The Castlemilk Moorit: the continuing story of the search for the golden fleece

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Fascinated by the - sometimes conflicting - stories around the development of the Castlemilk Moorit breed, some time ago we embarked on the adventure of searching for its origins. Having first dived into socio-historical sources, we are now happy to complement that analysis with our first findings of research into the genetic origin of the breed.

Most breeds of livestock have emerged by careful selective breeding according to well defined

objectives. Breeders of sheep have often been quite creative in that endeavour. Several sheep breeds are the result of cross-breeding and combine favourable traits of various origins. For instance, in the first decades of the last century Sir John (Jock) Buchanan-Jardine of Castle Milk Estate in Scotland created a fairly large lightbrown ('moorit') and horned sheep primarily for beauty (Figure 1). Seeing that he was strikingly successful in this objective, it would be rather unfair to complain about any absence of extensive breeding records and unclear breed origins. And to be fair, this happened more often: in the same period breeders on the Dutch Isle of



Figure 1. Castle Milk Park, 1930s. The sheep on the right is possibly a dark phase Soay. Photo courtesy of Sir Rupert Buchanan-Jardine, son of Sir John Buchanan-Jardine.

Texel imported so many British rams, that the ancestry of the Texel breed, an excellent producer of mutton but with decorative scores at the opposite side of the scale, is both uncertain and complex.

Nowadays, modern DNA technology can come to the rescue in reconstructing unknown ancestry. In human genetics, several cases have made the headlines, such as the prehistoric Neanderthal - Homo sapiens contacts, successive waves of immigration in Europe, the royal origins of bones, and criminals brought to justice. And if it works for humans, there is no reason to assume it wouldn't work for sheep.

To look at the ancestry of sheep we need genome-wide SNP DNA profiles, which are based on thousands of single nucleotide polymorphisms (ie: variable positions, the different variants are called 'alleles') in the sheep genome. Since 2012 50K and 600K (50,000 and 600,000 SNPs, respectively) profiles have become available for many different breeds and have generated a wealth of information on the history of sheep, on breed origins and on genes involved in breed-specific traits. Sheep from most European countries have already been investigated. However, from the British sheep, only the Welsh breeds and a small minority of the English and Scottish breeds have been studied.

In some ways more interesting even than its wild mammals, are the domesticated sheep of the islands, sometimes known as St Kilda sheep, but more accurately as Soay sheep, from the name of the island they inhabit. I notice that recent articles in Scottish newspapers have called these "brown Mouflon sheep," but that is an error.

An example of how easily Soays and Mouflons were confused which may have also occurred at Castle Milk Estate. © The Scottish Naturalist, 1930, St Kilda and some of its animal inhabitants by Dr. James Ritchie, p70. This is all the more remarkable seeing that British breeds, in particular those in the southern parts of the UK, have been exported to many countries of the world and, together

with the Spanish Merino, dominate sheep husbandry worldwide. Fortunately, for the purpose of our research into the ancestry of the Castlemilk Moorit breed specifically, several people and institutions were willing to collaborate to rectify this situation. Stephen Hall (now at the Estonian University of Life Sciences) contacted the Rare Breed Survival Trust and the Sheep Trust, who in 2018 sent 345 samples from their semen archives to the AgResearch company in New Zealand. DNA was extracted and 8K profiles were generated. Combining these with previous data yielded a comprehensive 4263 SNP dataset of 58 British breeds. An additional 50K profiles are planned, including those generated from more recent CM blood samples, taken in both UK and NL

flocks as well as a Manx Loaghtan flock in Belgium.

Bioinformatics has kept pace with the technical progress of modern genome science and delivered several methods of analysis. Broadly speaking, most methods rely on a comparison of the frequencies of the alleles. The principle is simple. Imagine that there is a breed, we call it Colchis, with a unique trait, a golden fleece. Finding golden hairs in the wool of other breeds would then be an



Figure 2. Supervised STRUCTURE analysis with prior population info set for Soay, Manx-Loaghtan, Shetland and Wilshire Horn .

indication of Colchis incrossing; the more golden hairs, the stronger the influence of the Colchis sheep. Now replace golden hairs with breed-specific allele frequencies of 4263 SNPs, account for more than one ancestor and there you have it: modern genetic analysis - although the calculations are a bit more complex than counting hairs....

We used a number of methods, with each based on the allele frequencies in a different way: supervised model-based clustering and f3 as well as f4 statistics. Most of the results are pretty consistent and differences between the methods have reasonable explanations.



Figure 3. *f4* analysis for Manx Loaghtan, Shetland, Soay and Wiltshire Horn (partial results). The 2 lines demarcate the confidence interval, with 0 indicating a breed not having been incrossed into the CM

The outcome is rather surprising. Whereas Mouflons are often mentioned as ancestors of the CM, even by Sir Rupert Buchanan-Jardine (Sir John's son), we did not find influence of any wild or feral sheep species. Most British breeds, including almost all kept by Sir John and his father on their estate, can be excluded as well. Four ancestors of Castlemilk Moorit are indicated: Soay, Manx-Loaghtan, Shetland and, unexpected, Wiltshire Horn.

Figure 2 shows the supervised model-based clustering implemented in the popular STRUCTURE program. The coloured blocks consist of many vertical lines, each representing an individual sheep from the four indicated ancestral breeds of the Castlemilk Moorit. Colours indicate the ancestry and more lines per colour shows a mixed breed composition. Adding other ancestors to this plot only generated either background or inconsistent signals. The percentages are only indicative, as our samples of the reference populations may only incompletely represent the animals used for breeding the CM and there may be unidentified (small) components from other breeds not taken into account.

The contributions of Soay, Manx-Loaghtan and the Wiltshire Horn to CM were unambiguously confirmed by f4 analysis (Figure 3), which is based on a correlation of the allele frequencies of the donor breed and the recipient (Castlemilk Moorit). However, according to the supervised Structure

the Shetland is the most important contributor of the Castlemilk Moorit, but has the lowest *f*4 signal of all four mentioned breeds. This might be explained by the inbreeding of Soay, Manx-Loaghtan and Wiltshire Horn, which caused many SNPs in these three breeds to become homozygous (ie with the same allele inherited from both parents) and generate relatively high f4 values (see Figure 3). However, we will need 50K data for more definite conclusions about the Shetland component of the Castlemilk Moorit.



Figure 4. Photograph of Western or Wiltshire (Horn) ram and ewes in the early 20th century. Source: "Roslin glass slides", University of Edinburgh Collections, license CC BY-NC-SA 4.0

A said, breed descriptions often mention the Mouflon, Manx Loaghtan and Shetland, sometimes also the Soay, but never the Wiltshire Horn as likely ancestors of the CM. We excluded a substantial contribution of the Mouflon and other breeds present at Castle Milk Estate. But all analyses consistently indicate an ancestral component of Wiltshire Horn, which may have passed on its size and possibly other characteristics to the CM. Considering the history of the Wiltshire Horn, we should bear in mind that it was a very different sheep in 1920s (Figure 4). It was then described as 'medium wool', yielding about a 2lb fleece but having no wool on the belly – both

attributes match the CM with about 1 kg in fleece and for the majority no wool on the belly. There are records of the presence of Wiltshire Horn sheep on Castle Milk Estate. We will probably never know if their involvement in the creation of the Castlemilk Moorit was planned by Sir John or just happened 'in the field'.

Hopefully our search for the CM ancestry will have a grand finale with the analysis of the much anticipated 50K SNP data. However, the above clearly shows already that DNA analysis has the power to confirm the expected, refute the commonly believed and reveal the great unknown.

31st August, 1934. Dear Sir. With reference to your letter of the 14th I have now had an opportunity of discussing the matter with Sir John, and as he is only getting the sheep for crossing purposes he does not wish to join the Society, at any rate at present. I do not think we ever had a ram from you. We had some ewes forwerly and crossed them with Shetlands. The result was that we got a very good fleece, which is what Sir John wishes. Yours faithfully, The Scorethry; Wiltshire Korn Sheep Socy.; 162, Ardington Road, Northampton.

A letter from the factor of Castle Milk Estate mentioning crossing Wiltshire Horn ewes with Shetlands



PRESERVATION.—Owned by Lady Collet, this flock of Loughtan sheep —a hardy Manx breed almost extinct—is pastured near Peel golf links, and is the only flock of this type of animal on the Island. Believed of Spanish descent the Loughtan sheep is unusual in having several horns.

> Mid-left a rather large ram originating from Castle Milk Estate in a flock of Manx Loaghtan and a few crossbreds