Relationships among and variation within rare breeds of swine

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ABSTRACT: Extinction of rare breeds of livestock threatens to reduce the total genetic variation available for selection in the face of the changing environment and new diseases. Swine breeds facing extinction typically share characteristics such as small size, slow growth rate, and high fat percentage, which limit them from contributing to commercial production. Compounding the risk of loss of variation is the lack of pedigree information for many rare breeds due to inadequate herd books, which increases the chance that producers are breeding closely related individuals. By making genetic data available, producers can make more educated breeding decisions to preserve genetic diversity in future generations, and conservation organizations can prioritize investments in breed preservation. The objective of this study was to characterize genetic variation within and among breeds of swine and prioritize heritage breeds for preservation. Genotypes from the Illumina PorcineSNP60 BeadChip (GeneSeek, Lincoln, NE) were obtained for Guinea, Ossabaw Island, Red Wattle, American Saddleback, Mulefoot, British Saddleback, Duroc, Landrace, Large White, Pietrain, and Tamworth pigs. A whole-genome analysis toolset was used to construct a genomic relationship matrix and to calculate inbreeding coefficients for the animals within each breed. Relatedness and average inbreeding coefficient differed among breeds, and pigs from rare breeds were generally more closely related and more inbred (P < 0.05). A multidimensional scaling diagram was constructed based on the SNP genotypes. Animals within breeds clustered tightly together except for 2 Guinea pigs. Tamworth, Duroc, and Mulefoot tended to not cluster with the other 7 breeds.

Key words: genetic diversity, genetic relationship, rare breed, SNP, swine

INTRODUCTION

Each year, many breeds face the threat of extinction as populations dwindle to nonviable numbers. According to the Food and Agriculture Organization (FAO), approximately 20% of documented livestock breeds are at risk of extinction (AGRG, 2006), with 300 breeds having become extinct in the past 15 yr (Cardellino, 2004). Our focus on swine arises from their greater threat of extinction compared to that of other endangered livestock breeds. Raising pigs is more management intensive than other livestock production systems; therefore, it is uncommon for farmers to keep pigs as a hobby. This puts rare breeds of pigs, compared with other livestock, at greater risk of being lost. In recent years, heritage breed producers have become increasingly interested in the genetics of their animals and ways to preserve diversity for future generations.

In contrast to commercial pigs, many heritage breeds of pigs are able to forage for themselves, thrive on limited resources, and care for their own young (Dohner, 2001). These breeds may contain genetic material for traits such as disease resistance or forage utilization, which could be of future use. To many, preserving these small populations seems inconsequential, but the extinction of even 1 breed means a certain amount of genomic information is lost forever.

Knowledge of relationships among breeds will better enable conservation organizations to prioritize...
breeds for conservation. Endangered breeds included in this study were the Guinea, Ossabaw Island, Mulefoot, Red Wattle, and Saddleback. The objectives of this study were to determine the relationships among rare breeds of pigs, their relationships with common breeds, and estimate genetic variation within rare breeds through the use of SNP data. This information can be used to identify heritage breeds most divergent from common breeds of swine and prioritize those for preservation.

**MATERIALS AND METHODS**

Seven heritage breeds and 4 commercial breeds were utilized in this study. Hair samples were collected from 10 Guinea, 10 Ossabaw Island, 5 Red Wattle, 4 Mulefoot, and 2 American Saddleback hogs and genotyped with the Illumina PorcineSNP60 BeadChip (GeneSeek, Lincoln, NE; Ramos et al., 2009). Except for the Mulefoot and American Saddleback hogs that had samples collected from a single farm for each, samples for each breed were collected from 2 or more farms that did not share close ties located in the central United States. Publicly available genotypes (20 individuals per breed) were obtained for British Saddleback, Duroc, Landrace, Large White, Pietrain, and Tamworth pigs (Goedbloed et al., 2012, 2013).

Files of SNP data were converted to pedigree (PED) and genetic map (MAP) files for analysis in PLINK (Purcell et al., 2007; Purcell, 2013). PLINK incorporates a method of moments approach using a hidden Markov model to infer identity-by-descent from identity-by-state data (Purcell, 2013) for all pairs of individuals used to construct a genomic relationship matrix (GRM) for each breed. Quality filtering of SNP listed the following criteria: minor allele frequency greater than 0.05 or call rate for individual greater than 0.90. These parameters are within the range of those used by Saatchi et al. (2011), McClure et al. (2012), Ramey et al. (2013), and Decker et al. (2014). After filtering, 29,887 SNP remained. The relatively low yield from heritage breeds may result from the chip having been designed for commercial breeds of pigs. However, Rolf et al. (2010) shows that no more than 10,000 markers are required for estimation of genomic relationship matrices.

Inbreeding coefficients were also calculated using PLINK after the data set was pruned to only include SNP in windows of 50 with linkage disequilibrium less than an 0.50 approximate linkage equilibrium (Purcell et al., 2007). By excluding SNP in linkage disequilibrium, bias due to selection causing homozygosity not directly related to relationship is eliminated. After pruning, 9,130 SNP were removed leaving a total of 20,757.

<table>
<thead>
<tr>
<th>Breed</th>
<th>n</th>
<th>Relationship</th>
<th>Inbreeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Saddleback</td>
<td>2</td>
<td>0.15 ± 0.12abcd</td>
<td>0.08 ± 0.05abc</td>
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<tr>
<td>British Saddleback</td>
<td>20</td>
<td>0.14 ± 0.01c</td>
<td>0.15 ± 0.02a</td>
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<tr>
<td>Duroc</td>
<td>20</td>
<td>0.21 ± 0.01d</td>
<td>0.25 ± 0.02b</td>
</tr>
<tr>
<td>Guinea</td>
<td>14</td>
<td>0.17 ± 0.02ed</td>
<td>0.26 ± 0.03bd</td>
</tr>
<tr>
<td>Landrace</td>
<td>20</td>
<td>0.03 ± 0.01a</td>
<td>0.15 ± 0.02a</td>
</tr>
<tr>
<td>Large White</td>
<td>20</td>
<td>0.05 ± 0.01ab</td>
<td>0.15 ± 0.02a</td>
</tr>
<tr>
<td>Mulefoot</td>
<td>4</td>
<td>0.69 ± 0.02h</td>
<td>0.39 ± 0.04de</td>
</tr>
<tr>
<td>Ossabaw Island</td>
<td>10</td>
<td>0.37 ± 0.02efg</td>
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<td>Pietrain</td>
<td>20</td>
<td>0.07 ± 0.01b</td>
<td>0.12 ± 0.02e</td>
</tr>
<tr>
<td>Red Wattle</td>
<td>5</td>
<td>0.49 ± 0.04fg</td>
<td>0.28 ± 0.03bc</td>
</tr>
<tr>
<td>Tamworth</td>
<td>20</td>
<td>0.46 ± 0.01f</td>
<td>0.41 ± 0.02c</td>
</tr>
</tbody>
</table>

*Within a column, values with no superscripts in common are significantly different (P < 0.05).*

Breed averages and standard errors for relationships between individuals and inbreeding coefficients were obtained by fitting relationships between individuals within breed and inbreeding coefficients to a model including breed as a categorical fixed effect (SAS Inst. Inc., Cary, NC).

Multidimensional scaling (MDS) was used to visually present values from a genetic distance matrix among individuals. The MDS plot is prepared in PLINK by using whole-genome SNP data to produce pairwise identity by state distance (Cox and Cox, 2001; Borg et al., 2013).

**RESULTS AND DISCUSSION**

**Coefficients of Relationship and Inbreeding**

Table 1 summarizes the average relationships (R) between individuals (n) within breeds and average the inbreeding coefficient (F) of individuals within breeds.

Mulefoot pigs showed the highest average level of relatedness (0.69) while Landrace had the lowest level of relatedness (0.03). As expected, commercial breeds (Landrace, Large White, and Pietrain) exhibited lower levels of R between individuals, on average, as compared to R between individuals of rare breeds, especially Ossabaw Island, Red Wattle, Mulefoot, and Tamworth. Duroc had a surprisingly high average R for a commercial breed and was similar to Guinea and Saddleback.

Similarly, average inbreeding was high for Ossabaw Island, Tamworth, and Mulefoot (all ≥ 0.39), low for Pietrain, Large White, Landrace, and Saddleback (all ≤ 0.15), and intermediate for the other 3 breeds (0.25 to 0.28). Ossabaw Island has the highest F value (0.47), which is expected considering all hogs of that breed originated from an isolated herd on Ossabaw Island.
Saura et al. (2013) estimated inbreeding in ancient Iberian pigs. Inbreeding estimated from PED information averaged 0.39, within the range of values from heritage breeds in the present study. Inbreeding coefficients among 10 breeds of indigenous Chinese breeds ranged from 0.07 to 0.20 (Wang et al., 2006).

Multidimensional Scaling

Multidimensional scaling plots provide a visual representation of breed differences and similarities (Kijas et al., 2012; Herrero-Medrano et al., 2013). Figure 1 shows relative relationships between breeds. Breeds clustered more closely together (i.e., Landrace, Pietrain, and Large White) share a more similar genetic makeup than breeds plotted farther apart. Such a plot can be used to evaluate which breeds might possess unique traits as compared to commercial breeds. For example, outlying breeds such as Tamworth, Red Wattle, and Duroc may carry unique alleles not seen in breeds sharing a similar genetic makeup.

Two Guinea hog outliers are evident from Fig. 1. One of these hogs, Samson, was described as being from an older bloodline and was found to be less related to the other Guinea hogs as was the other outlier. The other Guineas were from more recent generations, and they all group closely together.

From this plot, there appear to be 6 distinct groups or clusters of individuals. The first group consists of Pietrain, Large White, and Landrace. These breeds may share origin. The second group includes British Saddleback and American Saddleback. These plotted on top of one another and appear to have diverged little beyond their common origin. These are located near groups 1 and 3 but are obviously their own unique group, having no overlap with other breeds. The third group consists of 3 breeds, Mulefoot, Guinea, and Ossabaw Island. Individuals from these breeds are plotted exceptionally close together, and in some cases, are overlapping. As with group 1, these 3 breeds share some physical characteristics such as color and general conformation.

The fourth and fifth groups consist of Red Wattle and Duroc, respectively. These 2 breeds appear to be plotted closer to each other than to the rest of the breeds. While there is no overlap between these 2 breeds, they do share similar characteristics and may have had a common origin. The sixth group consists of the Tamworth breed. They are tightly grouped and plotted well away from all other breeds in this diagram.

In reference to this particular MDS plot, Red Wattle, Duroc, and Tamworth appear to be divergent from all other breeds. Since the Red Wattle and Duroc group close to one another, it is likely they share a similar origin. Tamworth draws the most interest, as it is distinct from other breeds on the plot and does not appear to fall in line with any other breeds. Tamworth individuals may possess alleles not carried by individuals in the other breeds.

Buchanan and Stalder (2011) present a phylogenetic trace of 34 breeds of pigs included in the Roslin Institutes pig biodiversity database. Breeds in the phylogenetic tree include 6 from the present study: Tamworth, Duroc, Landrace, British Saddleback, Pietrain, and Large White. Similar to results from the present study, Tamworth and Duroc were distant from other breeds, but surprisingly, Large White and Pietrain were relatively distant from Landrace, which is contrary to the results of the current study.

Ramírez et al. (2015) described relationships among modern pig breeds including Duroc, Guinea Hog, Landrace, Large White, Mulefoot, and Ossabaw in an MDS plot. As in the current study, Mulefoot, Ossabaw, and Guinea Hog clustered closely together and relatively close to Large White and Landrace. Duroc clustered separately from the other 6 breeds.

Herrero-Medrano et al. (2013) compared Meishan, Large White, Landrace, Chato Murciano, Berkshire, Tamworth, Iberian Pig, and Duroc in an MDS plot. Similarly to the present study, they reported that Large White and Landrace clustered closely together when compared with all pigs and when compared with only European breeds. Herrero-Medrano et al. (2013) reported that Tamworth clustered closely with Berkshire and Chato Murciano when all breeds were compared. When only European breeds are compared, Tamworth loosely clusters with Berkshire and Iberian Pig. Tamworth did not cluster closely with other breeds included in the present study; however, Berkshire, Chato Murciano, and Iberian Pig were not included in this study. Similarly to the present study, Herrero-Medrano et al. (2013) reported that Duroc clusters well away from all other breeds. Red Wattle clustered closest to Duroc among the breeds included in the present study, but Red Wattle was not included in the study reported by Herrero-Medrano et al. (2013).
IMPLICATIONS

Genotyping with the PorcineSNP60 BeadChip offers the opportunity to estimate the inbreeding and relationship coefficients of animals within breeds with no PED information and to estimate the relationships across divergent breeds. Knowledge of relationships or divergence among breeds offers the opportunity to determine which rare breeds may be most critical for preservation. Results from this study suggest that Tamworth pigs in particular cluster well away from other breeds and, thus, perhaps should be a priority for preservation. Red Wattle pigs cluster away from Pietrain, Landrace, and Large White, which cluster together, but not far from Duroc.

LITERATURE CITED

Animal Genetics Resources Group (AGRG). 2006. Protecting results from this study reveal the opportunities to determine the relationships among breeds. Animal Genetics Resources Group (AGRG). 2006. Protecting results from this study suggest that Tamworth pigs in particular cluster well away from other breeds and, thus, perhaps should be a priority for preservation. Red Wattle pigs cluster away from Pietrain, Landrace, and Large White, which cluster together, but not far from Duroc.