring their commitment to the goals of the programme is essential.

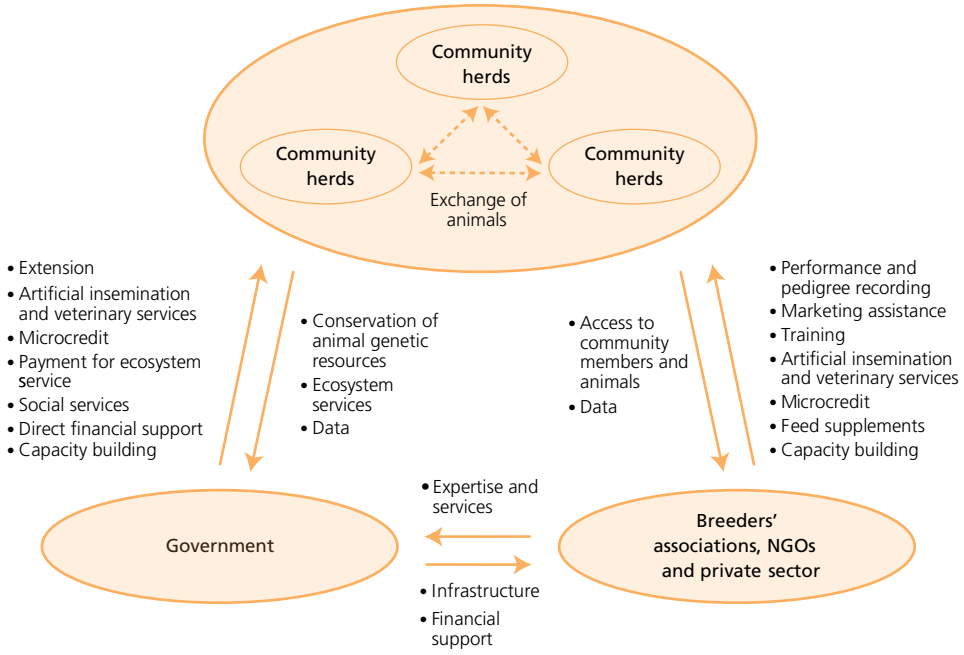
In some countries, mechanisms for livestock-keeper participation in conservation programmes are well developed, particularly via the activities of breeders' associations. Elsewhere, involving livestock keepers in organized conservation activities often remains very challenging. Initiatives to promote so-called community-based conservation programmes have been taken in various countries (FAO, 2003). Establishing a programme of this kind is normally a multifaceted task and requires careful assessment of the current and potential future roles of the targeted breed(s) in the livelihoods of local people. A top-down approach is unlikely to be successful. In other words, the livestock keepers potentially involved in the conservation activities will need to participate, from the start, in assessing the feasibility of the scheme and its relevance to their livelihoods and future objectives. New measures introduced to support the maintenance of the targeted breeds (e.g. breeding or marketing activities) will need to be planned in close collaboration with livestock keepers and other relevant stakeholders.

The long-term success of a community-based scheme is likely to depend on its being able to operate effectively with relatively little outside support (e.g. from government agencies).

Establishing or strengthening organizations within the community that are able to undertake the various tasks involved in implementing the programme (breeders' associations, marketing cooperatives, etc.) will therefore be essential. Nevertheless, as illustrated in Figure 4D1, some outside support from government or NGOs is likely to be necessary, particularly during the early phases of the programme. For example, at the start of a programme it may be necessary to create infrastructure such as new facilities for processing livestock products. Capacity-building to strengthen livestock keepers' abilities to undertake any new activities introduced as part of the programme is likely to be essential.

In many instances, particularly in developing countries, a livestock-keeping community that is a potential player in a conservation programme will have a very strong cultural tie to their breed and strong interactions are likely to exist between the community, the breed and the production environment. In such cases, the survival of the breed *in situ* will depend on the sustainability of these interactions. The community will often have indigenous knowledge on how to co-manage the animals and the local environment and have clear goals and ideas about selection. Documenting a community's role in the maintenance of AnGR diversity (and biodiversity more broadly) may encourage the development of policies that are favourable to the continued existence of the community and thus to the conservation of the breeds they keep. One approach that has been attracting increasing interest in recent years is to record such information in

FIGURE 4D1
Interactions among the potential stakeholders of a community-based conservation programme



Note: The ellipses indicate the major stakeholders. The bulleted lists indicate the goods and services exchanged between each pair of stakeholders, with the solid arrows indicating the flow of these goods and services.

the form of a biocultural community protocol, a formal document prepared on the basis of consultations between community members, lawyers and experts in indigenous knowledge (see Box 4D3).

Breeders' associations can contribute in many ways to conservation activities, as well as to other aspects of AnGR management. Promoting the establishment of well-organized and well-functioning breeders' associations, where they do

Box 4D3
Biocultural community protocols

Biocultural community protocols (BCPs) are a tool developed in response to the Nagoya Protocol on Access and Benefit-Sharing. The Protocol mandates governments to support indigenous and local communities, including women within these communities, to develop "community protocols in relation to access to traditional knowledge associated with genetic resources and the fair and equitable sharing of benefits arising out of the utilization of such knowledge."

BCPs are established through a facilitated process in which a community or group of livestock keepers reflect about the meaning and importance of their breeds and their production system, their own role in maintaining these resources and their vision and concerns for and about the future. The facilitators help the community to put these reflections down on paper, and provide information and advice about existing national rules and international legal frameworks that support the role of communities in *in situ* conservation and provision of ecological services.

BCPs make visible the linkages between breeds and the communities that have developed them. They establish breeds as the "prior art" of communities and therefore represent community claims over animal genetic resources. With regard to the implementation of the Nagoya Protocol, BCPs are potential tools in the process of establishing prior informed consent and mutually agreed terms when animal genetic resources sourced from indigenous and local communities are either utilized for research within the country or moved across international borders for that purpose.

BCPs also document community assets, including genetic resources, customary rights and traditional knowledge, and raise awareness about the value and potential of local production systems. They may also be important when public–private partnerships that involve livestock keepers are set up, and could be a first step towards payment for environmental services.

The process itself is extremely empowering for communities, as a means of self-reflection and understanding their existing rights. In addition, having at hand a written document that details their rights puts communities in a much better negotiating position with outside actors.

By October 2014, about eight livestock-keeping communities in India, Kenya and Pakistan had established BCPs. Interest in and demand for this approach are also increasing in other countries, especially in Africa and Latin America. A programme to develop more BCPs in India is ongoing.

Communities that have benefitted from the BCP process include the Brela pastoralists of Pakistan, who are nomadic and keep chickens and camels. The Brela camel breed is highly valued by the camel dairy industry in oil-rich countries because of its exceptional dairy potential. After going through the BCP process and becoming aware of the value of their genetic resource, the Brela pastoralists were able to double, triple and even quintuple the prices obtained for their female camels – increases of such a magnitude that sale of even one camel will provide sellers with enough income for the rest of their lives (Abdul Raziq Kakar and Rao Qadeer, personal communication).

Provided by Ilse Köhler-Rollefson and Evelyn Mathias.
For further information see UNEP and Natural Justice (2009) and the "Community Protocols" website maintained by Natural Justice (http://www.community-protocols.org/).

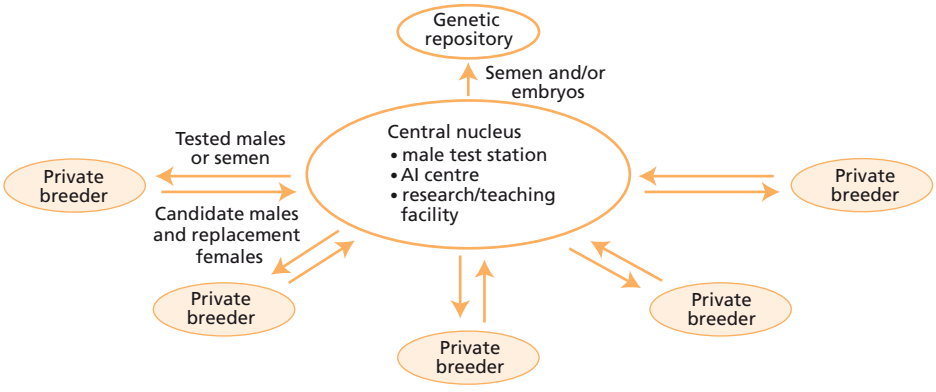
not already exist, is therefore an important objective. However, this can again be a challenging task. For example, potential members may lack the relevant organizational skills or there may be a lack of agreement over objectives for the management of the targeted breed. Elements that need to be considered in the establishment of a breeders' association include rules on eligibility for membership, procedures for registering animals and validating pedigrees, by-laws for the operation of the association (election procedures, composition of the board of directors, etc.), procedures for communication among the membership, procedures for conflict resolution and procedures for evaluating the performance of the association.

Where a range of different stakeholders are involved in conservation activities (e.g. both commercial farmers and hobbyists) and the animals are kept for a variety of purposes (e.g. for food production and for the management of landscapes and wildlife habitats) different objectives may result in different views about what breeding goals are appropriate (Lauvie *et al*., 2011). However, the populations concerned will often be too small to allow the simultaneous operation of several different conservation and/or selection programmes. In these circumstances, it is important to ensure effective communication among stakeholders and discussion of any tensions that may arise.

Breeding goals may change over time and this will affect the genetic variability of a breed population conserved *in vivo*. For example, as noted by Martyniuk *et al.* (2014), many dual-purpose (milk and beef) cattle breeds in Europe are no longer used primarily for mainstream food production and their numbers have decreased sharply. Animals belonging to these breeds are now used for a variety of purposes, mainly in suckler cow systems, where improving beef production from the offspring is an important objective. This has meant that the breeding goal (in the past a balance between milk and beef production) has shifted more towards beef production. This, in turn, means that genetic diversity in the populations maintained *in situ* will come to differ from that present in the original dual-purpose populations. This phenomenon calls for storage of genetic material from the original populations in a gene bank.

The maintenance of *ex situ in vivo* populations can also play an important role in conservation strategies. For example, they may provide a means of sustaining a breed whose population

FIGURE 4D2
A decentralized *ex situ* conservation programme involving institutional herds and private breeders



Source: FAO, 2013.

has declined to such an extent that it is difficult to maintain *in situ* or a breed for which there are few current options for promoting profitable production *in situ*. Establishing and operating an *ex situ in vivo* facility involves a substantial investment and provides little return in the short term. Programmes of this type are typically operated by governments, research institutes or NGOs and their long-term existence may be threatened by financial shortfalls.

One potential means of overcoming the constraints imposed by the cost of operating a centralized institutional farm is through the use of a dispersed model in which a breeding nucleus is linked to herds kept by NGOs and by private individuals who are willing to raise animals on a commercial or hobby basis. A network of several herds can provide a basis for an integrated conservation programme and systematic genetic improvement. The basic design of this type of model is illustrated in Figure 4D2. This approach is promoted in India as a means of conserving several of its indigenous cattle breeds.

5.2 Conserving genetic variability in small populations

The probability that a breed will survive depends greatly on the amount of genetic diversity it harbours. A high level of genetic diversity allows the population to adapt to changes in the production environment. It prevents the rise of inbreeding and its detrimental effects. In very small populations, i.e. breeds whose risk status is critical or endangered, the management of genetic diversity is crucial to survival, and breeding programmes should focus on this task (see Subsection 3 for an explanation of the risk-status categories). In populations that are somewhat larger, i.e. breeds whose risk status is vulnerable, there is more opportunity to implement programmes aimed at genetic improvement. However, maintaining genetic variation remains essential.

A strategy aimed at maintaining a breed's genetic variability needs to focus on managing the relationships among the breeding animals. Measures that can be taken include:

- involving as many animals as possible in the programme from the start in order to minimize genetic drift;
- increasing the number of males used for breeding;
- lengthening the generation interval;
- optimizing the contribution of each individual to the next generation;
- banking genetic material at the start of the programme and then at regular intervals, so that it can be used in subsequent generations; and
- in species with low reproductive rates, using embryo transfer to increase the population size.

It is also possible to adopt a mating strategy aimed at reducing inbreeding. This can involve:

- setting a limit to the degree of relationship between mates;
- using algorithms and software that determine the ideal set of matings for the entire population; and
- simple strategies that can be implemented even if no pedigree information is available (e.g. fixed rotation of males between herds).

Determining molecular coancestry using SNP-chip technology is a very effective tool in the management of genetic diversity within a population (Gómez-Romano, 2013). Several strategies for maintaining molecular genetic diversity in conserved populations have been developed (Fernandez *et al.*, 2011; Toro *et al.*, 2014). In general, molecular coancestry is a better descriptor of genetic relationships in a population than pedigree coancestry and is a better indicator of inbreeding and inbreeding effects. Pedigrees only indicate expected genetic relationships, whereas molecular coancestry provides information about the actual transmission of genes from parents to offspring. Moreover, pedigree registration occasionally includes errors (e.g. Kugonza *et al.*, 2012). Errors occur in genotyping as well, and these errors can affect the accuracy of estimates of genetic parameters (Hinrichs and Suarez, 2005). However, they tend to be less serious than incorrect assignments of parentage in pedigrees.

It is, however, important to pay particular attention to determining whether genetic similarity between animals at molecular level indicates identity by state or identity by descent (Powell *et al.*, 2010; Stevens *et al.*, 2011). Where maintaining diversity in a conserved population is concerned, identity by descent is of primary interest (Toro *et al.*, 2011).

Use of genomic technology in small conserved populations is very informative and highly recommended where possible (e.g. Pertoldi *et al.*, 2014). Clearly, however, costs and requirements for technical expertise will limit such applications, especially in developing countries. Accuracy of inference depends on the amount of genomic information available (e.g. the number animals genotyped and the number of SNPs per animal) (Toro *et al.*, 2011).

Further information on the various tools and approaches discussed in this subsection can be found in FAO's guidelines on *in vivo* conservation (FAO, 2013).

5.3 Potential strategies for increasing demand for at-risk breeds

Breeds may face the risk of extinction because their productivity is low and therefore keeping them provides inadequate economic returns. Breeding strategies can be a means of addressing this problem. Options include within-breed selection programmes (balancing between genetic progress in terms of increasing production and avoiding an increase in inbreeding) and strategies based on cross-breeding. The optimal approach will depend on the situation. As in all circumstances, any breeding strategy adopted must be well-matched to the production system (FAO, 2010). The size of the population is also an important consideration. If populations are too small, within-breed selection may not be a viable option. Genetic drift is likely to negate any potential for progress through selection.

Cross-breeding may not, at first sight, appear to be a good means of promoting the conservation of an at-risk population. However, there are situations where cross-breeding can be extremely useful. For example, if a breed population has become so small that it is non-viable, limited crossing with a genetically similar breed to increase the population size and increase genetic variability may be an option to consider. Moreover, cross-breeding strategies that involve ongoing maintenance of pure-bred populations (e.g. terminal crossing systems) may create a profitable means of utilizing breeds that in their pure-bred form are not sufficiently competitive to encourage livestock keepers to maintain them.

Aside from breeding strategies, a number of other methods can potentially be used to increase the value of at-risk breeds to livestock keepers (or other potential users) and hence promote their continued use. Techniques such as SWOT analysis (see Subsection 2) can help in the identification of appropriate strategies for specific breeds.

One potential, and relatively straightforward, approach is to provide practical support to livestock keepers that raise at-risk breeds. This can both increase the likelihood that the livestock keepers will be willing and able continue raising the targeted breeds and help ensure that they are appropriately managed in genetic terms. The type of support needed will clearly vary depending on the circumstances. Where an organized community-based conservation programme (see Subsection 5.1) is being implemented, the aim should be to tailor advice and support to the specific conservation activities being undertaken. More broadly, the provision of appropriate services that support the sustainability of diverse livestock-keeping communities – particularly smallholder and pastoralist communities – is likely to promote the continued use of the locally adapted breeds associated with these communities. In many circumstances there will be potential for increasing the profitability of livestock keeping by improving management at farm (or herd/flock) level (improving feeding, housing, disease control, etc.). Where "hobby farmers" (largely a developed-country phenomenon) are concerned, enthusiasm for keeping locally adapted breeds may not be matched by sufficient experience in breeding and in other aspects of animal husbandry. Advice on these matters may

therefore be needed. One option for disseminating breed-specific knowledge is to implement a "role model breeders" programme that enables the experience accumulated by long-standing and successful breeders to be passed on to others (see FAO, 2013 for further discussion of schemes of this kind).

Another means of increasing the profitability of keeping an at-risk breed is to increase the marketability of its products (see Box 4D4). This may enable lower production levels to be compensated for by higher per-unit prices. Particularly in developed countries, a lot of attention has been paid in recent years to the development of niche markets for the products of "non-mainstream" breeds (e.g. Ligda and Casabianca, 2013). In some cases, this involves marketing on the basis of some unique and desirable characteristic of the product itself (e.g. superior taste). In others, it involves some desirable aspect of the breed's production system (e.g. the appeal of buying a locally grown product). Initiatives of this kind can be facilitated by the existence of labelling schemes that increase consumer confidence in the provenance of the products (see Part 3 Section F Subsection 4.4 for a discussion of legal frameworks for schemes of this type).

As well as providing marketable goods and services, livestock also have the potential to deliver various other kinds of benefits within the

Box 4D4

Identifying keys to success in breed conservation and development in France: the VARAPE project

About 30 percent of French local breeds are considered to be endangered according to thresholds set by national legislation (fewer than 5 000 breeding females for cattle, 8 000 for sheep and goats, 1 000 for pigs). Most of these breeds declined until the 1970s, at which time the introduction of national conservation policies and programmes helped to stabilise or increase their population sizes.

The VARAPE project (valorization of rare breeds with short supply chains), which ran from 2012 to 2014 and targeted 13 breeds, was coordinated by France's Institut de l'Elevage, working in association with seven technical partners. Based on 13 breed surveys (involving inventories of production and marketing, and meetings with local committees) and 16 case studies, the project aimed to assess factors influencing the success of collective projects targeting the development of short supply chains for breed products.

One output was a diagnostic tool that can be used to formalize breed valorization projects and choose optimal organizational structures. Eight keys to success were identified:

- building a network involving all relevant stakeholders (farmers, processors, retailers, etc.);
- ensuring long-term coordination of the network;
- sharing a common vision and common objectives;
- highlighting links to history and culture;
- developing products and markets in a way that is consistent with the production capacity of the livestock keepers involved;
- establishing adequate quality indicators or labels;
- identifying relevant economic and technical indicators; and
- maintaining links with partners.

The results of the study showed that breed associations generally wanted to improve marketing structures, with the aim of increasing the number of livestock keepers raising the breed and improving the protection of their products from unfair competition (misleading labelling, etc.). They also showed that quality indicators (individual brands or schemes such as the European Union's Protected Designation of Origin or Traditional Specialities Guaranteed) need to be chosen according to the specific context of the breed, considering factors such as the size of the breed population and the type of product involved.

Provided by Lucie Markey and Christèle Couzy.
For further information on the VARAPE project (in French) see www.varape.idele.fr

ecosystems in which they are kept, for example by maintaining landscapes and wildlife habitats (see Part 1 Section D and Part 4 Section E for further discussion). Given that these benefits tend to be public goods, they generally cannot be marketed (i.e. directly sold to consumers) to provide additional income for livestock keepers. However, governments may be willing to pay for services of this kind. For example, so-called "conservation grazing" has become a significant tool in the management of wild biodiversity in a number of countries, mainly in developed regions. This trend has created opportunities to keep locally adapted breeds of grazing animals such as cattle, sheep, goats and horses in use and hence to promote their conservation. Locally adapted breeds are often the best suited to this role because of their ability to cope with the harsh environments (mountains, heaths, wetlands, etc.) where such services are often required.

Touristic value is another attribute that can potentially be exploited to promote conservation. This is more likely to be the case where the breed has some kind of distinctive appearance or is closely linked to local products or cultural traditions. Some communities hold festivals celebrating traditional customs associated with raising local breeds of livestock. Such events, although they may not provide direct economic support to livestock keepers, may improve the economic status of the communities in general (e.g. by promoting tourism) and can provide marketing opportunities for the breeds' products.

When possible, combining a number of different conservation activities is a logical approach. Box 4D5 describes a proposed programme to conserve Pantaneiro dairy cattle in Brazil. The programme aims to combine practical support for breeding with the marketing of a breed-specific product. In addition, opportunities have been identified to exploit specific genes from the Pantaneiro in breeding programmes for other breeds, as well as to leverage the ecosystem services provided by the breed in its traditional production environment.

6 Cryoconservation

As described in Part 3 Section D, recent years have seen an increase in the number of national gene banks and in the sizes of their collections (see also Boettcher and Akin, 2010; Pizzi *et al*., 2010). National gene banks are a relatively new element of AnGR management and there have been ongoing efforts to develop the protocols and facilities needed to increase their operational efficiency.

All the available scientific evidence indicates that cryopreserved biological material can be stored without deterioration for several thousand years (Mazur, 1985). The possibility of long-term storage opens opportunities to conserve and utilize animal genetic diversity in ways that were impossible in the past when *in vivo* conservation was the only option available. Cryoconservation programmes can serve a number of purposes. FAO (2012) identified the following major objectives:

- One common reason for gene banking is to provide the possibility of recreating breeds or breeding lines if they are lost as the result of a catastrophic event or deliberately allowed to go extinct for financial reasons (e.g. the discontinuation of a specialized research line). In such cases, having sufficiently large and genetically diverse collections of germplasm from the affected breeds can allow them to be reconstituted.
- Cryoconserved material can be used to introduce genetic diversity into *in vivo* populations for the purposes of reducing inbreeding levels and broadening diversity. It can also be used to provide flexibility to the livestock industry when selection goals are found not to be as desirable as initially thought.
- Gene bank collections are invaluable if breeds are threatened with extinction because of an extreme genetic condition such as high frequency of a genetic defect resulting from selection or genetic drift. Stored material

Box 4D5

Indigenous people and scientists team up to conserve Pantaneiro cattle in Brazil

Pantaneiro cattle have lived in Brazil's Pantanal Biome since their introduction by the Portuguese some 400 years ago. They are believed to be resistant to trypanosomosis, myiasis, worms and ticks. They are able to survive under the challenging ecological conditions of the Pantanal, which include both floods and droughts, as well as coarse native pastures and jaguar predation.

At the beginning of the twentieth century there were several thousand Pantaneiro cattle. However, the breed's population has since fallen to a few hundred. Intermixing with commercial breeds is the main threat to its survival. Today, only 500 pure-bred animals, split between two herds, are left. This small population size and the accompanying loss of genetic variation threaten to erode the breed's capacity to adapt and survive.

Commercial breeds have lost some alleles associated with fitness and survival in harsh environments. One example is the G1 allele of the bovine growth hormone gene, dubbed the "thrifty gene", which has become essentially extinct in commercial breeds, but can be found in some traditional cattle (Dani *et al.*, 2010), including the Pantaneiro.

As part of efforts to protect the Pantaneiro breed and the ecosystem to which it is adapted, as well as their own livelihoods and culture, indigenous people from the Pantanal region have teamed up with scientists from several Brazilian research institutes to develop the Pantanal Biome Cheese Project. As the true "Nicola cheese", a traditional local product of the Pantanal, is prepared with the milk of Pantaneiro cows, it is threatened with extinction along with the breed. However, it may also hold the key to the breed's conservation. The production and commercialization of Pantaneiro cattle and Nicola cheese may provide the Pantaneiro people with regular income, while also helping them conserve the local ecosystem.

One of the activities undertaken by the scientists working on the Pantanal Biome Cheese Project is to screen the Pantaneiro cattle for genetic polymorphisms associated with milk protein and fat composition, as well with the "thrifty" phenotype of these cattle. This molecular characterization will not only help identify valuable genetic resources for breeding, but will also serve as the basis for marker-assisted certification to ensure accurate identification of the genetic material of Pantaneiro animals and the breed's products. The scientists believe that a conservation programme that includes marker-assisted selection, distribution of genetic material such as semen and embryos, and marker-assisted certification of origin may help save the Pantaneiro cattle from extinction and also contribute to the conservation of the Pantanal Biome and the life and traditions of its people.

The Pantanal Biome Cheese Project capitalizes on the fact that the Pantanal Biome is a Biosphere Reserve included in UNESCO's World Heritage and the MAB-Man and Biosphere programme of the United Nations.

Photo credit: José Medeiros.

Provided by Sergio Ulhoa Dani and Marcus Vinicius Morais de Oliveira. For further information see Dani and Oliveira (2013) and http://biomacheese.blogspot.it/

from animals not carrying the deleterious allele can be used to decrease the frequency of the defect to a manageable level.

- Gene bank collections can be used to develop new lines or breeds, introgress desired characteristics from one breed into another or quickly reorient the evolution or selection of a population.
- Gene banks serve as a ready source of genetically diverse and specialized DNA for genetic diversity studies, genome-wide association studies, exploration of gene function and other types of research. Importantly, gene banks can, over time, provide multigenerational samples that contribute to increasing the accuracy of genomic selection. These latter benefits will be more easily realized if information on animals' phenotypes is maintained along with their genetic material (see Subsection 6.4).

6.1 Gene bank operations, infrastructure and institutional frameworks

A national gene bank should be designed in accordance with the needs and capacities of the country. Staffing a gene bank requires, in particular, expertise in genetics, cryobiology/reproduction and data management. The necessary physical infrastructure also needs to be developed. Figures presented by FAO (2012) illustrated that, in the case of small repositories, the cryopreservation component of a gene bank could potentially be established for less than US$50 000 in equipment costs. Greater access to commercial genotyping and potentially to large amounts of genomic data implies that a gene bank needs either to develop within-house capacity to conduct statistical analysis and interpret genetic and genomic data or contract out the analysis phase of genetic diversity studies. Hardware costs associated with the development of information systems are relatively minor. The largest recurrent costs in the operation of a gene bank are usually those associated with human resources.

A cryoconservation programme can involve the collection of various types of genetic material. Semen is the most commonly banked material. Embryos are more complicated and expensive to collect and store (Gandini *et al*., 2007). However, if a breed needs to be reconstituted, embryos have an advantage over semen in that they provide the full genetic complement of the reconstituted breed in a single generation. Reconstitution with semen requires several generations of backcrossing and will never achieve 100 percent reconstitution of the original genome. Moreover, the mitochondrial genome of the original breed is totally lost if only semen is stored. As well as semen and embryos, gene bank collections can include oocytes and various gonadal and non-gonadal tissues.

Because of the role of the private sector in maintaining breeds *in situ*, it is essential that gene banks have close links to individual breeders and to breed organizations or livestock-keeping communities. This allows stakeholders to communicate their needs and helps establish working relationships that facilitate the collection of samples.

Gene bank collections should be viewed dynamically, with samples entering and exiting the gene bank as a matter of routine and being used for a variety of purposes. This type of approach is relatively new in the livestock sector. Each gene bank should have a set of protocols and procedures for assessing requests for germplasm. One option is to establish an advisory committee (e.g. consisting of industry and public-sector representatives) to review and make recommendations concerning requests. Issues for consideration when reviewing such requests can include the availability of the respective genetic resource *in situ*, whether the gene pool needs to be expanded, current and projected inbreeding levels, selection options available to the breeders and the way in which the progeny obtained using the gene bank material are to be utilized. Depending on the policies or regulations of the country, the advisory committee may also be interested in knowing whether,

and if so how, germplasm from the progeny will eventually be made available to help replenish the gene bank.

Choice of breeds for inclusion in a gene bank collection can be politically sensitive. Gene bank managers should recall that while breeds do not need to be treated equally they should be treated equitably and reasonably.

Because of the increasing number of national gene banks (see Part 3 Section D) the question of potential international cooperation in gene banking is becoming increasingly prominent. Potential cooperative activities need to be evaluated on the basis of the needs and capabilities of the potential partners and the potential benefits that might be gained. Establishing linkages between gene banks is likely to be easiest at regional level, as there are likely to be shared interests, similar breed types and similarities in collection protocols. For example, groups of countries in the Americas and in Europe have identified common goals and interests. These are generally based on broad initiatives such as the development of shared databases (or at least some level of commonality among databases) and the exchange of experiences and technical know-how. Protocols used to cryopreserve samples or to genetically evaluate collections are another area of collaboration.

In the plant genetic resources sector, pairs or groups of countries have agreed to back up each other's gene banks by holding a complementary collection of some or all samples. However, for several reasons this approach has rarely been employed in the AnGR sector. Sanitary regulations restricting germplasm movement across national boundaries are a major limitation. It may, however, be possible to overcome constraints of this kind by classifying material "for gene bank storage only" (i.e. not for use within the importing country). If the material is not used in the importing country, then the risk of disease transmission will be low. Administratively, the most direct and effective means for a country to back up samples from another country is via a bilateral agreement. Such an approach also facilitates the identification of the specific needs of the cooperating countries and their rights, limitations and obligations with respect to storing and using the material.

6.2 Establishment and assessment of gene bank collections

Collection strategy

The establishment and ongoing operation of a gene bank collection require strategic decisions regarding what material to collect. Consideration needs to be given to the intended scope of the collection. For example, some countries have focused gene bank collections on at-risk breeds (Mariante *et al.*, 2009; Paiva *et al.*, 2014), while others are developing collections that include both at-risk and mainstream breeds (e.g. Pizzi *et al.*, 2010; Blackburn, 2009; Woelders and Hiemstra, 2011). While it is possible to argue that widely used transboundary breeds are not priorities for inclusion in conservation programmes, there are several reasons why countries may wish to include such breeds in their collections. For example:

- widely used transboundary breeds are likely to be important for the future of commercial agriculture and therefore need to be included in the gene bank to ensure a backup that can be drawn upon in case of need;
- large collections of material from such breeds have been shown to be invaluable in providing specific alleles or allelic combinations for use in industry or research; and
- collecting samples from such breeds will ensure that changes in allelic frequencies that may confer adaptation to environmental variables are captured and available for use as needed.

Regardless of what types of breed a country chooses to target, there will be a need to assess the genetic diversity captured and the quantity of germplasm accumulated and to optimize the collection in accordance with associated costs. Theoretical methods for prioritizing breeds (e.g. Boettcher *et al.*, 2010; Martin-Collado *et al.*, 2013) and animals

(e.g. Blackburn, 2009; Engelsma *et al*., 2011) have been developed. Blackburn (2009 and 2012) discusses practical approaches to building collections at both within-breed and between-breed levels. In practice, effective development of a collection requires flexibility in the selection of animals within a population and the capacity to adjust and adapt cryopreservation protocols to the given situation. For example, theoretical approaches to selecting the optimal set of gene bank donors typically lack the flexibility needed to account for real-life circumstances such as the death or poor fertility of an animal targeted for collection or the refusal of its owner to allow access.

In developing a collection there is need to determine the minimum quantities of germplasm and genetic variation needed to meet the objectives of the gene bank. In general, the primary objective will be to store enough germplasm to reconstitute a breed that is extinct (*in vivo*) to create a new population with an effective population size of 50 animals. Population reconstitution is generally the objective that requires the greatest quantity of germplasm. The quantity required will depend on a number of factors, including the type of germplasm stored, the species involved and the reproductive efficiency achieved (see FAO, 2012 for further information). In general, breed reconstruction requires fewer embryos than units of semen. Species with multiple offspring per pregnancy, such as chickens, rabbits and pigs, will require fewer doses of semen than species, such as cattle, horses and small ruminants, that produce one or few offspring. The higher the expected pregnancy and survival rates, the less germplasm is needed.

Once minimum quantities for a given cryoconservation objective have been achieved (i.e. sufficient numbers of donors and quantities of germplasm per donor have been acquired), gene banks can consider various approaches to the management of their collections. For example, the national gene bank in the United States of America has developed an index that gives equal weight to quantities of germplasm and number of donors and uses this index to monitor the inventories of breeds with material in the bank (Blackburn, 2012). The index provides a simple means of identifying breeds for which additional collection would be beneficial. Closer examination of the data contributing to the index can then determine whether a given breed simply requires collection of additional material (i.e. from the same animals or their close relatives) or whether genetically diverse material from new, unrelated donors is needed.

While meeting targets is a first objective in the development of a gene bank collection, gene bank managers may choose to expand the scope of their collections for a variety of reasons. Smith (in FAO, 1984) showed that the probability of capturing an allele in 10 or more units of semen is equal to $1 - (1 - P)^{2N}$, where P is the allelic frequency and N is the number of males sampled (equation modified by Blackburn, 2004). As this equation demonstrates, increasing the number of males collected raises the probability of capturing an allele, but with a trend of diminishing returns. For example, with an allele frequency of 0.005, sampling 100 males will result in a 63 percent probability of capturing the allele. With 300 males, this value jumps to 95 percent. However, increasing the number of males sampled to 500 will raise the probability only another 4 percentage points, to 99 percent. This suggests that big collections may be necessary in order to capture and preserve extremely rare alleles. For example, the United States of America's gene bank has a large collection of samples from Holstein cattle. This has allowed the cryoconservation of semen from bulls that carry rare Y chromosomes that are no longer present in the *in situ* population (Yue *et al.,* 2015).

Assessing and ensuring genetic diversity

There are several approaches that gene bank managers can use to assess the genetic diversity of the collection and to identify the animals in the *in vivo* population that they wish to sample to broaden the diversity of the collection. These approaches may use pedigrees, molecular markers and/or geographic location as indicators of diversity. In addition to genetic variability, there is a

need to consider variability in phenotypic or genetic measurements (e.g. breeding values) for economically important characteristics.

A broad array of analyses can be applied to pedigree information to estimate genetic parameters and compare the diversity of animals in the collection and in the *in situ* population. For example, Danchin-Burge *et al*. (2011) used the parameter "effective number of founders" to demonstrate that both the French and the Dutch gene banks have fully captured the level of genetic diversity present in the *in situ* Holstein population. They also showed that the effective number of Holstein-Friesian founders stored in the United States of America's gene bank substantially exceeds that of the current *in situ* population. With pedigree information available, the genetic coefficient of relationship between animals in the collection and the *in situ* population can be computed. This information can be extended, through various clustering routines, to determine the status of germplasm already in the collection (in terms of influential founders and their descendants) and identify groups of animals that might be targeted for procurement to increase the genetic variability in the collection (Blackburn, 2009; FAO, 2012; Blackburn, 2012).

The development of collection strategies can also be supported by the use of DNA markers (either microsatellites or SNPs) to assess differences among and within populations. For example, a comprehensive assessment of microsatellite genotypes among sheep breeds in the United States of America determined that the Warhill population should be classified as a strain of Rambouillet and not as a separate breed (Blackburn *et al*., 2011). As a result, collection strategies were adjusted. Numerous characterization studies have evaluated breed similarities and differences at the molecular level, both within and across countries (for a review, see Groeneveld *et al*., 2010). Countries should consider such results and consult with each other when developing gene banking strategies, particularly for transboundary breeds. As the functional role of genes marked by particular SNPs is determined, it will become possible to incorporate such information into strategies for the assessment and acquisition of gene bank collections.

Geographic approaches to planning and evaluating collections have been used for wild animal species and plant genetic resources (e.g. Hijmans *et al*., 2000). However, in the case of AnGR, the utility of developing or evaluating collections solely on the basis of geographic location seems to vary from situation to situation. At the breed level, pedigree or molecular data suggest that in some instances there are only slight to modest differences between geographically distant populations. For example, Maswashie and Blackburn (2004) found no evidence of substantial subpopulations of Navajo Churro sheep across the United States of America. Based on SNP data on African goat breeds, Huson *et al*. (2014) suggest that there is little genetic differentiation among goat breeds found in the various countries of East Africa.

Comparing average phenotypes or estimated breeding values (EBV) of animals with material stored in gene bank collections to those of *in situ* populations serves to gauge the completeness of the collection in terms of diversity and its utility for various functions. Whenever possible, highest and lowest values for animals in the bank should, respectively, be superior and inferior to the mean by at least one standard deviation. "Bounding" the breed's mean in this way helps ensure that two important goals are met: first, the choice of animals with both high and low values ensures that genetic variability is captured; second, the choice of animals with high (i.e. favourable) EBVs means that samples in the collection are likely to have industry relevance for two to five decades. If this approach is followed, taking a large number of traits into account and with periodic resampling, there is no reason for gene bank collections to become obsolete.

6.3 Cryobiology and reproductive physiology

At one time, the advice was that gametes for cryoconservation should be collected only at artificial insemination centres (FAO, 1998). However,

the experiences of the last decade show that this is not necessary, particularly for material to be utilized at country level (i.e. that is not going to be exported). Assuming the sanitary restrictions of the respective country allow (and if proper collection, cryopreservation and health procedures are followed), germplasm and tissue from nearly all livestock species can be acquired in the field with little to no negative consequences in terms of viability or veterinary hygiene. This provides additional opportunities to capture genetic diversity and reduce collection costs. Once germplasm has been collected, it can generally be stored for 24 to 36 hours while being transferred to a cryopreservation laboratory. Fresh semen from various species has been routinely moved from place to place prior to being used successfully for insemination, suggesting that semen transported in this way can also be cryopreserved and banked. For example, Purdy *et al*. (2010) found that ram semen could be held for 24 hours before cryopreservation and still achieve acceptable fertility and prolificacy levels when subsequently used for artificial insemination.

If traditional semen collection and processing are not feasible because of a lack of facilities or expertise near the area where the targeted animals are raised, or if genetically valuable animals die before collection is possible, collecting epididymal sperm from deceased or castrated animals may be a useful means of enhancing gene bank collections (Silvia *et al*., 2014). Testes collected from such animals are quite robust, and sperm remain viable after several hours of storage at body temperature or even longer if properly cooled. This allows collection on the farm or at the slaughterhouse and transport to a laboratory. Recent studies on the cryobiology of epididymal sperm from ibex (Pradiee *et al*., 2014) and goats (Turri *et al*., 2014) suggest that storing such material in gene banks is feasible.

Direct freezing of samples in the field may be an option, depending on the type of biological material involved. For example, Groeneveld *et al*. (2008) detailed a method used for collecting pig tissue from the field in Viet Nam. The equipment needed for field collections is relatively inexpensive. For example, samples can be cryopreserved in a simple Styrofoam box and then placed in a portable liquid nitrogen tank.

Cryopreservation involves freezing cells and tissues to -140 °C (the vapour phase of liquid nitrogen) or -196 °C (the liquid phase of liquid nitrogen). The process places cells into a suspended state of animation where most biological processes cease to function. Cells that have been successfully cryopreserved remain suspended until revived by thawing. The type of cell (e.g. whether sperm, embryo or blood), particularly cell size and cell membrane composition, affects the way cells need to be prepared for freezing and the freezing rates that need to be applied. For example, the cooling rate for bovine sperm (-19 °C to -25 °C/minute) is very different from that for embryos (-0.5 °C/minute) (FAO, 2012) and freezing protocols for semen differ among species.

Cells to be cryopreserved are suspended in a medium containing various sugars, lipids and – most importantly – cryoprotectant compounds such as glycerol. Glycerol was the first cryoprotectant agent identified (Polge *et al*., 1949) and is still the primary cryoprotectant used across species. The cryoprotectant compound reduces the formation of ice crystals, which can damage cells of all types. In recent years (i.e. since 2005/2006 when the first SoW-AnGR was prepared), cryopreservation research has continued to advance (e.g. Okazaki and Shimada, 2012; Woelders *et al*., 2012), particularly with regard to the preservation of oocytes and other non-traditional types of germplasm (Pereira and Marques, 2008; Mullen and Fahy, 2012) and the analysis of changes in the cell membrane before and after cryopreservation. As a result of this and other work, new media for cryopreservation are continually being evaluated and improved upon.

Genetic material from all livestock species can be cryopreserved and stored in a gene bank. However, the efficacy of the cryopreservation process and the ease with which germplasm or tissue can be used to generate animals varies substantially across species. Protocols for cryopreservation and

regeneration using either semen or embryos are well established for cattle. Cryopreservation of pig germplasm is also relatively straightforward. However, for sheep and goats, both cryopreservation protocols and regeneration procedures need to be improved. For both these species, infrastructure limitations impede the widespread use of cryopreserved material. Moreover, these species have smaller commercial industries, which means there is less investment in research.

The use of cryopreserved chicken semen has been particularly problematic: not because the sperm do not freeze well, but because the cryoprotectant glycerol is a contraceptive in the hen. Several means of addressing this problem – alternative cryoprotectants such as dimethyl sulfoxide (commonly known by the abbreviation DMSO) or intramagnal inseminations – have been developed and have sometimes been used (e.g. Long *et al*., 2014). However, results have not always been totally satisfactory for a number of reasons. The ground-breaking approach developed by Song and Silversides (2006; 2007a; 2007b) – involving the harvesting of gonads from day-old chicks, cryopreserving them and then transplanting the thawed tissue into chicks of three to seven days of age – represents a quantum step forward in the cryoconservation of avian genetic resources. Using this approach, entire breeds or lines can be reconstituted and ready for mating in approximately one year (see Box 4D6).

Lack of a stable, long-term and financially affordable source of liquid nitrogen can be a severe constraint to gene banking. Freeze-drying sperm does not require liquid nitrogen and allows sperm to be stored at 4 °C and transported at room temperature. Offspring have been obtained from oocytes fertilized with freeze-dried rat epididymal sperm stored at 4 °C for five years (Kaneko and Serikawa, 2012). However, further development is needed in order to make this approach viable in livestock species. Other innovative approaches to biobanking are being developed (see Box 4D7). For example, studies are being undertaken on the maintenance of nuclear and cellular viability in somatic cells and female gametes following freeze-drying. The development of dry biobanks of cells and gametes, which rely on protocols that are less costly and more environmentally friendly than current methods, could become a reality in the future (for a review see Loi *et al*., 2013).

6.4 Information systems and documentation

Another important aspect of gene banking is the development and management of a database and the provision of information on the collection to stakeholders. A gene bank information system needs to handle two major categories of data:

- information on the quantities and types of germplasm and tissue maintained in the collection; and
- information on the animals whose genetic material is stored – phenotypic and genetic measures and information on the production systems and environmental conditions in which the animals were raised (FAO, 2012).

If information on a gene bank's holdings is made publicly available on the internet stakeholders will be able to view the collection and make a request for samples or determine what germplasm they might like to contribute to the gene bank. Establishing a comprehensive database takes substantial effort and time. Pooling efforts internationally may be helpful. For example, Brazil and the United States of America have collaborated in the development of the Animal-Genetic Resources Information Network (Animal-GRIN),[4] a database used to manage their respective AnGR programmes.

Web software for the documentation of cryoconserved material in animal gene banks is widely used in Europe. The CryoWEB software (Duchev *et al*., 2010) can record basic information on donor animals, storage facilities, and stored samples and their sites of storage within a gene bank. In order to integrate information from national gene bank collections, the European Regional Focal Point for the Management

[4] http://nrrc.ars.usda.gov/A-GRIN/database_collaboration_page

Box 4D6

A study of the comparative costs of *in vivo* and cryoconservation programmes for chickens

A study estimated and compared the costs, over a 20 year period, of three different approaches to chicken conservation:

1. maintaining live populations;
2. semen cryopreservation followed by reconstitution of the population via backcrossing; and
3. ovary and semen cryopreservation followed by reconstitution of the population via ovarian transplantation and subsequent insemination.

The costs of keeping live populations vary greatly, but for the purposes of the study they were approximated on the basis of typical costs of maintaining a population at an institution in North America. It was assumed that no revenue was derived from the live populations. Costs of cryopreservation and population reconstitution were based on biological parameters derived from the literature. The costs for all three programmes were subdivided into the cost of preservation, the annual cost and the cost of recovering the population.

For populations maintained in living form, there are no costs for preservation and reconstitution. However, the annual costs are high and cumulative: the longer the live population is maintained, the higher the total costs. The costs of cryopreservation are low, and the annual costs of maintaining cryopreserved material are extremely low. The largest cost of a cryoconservation programme relates to recovery of the population.

In this example, keeping live populations was found to be the most cost-effective strategy for periods of up to three years. However, if the population was not going to be used within five years, cryoconservation was the most cost-effective strategy. The least expensive cryoconservation strategy was found to be the one based on storing both ovaries and semen. Over an extended period of time, the estimated savings relative to the costs of maintaining live populations were found to be more than 90 percent (see table). The low cost of cryoconservation suggests that avian genetic material should be cryoconserved, with individual populations reconstituted when needed.

This study focused on chickens and used parameters particular to that species and a particular institutional situation, so the results and conclusions are not universally applicable. However, the principal of estimating and comparing the costs of various conservation programmes by dividing the costs into costs of preservation, yearly maintenance costs and costs of recovery can be used for any mammalian or avian species in any situation.

Provided by Frederick G. Silversides.
For further information, see Silversides *et al.* (2012).

Estimated costs (US$) of different conservation programmes

Conservation method	Years of storage	Number of populations stored/recovered	
		10/1	10/10
Maintaining living birds	1	179 000	179 000
	5	957 000	957 000
	20	5 306 000	5 306 000
Storing semen followed by backcrossing	1	288 000	758 000
	5	298 000	769 000
	20	354 000	825 000
Storing semen and ovaries followed by ovary transplantation and insemination	1	109 000	218 000
	5	118 000	228 000
	20	172 000	281 000

Box 4D7

Use of induced pluripotent stem cells in *in vitro* conservation

Somatic reprogramming (Takahasi and Yamanaka, 2006) has brought about a revolution in the field of stem cell research. Pluripotent stem cells whose developmental potential includes germline colonization can now be obtained via a simple non-invasive biopsy. In other words, it is now possible to transmit the diploid genetic patrimony of an individual (male or female) directly from a somatic cell. While this has so far been demonstrated only in rodents, it is hoped and expected that further research will make it possible in many species. Considerable advances have already been made, particularly in the delivery of the molecular factors able to reprogramme somatic cells without affecting the stability and integrity of the genome, i.e. without generating genetically modified cells. Importantly, the prospect of using induced pluripotent stem cells in regenerative human medicine has greatly stimulated the development of methods for obtaining safe and high-quality cells.

One of the most interesting potential roles of induced pluripotent stem cells in *in vitro* conservation is in preserving, and eventually amplifying, the diploid gene pools of individual animals with extreme phenotypes. Somatic reprogramming would allow a large and diverse group of genetically different individuals to be sampled without killing the donors and without having to produce embryos that contain only half the interesting genetic patrimony. Moreover, the methodology is not limited to males (as is the case with the storage of semen), as female cells can also be stored and reprogrammed.

Further work will undoubtedly reveal differences between species, both in terms of the efficiency of reprogramming and the ease of germline colonization and contribution. Because of their phylogenetic proximity to the model species, the first livestock species in which these techniques can be used will probably be mammalian. The commercial and genetic value of exceptional phenotypes and individuals will help to stimulate the development of innovative methodologies.

It is impossible to know how long it will be before these techniques can be used routinely, as progress will depend on the level of research in each species. Nonetheless, collection of tissues and other sources of somatic cells in anticipation of further development may be a prudent strategy. Collection of such materials is usually simple and inexpensive, and can complement or replace the collection of semen and embryos. Once cryopreserved, the tissues and cells will remain viable indefinitely and can thus be kept until the technology needed to utilize them is well established.

Provided by Bertrand Pain.

of Animal Genetic Resources (ERFP) decided to develop the European Register of Cryomaterial as part of EFABISnet, a regional network of national AnGR information systems linked to FAO's worldwide system, DAD-IS (Hiemstra *et al.*, 2014) (see also Part 4 Section A). Information about national gene bank collections can be automatically uploaded from national databases (CryoWEB) to the European Farm Animal Diversity Information System (EFABIS). ERFP members have also recently established the European Gene Bank Network for Animal Genetic Resources (EUGENA – Hiemstra *et al.*, 2014), which allows for sharing of cryoconservation information at all levels (i.e. not only the content of national gene banks), thus allowing the optimization of conservation efforts at regional level (see Box 3D8).

Information systems for gene banks can be made even more powerful if they are integrated with systems used in *in vivo* conservation. The benefits of integrated databases increase in systems where stored materials are regularly used in the management of the *in vivo* populations.

Box 4D8
Bilateral agreement on sanitary issues in germplasm exchange – an example

Health regulations are a major issue confronting regional gene bank development. As national gene banks may collect germplasm without the intention of distributing it to other countries, collections may include material collected and cryopreserved without the rigorous testing that would be needed to allow it to be exported. Thus, if countries wish to set up a regional gene bank, there may be a need to develop alternative protocols for exporting genetic material.

Arrangements for transboundary exchange of genetic material were required when Jersey cattle breeders from the Island of Jersey wanted the United States of America's gene bank to back up their breed population. In this instance, the breeders had been collecting and storing semen from their cattle since the 1960s. While health tests were performed on the cattle at the time of collection, there were no veterinary certificates that could be used to acquire permits to import samples into the United States of America.

Another complicating issue was that Jersey and the United States of America had no agreements in place to verify the health status of each other's livestock populations (similar to those existing between the United States of America and the European Union). The solution was for the relevant agency in the United States of America to issue a special permit allowing the samples to enter the country but not to be used for breeding purposes. This solution was acceptable to all parties as the intention of the transfer was to provide a mechanism for keeping the samples safely so that in the event of need the genetics could be reintroduced to Jersey. Transmission of disease into American livestock populations was considered to be practically impossible given that no live animals would be produced in the territory of the United States of America.

Provided by Harvey Blackburn, National Coordinator for the Management of Animal Genetic Resources, United States of America.

6.5 Legal aspects of gene banking

Gene banks need to establish policies that ensure they comply with national laws. The two primary areas that need to be considered are interactions with the owners of the livestock from which samples are obtained and compliance with relevant national or international health standards. In the former case, the main issue is normally the question of private property rights over the material as it is collected, stored and distributed. National animal-health regulations may determine which animals can be used as sources of germplasm and how the collected germplasm can be used. Where international transfers are concerned, the country's overall health status will determine the type of testing needed before, during and after collection in order to allow the movement of samples through the normal protocols of international animal germplasm transfer. If countries wish to develop bilateral backup collections of germplasm (e.g. Box 4D8), they will need to evaluate whether current World Organisation for Animal Health (OIE) regulations will allow the required exchanges to take place or whether waivers will be needed (Blackburn and Boettcher, 2010).

In 2010, member countries of the Convention on Biological Diversity adopted the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (see Part 3 Section F). The protocol, which entered into force in October 2014, may influence the way that livestock germplasm is exchanged internationally and could potentially impede the exchange of AnGR between the national gene banks of countries that are signatories to the agreement.

PART **4**

7 Conclusions and research priorities

Conservation of livestock breeds can have many objectives, and various types of activity can be employed to address them. Comprehensive planning is required in order to identify the breeds with the greatest priority for conservation and to identify the most appropriate strategies for their management. Over recent years, substantial strides have been made in the development and improvement of conservation methods. Both *in vivo* and *in vitro* conservation have their advantages and shortcomings as standalone activities, so a strategy that employs both methods is usually optimal.

In the field of *in vivo* conservation, new methods allow more effective incorporation of economic and social factors into national conservation strategies. A desire to decrease direct public subsidies and make breeds more financially self-sustainable has led to a greater focus on the development of niche markets for breed-related products and spurred interest in methods of capturing other values of locally adapted breeds, such as their contributions to landscape maintenance and agricultural tourism. These approaches based on promoting financial self-sustainability both allow and obligate individual livestock keepers to play the major role in breed management. However, while developments of this kind are providing new opportunities, it should be borne in mind that they do not necessarily provide a strong guarantee that the targeted breeds will survive. For example, niche markets can often be unstable.

An unprecedented number of national gene banks have now been established and more are planned. Effectively building gene bank collections requires countries to improve their capabilities in cryopreservation, reproductive physiology, quantitative and molecular genetics and – above all – effective and openly accessible information systems. With the explosion in the availability of genomic information, there will be a greater need for gene banks to expand their collections to assist in conservation efforts and to serve as a reference of genomic information for various populations. Increasing the efficacy of cryopreservation protocols will facilitate cryoconservation and genetic utilization of stored material in *in situ* populations.

Effective decision-making in conservation strategies requires access to a range of data on breeds and their production environments, as well as appropriate methods for integrating these data into decision-making processes. For example, detailed DNA analysis may reveal the genetic uniqueness of a breed through the presence of rare alleles and rare haplotypes. This will improve estimates of breeds' conservation values and may indicate opportunities for sustainable use in pure- or cross-breeding programmes. New molecular approaches can facilitate the operation of such breeding programmes. Collecting data of this type is the task of characterization studies and inventory and monitoring programmes. Research priorities in these fields are discussed in Part 4 Sections A and B and needs for capacity development in Part 3 Section B.

With regard to decision-support tools in the field of conservation, research priorities include:

- improving methods for estimating breeds' extinction probabilities;
- developing user-friendly methods for prioritizing AnGR for inclusion in conservation programmes, and decision tools to guide resource allocation in conservation programmes, including methods that can effectively combine information of varying degrees of uncertainty; and
- further developing methods for incorporating genomic information into conservation planning.

Research is also required into the socio-economic, infrastructural, technical and policy factors that influence success in establishing and sustaining conservation programmes.

With regard to *in situ* conservation, research priorities include:

- developing strategies through which conservation activities can be implemented in ways that maximize livestock keepers' livelihoods,

including through value-addition methods such as niche marketing and agritourism;
- developing strategies through which genomics and other advanced tools and methods can be efficiently used to improve the genetic merit of conserved breeds while maintaining sufficient genetic variability;
- developing strategies through which breed conservation can be combined with efforts to promote the provision of services such as the maintenance of landscapes and wildlife habitats, as well as developing methods to estimate the value of these services and identify the beneficiaries; and
- determining how organizational structures can be improved so as to allow better integration and coordination among actors involved in conservation.

In the field of *ex situ in vivo* conservation, priorities include:
- identifying approaches that can enable programmes, particularly those in developing countries, to become more self-sustaining and hence less vulnerable to collapse if state support is withdrawn.

In the field of *in vitro* conservation, research priorities include:
- further developing strategies to increase and improve the utilization of stored material in *in situ* populations;
- developing information management systems that allow better monitoring and assessment of gene bank collections;
- designing comprehensive database structures and portals that are dynamic and thereby allow a broad range of users to access gene bank holdings and make requests for material;
- refining cryopreservation and freeze-drying protocols to increase the efficacy of collecting and storing germplasm;
- enhancing reproductive biotechnologies to improve the efficiency and reduce the costs of regenerating live animals from stored germplasm and cell lines;
- developing approaches for quantifying genetic differences among animals within the collection and comparing the status of the collection to *in situ* populations;
- improving methods for optimizing ongoing sampling and storage of genetic material in systems where the primary objective is to provide a backup to ongoing genetic improvement programmes;
- increasing the efficiency of reproductive technologies (in terms of the number of live animals produced per unit of material stored) in order to improve the cost-effectiveness of *in vitro* conservation programmes; and
- identifying policy, legislative and zoosanitary frameworks (and strategies for their implementation) that will facilitate the storage of germplasm in gene banks and access to such material.

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