

U. S. Swine Genetic Resources and the National Animal Germplasm Program

H. Blackburn¹, C. Welsh¹ and T. Stewart²

¹USDA-ARS-NCGRP-National Animal Germplasm Program, Ft. Collins, CO.

²Purdue University, West Lafayette, IN.

Introduction

Swine genetic resources underpin swine production and profitability. Swine genetic diversity is an essential element in breeding pigs to meet current and future consumer demands, which has been shown to change dramatically over time. Therefore, access and utilization of genetic variability are primary drivers in sustaining the swine breeding industry.

Currently, in the U.S. the majority of swine breeds come from Europe, with some Chinese breeds having been imported directly to the U.S. or via European breed formation. U.S. breeders have effectively utilized genetic diversity to meet consumer and production system demands. However, as selection emphasis shifts from production characteristics to issues involving animal health and welfare there may be a need to access additional genetic variability for introgression into current populations or to serve as biological models, as the Meishan was used for reproductive characteristics. The recent finding that pigs have multiple origins of domestication underscores the existence of potentially significant genetic variation for industry to tap (Larson et al., 2005). Therefore such variation is an economically important resource to utilize, manage and conserve for potential use.

In addition to preparing for future changes in consumer demand, there is a need to protect existing genetic diversity from epidemic diseases. For example, the 2001 outbreak of foot and mouth disease (FMD) in the United Kingdom (UK) cost 3.1 billion pounds. In that outbreak 6 million animals were culled causing a 20 to 40 percent decrease in breeding females for some breeds (Roper personal communication). The FMD event in the UK underscores how such disease outbreaks can indiscriminately spread and result in significant private and public sector financial losses. In addition to disease challenges, the breeding industry's natural tendency to select individuals of high economic merit with high levels of accuracy reduces genetic diversity and therefore presents another challenge for conservation efforts.

National Animal Germplasm Program

The U.S. National Animal Germplasm Program (NAGP) was initiated to provide genetic security for domestic livestock populations. It accomplishes this mandate by collecting and storing diverse genetic resources and by assessing the genetic variability of breed populations for all livestock sectors. The NAGP takes such actions with the assistance of its Swine Genetic Utilization and Preservation Committee (SGUP) which is comprised of federal, university and industry scientists, representatives from breed associations and companies servicing the swine industry are also members of the committee. One of the first decisions this committee took was that the primary conservation activity would be to collect and store cryopreserved semen from all

swine breeds and that the collection would be refreshed at regular intervals to insure that industry relevant boars would always be available in the collection.

Collection Goals

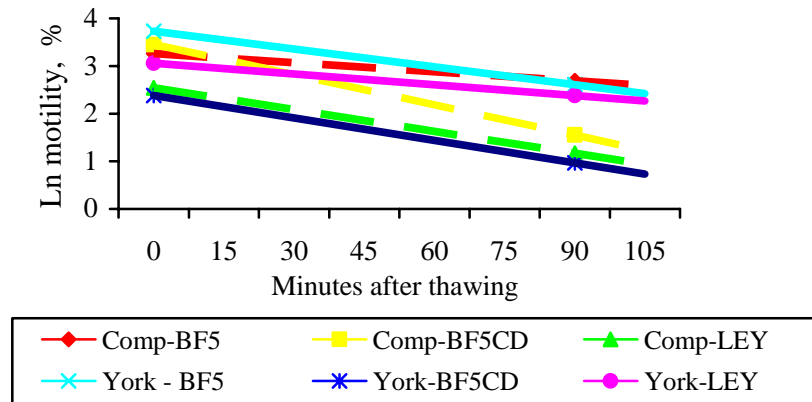
Prior to the initiation of NAGP there were no public reserves of swine germplasm which could serve as a national reserve. Therefore, the NAGP-SGUP set collection goals and devised a collection strategy. A collection goal for the Core collection was set at 18,750 0.5ml straws of semen per breed. The Core collection will enable 150% regeneration of a breed. The SGUP also concluded that the Core collection should be comprised of 100 boars for major breeds. For minor and rare breeds it may not be possible to acquire samples from 100 unrelated boars. For such breeds the criteria on boar number may be relaxed and larger quantities of semen per boar cryopreserved. For research lines or industry populations this minimum Core collection was reduced to 5,000 0.5 ml straws with an unspecified number of boars.

Acquisition of Germplasm

There are two primary aspects of acquiring germplasm for the collection: identification of the boar to collect, and the logistics of collecting the boar and shipping and freezing the semen. To acquire semen from designated boars a set of logistical issues need to be addressed. First, the boar has to be located and semen collected. To date the largest proportion of boars collected were located in commercial boar studs. Upon collection semen is shipped for next day delivery in a shipping container that maintains the sample temperature at 15° C. Upon arrival the sample is processed and cryopreserved. Due to the steps involved with collecting boars and shipping their semen to NAGP, there is a need for significant lead time (preferably two weeks) to arrange for collecting, shipping and freezing.

Upon receipt of a shipment at NAGP all samples are evaluated for motility using computer assisted sperm analysis (CASA). If samples have exceptionally low motility scores they are not frozen. Assuming the samples are viable they are cryopreserved. A number of protocols have been developed to cryopreserve boar semen. NAGP evaluated several of these protocols with Yorkshire and composite boars. The extenders tested included: LEY (Almid and Johnson, 1988), BF5 (Pursel and Johnson, 1975), and BF5CD (Zeng and Terada, 2000) which was the BF5 extender with the addition of cholesterol loaded cyclodextrin to potentially improve the condition of the sperm cell membrane. Post-thaw analysis with CASA was performed immediately after thawing and at 15 minute intervals for 105 minutes post-thaw. Figure 1 depicts the change in motility over the times tested and demonstrates how the BF5 extender provided better post-thaw motility for both Yorkshire and composite boars (Blackburn, 2003).

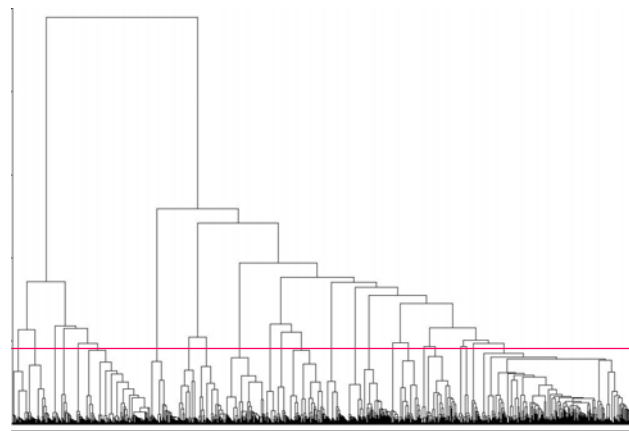
Figure 1. Change in the natural log of percent post-thaw motility over time for Yorkshire and composite boar semen frozen with three different extenders.



Boar selection

Initially boar selection was based upon availability in a boar stud. However, once breed collection was started, boar selection shifted to using association pedigrees to provide a cost effective way of assessing genetic diversity. The procedure developed involves utilizing pedigree files to compute the genetic relationships between available boars and those boars already in the collection. The genetic relationships are then used in the Ward's minimum variance method of cluster analysis found in SAS (Ouendeba et al., 1995). A target of approximately 20 clusters per breed has been set so that each cluster would contain approximately five boars. As part of the SAS Ward cluster analysis a pseudo-t test is used to find a break in the phylogeny at or around twenty clusters. Figure 2 provides the cluster analysis for the Yorkshire breed and Figure 3 gives the number of boars from each cluster that have been collected and placed in the repository.

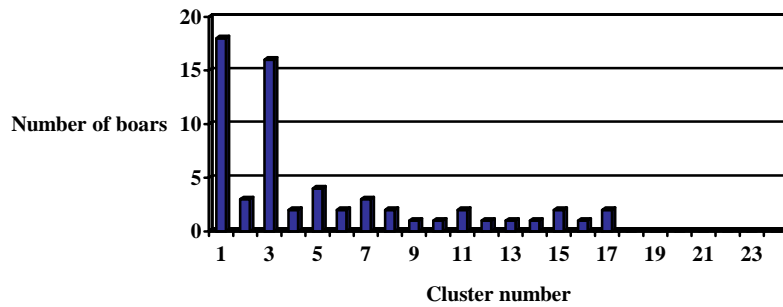
Figure 2. Cluster of Yorkshire boars, horizontal line demarks the pseudo-t test break in the clusters.



Several issues are illustrated in Figure 3. First, it should be noted that there are a large number of repository boars are in clusters 1 and 3. This accumulation was due to boars being acquired prior

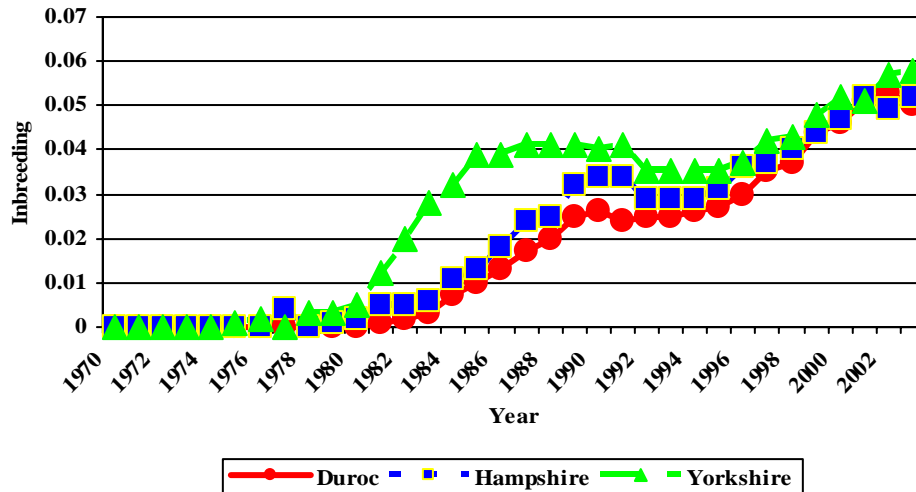
to initiating the clustering approach and demonstrates the difficulty in acquiring boars from commercial studs without any pedigree information. Also cluster 1 and 3 are relatively large clusters (700 and 123 hd, respectively) with low average relationships (0.09 and 0.04, respectively vs an average within cluster relationship of 0.24) among the animals in the cluster that underscores a need for greater representation in the collection. Second, to acquire samples from small clusters (e.g., clusters 20 through 24 have less than 45 boars) may be difficult due to these boars not being present in commercial studs, and therefore requiring on farm collections which introduces additional logistical hurdles.

Figure 3. The number of boars in the population and in the repository by cluster.



An ancillary product of the cluster analysis is the ability to calculate inbreeding trends for the breed and from that the effective population size which serve as a measure of genetic diversity. Figure 4 illustrates how inbreeding has increased for Yorkshire, Hampshire and Duroc breeds, using 1970 as the base year.

Figure 4. Inbreeding trends for Yorkshire, Hampshire and Duroc breeds.



Interestingly, the 2003 inbreeding levels for all three breeds are similar to Jersey and Holstein cattle (Weigel, 2001) where there has been a considerable discussion about the loss of genetic

diversity over the past five years. Using the data from 1994 to 2003, the rate of change in inbreeding level for these three pig breeds is slower than what has been observed in dairy cattle. Using this same period of time effective population sizes were computed and found to be 71, 74, and 61 for the Yorkshire, Hampshire, and Duroc, respectively. While these effective population sizes are larger than those reported for Holstein and Jersey cattle (39 and 30, respectively) they are still under the 100 hd level which some (Meuwssen, 1999) have identified as critical levels for maintaining genetic diversity.

Swine Collection

Since 2000 boar semen has been shipped to the NAGP where it has been frozen and stored. A wide range of donors have contributed semen from their boars and specialists from industry and universities have assisted in collecting and shipping these samples. Core collection requirements have been met for several industry lines and the Meishan breed. Among the major breeds, Yorkshires are closest in meeting Core Collection requirements in terms of the quantity of germplasm in the repository and the targeted number of boars. However as Figure 3 indicates there are a number of clusters that are not represented or are underrepresented at this time. Collection status for the other major breeds is approximately at 25%, with the exception of Landrace, 13.7% (Table 1). For minor or rare breeds the collection status is much smaller, in part due to these boars not being found in commercial boar studs. However, of the ten rare breeds identified by the U.S. country report (Blackburn et al., 2003) five have representation in the collection.

Clearly significant work remains in developing cryopreserved collects so that security is offered to all breeds and industry populations. Acquisition of samples through commercial studs will continue. For rare breeds accessibility will continue to be a major issue. To start addressing this in August 2005 a workshop targeting the collection of rare breeds was sponsored by the University of Missouri, Purdue University, American Livestock Breeds Conservancy, and NAGP. The goal of the workshop was to develop sufficient expertise to promote on farm boar collection of rare breeds. Approximately 30 participants attended and as a result of the workshop several owners have attempted to collect and ship boar collections to the NAGP.

Accessing the collection

The collection of germplasm by NAGP is not meant to be a static collection that is not utilized. In addition to providing long term protection via the Core collection, additional quantities of germplasm will be acquired and available for use. Figure 5 presents the process of requesting material from NAGP. To access this material a requestor must first submit a request that is reviewed by NAGP-SGUP and that group is to provide NAGP with a recommendation about the potential release. A primary factor NAGP-SGUP has to take into consideration is if the requested genetics can be obtained from industry sources, if there are such resources already available in the industry the requestor will be advised to acquire the material directly from those sources. To date no swine germplasm has been released from the repository, however semen from 370 bulls has been released for DNA analysis.

Conclusions

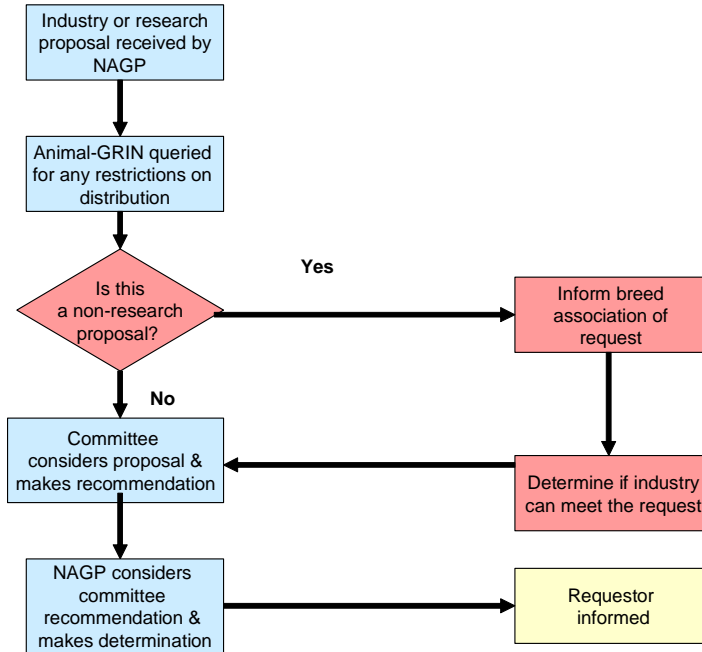
Since the initiation of the NAGP, and particularly the SGUP, several milestones have been achieved. These include: a multi-institutional framework has been developed via SGUP to assess swine genetic diversity and collection strategies; mechanisms for acquiring germplasm, in terms of identifying animals and acquisition of semen samples have been established and are in practice; a baseline understanding of genetic diversity has been developed as part of FAO's report on the State of the World's Animal Genetic Resources; germplasm has been acquired, cryopreserved, and documented for most breeds of swine in the US; and a mechanism for releasing material has been put in place. Clearly over the next five years: breed and line collections will have to be further developed; a better understanding of swine genetic diversity (and how to use that diversity) will have to be addressed; and there still is a need for more effective cryopreservation protocols for semen and embryos.

Table 1. Summary of the swine germplasm collection.

| <i>Group</i> | <i>Number of breeds or lines</i> | <i>Boars, hd</i> | <i>Number of 0.5 ml straws (% of Core Collection goal)</i> |
|----------------------|----------------------------------|------------------|------------------------------------------------------------|
| Major Breeds | 5 | 144 | 37,491 |
| Berkshire | | 18 | 4,595 (24.5) |
| Duroc | | 22 | 5,505 (29.4) |
| Hampshire | | 24 | 4,231 (22.6) |
| Landrace | | 10 | 2,570 (13.7) |
| Yorkshire | | 70 | 20,590 (109.8) |
| Minor Breeds | 3 | 11 | 1,893 |
| Chester White | | 5 | 953 (5.1) |
| Poland China | | 1 | 105 (0.5) |
| Spotted Poland China | | 5 | 835 (4.4) |
| Industrial lines | 12 | 309 | 25,251 |
| Rare Breeds | 5 | 34 | 9,287 |
| Hereford | | 22 | 4,871 (26.0) |
| Mulefoot | | 1 | 722 (3.8) |
| Red Wattle | | 1 | 997 (5.3) |
| Saddleback | | 1 | 504 (2.7) |
| Tamworth | | 9 | 2,193 (11.7) |
| Chinese | 3 | 83 | 7,144 |
| Fengjing | | 22 | 545 (10.9*) |
| Meishan | | 41 | 6,075 (121.5*) |
| Ming | | 20 | 524 (10.5*) |

* Core requirement for research populations is 5,000 0.5 ml straws instead of the 18,750 for breeds.

Figure 5. Process for reviewing industry or research requests for animal germplasm and/or tissue.



References

Almid, T. and L. A. Johnson. 1988. Effect of glycerol concentration, equilibration time and temperature of glycerol addition on post-thaw viability of boar spermatozoa frozen in straws. *J. Anim. Sci.* 66:2899-2905.

Blackburn, H. D., 2003. Evaluation of post-thaw boar semen characteristics of two genotypes using three extenders. *J. Anim. Sci.* 81(Suppl. 1):241-242.

Blackburn, H. D., T. S. Stewart, D. Bixby, P. Siegel, and E. Bradford. 2003. United States of America Country Report for FAO's State of the World's Animal Genetic Resources. Available: <http://dad.fao.org/en/Home.htm>. Accessed 11-22-2005.

Larson, G., K. Dobney, U. Albarella, M. Fang, E. Matisoo-Smith, J. Robins, S. Lowden, H. Finlayson, T. Brand, E. Willerslev, P. Rowley-Conwy, L. Andersson, A. Cooper. 2005. Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* 307:1618-1621.

Meuwissen, T. H. E. 1999. Operation of conservation schemes. Pages 91-112 in *Genebanks and the Conservation of Farm Animal Genetic Resources*. J. K. Oldenbroek, ed. DLO Inst. For Anim. Sci. and Health, Lelystad, The Netherlands.

Ouendeba, B., G. Ejeta, W. Hanna, and A. Kumar. 1995. Diversity among African pearl millet landrace populations. *Crop Science*, 35:919-924.

Pursel, V. G. and L. A. Johnson. 1975. Freezing of boar spermatozoa. Fertilizing capacity with concentrated semen and a new thawing procedure. *J. Anim. Sci.* 40:99-102.

Weigel, K. A. 2001. Controlling inbreeding in modern breeding programs. *J. Dairy Sci.* 84(E. Suppl.):E177-E184.

Zeng, W. X. and T. Terada. 2000. Freezability of boar spermatozoa is improved by exposure to 2-hydroxypropyl-beta-cyclodextrin. *Reprod. Fertil. Dev.* 12:223-228.