

The Facilitating Roles and Uses of Gene Banks in Addressing the Global Plan of Action

H. D. Blackburn*

Introduction

Gene banks storing animal germplasm and tissues are a relatively new construct to conserve animal genetic resources (AnGR); especially when compared to the plant community which started the formal development of gene banks during the 1950's. Perhaps the oldest gene bank for AnGR was established by Brazil in the 1980's. The 1990's were pivotal in broadening and raising the awareness about the loss of AnGR in general and in the formation of national gene banks (Danchin and Hiemstra; 2003; and Blackburn, 2004). While no formal census of national gene banks has been performed it is common knowledge that national gene banks are now operating in all the FAO geographic regions. Due to the emergence of active gene banks at the national level it is well to explore their role in addressing the Global Plan of Action for Animal Genetic Resources (GPA) developed and adopted at the 2007 International Technical Conference in Interlaken, Switzerland.

At the conference in Interlaken 109 countries discussed and agreed upon a GPA for the conservation and utilization of AnGR (FAO, 2007). Also during this meeting the Interlaken Declaration, a consensus political agreement, calls upon countries to initiate sustainable conservation strategies for AnGR. A basic premise underscoring both of these documents is that each country has a responsibility to establish and conserve its AnGR. While many of the priority areas (PA) identified in the GPA can be addressed by gene banking activities, it is PA #3, Conservation, that pertains most directly to gene bank establishment, operation and function. Specifically, strategic priorities (SP):

- 7, establish national conservation policies;
 - 8, establish or strengthen in-situ conservation programmes;
 - 9, establish or strengthen ex-situ conservation programmes;
 - 10, develop and implement regional and global long-term conservation strategies; and
 - 11, develop approaches and technical standards for conservation;
- are relevant to the development, formation and use of gene banks.

SP-7 Establish national conservation policies. The GPA states that countries have a responsibility to conserve their AnGR and implement an appropriate set of policies supporting conservation. The decision to develop and make operational a gene bank impacts not only *ex-situ* conservation but also has carry-over effects on policies for *in-situ* conservation. Therefore while countries are developing such policies the option for developing gene banks and how they are to be used is needed.

* National Animal Germplasm Program, National Center for Genetic Resources Preservation, ARS, USDA, Fort Collins, CO 80521, USA

SP-8 Establish or strengthen in-situ conservation programmes. Creation and use of gene banks can support in-situ conservation programs. For example, the semen and/or embryos collected in a gene bank can be used in mating strategies to better control increases in inbreeding levels and to provide an emergency backup to in-situ populations in the event that some disaster or disease decimates the population. Additionally, gene banks can be used to help maintain rare breed diversity.

SP-9 Establish or strengthen ex-situ conservation programmes. The principal action for this SP is the establishment and operation of national gene banks. As part of gene bank operations there is the establishment of collection priorities, develop modalities for using the genetic material stored, and to develop mechanisms for replenishing material taken from the repository.

SP-10 Develop and implement regional and global long-term conservation strategies. The GPA calls for the development of conservation plans: for global and transboundary breeds; for integrated support arrangements to protect populations at risk from emergency or disaster situations; global networks of gene banks to harmonize approaches and the potential exchange of AnGR; and facilitate the collection of core collections of AnGR at the appropriate regional and species levels.

SP-11 Develop approaches and technical standards for conservation. Due to the relative youth of animal gene banks there is a need to develop: approaches to reconstitute populations; policy for use of germplasm stored in the repository to augment in-situ conservation activities; disseminate information and technologies that aid in-situ and ex-situ conservation; and determine the appropriate level of managing animal health issues.

Gene bank development

During the 1990's it was often suggested that gene banks were only a secondary mechanism for conserving AnGR. In addition, the conventional wisdom, at that time, was that developing countries may not have the necessary infrastructure, human resources and financial capacity to operate gene banks. However, during the past decade there have been a number of gene banks initiated in developing countries. For example, countries as diverse as Tunisia, Uganda, and Mexico have made national gene banks operational. Furthermore, in 2009 the first international conference on gene banking for animal genetic resources was held in Tunisia and attended by 50 participants from 25 developing countries that either had gene banks for livestock or were in the process of establishing them.

As countries develop and implement gene banks it is apparent that these structures and activities will be unique to the country. The size of the physical structure, storage capacity, size of the collection (e.g., number of breeds and tissue types), staffing and interactions with the livestock industry will vary. This implies that in the establishment of gene banks that a relatively broad consultation among national stakeholders should take place to insure the breath of issues are considered.

Costs for development and conservation. In a forth coming FAO publication on gene banking, costs for establishing gene banking activities for various sizes have been developed. In general, equipment costs for small (300,000 straws), medium (400,000 to 600,000 straws), and large (>500,000 straws) repositories ranged from \$11,500 - \$66,500, \$111,000 - \$211,000, and \$210,000 – \$221,000, respectively. The costs presented would suggest that countries could initiate gene banking activities for a relatively small amount of financing. In addition, Groeneveld et al. (2006) presented the collection and equipment costs to initiate a tissue/DNA gene bank in Viet Nam and those costs (~\$5,000) were lower than what has been presented here. Furthermore, when compared to maintaining in-situ populations gene banking may be the more cost effective approach, however similar figures for in-situ have not been reported.

Gene banks and the germplasm they collect and hold are a public good, however the issue of cost effectiveness of such collections are often raised. The return on investment of such collections is difficult to assess given the long term nature of the enterprise and the potential magnitude of problems they might be called upon to address. One approach to make an initial assessment of gene banks cost effectiveness is to use known costs (e.g., liquid nitrogen, electricity, staffing) for collecting and maintaining germplasm samples from which the future value can be calculated. This has been performed for the US situation using a 50 year time horizon and a discount rate of 4 and 6%, which is common for public goods projects. As Table 1 illustrates the level of expense is relatively modest and it would appear that gene banking is cost effective in conserving AnGR.

Table 1. Future value of germplasm stored for 50 years.

Discount rate	0.04	0.06
1 straw	\$ 24.87	\$ 42.98
1 breed*	\$ 74,610.00	\$128,940.00

*Assumes 3,000 straws per breed.

Examples of gene banks supporting animal genetic resources

The livestock community has had limited experience with gene banks. However, case studies are increasing about their use in securing AnGR resources and enriching our understanding of AnGR. To date several gene banks have established substantial collections of germplasm for most livestock species of interest (e.g., France, Netherlands, and USA). Additionally, many of these gene banks are starting to operate collaboratively on a number of projects. For example, comparing the similarity/dissimilarity between major breeds will be reported at this meeting (Danchin-Burge et al. 2010). To further elaborate on how gene banks can contribute to a country's GPA several examples from the USA are presented.

Reconstitution of pig populations. A major issue for gene banks is whether they can successfully be used to reconstitute populations. The following illustrates how a research population was reconstituted. Purdue University had developed a line of pigs that combined the Halothane and Napole genes in the homozygous or heterozygous state. As a result of budgetary constraints the population was discontinued in 2003. However, before this was to

occur semen was collected, cryopreserved, and stored in the gene bank from 3 boars having a copy of each recessive allele. In 2007 the university decided to reestablish the population. Cryopreserved samples were sent to the university where 7 sows were inseminated and each (100% conception) produced on average 7.7 pigs. The resulting boars were genotyped and the double heterozygotes were used to continue reconstituting the line. By 2009 the F₂ generation had been produced and consisted of double homozygous and homozygous/heterozygous boars. These boars have had semen samples collected, cryopreserved and placed in the repository. In a relatively short time the population was reestablished, additionally during the interim (2003 – 2007) the university was spared the expense of maintaining this population.

Introduction of genetic variability to in-situ populations. Gene banks need to provide rare breed germplasm to breeders in support of their in-situ breeding strategies. There have been several instances where the livestock industry has requested and received germplasm from the repository to broaden the genetic base of relatively small populations. The latest of which was in 2008 when semen from two Milking Shorthorn bulls (born in 1954 and 1959) were requested by a Shorthorn breeder to add diversity to their breeding program. From the matings five calves were produced and semen from the resulting bull progeny will be collected, cryopreserved and a portion sent back to the repository. This example illustrates how the repository can function in non-emergency conditions to incorporate genetic variability into the in-situ population.

Aged Bull Semen. Despite the long term use of cryopreserved bull semen the issue of sample longevity in liquid nitrogen has never been evaluated in a designed experiment. To address this question Carwell et al. (2009) accessed repository samples from 25 bulls cryopreserved between 1960 and 2004 which were used to inseminate Angus cows, via AI. Two interesting results emerged from this experiment. First, there were no significant differences in conception rate for the aged semen, indicating that sperm viability does not decrease over time (Table 2). Second, and perhaps more interesting, are the calves generated by this experiment and their range of differences in body size (e.g., birth weights ranging from 21 to 48 kg) and potential performance which provides a considerable biological range for researchers to consider in evaluating growth, feed efficiency and carcass characteristics.

Table 2. Conception rates using cryopreserved semen from Angus bulls born in different time periods.

Item	1960-1975	1976-1991	1992-2002
Bulls, #	5	11	9
Conception rate, %	47	52	49

Information system development. An integral component of gene banking activities and AnGR conservation activities is the development and implementation of a database that provides both gene bank managers and gene bank customers with information on the collection and the relationship to the in-situ population. This implies that the database needs to function as a inventory management tool for the gene bank, providing for example, the storage location of an animals germplasm and the viability of that germplasm. It also needs to provide potential users with pedigree, production system, and phenotypic and/or genotypic

information on individual animals stored in the collection. Comprehensive databases take considerable time and effort to construct and make operational. For this reason Brazil, Canada, and the USA have embarked upon the joint development of a database that will not only facilitate the management of their own collection, but it will also allow cross country comparisons.

Addressing broader industry concerns. For gene banks to function at full capacity they must adopt or develop genetic and cryopreservation protocols that facilitate the development of comprehensive germplasm collections. The primary mechanism for genetic conservation in gene banks is the cryopreservation of gametes. While substantial levels of success have been achieved in cryopreserving cattle gametes the efficacy for other species is substantially lower. These lower levels impede collection development by the gene bank, inhibit the employment of effective mating strategies for rare and endangered breeds, and also slow genetic progress in the industries at large. From a genetics perspective many breeds lack basic information concerning their level of genetic diversity. Such knowledge voids impede collection development and breeders from making informed choices when planning mating strategies. Clearly there is a broad intersection of interests between the gene bank and the various components of the livestock industry to which the gene bank can directly or indirectly facilitate the development of solutions.

Table 3 illustrates a common set of problems that both U.S. industry and the national gene bank have had and the conclusion developed from one or more joint experimental activities. Not only do these results illustrate how common problems could be addressed, but the examples also serve to illustrate how gene banks can engage in shorter term problems facing the livestock industry and thereby underscore another important role gene banks can serve.

Conclusion

The GPA provides nations with a road map to assist in implementing AnGR conservation and utilization strategies. In the drafting and negotiation of the GPA careful attention was made to insure that the PA and SP contained elements applicable to all countries. It is within this context that gene banking activities were developed and accepted as part of the GPA.

While the development and implementation of gene banking activities is relatively new the examples provided illustrate that not only can gene banks serve as a collection of germplasm to use in emergency situations but that gene banks can fill a broad array of roles that serve conservation efforts as well as AnGR utilization. As a result of the increased scope for gene banks utilization it is suggested that they provide a logical focal point from which activities to initiate and structure national AnGR activities. In essence they provide national livestock sectors with a functioning activity to associate with the conservation and use of AnGR.

Perhaps one of the greatest challenges facing the livestock sector and conservation of AnGR is how to balance exponential growth and demand for livestock products (FAO, 2009), which entails rapid transformation of existing AnGR, and the maintenance of genetic diversity. Currently it appears that these changes will apply greater pressure on indigenous AnGR to increase productivity to commercial scales or be put aside by breeders in favor of

breeds that can meet production and profitability demands. As a result of these economic realities it becomes increasingly clear that gene banks have a vital role to play in conserving breeds and unique gene combinations for future use in production systems or for understanding underlying genetic mechanisms.

Table 3. Examples of common industry and gene bank issues.

<i>Species</i>	<i>Industry issue</i>	<i>Gene bank issue</i>	<i>Approach</i>	<i>Result</i>
Sheep ^a	Lack of AI collection facilities	Lack of AI collection facilities	Shipment of fresh extended semen	No difference in fertility between semen that was: fresh, freshly frozen, or held for 24 hr prior to freezing.
Swine ^b	Reduce cost of fresh semen	Optimal insemination dose	Evaluate fertility with varying doses	One insemination is as effective as two and 1 billion motile sperm are as effective as 4 billion motile sperm.
Swine ^c	Quality of fresh semen	Quality of fresh semen	Determine optimal temperature & pH	Recommendations on optimal shipping temperature and pH disseminated to commercial boar stud managers.
Chicken ^d	Reduce AI and frozen semen costs	Maximum use of cryopreserved semen	Diminishing returns from one AI calculated	AI with fresh semen should be performed every 6 d (vs 4 d); and AI with frozen semen for regeneration to be performed every 17 d.
Sheep, cattle, swine ^e	Level of inbreeding unknown	Level of inbreeding unknown	Compute inbreeding levels for targeted breeds	Results provided to breed societies on the status and trends in inbreeding levels/rates and N_e .

^aPurdy et al. (2010); ^bSpencer et al. (2008), Fisher et al. (2010); ^cPurdy et al. (2008); ^dBlackburn et al. (2009); ^eMaiwashe and Blackburn (2004), Cleveland et al. (2005), Welsh et al. (2010).

References

- Blackburn, H. (2004). *Repro. Fert. Devel.* 16:27-32.
- Blackburn, H. (2009). *Livestock Sci.* 120:196-203.
- Blackburn, H., F. Silversides, and P. Purdy. (2009). *Poult. Sci.* 88:2192-2198.
- Carwell, D, J.A. Pitchford, G. Gentry Jr., H. Blackburn, K.R. Bondioli and R.A. Godke. (2009). *Reprod. Fert. Devel.* 22:167-168.
- Cleveland, M., H. Blackburn, R. Enns, D. Garrick. (2005). *J. Anim. Sci.* 83:992-1001.

- Danchin, C. and S. J. Hiemstra. (2003). In *Workshop on cryopreservation of animal genetic resources in Europe*. pp 156.
- Danchin-Burge, C., H. Blackburn and S. J. Hiemstra. (2010). In *Proc 9th WCGALP*. (In Press).
- FAO. (2007). Global plan of action for animal genetic resources and the Interlaken Declaration.
- FAO. (2009). The state of food and agriculture: Livestock in the balance.
- Fisher, K., T. Stewart, P. Purdy, H. Blackburn, W. Singleton, B. Sparks, P. Gunn, G. Bridges. (2010). *J. Anim. Sci.* (In Press).
- Groeneveld, E., N. Huu Tinh, N. Thi Vien, B. Phu Nam Anh, and L. Thi Thu Ha. 2006. In *Proc 8th WCGALP*. 33:28-31.
- Maiwashe, A. and H. Blackburn. (2004). *J. Anim. Sci.* 82:2900-2905.
- Purdy, P., E. Moce, R. Stobart, W. Murdoch, G. Moss, B. Larson, S. Ramsey, J. Graham, H. Blackburn. (2010). *Anim. Repro. Sci.* 118:231-235.
- Purdy P., R. Knox, W. Singleton, K. Spencer, S. Spiller, T. Stewart, N. Tharp, C. Welsh, Blackburn, H. (2008). In *Proc Midwest Boar Stud Managers Conference*.
- Spencer, K., P. Purdy, H. Blackburn, S. Spiller, C. Welsh, T. Stewart, S. Breen, J. Taibl, B. Yantis, and R. Knox. (2008). *J. Anim. Sci.* 86, E-Suppl. 2:525.
- Welsh, C., T. Stewart, C. Schwab, and H. Blackburn. (2010). *J. Anim. Sci.* 88:1610-1618.