THESIS

EVALUATING THE GENETIC ARCHITECTURE OF THE JAPANESE WAGYU BREED WITHIN THE UNITED STATES

Submitted by

Kathryn Heffernan

Department of Animal Sciences

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Master's Committee

Advisor: Scott E. Speidel

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ABSTRACT

EVALUATING THE GENETIC ARCHITECTURE OF THE JAPANESE WAGYU BREED WITHIN THE UNITED STATES

The objective of this thesis is to better understand the genetic architecture of Japanese Wagyu cattle in the United States to improve on current breeding strategies, as well as help to develop new strategies to increase the genetic diversity of the U.S. Wagyu population. For this study, pedigree data (n = 48) were collected on full-blood Japanese Wagyu sires and dams, and high-density genotype data (n = 414) were collected on Japanese Wagyu, Ayrshire, Brahman, Brangus, Devon, Holstein, Milking Devon, Milking Shorthorn, Shorthorn, Simmental, South Devon, Angus, Jersey Island, and Brown Swiss cattle.

Inbreeding coefficients were calculated from the pedigree data and averaged 0.19, with a minimum coefficient of zero, max coefficient of 0.43, and a standard deviation of 0.08. Using the genotypes, a principal component analysis (PCA), an admixture graph, and a phylogenetic tree were computed to ascertain the population structure and breed composition of modern Japanese Wagyu cattle in relation to other breeds. The majority of the Japanese Wagyu had an elongated dispersal that overlapped with the origin of the PCA graph. Japanese Wagyu also averaged 85.5% indicine influence as indicated by the admixture graph, and the phylogenetic tree showed Japanese Wagyu as the closest branch to the root breed Brahman. These results suggest that Japanese Wagyu are an inbred population. While mostly *Bos indicus*, these results suggest that Japanese Wagyu are admixed with low percentages of *Bos taurus* breeds such as Simmental and Brown Swiss.

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TABLE OF CONTENTS

ABSTRACTii
ACKNOWLEDGEMENTS iii
LIST OF TABLES vi
LIST OF FIGURES vii
CHAPTER 1: INTRODUCTION AND OBJECTIVE 1
CHAPTER 2: REVIEW OF LITERATURE
SECTION 1: HISTORY OF JAPANESE WAGYU
BACKGROUND
INTRODUCTION TO THE UNITED STATES
SECTION 2: INBREEDING 5
ISOLATION
EFFECTIVE POPULATION SIZE
PEDIGREE ANALYSIS7
GENOTYPE ANALYSIS 12
SECTION 3: COMPARISON OF WAGYU TO OTHER BEEF BREEDS 18
BACKGROUND18
MARBLING AND CARCASS COMPOSITION 18
LITERATURE CITED
CHAPTER 3
INTRODUCTION
MATERIALS AND METHODS

I	RESULTS AND DISCUSSION	36
(CONCLUSIONS	39
J	LITERATURE CITED	40
CHAPT	TER 4	45
]	INTRODUCTION	45
I	MATERIALS AND METHODS	46
I	RESULTS AND DISCUSSION	51
(CONCLUSIONS	62
]	LITERATURE CITED	63
APPEN	DIX: List of cattle sampled for high-density genotyping	66

LIST OF TABLES

Table 2.1: Table of foreign breeds crossed with native cattle in each prefecture. (Namikawa, 1980). (https://tbrwagyu.com/wp-content/uploads/2017/08/Breeding-History-of-Japenese-Beef-Cattle.pdf)
Table 2.2: Average additive relationship coefficients within and among 25 subpopulations andthe average inbreeding coefficients of each subpopulation (Honda et al.,2002)
Table 2.3: A comparison of inbreeding coefficients estimated using pedigree or genotype data foreight full-blood Wagyu sires registered with the American Wagyu Association(Scraggs et al.,2014)
Table 3.1: Summary Statistics of coefficients of inbreeding (COI) for 30 fullblood Wagyu damsand 18 AI sires
Table 4.1: Description of the data with 14 cattle populations used in the analyses

LIST OF FIGURES

Figure 2.1: Number of full-blood Wagyu registered with the American Wagyu Association by year of birth between 1994 to 2011 (Scraggs <i>et al.</i> , 2014)
Figure 2.2: A Cluster Analysis of 1,104 Wagyu cattle based off of pedigree data done by Dr. Harvey Blackburn at the USDA-NAGP (2019)
Figure 2.3: Arithmetic mean, standard errors and a fitted regression line for overall inbreeding coefficients (FIT) estimated from pedigree records of full-blood Wagyu cattle registered with the AWA by year of birth between 1994 and 2011 (Scraggs <i>et al.</i> , 2014)
Figure 2.4: Figure of the official beef marbling standard, including beef color standard and beef fat standard, from the Japan Meat Grading Association. (<u>https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6039323/</u>)
Figure 2.5: The Beef Marbling Score (BMS) is directly related to the percentage extractable lipid. Cattle in the U.S. that grade Choice occupy the lowest portion of the curve. Only Japanese Black cattle produced under Japanese production conditions achieve BMS of 12 (Cameron <i>et al.</i> , 1994; Smith <i>et al.</i> , 2004). (https://www.researchgate.net/publication/283487914_The_Production_of_High- Quality_Beef_with_Wagyu_Cattle)
Figure 2.6: MUFA:SFA ratios of fat from Japanese Black cattle raised in the Hyogo prefecture of Japan; from Wagyu steers fed high-roughage diets for 550 days in Texas; from Angus steers fed high-roughage diets for 550 days in Texas; from Angus x Hereford crossbred steers fed a standard finishing diet for 150 days (Smith, 2015)
Figure 2.7: Body weights of Angus and Wagyu steers fed to either the U.S. endpoint (1st group corn and 2nd group hay) or the Japanese endpoint (3rd group corn and 4th group hay). Boxes indicate the weights at which each of the groups were sampled (Lunt <i>et al.</i> , 2005)
Figure 2.8: Marbling scores of Angus and Wagyu steers fed either corn- or hay-based diets 24
Figure 3.1: A heat map visualizing the inbreeding coefficients calculated between 30 fullblood Wagyu dams and 18 AI sires
Figure 4.1: Plot of the residuals from the fit of the model representing the pairwise admixture likelihoods between the 14 cattle populations analyzed. The color scale represents the standard

error of the pairwise admixture maximum-likelihoods between
breeds
Figure 4.2: The first three principal components (PC) using 14 cattle populations. Values
between the parentheses at each axis show how much variation is explained by each principal
component
Figure 4.3: Image capture of five angles of a 3D representation of the first three principal
components (PC) using 14 cattle populations. The axis values match those shown in Figure 4.2
of the 2D PCA
of the 2D FCA
Figure 4.4: The first three principal components (PC) using 119 Japanese Wagyu cattle and
distinguishing between full-blood and purebred Wagyu cattle. Values between the parentheses at
each axis show how much variation is explained by each principal component
Figure 4.5: Image capture of five angles of a 3D representation of the first three principal
components (PC) using 119 Japanese Wagyu cattle and distinguishing between full-blood and
nurebred. The axis values match those shown in Figure 4.4 of the 2D PCA 55
pareored. The axis values match those shown in Figure 1.1 of the 2D Forthermore 55
Figure 4.6: Plot of ADMIXTURE cross-validation error from K=1 through K=15. We chose K=7
to analyze the SNP data as the value that minimizes error
······································
Figure 4.7: Plot of model-based clustering (ADMIXTURE) results from $K = 2$ to 15 using 14
cattle populations. Each individual is represented by a vertical bar. Different colors indicate
different clustering groups
Figure 4.8: Population tree with 14 cattle populations from the Treemix 1.13 software using
Brahman as the root and showing 7 migration events. The events are represented as a color scale
based on their migration weight. The drift parameter models the strength of genetic drift along
the tree

CHAPTER 1

INTRODUCTION AND OBJECTIVE

Japanese Wagyu meat is one of the fastest growing food trends today. A recent report on industry trends forecast the Wagyu beef market to grow by USD 2.43 billion, progressing at a compound annual growth rate of 6.15% from 2020 to 2025 (Technavio, 2021). Meat produced from full-blood Wagyu cattle is highly marbled and fat within the steak is softer, with Wagyu crosses being shown to have decreased marbling and quality (Smith, 2015). This leads selection decisions to focus on maintaining fullblood Wagyu genetics so as to retain a high value carcass.

However, while Wagyu meat is a popular commodity, little is known about the breed itself. Imported breeds were crossed with native Japanese cattle to create the Japanese cattle breeds today, but the degrees of influence of these imported breeds differed across prefectures, and even among regions within prefectures (Namikawa, 2018). So while it is known Japanese Wagyu cattle were created using crossbreeding strategies, the breeds and the extent to which they influenced the breed composition are unknown. At present, information that has been reported on the breed are high inbreeding levels and a small effective population size, especially within the United States (Decker *et al.*, 2012; Nomura *et al.*, 2001).

Due to lack of available information and the presence of potential problems with breed management, a cooperator breeding herd approached Colorado State University to help build a nucleus population of full-blood Wagyu cattle for their breeding program within the United States. The inbreeding levels were of main concern and were analyzed through pedigree data provided by the American Wagyu Association (AWA). However, pedigree data has been found to underestimate homozygosity by descent among individuals (Scraggs *et al.*, 2013) so genomic data were also collected. With both pedigree and genomic data showing high rates of inbreeding

1

within the herd, we wanted to develop a strategy to increase genetic diversity within the herd while maintaining carcass quality. To accomplish this, more information was needed on breed composition and population structure in relation to other cattle breeds. Therefore, the objective of this thesis is to better understand the genetic architecture of Japanese Wagyu cattle in the United States to improve on current breeding strategies, as well as help to develop new strategies to increase the genetic diversity of the U.S. Wagyu population.

CHAPTER 2

REVIEW OF LITERATURE

SECTION 1: HISTORY OF JAPANESE WAGYU

Background

The Japanese Wagyu breed was fixed from a mongrel population through selection and was domesticated by immigrants who brought their animals over from the Asian continent via the Korean peninsula, accompanying the introduction of rice cultivation (Noda *et al.*, 2018). The breed type that was imported was the Hanwoo, and at the time, the primary need was a labor source, so cattle were selected for based on their use in farming, mining, forestry, and transportation as opposed to as a food source (Namikawa, 1992). Buddhist leaders eventually prohibited the eating of flesh, halting plans to use cattle for meat consumption (Lunt, 1991). The Meiji Restoration, which was a political event that restored practical imperial rule under Emperor Meiji, began in 1867 and sanctioned the consumption of beef again, allowing for cattle to be crossbred and consumed for their meat by 1868 (Smith, 2015). The demand for cattle as draft animals persisted until 1960 when machines replaced them as more efficient labor and the mass production of chemical fertilizer was made available (Namikawa, 1980).

At the start of the decline in a need for draft animals in the early 1900s, the focus shifted to meat quality. Japan started to crossbreed native cattle with imported breeds such as Braunvieh and Simmental cattle from Switzerland, Ayrshire, Devon and Shorthorn cattle from the United Kingdom, and Holstein cattle from Germany and the Netherlands to increase the size and meat yield of draft and beef cattle. Following a decline in meat quality and a sharp increase in body size, Japan stopped crossbreeding and started to intra-breed, resulting in the establishment of the

3

Japanese Black, Japanese Brown, and Japanese Polled breeds in 1944. Japanese Shorthorn was not formally established until 1957 (Gotoh *et al.*, 2018; Table 2.1).

Name of Modern breed	Prefecture	Crossed Foreign Breeds					
Japanese Black	Kyoto	Brown Swiss					
	Hyogo	Shorthorn, Devon, brown Swiss					
	Okayama	Shorthorn, Devon					
	Hiroshima	Simmental, Brown Swiss, Shorthorn, Ayrshire					
	Tottori	Brown Swiss, Shorthorn					
	Shimane	Devon, Brown Swiss, Simmental, Ayrshire					
	Yamaguchi	Devon, Ayrshire, Brown Swiss					
	Ehime	Shorthorn					
	Ohita	Brown Swiss, Simmental					
	Kagoshima	Brown Swiss, Devon, Holstein					
Japanese Brown	Kochi	Simmental, Korean Cattle					
	Kumamoto	Simmental, Korean Cattle, Devon					
Japanese Poll	Yamaguchi	Aberdeen-Angus					
Japanese Shorthorn	Aomori	Shorthorn					
	Iwate	Shorthorn					
	Akita	Shorthorn, Devon Ayrshire					

Table 2.1: Table of foreign breeds crossed with native cattle in each prefecture. (Namikawa, 1980). (https://tbrwagyu.com/wp-content/uploads/2017/08/Breeding-History-of-Japenese-Beef-Cattle.pdf)

Introduction to the United States

The Wagyu population outside of Japan is derived from fewer than 200 exports to the U.S. that were imported between the years of 1993 and 1997 after the first imports in 1976. In 1976, four bulls were sent from Japan by Texas cattleman Morris Whitney. Colorado State University collected semen samples from these four sires which were then bought by Wagyu Breeders Inc. Due to the lack of Wagyu females in the U.S. at the time, the four sires were mated to Angus, Holstein, Hereford and Brangus cows. It was estimated that there were less than 300 crossbred females of breeding age by 1991 that were ³/₄ Wagyu or higher. In 1992, an agreement was made between the U.S. and Japanese government to allow Japanese Wagyu dams to be exported from Japan. Following this agreement, additional bulls and dams arrived in the U.S. in

1994, 1995, 1997 and 1998. Since 1998, no further exports of Japanese Wagyu have been allowed from Japan. Approximately 30 bulls and 200 dams were exported to the U.S. within that time, creating a limited population of full-blood Japanese Black cattle in the United States (Scraggs, 2014; Bennett, 2013).

SECTION 2: INBREEDING

Isolation

The decrease in effective population size, or the decrease in genetic diversity, is associated with many unfavorable events, such as inbreeding depression in fitness-related traits and an increased fluctuation in selection response (Falconer & Mackay, 1996). The Japanese Wagyu breed became a highly inbred breed starting at their conception. Hanwoo cattle were first imported into the Shikoku region and remained isolated there due to the mountainous terrain and lack of passable roads. They were substantially isolated over the course of 200 years due to Japan being an island country, as well as historical events that exacerbated the issue. A notable one being the national isolation by the Shogun in 1635. This lasted for two centuries till 1854, with the only exceptions being foreign trade with China and the Netherlands. Within that period, there was no introduction of new genes to the cattle population (Namikawa, 1992).

Effective population size

The selection of sires within the Wagyu breed was mainly restricted to those sires residing within the same Japanese prefectures as the cows to which they were mated during most of the twentieth century (Nomura, 1996). This changed with the implementation of genetic improvement programs using BLUP methodology (Sasaki *et al.*, 2006) and the use of artificial insemination. The use of these technologies led to the heavy use of a few prominent sires, and

5

the offspring from five sires alone represented 42% of all registered Wagyu cattle (Nomura *et al.*, 2001). The popularity of these sires reduced the genetic diversity within the Wagyu breed and resulted in a sharp increase in the rate of inbreeding and a drop in the effective population size from 32 in 1986 to 14 in 1993 (Nomura *et al.*, 2001). Effective population size 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). In a population that undergoes a bottleneck event, or when a small group of animals within the population establishes a new population, the effective population size is closer to the number of animals at the point of maximum contraction than the number of animals at its maximum (Sjodin *et al.*, 2005). Scraggs *et al.* (2014) assessed the effective population size for the U.S. Wagyu population between the years 1994 to 2011 (Figure 2.1).



Figure 2.1: Number of full-blood Wagyu registered with the American Wagyu Association by year of birth between 1994 to 2011 (Scraggs *et al.*, 2014).

A total of 29 males and 207 females were imported from Japan into the U.S., with the first 14 full-blood Wagyu animals being born in the U.S. in 1994. The Wagyu population, as measured by the number of calves registered by birth year, steadily increased from 14 animals born in 1994 to a maximum of 698 animals born in 2009. The number of full-blood animals registered with the American Wagyu Association (AWA) by birth year has steadily declined since 2009, with a drop of 315 registered animals in 2011. While this indicates growth of the U.S. Wagyu herd, it is not indicative of the amount of genetic diversity within registered full-blood Wagyu cattle. The effective population size estimate, a more accurate estimation of genetic diversity, had an arithmetic mean of 17.0 + 3.19 animals and geometric mean of 13.2 + 2.47 animals between 1994 and 2011, with an increase of 47.59 in 2002 and a drop of 2.3 in 1996 (Scraggs *et al.*, 2014).

Pedigree Analysis of Inbreeding

Both Nomura *et al.* (2001) and Scraggs *et al.* (2014) reported similar results and found that during the past decade, the disappearance of genetic subdivision has proceeded rapidly and the decline of genetic diversity in the Japanese Wagyu breed is an increasing problem. Pedigree analyses have been reported on the genetic structure of the breed to help design mating strategies to increase genetic diversity. Due to the isolation of the breed, as well as the limited migration between prefectures, the breed was genetically subdivided into several local subpopulations (Nomura & Sasaki, 1988). In a Ward's Cluster Analysis based on pedigree data of 1,104 Wagyu cattle conducted by Dr. Blackburn (2019) at the United States Department of Agriculture-National Animal Germplasm Program (USDA-NAGP), the Wagyu population is represented by six clusters (Figure 2.2).

7



^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 2.2: A Cluster Analysis of 1,104 Wagyu cattle based off of pedigree data done by Dr. Harvey Blackburn at the USDA-NAGP (2019).

This supports the hypothesis that the prefectures from which the Japanese Wagyu breed originated are each a unique genetic line. Cluster analysis involves sorting data objects into groupings based on similarity of Clustering Genes through Replicates (CGR). In the Ward's Cluster Analysis, 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, 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:

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

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

$$\Delta(A,B) = \sum_{i \in A \cup B} \| \overrightarrow{x_i} - \overrightarrow{m_{A \cup B}} \|^2 - \sum_{i \in A} \| \overrightarrow{x_i} - \overrightarrow{m_A} \|^2 - \sum_{i \in B} \| \overrightarrow{x_i} - \overrightarrow{m_B} \|^2$$
(2)
$$= \frac{n_A n_B}{n_A + n_B} \| \overrightarrow{m_A} - \overrightarrow{m_B} \|^2$$
(3)

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

Honda *et al.* (2002) also reported on average additive relationships within and between prefectures of 2000 Japanese Black cattle (Table 2.2).

Table 2.2: Average additive relationship coefficients within and among 25 subpopulations and the average inbreeding coefficients of each subpopulation (Honda *et al.*, 2002).

-	<u> </u>			<u> </u>						-	-		· ·					/							
	HK†	AM	IW	MG	AK	YT	FK	IB	TC	GM	NN	GF	HY	TT	SM	ок	HR	YC	SG	NS	KM	OT	MZ	KG	ON
HK	0.12																								
AM	0.11	0.12																							
IW	0.11	0.12	0.13																						
MG	0.11	0.12	0.12	0.17																					
AK	0.12	0.12	0.12	0.13	0.15																				
YT	0.12	0.13	0.13	0.15	0.14	0.16																			
FK	0.11	0.12	0.12	0.12	0.12	0.13	0.13																		
IB	0.11	0.11	0.11	0.12	0.12	0.13	0.11	0.12																	
TC	0.12	0.12	0.12	0.12	0.13	0.14	0.12	0.12	0.15	0.10															
GM	0.11	0.12	0.12	0.11	0.12	0.13	0.12	0.11	0.13	0.13	0.10														
NN	0.11	0.12	0.11	0.12	0.12	0.13	0.12	0.11	0.13	0.12	0.12	0.10													
GF	0.12	0.12	0.12	0.13	0.15	0.14	0.12	0.12	0.13	0.12	0.15	0.18	0.41												
HY	0.15	0.17	0.16	0.17	0.15	0.19	0.15	0.15	0.14	0.15	0.15	0.17	0.41	0 10											
11 CM	0.08	0.08	0.09	0.07	0.08	0.08	0.09	0.07	0.09	0.09	0.09	0.09	0.07	0.18	0.20										
OK	0.12	0.09	0.09	0.09	0.12	0.09	0.09	0.09	0.12	0.09	0.11	0.00	0.08	0.09	0.20	0.15									
UD	0.09	0.08	0.07	0.07	0.09	0.08	0.00	0.07	0.09	0.00	0.06	0.09	0.08	0.07	0.04	0.13	0.10								
VC	0.00	0.00	0.05	0.08	0.00	0.12	0.05	0.00	0.12	0.05	0.00	0.00	0.08	0.09	0.04	0.03	0.05	0.12							
SG	0.11	0.12	0.12	0.11	0.12	0.12	0.12	0.11	0.12	0.13	0.12	0.13	0.14	0.10	0.10	0.08	0.05	0.12	015						
NS	0.10	0.11	0.11	0.11	0.11	0.12	0.11	0.10	0.11	0.11	0 11	0.12	0.14	0.09	0.10	0.08	0.05	0.11	0.13	0.12					
KM	0.10	0.11	0.11	0.10	0.11	0.12	0.11	0.11	0.11	0.12	0.11	0.12	0.14	0.09	0.09	0.07	0.05	0.11	0.13	0.11	0.12				
OT	0.08	0.08	0.08	0.08	0.09	0.08	0.08	0.08	0.10	0.09	0.08	0.09	0.10	0.07	0.09	0.07	0.04	0.08	0.09	0.09	0.10	0.20			
MZ	0.09	0.10	0.10	0.09	0.09	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.13	0.08	0.08	0.06	0.05	0.10	0.11	0.10	0.12	0.08	0.16		
KG	0.08	0.09	0.09	0.09	0.08	0.09	0.10	0.08	0.08	0.11	0.10	0.10	0.12	0.09	0.05	0.05	0.04	0.10	0.12	0.11	0.12	0.07	0.10	0.19	
ON	0.11	0.11	0.10	0.10	0.12	0.11	0.11	0.10	0.12	0.11	0.11	0.11	0.13	0.08	0.11	0.08	0.05	0.10	0.11	0.10	0.10	0.08	0.09	0.09	0.12
θ‡	0.06	0.06	0.06	0.09	0.08	0.08	0.06	0.06	0.07	0.06	0.06	0.09	0.21	0.09	0.10	0.08	0.09	0.06	0.07	0.06	0.06	0.10	0.08	0.10	0.06
F§	0.05	0.05	0.05	0.07	0.05	0.06	0.05	0.05	0.04	0.04	0.05	0.06	0.17	0.07	0.07	0.06	0.07	0.05	0.04	0.04	0.05	0.04	0.05	0.05	0.04

[‡]Inbreeding coefficient expected under random mating within subpopulation. [§]Average inbreeding coefficient within subpopulation

The coefficients in the last two rows are half the average additive relationships and the average inbreeding coefficients within each subpopulation respectively. The average additive relationship coefficient and the inbreeding coefficient of the subpopulation are much higher than the others due to the Hyogo prefectures history of completely closed breeding (Mukai *et al.*, 1989). The coefficient θ gives an estimate of F_{ST} of Wright's F-statistics (Wright 1951, 1969). In all of the subpopulations, the coefficient θ exceeds the actual inbreeding coefficient F, which suggests that genetic subdivisions within subpopulations have essentially disappeared. The mean of the average additive relationship coefficients within subpopulations (0.16) was significantly (P < 0.01) larger than the mean of the average additive relationship coefficients (0.103) between all the possible pairs of subpopulations (Honda *et al.*, 2002). From the estimates of F-statistics in 1985-1997, Nomura *et al.* (2001) showed that the genetic homogeneity within the Japanese

Black population has rapidly increased during this period, though the results of Honda *et al.* (2002) and Blackburn (2019) imply that genetic subdivision still remains in the current breed.

Scraggs *et al.* (2014) estimated levels of inbreeding using pedigree information for fullblood Wagyu cattle registered with the AWA between the years 1994 and 2011 (Figure 2.3).



Figure 2.3: Arithmetic mean, standard errors and a fitted regression line for overall inbreeding coefficients (F_{IT}) estimated from pedigree records of full-blood Wagyu cattle registered with the AWA by year of birth between 1994 and 2011 (Scraggs *et al.*, 2014).

The mean inbreeding coefficient between 1994 and 2011 was estimated at 4.80 +/- 2.5%. The mean inbreeding coefficient was estimated at 3.2 in 1994 when the first full-blood Wagyu animals were born in the U.S. and increased until it reached its maximum of 7.14 in 1996. The largest inbreeding coefficient estimated for a single animal was 37.5% for an animal born in 2007 (Scraggs *et al.*, 2014).

Genotype Analysis of Inbreeding and Population Structure

While analyzing inbreeding with pedigree information is a commonly used method, inbreeding values found through pedigree data tend to underestimate homozygosity (Decker *et al.*, 2012). Scraggs *et al.* (2014) compared genomic and pedigree inbreeding coefficients of eight sires in the U.S. Wagyu population (Table 2.3).

Sires	Registration number	Inbreeding coefficient based on pedigree (%)	Inbreeding coefficient based on genotype (%)
Kitaguni Junior	FB2422	0	1.66
T.F. Itomichi 1/2	FB2126	0	5.67
T.F. Kikuhana	FB2127	18.7	6.37
Kitateruyasudoi	FB0686	9.38	25.91
Michifuku	FB1615	3.9	10.22
Sanjirou	FB2501	9.3	16.17
Takazakura	FB2892	0	0.99
JVP Fukutsura 068	FB2101	4.7	19.23
Average		5.75	10.83

Table 2.3: A comparison of inbreeding coefficients estimated using pedigree or genotype data for eight full-blood Wagyu sires registered with the American Wagyu Association (Scraggs *et al.*, 2014).

The pedigree estimated inbreeding coefficients consistently registered a lower rate of inbreeding than genotype estimated inbreeding coefficients, except for one (FB2127) whose pedigree inbreeding coefficient was about a three times higher rate. The disparity of observations between pedigree and genomic data is likely because pedigree estimates of inbreeding assume that F_{IT} is set to zero for all animals at the base of the pedigree. It could also be due to a result of pedigree errors and incomplete pedigree information (Scragss *et al.*, 2014). Genomic data can

also be used to analyze population structure and breed composition. Three of the most common approaches to analyzing population structure are principal component analyses (PCA), admixture graphs and phylogenetic trees.

Principal component analysis (PCA) uses a dimensionality-reduction method to visualize data which transforms a large set of variables into a smaller one while retaining most of the information from the original dataset. PCA uses new variables called principal components (PC) that are computed as linear mixtures of the initial variables. These new variables capture the maximum amount of information from the original data and are new axes that provide the best angle to evaluate the data so differences between observations are better visualized. The axes are chosen so that the projection of samples along the first axis (or first PC) explains the greatest possible variance in the data among all possible axes while the projection of samples along the second axis maximizes the variance for all possible axes uncorrelated with (or perpendicular to) the first. This continues for all subsequent components (McVean, 2009).

The analysis can be broken down into five steps: 1) Standardize the range of continuous initial variables, 2) Compute the covariance matrix to identify correlations, 3) Compute the eigenvectors and eigenvalues of the covariance matrix to identify the principal components, 4) Create a feature vector to decide which principal components to keep, and 5) Recast the data along the principal components axes (Jaadi, 2021).

To standardize the initial variables, the first step is to zero-center the data to create a new matrix \mathbf{X} ,

$$X_{si} = Z_{si} - \frac{1}{n} \sum_{j=1}^{n} Z_{sj}$$

where \mathbb{Z} consists of a *L x n* binary matrix, and *L* represents the number of SNPs (McVean, 2009). Next is to compute the covariance matrix. The covariance matrix

$$C = \frac{1}{n-1} X X^T$$

is a $p \ x \ p$ symmetric matrix, where p is the number of dimensions that has as entries the covariances associated with all possible pairs of the initial variables, such that the *i*th principal component (the *i*th row of p) is the *i*th eigenvector of **C** (McVean, 2009). Next is to compute the eigenvectors and eigenvalues of the covariance matrix to identify the principal components. Eigenvectors of the covariance matrix are the directions of the axes where there is most variance and that we call Principal Components. Eigenvalues are the coefficients attached to those eigenvectors which give the amount of variance carried in each PC (Jaadi, 2021). For genomewide SNP datasets, the analysis uses singular value decomposition (SVD) to find the PC's. SVD, which exists for any $L \ x \ n$ matrix rewrites the original data in terms of three other matrices

$$X = U \sum V^T$$

where U is an orthogonal matrix of dimension L x n, Σ is a diagonal matrix of dimension n x n, and V is another orthogonal matrix of dimension n x n. This is done by setting v_i , the *i*th column of V, to be the *i*th eigenvector of the matrix

$$M = X^T X$$

with the vector being

$$u_i = \frac{1}{\sigma_i} X v_i$$

where σ_i is the square root of the corresponding eigenvalue on the *i*th diagonal entry of Σ , and u_i is the *i*th column of U (McVean, 2009). After computing each eigenvector, order them by their eigenvalues in descending order to find the PCs in order of significance. Discard those of lesser significance and with those remaining form a matrix of vectors *F*, called a *Feature vector*. The last step is to use the feature vector to reorient the data from the original axes to ones represented by the PC's

$$Final = X^T F^T$$

Where X^T is the transpose of the original dataset and F^T is the transpose of the feature vector (Jaadi, 2021).

Admixture occurs when individuals from two or more previously isolated populations interbreed. The previously isolated populations are referred to as ancestral or parental and the newly formed population is referred to as admixed, often referred to as hybridization when not talking about humans. Admixture mapping is a method for capitalizing on recent admixture to correlate ancestry at genetic loci with a phenotype and relies on a value K to compute. K is the belief of the number of ancestral populations involved in your data (Shriner, 2014). While there are many different approaches to calculating the admixture of a population, this paper will focus on an approach used by the model-based clustering software ADMIXTURE v1.3.0 as that was used in this research.

To identify the value of K for which the model has the best predictive accuracy, ADMIXTURE uses a cross-validation procedure which partitions all the observed genotypes into roughly equally sized folds. The procedure then masks all genotypes for each fold in turn. For each fold, the resulting masked dataset \tilde{G} is used to calculate estimates

$$\tilde{\theta} = (\tilde{Q}, \tilde{P}).$$

15

Each masked genotype g_{ij} is predicted by

$$\widehat{\mu_{ij}} = E[g_{ij} | \widetilde{Q}, \widetilde{P}] = 2 \sum_{k} \widetilde{q_{ik}} \widetilde{P_{kj}}$$

and the prediction error is estimated by averaging the squares of the deviance residuals of the binomial model described by McCullagh and Nelder (1989),

$$d(n_{ij}, \hat{\mu}_{ij}) = n_{ij} \log(n_{ij}/\hat{\mu}_{ij}) + (2 - n_{ij}) \log[(2 - n_{ij})/(2 - \hat{\mu}_{ij})],$$

across all masked entries over all folds. The smallest estimated prediction error then suggests the most suitable K (Alexander *et al.*, 2015).

Evolutionary trees are illustrative representations of evolutionary relationships among taxa (Choudhuri, 2014). While there are many types of evolutionary trees, this paper will focus on phylogenetic, or species, trees as that was used for this research. The focus was to analyze the topology of relationships between different cattle populations and test for gene flow between different groups. The Treemix software used in the analysis accomplishes this by building a maximum likelihood tree of populations as proposed by Pickrell *et al.* (2012).

For a multiple population analysis, first step is to calculate the covariance between any two populations with respect to the ancestral allele frequency,

$$Cov(X_1, X_2) = c_2 x_A [1 - x_A]$$

 $Cov(X_3, X_4) = c_1 x_A [1 - x_A]$
 $Cov(X_1, X_2) = 0$

Where X_1, X_2, X_3 , and X_4 denote the allele frequencies of the populations, x_A is the allele frequency of one of the alleles at the SNP in an ancestral population, and *c* is a factor that reflects the amount of genetic drift that has occurred between the ancestral population and the descendant population. Next is to calculate the vector of all allele frequencies,

$$\vec{X} \sim MVN(\vec{x_A}, V)$$

where \vec{X} is the vector, *MVN* represents the multivariate normal distribution, and *V* is the variance-covariance matrix of allele frequencies between populations implied by the tree. To include migration, populations get ancestry from multiple parental populations with weighted contributions from each parental population,

$$X_3 = wX_v + (1 - w)(X_z + \varepsilon_3)$$

where X_3 is the allele frequency of a population, Y and Z are parental populations, X_y and X_z are allele frequencies of those parental populations, and $\varepsilon_3 \sim N(0, c_3 x_A [1 - x_A])$. 1 – w, w, and 1 are all weights for three events of drift: drift since Z but before the population mixture, drift since Y but before the population mixture, and drift since the mixture. Set the edge with the largest weight as the non-migration edge, and the other(s) as the migration edge(s). The variance of X_3 can now be written as:

$$Var(X_3) = Var\left(wX_y + (1 - w)(X_z + \varepsilon_3)\right)$$
$$= w^2 Var(X_y) + (1 - w)^2 [Var(X_z) + Var(\varepsilon_3)]$$
$$+ 2w(1 - w)Cov(X_y, X_z)$$

Final step is to estimate the population history of graph G. Estimate the sample covariance matrix \widehat{W} :

$$\widehat{W}_{ij} = \frac{\sum_{k=1}^{n} [(\widehat{X}_{ik} - \widehat{\mu}_k)]}{n}$$

Where $\hat{\mu}_k = \frac{1}{m} \sum_{i=1}^m \hat{X}_{ik}$. Then calculate \widehat{W} separately for each *p* block with *K* SNPs per block and write a composite likelihood for \widehat{W} :

$$L(\widehat{W}|W) = \prod_{i=1}^{m} \prod_{j=i}^{m} N(\widehat{W}_{ij}|G, \widehat{\sigma}_{ij}^{2})$$

where $N(\widehat{W}_{ij}|G, \widehat{\sigma}_{ij}^2)$ is a Gaussian density with mean W_{ij} and variance evaluated at \widehat{W}_{ij} (Pickrell *et al.*, 2012).

SECTION 3: COMPARISON OF WAGYU TO OTHER BEEF BREEDS Background

As mentioned previously, Japanese Wagyu cattle have a small effective population size and are highly inbred, especially when compared to other cattle breeds. The effective population size of Wagyu averaged 17 animals between 1994 and 2011 (Scraggs *et al.*, 2015). In comparison, estimates of the effective population size for Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, and Angus breeds were 161, 61, 65, 39, 38, and 94 animals respectively (Weigel & Lin, 2002; Decker *et al.*, 2012). The estimated mean inbreeding coefficient for Wagyu between 1994 and 2011 was 5% (Scraggs *et al.*, 2015). 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. While this disparity in genetic diversity between breeds has been caused by selection pressure mainly focused on carcass characteristics, this has also led to increased carcass quality and marbling that has yet to be matched.

Marbling and Carcass Composition

Beef cattle raised in Japan show an increased ability to accumulate marbling and their grading system is very different from the USDA quality grading system. Carcasses receive a Beef Marbling Score (BMS) based on the amount of visible marbling in the loin muscle between the 6th to 7th thoracic rib interface, as opposed to in the U.S. which are graded at the 12th and 13th interface (Figure 2.4; JMGA, 1988).



Figure 2.4: Figure of the official beef marbling standard, including beef color standard and beef fat standard, from the Japan Meat Grading Association. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6039323/)

Another difference between Japanese and U.S. grading systems is the scale. The U.S. marbling score ranges from Practically Devoid to Abundant, or 1% to 12% intramuscular lipids. The Japanese BMS values range from 1 to 12, or 1% to 35% intramuscular lipids (Cameron *et al.*, 1994; Smith *et al.*, 2004). Choice cattle occupy the lowest portion of this curve with Japanese Black cattle occupying the upper portion (Figure 2.5).



Figure 2.5: The Beef Marbling Score (BMS) is directly related to the percentage extractable lipid. Cattle in the U.S. that grade Choice occupy the lowest portion of the curve. Only Japanese Black cattle produced under Japanese production conditions achieve BMS of 12 (Cameron *et al.*, 1994; Smith *et al.*, 2004). (https://www.researchgate.net/publication/283487914_The_Production_of_High-Quality_Beef_with_Wagyu_Cattle)

The capacity of Wagyu cattle to accumulate increased marbling is due to their unique distribution of marbling adipocytes in their muscles. It is rare to obtain a field devoid of adipocytes in sections of ribeye muscle from Japanese Black cattle, whereas marbling adipocytes are rarely observed in microscopy samples of muscle from North American breed types. Wagyu marbling adipocytes cluster in large groups unlike other breed types where marbling adipocytes are arranged like strings of pearls (Smith, 2015).

Fatty acid composition is another important factor in comparison of Japanese Wagyu cattle to other breeds as that directly influences beef palatability (Dryden & Marchello, 1970; Westerling & Hedrick, 1979). The melting point of beef fat is determined by the ratio of monounsaturated fatty acids (MUFA), with melting points below 20°C, to saturated fatty acids (SFA) with melting points around 70°C. The ratio is calculated by summing all of the MUFA and SFA, and the sum of the MUFA is then divided by the sum of the SFA. The higher the ratio, the softer the fat, which is the more desirable meat quality. Lipids extracted from the fat of Japanese Black cattle raised in Japan have melting points as low as 24°C, with a very high MUFA:SFA ratio (Smith, 2015). Cattle in Japan are fed around 19 months past weaning and this may contribute to the high MUFA:SFA ratio seen in Japanese Black cattle in comparison to Japanese Shorthorn and Holstein cattle (Tanaka, 1985).

A study conducted at Texas A&M in which Japanese Wagyu and Angus steers were fed high-roughage diets for 550 days showed that the highest MUFA:SFA ratios typically were observed in fat and lean from Japanese Black cattle that achieved the highest BMS scores, which suggests a genetic relationship between fatty acid composition and marbling (Lunt *et al.*, 1993; May *et al.*, 1993). Both long-fed groups of steers had MUFA:SFA ratios greater than typical Angus x Hereford crossbred steers, but their ratios were far below those of Japanese cattle produced in Japan (Figure 2.6).



Figure 2.6: MUFA:SFA ratios of fat from Japanese Black cattle raised in the Hyogo prefecture of Japan; from Wagyu steers fed high-roughage diets for 550 days in Texas; from Angus steers fed high-roughage diets for 550 days in Texas; from Angus x Hereford crossbred steers fed a standard finishing diet for 150 days (Smith, 2015).

The Angus steers were selected from the top Angus sires of the time so the Angus breed was well represented, grading USDA Prime with 14.5% extractable lipid in their ribeye muscle. However carcasses from the American Wagyu cattle contained 19% lipid in the ribeye. Based on the Japanese grading system, the Angus cattle achieved a BMS value of 4.5 while the Wagyu cattle achieved a BMS of 7.3 (Lunt *et al.*, 1993). In a second investigation, it was confirmed that Angus cattle cannot achieve a BMS value greater than five (Cameron *et al.*, 1993).

In a more recent study, Lunt *et al.* (2005) compared the performance of Angus and Wagyu steers raised into either a typical U.S. endpoint (525 kilograms; 1,100 pounds) or a Japanese endpoint (650 kilograms; 1,400 pounds), while being fed either corn- or hay-based diets. The main purpose was to determine if Wagyu steers would out-perform Angus steers if the cattle were fed to a typical U.S. endpoint, or if the Wagyu steers would have to be raised to the Japanese endpoint in order to demonstrate superior carcass quality (Figure 2.7).



Figure 2.7: Body weights of Angus and Wagyu steers fed to either the U.S. endpoint (1^{st} group corn and 2^{nd} group hay) or the Japanese endpoint (3^{rd} group corn and 4^{th} group hay). Boxes indicate the weights at which each of the groups were sampled (Lunt *et al.*, 2005).

The corn-fed Angus steers had a high rate of gain at the beginning of the trial, weighing 90 to 100 kilograms more than the corn-fed Wagyu steers at the first and third slaughter dates. Hay-fed Angus steers grew slightly faster than hay-fed Wagyu steers. The hay-fed Angus calves were 40 kilograms heavier than the hay-fed Wagyu steers at weaning and 50 to 60 kilograms heavier than Wagyu steers at the second and third slaughter dates (Lunt *et al.*, 2005).

In general, marbling scores were lower in hay-fed steers than in corn-fed steers at both the U.S. and Japanese weight endpoints, but corn-fed Angus steers had higher marbling scores than corn-fed Wagyu steers at the U.S. endpoint. However, by the time the cattle reached the Japanese endpoint, both corn-fed and hay-fed Wagyu steers had much higher marbling scores than Angus steers (Figure 2.8; Chung *et al.*, 2006).



Figure 2.8: Marbling scores of Angus and Wagyu steers fed either corn- or hay-based diets (Chung *et al.*, 2006).

On the hay-based diet, marbling scores in Wagyu steers were the same as those in hayfed Angus steers at the U.S. endpoint and exceeded those of hay-fed Angus steers at the heavier weight of the Japanese endpoint. The marbling score of the hay-fed Wagyu fed to the Japanese endpoint was nearly as high as the marbling score of the corn-fed Angus steers raised to the same endpoint. One of the conclusions drawn from this study was that Wagyu steers can achieve superior marbling scores when fed hay- or pasture-based diets, which is a trait that is missing from other beef breeds raised in the U.S. (Chung *et al.*, 2006).

Due to no other U.S. beef breed being able to match the carcass quality of beef cattle as seen in Japan, the breeding strategy was to continue to breed within the U.S. Wagyu population. There are four old, inbred lines of Japanese Black cattle: the Takenotani-zuru, Bokura-zuru, Iwakura-zuru, and Shusuke-zuru (Mitsumoto *et al.*, 1989). Three of these inbred lines of cattle bloomed in the U.S: Tottori, Shimane, and Hyogo cattle. Hyogo cattle were selected for carcass quality, which is reflected in their greater amounts of intramuscular lipids at the 6th and 12th ribs, Tottori cattle were selected for their large size and strong back line, which negatively selected for carcass quality, and Shimane were selected for traits similar to Tottori but Shimane cattle retained a better carcass quality (Smith, 2015). High selection pressure for desirable marbling traits as seen in fullblood Japanese cattle along with a small effective population size has led to an increased risk of inbreeding depression within the breed population in the United States.

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CHAPTER 3

CASE STUDY OF INBREEDING WITHIN WAGYU CATTLE USING RESOURCES OF THE AMERICAN WAGYU ASSOCIATION, NATIONAL ANIMAL GERMPLASM PROGRAM AND A COOPERATOR BREEDING PROGRAM IN WYOMING

INTRODUCTION

Wagyu is a Japanese beef breed that was derived from native Asian cattle; though there were times in history Wagyu cattle were crossed with both beef and dairy breeds (Namikawa, 1980). Wagyu originated from six of the 47 political units, or prefectures, in Japan, and within the breed, each unit generated their own unique genetic line using different breeding objectives (Gaskins, 2008). In 1976, four Japanese Wagyu bulls were imported to the U.S. for research into meat quality and in 1992, an agreement was made between the U.S. and Japanese government to allow Japanese Wagyu dams to be exported from Japan. Following this agreement, additional bulls and dams arrived in the U.S. 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 U.S. within that time, creating a limited population of full-blood Wagyu cattle in the United States (Bennett, 2013).

Currently, Wagyu beef sales are increasing in the United States (Industry Research, 2020). As a result, three predominant breeding strategies have developed: 1) full-blood herds to maintain meat quality similar to that found in Japan, 2) a purebred upgrading program, and 3) cross breeding with Angus.

Extreme marbling is associated with full-blood Wagyu cattle and is assumed to be inherited. Utrera (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 often practiced (Lloyd *et al.*, 2017). Linebreeding is the mating of closely related individuals to concentrate desirable traits in a population (Lush, 1933). One of the challenges with continued linebreeding is that there is a high risk of inbreeding depression occurring, which is a decrease in mean performance due to the mating of relatives (Hieber, 2020). Inbreeding depression is at an especially high risk of occurring in breeds with a small effective population size. Effective population size (Ne) 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 (2014) estimated effective population size of full-blood Wagyu cattle registered in the United States from 4132 full-blood Wagyu cattle pedigrees for the period 1994 to 2011. The Ne averaged 17 between the years 1994 and 2011 with an increase to 58.1 in 2011. A minimum effective population size of 50 has been recommended by the Food and Agriculture Organization since an Ne of 50 will result in an inbreeding rate of one percent per generation which will safely conserve a population (FAO, 1992). Wu (1990) and Meuwissen (1994) recommended a minimum population size of 100 or more for livestock populations.

A frequently used breeding strategy is to crossbreed Wagyu with Angus or develop purebreds (Liu *et al.*, 2021). Instead of being able to advertise their product as full-blood Wagyu, breeders using these strategies would either market their product as purebred Wagyu or crossbred Wagyu. Full-blood Wagyu are considered to be 100% traceable to Japanese-derived herds with no evidence of crossbreeding or up-grading. Subsequently, purebred Wagyu are defined as being 87% pure Japanese Wagyu genetics (Lone Mountain Wagyu, 2018). While both

breeding strategies could decrease the risk of inbreeding depression, full-blood Wagyu meat has been profiled to have a unique umami flavor that other beef cattle breeds lack (Postal, 2018). Because of this unique flavor profile, consumers associate full-blood Wagyu with a higher quality of meat, as opposed to purebred.

A 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. A nucleus breeding system is a system where the traditional breeding population is divided into a small elite "nucleus" and a large "main" or multiplier population. The nucleus population is regenerated each generation by selective 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 fullblood Wagyu within and outside of Japan have small effective population sizes, the objective of this study was to estimate inbreeding levels between full-blood sires stored in the USDA-National Animal Germplasm Program (NAGP) semen repository and the full-blood dams from a herd established by a cooperator ranch 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).

MATERIALS AND METHODS

Data collection and editing

Pedigree data for the calculation of inbreeding coefficients were obtained for 30 American Wagyu Association (AWA)-registered full-blood Japanese Wagyu dams from a cooperator herd (Brush Creek Ranch, Saratoga, WY) with ages ranging from two years of age to 13 years 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 fourgeneration pedigree was created in Microsoft[®] Excel (2019) for individuals in these two groups. Inspection of the pedigree showed sire NR251A and dam NR251B were present in 18 of the 48 pedigrees. It was found that this could have been any sire or dam from Japan that created offspring imported to the United States, so both were recorded as "unknowns" in the analysis. The pedigree was then ordered from oldest to youngest.

Statistical Analysis

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

A = LDL'

where L was a lower triangular matrix containing the fraction of the genes that the individual animals inherit from their ancestors, and D was 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:

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

where A_{ii} was the *i*th diagonal element of **A**, which was equivalent to the inbreeding coefficients of animal *i* plus one (Meuwissen *et al.*, 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.

RESULTS AND DISCUSSION

Inbreeding coefficients were calculated for the 30 fullblood Wagyu dams and the 18 AI sires and summarized in Table 3.1, with a heat map shown in Figure 3.1, resulting in a total of 540 inbreeding coefficients between the 48 cattle.

Table 3.1. Summary Statistics of coefficients of inbreeding (COI) for 30 fullblood Wagyu dams and 18 AI sires

Trait ¹	Number	Mean	SD^2	Min	Max
COI	540	0.18979	0.08201	0	0.43173

 $^{1}COI = coefficient of inbreeding$ $^{2}SD = standard deviation$



Sires

Figure 3.1. A heat map visualizing the inbreeding coefficients calculated between 30 fullblood Wagyu dams and 18 AI sires. ^ACOI = coefficient of inbreeding.

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 Figure 3.1. The average inbreeding coefficient for this population was 0.190, with a range from zero to 0.43. This means that 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 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 presented. American Angus, Charolais, and Limousin cattle inbreeding trends never increase above 1% over those six years, 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 recommended level (Clodagh, 2018).

The information herein was compiled after the cooperating breeding program shared interest in learning about familial relations within their nucleus population of full-blood Wagyu. Due to the small effective population size, genetic drift, or the change in allele frequency due to chance, needs to be taken into account when developing a strategy to manage inbreeding. 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 the profile of a relatively rare breed with a small population size, high selection intensities are suggested to achieve selection goals at the cost of slower rates of genetic gain per generation (Blackburn *et al.*, 2018).

When studying inbreeding levels in Wagyu cattle using BovineSNP50 BeadChip genotype data, using 54,609 evenly spaced SNPs, and a ten-generation pedigree, it was reported that the inbreeding values estimated from the pedigree tend to underestimate homozygosity by descent among individuals (Scraggs *et al.*, 2013). With the use of genotypes, inbreeding coefficients could be more accurately estimated.

CONCLUSIONS

In this study, Wagyu cattle have high inbreeding coefficients, which supported our hypothesis. Along with a small effective population size, further use of linebreeding will greatly increase the risk of inbreeding depression occurring in U.S. Wagyu cattle. Further inbreeding analysis should be done within Wagyu cattle using genotypes to mitigate this challenge and more effectively conduct genetic improvement.

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CHAPTER 4

POPULATION STRUCTURE OF JAPANESE WAGYU CATTLE IN THE UNITED STATES

INTRODUCTION

Very little is known about the breed composition of Japanese Wagyu cattle. All modern cattle today, including Japanese Wagyu, are not direct descendants of old native cattle but descendants of mongrels between native and various kinds of imported cattle (Namikawa, 2018). While Dr. Namikawa (2018) did report on what imported breeds were crossed with native Japanese cattle to create the modern Japanese cattle breeds, the degree(s) of influence of these imported breeds differed across prefectures, and even among regions within prefectures. The crossbreeding practices at the time lacked consistency and the reason these breeds were selected was sparsely documented. So while it is known Japanese Wagyu cattle were created using crossbreeding strategies, the breeds and the extent to which they influenced the U.S. breed composition are unknown, and analyzing genetic relationships and studying genetic structure is an important step in understanding the demography and ancestral history of cattle populations (Manomamohan *et al.*, 2021).

Levels of crossbreeding within a population are often determined with pedigree information and herd data, but single nucleotide polymorphism (SNP) chip data provide more accurate information, and also allow for estimation of individual levels of continental and local admixture in populations due to past and recent migration events (Frkonja *et al*, 2012). Various genomic tools have been developed over the years to use SNP to analyze variation among DNA sequences to reconstruct population history in many species with model-based clustering becoming an effective approach to visualize genetic ancestry (McTavish *et al.*, 2013; Lawson *et*

al., 2018). Three of the most common approaches to analyzing population structure are principal component analyses (PCA), admixture graphs and phylogenetic trees. These approaches and their results are often presented together to ascertain if the assumptions of each approach are parallel and to validate features of the results of each analysis in the results of the other approaches (Lawson *et al.*, 2018).

In this study, we used high-density SNP genotypes from Japanese Wagyu cattle and various beef and dairy cattle breeds within the United States and ascertained the population structure and breed composition of modern U.S. Japanese Wagyu cattle in relation to other breeds. The objective was to investigate relationships between breeds as well as the proportion of admixture within the current Japanese Wagyu population in the United States to expand on our knowledge of the breed and help inform future breeding strategies to increase genetic diversity.

MATERIALS AND METHODS

Samples.

Cattle (Appendix; n = 414) were genotyped with Illumina BovineHD BeadChip (777,964 SNPs). The dataset consisted of 14 breeds, both beef and dairy. Cattle sampled were Japanese Wagyu, Ayrshire, Brahman, Brangus, Devon, Holstein, Milking Devon, Milking Shorthorn, Shorthorn, Simmental, South Devon, Angus, Jersey Island, and Brown Swiss (Table 4.1). The Brown Swiss, Jersey Island, Brangus, Brahman, Devon, Milking Devon, Milking Shorthorn, Shorthorn, Simmental, South Devon and 32 of the Japanese Wagyu samples were acquired from National Animal Germplasm Program's (NAGP-ARS-USDA) genetic resource semen collection (Fort Collins, CO, United States). The Angus samples were genotyped by the USMARC research center (ARS-USDA, Clay Center, NE, United States).

Breed	Number of samples	Acquired from	Description
	collected		•
Japanese Wagyu	119 ^a	NAGP-USDA, Fort Collins, CO	Consisted of samples from both full-blood and purebred
		Brush Creek Ranch, Saratoga, WY	Japanese Black bulls, donor dams, and heifers, as well as
		Bar R Cattle Co., Pullman, WA	Japanese Red steers.
	2	ST Genetics, Navasota, TX	
Ayrshire	2	GENEX Cooperative, Shawano, WI	Only two samples were available for HD analysis.
Brahman	36	NAGP-USDA, Fort Collin, CO	The same samples were used by Paim et al. (2020) when studying the genomic architecture during composite breed development.
Brangus	20	NAGP-USDA, Fort Collin, CO	The same samples were used by Paim et al. (2020) when studying the genomic architecture during composite breed development.
Devon	5	NAGP-USDA, Fort Collin, CO	Consisted of samples from AI sires born from the 1990s.
Holstein	11	Penn State, State College, PA	Consisted of four females and seven males. Cattle originated
		DI. Chad Dechow	from two sites born in the 1950s
Milking Devon	9	NAGP-USDA, Fort Collin, CO	Consisted of samples from artificial insemination sires born
	14		from the 1980s through the 2000s.
Milking	14	NAGP-USDA, Fort Collin, CO	Consisted of samples from artificial insemination sires born
Shorthorn	16	NACD USDA Fort Collin CO	from the 1950s through the 2000s.
Shorthorn	10	NAGE-USDA, Foit Collin, CO	from the 1960s through the 1990s
Simmental	15	NAGP-USDA, Fort Collin, CO	Consisted of samples from AI sires born from the 1970s.
South Devon	3	NAGP-USDA, Fort Collin, CO	Consisted of samples from AI sires born from the 1990s.
Angus	95	ARS-USDA, Clay Center, NE	The same samples were used by Paim et al. (2020) when
0			studying the genomic architecture during composite breed
Jersev Island	49	NAGP-USDA, Fort Collin, CO	Consisted of samples taken from cattle from the Island of
	-		Jersey.
Brown Swiss	20	NAGP-USDA, Fort Collin, CO	The samples were used by Porto-Neto et al. (2013) when
			studying the genomic divergence of zebu and taurine cattle.

Table 4.1. Description of the data with 14 cattle populations used in the analyses.

^aNumber of samples left after quality control.

The rest of the semen samples were contributed by breeders from the United States or purchased. DNA was extracted and genotyping was completed by Neogen Corporation's GeneSeek Operations (Lincoln, NE, United States). The decision to acquire samples from Simmental, Shorthorn, Brown Swiss, Ayrshire, Devon and Holstein samples were heavily influenced by Dr. Namikawa's (2018) reports on imported foreign breeds that were crossbred with native Japanese cattle. While there was an effort made to acquire additional samples, only two Ayrshire samples were available for analysis. The sampled Japanese Wagyu consisted of Japanese Red steers, Japanese Black donor dams and heifers, and Japanese Black bulls, both fullblood and purebred. Due to the evidence of heavy dairy influence, the Angus samples were selected to analyze the Japanese Wagyu samples against a beef breed. The Brahman samples were selected to analyze any indicine influence, and the Brangus samples were added as an intermediate composite between the Angus and the Brahman.

Quality Control

Due to the diversity of the breeds evaluated, along with the limited number of samples of within breeds, quality control and filtering of genomic data was only conducted on the main breed of interest, the Japanese Wagyu samples. All quality control was conducted in SNP and Variation Suite v8.9.1 (Golden Helix, Inc., Bozeman, MT). Samples and SNP with a call rate of <0.90 were removed. SNP with minor allele frequency <0.01 were removed and only autosomal SNPs were used. Markers that violated Hardy-Weinberg Equilibrium (p<0.001) were removed. The final number of SNPs after quality control was 593,592 SNPs. This filtered Japanese Wagyu dataset was then merged back into the dataset with the remaining breeds and that combined dataset was analyzed.

Genetic Diversity

Three analyses were used to evaluate the genetic diversity among these cattle: principal component analysis (PCA), Admixture, and a phylogenetic tree. The PCA was executed using SNP and Variation Suite v8.9.1 and the plotting of the first three components was visualized in R Studio using the ggbiplot package. The parameters for PCA analysis were set to find the first 10 components due to the calculated eigenvalues.

Genetic relationships among breeds and the level of admixture were evaluated using a model-based clustering software ADMIXTURE v1.3.0 (Alexander *et al.*, 2009). The cross-validation procedure (10-fold) was executed to estimate prediction errors for each K value, or the number of assumed populations (from 2 to 15). The value of K that minimizes the estimated prediction error, otherwise known as the optimal K value, represented the best predictive accuracy for the dataset. The plotting of the matrix of the maximization of the log-likelihood estimates were visualized in R Studio using the barplot command in the graphics package. Due to the small sample size of some of the populations and for best visualization of the results, only Japanese Wagyu, Brahman, Brangus, Simmental, Angus, Jersey Island, and Brown Swiss were highlighted in the plot.

The tree-based analysis was used to reconstruct historical relationships between the sampled populations and to test for the presence of gene flow using the Treemix 1.13 software (Pickrell & Pritchard, 2012). The program was executed with animals partitioned into 14 populations; one for each breed. To determine what breed to use as the root, a plot of the residuals from the fit of the model to the data was created, which represented pairwise admixture maximum likelihoods between populations that may not have been presented in the tree graphic because they were not well-modeled (Figure 4.1). Colors within the plot represent standard error

(SE) of the pairwise admixture maximum likelihoods between breeds with the color white equating to zero SE. Slight discoloration between the same breed could be the result of the effect of a small sample size. With Brahman (*Bos indicus*) being found to be an outlier, it was determined to be used as the root. Various runs of migration events were analyzed (3, 5, and 7), all of which showed Brangus distorting the placement of Angus on each tree. This in a decision to remove Brangus from the tree-based analysis.



Figure 4.1. Plot of the residuals from the fit of the model representing the pairwise admixture likelihoods between the 14 cattle populations analyzed. The color scale represents the standard error of the pairwise admixture likelihoods between breeds.

RESULTS AND DISCUSSION

The eigenvalues for the PCA showed ten as a reasonable number of principal components to be evaluated, which explained 78.73% of the variation within the data. Only the first three principal components were plotted, as they explained the majority of the variation out of the ten components (Figure 4.2). A three-dimensional representation of the PCA was also created to present a clearer picture of the interactions between data points (Figure 4.3).



Figure 4.2. The first three principal components (PC) using 14 cattle populations. Values between the parentheses at each axis show how much variation is explained by each principal component.



Figure 4.3. Image capture of five angles of a 3D representation of the first three principal components (PC) using 14 cattle populations. The axis values match those shown in Figure 4.2 of the 2D PCA.

In these first three principal components, each breed formed distinct clusters, with Shorthorn appearing to have a wider dispersal than the rest. Due to the high similarity between certain groups and to make the graph easier to comprehend, Milking Shorthorn was merged with Shorthorn and Milking Devon along with South Devon was merged with Devon for the PCA analyses. Japanese Wagyu clustered into two distinct groups: samples taken from the NAGP repository clustered closely with samples from the cooperating breeding herd (Brush Creek Ranch) and samples taken from present-day industry bulls from around the U.S. The Wagyu industry bulls clustered the most distant from the graph's origin, which we hypothesize was due to their high inbreeding levels. The Japanese Wagyu from the NAGP repository had an elongated dispersal that overlapped with the origin of the graph, indicating an admixed population.

Paim *et al.* (2019) found similar dispersal patterns in New World Angora goat populations. Within the Angora breed, the admixed Argentinian population was placed closer to the origin of a PCA graph, while the South African Angora with the higher inbreeding levels were the most distant from the origin, and the USA Angora were in an intermediate position. A weak genetic structure was also observed between breeds that were closely placed on the PCA graph and share genomic clusters. This suggested that genetic drift and selection had not separated those populations from old world progenitor groups (Paim *et al.*, 2019). Extrapolating from that observation, the distant clustering of present-day Japanese Wagyu could suggest that some of the modern-day Japanese Wagyu population have separated enough from their ancestral breed to become a distinct subpopulation through selection and genetic drift.

Due to the Japanese Wagyu being the breed of interest, a separate PCA was generated which focused on just full-blood versus purebred Japanese Wagyu cattle to determine whether this could be a factor of the separate clustering within the breed.



Figure 4.4. The first three principal components (PC) using 119 Japanese Wagyu cattle and distinguishing between full-blood and purebred Wagyu cattle. Values between the parentheses at each axis show how much variation is explained by each principal component.

As for the previous PCA, ten was shown to be a reasonable number of principal components to be evaluated which explained 96% of the variation within the data and only the first three principal components were plotted, as they explained the majority of the variation out of the ten components (Figure 4.4). A three-dimensional representation of the PCA was also created to present a clearer picture of the interactions between data points (Figure 4.5). In these first three principal components, the purebred cattle did not distinctly cluster away from the full-blood cattle. The purebred cattle remained within the group of NAGP and cooperating breeding herd cattle, which was primarily dispersed around the origin of the graph. The present-day industry bulls remained their own distinct cluster away from the origin of the graph.



Figure 4.5. Image capture of five angles of a 3D representation of the first three principal components (PC) using 119 Japanese Wagyu cattle and distinguishing between full-blood and purebred. The axis values match those shown in Figure 4.4 of the 2D PCA.

Gobena *et al.* (2018) found similar dispersal patterns between purebred Angus, purebred Brahman, and first- and multiple-generation Brangus cattle. Multiple-generations of Brangus – Brangus matings were more distant from first generation Brangus. First generation Brangus cattle showed less variation and were located along the line that connected the two clusters formed by the purebred breeds, which is expected in the case of recent two-way admixture (Patterson *et al.*, 2006). The pattern of variation seen in the cattle born from Brangus – Brangus mating was hypothesized to be due to the extended number of generations since the initial crossing of the parental breeds (Gobena *et al.*, 2018). This further supports the hypothesis that modern-day Japanese Wagyu formed a distinct subpopulation due to the number of generations of selection and genetic drift since the initial admixture event.



Figure 4.6. Plot of ADMIXTURE cross-validation error from K=1 through K=15. We chose K=7 to analyze the SNP data as the value that minimizes error.



Figure 4.7. Plot of model-based clustering (ADMIXTURE) results from K = 2 to 15 using 14 cattle populations. Each individual is represented by a vertical bar. Different colors indicate different clustering groups.

A plot of model-based clustering results were calculated using 14 cattle populations: Japanese Wagyu, Ayrshire, Brahman, Brangus, Devon, Holstein, Milking Devon, Milking Shorthorn, Shorthorn, Simmental, South Devon, Angus, Jersey Island, and Brown Swiss. A cross-validation procedure through the program ADMIXTURE showed K = 7 had the best predictive accuracy to evaluate the data (Figure 4.6).

The column in the middle of the plot consisted of the breeds that had a significantly reduced sample size (Devon, Holstein, Milking Devon, Milking Shorthorn, Shorthorn). Due to this and to conserve space on the plot, they were not individually labeled and instead labeled "Pooled Breeds" (Figure 4.7). At K = 2, all cattle were estimated to have both indicine and taurine influence with heavy taurine influence (represented by the color pink) shown in Angus cattle which averaged at 99.4%, and heavy indicine influence (represented by the color brown) shown in Japanese Wagyu and Brahman cattle which averaged at 85.5% and 95.6% respectively. When evaluating only Japanese Wagyu, there was a distinct difference in the amount of indicine introgression between the samples taken from the NAGP repository and the samples contributed by U.S. breeders, averaging at 87.1% and 55.7% respectively. This corroborates what was observed in the PCA analysis with split and distinct clustering of the two Japanese Wagyu groups.

A similar split was documented in the Jersey breed in a genetic investigation of the foundation of Jersey Island cattle as compared to the intensively selected United States cattle. The dataset analyzed consisted of 49 Jersey sires born between 1964 and 2004 from the Island of Jersey and comparable U.S. Jersey sires spanning the same years. The results provided insights into the divergence of three major subsets of the breed over decades of isolation as well as in response to selection (Huson *et al.*, 2020).

At K = 3, the indicine influence in Japanese Wagyu cattle was almost unobservable and the Wagyu breed consistently showed a complex ancestry across a range of K values and amongbreed genetic structure predominated as K increased. Paim *et al.* (2020) described similar relationships in the dynamics of genomic architecture in Brangus cattle. Admixture analysis was performed on Angus, Brahman, and Brangus cattle breeds and the results at K = 3 suggested that Brangus, while a composite breed of Angus and Brahman, became a unique breed after six generations. However genomic inbreeding will likely increase with advancing generations due to the Japanese Wagyu's origin as a composite breed (Paim *et al.*, 2020).

K = 9 and K = 15 were selected along with K = 2, K = 3, and the optimal K = 7 to demonstrate these patterns and how they change over time. McTavish *et al.* (2013) reported similar K = 2 results with Hanwoo and Wagyu and suggested a hybrid indicine-taurine origin. While their proportion of indicine influence was much smaller, they only sampled a total of 17 animals for both groups, which could be the reason for the discrepancy.

For the phylogenetic tree, different runs with different numbers of migration events were analyzed and the model that best fit the data supported seven as the appropriate number of migration events (Figure 4.8).



Figure 4.8. Population tree with 14 cattle populations from the Treemix 1.13 software using Brahman as the root and showing 7 migration events. The events are represented as a color scale based on their migration weight. The drift parameter models the strength of genetic drift along the tree. ^H The most recent common ancestor of all populations that are represented on the tree.

Migration events are represented by colored arrows leading from one breed to another with the weight of the event represented as a color scale. The first event happened between Brahman and Milking Shorthorn with a migration weight of zero, followed by events between Japanese Wagyu to Simmental, Milking Devon to Devon, Devon to Simmental, Ayrshire to Shorthorn, Angus to Shorthorn, and finally Shorthorn to South Devon. Japanese Wagyu was the closest branch to the root breed Brahman, which paralleled the Admixture K = 2 suggesting that Brahman and Japanese Wagyu as being the most similar with the two highest percentages of indicine influence. However, while there is similar indicine influence, Brahman and Japanese Wagyu are not comparable in other regards and are dissimilar when it comes to carcass characteristics. Shahrai *et al.* (2021) found that Japanese Wagyu have the highest percentage of intramuscular fat (IMF), or marbling, at 33.9% followed by Angus at 20.87% and Brahman at 12.17%. In a study that analyzed genetic effects on beef tenderness in *Bos indicus* composite and *Bos taurus* cattle, beef from the *Bos indicus* composites was tougher and aged slower, and these traits were associated with Brahman breed effects (O'Connor *et al.*, 1997). This suggests that while close in comparison to *Bos taurus* breeds due to their indicine influence, comparison within *Bos indicus* breeds could generate different results. Further investigation is recommended to analyze the differences in modern Japanese Wagyu and *Bos indicus* populations and the reasons behind their dissimilarities. These future investigations should also strive to include Japanese Wagyu cattle from more varied locations and potentially more cattle breeds as estimates based on genomic data could be biased or lose accuracy due to sample selection, which could be described as a failure to include sufficient samples to represent all parental breeds in the analysis (Patterson *et al.*, 2006).

Due to its small effective population size and high risk of inbreeding depression, future mating plans of Japanese Wagyu cattle should have increased emphasis on maintaining a substantial population size at the expense of genetic uniqueness to ensure future adaptability. These matings could be informed with the results of future investigations that focus on the potential *Bos indicus* cattle breeds that Japanese Wagyu cattle consist of. Based on pedigree analyses, it was demonstrated that recent inbreeding has more detrimental effects compared to ancestral inbreeding, especially for production traits (Doekes *et al.*, 2019). So if the focus of a mating plan is to maintain the increased marbling trait that is represented in full-blood Japanese Wagyu cattle, our results suggest a breeding strategy could utilize matings between modern-day Japanese Wagyu cattle and older Japanese Wagyu genetics as to retain the desired trait while

mitigating the deleterious effects of inbreeding as much as possible while mating within the breed.

CONCLUSION

These genotype analyses together corroborated the hypothesis that Japanese Wagyu is an admixed population. While initially it was thought that Japanese Wagyu would be derived from a similar ancestor population as Angus cattle with high *Bos taurus* influence, this research suggested that Japanese Wagyu originated from an ancestor population of *Bos indicus* influences and admixed at low percentages with numerous other *Bos taurus* breeds.

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APPENDIX

LIST OF CATTLE SAMPLED FOR HIGH-DENSITY GENOTYPING

Sample ID	Breed	Sample ID	Breed	Sample ID	Breed
8867	Wagyu	171	Wagyu	NE00993069	Wagyu
8868	Wagyu	196	Wagyu	AF00546005	Wagyu
8869	Wagyu	264	Wagyu	NE00993070	Wagyu
8870	Wagyu	281	Wagyu	NE00993074	Wagyu
8882	Wagyu	735	Wagyu	AF00545903	Wagyu
8883	Wagyu	783	Wagyu	AF00545908	Wagyu
8884	Wagyu	1071	Wagyu	AF00545905	Wagyu
8885	Wagyu	1201	Wagyu	AF00545910	Wagyu
10071	Wagyu	1283	Wagyu	AF00545902	Wagyu
10072	Wagyu	1454	Wagyu	AF00545907	Wagyu
10073	Wagyu	2105	Wagyu	AF00545904	Wagyu
10074	Wagyu	2128	Wagyu	AF00545906	Wagyu
14144	Wagyu	2451	Wagyu	AF00545901	Wagyu
14145	Wagyu	7037	Wagyu	AF00545909	Wagyu
14146	Wagyu	9038	Wagyu	271KB8940	Wagyu
14147	Wagyu	9276	Wagyu	271KB8931	Wagyu
14148	Wagyu	15110	Wagyu	54KB140	Wagyu
14149	Wagyu	NE00993077	Wagyu	54KB139	Wagyu
14211	Wagyu	NE00993078	Wagyu	99KB8941	Wagyu
14212	Wagyu	AF00546008	Wagyu	90KB1951	Wagyu
14213	Wagyu	NE00993072	Wagyu	90KB1952	Wagyu
17689	Wagyu	NE00993062	Wagyu	271KB8910	Wagyu
17690	Wagyu	NE00993075	Wagyu	090KB1961	Wagyu
17691	Wagyu	AF00546006	Wagyu	271KB8908	Wagyu
17692	Wagyu	NE00993071	Wagyu	271KB8920	Wagyu
17693	Wagyu	NE00993076	Wagyu	54KB118	Wagyu
17694	Wagyu	NE00993061	Wagyu	271KB8934	Wagyu
17695	Wagyu	NE00993073	Wagyu	271KB8930	Wagyu
17696	Wagyu	NE00993065	Wagyu	271KB8921	Wagyu
17697	Wagyu	AF00546009	Wagyu	551KB8921	Wagyu
17698	Wagyu	AF00546010	Wagyu	203KB01327	Wagyu
48828	Wagyu	NE00993059	Wagyu	46-7787W	Wagyu
1	Wagyu	NE00993058	Wagyu	89-7891W	Wagyu
22	Wagyu	NE00993064	Wagyu	65-5248	Wagyu
23	Wagyu	AF00546007	Wagyu	90-7815W	Wagyu
118	Wagyu	NE00993063	Wagyu	67-7246U	Wagyu

Sample ID	Breed	Sample ID	Breed	Sample ID	Breed
120	Wagyu	NE00993066	Wagyu	3-737	Wagyu
140	Wagyu	NE00993067	Wagyu	45-7883W	Wagyu
141	Wagyu	NE00993068	Wagyu	77-7385U	Wagyu
1-7417U	Wagyu	14118	Brangus	8138	Milking Devon
43-97284	Wagyu	3982	Brangus	8139	Milking Devon
001AY00345	Ayrshire	14108	Brangus	8140	Milking Devon
001AY00347	Ayrshire	14128	Brangus	57664	Milking Devon
4552	Brahman	3977	Brangus	57665	Milking Devon
6011	Brahman	4544	Brangus	2789	Milking Shorthorn
7683	Brahman	14116	Brangus	2796	Milking Shorthorn
7684	Brahman	17945	Brangus	7635	Milking Shorthorn
8853	Brahman	14121	Brangus	7636	Milking Shorthorn
8857	Brahman	17984	Brangus	7637	Milking Shorthorn
8858	Brahman	R9694111	Brangus	9933	Milking Shorthorn
9235	Brahman	14115	Brangus	9935	Milking Shorthorn
9236	Brahman	17985	Brangus	9939	Milking Shorthorn
9237	Brahman	3981	Brangus	15201	Milking Shorthorn
9238	Brahman	17986	Brangus	19726	Milking Shorthorn
9249	Brahman	9965	Brangus	55407	Milking Shorthorn
9255	Brahman	8741	Brangus	55565	Milking Shorthorn
14135	Brahman	14127	Brangus	10346	Milking Shorthorn
15433	Brahman	17946	Brangus	55577	Milking Shorthorn
15558	Brahman	9962	Brangus	2996	Shorthorn
15816	Brahman	1169	Devon	2999	Shorthorn
15818	Brahman	7834	Devon	3095	Shorthorn
15820	Brahman	8583	Devon	3155	Shorthorn
15822	Brahman	8984	Devon	3402	Shorthorn
15823	Brahman	17999	Devon	4711	Shorthorn
15824	Brahman	1	Holstein	4715	Shorthorn
15826	Brahman	5	Holstein	8652	Shorthorn
15827	Brahman	8	Holstein	8653	Shorthorn
15828	Brahman	2869	Holstein	8654	Shorthorn
15829	Brahman	2875	Holstein	3156	Shorthorn
15831	Brahman	2873	Holstein	6396	Shorthorn
15833	Brahman	2872	Holstein	6401	Shorthorn
15835	Brahman	3431	Holstein	6431	Shorthorn
15838	Brahman	3432	Holstein	6446	Shorthorn
15844	Brahman	3434	Holstein	6448	Shorthorn
15845	Brahman	3436	Holstein	1666	Simmental
15847	Brahman	8105	Milking Devon	1671	Simmental

Sample ID	Breed	Sample ID Breed		Sample ID	Breed
15850	Brahman	8129	Milking Devon	1681	Simmental
20427	Brahman	8130	Milking Devon	8592	Simmental
20430	Brahman	8135	Milking Devon	8766	Simmental
8769	Simmental	14201376	Angus	13058662	Angus
8772	Simmental	14187839	Angus	14327307	Angus
8773	Simmental	12396224	Angus	15034593	Angus
8774	Simmental	13839683	Angus	14311951	Angus
8775	Simmental	13588640	Angus	13818764	Angus
8779	Simmental	14088423	Angus	14769662	Angus
8780	Simmental	13875838	Angus	15585939	Angus
8782	Simmental	15313140	Angus	13880818	Angus
8784	Simmental	15150605	Angus	15349689	Angus
8795	Simmental	13512009	Angus	13791489	Angus
7833	South Devon	13850307	Angus	15163839	Angus
26512	South Devon	13806971	Angus	15543702	Angus
10078	South Devon	15148659	Angus	12075716	Angus
14660859	Angus	13395329	Angus	14737017	Angus
13739532	Angus	12760345	Angus	13448453	Angus
13062750	Angus	13050780	Angus	14551206	Angus
13752642	Angus	12500199	Angus	14740749	Angus
15539210	Angus	13395344	Angus	14844714	Angus
14474596	Angus	13936986	Angus	11951654	Angus
13828202	Angus	13320150	Angus	13896250	Angus
15012747	Angus	13582536	Angus	13761928	Angus
11567326	Angus	13360311	Angus	13498476	Angus
13776378	Angus	14844711	Angus	12309327	Angus
11973367	Angus	13447282	Angus	13054003	Angus
11935889	Angus	12783540	Angus	12270349	Angus
15262910	Angus	13119152	Angus	11601330	Angus
14560689	Angus	15355003	Angus	11520398	Angus
13433023	Angus	13009379	Angus	11373742	Angus
15603560	Angus	15147477	Angus	12007667	Angus
12048084	Angus	13286230	Angus	12173581	Angus

Sample ID	Breed	Sample ID	Breed	Sample ID	Breed
11447335	Angus	JE_ISL-ISLE_JE_13	Jersey	JE_ISL-ISLE_JE_43	Jersey
10705768	Angus	JE_ISL-ISLE_JE_14	Jersey	JE_ISL-ISLE_JE_44	Jersey
11391800	Angus	JE_ISL-ISLE_JE_15	Jersey	JE_ISL-ISLE_JE_45	Jersey
12588758	Angus	JE_ISL-ISLE_JE_16	Jersey	JE_ISL-ISLE_JE_46	Jersey
11741667	Angus	JE_ISL-ISLE_JE_17	Jersey	JE_ISL-ISLE_JE_47	Jersey
11788302	Angus	JE_ISL-ISLE_JE_18	Jersey	JE_ISL-ISLE_JE_48	Jersey
10776479	Angus	JE_ISL-ISLE_JE_19	Jersey	JE_ISL-ISLE_JE_49	Jersey
11994601	Angus	JE_ISL-ISLE_JE_20	Jersey	7469	Brown Swiss
11647343	Angus	JE_ISL-ISLE_JE_21	Jersey	7488	Brown Swiss
11569005	Angus	JE_ISL-ISLE_JE_22	Jersey	7491	Brown Swiss
11869992	Angus	JE_ISL-ISLE_JE_23	Jersey	7494	Brown Swiss
11223766	Angus	JE_ISL-ISLE_JE_24	Jersey	7497	Brown Swiss
12530601	Angus	JE_ISL-ISLE_JE_25	Jersey	7501	Brown Swiss
12077888	Angus	JE_ISL-ISLE_JE_26	Jersey	7502	Brown Swiss
12422806	Angus	JE_ISL-ISLE_JE_27	Jersey	7507	Brown Swiss
12170393	Angus	JE_ISL-ISLE_JE_28	Jersey	7509	Brown Swiss
12284312	Angus	JE_ISL-ISLE_JE_29	Jersey	7504	Brown Swiss
12448729	Angus	JE_ISL-ISLE_JE_30	Jersey	7505	Brown Swiss
JE_ISL-ISLE_JE_1	Jersey	JE_ISL-ISLE_JE_31	Jersey	7506	Brown Swiss
JE_ISL-ISLE_JE_2	Jersey	JE_ISL-ISLE_JE_32	Jersey	7483	Brown Swiss
JE_ISL-ISLE_JE_3	Jersey	JE_ISL-ISLE_JE_33	Jersey	7499	Brown Swiss
JE_ISL-ISLE_JE_4	Jersey	JE_ISL-ISLE_JE_34	Jersey	7468	Brown Swiss
JE_ISL-ISLE_JE_5	Jersey	JE_ISL-ISLE_JE_35	Jersey	1498	Brown Swiss
JE_ISL-ISLE_JE_6	Jersey	JE_ISL-ISLE_JE_36	Jersey	7465	Brown Swiss
JE_ISL-ISLE_JE_7	Jersey	JE_ISL-ISLE_JE_37	Jersey	7498	Brown Swiss
JE_ISL-ISLE_JE_8	Jersey	JE_ISL-ISLE_JE_38	Jersey	7466	Brown Swiss
JE_ISL-ISLE_JE_9	Jersey	JE_ISL-ISLE_JE_39	Jersey	7464	Brown Swiss
JE_ISL-ISLE_JE_10	Jersey	JE_ISL-ISLE_JE_40	Jersey		
JE_ISL-ISLE_JE_11	Jersey	JE_ISL-ISLE_JE_41	Jersey		
JE ISL-ISLE JE 12	Jersey	JE ISL-ISLE JE 42	Jersey		