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GENETIC ORIGIN OF ARAPAWA SHEEP AND ADAPTATION TO A FERAL LIFESTYLE

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SUMMARY

This work aimed to investigate the population history and patterns of genetic diversity present within the isolated population of New Zealand Arapawa sheep. In order to identify genetic regions associated with reversion to a feral lifestyle, a selection sweep analysis was performed comparing 40 Arapawas to related breeds using Wright's fixation index (F_{ST}). Comparisons were graphed as the moving average of 5 F_{ST} values. A threshold of 0.25 was used to identify significant regions; 8 genomic regions were identified for the Arapawa and Florida Gulf Coast Native, 9 for the Arapawa and Castellana and 3 for the Arapawa and Australian Merino breed pair comparisons. One region on chromosome 2 was identified in all three comparisons with two underlying genes, CFDP2 and NAB1. Other genes identified were RXFP2, IFT88, SLC9A3, HERC2, NIPA1, NIPA2 and DACH2. The current work confirms Arapawa sheep are an important reservoir of unique gene variants available to the New Zealand sheep industry.

INTRODUCTION

The feral sheep of Arapawa Island are thought to be the oldest feral flock in New Zealand. Their isolated island location makes them an excellent example of natural selection accompanying reversion to a feral existence. Anecdotal information suggests the Arapawa was derived from an Australian Merino flock introduced and farmed on the island in 1867. However, recent research suggests the New Zealand feral Arapawa sheep are most closely related to the Gulf Coast Native (GCN) breed, which in turn comprises a significant component of the Castellana (Young *et al.* 2011). Further studies into the GCN have now found two separate lines of the GCN, the Florida and the Louisiana (Kijas *et al.* 2012a).

Phenotypically, Arapawa sheep are unique, differing from domestic sheep in New Zealand as summarised by Orwin and Whitaker (1984). Arapawa sheep have predominantly black skin and wool colouration, with white on the distal part of the tail and a white crown which can extend down the face and throat. Ewes are generally polled, with some growing small scurs; males have large curled horns with approximately 10% being polled. Arapawa sheep are small bodied with long legs and males weigh 51kgs and females 38kg in the wild. Fleece weight is low at approximately 2kgs per year, with shedding occurring in some animals. The wool has high bulk and fibre diameter of approximately 22 µm. Ewes can ovulate throughout the year, lambs are born small with a hairy coat which is later shed. As both the Arapawa and particularly the GCN are reportedly naturally resistant to parasites and footrot, these animals may be a reservoir of unique gene variants for sheep breeds more commonly farmed in New Zealand.

The current work attempts to identify which of the two GCN lines is most closely related to the Arapawa, and gene regions that have been subject to selection sweeps as part of the Arapawa reversion to a feral lifestyle.

MATERIALS AND METHODS

Resource. The 40 Arapawa animals described by Young *et al.* (2011) were sourced from New Zealand flocks. The 56 Australian Merinos, 23 Castellana and 95 GCN (40 Florida and 55

Louisana origin) were sourced from the ovine HapMap project (Kijas *et al.* 2012b). All animals were genotyped using Illumina's OvineSNP50 Beadchips (Kijas *et al*. 2012b). All genotypes were quality checked before analysis. The SNPs were discarded if the minor allele frequency was <0.02 in a population comparison or if the call rate was less than 95%.

STRUCTURE. Model based clustering using SNP genotypes from 132 individuals was performed using the program STRUCTURE (Pritchard *et al.* 2000). Three runs were performed at K = 2 - 4, where K is the number of assumed subpopulations. The admixture model was applied and runs comprised 5000 burn-in replications followed by 5000 run lengths.

Wright's F_{ST}. SNPs not aligned on Ovine genome v3 were discarded. Wright's fixation index (F_{ST}) was calculated for each breed pair as $(H_T - H_S)/ H_T$, where H_T is the expected heterozygosity for the overall breed pair population, and H_S is the expected heterozygosity of the subpopulation. The F_{ST} is the extent of genetic difference between subpopulations. Smoothed estimates were calculated as the moving average with a window of 5 (WIN5) SNPs and plotted for Arapawa versus each of the other breeds (Florida GCN, Castellana and Australian Merinos). A threshold level of a WIN5 F_{ST} value greater than 0.25 was chosen and the identified regions were examined using Ovine genome v3 to identify underlying genes.

RESULTS AND DISCUSSION

Structure and principal components. Model based clustering was used to examine the relationship between three populations: the Arapawa and two lines of the Gulf Coast Native breed, the best solution (K = 3) is shown in Figure 1. The Florida GCN line had the highest proportion of common ancestry with Arapawa, contributing 27% of the dark grey component and only 6% of the black. The light grey component (67%) of the Arapawas has been contributed from elsewhere. The Louisiana native is believed to be derived from animals introduced by explorers from Latin America, whereas the Florida natives are most likely founded from sheep arriving with settlers on the east coast (Kijas *et al.* 2012a). This supports the theory stated in Young *et al.* (2011) that it was possible that Arapawa sheep were introduced to New Zealand by whalers in the early 19th Century.

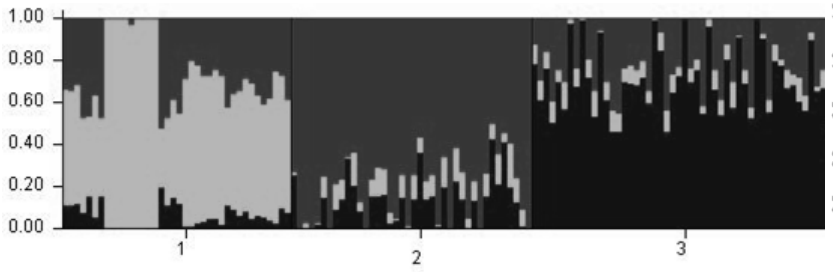

Figure 1. Admixture analysis using 5000 SNP, for Arapawa (1), Florida GCN (2) and Louisiana GCN (3). For each animal, the proportion of 3 genomic components (light grey, dark grey and black) is given on the Y axis.

Selection sweep. Genotypes from breed pairs were used to search for genomic regions with signatures of selection (Arapawa versus Florida GCN, Castellana or Merino). A number of significant regions identified in the comparisons with Arapawa e.g. Figure 2 shows the moving window of 5 (WIN5) F_{ST} values across the genome for the Arapawa versus Florida GCN comparison. This comparison identified 8 significant peaks (> 0.25 WIN5F_{ST} value) on chromosomes 1, 2, 9, 10 and X. Table 1 lists significant regions from all the comparisons and the major genes identified within.

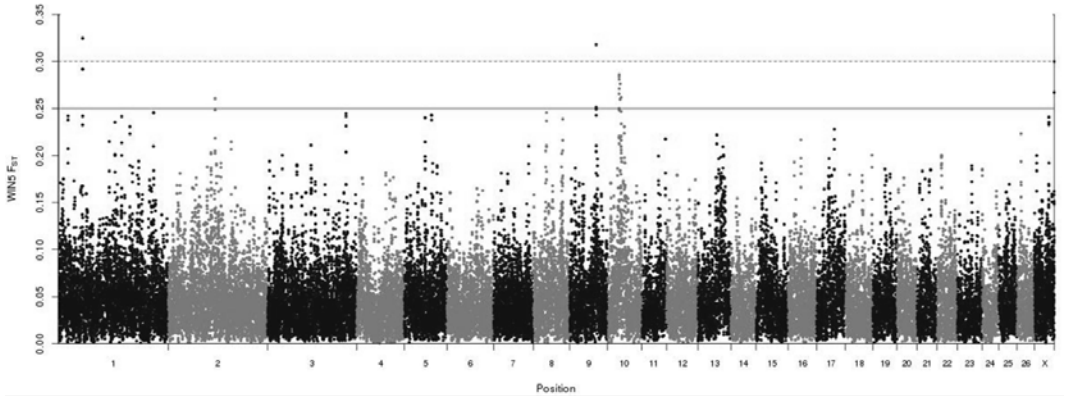

Figure 2. Manhattan plot of the moving window of 5 (WIN5) F_{ST} values between Arapawa and Florida GCN. Ordered on Ovine genome v3, WIN5F_{ST} = 0.25 (solid line), WIN5F_{ST} = 0.30 (dash line).

Table 1. The number of regions, chromosomes and significant known genes found under the peaks with WIN5 F_{ST} values > 0.25 for each breed comparison with Arapawa.

Comparison	Regions	Chromosomes	Genes
Florida GCN	8	1,2,9,10,X	NAB1, CFDP2, RXFP2
Castellana	9	1,2,4,6,9,10,13	HERC2, NIPA1, NIPA2, NAB1, CFDP2, RXFP2, IFT88
Merino	3	2,16	NAB1, CFDP2, SLC9A3

The polled/horns gene relaxin/insulin-like family peptide receptor 2 (RXFP2) was identified in both the comparisons of Arapawa to Florida GCN and Castellana. This region acts as a positive control, as this gene is known to be associated with polledness in sheep (Kijas *et al.* 2012b). Most Arapawa and Merino rams are horned and the GCN Florida and Castellana breeds are predominantly polled. The results suggested that there had been natural selection for horns once animals were introduced to Arapawa Island, as the selection sweep was notable, based on diversity reduction.

The gene SLC9A3 (solute carrier family 9, subfamily A, member 3) has been associated with pH regulation in mice. (Schultheis *et al.* 1998). This gene was identified in the Arapawa/ Merino comparison, with reduced genetic diversity in Merinos. Merino meat has a higher ultimate pH than crossbreds (Young *et al.* 1993) and high pH has been associated with undesirable flavours (Hopkins and Fogarty 1998). It would be interesting to assess the meat quality of the Arapawa as it may be leaner like the Merino, yet with a lower pH.

The genes: HECT and RLD domain containing E3 ubiquitin protein ligase (HERC2), non-imprinted in Prader-Willi/Angelman syndrome 1 and 2 (NIPA1 and NIPA2) are within the region associated with the paternally imprinted Prader-Willi syndrome and the maternally imprinted Angelman syndrome (Cassidy *et al.* 2012). In this analysis, selection sweep was towards homozygosity in the Arapawas. HERC2 has also been associated with hair colour in cattle (Han *et al.* 2008) and in a long haplotype block in Spanish Churra sheep (Garcia-Gamez *et al.* 2012).

The intraflagellar transport 88 (IFT88) gene located on chromosome 10 is a homolog to mouse TG737 associated with recessive polycystic kidney disease (Moyer *et al.* 1994). Reduced genetic diversity was observed for Arapawa in this region.

One region on chromosome 2 was identified in all three breed pair comparisons, with reduced diversity observed for Merino and Castellana. Two genes were identified in this region. The gene craniofacial development protein 2 (CFDP2/*p97bcnt*) created by gene duplication of *bcnt/cfdp1* (Iwashita *et al*. 2006). There are 48 copies of CFDP2 in Ovine genome v3 and association of intra-muscular fat with a copy on chromosome 14 was found in a genomic selection study of carcass and meat quality traits in Australian sheep (Daetwyler *et al.* 2012). The second gene in this region, NGFI-A binding protein (NAB1), is highly expressed in cardiac muscle and is implicated as a regulator of pathological cardiac growth (Buitrago *et al.*, 2005).

CONCLUSION

The Arapawa shared the highest proportion of common ancestry with the GCN Florida, supporting previous evidence that they were introduced to New Zealand by Whalers in the 19th century. Nine genes were identified as under selection from the F_{ST} analysis, seven are described above. The phenotypes associated with reversion to a feral lifestyle are unknown for most genes identified as under selection. The exceptions are horns and coat colour. However, this study provides a list of candidate genes for future studies of domestication.

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