

Article

Conservation and Utilization of Livestock Genetic Diversity in the United States of America through Gene Banking

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Abstract: A germplasm collection curated by the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Animal Germplasm Program contains of over one million samples from over 55,000 animals, representing 165 livestock and poultry breeds. The collection was developed to provide genetic conservation and security for the U.S. livestock sector. Samples in the collection span 60 years, suggesting a wide range of genetic diversity and genetic change is represented for rare and major breeds. Classifying breeds into four groups based upon registration or census estimates of population size of < 1000, < 5000, < 20,000, and > 20,000 indicated that 50% of the collection is comprised of rare breeds in the < 1000 category. As anticipated, collections for breeds in the < 20,000 and > 20,000 are more complete (86% and 98%, respectively) based upon an index combining the number of germplasm samples and the number of animals. For the rarest breeds (< 1000), collection completeness was 45%. Samples from over 6000 animals in the collection have been used for adding diversity to breeds, genomic evaluation, reconstituting populations, or various research projects. Several aspects of collecting germplasm samples from rare breeds are discussed. In addition, approaches that could be used to enhance the status of rare breeds via the repository use are presented. However, given the array of obstacles confronting rare breeds, the gene bank may be the most secure prospect for the long-term conservation of rare breed genetics.

Keywords: gene bank; biorepository; genetic diversity; livestock

1. Introduction

The task of conserving rare and/or minor breeds has been a global challenge for the past 20 to 30 years, as noted by the development of the World Watch List for Domestic Animal Diversity [1] and subsequent State of the World Reports [2,3]. Some successes to this end have been achieved, as evidenced by the increase in population size (or breed registration numbers) for some populations. Nonetheless, globally, improvement in genetic security has been marginal or rare for in situ populations [3]. In part, rare breeds are subjected to endangerment principally due to small population sizes which, in turn, amplify the effects of genetic drift and selection [4–6]. Compounding these biological aspects, there is an insufficient number of breeders, as well as a lack of breeder longevity and the organization [7,8] needed for concerted action to improve rare breed competitiveness with numerically-larger breeds. Furthermore, as market structures become more complex and consolidated, market entry may become more difficult.

The above challenges can be viewed from the perspective of national agricultural policies that are focused upon promoting economic growth and food security. Norton [9] explains that at the producer level, agricultural policies should address three basic needs: incentives to produce (not to be confused

with subsidized production), a secure resource base, and access to markets for outputs and inputs. The development of animal germplasm collections serves the basic purposes of securing a country's genetic resource base [10] and making those resources available to stakeholders.

In the United States, the USDA's National Animal Germplasm Program (NAGP) was initiated in 1999 to address the issue of conserving livestock, poultry, and aquatic genetic resources [11–13]. This study details elements of the progress to date and future activities that not only increase genetic security, but may also increase the utilization of genetic resources.

2. Materials and Methods

The effective gene banking of breeds requires genetic assessments of populations (within and among breeds), cryopreservation protocols for varying gametes and/or tissues, and a publicly-accessible database that provides a mechanism by which to monitor the inventory and inform stakeholders about which samples are maintained in the collection. Genetic assessments have largely been performed using either pedigree and/or genomic information. Pedigree evaluations (computation of coefficients of genetic relationships) are used with Ward's clustering technique, as demonstrated by Ouendeba, et al. [14].

Germplasm acquired for the collection is achieved through a variety of approaches, and is dependent upon industry infrastructure. For example, beef and dairy cattle germplasm is primarily cryopreserved at private sector studs throughout the nation, while boar germplasm is collected and shipped and then cryopreserved at the NAGP laboratory. In addition, other tissue types which may be useful in regenerating populations have been collected and cryopreserved (embryos, ovaries, and testes).

A comprehensive information system, i.e., Animal-GRIN [15], that is publicly accessible, has been developed in collaboration with the Brazilian and Canadian national genetic resource programs. In addition to monitoring samples in the collection, the database provides information on breeds and specific animals, including phenotypic, genotypic, management, and production system information. Animal pedigrees are also maintained, allowing the user to evaluate the genetic relationships among the collection animals within a breed.

3. Results

3.1. Current Collection

The USDA National Animal Germplasm Program germplasm and tissue collection is quite broad, consisting of 1070,258 samples from 55,094 animals that represent 40 species, 168 breeds, and 350 unique subpopulations. In 1999, the collection was initiated. Some of the samples in the collection were frozen in the 1950s, thereby enabling the collection to be used to evaluate the historical changes that have occurred [13] among various breeds. The following describes several of the unique attributes of the collection that exemplify the breadth of genetics acquired to date. In the late 1960s and 70s the U.S. beef industry imported new continental breeds of cattle (e.g., Simmental, Salers, Limousin). Samples from many of the original imported bulls reside in the collection. The same holds true for the three pig breeds imported from China in the late 1980s. As might be expected, the collection contains many historically-important animals, for example > 99% of Holstein AI sires born in 2010 can be traced to just two bulls, i.e., Chief and Elevation [16]; samples from both of these bulls are in the collection. The collection also contains multigenerational samples which make possible any variety of research projects possible where such a data structure is useful.

Sampling breeds is a continuous process, so changes in genetic variability over time can be captured [13]. For commercially-important breeds with aggressive selection programs, resampling needs to occur at approximately four- to five-generation intervals, where generation intervals range from one (poultry) to four (beef cattle) years. However, most rare breeds lack consistent and intensive

selection pressure; therefore, their generation intervals tend to be longer, and any resampling of those breeds can occur at much longer intervals, e.g., 10 to 20 generations.

Table 1 lists the breeds represented in the collection. The breeds are partitioned into four categories, based upon annual registrations or census information: < 1000 animals, 1000 to 5000 animals, 5001 to 20,000 animals, and > 20,000 animals. These categories deviate from previously-derived classifications. Breeds listed within the same category tend to have a similar set of issues in relation to their conservation and how the gene bank may be used. For example, breeds with less than 1000 head tend to have smaller numbers of breeders, small flock or herd sizes, a rapid turnover of breeders entering and exiting their production, disparate selection programs, less selection intensity, and are most vulnerable to genetic drift. The next largest category of breeds, i.e., having < 5000 animals or registrations per year, contends with a similar set of challenges, but it does have more flexibility in terms of resolving the aforementioned challenges. The third and fourth groups, while numerically large and with the potential to overcome many of the biological and breeder issues, may still have genetic resource issues, for example, relatively small population sizes and the emergence of lethal mutations due to high relatedness [16,17]. However, these groups tend to have organizations and breeders that can leverage resources to address a broad array of problems.

Table 1. Categorization of mammalian breeds by species in the collection based upon annual registrations or census information.

Registration /Census Category (Animals)	Beef Cattle	Dairy Cattle	Sheep	Goats	Pigs
<1000	23—Belgian Blue, Blonde d’ Aquitaine, Chianina, Criollo, Devon, Florida Cracker/Pineywoods, Galloway, Marchigiana, Masona, Murray Grey, Normande, Parthenais, Red Poll, Romagnola, Romosinuano, Senepol, South Devon, Tarentaise, Tuli, White Park, Nelore, Tabapua, Indu Brazil	7—Dutch Belted, Herens, Kerry, Meuse-Rhine-Yssel, Milking Devon, Norwegian Red, Randall Lineback	24—Barbados Blackbelly, Black Welsh Mountain, Bluefaced Leicester, Border Leicester, Cotswold, Delaine Merino, Finnsheep, Gulf Coast Native, Hog Island, Icelandic, Jacob, Karakul, Leicester Longwool, Lincoln, Merino, Navajo Churro, North Country Cheviot, Romadale, Santa Cruz Island, Scottish Blackface, Shetland, Soay, Saint Croix, Texel	4—Kiko, Sable, San Clemente, Savanna	10—Fengjing, Gloucestershire Old Spot, Guinea Hog, Mangalitsa, Meishan, Minzu, Mulefoot, Ossabaw Island, Red Wattle, Saddleback
<5,000	12—Belted Galloway, Braunvieh, British White, Highland, Piedmontese, Pinzgauer, Texas Longhorn, Wagyu, Braford, Simbrah, Red Brangus, Santa Gertrudis	4—Ayrshire, Dexter, Guernsey, Milking Shorthorn	6—Columbia, Corriedale, Polypay, Romanov, Shropshire, Targhee	6—Lamancha, Myotonic, Nigerian Dwarf, Oberhasli, Saanen, Togenburg	2—Tammworth, Hereford
<20,000	6—Limousin, Maine-Anjou, Salers, Shorthorn, Beefmaster, Brahman	1—Brown Swiss	7—Dorper, Dorset, Hampshire, Katahdin, Southdown, Suffolk, Rambouillet	2—Alpine, Nubian	3—Chester White, Pietrain, Poland China
>20,000	7—Angus, Charolais, Gelbvieh, Hereford, Red Angus, Brangus, Simmental	2—Holstein, Jersey	0	3—Angora, Boer, Spanish	6—Berkshire, Duroc, Hampshire, Landrace, Spotted, Yorkshire

Table 2 summarizes several aspects of the mammalian collection status based upon the four population categories identified in Table 1. Stakeholders have used breeds in all census categories for multiple purposes including corrective mating, genomic evaluation, research purposes, rebuilding a breed by adding lost genetic diversity, and population reconstitution. Samples from over 6000 animals have exited the collection for the purposes listed, indicating that collection use has exceeded the overarching goal of genetic conservation for worst-case scenarios.

Table 2. Summary of gene bank collection use, collection frequency, collection completeness, and other attributes of the collection of mammalian species.

Registration /Census Category (Animals)	Collection Use	Frequency of Collection	Average Collection Completeness (Minimum Target Goal *), %	Collection Issues	Special Attributes
<1000	C, G, E, R, B	Gaps in time between collections	45.3	Small population sizes, geographic separation of breeders	original imports, small farmer
<5000	C, G, E, R, B	Gaps in time between collections	75.1	Small population sizes, geographic separation of breeders	original imports, small farmer
<20,000	C, G, E, R, B	Continuous	86.1	Short generation intervals, fine scale sampling possible	Time span, original imports
>20,000	C, G, E, R, B	Continuous	97.7	Short generation intervals, fine scale sampling possible	Time span, original imports

C = corrective mating; G = genomic evaluation; E = reconstituting breed, R = research projects, B = rebuilding breed by adding diversity or breed admixture. * Minimum target goal is an index, ranging from 0.0 to 1.0, which combines germplasm quantity and minimum number of animals needed to reconstitute a breed to 150% with an effective population size of 50.

Table 3 provides an overview of the collection's 21 chicken breeds. Seventy-one percent of the breeds have < 5000 animals. In the U.S., these breeds are typically denoted as fancier, and are maintained by fewer than 10 breeders. Avian species represent a unique challenge, in that the male is homogametic and the egg is too large to be cryopreserved. As a result, a collection of only semen lacks the mitochondria DNA (as with mammals), but is also missing an entire chromosome. To broaden the captured poultry genome, ovaries and testes from chicks one to three days of age have been collected and cryopreserved. These tissue types can then be thawed and transplanted into recipient chicks as a means of rebuilding populations. Somatic cell tissues have been collected for future DNA analyses.

Table 3. Chicken breed collection summary, including quantity of samples, types of germplasm and tissue stored, and collection completeness.

Registration/Census Category (Animals)	Breed	Animal Number	Units of Germplasm	Semen	Ovaries	Testes	Somatic Cells	Collection Completeness (%)
<1000	Crevecoeur	100	170		52	48	70	100
<1000	Aseel	97	161		56	41	64	100
<1000	Transylvania Naked Neck	2	46	46				6
<1000	Redcap	4	8		2	2	4	9
<1000	Spanish	7	8	8				10
<5000	Sumatra	2	11	11				4
<5000	Buttercup	84	140		52	31	57	100
<5000	Polish	28	84		15	13	56	64
<5000	Java	40	80		20	20	40	92
<5000	Jersey Giant	40	77		32	18	27	100
<5000	Andalusian	70	109		46	24	39	100
<5000	New Hampshire	57	334	81	40	8	205	100
<5000	Old English Game	8	252	252				31
<5000	Phoenix	85	191	27	45	38	81	100
<5000	Buckeye	74	118		52	22	44	100
<20,000	Fayoumi	68	135	108	19	8		100
>20,000	Silkie	3	37	37				7
>20,000	Leghorn	4985	9415	4893	1959	1898	665	100
>20,000	Cornish	777	1099	322	583	194		100
>20,000	Rhode Island Red	78	188	14	53	23	98	100
>20,000	Plymouth Rock	178	896	562	88	34	212	100

The index for average collection completeness was derived and based upon the minimum number of germplasm samples needed to reconstitute a breed to 150% with an effective population size of 50, as calculated by Food and Agriculture Organization (FAO) [18]. For easy comparison, the NAGP has developed an index combining animal number and germplasm quantity in the numerator and FAO target animal numbers and germplasm in the denominator. The average index value for each population group indicates that the collection is well established, in general, and especially for breeds within the three largest population categories. While the category for breeds with < 1000 registrations per year had the lowest level of completeness, the index tends to be biased downward based upon the following. Across mammalian species, there are 18 to 20 breeds that were imported into the U.S. which have not become established and viable breeds in terms of population size and number of breeders raising them (e.g., Fengjing, Mangalitsa, Blonde D' Aquitaine, Herens, Meuse-Rhine-Yssel, and Shetland). While the genetic base is limited, they still add unique genetic resources to the collection. In addition, breeds with the smallest populations (e.g., San Clemente goats) will likely never have sufficient population sizes to enable unique germplasm to be collected to have sufficient genetic diversity for reconstitution. In such cases, the collection strategy is to collect groups of animals over long periods as a mechanism to capture genetic change as it occurs. Poultry collection completeness by averaging index values was 72% among all chicken breeds. Of breeds with < 5000 birds, eight had a collection completeness of 100%, and the remaining five had a collection completeness of 64% or less (Table 3).

Sufficiently securing genetic variability for breeds in larger population groups requires a more frequent collection strategy to keep pace with faster genetic changes in order to keep the collection refreshed and relevant for the breeds in these categories. The goal of such a strategy is to minimize the time needed to produce progeny relevant to current industry needs [19,20]. In addition, with their available infrastructure, large breed associations can utilize pedigree data for the analysis of collection gaps, as previously described.

Populations with < 1000 represent a challenge in executing collection activities, principally due to a lack of infrastructure for germplasm collection (semen or embryos) and on-farm expertise of breeders. To date, we have used an array of approaches to accomplish collection goals. For example, collecting germplasm from animals at national shows, developing specific collection workshops where breeders may bring their animals to be collected, and targeting on-farm collections for individual breeders known to have important herds or flocks of a rare breed. More recently, with the development of techniques for cloning in mammals, collection activities will migrate, in part, to collecting ear notches harvested by breeders. We anticipate this activity to substantially broaden the diversity acquired for rare mammalian breeds. Chicken collection activities will likely shift to harvesting primordial germ cells in combination with using gene-edited birds in the reconstitution process.

3.2. Analyses of Breed Diversity

Effective population size (N_e) is a basic statistic that can be useful in assessing the genetic variability of a breed. From several sources, indicative breed effective population sizes are summarized in Table 4. In general, N_e tends to be less than 100 animals, which has been suggested as a benchmark for assessing breed diversity, although FAO [18] has suggested 50 animals be used as a minimum goal. Further understanding the genetic diversity in the collection and how it might be used is an ongoing process as new information and techniques become available. A series of pedigree and genomic studies have been performed to assess various species and breeds among species [7,8,21–24]. In general, within-breed genetic analysis has tended to suggest a contraction of genetic diversity among most breeds in the collection. Figure 1 demonstrates how N_e has decreased over time for pig breeds based upon molecular data and the computation of linkage disequilibrium [24].

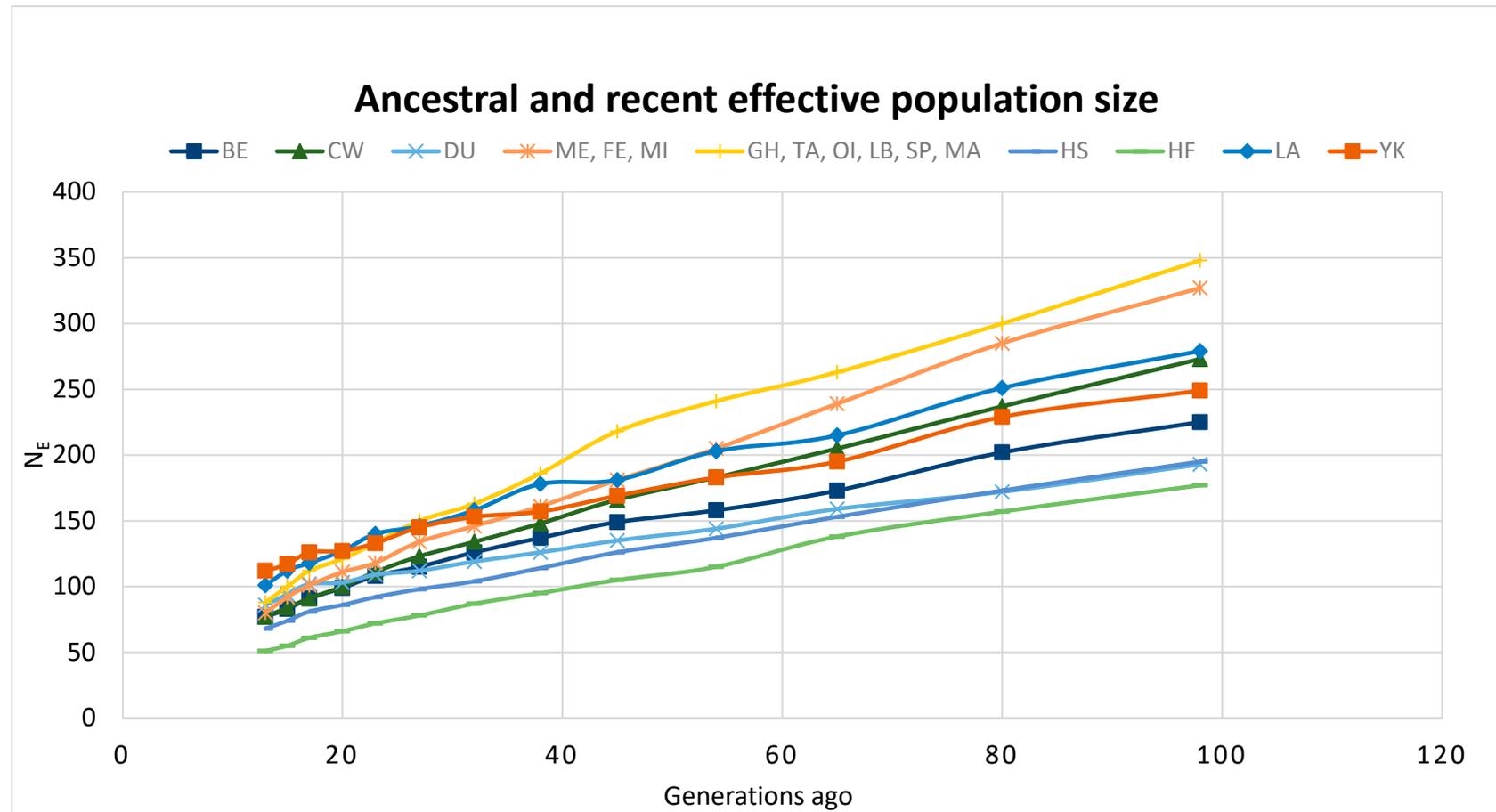


Figure 1. Change in effective population size of pig breeds in the gene bank across generations. BE = Berkshire; CW = Chester White; DU = Duroc; ME, FE, MI = Meishan, Fengjing, Minzhu; GH, TA, OI, LB, SP, MA = Guinea Hog, Tamworth, Ossabaw Island, Large Black, Spotted, Mangalista; HS = Hampshire; HF = Hereford; LA = Landrace; YK = Yorkshire. From Faria, et al., 2019 [24].

Table 4. Indicative effective population sizes for mammalian species in the gene bank.

Species	Breed	Ne	Method of Computation
Goats	Angora	121	molecular
	Boer	59	molecular
	LaMancha	41	molecular
Pigs	Spanish	105	molecular
	Duroc	86	molecular
	Berkshire	77	molecular
	Chester White	77	molecular
	Hampshire	68	molecular
	Hereford	51	molecular
	Landrace	101	molecular
	Yorkshire	112	molecular
Cattle	Brangus	90	pedigree *
	Hereford	85	pedigree *
	Limousin	80	pedigree *
	Holstein	57	CDCB *
	Ayrshire	185	CDCB *
	Brown Swiss	72	CDCB *
	Guernsey	263	CDCB *
	Jersey	79	CDCB *
Sheep	Black Welsh Mountain	32	pedigree *

* Computation of $N_e = 1/(2 \text{ DF})$, [4]; CDCB = Council on Dairy Cattle Breeding, <https://www.uscdcb.com/>, method unknown.

When molecular markers have been used, particularly for sheep [22] and goats [23], the U.S. populations tend to be genetically diverse, with relatively high levels of observed heterozygosity. In addition, these studies suggest that when compared to breeds near the origin of domestication, little genetic diversity has been lost over the course of time [23].

Further work combining molecular (60K single nucleotide polymorphisms, SNP) and pedigree analysis in conjunction with Ward's method for clustering [13,14] was performed using Duroc and Yorkshire pigs. The results to date show that ~99% of the in situ alleles have been captured [25] in the collection. Encouragingly, these results suggest that comprehensive collections of genetic resources can be developed for future utilization.

3.3. Future Work to Understand Genetic Diversity and Enhance Collections

Setting aside collection completeness, which techniques or technologies are to be developed and used to strengthen germplasm collections? Experience to date suggests that there is a greater need to understand the interactions between environments and genetic resources through landscape genetics. Landscape genetics provides a basis for the evaluation of changes in the genetic structure of populations as impacted by environmental conditions [26]. The livestock sector has long known that interactions exist between genetics and the environment [27–29], and that these are important for determining animal productivity. However, little has been done to develop the magnitude of these interactions, or more specifically, to determine where the geographic intersection between differing performance levels caused by genetic and environmental interactions (GxE) are located. Such information is useful for livestock producers as combinations of breed, management systems, and environmental factors can be combined in attempts to evaluate and increase profitability [30]. The study of landscape genetics may provide a more comprehensive approach for evaluating GxE.

Initial steps: The gene bank collection has, to date, sampled breeds from a wide geographic distribution, as can be seen in the online database, Animal-GRIN. The collected samples can be useful in evaluating landscape genomics. Combining genetic/genomics and geographic differences can assist in evaluating genetic differences within and among populations. Pavia, et al. [31], using the beef cattle collection of the gene bank, demonstrated that regional climate (temperature, humidity, normalized

difference vegetative index, and the computed temperature and humidity index) similarities and dissimilarities could project geographic ranges for different breed types (*Bos taurus* continental, *Bos taurus* British, and *Bos indicus*) within the U.S. By linking geographic information systems and individual animals within breed types from gene bank collections and identifying geographic regions where breeds could be successfully used, additional acquisitions to the gene bank can be targeted.

We envision enhancing our understanding of genetic diversity in the context of landscape genetic analysis. Combined with the ability to combine phenotypic, genotypic, and environmental parameters like temperature, precipitation, and average thermal heat index, it will be possible to make any number of mapping overlays that the gene bank managers and/or researchers may require to evaluate breed or specific genotypic differences. For example, using SNP associated with an animal's ability to withstand environmental stressors, such as heat stress or altitude, can be important for maintaining within- and among-breed genetic diversity. By partitioning the U.S. into five geographic zones and assigning Hereford cattle to their respective regions, it was possible to determine how allelic frequencies for the identified SNP differed among regions [32]. This underscores the importance of knowing how subpopulations within a breed differ, and how those differences might be used to develop populations which are more resilient to environmental stress.

The utilization of landscape genetics, as described above, may prove to be of benefit to all breeds, but may have important consequences for breeds with less than 5000 animals. When these smaller populations are genotyped, that information can be combined with data from larger breeds. It may then be possible to perform fine scale analyses on the SNP of interest to quantify differences or similarities between small and large populations.

3.4. Further Gene Bank Use

As noted in Table 2, there are a variety of ways the gene bank can be used to advance different private or public sector objectives. To date, the owners of rare breeds have used the gene bank to reintroduce lost genetic variation for Dexter and Milking Shorthorn cattle breeds. In releasing samples to accomplish any given community's need, a new issue emerged: how to prevent overuse of the released genetics. Obviously, the cooperation and awareness of breed associations and breeders are needed to address potential over use. Recent work with the Livestock Conservancy involving the use of imported Large Black pig semen is like releasing germplasm from the gene bank. In that situation, contracts between the Livestock Conservancy and breeders using the semen set the stage for minimizing inbreeding and setting reasonable prices for progeny.

Because the effective population size tends to be low for many rare breeds, samples in the gene bank can be used to cross breeds and, in subsequent generations, backcross to the breed of interest until the desired percentage of breed composition is reached. In such a process it could be more advantageous to use breeds that are genetically similar. For example, Cotswold, Leicester Longwool, and Lincoln are all numerically-small breeds (Table 1), but their genetic distance from each other is relatively close, which suggests that the breeds might be mutually supportive [21]. In addition, Faria, et al. [24] showed that by combining all pig breeds in the gene bank, the current N_e would be 225 animals, suggesting that ample genetic diversity exists for any variety of purposes.

A major concern that rare breed groups express is a lack of genetic diversity that can result in high inbreeding levels. As a result, breeders often neglect selection which, in turn, increases the likelihood that genetic drift will randomly alter the breed [4]. Blackburn, et al. [6] demonstrated how breeders could effectively employ selection while minimizing inbreeding. In addition, the gene bank collection of germplasm can be used in support of selection efforts by reducing inbreeding levels of the in situ population.

4. Conclusions

Animal agriculture contributes over 60% of farm gate receipts in the U.S. The continued advancement of production efficiency will require the effective use of genetic variation. Therefore,

animal agriculture needs greater engagement in terms of the conservation of animal genetic resources in general. Breeders of rare breeds find themselves in a problematic position, in that the lack of consistently measured information on phenotypic performance and genotypic evaluation suggests that the value of these populations is largely unknown. While it is often assumed that such breeds are an untapped genetic resource containing rare alleles not found elsewhere, definitive examples are largely missing from the literature. Until this question can be conclusively resolved, it will remain a challenge for rare breeds to increase in population size and in terms of their general viability among the livestock sector. That said, we know that the livestock sector is dynamic, and breed preferences change over time; the change in population size and industry preference for Hampshire and Berkshire pigs is such an example. However, until these outstanding issues have been sufficiently addressed, national gene banks may be the best hope for rare breed preservation.

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