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Genebank development for the conservation of livestock genetic resources in the United States of America ☆

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Abstract

The USDA established the National Animal Germplasm Program (NAGP) to conserve livestock genetic resources in 1999. The NAGP is primarily concerned with the development of cryopreserved germplasm and tissue samples from U.S. livestock species. To execute the program's mission, subcomponents dealing with genetic diversity, cryopreservation, and database development have been initiated. An early decision was made to develop collections on all U. S. livestock breeds. Since that decision, collections on 119 breeds have been initiated and ten of the collections have reached the collection goal. An example of selecting animals for the collection is given using Jersey cattle. The selection procedure utilizes the coefficient of genetic relationship as a basis to cluster candidates for the collection and as a method for assessing how complete a breed's collection may be. By using this approach it was determined that 86% of the clusters were presently represented in the collection. With this information collection efforts could be planned to fill in the missing gaps. Performance information from a sample of the in-situ population and the collection were compared. This analysis indicated that there were no significant differences between the means for the Core Collection and the in-situ population sample for milk production, milk protein and net merit index. A significant difference was found for milk fat. While the total collection effort is not complete, samples have been distributed from the repository to perform QTL and genetic distancing studies, creation of a research population, and to introduce genetic variation into a rare cattle breed. Progress has been made in starting breed collections however, significant efforts are needed to: acquire additional accessions for the collection, develop the information system, and quantify levels of genetic diversity within and among breeds.
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Keywords: Animal genetic resources; Conservation; Cryopreservation

1. Introduction

US breeders have a relatively large number of livestock breeds and specialized lines within breeds. These genetic resources were imported from the 1500s to 1900s (Dohner, 2001). In the U.S., animal ownership and breeding choices are private sector activities which do not warrant governmental intervention. This latitude has facilitated the enormous strides in animal productivity while at the same time allowing some producers to pursue the production and selection of rare and minor breeds of livestock. As with other countries there has been a growing awareness about contractions in livestock genetic diversity and the need for a set of actions that ensure the livestock industry has ample genetic resources

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for future use. Some of the primary issues creating this situation were identified in the US Country Report for FAO's State of the World's Animal Genetic Resources (Blackburn et al., 2003) and include: industry emphasis on a few breeds, reduced breeder longevity, and increased inbreeding levels in major, minor and rare breeds. The objectives of this paper are to further discuss the US situation and the steps taken to address conservation of animal genetic resources.

1.1. National genebank establishment

Contraction of genetic resources has been a concern for portions of the livestock industry and public sector groups for some time, but it was not until 1990 that national legislation was passed that provided the US Department of Agriculture (USDA) with a mandate to conserve animal genetic resources. This legislation provided further impetus for public and private sector initiatives to explore necessary actions (NRC, 1993a). By 1999 a decision had been taken within USDA to initiate a livestock conservation program, through a newly formed National Animal Germplasm Program (NAGP). To assist in the new program's formulation the American Dairy Science Association sponsored, as part of their Discover Conference series, a meeting in late 1999 (and again in 2004) with public and private sector participants to plan and initiate conservation activities. The meeting resulted in the formulation of six committees (aquatic species, beef cattle, dairy cattle, poultry, small ruminants, and swine) to assist in the identification and acquisition of germplasm and tissue for a genebank.

The development and application of a genebank for livestock species was a relatively new activity for public sector organizations. However, the plant community has used genebanks as an integral component of genetic resource conservation strategies and breeding programs (NRC, 1993b), illustrating that a wide variety of germplasm can be collected and preserved. For example, in the USDA/ARS National Plant Germplasm System there are approximately 500,000 different accessions in the collection. As Gollin and Evenson (2003) point out for livestock, the trade off between ex-situ/cryopreserved collections, in vivo conservation efforts, or the implementation of both is primarily a financial consideration to the extent the two are substitutes.

For the US, development of ex-situ/cryopreserved collections has several appealing properties. It allows the conservation of all breeds, eliminating the need for a decision as to what breeds to conserve and what breeds not to conserve, and it provides equity in the treatment of various livestock breeders and their breeds. An often cited reason for not starting cryopreserved collections is concern that samples may lose utility as gene frequencies change. NAGP has decided to overcome this potential shortcoming by repeated sampling over time to capture changes in gene frequency and to keep the collection relevant to current industry standards. While cost comparisons of ex-situ/cryopreservation and ex-situ/in-vivo have not been performed, there has been a significant decrease in the maintenance of live animal populations by public sector entities primarily due to recurrent costs for population maintenance. While this trend is disturbing, the disappearance of these breeds and lines draws into question the validity of the argument that maintaining live animals serves as an encouragement for their utilization. Furthermore, in the US there has been little new public sector funding to encourage maintenance of live animal populations.

1.2. In-situ populations

Due to the strong private sector role in animal production and breeding and the cost associated with maintaining live animal populations, the NAGP focus is primarily on ex-situ/cryopreservation efforts. NAGP collaborates with breeders raising rare breeds and assisting breed associations by determining levels and trends of inbreeding (Maiwashe and Blackburn, 2004; Cleveland et al., 2005). In addition, NAGP interacts with non-governmental organizations that interface directly with producers raising rare and minor breeds of livestock.

2. Collection development

Early in the formation of the NAGP it was realized that while the primary purpose of the germplasm/tissue collection was to secure genetic resources for total breed regeneration, the repository could also collect additional material, for little additional cost, that could serve other purposes, such as research identifying QTLs and the introduction of genes and genotypes currently not available in the commercial market. To facilitate the different uses described, a breed's collection is subdivided into four sub-collections described by Blackburn (2004) and presented in Table 1. Given this categorization the procedure for placing germplasm in the various categories follows an order of Restricted, Core, Evaluation, and Working Collections. Assignment of animals to the Core Collection (CC) takes into consideration genetic diversity, capturing rare alleles, and obtaining sufficient quantities of germplasm to regenerate the breed. The Restricted Collection contains material that the original owner donated to the repository and has control over its

Table 1
Within breed components of the U. S. National Animal Germplasm Program germplasm collection

Category	Function	Accessing germplasm
Core	Provide sufficient quantities and diversity of germplasm for 150% of breed regeneration [a]	National, industry or breed emergency
Evaluation	Sufficient material to evaluate germplasm quality over time, and genetic diversity	As needed by NAGP
Working	Germplasm for industry and research utilization for new or experimental line development or DNA studies	Requestor submits a proposal to NAGP
Restricted	Provides a security backup for private sector germplasm	Permission from germplasm owner

[a] 150% regeneration is the amount of germplasm necessary to regenerate a breed 1.5 times from cryopreservation using semen, embryos, or a combination of both. From Blackburn (2004).

release for a limited time, based upon an agreement with ARS.

The sources of NAGP germplasm have been highly variable depending upon the species. For example, much of the dairy cattle collection has been derived from commercial artificial insemination (AI) centers. The beef cattle collection samples have come from individual breeder donations and commercial AI companies. Small ruminant, chicken and swine germplasm samples have been received from all sectors and were frozen by the NAGP. In the initial phase of collecting any breed, the acquisition process has been opportunistic, that is germplasm acquisition was not focused upon specific animals. However, as breed collections have grown, more emphasis has been placed upon selecting animals that will add genetic diversity to the collection.

To date, approximately 425,000 samples from 141 breeds have been accessioned into the repository (Table 2). Across species groups, significant progress has been made in collecting samples from all existing breeds in the US. As expected, beef and dairy cattle comprise almost 50% of the collection due to the wider use of assisted reproductive technologies in those industries. Even though frozen semen is not typically used in the swine and sheep industry, significant progress has been made in collecting samples from a large portion of the breeds present in the US. The distribution of animals represented in the collection, when viewed in combination with the number of samples collected, illustrates to a large degree the reproductive differences between the various species and the germplasm requirements for breed reconstitution.

2.1. Cryopreservation

Procedures for cryopreserving gametes for all livestock species exist, although there is extreme variation in fertilization rates across species when cryopreserved germplasm is utilized. As a result, it becomes necessary to adjust collection strategies for the various reproductive inefficiencies of the species being collected. In developing the U.S. collection the primary emphasis has been on acquiring semen and to opportunistically acquire cattle, sheep and pig embryos. This approach allows for a more rapid collection of germplasm and a broader sampling of genetic diversity at a lower per unit cost.

Species other than cattle represent unique challenges for germplasm collection due to the low utilization of assisted reproductive technologies. Therefore, procedures to facilitate semen collection for swine and sheep have

Table 2
Summary of cryopreserved germplasm and tissues in the NAGP repository

Species group	Total no. of U.S. breeds	No. breeds (no. rare breeds) collected	No. samples (% of total)	No. animals (% of total)	No. commercial or research lines
Beef cattle	48	40 (10)	107,199 (25)	1915 (22)	3
Chickens	55	7 (1)	4993 (1)	1135 (13)	83
Dairy cattle	11	11 (3)	72,539 (17)	2204 (25)	1
Freshwater finfish	11 [a]		13,200 (3)	424 (5)	6
Goats	14	12 (4)	8472 (2)	269 (3)	0
Marine finfish	5 [a]		534 (0)	10 (0)	0
Oysters	1 [a]		5714 (2)	180 (2)	1
Pigs	18	18 (8)	149,333 (35)	976 (11)	19
Screwworm			19,350 (5)	10 (0)	5
Sheep	41	39 (17)	43,581 (10)	1584 (18)	3

[a] Number of species in the collection.

been developed (http://www.ars-grin.gov/animal/). These procedures entail shipping fresh extended semen to the laboratory for cryopreservation within 24 to 36 h from collection. Fertility trials for sheep show no significant differences between freshly frozen semen and samples held for 24 h prior to cryopreservation. Producers having the ability to ship fresh extended samples coupled with the NAGP's ability to cryopreserve samples, overcomes industry infrastructure limitations and allows a broader sample of genetic resources to be collected, facilitates producer interaction, and technology transfer.

Poultry represent a unique challenge due to the low fertility rates using cryopreserved semen when glycerol is used as a cryoprotectant and the lack of a protocol for storing female gametes. The proposed semen cryopreservation method by Woelders et al. (2006) appears to be promising but needs additional confirmation by other laboratories. Several approaches are being evaluated that would permit the reconstitution of females and the preservation of primordial germ cells (PGC) is one such approach (Petitte et al., 1990). However, to date the preservation of PGCs and generation of chimeric birds has been inefficient (Song, 2003). Moore et al. (2006) have developed near optimal cryopreservation protocols for PGCs, but significant effort is still needed to increase the efficiency of PGC use in reconstituting populations. Song and Silversides (2006) have recently been freezing and transplanting ovarian tissue, and this approach may also address the current deficiencies in preserving the female portion of the chicken genome.

2.2. Genetic diversity

NAGP has been engaged in the measurement of genetic diversity using microsatellites for the purpose of better understanding breed differences, making collection decisions on non-pedigreed and feral populations, and breed reconstitution. Briefly, Muigai et al. (2002) found that the major genetic influence of the Barbados Blackbelly and St. Croix hair sheep is likely European and not African. This result has potentially interesting applications for reconstituting hair sheep breeds. The genetic distancing study of MacNeil et al. (2006) provided important insights about cattle breeds originating from the Iberian Peninsula and their relationship to western European and British breeds. In particular the quantifiable difference between the Florida Cracker and Pineywoods cattle breeds is important in planning collection activities. It is anticipated as more SNPs become available, evaluating the collection for specific SNPs will increase in importance and could potentially drive sample requests.

2.3. Genetic aspects for collection development

Criteria must be determined for selecting individuals to be in a breed's collection. Selecting animals within a breed can be accomplished by a range of approaches (e.g., geographic location, pedigree analysis, molecular information, random selection) and NAGP has used and integrated several of these approaches based upon available information.

One cost-effective mechanism for assessing and selecting animals to add to the collection involves using pedigrees from association registration records to calculate the average genetic relationship between candidate animals. Several algorithms to select animals have been evaluated (Lamberson et al., 2002). During the algorithm evaluation process, it became apparent that approaches ranking animals by least relatedness to the other animals in the breed would not be workable due to never knowing if a selected animal could be sampled. As a result clustering, based upon genetic distance derived from computed genetic relationships, was evaluated. Clustering approaches overcome the sampling issue mentioned above because if one animal in the cluster cannot be sampled there are other animals from that cluster that potentially can. Various methods of clustering in SAS (2003) were evaluated. After analysis and considering Ouendeba et al. (1995) the Ward's minimum-variance method was selected. The Ward's method computes genetic distance as the ANOVA sum of squares between the two clusters summed up over all variables. This method tends to join clusters with a small number of observations and is biased toward producing the clusters of the same size; however, in our work we have not found this to be a limitation.

2.4. Building a core collection

An example of how clustering would work in developing the Core Collection (CC) for Jersey dairy cattle follows. In this example the repository had acquired samples from 537 Jersey bulls that were born from 1958 to 2004. The complete pedigree file starting in 1950 was obtained from USDA's Animal Improvement Production Laboratory and the American Jersey Cattle Association; with these data genetic relationships were calculated using the Animal Breeders Tool Kit (Golden et al., 1992). Due to limitations on matrix size in the clustering algorithm 3350 bulls were selected from the entire pedigree file. The selected bulls included the 537 repository bulls plus non-repository bulls that had sired at least one progeny in 2004 or 2005. Based on a pseudo-*t* test and inspection of the phylogeny tree the bulls were

Table 3
Number of Jersey bulls per cluster, mean relationship within a cluster, mean relationship of a cluster to other clusters, and the number of bulls per cluster in the repository

Cluster	*N*	Mean genetic relationship within cluster	Mean genetic relationship to other clusters	No. Bulls in repository by cluster
1	350	0.184	0.125	83
2	98	0.205	0.108	30
3	451	0.050	0.064	105
4	50	0.232	0.063	0
5	115	0.348	0.128	8
6	214	0.208	0.121	36
7	193	0.254	0.126	13
8	198	0.106	0.060	13
9	342	0.209	0.125	42
10	161	0.198	0.113	37
11	116	0.360	0.131	17
12	126	0.223	0.119	35
13	142	0.194	0.071	3
14	116	0.327	0.129	11
15	62	0.258	0.098	0
16	104	0.197	0.083	1
17	156	0.258	0.124	35
18	105	0.327	0.128	22
19	70	0.305	0.129	18
20	86	0.281	0.099	14
21	49	0.215	0.055	0
22	46	0.220	0.097	10

clustered into 22 groups. The number of bulls and mean cluster relationships are presented in Table 3. Inspection of between cluster relationships shows that cluster number 4, 8 and 21 had the lowest relationships to other clusters and that cluster 11 had the highest. Three of the clusters were not represented in the repository. Given this information, steps were initiated to acquire samples from the missing clusters. Relatively high (approaching or exceeding half-sibs) within cluster relationships for 16 of 22 clusters were found. Having a relatively high within cluster relationship simplifies the selection of individuals from a cluster, and if a sample cannot be obtained from an animal a replacement can potentially be found.

Once a set of animals have been clustered a CC for the breed can be constructed. There are several potential methods to accomplish this goal: select bulls from the collection based on their lowest relationship to the population, select a predetermined number of bulls from each cluster, select bulls based on birth years, or select animals based upon estimates of predicted transmitting ability (PTA) for milk and/or linear measurements. It was decided to make the selection based upon the lowest relationship. Therefore, from the 537 bulls in NAGP, 253 of the least-related bulls were selected for the CC. The primary reason for this relatively large number of bulls being added to the CC is to achieve the minimum quantity of semen (5250 straws). However, by placing such a large number of bulls into the CC the probability of capturing potentially rare alleles from the population is increased (Smith, 1984). Using Smith's (1984) equation (probability of capturing a rare allele $=1-(1-p)^{2n}$ where p is the allele frequency and n equals the number of animals) and assuming the frequency of a rare allele is 0.005, the probability of capturing that allele in the Jersey CC with 253 bulls would be 0.92 (given 10 units of semen per bull in the repository).

After a set of animals are selected for a CC, further analysis can be conducted to determine how well the CC is representing the breed. From the perspective of genetic relationships and the clustering approach, the Jersey CC contained 86% of the designated clusters. Further comparisons were made between the CC and a sub-sample of the U.S. in-situ population by using the predicted transmitting abilities (breeding values) that have been computed for various production characteristics. In the ANOVA, birth year group and classification (CC vs. in-situ population) were the model's main effects. The data analysis (Table 4) suggests that there are no significant differences between the CC and the in-situ population for most traits, with the exception of milk fat. While these results suggest that the CC contains a satisfactory representation of the in-situ Jersey population there are opportunities to search for and collect samples from bulls that may have milk fat levels that are outside the range of those in the present collection.

2.5. Information system

The third area of work necessary to conserve genetic resources is an effective information system. Information on animal genetic resources is maintained as a component of the ARS Germplasm Resources Information

Table 4
Comparison of in-situ and core collection least squares means for predicted transmitting abilities

Trait	In-situ ($n=2817$)		Core collection ($n=253$)	
	Mean	Standard error	Mean	Standard error
Fluid milk	−635.4	29.69	−615.5	50.13
Milk fat	−20.89	1.12	−14.23 **	1.90
Milk protein	−20.07	0.92	−19.16	1.55
Net merit	−130.96	6.77	−122.57	11.40
Jersey Performance Index	−70.85	3.36	−79.42	5.58

** Least squares means in the same row are different by $p<01$.

Network (GRIN). The database encompasses the areas of: germplasm inventory, phenotypic and genotypic descriptors of the samples in the repository, animal pedigree information, and breed population status. To facilitate database utilization, web-pages are dynamically interfaced with the database. This approach assures the information derived from the database is current. When exploring the inventory, the drill-down concept is utilized so that when a user finds a sample of potential interest they can query the database for additional details. For illustrative purposes, Table 5 demonstrates the type of information currently maintained for a specific animal.

The first version of the database has been developed and can be accessed via the web address: http://www.ars-grin.gov/animal/. The design of the second version of the database has been initiated. The primary focus for version-2 will be an increase in the number of tools available for the user. In addition, Brazil and Canada will participate in version 2 development and this will increase the scope of user applications.

3. Collection utilization

The primary focus of the collection is to provide a secure reserve of germplasm that can be used to introduce genetic variation into livestock populations of interest when deemed appropriate. However, after initiating collection development it became apparent that such a collection has a range of purposes beyond genetic conservation. For example, providing samples for genomic research (e.g., QTL detection), or as a source of standardized genotypes that can be utilized in evaluating commercially developed genotyping kits. Increasing the number of collection applications only serves to strengthen support, understanding and utilization of the collection.

The ability to release germplasm from the repository is necessary to make the repository fully functional. Fig. 1 illustrates the process developed for releasing material from NAGP. For any particular release the appropriate species committee is consulted and a decision reached. As part of the release process, the requestor is required to supply the repository with germplasm samples from any resultant progeny, or in the case of genomic studies, to provide NAGP with the genomic information derived from the released samples. This information will be placed into the database and made publicly available. To date, cattle, pig and trout samples have been released to: perform QTL and genetic distancing studies; generate an experimental research line; and to introduce needed genetic variability into a rare cattle breed.

Table 5
Example of individual animal information stored in the animal-GRIN database

Individual details — NAGP 357					
Taxonomy			*Phenotypic observations*		
Pig — *Sus scrofa* — Yorkshire			Descriptor name	Value	Date
			21-day litter weight	73.9 kg	
Identifiers			Backfat	10.16 mm	
NAGP ID	357		Days to market	173 days	
Germplasm source number	8222		Loin eye area	53.61 cm^2	
Name	Terminator		Market weight	113.4 kg	
Name	MSU0 HITMAN 45-10		Number born alive	12	
Registration number	385962010		Number of teats	16	
			Percent lean	61.2%	
Description					
Gender	M		*Genotypic observations*		
Purpose	Meat		Descriptor name	Value (accuracy)	Date
Birth date	2000-09-07				
Origin	United States, Michigan		21-day litter weight EPD	+.05	07-2005
Breeder	Michigan State University		EPD		
			Backfat EPD	−.04	07-2005
Other observations					
Descriptor name	Value	Date	Days to 113 kg EPD	+2.28	07-2005
Age at Sampling	1.6 years				
Cluster Number	1	12-2004	Number Born Alive EPD	+.04	07-2005
Halothane Gene	NN				
Inbreeding	.02		*Germplasm counts*		
Coefficient			Semen		321
Why collected	Industry standard for year	Total: 321			Total: 321
Why collected	Baseline collection				

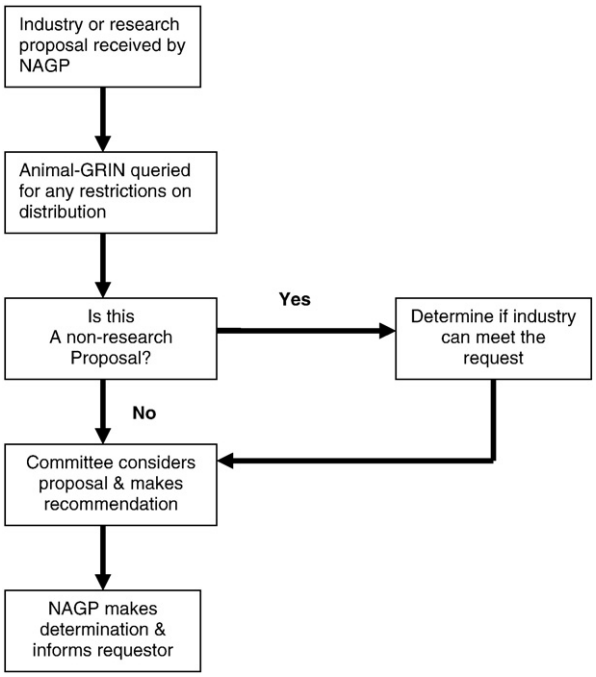

Fig. 1. Process for reviewing industry or research requests for animal germplasm and/or tissue.

3.1. Regeneration

Complete breed or line regeneration is a significant issue due to the time requirement, especially for cattle. As Boettcher et al. (2005) have shown there are trade-offs between semen and embryos for mammalian species in terms of time to regenerate a breed and cost, with regeneration via semen having the cheapest collection cost but slowest regeneration time. However, in reconstituting a population the probability of needing to reconstitute the entire population is also a consideration and therefore several types of reconstitution strategies need further exploration and quantification. The most obvious of these is the collection and use of oocytes. Oocytes offer an opportunity over embryos to mix genotypes within a breed, via IVF, as may be needed by potential users, therefore giving the collection greater flexibility in some reconstitution strategies. Oocytes from prepubertal animals could also be used in conjunction with various assisted reproductive technologies potentially reducing reconstitution time by 50%.

4. Conclusions

The U.S. NAGP genebank has grown faster than envisioned. Contributing to the collection's growth have been the contributions and involvement of private sector breeders and the public sector. The speed at which this collection has been developed suggests that countries can make significant strides in cryopreserving all breeds of interest in the national population thereby eliminating the need for deciding which breeds to conserve and which breeds not to protect. As a result all breeds have the opportunity to be treated equitably.

During the development of the repository, differences between theoretical expectation and field execution have become clear not only in the U.S. but also in reports from other countries (Danchin-Burge et al., 2006). One of the principal aspects limiting collection strategies is the logistical dimensions which range from contending with broad geographic locations, ownership considerations, and the ability to acquire a germplasm collection from animals of interest.

Due to the collection efforts to date the level of genetic resource protection has increased significantly. However, significant efforts lie ahead in completing the collection process, understanding the diversity captured, and providing information about the collection to potential users. Key areas of future program development include: continued collection development for all breeds; development of baseline genetic diversity levels for all breeds; improved cryopreservation protocols across species and tissues types; and increasing the capacity of the information system to facilitate collection utilization, understanding breed diversity, and providing users with a set of tools to assist in managing animal genetic resources.

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