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## BRIEF COMMUNICATION: Arapawa: a novel New Zealand sheep breed of distinct origin

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### INTRODUCTION

Arapawa sheep are a feral breed found on Arapawa Island in the Marlborough Sounds, New Zealand. Their origin is not documented, but it is thought to be one of the oldest feral flocks in New Zealand dating back to approximately mid 1800s (Orwin *et al.*, 1984). There are numerous theories of how Arapawa sheep came to be on the island and what breed they originated from. The most commonly recognised origin is that the sheep have derived from an Australian Merino flock that was introduced and farmed on the island in 1867. Another suggestion is that Arapawa sheep are descendants from mixed breed European sheep that were brought to the island to be farmed by whalers who originally occupied the island in the 1820s. Perhaps the most curious theory is that the sheep were dropped off on the island by Spanish galleons in the 1500s (New Zealand Rare Breeds Conservation Society, 2002).

Phenotypically, Arapawa sheep are unique from other domestic sheep in New Zealand as described by Orwin *et al.* (1984). Arapawa sheep have predominantly black skin and wool colouration, typically with a white crown which can extend down the face and throat, with a white area on the distal part of the tail. However, totally white individuals also exist. Ewes are generally polled with some growing small scurs. Males characteristically have large curled horns with approximately 10% being polled. Arapawa sheep are small bodied with long legs. On average males weigh 51 kgs and females 38 kg in the wild. Fleece weight tends to be low at approximately 2 kg per annum with shedding occurring in some animals. The wool is highly crimped with a mean fibre diameter of approximately 22 µm (Orwin, *et al.*, 1984). As a feral breed they are reputedly very resistant to footrot, flystrike and parasites (Litherland *et al.*, 1992). Ewes can ovulate throughout the year, lambs and are born small with a hairy coat similar to that of a Merino (Schinckel., 1954), which is later shed.

New Zealand has significant barriers to importation of new breeds. It is therefore of interest

to determine the origin of these sheep. Potentially they may be a reservoir of unique gene variants for mainstream farmed animals. They also act as an excellent documented reversion to a feral existence because their island location has restricted ongoing gene transfer from domesticated sheep. This isolation allows investigation of genes that have been naturally selected during this process, provided matching animals of a related domesticated control breed are available. Finally, as with all feral breeds the true level of inbreeding is unknown. This is important in long term conservation strategies. The current work attempts to identify their origin and current inbreeding level and is part of a longer term project to identify what genetic selection has occurred during the reversion to a feral existence.

### MATERIAL AND METHODS

Blood samples were collected from a total of 60 Arapawa animals that were “purebred” animals descended from original wild capture. They consisted predominantly of ewes from six farms located throughout Southland, Otago and Canterbury. Their coat colouration was predominately black with white points, but a proportion of animals displayed a variety of colouration patterns. DNA was extracted from the blood samples according to a standard protocol (Montgomery & Sise, 1990). Forty of the least related animals were genotyped with the Illumina OvineSNP50 Beadchip. These were then compared with genotypes of 1,000 European derived sheep from the HAPMAP (2002) project using principal component analysis (PCA). Uninformative or unreliable single nucleotide polymorphism (SNP) markers (call rate  $\leq 99\%$ , MAF  $\leq 2\%$ , Hardy-Weinberg  $P < 1e-6$ ) were discarded, and the remaining SNPs used in this analysis. A region was defined as homozygous when the value of a spline using PROC TRANSREG of SAS with 20 knots per chromosome fitted to the relationship between SNP homozygosity and genome position, exceeded 0.95 for at least two adjacent SNPs. The length of the homozygous region was taken as the length spanned by SNPs where the spline exceeded 0.95.

**TABLE 1:** Summary data from the SNP50 Beadchip for 48,706 single nucleotide polymorphisms analysed in samples of blood taken from Arapawa sheep.

Parameter	Number
Number of sheep	40
Number of fixed alleles	1.45
Average minor allele frequency	0.263
Standard deviation of minor allele frequency	0.145
Hardy-Weinberg P values less than 0.000001	0

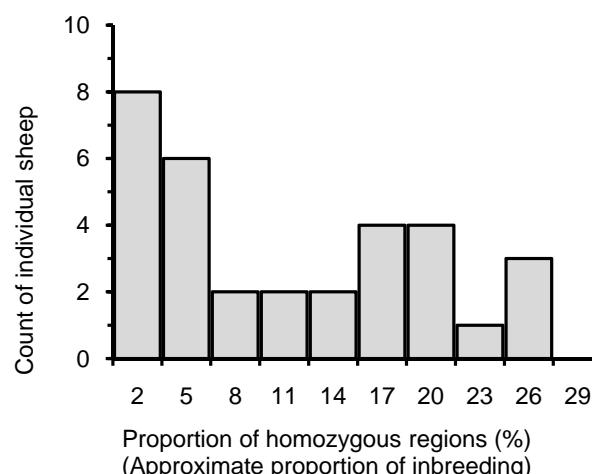
## RESULTS

After data cleaning, 48,706 single nucleotide polymorphisms were available for analysis (Table 1). Their mean minor allele frequency was 0.26 with a standard deviation of 0.14 after discarding uninformative markers including fixed alleles. This is a slightly lower genetic diversity than for many breeds where the values for sheep are typically closer to 0.31 (J.C. McEwan, Unpublished data) and suggest that small numbers and isolation have reduced genetic diversity.

Inbreeding estimates based on the genotype data, reflect the result of inbreeding over many generations. These had a wide range between 0 and 28.8%, averaging 11% (Figure 1). The distribution appears bi-modal, indicative of uncontrolled natural mating of close relatives.

The PCA plot that best represented Arapawa from other breed patterns was constructed from the first and fifth dimensions. The first dimension separates Northern from Southern European breeds while the fifth dimension separates the various Iberian and Merino derived breeds. The plot shows that the Gulf Coast Native clustered in a similar

**FIGURE 1:** Percentage of homozygous regions, as an estimate inbreeding, for 32 individual Arapawa sheep that were considered to be unrelated from six flocks.

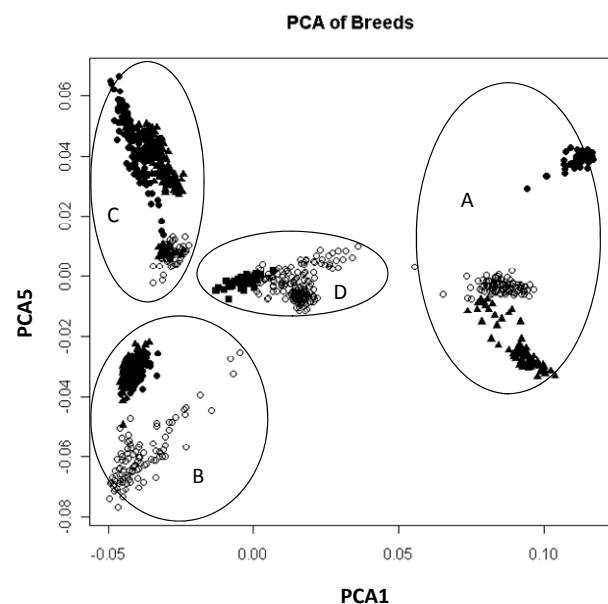


position to that of the Arapawa, with the Castellana breed nearby. On the plot these two breeds are intermediate between Merino/southern European and English/Northern European breeds, suggesting the Arapawa are primarily European origin, but distinct from the Australian Merinos.

## DISCUSSION

In a small population, such as the Arapawa sheep, inbreeding is a major consideration in the conservation of the breed. Historically, data regarding inbreeding were unavailable for this breed. The single nucleotide polymorphism chip results offer potential to identify both the level of inbreeding and also the level of co-ancestry between individuals. Using this information will allow a breeding plan to be designed to reduce future inbreeding. In a normal domestic sheep population an inbreeding value of around 3% is observed using this method (K.G. Dodds, Unpublished data) whereas the Arapawa was higher values, averaging 11%. Inbreeding was also variable and reflected the unmanaged matings of the natural population and the disorderly nature of conservation since they have

**FIGURE 2:** Plot of principal component analysis of sheep breeds from the HapMap data set.



In region A: Open circle = Poll Dorset; Filled triangle = Scottish Texel; Filled circle = Border Leicester.

In region B: Filled triangle = Australian Merino; Filled circle = Poll Merino; Open circle = Rambouillet.

In region C: Filled circle = Churra; Filled triangle (upper group) = Lacaune; Open circle = Ojalada; Open circle = Rasa Aragonesa (lower group); Open circle = Finn sheep. Ojalada and Rasa Aragonesa overlap each other.

In region D: Black square = Arapawa; Filled triangle = Castellana; Open circle = Gulf Coast Native.

been removed from the island. However, given their history of possibly less than one hundred individuals isolated for over 150 years it is perhaps surprising that the level observed is not significantly higher. These inbreeding values show there is still reasonable genetic diversity but suggest future mating management is desirable.

Genetically the closest breeds to Arapawa (Figure 2) are the Gulf Coast Native and the Castellana, with the breeds overlapping in the case of the Gulf Coast Native. Surprisingly, the pattern for the Arapawa did not match either the improved Merino or Merino-derived breeds in the sample, including several strains from Australia. Previously, many have suggested that the most probable historical origin was a shipment of early Australian Merinos. The Gulf Coast Native sheep have similar characteristics to the Arapawa breed with most rams, and some ewes, being horned with little wool on their faces and legs. Most are white with a few being black or brown (American Livestock Breeds Conservancy, 2009).

The Gulf Coast Native in the southern United States was derived from an admixture of Spanish breeds first arriving in the 1500s including most likely the Castellana breed as pure bred Merinos were too valuable to export (American Livestock Breeds Conservancy, 2009). There were subsequent infusions of other breeds into the Gulf Coast Native, probably including those of northern European origin. This is evident in the wide scatter observed along the first principal component axis. The direct importation of the Castellana or a closely related Iberian breed, is an alternative origin for the Arapawa. These results are consistent in both cases with an Iberian or Iberian composite animal being introduced by Spanish or other sailors, although they do not definitely prove such an origin. The final possibility is that they are a hybrid of English and Merino breeds introduced to the island after 1867 that have subsequently undergone natural selection for their colouration and other traits. However, this hypothesis is unlikely based on the Arapawa breed's plot location and spread being off the axis connecting Australian Merino and Northern European breeds. Similarly, the breed scatter is consistent with a relatively homogenous, possibly Iberian, origin. This new information restricts theories of how the sheep came to be on the island. The possibility still remains that they potentially pre-date the importation of Australian Merinos to the island. This could be confirmed by examining haplotypes unique to certain breeds. Unfortunately the genotyping density used in this study is insufficient to fully explore possible admixture scenarios. This work will be undertaken in the future. Hence the legend that Arapawa sheep were released on to the island by the Spanish in the 1500s

remains a possibility. Now that we have identified the likely origin of the breed, the next step is to compare these animals with their domesticated close relatives in order to identify chromosomal regions that have been under selection in their reversion to a feral lifestyle.

In conclusion, we have used new genomic technologies to examine the origin of a feral sheep breed. The results are of immediate use in identifying the current level of inbreeding and co-ancestry. This will aid future conservation efforts. Light has also been thrown onto the possible origin of Arapawa sheep. Two theories are plausible at this stage, deposition of Iberian sheep at an early date perhaps by a Spanish ship or alternatively a Merino-Northern European composite that has subsequently developed similar phenotypic traits via natural selection. The current evidence favours the first, but does not exclude the latter possibility. These two theories will be tested based on their haplotype composition during future work.

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