# Case study of inbreeding within Japanese Black cattle using resources of the American Wagyu Association, National Animal Germplasm Program, and a cooperator breeding program in Wyoming

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## INTRODUCTION

Wagyu is a Japanese beef breed that was derived from native Asian cattle, although there were times in history when Wagyu cattle were crossed with both beef and dairy breeds (Namikawa, 1980). Wagyu originated from 6 of the 47 political units in Japan, and within the breed, each unit generated its own unique genetic line using different breeding objectives (Gaskins, 2008). In 1976, four Japanese Wagyu bulls were imported to the United States for research into meat quality. In 1992, an agreement was made between the United States and Japanese government to allow Japanese Wagyu dams to be exported from Japan. Following this agreement, additional bulls and dams arrived in the United States in 1993, 1994, and 1998. After 1998, no further exports of Japanese Wagyu have been allowed from Japan. Approximately 30 bulls and 200 females were exported to the United States within that time, creating a limited population of full-blood Japanese Black cattle in the United States (Bennett, 2013).

Extreme marbling is associated with full-blood Wagyu cattle and is assumed to be inherited. Utrera and Van Vleck (2004) reviewed 72 scientific papers that were published from 1962 to 2004 to summarize heritability estimates for carcass traits of beef cattle and calculated an unweighted mean estimate of 0.37 for marbling heritability across beef breeds. To accomplish the breeding strategy of upholding the quality of meat similar to that of Japan, linebreeding within Wagyu breeding programs is a common strategy (Lloyd et al., 2017). Inbreeding depression is at an especially high risk of occurring in breeds with a small effective population size (Ne), which is defined as the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift (Wright, 1950). Scraggs et al. (2014) estimated the NE of full-blood Wagyu cattle registered in the United States from 4,132 full-blood Wagyu cattle pedigrees for the period 1994 to 2011. The Ne averaged 17 animals between the years 1994 and 2011 with an increase to 58.1 in 2011. A minimum Ne of 50 has been recommended by the Food and Agriculture Organization since a Ne of 50 will result in an inbreeding rate of one percent per generation which will safely conserve a population (FAO, 1992).

The main goal for the cooperating breeder described herein was to build a nucleus population of full-blood Wagyu for their breeding program, which makes knowledge of the inbreeding coefficients of the herd crucial. The nucleus population is regenerated each generation by controlled breeding involving assortative mating. This

together with intensive combined index selection is intended to maximize short- and long-term genetic gains in the nucleus (James, 1977). Being that full-blood Wagyu within and outside of Japan have small Nes, the objective of this study was to estimate inbreeding levels between full-blood sires stored in the United States Department of Agriculture (USDA)-National Animal Germplasm Program (NAGP) semen repository and the full-blood dams from a herd established by a co-operator in Wyoming, with our hypothesis being the inbreeding would be greater than 6% for each group. The USDA-NAGP operates a repository for livestock, aquatic, poultry, and insect genetics whose mission is to protect U.S. animal genetic resources from loss due to epidemic or loss of genetic variation. The collections are tools for research and industry use to increase understanding of animal genetic resources and solve animal genetic issues (ARS, 2019). Ward's Cluster Analysis has been used by this group to visualize and understand the genetic architecture of relatedness within breeds of livestock (Blackburn, 2018).

## MATERIALS AND METHODS

### Data Collection and Editing

Pedigree data for the calculation of inbreeding coefficients were collected on 30 American Wagyu Association (AWA)-registered full-blood Japanese Wagyu dams from the cooperator herd (Brush Creek Ranch, Saratoga, WY) with ages ranging from 2 to 13 yr of age. Pedigree data were also collected for 18 full-blood Japanese Wagyu sires registered and provided by the AWA, all of which had semen stored at the USDA-NAGP located in Fort Collins, Colorado. Birth years ranged from 1973 to 2017 for these sires. A four-generation pedigree was created for individuals in these two groups. The pedigree was then ordered from oldest to youngest. A Ward's Cluster Analysis done in collaboration with the USDA-NAGP was executed using the statistical software SAS (Cary, NC, SAS Institute Inc., 2020) with pedigree data from 1,104 Wagyu cattle provided by the AWA, using the coefficient of genetic relationship between each animal, to develop clusters of animals with similar genetic relationships. This was used to gain knowledge on the genetic structure of the breed and assist in further developing germplasm collections (Blackburn, 2018).

### Statistical Analysis

The formula used to calculate the inbreeding coefficients was based on the additive genetic relationship matrix $A$, which was described in Henderson 1976 by Henderson:

$$\mathbf{A} = \mathbf{LDL}\prime$$

where $L$ is a lower triangular matrix containing the fraction of the genes that the individual animals get from their ancestors, and $D$ is a diagonal matrix containing the within-family additive genetic variances of animals. From the breakdown of the original formula, we used the Quaas (1976) method:

$$A_{ii} = \sum\nolimits_{j=1}^{i} L_{ij}^2 D_{jj}$$

where $A_{ii}$ is the $i$th diagonal element of $\mathbf{A}$, which was equivalent to the inbreeding coefficients of animal $i$ plus one (Meuwissen and Luo, 1992). These statistical analyses were accomplished using the statistical software package the Animal Breeder's Toolkit (Golden et al., 1992). To better visualize the data, a heat map was generated for the inbreeding coefficients between the 30 dams and the 18 sires using heatmap.2 command in the Various R Programming Tools for Plotting Data package in R (R Core Team, 2018), which is a graphical representation of data that uses a system of color coding to represent different values

Cluster analysis involved sorting data objects into groupings based on the similarity of Clustering Genes through Replicates. In this study, the coefficient of genetic relationship as a measure of genealogical structure was used to cluster the breed. Wright's coefficient of inbreeding (Wright, 1922) was defined as:

$$F = \sum [(1/2)^{n_1+n_2+1}(1 + F_A)]$$

for all common ancestors, where $n_1$ equaled the number of links between one parent of an individual and any ancestor both parents have in common, $n_2$ equaled the number of links between the other parent and the same common ancestor, and $F_A$ was the same coefficient ($F$) for the common ancestor of the parents (Chepko-Sade and Olivier, 1979). Starting with $N$ clusters each containing one object, Ward's method then computed the total within-cluster sums of squares (SSE) to determine the next two groups merged at each step of the algorithm. The SSE was defined as:

$$SSE = \sum_{i=1}^{k} \sum_{j=1}^{k} \left( y_{ij} - \bar{y}_{i.} \right)^2$$

where $y_{ij}$ is the $j$th object in the $i$th cluster and $n_i$ is the number of objects in the $i$th cluster (Ward, 1963; Ferreira and Hitchcock, 2009). The distance between two clusters, $A$ and $B$, was how much the SSE will increase when algorithms were merged:

$$\Delta(A,B) = \sum_{i \in A \cup B} \| \vec{x_i} - \overrightarrow{m_{A \cup B}} \|^2 - \sum_{i \in A} \| \vec{x_i} - \overrightarrow{m_A} \|^2 \quad (1)$$

$$- \sum_{i \in B} \| \vec{x_i} - \overrightarrow{m_B} \|^2 \quad (2)$$

$$= \frac{n_A n_B}{n_A + n_B} \| \overrightarrow{m_A} - \overrightarrow{m_B} \|^2 \quad (3)$$

where $\overrightarrow{m_j}$ is the center of the cluster $j$, and $n_j$ is the number of points in the cluster. $\Delta$ is the merging cost of combining clusters $A$ and $B$ (Moore, 2009).

## RESULTS AND DISCUSSION

Inbreeding coefficients were calculated for the 30 full-blood Wagyu dams and the 18 Artifical Insemintation (AI) sires and summarized in Table 1 with a heat map shown in Fig. 1, resulting in a total of 540 inbreeding coefficients. One of the original four imported Japanese Black bulls had an inbreeding coefficient of zero with all 30 dams, which is represented by the entirely white column in Fig. 1. The average inbreeding coefficient for this population was 0.190, with a range from 0 to 0.43. On average, this population of Wagyu cattle was expected to have 19% more homozygous gene pairs than a non-inbred individual from the same population. McParland et al. (2007) calculated mean inbreeding coefficients for Irish beef cattle breeds Hereford, Simmental, Angus, Limousin, and Charolais in 2004 to be 2.19%, 1.35%, 1.31%, 0.57%, and 0.54%, respectively. According to a presentation given at the Irish Cattle Breeding Federation (ICBF) Genetics Conference in 2018 on "Inbreeding Trends in Pedigree Beef Cattle", an inbreeding coefficient of 6.25% is widely viewed as the maximum level acceptable for livestock, which is the typical coefficient that indicates

the parents have a common grandparent. In the same presentation, trends of inbreeding levels of five other beef cattle breeds, from 2009 through 2017, were shown. American Angus, Charolais, and Limousin cattle inbreeding trends never increase above 1% over those 6 yr, and Hereford and Simmental fluctuate between 1.5% and 2%. In comparison, the coefficients of inbreeding of this population of Wagyu cattle were, on average, nine times more inbred than other beef cattle breeds, as well as more than double the maximum acceptable level (Clodagh, 2018).

In the Ward's Cluster Analysis shown in Fig. 2, the 1,104 Wagyu cattle that were provided by the AWA were represented in six clusters. Cluster 2 had four subclusters, cluster 3 had three subclusters, clusters 4 and 6 had two subclusters, and clusters 1 and 5 had zero subclusters. The results of the Cluster Analysis are summarized in Table 2. The second cluster had the most cattle with $n = 337$, and the fifth cluster had the least cattle with $n = 123$. Cluster 1 had the highest coefficient of relationship mean with $F_x = 0.20598$, and cluster 2 had the lowest coefficient of relationship mean with $F_x = 0.09637$. Of the six clusters, four had individuals represented in the USDA-NAGP collection with cluster 4 being principally comprised of Japanese Brown cattle. Results of the Ward's Cluster Analysis support the information presented in the introduction that the six prefectures from which the Wagyu breed originated are each a unique genetic line. Cluster analyses can be used by breeders to plan matings by mating cattle within or across clusters depending on individual goals and provide easier methods to compare genetic relationships of potential mates (Blackburn et al., 2020).

The information herein was compiled after the cooperating breeding program reached out for help with matings and to help them build a nucleus population of full-blood Wagyu. Due to the small Ne, genetic drift needs to be considered when developing a strategy to accomplish this goal. While dampened in large populations, genetic drift can create large and substantial changes in small populations (Blackburn et al., 2014), making it an important factor for breeders to consider with relatively small herds and especially breeders involved in raising rare breeds of livestock. Since Wagyu cattle fit this description, high selection intensities are suggested to achieve selection goals at the cost of slower rates of genetic gain (Blackburn et al., 2020).

When studying inbreeding levels in Wagyu cattle using BovineSNP50 BeadChip genotype data, with 54,609 evenly spaced single nucleotide polymorphisms (SNPs), and a 10-generation pedigree, it was
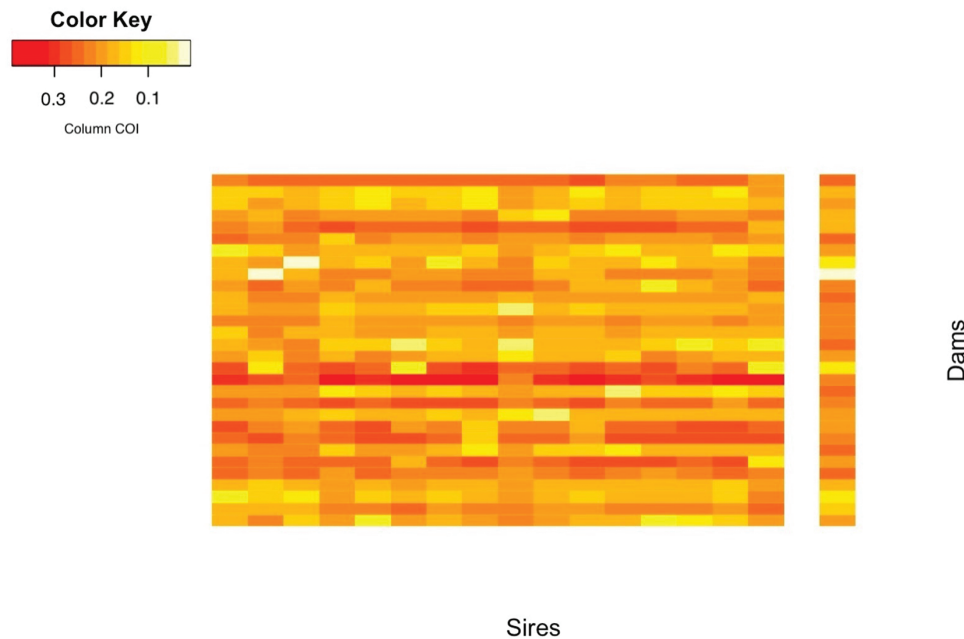
**Table 1.** Summary statistics of coefficients of inbreeding (COI) for 30 full-blood Wagyu dams and 18 AI sires

| Trait | Number | Mean | SD | Min | Max |
|-------|--------|------|-----|-----|-----|
| COI | 540 | 0.18979 | 0.08201 | 0 | 0.43173 |

**Figure 1.** A heat map representing coefficients of inbreeding (COI) between 30 full-blood Wagyu dams and 18 full-blood Wagyu sires.



[A] number of bulls in the USDA-NAGP collection; [B] number of full-blood bulls in the USDA-NAGP collection; [C] number of animals in the cluster.
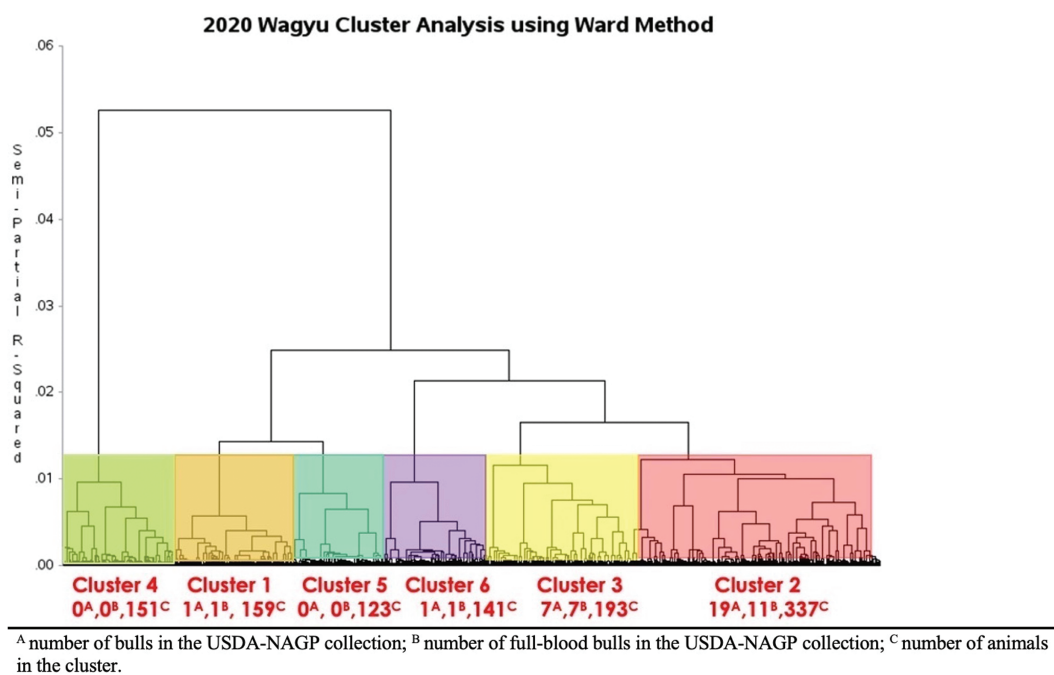
**Figure 2.** A cluster analysis of 1,104 Wagyu cattle based on pedigree data done by Dr. Harvey Blackburn at the USDA-NAGP.

**Table 2.** Summary statistics of coefficient of relationship ($F_x$) within clusters of the Ward's Cluster Analysis

| Cluster | Number within cluster | Mean | Variance |
|---|---|---|---|
| 1 | 159 | 0.204 | 0.007 |
| 2 | 337 | 0.096 | 0.005 |
| 3 | 193 | 0.112 | 0.007 |
| 4 | 151 | 0.144 | 0.010 |
| 5 | 123 | 0.204 | 0.008 |
| 6 | 141 | 0.153 | 0.007 |

reported that the inbreeding values estimated from the pedigree tend to underestimate homozygosity by descent among individuals (Scraggs et al., 2014). With genotypes, inbreeding coefficients could be more accurately estimated.

**CONCLUSIONS**

In this study, Wagyu cattle have high inbreeding coefficients. Along with a small Ne, further use of linebreeding will greatly increase the risk

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of inbreeding depression occurring in U.S. Wagyu cattle. Further inbreeding analysis should be done within Wagyu cattle using genotypes.

## ACKNOWLEDGMENTS

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