CAST® Issue Paper
Number 65
September 2019

Protecting Food Animal Gene Pools for Future Generations

A paper in the series on The Need for Agricultural Innovation to Sustainably Feed the World by 2050

Using different preservation techniques, breeds such as the Mulefoot hog, Buckeye chicken, San Clementine goat, Gulf Coast sheep, and Narragansett turkey can be preserved to ensure genetic diversity in livestock and poultry around the world. (Photo collage by Megan Wickham. Photos courtesy of the USDA, Wikimedia Commons, and Curtis Youngs.)

Abstract

The world's population is expected to reach more than 9 billion by 2050, creating a grand societal challenge: ramping up agricultural productivity to feed the globe. Livestock and poultry products are keys to the world supply of protein, but genetic diversity of livestock is fading. The number of breeds has declined as farming practices have focused on a small number of high-producing breeds to meet low-cost market demands. In fact, up to 25% of global livestock breeds are either at risk of being lost, or have already been lost. In the face of the mounting depletion in genetic diversity among livestock species, there is an urgent need to develop and maintain an intensive program of sampling and evaluation of the existing gene pools. Genetic diversity can be preserved through living populations or cryopreserved for future use. Living populations can adapt to changes in the natural or production environment, provide value in research, and contribute to specialty

This material is based upon work supported by the U.S. Department of Agriculture's (USDA) Agricultural Research Service (ARS) and Animal and Plant Health Inspection Service (APHIS) Agreement No. 59-0202-5-002. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of USDA–ARS, USDA–APHIS, any other USDA agency, or the USDA.

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markets. Cryopreservation offers rare and major breeds a benefit—whether to reconstitute lost bloodlines or to serve as a safety net in case of catastrophic loss of a diminished population. This paper addresses several important challenges regarding the effective protection of remaining livestock and poultry genetic diversity: (1) characterizing the animal populations to identify unique attributes that will influence the collection and conservation of breeds; (2) improving cryopreservation technology for a variety of germplasm and cell types that targets biological differences impeding success among species; (3) expanding the content, accessibility and cross-talk among databases housing breed and genetic resource information; and (4) developing private-public partnerships among rare breed associations/curators, agricultural universities, federal agencies and non-governmental organizations to ensure the long-term operational continuity of germplasm repositories.

Introduction

Seven domesticated species (cattle, sheep, goats, pigs, chickens, turkeys, and ducks) account for most of the world's livestock and poultry food production (FAO 2019). It is from this handful of domesticated species that the world's population, expected to reach 9.6 billion in less than 35 years (Searchinger et al. 2014), will be largely nourished. Animals are an important source of human dietary protein, supplying one-third of the protein consumed in the world, as well as micronutrients vital for infant brain development.

The United States has one of the most vibrant livestock sectors in the world. Livestock breeders produce the genetic resources necessary to address domestic consumption and supply genetic resources to the world. For example, in 2017 the United States exported approximately $175 million of bull semen for use in other countries compared to domestic sales of approximately $22 million (https://www.naab-css.org/semen-sales). The productivity of the U.S. livestock sector, however, is based upon ready access to and use of highly specialized genetic resources, and there is cause for concern at multiple levels. The highly specialized livestock industries in North America are dominated by a small number of productive breeds for which there is a concomitant downward trend in the effective number of breeding animals and a general contraction of genetic diversity, particularly in the commercial dairy and poultry breeds. This problem has been noted in the recent National Genetics Resources Advisory Council's Animal Genetic Resources summary, where it was stated that "all livestock and poultry species and breeds share a common genetic resource problem to varying degrees: there is a global contraction in genetic diversity" (USDA-NGRAC 2018). The contraction is more evident when moving beyond the handful of productive breeds, where up to 20% of livestock and 79% of poultry breeds are classified as "at risk" for extinction (FAO 2007).

Two examples of American breeds that are considered rare, but that possess economically important traits, are the Gulf Coast sheep and the Narragansett turkey. The Gulf Coast sheep breed is resistant to internal parasites, foot rot, and other common sheep diseases; however, there are fewer than 200 pedigree registrations[1] per year in the United States and an estimated global population of less than 2,000 individuals. Parasitism is widely recognized as the most important health issue of sheep and goats, and the costs of prevention, treatment, and lost production worldwide are proposed to be tens of billions of dollars (Roeber et al. 2013); thus, genetic resistance to parasites is an important prevention strategy. The Narragansett turkey, named for the bay in Rhode Island where it was developed in the 1800s, not only is one of the oldest turkey breeds but also possesses genes

[1] Registration identifies an animal as the product of a known sire and dam, born on a known date. Among livestock, primarily breeding animals have their pedigrees registered with associations that track each breed; thus, annual registrations are a strong indicator of breeding population size and are used by conservation organizations in most countries.

for important economic traits—such as early maturation, egg production, meat quality, and disposition—and has broader genetic variation than commercial turkey lines (Aslam et al. 2012; Kamara et al. 2007). There are no more than seven primary Narragansett breeding flocks in the United States, and the estimated global population is less than 5,000.

Although the rare livestock and poultry breeds are currently not major contributors to modern U.S. agriculture, one can examine the recent outbreaks of highly pathogenic avian influenza in the United States (or outbreak of African swine fever in China) and realize that over-reliance on a few highly-productive breeds could endanger the global food supply if these productive breeds are highly susceptible to a new pathogen. The 2015 outbreak of avian influenza cost the layer chicken, broiler chicken, and turkey industries in the United States an estimated $3.3 billion. The United Nation's Convention on Biological Diversity and its Nagoya Protocol (https://www.cbd.int/abs/about/), which provides countries a mechanism to regulate exchanges of genetic resources, are signals of countries viewing livestock genetic resources from a new perspective. As a result, genetic resources from outside the United States may become more difficult to obtain. This, in turn, increases the need to conserve and manage existing U.S. livestock and poultry genetic resources.

In addition to rare or minor livestock breeds, there are unique research lines at U.S. research and educational institutions that contain valuable phenotypes for various traits and have served as important research models for the livestock and biomedical communities. For example, the Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA) developed Line 1 Hereford cattle in the late 1940s which was selected for certain characteristics, including exceptionally fast growth and weight gain. It was not possible to predict at that time that, in the 1970s, the Line 1 Hereford genetics would be responsible for transforming the commercial Hereford breed as producers sought to address consumer preferences for leaner beef. Thirty years later, a cow from the Line 1 Hereford population, "Dominette 01449", was the first animal to be sequenced for the construction of the bovine genome (The Bovine Genome Sequencing and Analysis Consortium et al. 2009), due to the uniformity of this unique stock after 75 years of selective breeding. As another example, the University of Nebraska developed a unique line of pigs with superior reproductive qualities for ovulation rate and embryonic survival (Johnson et al. 1999) which not only helped elucidate the molecular basis of ovarian physiology in swine (Caetano et al. 2004), but also could be sought by swine producers in the future, similar to the Line 1 Hereford. The Avian Disease and Oncology Laboratory of the ARS maintains more than 30 lines of layer chickens, some of which have been developed with resistance to Marek's disease, a highly contagious viral disease-causing lymphoma in chickens (Chang et al. 2010; Mitra et al. 2012). These unique populations take substantial time and resources to develop and provide invaluable answers to many basic biological questions. Their elimination is a major cause for concern as they are not easily replaced. Unfortunately, the long-term value of these populations is often not realized until after they are gone. A case in point was a research broiler line selected in the 1980s for the duration of fertility after insemination with cryopreserved/thawed semen (Ansah and Buckland 1983; Ansah, Seugura, and Buckland 1985; Bentley, Ansah and Buckland 1984) but that is now gone. With today's genomic tools, these genetic traits would have been invaluable for addressing the challenges associated with poultry semen cryopreservation (Long 2006), and for increasing the understanding of cryopreservation at the cellular level.

Long-term preservation of semen and embryos in a deep-frozen state provides a measure of insurance against the loss of genetic diversity, whether among or within specialized lines, breeds or species. A CAST (1984) Task Force Report succinctly made the case for developing a national germplasm repository in the United States. The USDA-ARS's National Animal Germplasm Program (NAGP) was formed in 1999 as a result of 1990 Farm Bill, which called for the Secretary of Agriculture to develop a National Genetic Resources Program to be administered by the ARS. The NAGP repository currently houses cryopreserved germplasm from the major livestock (cattle, goat, sheep, and pig), and poultry (chicken and turkey) species, as well as major aquatic species. This collection includes a number of rare and minor breeds such as the Gulf Coast sheep but lacks many of the other minor breeds (e.g., Narragansett turkey). There are additional gaps in the germplasm collection for other rare and minor breeds for all of the major livestock species. In the face of the mounting depletion of genetic diversity among livestock species, there is an urgent need to expand the sampling program, sustain the preservation effort, and evaluate the remaining livestock and poultry gene pools.

National germplasm repositories are the responsibility of national governments. Accordingly, 128 countries have established gene banks (FAO 2015) and we estimate that samples from more than 100,000 animals have been collected to date (Paiva, McManus, and Blackburn 2016). Limited finances, combined with low priority in national livestock policies, were cited as the most common factors hindering the establishment and operation of germplasm repositories.

The objectives of this paper are:

1. Identify and define what types of traditional livestock resources (e.g., commercial lines, minor breeds, research strains, transgenic stocks) should be considered for long-term germplasm preservation;
2. Describe the state-of-the-art preservation methodologies for those animal genetic resources with special emphasis on gaps in knowledge and application;
3. Describe the existing operations for livestock animal germplasm preservation within the United States;
4. Identify the resources needed to develop a highly efficient and fully optimized national germplasm repository, including (a) a contemporary comprehensive census of existing breeds and strains in the United States, (b) a strategic plan for prioritizing germplasm collection, and (c) research programs targeted to improve gamete and tissue storage and use for poultry, small ruminant, swine, and other

species in which cryopreservation is less than optimum; and

5. Frame a realistic plan to develop the necessary resources to maintain the long-term operational continuity of a highly efficient and fully optimized national germplasm repository.

Genetic Diversity of Today's Livestock Breeds

Domestication and the Rise of Animal Agriculture

Domestication of livestock and poultry began about 12,000 years ago and changed human culture and ecology profoundly (Larson et al. 2014). The exact sequence of events leading to domestication probably varied by species. Figure 1 shows the primary locations of domestication events for agriculturally relevant species.

Early domestications provided a regular supply of meat, first for settled communities and later in conjunction with nomadic pastoralists. Domesticated sheep and goats provided fiber and milk in addition to meat. As the first draft animals, cattle represented a significant development for agriculture. The diversity of pigs and cattle indicates several domestication events (e.g., taurine vs. zebu cattle) and regionally different husbandry practices (Larson et al. 2007; McCann et al. 2014). Poultry likewise underwent multiple domestication events in various centers of human development. Chickens were domesticated in Southeast Asia; domestication of ducks followed 2,000–3,000 years later. Turkeys, originally found only on the American continent, were first domesticated in Mexico and later by the Pueblo cultures of North America.

Domesticated species share common behavioral traits that lent themselves to co-existence with humans. Simple mating strategies and plasticity in food requirements made them compatible to human ecologies. These attributes made them easy to manage and reproduce and provided human caretakers with animals for food production. Both unintentional and intentional selection for domestication strengthened behavioral and phenotypic changes in these livestock and poultry species.

Major centers of livestock domestication as inferred from archaeological and molecular genetic evidence

Figure 1. Domestication centers are: (1) turkey; (2) guinea pig, llama, alpaca, Muscovy duck; (3) rabbit; (4) donkey; (5) taurine cattle, pig, goat, sheep; (6) dromedary, (7) zebu cattle, river buffalo; (8) Bactrian camel; (9) horse; (10) reindeer; (11) yak; (12) pig; (13) chicken; (14) swamp buffalo; (15) Bali cattle. Source: FAO 2015. p. 10. Reproduced with permission.

Signatures of Selection: Shaping Breeds for Optimal Food Production

Livestock and poultry have long been selected to suit needs and desires of humans. Over time, differences emerged in livestock populations of different regions due to combinations of distinct foundation animals, genetic isolation, genetic drift, and selection. These influences explain most differences among livestock populations. Prior to humans, natural selection assured that populations were adapted to various local environmental conditions. Subsequent selection by humans resulted in breeds suited for specific roles, such as milk production or draft power. The result of these combined selection influences was an intricate patchwork of populations that varied from region to region.

Genetic selection of livestock as we know it today did not become routine until the 18th century in England and northern Europe (Wood and Orel 2001). Breeders established and documented the fundamental concepts of measuring production, comparing animals within a herd and across herds to each other, and breeding only "the best to the best" as a strategy to improve herds. The resulting "improved" breeds such as Leicester Longwool sheep and Shorthorn cattle soon spread across the world to influence livestock populations in many countries. More importantly, the principles used by English breeders were rapidly adopted by other breeders interested in livestock genetic improvement. Furthermore, these principles inspired Austrian monk Gregor Mendel to conduct his foundational work in plant genetics (Wood and Orel 2001).

Driven by the industrial revolution, the mid-to-late 1800s saw a dramatic upsurge of deliberate breed development and standardization. Livestock exhibition grew to be a passion. At the same time, the opening of trade routes to the Far East brought novel breeds to Europe and America, and breeders experimented with those breeds. These experiments led to new genetic combinations and subsequent breed development. Breed standardization and development left their marks on American agriculture as a few of the newer breeds replaced many distinct older local types of livestock (Leavitt 1933; Lemmer 1947). For example, muscular Hereford and Angus cattle became specialty beef breeds, and eventually replaced most of the dual-purpose Shorthorns and "native"

cattle of Spanish origin, especially as stockyards grew increasingly important to fatten cattle in the final weeks before slaughter.

Mechanization and social changes led to further shifts and consolidation in livestock agriculture. Tractors and trucks replaced draft animals. Trains and refrigeration made it possible to transport livestock and food over previously unimaginable distances. Economic shifts in the 1930s and 1940s caused the U.S. government to invest in research to improve animal productivity. Farms became ever larger because of enhanced efficiency needs, and grocery stores created consumer demand for large supplies of uniform, low-cost products.

Selection of livestock and poultry for improved production made this new intensive form of agriculture possible. Breeds became specialized—no longer would one type of chicken be used for egg and meat production or one breed of cattle for meat, milk, and draft. Specialty breeds were developed for improved yields. New husbandry methods were developed that allowed more animals to be produced using less land and feed (Capper, Cady, and Bauman 2009; Pelletier, Ibaruru and Xin 2014). Specific breeds excelled in these ever-more concentrated livestock production systems, whereas other breeds were deemed inferior.

Today, breed improvement and selection are global as well as local, spurred by growing demand for livestock products in conjunction with income growth (FAO 2007). Highly specialized breeds or new composite breeds (formed by mating two or more different breeds) are selected for productivity and other economically valuable traits. Genetic change is now being accelerated by sophisticated statistical analyses, ultrasound measurements of body composition, genomic analyses, and assisted reproductive technologies. The United States is a leader in providing genetic resources on a global scale (FAO 2007; Gollin, Van Dusen, and Blackburn 2009), and U.S. husbandry methods have been exported concurrently with germplasm resources. At the same time, local and regional breed populations have contracted, and losses in these breeds represent losses of unique genetic variations with potential value for future food production systems that cannot always be predicted. For example, U.S. swine farmers in the 1920s probably did not envision the transition from lard-type hogs to lean meat hogs.

Whereas much of the world is focusing on least-cost production of livestock products, a new two-tier approach to consumer demand for livestock products is emerging within the United States and Europe. While the larger tier focuses on large-scale, least-cost production, newer and smaller niche markets desire locally raised food products, such as eggs from pasture-raised chickens and meats from slow-growing animals raised in less intensive production systems. These niche markets provide an entry into the marketplace for a much wider array of breed types, and new opportunities are opening to breeders of less common or rare breed animals (e.g., meat from the Berkshire breed of pig for high value charcuterie dishes that can fit these alternative consumer requirements). Demands from the niche market consumers are being met primarily by small farmers. Significant efforts are needed to increase those farmers' access to and use of genetic, reproductive, and information technologies appropriate to the markets that they are supplying.

Then and Now: What Has Been Lost

Agricultural books of the early 20th century featured a dozen breeds per species from which the farmer could choose, in addition to locally available breeds. Locally adapted Spanish cattle that were once the mainstay of beef production in California are now extinct. While three other cattle breeds that share a Spanish origin still exist in the United States (Texas Longhorn, Pineywoods, and Florida Cracker), their numbers are drastically reduced from the hundreds of thousands that once roamed Texas and the Deep South (Simon 2006; Sponenberg and Olson 1992). The vast array of Linebacked cattle from the northeast United States has declined to a single line saved from 12 animals (genetic variation is lost through bottlenecks such as this). Hog breeds like the Mulefoot came very close to extinction but now are the target of conservation programs. In each case, intensification of livestock production and a new array of disease and parasite preventions and treatments diminished the value of environmental adaptation and enabled larger, faster growing breeds to capture the agricultural market. Yet, emergence of multi-resistant parasites, inbreeding in dominant livestock breeds (such as Holstein dairy cattle), and ever-changing environmental stressors and production requirements emphasize the need to conserve animal genetic resources with adaptive potential.

Poultry losses are more difficult to assess; 50% or more of the genetic diversity in ancestral chicken breeds is lacking in commercial pure lines (Muir et al. 2008). Nonindustrial turkey breeds have been hit especially hard, with almost no production-oriented breeding occurring until the 1990s. Historic sheep breeds adapted to Florida and the Gulf Coast conditions are now greatly reduced from their previously high numbers (Dohner 2001). These are sheep adapted to survive humid conditions and heavy internal parasite loads, traits that would be difficult to reestablish if those breeds are lost. In fact, most similarly adapted strains of Spanish goats previously found in the southeastern United States are now extinct and their adaptation to humid subtropical environments has been lost. In the Southwest, the Navajo-Churro sheep was saved from extinction only by the active engagement of several breeders (see Textbox 1). Well-planned efforts to preserve existing livestock and poultry genetic resources and to characterize their adaptations, productivity, and their genomes can prevent such losses in the future.

Intensification of global animal agriculture has depleted the historical numbers of breeds to a relatively few breeds selected for high-production traits to meet low-cost market demands. Development of these specialized breeds has been facilitated by intensive management systems such as enhanced nutritional regimens, indoor rearing (some species), and improved health care. Modern examples of breeds that are markedly different from their ancestors, despite the breed name remaining the same, include swine breeds such as Duroc, Hampshire, Chester White, and Yorkshire, which substantially changed from their original

Textbox 1. Preventing Extinction of a Breed: The Navajo Sheep Project

Navajo-Churro sheep are a distinctive breed descended from Churra sheep from Spain. They have been part of the Navajo, Hispanic, and Anglo cultures in the Southwest United States for more than 400 years. The fleece type, with its durability and mixture of short fine fibers and longer coarse fibers, is superbly suited to the textiles produced in the local region, famous throughout the United States for their unique qualities and cultural relevance. Navajo sheep numbered more than 550,000 in 1930; however, the breed nearly became extinct from the 1950s to 1970s following severe drought, government stock reduction programs, and crossbreeding programs. By 1977, fewer than 500 head of traditional-type Navajo sheep remained. The launching of the Navajo Sheep Project prevented total extinction. This and other conservation programs saved this unique breed. In 1986, a registry system was launched through the efforts of individual breeders and non-governmental organizations, and ongoing inspection of each generation assures that breed type remains traditional. Census numbers are now close to 3,000 head, and the breed can be found widely throughout the United States (Maiwashe and Blackburn 2004). Adapted from Sponenberg and Taylor (2009).

conformations due to shifts in consumer dietary preferences (i.e., desire for lean meat). Nevertheless, the Hampshire, Yorkshire and, in particular, Duroc are among the most genetically diverse pig populations in the United States, and this diversity should allow for plasticity within these breeds as agriculture and consumer demands continue to evolve (Faria et al. 2019).

In general, across livestock and poultry species, performance for some traits (foraging, climate adaptation, mothering ability, longevity, and fertility) has decreased in some breeds because of selection pressures and elements of intensified production systems. Although the pace of modernizing animal agriculture has slowed somewhat, existing breeds are still undergoing contraction. Beef breeds such as Limousine, Gelbvieh, and Salers have shown a reduction in purebred registration numbers because they are being crossbred to be competitive in commercial production systems. The dairy industry in the United States relies almost entirely upon the Holstein breed, and inbreeding in Holstein cattle is accelerating in the United States (Dairy Cattle Reproduction Council 2019); the effects of inbreeding on reduced fertility and productive lifespan are being felt even as milk production per cow continues to increase. Effective population sizes in Holstein and Jersey dairy cattle dropped to 39 and 30 animals, respectively (Weigel 2001), and the existence of only two Y chromosome lineages in the Holstein, suggest only two sire lines are present for the breed (Yue, Dechow, and Liu 2015). Genetic contraction also has occurred in swine breeds, where composite populations of pig breeds have resulted in smaller purebred populations (Welsh et al. 2010). The entire U.S. sheep industry has contracted over the last six decades, reducing numbers for all breeds. Goats have escaped many losses, and although numbers are greatly reduced for San Clemente, Angora, and Spanish goats, they remain as viable locally adapted breed resources (do Prado Paim et al. 2019). For chickens, the culmination of intensive production systems has led to the development and use of very distinct, specialized strains that have been selected for traits linked to commercial production, such as reduced broodiness to provide a more continuous production of eggs. Consumer preference (e.g., production of cage-free eggs, reduced use of antibiotics) is now causing some reversal in this trend, requiring breeding companies to shift selection criteria for production in less-intensive environments, more similar to the historical breeds.

One of the ways genetic diversity of livestock and poultry can be represented is in breeds; therefore, breed disappearance means a loss of easily used genetic diversity (Bixby et al. 1994; FAO 2007). Moreover, because many breeds are closely tied to the history of specific communities and locations, breed losses also represent losses of the cultural landscape. Although disappearance of a few breeds may not entail a complete loss of genes that are shared within a species, loss of several breeds reduces genetic variation and increases the theoretical likelihood of permanently losing specific genes and gene combinations that often define a breed. Putting a value to the loss of these genetic variants is always incomplete because one cannot predict whether these genes or gene combinations will be needed for future environments, production changes, or biomedical research. This is especially true in light of the "genomics era" where all of the major livestock species now have a reference genome sequence, and comparisons of DNA sequences of breeds with different phenotypes could provide much needed information to drive selection forward for specific traits (e.g., sheep breeds with known resistance to parasites). With the loss of many therapeutics for use in animal production systems, genetic resistance to disease has more relevance today than ever before. Additionally, newly developed gene-editing methods would allow the introduction of desirable variants identified in minor breeds into more mainstream breeds. It is critical that genetic resources be conserved to maintain as many options as possible to meet a wide variety of future needs that are often unknown at present,

Protecting Remaining Food Animal Gene Pools in North America

On-farm Conservation: The Value of Live Populations

Farm animal populations consist of

living individuals, and there are advantages of keeping genetic material in this form. Living populations are advantageous because they can adapt to changes in the natural or production environment, such as the emergence of new disease challenges, alterations of husbandry practices, changes in consumer preferences and climate change (FAO 2010). As populations respond genetically to their environment, producers in turn maintain or develop knowledge concerning husbandry and use, including unexpected discoveries such as human disease models (Torres et al. 2010).

Live populations of animals can contribute a part or all of the cost of supporting themselves through the production of meat, milk, eggs, or fiber (or simply through their cultural value), but annual expenditures are greater than for maintenance of the same genetic resources in a cryopreserved form. Living animals in a conservation program can be used to develop specialty markets to support economies in rural areas, and their characteristics can be integrated immediately into a production system (Sponenberg, Beranger, and Martin 2014).

Living populations provide value in research because the physical traits (phenotypes) can be observed and studied. Highly inbred and selected research lines have contributed to basic scientific knowledge in agriculture, medicine, physiology, and genetics. Such stocks have been subjected to long-term selection, and in some cases reconstituting these stocks from cryopreserved material would require both a relatively high level of expertise and cost. Unfortunately, many of the specialty research lines are unable to generate enough income to be economically self-sufficient and thus their continued existence is often vulnerable to short-term budget considerations.

Maintenance of living populations is complementary to a cryopreservation program, where the genetics of breeds and lines are conserved in frozen form. Cryopreservation offers rare and major breeds a benefit—whether to reconstitute lost bloodlines or to serve as a safety net in case of catastrophic loss of a diminished population. Reconstitution of cryopreserved genetic material requires live animals as hosts and, while the host or recipient animal can be from a readily available commercial line or breed, more effort will be required to reconstitute the lost line if the recipient animals are genetically divergent. For example, if there are no living females from a research poultry line, multiple breedings known as "backcrosses" are required to restore the line with commercially available hens. For example, five to seven backcrosses would yield 96.9 to 99.2% of the original line's genome, at which point the reconstituted line will still contain up to 3.12% of the genetics of the unrelated recipient hens. Importantly, living animals are also necessary to verify the effectiveness of cryopreservation techniques. Verification includes the routine evaluation of cryopreserved material and the validation of techniques for specific lines because there is well-documented variation among species, lines, and individuals in the ability of biological material to survive cryopreservation, as well as from a wide variety of non-biological sources including research techniques (Bacon et al. 1986; Blesbois et al. 2007; Kishida et al. 2015; Long et al. 2014; Windsor 1997;). This verification should include the final proof of success, the production of viable and healthy live animals, and not just the identification of fertility or pregnancy rates. Moreover, living animals also are needed as a resource for research to develop new and/or more effective methods of cryopreservation.

The possibility of using and studying living animals in various production environments and for research means that maintaining living populations is nearly always the preferred conservation strategy if financial concerns are removed. Most countries have adopted conservation strategies that include living populations, primarily through public-private partnerships. In cases where a particular species, breed or line cannot be cryopreserved, maintenance of living populations is the only preservation strategy currently available.

Ex Situ Preservation: Current Methods of Cryopreservation

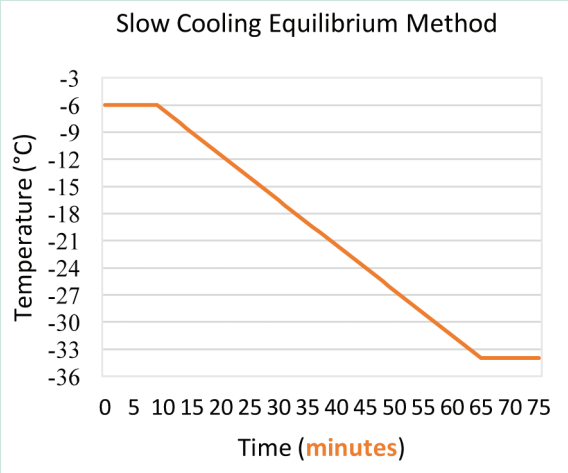
Genetic material from livestock and poultry can be cryopreserved in several forms: male gametes (spermatozoa), female gametes (oocytes), embryos, embryonic cells, gonadal tissue, primordial germ cells (PGC), and somatic tissues. There are two main approaches to cryopreservation of cells or tissues: slow "equilibrium" cooling and ultra-fast "non-equilibrium" cooling, known as vitrification (see Textbox 2; Youngs et al. 2010; Youngs 2011). The goal of slow-cooling procedures is to prevent cell damage caused by ice crystal formation by removing intracellular water (e.g., cellular dehydration) via diffusion across the cell membrane. Slow cooling has been used extensively for sperm and other cells, but it cannot be used with equal efficiency for other tissues and organs (Liu, Cheng, and Silversides 2013a) because tissues and organs contain a wide variety of cell types that often have different characteristics (Mazur 1970). A slow-cooling protocol that results in high survival of one cell type may lead to the death of other types of cells in that same tissue. Vitrification procedures, on the other hand, turn water inside the cell to a solid without allowing ice crystals to form (Liu, Cheng, and Silversides, 2013a). The ultra-rapid cooling of biological materials converts intracellular and extracellular aqueous components to a glass-like state almost instantaneously. Irrespective of the cryopreservation method, cryobiologists have calculated that cells and tissues may be stored indefinitely at the temperature of liquid nitrogen (-196°C). For example, cryopreserved ram semen has maintained its fertilizing ability for 35 years (Salamon et al. 2004).

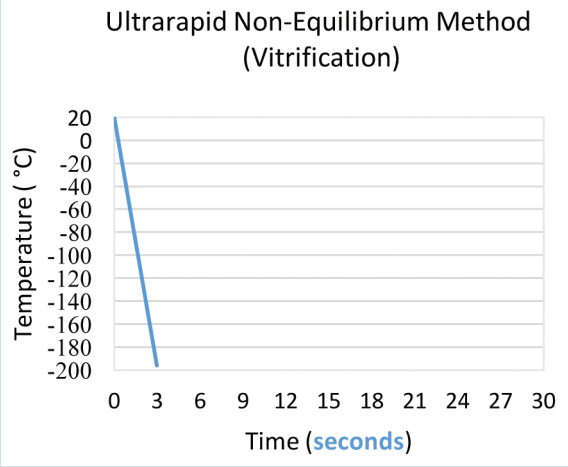
Male Genetic Material: Spermatozoa

Cryopreservation of spermatozoa (hereafter, sperm) has taken place for decades, and substantial efforts have been made toward developing protocols, primarily because of the relative ease of semen collection. The discovery that glycerol could protect rooster sperm against damage caused by cold temperatures (Polge, Smith, and Parks 1949) paved the way for semen cryopreservation procedures for a wide range of species, including livestock, companion animals, aquatic species, and humans. The greatest progress in commercial use of sperm cyropreservation has been achieved

Textbox 2. Embryo Cryopreservation Methods: Slow Cooling Versus Vitrification

The Water/Ice Problem:
Preimplantation embryos from mammalian livestock species consist of approximately 80% water. This high water content represents a significant obstacle to the successful preservation of embryos at subzero temperatures because water turns into ice crystals when cooled below 0°C (32°F). Ice crystals that form inside the cells of an embryo cause significant damage to the cells, usually leading to death of the embryo.





Two Methods of Freezing:
To reduce the likelihood of ice crystals forming inside the cells of an embryo, embryos must be dehydrated prior to cooling. Dehydration is accomplished by placing embryos into a solution containing a compound that draws water out of the embryo. As illustrated in the top graph, the slow-cooling equilibrium method consists of a 10-minute equilibrium period, followed by a gradual cooling period for nearly an hour, and then a final 10-minute equilibrium period. In contrast, the bottom graph illustrates ultra-rapid, nonequilibrium cooling (vitrification) and that involves placement of the embryo in a very small volume of the dehydrating solution directly into liquid nitrogen (-196°C [-320°F]) or into liquid nitrogen vapor (-180°C [-292°F]) for a few seconds before plunging into liquid nitrogen.

by the dairy and beef cattle industries, where semen cryopreservation has been optimized, standardized, and automated for commercial production. The National Association of Animal Breeders (NAAB) indicates that more than 23.1 million units (i.e., straws) of cryopreserved dairy cattle semen and more than 2.5 million units of cryopreserved beef cattle semen were sold by the NAAB members during 2017. A typical straw of bovine semen (Figure 2) contains many millions of sperm, and the rate of cell survival after freezing and thawing is not critical so long as a sufficiently large number of functional sperm cells are deposited into the female at the time of insemination. For example, even when fewer than 20% of thawed bovine sperm cells are alive, fertility rates as high as 68% are routinely observed (Vishwanath 2003).

Semen cryopreservation procedures for other domestic mammalian livestock species are not as well developed as those

Figure 2. A straw such as this one can be used for long-term cryopreservation of semen.

for cattle, but numerous successes have been reported in the scientific literature for sheep and goats. In sheep, one of the historical challenges with frozen-thawed semen was the inability to obtain high pregnancy rates following cervical insemination because of the difficulty in passing through the ewe's cervix (Salamon and Maxwell 1995a,b). The use of laparoscopic artificial insemination, where semen is placed directly into the uterus via a fiber optical endoscope, has improved conception rates from cryopreserved semen to as high as 80% (Cseh, Faigl, and Amridis 2012). This insemination method is used extensively for sheep in some countries outside of North America; however, the U.S. sheep industry perceives the use of cryopreserved semen as having a minimal benefit-cost ratio, where the improved conception rate is a marginal benefit compared to the added cost of laparascopic insemination. As a result, it is used only in very elite flocks. Goat semen has been cryopreserved for several decades, and there have been reports of acceptable pregnancy rates of 70 to 76% (Bispo et al. 2011).

North American swine producers routinely use fresh semen that has been chilled (not frozen) for artificial insemination (Flowers 2015). The commercial swine industry has not yet embraced the use of frozen semen because it typically yields lower pregnancy rates and reduced litter sizes. Boar sperm are sensitive to a phenomenon known as cold shock, have low tolerance to osmotic stress, and there are dramatic differences among boars in the ability of sperm to survive the freeze/thaw procedure (Benson et al. 2012). Use of a nonsurgical intrauterine artificial insemination method (Kraeling and Webel 2015) could enable more widespread use

of thawed boar semen, especially when used in conjunction with methods to control the time of ovulation in sows.

Although the first live chick was produced from thawed rooster semen in the 1940s (Shaffner, Henderson, and Card 1941), semen freezing technology has subsequently developed more successfully for other animal groups than for poultry. Bird sperm physiology and the requirements for fertilization are different from those of mammals (Liu, Cheng, and Silversides 2013a; Long 2006). Sperm must be able to be stored in the sperm storage ducts in the lower end of the hen oviduct and subsequently released to coincide with hen ovulation. Cryopreserved rooster semen may retain less than 2% of the fertilizing ability of fresh semen (Wishart 1985). Lower fertility rates of frozen/thawed semen, combined with inconsistent results, have greatly hindered the use of cryopreserved semen by the poultry industry. Cryopreserved chicken semen can yield fertility rates ranging from 0% to 90%; while the range for turkeys (0% – 65%) is lower (Blesbois et al. 2007) and results often are not repeatable (Long et al. 2014). High fertility is not needed for conservation and recovery of avian lines (Blackburn, Silversides, and Purdy 2009; Blesbois et al. 2007), but there is variation among genetic lines in the sperm's ability to survive the freeze/thaw cycle (Ansah and Buckland 1982; Long et al. 2014) and some lines may not be recoverable from cryopreserved semen.

Another difference between avian and mammalian sperm is the sex chromosome composition. In mammals, males have an "XY" sex chromosome composition, and individual sperm contain either an "X" or a "Y" chromosome, which determines genetic sex of the embryos at the time of fertilization. In birds, the sex chromosomes are labeled as "W" and "Z"; females are "WZ" and males are "ZZ", and the presence of a "Z" or "W" chromosome in the oocyte determines genetic sex at the time of fertilization. Stored semen lacks the female "W" chromosome and maternal mitochondrial DNA which, from a practical perspective, are necessary to maintain the complete genetic complement of avian genetic lines. Recovering a line or breed from any species using only thawed semen requires multiple generations of backcrossing to reduce the "contaminating" DNA from the recipient female, and for birds this cost is compounded by the need for concentrated semen to overcome the issues of fertility (Silversides et al. 2012). Despite the limitations of cryopreserving semen for birds, that technique is the basis of several significant avian cryoconservation programs in the absence of other technologies (Blackburn 2006; Blesbois et al. 2007; Woelders, Zuidberg, and Hiemstra 2006).

Female Genetic Material

Oocytes

Oocytes are not as accessible as sperm because the female gonad (ovary) resides within the internal body cavity, and surgical procedures are often required to recover oocytes. Avian oocytes are even less accessible than mammalian oocytes because the egg of the hen includes a large and fragile yolk, and non-fertilized avian oocytes degrade during the 23 hours between ovulation and egg lay. Oocytes are not as plentiful as sperm. For example, a cow has an average of 133,000 oocytes at puberty (Erickson 1966), and this finite number will not increase during the cow's lifetime. In contrast, a bull produces 6.0–7.5 billion sperm per day (Senger 2012). The mammalian oocyte is more difficult to cryopreserve than sperm, primarily due to its larger size; nevertheless, mammalian oocytes can be recovered, frozen, thawed, fertilized in vitro and resulting embryos can be transplanted into the uterus, with good success in some species (e.g., >50% pregnancy rate in cattle). For poultry, an additional barrier to the use of cryopreserved oocytes is that, while minimal embryonic development occurs in the female reproductive tract (30,000 to 60,000 cells), most embryonic development is largely external (i.e., occurs within a hard-shelled egg after the egg is laid by the female). The complexity of this developmental process limits the ability to reintroduce a fertilized avian oocyte into a hard-shelled egg.

Without question, the technology to cryopreserve oocytes from domestic mammalian livestock species is far less developed than that for sperm and for embryos. Numerous efforts to cryopreserve oocytes (Arav 2014; Dinnyes, Liu, and Nedambale 2007; Hwang and Hochi 2014; Mullen and Fahy 2012; Somfai, Kikuchi, and Nagai 2012; Zhou and Li 2013) highlight the need for substantial improvements in methodology. Although a limited number of healthy mammalian offspring have been produced using vitrified oocytes, it is not yet a routine procedure. Comparable results for poultry oocytes have not been achieved for reasons described above.

Embryos and embryonic cells

Cryopreservation of embryos from domestic mammalian livestock species has been possible for several decades. The first reports of live offspring after the transfer of cryopreserved cattle, sheep and goat embryos (Bilton and Moore 1976; Willadsen et al. 1974; Wilmut and Rowson 1973) were published in the 1970s and in the 1980s for pig embryos (Hayashi et al. 1989). Cryopreservation procedures have become routine for most domestic mammalian livestock species. This is particularly true for cattle, where more than 59% of embryos recovered from live cattle and transferred globally in 2017 had been cryopreserved (Viana 2018). Most embryo cryopreservation methodologies were developed for embryos recovered from live animals. Technology now permits the production of embryos in the laboratory through in vitro fertilization, but survival of laboratory-produced embryos is after transplantation can be 40% lower than in vivo-derived embryos (Farin and Farin 1995; Papadopoulos et al. 2002). Vitrification seems well-suited for cryopreservation of embryos produced in the laboratory, and it has been performed for many years with animal-derived embryos from cattle, sheep, goats, and pigs (Kobayashi et al. 1998, Massip et al. 1986; Schiewe, Rall, and Wildt 1990; Yuswiati and Holtz 1990;). Relatively high pregnancy rates (60 to 80%) have been reported from the transfer of thawed embryos for these species and such pregnancies proceed normally thereafter.

One advantage of using cryopreserved embryos (versus sperm or oocytes) is cost saving during the reconstitution process because the embryo represents a

"complete" genetic package of the breed (Gandini et al. 2007). Moreover, embryos from a rare breed can be transferred to the uterus of a common-breed female surrogate to accelerate the production of purebred progeny of the rare breed. The same result can be obtained with semen only if insemination occurs with a rare breed female (which may not always be available); otherwise multiple generations of backcrossing are needed to reconstitute "pure" populations. Reconstituting a mammalian breed only with semen also suffers from the lack of capturing the maternal genetic contribution of mitochondrial DNA that is strictly maternally inherited.

As for avian species, bird embryos cannot be cryopreserved because of the structure of the embryo in relation to the yolk, the number of embryonic cells present when the egg is laid, and the size and structure of the egg itself. Although avian embryos cannot be cryopreserved, avian embryonic cells can be isolated, grown in culture, cryopreserved, and used to produce germline chimeras (Naito 2003; Petitte 2006). In principle, these embryonic cells could be used for cryopreservation; however, the technology was developed primarily to provide access to germplasm for the genetic manipulation of poultry (van de Lavoir et al. 2006; Li and Lu 2010) which requires much lower efficiencies than those needed for genetic conservation. Several steps of the process of cryopreservation and reconstitution of avian embryonic cells work well, but the procedures needed are complex, and more than 25 years of intensive investigation by researchers around the world have not produced a technique for reconstitution that is sufficient for use in a cryopreservation program (Silversides and Liu 2012).

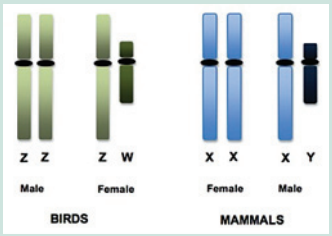
Gonadal material

During embryonic development, primordial germ cells that will eventually produce the next generation of sperm and oocytes migrate to and establish themselves within the gonads. These immature gonads can be surgically removed, cryopreserved and, after thawing, transplanted to recipient animals. Both of these processes allow conservation of the germ cells in their natural microenvironment with the subsequent development and production of fertile gametes. Gonadal transplantation has been successful in a number of mammalian species—including humans (Comizzoli and Wildt 2014; Silber 2012)—and also can be used for functional recovery of cryopreserved avian gonadal tissue (Silversides and Liu 2012). Harvesting of gonadal tissue could prove vital to enabling the rescue of genetic material from animals that die unexpectedly.

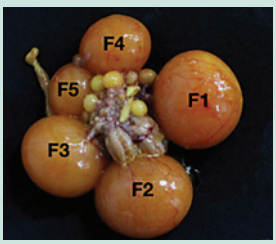
Cryopreservation of gonadal material could become a primary approach to cryopreservation of poultry germplasm because of the difficulties of cryopreserving poultry sperm, oocytes, and embryos (Textbox 3). Surgical ovariectomy of immature chickens followed by implantation of donor ovarian tissue into the normal anatomical location, enabled transplanted tissues to survive and resume development in recipients of the same age (Song and Silversides 2006). Further work in Japanese quail demonstrated that ovarian tissue from adults could be recovered in chicks (Liu, Cheng, and Silversides 2015). An important aspect of gonadal transplantation is the use of immunosuppressants to prevent rejection of the donor tissue. These techniques have been used to produce donor-derived offspring from the transplantation of fresh ovarian tissue from chickens (Song and Silversides 2007a), ducks (Song et al. 2012), and Japanese quail (Liu et al.

Textbox 3. Overview of Ovary Cryopreservation and Transplantation in the Chicken

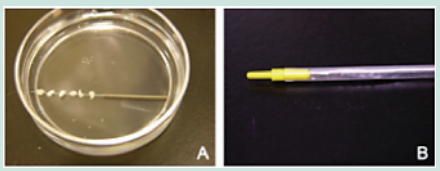
The Decider



Unlike mammals where the Y chromosome from males determines genetic sex, the genetic sex of birds is determined by the W chromosome from females; thus, females are needed for 100% recovery of a line.



Recovering oocytes for cryopreservation from mature ovaries (shown on the left) is not an option for birds; however, immature ovarian tissue (which contains a large pool of premature oocytes) can be frozen whole and subsequently transplanted into recipient birds.



Donor (day old) | Needle-in-straw vitrification* | Recipient (day-old)

The ovary is recovered from a day-old chick, frozen using a process known as vitrification, sealed in a specialized straw, and stored in liquid nitrogen (-196 °C). The recipient bird is ovarectomized prior to transplantation of the donor ovary. Immunosuppressants are administered for up to two months to prevent rejection of the donor ovary. For quails, the optimal age for ovary recovery and transplantation is one week after hatch; while the optimal age for the turkey has not yet been determined.
*Adapted from Liu et al., 2012
(Song and Silversides 2007; Song and Silversides 2008)

2010; Song and Silversides 2008a).

For avian testicular tissue, transplantation to a site other than the normal anatomical location of the testes is required because the male gonad is more intrinsically associated with the reproductive tract than the ovary and, to date, this limitation has not been overcome. A transplant location under the skin on the back of the chicken can support testicular growth (Silversides, Robertson and Liu 2013a) and live, donor-derived offspring have been produced by sperm recovered from transplanted testicles, although this sperm was fresh, not cryopreserved (Song and Silversides 2007b).

Primordial germ cells and somatic cells

Precise genetic editing (via CRISPR/Cas9 or similar enzyme systems) is becoming more and more feasible in livestock to produce animals with desired traits such as a lack of horns or resistance to disease (Tait-Burkard et al. 2018). As these gene editing techniques are more widely used, new opportunities are becoming available for the use of cryopreserved chicken primordial germ cells. These cells can be injected into early age embryos (2.5 days old) and will migrate to the gonads to become the eventual oocytes. Recently, gene-editing technology was used to create sterile hens that do not lay eggs but are otherwise healthy (Taylor et al. 2017). The next step would be to transplant primordial germ cells from a rare breed into the sterile hen, although this methodology has not yet been developed. If successful, this approach could overcome some of the limitations with cryopreservation in birds.

Somatic cells (such as skin cells) can be cryopreserved, usually without difficulty, and contain a complete complement of genetic information. For some mammalian species (e.g., cattle, sheep, goats, pigs), these cells can be used to produce offspring by somatic cell nuclear transfer (a.k.a. cloning); this is not currently an option for birds. The major advantages of somatic cells over germinal tissue are their greater availability, greater ease for cryopreservation, and the possibility to introduce selected genetic modifications. Healthy offspring have been produced in a multitude of mammalian livestock species, and further refinements in methodology will enable more consistent and repeatable results to be obtained.

Categorizing and Prioritizing Animal Populations for Cryoconservation

Despite the losses described above, there is still tremendous biological diversity among agriculturally important food animal species in North America. A strong need exists to characterize this remaining biological diversity to identify uniqueness that will influence the collection and conservation of breeds. Maintaining the diversity of livestock breeds is important to enable animal production systems to adapt to changing production environments caused by factors such as climate change and urbanization of farmlands (ERFP 2014), as well as to address changing consumer demands (e.g., niche markets). Any loss of existing animal genetic resources could hamper future efforts to feed the world. The FAO has estimated that 17% of the world's remaining livestock and poultry breeds are at risk of extinction (FAO 2015), so action is needed now.

In North America, the critical importance of characterizing and cryopreserving livestock genetic animal resources has been recognized by the U.S. Department of Agriculture, Agriculture and Agri-Food Canada, and The Livestock Conservancy. While Canadian and U.S. conservation policies dictate cryopreservation efforts for all breeds, the cryopreservation program and in-situ efforts in Brazil have focused solely on heritage breeds. In the United States, prioritization for germplasm collection has considered among-breed and within-breed genetic variability, societal values (especially regarding heritage breeds), genomic information, pedigrees, and geographic location. This approach has enabled the current germplasm collection to acquire a broad array of genetic resources. Evaluation of the number of samples per breed and the relatedness of those samples among animals within the breed readily reveals new priorities for collection.

Collection gaps exist in the U.S. cryopreservation program at the breed and within-breed levels. Information is needed at the pedigree and genomic levels to select animals or groups of animals to fill these gaps. Prioritizing conservation within breeds must also consider bloodlines and pedigrees to acquire the needed genetic diversity. For breeds that are critically endangered, or that suffer inbreeding due to a small number of founders, individual animals may represent extremely rare diversity needed for breed survival. Similar situations exist within commercially important populations (e.g., Line 1 Herefords and Wye Plantation Angus) that have made important contributions to the Hereford and Angus breeds at varying points in time due to the selection strategies used within those populations.

Genomic technologies aid in quantification of diversity and allelic combinations for economically important production and adaptation traits. Genomic information would enable better prioritization of which samples to be cryopreserved. This is particularly true when one considers the number of ongoing genome-wide association studies that are identifying polymorphisms associated with performance traits such as meat quality (Sanchez et al. 2014) and milk production (Fang et al. 2017). Genomic information will also safeguard against the unintentional narrowing of genetic diversity among North American livestock populations that could occur as a result of intense genetic selection for one or a handful of traits. Nevertheless, an over-reliance on genomic technologies may be problematic because it assumes a perfect understanding of the traits needed for the future.

A range of genetic diversity studies have been completed for livestock and chicken breeds that have provided important insights regarding the genetic variability within species; however, many of these performed prior to 2010 used the now outdated technology of microsatellite markers. The development of single nucleotide polymorphism (SNP) marker panels and whole genome sequencing allows for a fuller understanding of genetic variability within and among breeds. For example, these genomic tools enable identification of breeds or subpopulations within breeds that are best suited for

specific environments and could identify productive roles for minor and rare breeds. Quantifying the existing genetic diversity will assist with informed decisions on what populations should receive priority.

Challenges

There are several important challenges regarding the characterization of animal populations for cryopreservation. Firstly, a common agreement of basic data to be gathered across breeds within a species must be identified, in a manner analogous to the Dairy Herd Improvement Association standardized production record keeping systems, which facilitated significant improvement in milk production.

Secondly, financial support (in all breeds except those with large numbers of animals) is needed to develop and better use breed-specific genetic improvement technologies, as well as to identify unique genetic traits or alleles that may aid in adaptation, disease resistance, survival, and other biologically important traits.

Thirdly, research must be supported to develop viable and affordable cryopreservation techniques for species that cannot be adequately prioritized until robust methods are in place. Heightened research efforts are needed to overcome this present limitation to cryopreservation efforts. Additionally, applied research should be directed at approaches that can be efficiently scaled up for commercial use including throughput, quality management, labeling and storage systems (Torres and Tiersch 2018).

Finally, enhanced partnerships among agricultural universities, federal government laboratories (e.g., the U.S. Animal Genomics and Improvement Laboratory), and breed associations are needed to facilitate more rapid characterization of North American food animal populations. For many of the major breeds (across species), long-standing and productive partnerships already exist; however, these relationships need to be encouraged and expanded where appropriate. Such partnerships generally do not exist for minor (rare) breeds, and a mechanism to build, strengthen and maintain these relationships is needed. This is especially important because rare and minor animal breeders typically lack infrastructure to collect production data and to perform the needed genetic analyses. One such mechanism could be a public-private partnership where researchers conduct controlled research studies (with appropriate federal funding) necessary to characterize the rare breeds.

Once sufficient characterization has been accomplished, the task of prioritization will become easier. Priority must be given to populations with (1) important unique genetic attributes (e.g., disease and parasite resistance, survivability, adaptability), (2) rare alleles and divergent genetics, and (3) outstanding genetic performance for production traits. Animals within populations probably will not embody all of these priorities. It is important to preserve breeds with unique genetic traits, even though they may be less productive under current agricultural production systems. The poultry industry provides a good example of this, as global breeding companies have developed strains that perform well in the tropics and for free-range production systems (Aviagen 2019) These same animals would be less productive in U.S. broiler houses, but without a broad array of foundational genetics, these new strains could not have been developed.

Characterization of breeds and individuals for conservation is a challenging task, and much remains to be done. Nevertheless, conservation should move forward with a focus on breeds at greatest risk of extinction. The framework provided by The Livestock Conservancy's Conservation Priority List is the most commonly used reference for on-farm conservation, whereas the goal of the NAGP is to preserve genetic materials from all livestock and poultry breeds. Resource constraints also dictate that some conservation will take place on an ad hoc basis as opportunities become available.

Germplasm Repositories: Existing Government and Private facilities

The USDA-ARS established the NAGP in 1999, and thereby began development of livestock, poultry and aquatic gene banking for species of agricultural importance. The role of the U.S. Government in maintaining collections of genetic diversity for agricultural purposes has been acknowledged by the global community. In 2007, 109 nations (including the United States) adopted the Interlaken Declaration and the Global Plan of Action for Animal Genetic Resources. The Global Plan of Action details key priorities needed to advance the conservation and utilization of genetic resources (FAO 2007; 2015). As of 2014, 128 countries have established gene banks for animal genetic resources. A collection summary for five of these countries appears in Table 1.

Since its initiation, the NAGP has developed into the world's largest and most comprehensive repository for farm animal genetic resources (Danchin-Burge, Hiemstra, and Blackburn 2011; Paiva, McManus, and Blackburn 2014). Currently, the collection contains more than 1,000,000 samples (98% is cryopreserved semen) from 54,790 animals that represent 169 breeds and 349 subpopulations (USDA 2019a). The breeds and special populations in the collection consist of commercially important breeds, rare breeds, and research populations. Of the collected breeds, 46% have reached the minimum collection goals in terms of number of animals and samples; while 36% of the breeds on the Livestock Con-

Table 1. Gene bank semen collection sizes for selected countries in 2014.

Country	Species	Breeds	Animals	Number doses
Brazil	12	25	416	71,867
Canada	9	31	3,077	261,083
France	9	181	4,337	352,068
Netherlands	7	59	5,691	309,088
United States	38	149	16,397	709,657

From: Paiva, McManus, and Blackburn (2014)

servancy's priority list (of rare breeds) have reached minimum germplasm collection goals, underscoring the fact that germplasm collection must be a continual process.

Prior to the establishment of the NAGP there was no organized U.S. governmental collection of cryopreserved germplasm. The Livestock Conservancy had a small collection consisting of cryopreserved semen and embryos from 68 animals representing 11 breeds, which was subsequently donated to the NAGP. Within the private sector, two types of cryopreserved germplasm collections exist: (1) major companies who sell semen and embryos to producers; and (2) individual breeders who develop their own collections. These private and public collections share a common weak point—collection longevity. At the corporate level, where storage and space costs are issues, samples are often destroyed when they are no longer deemed to have market value. For public collections universities often dispose of the collection once the faculty member using the collection retires.

Germplasm collection longevity is particularly an issue after changes in company ownership. Among private breeders and university faculty members, lifetime collections have been destroyed when administrators perceived the collections as too costly to maintain. Fortunately, some research-oriented collections have been donated to the NAGP upon retirement of the scientists, including important lines of Holstein and Hereford cattle. Additionally, more than 3,5000 samples from private and commercial collections have been donated to the NAGP. These private sector donations have formed the backbone of germplasm collection and are quite valuable because they: (1) provide a snapshot of breed performance at specific points in time; (2) are often from some of the most influential males among the breeds; and (3) increase the genetic variability of the collection. As a case in point, ABS Global donated to the NAGP more than 40 years' (1960–2005) of cryopreserved semen from dairy and beef bulls (more than 200,000 units of semen from more than 5,000 bulls).

The dual purposes of developing national animal genetic resource collections are to: (1) secure genetic variability from a wide range of genetic resources in perpetuity, and (2) provide genetic resources to regenerate populations or introduce genetic variability into breeds when needed. Moreover, germplasm can serve industry, research and policy needs (Blackburn et al. 2014). To date, samples from more than 6,000 animals have been released from the NAGP repository. These samples have been used to regenerate populations that were no longer in existence introduce genetic variability into rare breeds, perform reproductive studies, and conduct wide-range genomic studies (see Textbox 4). A recent example of a germplasm release followed the finding that current Holstein bulls used in artificial insemination descend from

Textbox 4. Importance of Assisted Reproductive Technology for Gene Bank Use

Assisted reproductive technologies (ART) for livestock and poultry have been developed with varying degrees of success. Factors contributing to this variability may include the cryopreservation processes, varying degrees of field expertise, and biological differences that exist among breeds or management practices. For example, weaning age differs substantially between indoor swine operations vs. outdoor rearing systems. Such difference could impair the effectiveness of breeding sows at the time of first estrus after weaning. The following details two experiences with ART and swine.

The Success: Purdue University developed and maintained a line of pigs homozygous for genes controlling meat quality (napole and halothane conditions). Thinking the research use of the line was complete, the decision was made to cryopreserve boar semen for storage at the NAGP and dispose of the line. Three years later, Purdue University needed to rebuild the population after receiving extramural funding. University researchers inseminated Yorkshire sows with the thawed semen from the NAGP collection and obtained a 100% pregnancy rate. This was documented in more than 10 publications, and a second population of the pigs was established at another university.

The Failure: Large Black pig breeders in the United States imported cryopreserved semen from the United Kingdom to broaden the breed's genetic base. Working across four herds with faculty from three different universities, the pregnancy rate after insemination was zero.

Lesson learned: The team involved in the Large Black project hypothesized that protocols developed for intensively managed production systems, and for certain breeds, did not transfer well into other production settings for pigs of other breeds. If this is indeed the case, rare breed producers may not be able to use typical ART for production purposes; as a result, this limits their ability to use a diverse set of genetic resources and lowers production levels. A research study is now exploring this hypothesis. The research community has an important role to play in understanding and modifying technologies for producers from all sectors of the livestock industry.

Large Black pig (left, ARS photo) and Purdue University napole and halothane piglets (right, photo courtesy of Terry Stewart).

Figure 3. Holstein bull calves, born from frozen semen stored at the NAGP repository, have unique Y chromosomes missing from the current population.

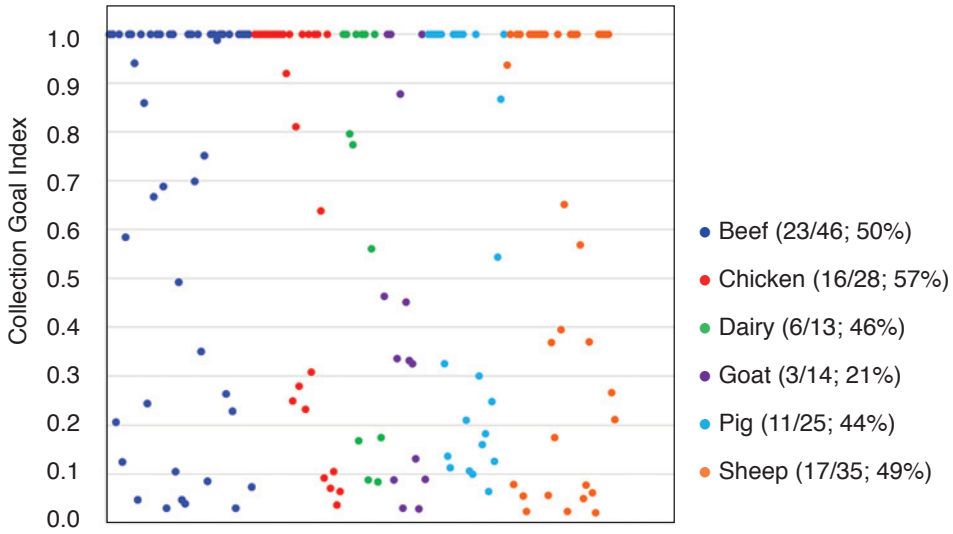


Figure 4. Status of breed collections (each dot represents a breed) by species based upon a collection goal based on the amount of germplasm and number of animals in the collection. An index value of 1.0 indicates that the minimum collection goal of a breed has been met. In parentheses are the number of breeds meeting minimum collection goals/ number of breeds per species group (Blackburn 2018).

only two paternal lines, suggesting only two Y chromosome variants are present in the population (Yue, Dechow, and Liu 2014). Fortunately, the NAGP collection contained samples from bulls with two additional paternal lines. Samples from these bulls were used to produce bull calves with the intention of incorporating lost Y chromosomal materials back into the Holstein breed (Figure 3). In addition, the progeny are being used in efforts to re-sequence the bovine Y chromosome and evaluate fertility aspects of bull semen at Pennsylvania State University and a commercial bull stud.

Challenges

Use of the NAGP's germplasm collection by various stakeholder groups could be expanded if research populations were curated with sufficient germplasm to reconstitute populations on demand for specific projects. The plant community has had a long history of using germplasm collections for such purposes. This tradition, however, does not exist within the livestock research community. Given the challenges within public institutions in maintaining livestock populations, the opportunity exists to promote greater use of the NAGP collection for this purpose. Three factors that can contribute to achieving this goal are: (1) raising awareness within the research community that the collection is available for use, (2) making funding sources aware of the potential use so they can encourage the research community to provide and use this material, and (3) funding the cost of reconstituting a population. The cost challenge is significant as animals must be raised to reproductive age and multiple generations may be necessary to reconstitute a specific population.

Enhancing the National Collection of Cryopreserved Genetic Resources

Prioritizing Germplasm Collection Efforts

Gene banks have been established across the globe to protect livestock and poultry industries from loss of genetic diversity that could subsequently hinder their capacity to adapt to new environmental or market pressures. The U.S. livestock and poultry germplasm collection at the NAGP encompasses 169 breeds and 336 research and industry-based populations. Although a broad array of genetic resources used by the livestock and poultry industries has been captured, the NAGP collection is not a complete representation of the available breeds and genetic lines. There is a strong and urgent need to enhance the NAGP collection, especially for poultry.

Having a breed represented in the NAGP collection may not be sufficient to reestablish the breed in the event of a catastrophic loss in the industry. For each breed, a "minimum collection goal" can be calculated as the minimum number of animals that would be needed to reconstitute the breed in the event of a total industry loss. Currently, only 47% of the breeds in the NAGP collection have met a minimum collection goal to reestablish the breed (Figure 4). This situation places many breeds at risk.

There are three main priorities for enhancement of the NAGP collection. One main priority is to add new breeds to the NAGP collection, even if the number of animals falls short of the minimum collection goal for the breed. A second main priority is to complete the existing breed collections, encompassing all breeds available. Given that 70 to 80% of the genetic variation of a species is

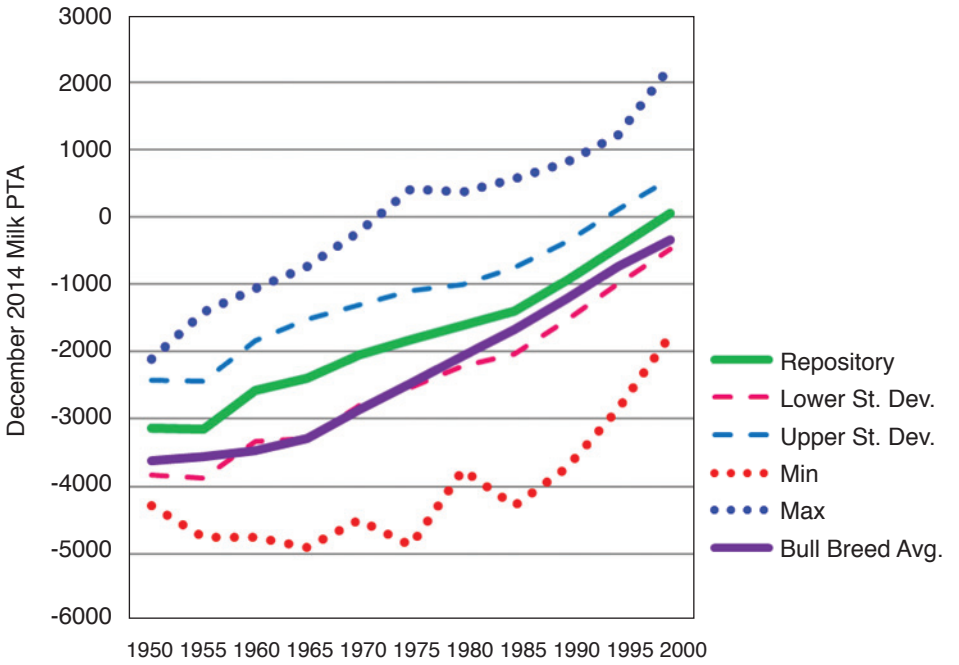


Figure 5. The Predicted Transmitting Ability (PTA) is a measure of the sire's genetic influence on the daughter's ability to produce milk over time. In this graph, the PTA of semen from living bulls used for artificial insemination (purple line) approximates the average PTA of semen from the NAGP repository (green line), demonstrating that the germplasm collection encompasses the variability of the in-situ population. The dashed lines represent one standard deviation above (blue) and below (red) the repository PTA mean; while the dotted lines represent the repository minimum (red dots) and maximum (blue dots) PTAs (from Blackburn, 2018).

attributed to individual animal differences (Blackburn et al. 2011; Lawson et al. 2007; Peter et al. 2007), selection of animals within a breed is critical to establish a functional germplasm repository. Guidelines for identifying animals for germplasm acquisition have been developed (FAO 2012) and are being used in the United States. Animals are selected based on genetic relationship to other animals within the breed and collection, phenotypic information, and computational or molecular genetic information when available (Blackburn 2012; FAO 2012; Smith 1984;). In situations involving rare breeds, collecting samples across geographic regions has and can be employed (Maiwashie and Blackburn 2004). Furthermore, by sampling across ecosystems a broader range of genetic diversity can be acquired, as shown by a fine structure genomic analysis of cattle (Blackburn et al. 2017) and pigs (Faria et al. 2019).

The third main priority is to refresh NAGP breed collections to ensure that changes in allelic frequencies (which may have resulted from intense genetic selection or widespread loss) have been captured. This need particularly applies to major breeds that are making genetic changes more quickly than rare or minor breeds. When germplasm collections encompass high-performing and low-performing animals, a substantial range of genetic variation has been captured (Figure 5). Moreover, it shows that a highly productive dairy bull collected in 1980 remained above breed average for approximately 15 years (until 1995; three to four generations). Similar trends have been computed for pig breeds and support the concept of refreshing the collections frequently. Furthermore, genomic approaches are accelerating genetic change by shortening generation length; therefore, refreshing the collection every three generations would be prudent.

Future Directions

Livestock breeds have never been naturally occurring populations; they have been constructed by livestock producers to meet the needs they or society deem important. In conserving breeds and subpopulations it is noteworthy to recall that, for centuries, breeds and subpopulations have been developed, recombined, and discarded as conditions dictated. Charles Darwin noted that livestock "breeders habitually speak of an animal's organization as something plastic which they model as they please" (Wood and Orel 2001). He also observed that the Berkshire pig of the 1780s was quite different from that of 1810 (Wilkinson et al. 2013), suggesting breed development has always been a dynamic process.

When assessing breeds and their conservation, two points are important to note: (1) breeds are a subpopulation of a species; and (2) as breeds evolve their genetic structure tends to become more similar due to genetic drift and selection. However, when viewed in total (i.e., a pooling of all breeds within a species) many species appear to have retained much genetic variation; for example, total genetic diversity of sheep and goat breeds near the center of domestication in Turkey and in the United States remain similar (Blackburn et al. 2011; Carvalho et al. 2015; do Prado Paim et al. 2019). It is important to keep in mind that breeds represent an array of allelic combinations in an easy-to-use form, and this may be their major value. Therefore, conservation at the breed level can be useful to incorporate large segments of a genome into another breed by combining two breeds. Within this context, the following points provide a basis for further enhancement of germplasm collections:

1. Continue germplasm collection for major and minor breeds of livestock, targeting current collection gaps;
2. Increase collection of breeds with contracting census numbers, including those not on the Livestock Conservancy's list of endangered breeds (e.g., Salers, Senepol, and Santa Gertrudis cattle; Columbia and Targhee sheep);
3. Maintain an expanded germplasm collection. Experience to date with U.S. plant and animal germplasm collections suggests the larger the

germplasm collection, the more useful it is (particularly in view of advances made with genomics). Germplasm collection use for the Holstein Y chromosome study and by the American Angus Association demonstrated the need for and advantages of large, comprehensive germplasm collections;

4. Incorporate new tools such as geographic information systems into collection activities to support acquisition of alleles that can confer an ability to perform under various environmental stressors (e.g., heat stress, heavy internal parasite load)

Targeted Cryopreservation Research for Species in Need

Major differences exist in the efficacy of cryopreservation procedures not only among the major domestic species (cattle, swine, sheep, goats, chickens, and turkeys) but also among the types of cells or tissue (e.g., sperm, oocytes, gonads, or embryos). Sperm are the most accessible gamete and remain the predominant cryopreserved cell type; however, extreme differences exist in the viability of frozen/thawed sperm not only among species but also within animals of the same species. Today the bovine is the only farm animal species for which cryopreservation of sperm is commercially routine. The success of semen cryopreservation in sheep and goats is lower than that of cattle, but better than swine. Among the major mammalian food animal species, the pig poses the greatest challenge for semen cryopreservation.

Potential targets for improving cryopreservation technologies for all mammalian livestock species include the prevention of (1) premature acrosome reaction, (2) DNA fragmentation, and (3) damage to the sperm plasma membrane (Holt and Penfold 2014). The biological basis for male-to-male differences in post-thaw survival of cryopreserved sperm also needs to be better understood to reduce the number of males that are "discarded" due to poor post-thaw sperm survival. Likewise, robust processes for successful artificial insemination of swine and small ruminants using cryopreserved semen in a variety of production settings are needed, incorporating enhanced methods of estrus synchronization, semen thawing, and semen dosage. For these techniques to be successfully applied to reconstitute breeds, such techniques must also work when semen quality or quantity is compromised. For breeds with less than 200 animals (small population size), research on cryopreservation of sperm obtained from the epididymis or seminiferous tubules of deceased animals would be beneficial. Avian semen can be cryopreserved (Silversides and Liu 2012), but fertility from cryopreserved avian semen is highly variable. The ability of avian semen to withstand cryopreservation is not only species-specific but also line specific (Long et al. 2014; Silversides and Liu 2012), and fertility from cryopreserved semen for many avian species or lines is either not sufficiently high for cryopreservation or has not been investigated.

Several reviews have been published regarding the cryopreservation of oocytes from cattle, sheep, and pigs (Hwang and Hochi, 2014; Mullen and Fahy 2012; Somfai, Kikuchi, and Nagai 2012; Zhou and Li 2013). In general, the developmental competence of cryopreserved oocytes lags far behind that observed with either cryopreserved semen or embryos. Oocytes are different than sperm or preimplantation embryos with respect to cryopreservation: the oocyte is a larger cell, its membrane does not permit easy movement of water in and out of the cell, it possesses a comparatively high lipid content, and its internal skeletal structure is more susceptible to chilling damage (Arav 2014). As is the case with the male, it is feasible to collect gametes from recently deceased females. The ovaries contain tens of thousands of immature oocytes at the time of puberty and, through the use of in vitro oocyte maturation, in vitro fertilization, and in vitro culture technologies, it is possible to produce live offspring from a recently deceased female. This approach could prove highly valuable when females of a rare breed die unexpectedly.

Gonadal transplantation has been demonstrated for several mammalian species, including mice (Sztin et al. 1998), rats (Dorsch et al. 2004), sheep (Gosden et al. 1994), rabbits (Almodin et al. 2004), and humans (Donnez et al. 2004). Presumably these techniques could be developed for cryopreservation of other agriculturally important mammalian species, but research is needed to confirm that. For birds, the feasibility of transplanting avian gonads has been demonstrated for chickens, Japanese quail, and ducks (Silversides and Liu 2012); however, the methods have not been replicated successfully by other groups (Liptoi et al. 2013) and have not been developed for turkeys.

Cryopreservation of preimplantation embryos is one strategy for conserving mammalian germplasm, and it is considered advantageous because each embryo represents a complete individual that needs only a surrogate uterus to be reconstituted; however, there is a greater cost associated with the collection of embryos compared with semen (FAO 2012; Gandini et al. 2007). Cryopreserved embryos are used extensively by the cattle industries and, although similar technologies are available for other species, they are not yet optimized for widespread commercial use.

Priority Research Areas

- **Spermatozoa**
  - Cattle - Despite commercial success, there is still tremendous opportunity to improve cryopreservation procedures because approximately 50% of frozen bovine sperm fail to survive the freeze/thaw cycle. This is an important conservation issue when the number of living males of a particular breed or species is limited.
  - Swine – Boar sperm are extremely sensitive to temperatures below 15°C. One publication has shown promising results with thawed boar semen (73% pregnancy rate and an average of 10.8 fetal piglets per sow; Spencer et al. 2010); however, additional research is needed to confirm these findings. Use of thawed boar sperm is further complicated by the relative inability to effectively synchronize estrus in sows to allow precise timing of insemination relative to multiple ovulations. Research to develop effective timed artificial insemination protocols is needed.
  - Small ruminants - The sperm plasma membrane of the ram is relatively

fragile, and ovine sperm are sensitive to cold shock. Use of thawed ram semen is hindered due to the anatomical nature of the ewe's cervix which requires intrauterine deposition of thawed semen to attain acceptable pregnancy rates. Buck semen contains an enzyme known as phospholipase A that prevents use of egg-yolk based semen extenders unless the seminal plasma is removed; however, removal of seminal plasma thus far has proven detrimental to goat sperm. Research is needed to develop methodologies to overcome ram sperm plasma membrane fragility and buck semen extenders that do not require removal of seminal plasm before freezing.

  - Poultry - The morphology of avian sperm is very different from that of mammalian sperm and requires protocols specific to birds. To date, the ideal conditions for cryopreservation are under development with some recent advances (Thélie et al. 2019). Extremely rapid cooling (vitrification) of sperm does not require the same degree of pre-freezing cellular dehydration and may be an effective method of preserving the ability of sperm to fertilize avian eggs (Liu et al. 2013). Research is needed to identify genetic factors causing line and individual differences in freezing semen so that these limitations may be overcome.

- **Oocytes**. The technology for cryopreservation of oocytes has substantial opportunity for improvement. To preserve fertility of oocytes after cryopreservation, methods must be developed that will maintain structure and subsequent function of the internal cellular structures (e.g., cortical granules, microtubules, and mitochondria) during freezing and thawing. There is also a need to investigate procedures to modify the pre-freeze cholesterol and lipid content of oocytes and their membranes to achieve higher survival rates.
- **Gonadal material**. Gonadal transplantation and immunosuppression procedures could be expected to be similar for a variety of avian species, but anatomical differences mean that optimization of the techniques is species-specific. The production of offspring from transplanted ovaries is straightforward because the normal reproductive apparatus is still being used to produce an egg. However, the testicles cannot be replaced in their normal position, and the exudate from the transplant that contains sperm must be harvested and used for insemination. Techniques and timing of insemination have not been optimized for chickens or quail and have not been investigated for other avian species, but they are expected to be dependent on the anatomical, physiological, and perhaps even behavioral reproductive specificities of each species.
- **Embryos and embryonic cells**. Cryopreserved in vivo-derived embryos from ruminant livestock species (cattle, sheep, and goats) typically yield 60 to 75% pregnancy rates; however, in vitro produced (IVP) embryos from those same species (cryopreserved with the same method) typically yield a pregnancy rate of only 25 to 50% (Youngs 2011). With the exponential growth of IVP technology in cattle, these differences must be resolved. For porcine embryos, the greatest research needs include development of more field-practical methods for embryo cryopreservation that do not require handling of embryos individually during vitrification and more robust/embryo tolerant methods of warming. Although avian eggs and embryos cannot be cryopreserved, the potential cryopreservation of primordial germ cells has seen some success (Taylor et al. 2017) and warrants further investigation.
- **Somatic cells**. Skin cells can be easily harvested, making them readily available for cryopreservation. Technologies for reproduction of animals from somatic cells are now well established for cattle and horses, and to a lesser extent for swine. Development of somatic cell nuclear transfer techniques for all agricultural mammals, and for cell types other than fibroblasts, could greatly facilitate conservation of genetic resources.

Germplasm Repository Infrastructure: Information System Development

In the broadest sense, collections of animal genetic resources are an accumulation of information. Examples of this information on a specific animal can include what the animal looked like (via photographs), where the animal was raised, under what conditions the animal was raised, how it performed for various traits of interest, what was its genetic composition and pedigree, what type and how many samples are in the repository, and how the samples were cryopreserved. Equally important is how accessible this broader information is to an array of users from research laboratories and industry.

Of the three components of gene banking—genetic diversity, cryobiology, and information systems—information systems are critically important and should not be overlooked. For large and substantial collections like the NAGP collection, information systems are indispensable for users to view the collection and make decisions about which samples will serve their research or reconstitution purposes.

Since 2005, the Animal-Genetic Resources Information Network (Animal-GRIN) has been the primary vehicle for storing information about animals in the NAGP's collection. A second version of the database, launched in 2016, was a joint effort between Agrifoods Canada, the Brazilian research organization EMBRAPA, and the NAGP. The unique feature of this updated version is that it allows all three countries to view information about genetic resources in each country.

Animal-GRIN (https://nrrc.ars.usda.gov/A-GRIN/database_collaboration_page_dev) is publicly accessible, dynamic, and searchable (Irwin, Wessel, and Blackburn 2012). Descriptors in the database encompass animal identification, germplasm or tissue type, phenotypes, genetic information, pedigree information, environment, and management. Tools have been developed for: donating and requesting germplasm or genotypes; comparing genetic relationships between animals of the same breed; collection completeness for a breed or line; pedigree

trees and viewing changes in the collection.

In addition to the features mentioned above, the NAGP has developed a genomics component of Animal-GRIN that facilitates storage of genomic information pertaining to farm animal genetic resources. Currently 2,083 animals from 41 breeds of cattle, sheep, goats, and pigs have single-nucleotide polymorphism genotypes in the genomic portion of the database. Depending upon species, the size of test panels per animal range from 50,000 to 770,000 SNPs. As the animals represented in the repository are genotyped, the information can be stored and be made publicly accessible. The construction of the genomics element is an opportunity to leverage previous investments in genotyping for future research activities and industry utilization of genomic phenotypic and environmental information. It also represents a unique structure where animal samples, phenotypes, and genotypes can be obtained through a single information platform. However, continuing to add information for the animals in the collection will require further investments in genotyping.

Nevertheless, there are opportunities to incorporate functionality for future needs:

1. An increased understanding and quantification of the interactions between genetics, environment and management are highly desired by the agricultural community. Adding geographic information systems capabilities to Animal-GRIN will become an important tool in this research (Blackburn et al. 2017).
2. Photographic images of animals with samples in the database are being acquired. Adding the potential capability of phenotyping animals (e.g., body weight) using these photographs, as that technology advances, would significantly increase the information about animals in the collection, especially for breeds where phenotypes are not routinely measured.
3. Accessibility would be enhanced by development of apps for mobile platforms, bringing the Animal-GRIN information into the field.
4. The USDA-supported genotyping results and, where feasible tissue samples should be added to the genomics database where they can be made available for the community after the initial project has been completed to capitalize on investments made in genotyping and to improve long term archiving of data.

The Insurance Policy: Long-term Operational Continuity of Repositories

Genetic resources are inextricably linked to food security and the livestock sector's economic vitality. For these reasons, operation of a national gene bank and genetic conservation program for livestock and poultry is a federal responsibility as authorized by Congress. It is recognized that such activities are for the public good given their contribution to food security, economic growth, and the opportunity to engage and address the concerns of livestock producers (especially small-scale farmers). Due to the long-term nature and the food security aspects of gene banking, it is best addressed by the federal government (e.g., USDA-ARS).

The NAGP is entering its 20th year thanks to the long-term support provided by the ARS. It mirrors, but lags behind, the more than 50 years that the ARS has operated its plant germplasm system. Initial costs for collecting and holding cryopreserved samples may be high, but costs are minimal when amortized over 50 years versus maintaining live populations (FAO 2012; Silversides, Blackburn, and Purdy. 2012; Smith 1984). Relatively low and static funding levels for the NAGP program have created a challenge for sustained collection. Funds are needed to allow collection of samples in the field or for owners to transport their animals to the NAGP laboratory for collection. Resources are also needed for validation of viability of samples already collected and the development of workable cryopreservation methodologies.

Strengthening the System

There are opportunities to strengthen and expand the NAGP's capacity and support long-term continuity of the program. These include the following:

1. Research to improve ART is sorely needed because reconstitution of a breed requires a complete package of tools (e.g., synchronization of estrus, sperm cryopreservation, artificial insemination techniques). Further research on ART and cryopreservation methodologies are particularly needed for species where the success of reconstitution from cryopreserved materials remains low. Each species needs techniques sufficiently robust to be applied to diverse future needs, including the possibility that cryopreserved materials may be needed in populations on farms with little or no access to today's most commonly used technologies and infrastructure.
2. While the NAGP collection is the most comprehensive collection of animal germplasm in the world, there are still gaps to be filled for many breeds. In addition, there are opportunities to expand existing breed collections to increase their use in genomic studies. Importantly, cryopreserved samples need to have a demonstrated capacity for some level of fertility, preferably live offspring, otherwise, the samples have limited value.
3. Storage of all types of information (phenotypes, genotypes, production systems, environments) in Animal-GRIN Version 2 is an important mechanism for increasing the use and value of the collection which in turn supports operational continuity. Further development of database capacities to accommodate geographic information systems, and addition of tools to facilitate accessibility and data analysis are critical areas of enhancement.

Conclusions

Humanity depends on a tiny fraction of global animal species that have been domesticated for production of food. It is readily apparent that we cannot continue to rely solely on a few breeds to provide the genetic diversity that is integral to sustaining food production for future generations, as we cannot predict what traits will become important in the face of weather extremes, niche markets, and consumer-driven demands (Rexroad et al. 2019). Failure to conserve animal genetic resources may cripple our capacity to

identify traits of value for the future. Disappearance of breeds is likely to induce losses of entire genetic combinations, and it is these combinations that stand to serve as ready-made matches for breeders to use. By losing breeds we make finding potential solutions to future production demands much more difficult, and recent history indicates that predicting future demand is problematic. Conserving breeds saves these options and keeping them in the agricultural landscape is a reminder that these options exist. The most effective conservation of these resources ideally involves living animals as well as cryopreserved reservoirs of their genetic material. On-farm conservation and cryopreservation of animal germplasm are complementary strategies for conservation of genetic diversity in livestock and poultry. Each strategy mitigates a different array of specific risks to agrobiodiversity, and one strategy should not proceed at the expense of the other; concurrent efforts are needed. An increased scope of intensified sampling, cataloging and evaluation of the existing gene pools in livestock and poultry, concomitant with more resources to fully develop cryopreservation methodologies and discover novel, more efficient methodologies, are paramount to meet the expected increases in human population in the face of unknown—but certain—global challenges.

Task Force Recommendations

1. Commit resources (capital, personnel, facility, information technology) necessary to characterize the genetic diversity of existing livestock and poultry populations in the United States, including both phenotypic and genotypic data, and enhance a cloud-based platform to house the data that is publicly accessible and interfaces with the USDA-NAGP, Animal-Genetic Resources Information Network, and the Livestock Conservancy Program Priority listings. Expand the information network to accommodate environmental descriptors in a geographic information system format. This effort will require large-scale coordination of breed associations, universities, and small/local farming operations, and should be led by USDA-ARS in partnership with State Departments of Agriculture, land-grant universities, the Livestock Conservancy and the USDA-National Agricultural Statistics Service.
2. Engage private sector philanthropic awareness and expand funding opportunities across the federal government for research to develop the most effective cryopreservation strategies for domesticated livestock and poultry species, including species-specific protocols for routine use with a high success rate for recovering the desired population. Improvement of cryopreservation efficacy for species with low success should receive particular emphasis. Funding priorities should be directed toward populations with contracting census numbers, extreme phenotypes, proven research or industrial benefit and/or potential commercial value. Interagency working groups (USDA-NIFA; USDA-ARS; National Science Foundation; National Institutes of Health; US Fish and Wildlife Service) should lead this effort and partner with the private sector.
3. Support the conservation of in situ populations, particularly for those species such as poultry in which cryopreservation methods are suboptimal, through funding opportunities related to maintaining important genetic stocks for research, small farms, urban development and/or sustainable agricultural practices. Specific funds, not tied to a particular research project, should be available for in situ conservation. If unique livestock or poultry lines housed by universities are to be discontinued, such lines designated for elimination must be offered to other institutions or individuals. Furthermore, guidelines should be developed that require appropriate genetic material, preferably germplasm, gonads or embryos, to be archived at the USDA-NAGP prior to de-population of the live animals. Acceptance of these guidelines could be made a requirement of receiving federal or state funds.
4. Evaluate cryopreserved germplasm, whenever possible, for the potential to generate offspring (not just fertility) and use data to inform the minimum collection score to provide the best protection of genetic resources for future use. There are no formal mechanisms in place for this type of testing, yet it is an important component of the success of ex situ conservation.
5. Expand investment in permanent staffing and programmatic support of the NAGP to increase the procurement, management and utilization of genetic resources. More staff are needed with technical expertise in the areas of cryopreservation, genetics, assisted reproductive technology and information technology; additional funding also should be provided to the NAGP and universities to support census and outreach activities, as well as evaluation of cryopreserved materials. These measures will help to ensure the long-term operation of the NAGP and its critical role in protecting genetic resources for the future.

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ISSN 1070-0021

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Citation: Council for Agricultural Science and Technology (CAST). 2019. *Protecting Food Animal Gene Pools for Future Generations—A paper in the series on The Need for Agricultural Innovation to Sustainably Feed the World by 2050*. Issue Paper 65. CAST, Ames, Iowa.

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