

Conservation of Animal Genetic Resources: the Next Decade

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ABSTRACT: After 20 years, progress has been made in conserving animal genetic resources; but how it will be in ten years? Viewing gene banks and *in situ* conservation in the context of food security, climate change, and product demand suggest a more efficient use of these practices to support sustainable production. Gene banks should become more dynamic, incorporate different biological materials and increase collection use. Accomplishments have been obtained for *in vivo* approaches but gaps are substantial and *ex situ* – *in vivo* conservation is costly. For cost effective conservation gene banks should be leveraged by *in situ* programs; this will require emphasis to be placed upon database development and accessibility. With effective databases in place it will be possible to better integrate these two conservation avenues and address future sectorial issues.

Keywords: Conservation genetics; *ex situ*; *in situ*

State of World Animal Genetic Resources - new drivers

Over the past twenty years substantial efforts and progress have been made in conserving animal genetic resources for food and agriculture (AnGR). Across continents and regions: national programs, NGOs (e.g., breed associations), and producer based activities have been initiated for *in situ* and *ex situ* (gene banking) conservation. These activities have been as diverse as the countries implementing such programs and the area of conserving AnGR itself. Exemplary efforts include: development of *in situ* conservation of rare breeds across all regions and recognition of locally adapted breeds by governments. Globally there exist both well-established and newly formed national gene banks operating and providing genetic security for commercial and rare breeds. Also, at the global level a more formal structure among nations leveraging their mutual interests in AnGR conservation through direction given via FAO has become prominent. High points of this mutual action were: the development of the country driven State of the World's Animal Genetic Resources report; the Global Plan of Action and the Interlaken Declaration, which 107 nations are implementing (FAO, 2007). Countries have just concluded their reports for the 2nd State of the World's (SOW) Animal Genetic Resources and the document should be finalized in 2015, from which potential adjustments in the Global Plan of Action should be made. While we do not know the conclusions of the second SOW for AnGR the efforts from country negotiations, or the opportunities, that might develop in the future, the objective of this paper explores *ex*

situ, *in situ* and future directions the community might consider taking over the next decade.

Global drivers of population growth, income, and climate change are already impacting the way AnGR are used globally and it is expected that additional changes will be required to meet the projected 2.4% annual increase in meat consumption from 2013 to 2022 in developing countries (USDA, 2013). Increased animal productivity (unit of output/animal) should be a primary format for addressing food security needs in a sustainable manner. Unfortunately, for the least developed countries, spread across regions, animal productivity has been stagnant or decreasing, especially for ruminant species (Figure 1, FAOSTAT). This finding suggests greater emphasis is needed on a variety of mechanisms to increase animal productivity and that if the livestock sectors of the least developed countries are to contribute to improving food security and meeting national demands. A substantial alteration in production practices is needed, especially for ruminant species.

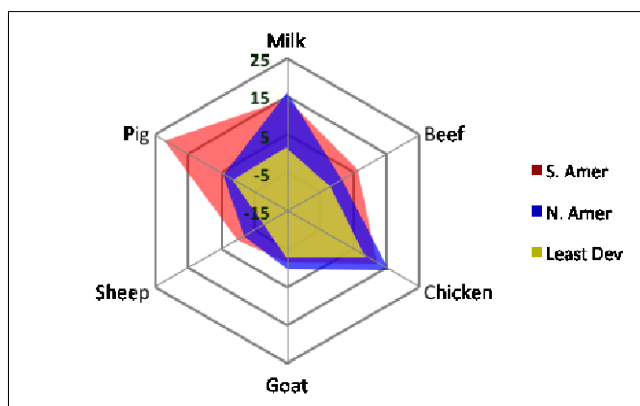


Figure 1. Animal productivity comparison among North America, South America and Least Developed countries following FAOSTAT.

In addition, the climate is expected world-wide to become highly dynamic and extreme, which in turn will impact food security (e.g. Jones and Thornton, 2009; Godfray and Garnett, 2014). Seo and Mendelsohn (2008) have shown that under various climate change scenarios there could be a shift from cattle production to small ruminant production in part of Sub Saharan Africa. Such a shift would likely mean a substantial loss of capital for producers in that region. In preparation, either new or

existing genotypes of interest will have to be developed, which of course will require access and utilization to a broad array of genetic variation.

Juxtaposed to these challenges is the ever increasing information on livestock genomes and tools that can be used to bring genomic knowledge to bear on the previously stated problems. While genotyping costs have been greatly decreasing over time perhaps the more important driver is our ability to manage, manipulate and understand the big data being generated.

Ex Situ Conservation via Gene Banks

Gene banks are the primary mechanism for *ex situ* conservation and may also be the most dynamic element in the conservation of AnGR. Globally we estimate national gene banks have been established in 50 countries and are dispersed across all geographic regions. Since the early 2000's national gene banks have accumulated substantial inventories of germplasm and tissues. Review of varying websites suggests the minimum size of this global collection likely exceeds 34,000 animals and over 3,300,000 germplasm/tissue samples across species (not counting the stores for sale at AI companies). From a sample of 15 gene banks (Table 1) the collection size does not appear to be strongly influenced by development status, but adding information from African and more Asian gene banks would add clarity to the evaluation (Figure 2). While most national collections have focused upon the primary agronomic species (Figure 3) we note that material from additional species important to specific countries has also been collected (e.g., rabbits, bison, equine, ducks, geese, aquatic species). The dynamic nature of this element of conservation programs has some interesting long-term impacts which will be discussed.

Table 1. Exemplary *in vitro* collection sizes from selected countries*.

Country	Species	Breeds/ lines	Animals	Number doses/ straws*
Austria	5	29	491	12,095
Brazil	12	25	416	71,867
Canada	9	31	3,077	261,083
Colombia	3	16	400	47,800
Finland	2	6	268	399,600
France	9	181	4,337	352,068
India	8	38	276	123,483
Italy	4	30	1,230	296,945
Netherlands	7	59	5,691	309,088
Norway	2	18	1,071	283,850
Poland	2	9	214	53,382
Portugal	3	36	399	195,046
Sweden	1	7	256	75,744
Ukraine	4	30	209	130,805
United States	38	149	16,397	709,657
Total	-	-	34,732	3,322,513

*Data from public and available material

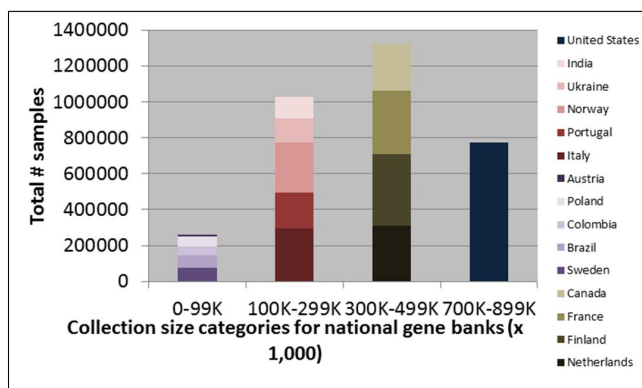


Figure 2: Comparison of national gene banks by size categories.

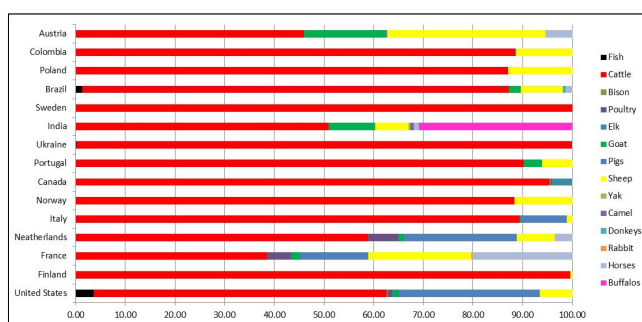


Figure 3. Comparison of national gene banks by within species distribution.

In addition to acquiring germplasm/tissue, gene bank collections in a number of countries have been accessed and used for a range of purposes. For example, in the US samples have been released to reconstitute a needed local pig population; enhance the genetic variability for *in situ* populations of several rare breeds; provide breeders of larger breeds with genetic variability to use in their breeding programs, and for molecular genetic and reproductive physiology research (Figure 4). Notably, in 2012 alone, material from 15% of the animals in the US collection were used for the above-mentioned purposes.

With the emergence of gene banking, FAO (2012a) published guidelines for establishing gene banks, along with cryopreservation protocols. A key change in that report from the previous edition was the development of new guidelines concerning the quantities of germplasm needed to reconstitute breeds from a gene bank. These recommendations illustrated that substantially smaller quantities of germplasm are needed than those previously published (FAO, 1998). Another early concept that had been advanced was that gene banking was actually more expensive than maintaining *in vivo* populations (FAO, 1998). This concept had not been supported in the past (Smith, 1984; NRC, 1993) and recent results indicate the situation remains unchanged. For example, Silversides et al. (2012), using actual cost data, demonstrated that developing cryopreserved collections of chicken lines and

reconstituting those lines after 20 years was 98% cheaper than maintaining *ex situ* - *in vivo* populations. Furthermore, those results showed that *ex situ* - *in vivo* was only cost effective if a chicken line was going to be used in the very near term (1-3 years). The magnitude of cost savings plus the elimination of depressed levels of performance for *in situ* populations suggest a critical need for greater gene banking efforts.

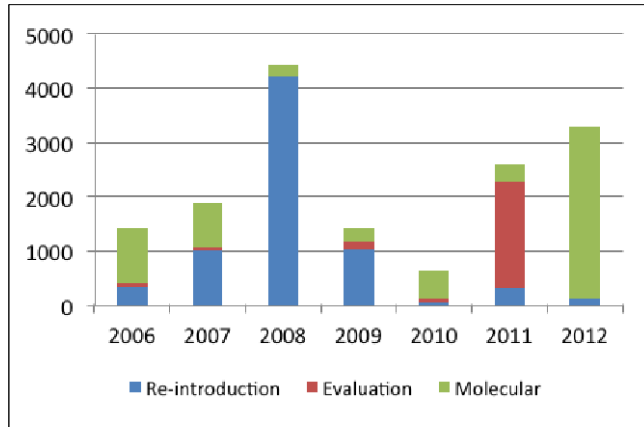


Figure 4: Number of samples released from the U.S. gene bank for molecular studies, germplasm evaluation and re-introduction of genetic resources into *in-situ* populations from 2006 to 2012.

Danchin et al. (2011) reported that national gene bank collections captured comprehensive collections of Holstein and in one instance the collection was more diverse than the *in vivo* populations. The ability to acquire samples cryopreserved in the past facilitates a gene bank's acquisition of additional genetic diversity. In fact, due to the longevity of stored animal germplasm many gene banks have samples from animals that were cryopreserved in the late 1950s or early 1960s (Blackburn, 2012). In earlier works it was frequently suggested that samples in a repository would lose their usefulness over time due to industry making genetic changes to *in situ* populations. Comparing the breed average EPDs (14-15 traits depending on breed) for Angus, Hereford, Charolais, and Brangus to the US gene bank averages for those breeds from 1960 to 2010 it was found that mean EPDs of the gene bank closely mirrored the *in situ* populations for the respective breeds (Figure 5). Interestingly, when the standard deviation and/or maximum values of the collection were considered, it suggests animals that have stored germplasm exceeded the breed averages by 30 to 50 years, depending upon breed and trait. This unexpectedly long time frame suggests samples have a substantial longevity and, when combined with periodic resampling the germplasm collection can remain on par with the performance of the *in-situ* population. These results and conclusion sharply differ from Leroy et al. (2011). But that study used simulation data with a hypothetical population and therefore was subject to the assumptions made. The results present here are actual values from the US gene bank collection and

compared to the *in situ* values for the 4 breeds over a substantial period of time. Actual results will depend on the characteristics of the corresponding *in situ* breeding program and its genetic trend.

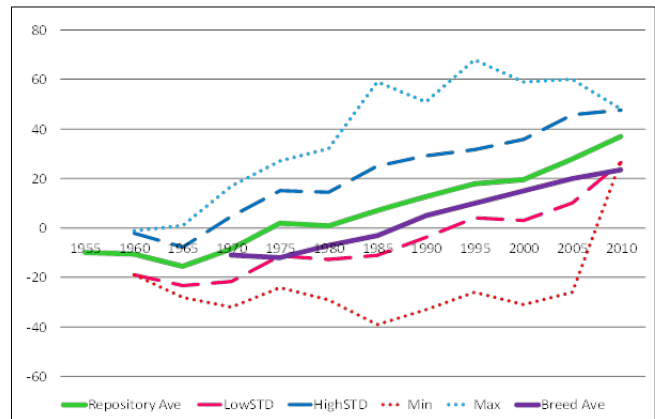


Figure 5. Angus breed average EPD for carcass weight compared to U.S. repository average, lower and upper standard deviation, minimum and maximum EPDs.

Future Roles of *Ex situ* Gene Banks. Gene banks, as well as collections of animal germplasm and tissues, have substantially increased during the last decade and germplasm collections have been initiated for all major livestock species and a multitude of breeds. But it is not clear from available data if minimum quantities of germplasm and numbers of animals per breed have been placed in the gene bank. For most collections, continued efforts are likely to be required. Nevertheless, as countries continue to develop collections, there is an opportunity to ensure that future collection activities accommodate the primary objective of conservation and security as well as to incorporate new types of tissues and areas of collection use; for example, ovaries for transplantation or skin or fibroblast tissues for either DNA extraction or cloning.

Coordinated releases of Germplasm/tissue from Brazil and US gene banks to date suggest gene banks can easily accommodate a second mission of providing samples for DNA analysis of varying types, assuming sufficient planning. Such a role seems a logical step for gene banks to take.

In addition to providing samples, it would be appropriate for gene banks to serve as a repository of genomic information. This would then make the database maintained by the gene bank particularly valuable since it would contain phenotypes, management systems, environmental descriptors and genomic information. Currently, Brazil, Canada and the US have initiated efforts to incorporate genomic information into their joint database Animal-GRIN (<http://nrc.ars.usda.gov/A-GRIN/>) and to make it publically available through the internet.

There has been mention of a global site to back up animal gene banks, similar to the Global Crop Trust's Svalbard "Doomsday Vault". Such an approach is certainly feasible but is it desirable? A more direct approach may be for countries to work at the regional level to collect tissues

and germplasm and provide cross-country security. A regionally-based approach could foster greater collaboration among neighboring countries on a number of fronts and thereby serve to strengthen national systems. In addition, there is a greater likelihood that countries within a region will share similar bureaucratic structures and economic interests, making it easier to move germplasm from one country to another.

A unique aspect of AnGR is that germplasm samples in the repository may be similar or identical to the resources being bought or sold among private sector entities. As a result, gene banks must take steps to insure they do not interfere with the exchange of material by the private sector (either within country or among countries). Given the close association between AnGR in the gene bank and that which may be available in the private sector, the first option for country exchange should be through the marketplace. However, if needs cannot be met via markets, germplasm exchange between countries will likely, but not necessarily, need to develop a more formalized agreement. As needs by both exporter and importer are likely to be diverse, it would seem that such agreements would best be formed on a case-by-case basis and maintain the spirit of a private treaty contract.

***In Vivo* Conservation**

Globally, *in vivo* programs are a varied combination of public *ex situ* (government, research and educational institutes) and private *in situ* (breeder associations, farmers, breeding companies) stakeholder actions. Many countries have had long running *in vivo* programs which had a dual purpose roll of serving as breed comparison studies and conservation herds. These herds and flocks have been useful in quantifying various phenotypic and molecular comparisons (e.g., van Marle-Koster et al., 2013; Carneiro et al., 2010). In addition, there has been an increase in our knowledge about breeds based upon census data as reported to FAO (Table 2). The number of breeds records increased by 11% from 2007 to 2012 (FAO 2012b), however we still do not have information from 55% of all breeds and with a substantial gap in documenting breeds in Africa (Table 2). Obviously a lack of such information increases the difficulty of implementing the most basic *in situ* strategies, let alone activities that improve animal performance.

In the 2014 US Country Report for the 2nd State of the World's AnGR it was reported that breeds previously considered at-risk of being lost have recovered and increased in population size. In Brazil, two locally adapted cattle breeds were officially recognized by the Department of Agriculture after the last SOW AnGR report. This action allowed producers to get agricultural financial loans with low interest rates as well the animals now can be sent to the AI facilities to have their semen collected and sold commercially. Given the level of effort in other regions it might be assumed that similar successes have been observed. Among developed countries there are successful

in situ conservation programs in part using protected geographical indicators strategy (e.g., European Community) which has been generating revenues to the farmers. However, it is still not clear how the new Common Agricultural Policy (CAP) will affect the *in situ* conservation of AnGR in Europe.

Table 2. Breeds with population data by World region.

Region	Major Groups	2007**		2012**		Relative Diff. (%)
		% Br. pop.data	N	% Br. pop.data	N	
Africa	Avian	21.37	351	22.80	421	6.72
	Mam.	32.52	1596	37.78	1670	16.19
Asia	Avian	41.33	600	43.98	673	6.41
	Mam.	31.95	1931	60.18	1949	88.36
Europe/Caucasus	Avian	58.37	1482	64.90	1638	11.19
	Mam.	67.20	3488	79.99	4332	19.02
Latin America/Caribbean	Avian	13.69	358	16.43	420	20.03
	Mam.	12.93	1640	18.65	1727	44.24
Near & Middle East	Avian	23.33	60	35.21	71	50.91
	Mam.	42.47	299	43.65	307	2.76
North America	Avian	97.80	91	93.69	111	-4.20
	Mam.	61.35	326	70.72	304	15.28
Southwest Pacific	Avian	15.15	132	13.51	148	-10.81
	Mam.	22.11	398	36.17	423	63.59

*% Br Pop. Data= percentage of breeds with recorded population data; N=total number of breeds per region; Relative Diff. (%)= relative difference observe between the 2007 and 2012 surveys; Mam=mammalian.** FAO (2007); FAO (2012)

Numerous efforts have been started to explore using environmental descriptors (e.g., Scherf, 2008; Scholtz et al., 2010) that would better facilitate the evaluation of genetic by environmental interactions. Combining this information with improved phenotypic characterization (FAO, 2012) could serve as a stimulus to *in situ* conservation efforts. Furthermore, overlaying genetic and geo-referenced data sets will be a strategic tool to monitor and planning *in situ* activities. Currently, landscape genetics; which is a combination of landscape ecology and population genetics aimed at providing information on the interaction between landscape and evolutionary processes, including gene flow, genetic drift, adaptation and selection (Manel et al., 2003; Holderegger and Wagner, 2008); have emerged as useful and unexploited technologies. In addition, the results may reveal attributes such as genetic adaptation to specific environmental stressors such as diseases, parasites and extreme heat, vegetation type, lack of water or combinations of these. Recent preliminary studies in Brazil (Hermuche et al., 2012; 2013a, b) used these tools to identify the patterns of region utilization for sheep production within Brazil based on environmental criteria and breed distribution data. The results have been used as a further criterion to optimize *in situ* and *ex situ* conservation schemes (McManus et al. 2013).

Future roles and needs of *in situ* schemes: While a few breeds may have been recently removed from at risk categories, attention must now be paid to how to build upon

these successes. One obstacle to address is genetic drift, as newly recovered breeds are still likely to have small population sizes. Therefore, genetic drift can be important because it can limit or negate any selection pressure that breeders may wish to employ. Nicholas (1980) developed an approach to determine, given a probability of success, the population size needed to meet a prespecified goal. Using this approach and assuming relatively low levels of intensity of selection and heritability (to represent traits such as resistance to internal parasites) we found that a population size of 6000 would be required to alleviate effects of genetic drift. Such a large population for a rare or minor breed is substantial. It suggests that breeders will have to work in unison, become more organized and/or settle for slower rates of genetic change, thereby risking any market position they may have gained.

The collective livestock developmental experience over the past 50 years shows us that increasing livestock productivity is a function of the entire system and that no one element or discipline can provide a quick and easy solution. This is obviously the case for the use of genomic tools. Research has been initiated to use genomics to facilitate selection for traits that confer some sort of resistance to environmental stressors (e.g., Dikmen et al., 2013). Selection for the slick haired trait in cattle is an important example of how a trait can better buffer livestock to environmental challenges. Work also continues on prolificacy genes in sheep which could contribute to increasing productivity levels of small ruminants. While these findings can certainly boost productivity, their full benefit will only occur when these approaches are combined with other technologies (e.g., nutrition and health). Looking from the conservation perspective, the monitoring and maintenance of these gene-assisted selection practices for *in situ* schemes can reduce the costs to breeding animals once the use of reproductive biotechnologies will be optional. In addition, this can be used to increase the value of the animals in the markets and raise the farmers' revenues.

Developing niche markets for rare and minor breeds seems to be gaining traction, particularly in developed countries (Blackburn, 2007). This approach offers a greater level of sustainability than implementing subsidies for maintenance of rare or minor breeds. Furthermore, with the development of niche markets that provide higher rates of return it can stimulate new producers to enter the market (e.g. Ligda and Casbianca 2013). The development of such markets suggests that governments and research institutes can divest their maintenance of *in situ* conservation populations and reserve their activities to one of technical support. The downside of some *in situ* programs appears to be the dependence upon government subsidies, for producers to be successful and offset lost revenues incurred by raising less productive minor or rare breeds (e.g. results showed by Gandini et al., 2010).

In developing countries, there are a substantial number of *in vivo* schemes maintained by governments; however, maintenance of live animals just for conservation purposes is becoming impracticable in terms of costs when compared with *in vitro* schemes. We suggest that public

sector *in vivo* conservation will decrease in the next ten years and transferred to private sector and NGO organizations. Assuming this scenario as true, its success will be totally based in the existence of a systematic data collection and the integration with the National or Regional Centres of Genetic Resources (see for example Fimland and Oldenbroek 2007).

Combining *Ex situ/ In situ* Efforts

Progress has been made on both *in situ* and *ex situ* conservation schemes. Across multiple regions breeds have either been taken off priority lists or experienced a decrease in their threat status. That said, effective *in situ* efforts are difficult to establish and maintain and the ability to increase population sizes, control inbreeding and promote effective genetic progress for traits of interest have not been fully realized. The available data suggests that *ex situ – in vivo* may be the most costly approach of sustaining rare populations, and *in situ* programs are only successful when sufficient subsidies are in place. However, throughout history market forces have always driven the success or failure of various breeds of livestock. It has been suggested that rare breeds may be able to sustain or grow in population size as consumer preferences change and become more price insensitive (Blackburn, 2007). Given such a scenario more producers may opt for using rare breeds if the economic incentive is great enough.

Given the high costs and unpredictability of some *in vivo* conservation approaches, it would seem that greater emphasis should be placed on *in vitro* collection development by national gene banks. Principally, this strategy opens up opportunities for rural development and producers of all scales. By successfully gene banking livestock populations, producers, governments and NGOs are free to pursue actions that result in increased productivity per animal without the fear that genetic resources will be lost by political instability, environmental or economic issues.

There is a common view that *in situ* and *ex situ* conservation methods are complementary. However, there are few examples in practice of those programs running in parallel. A key to promote this integration is the use of information systems containing information of gene bank samples, phenotypes, genotypes, GIS and live populations.

Conversely, public and private sectors should be able to overcome some actual limitations by using *in vitro* collections in order to guarantee that this conservation strategy is stronger and contributes to food security in the next ten years. For example, there is an unbalanced efficiency of reproductive technology by livestock species (FAO 2012a) and more focused research must be performed to improve cryopreservation rates. Furthermore, the different sanitary regulations among countries and the existence of non-tariff barriers face a major limitation for collections enrichment and material exchange.

Conclusions

Substantial progress has been made in conserving animal genetic resources for food and agriculture in the past 20 years. For the next ten years we believe *in vitro* collections should receive increased attention and be made more dynamic in order to incorporate different biological materials and increase the scope of their mandate to provide a wider variety of services (provision of germplasm, DNA, and information). To accomplish the expanded mission there is an urgent need to develop, implement and integrate information systems to optimize information flow; assist breeders with breeding practices; and facilitates exchange between countries. Governmental subsidies to keep *in vivo* populations are likely to contract in both developed as well in developing countries. Therefore, countries should speed up the generation of basic knowledge about their breeds. Our collective experiences indicate the global community has responded to the challenge of conserving AnGR. The low lying fruit has been harvested and over the next ten years country programs will have to become more strategic in how they advance their activities so as to increase productivity to meet the drivers of climate change, increased demand for livestock products and incorporation of new technologies.

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