DNA Parentage Verification Testing Proves Pattern Mutation in North American Icelandic ewe

by Laurie Ball-Gisch
The Lavender Fleece
3826 N. Eastman Rd.
Midland, Michigan 48642

One of the reasons people are initially attracted to Icelandic sheep is because of their myriad colors and patterns. For new breeders, understanding how the colors & patterns exhibit and express themselves is often confusing. It may take them a year or so to really understand the genetics of color.

Icelandic sheep breeders are indebted to Dr. Stefan Adalsteinsson for publishing his doctoral thesis “Colour Inheritance in Icelandic sheep and relation between colour, fertility and fertilization” (1970). This thesis formed the basis by which the Icelandic sheep breeders of North America were able to understand, codify and register the lambs born on their farms (see sidebar). Indeed, Dr. Adalsteinsson’s work was so important in the field of sheep color genetics, that many published works cite his findings, and he has co-authored many articles with other specialists in the field.

By understanding how color genes are inherited, breeders can maintain some measure of control over the colors and patterns of the lambs they produce. One can even breed for predictable colors & patterns. For instance, breeding a solid moorit spotted ram to a solid moorit spotted ewe will always produce a solid moorit spotted lamb. Homozygous white sheep will always produce white lambs, but those lambs can carry hidden patterns behind the white dominant. When we breed a white sheep to a colored sheep, we are assured that the lamb has inherited one of two patterns from its colored parent. We can predict what the possible hidden patterns of the lambs are. One of my biggest surprises so far was to breed a white ewe to a white ram and have them produce a black spotted lamb -- prior to this lamb's birth, I didn't know either parent carried the spotting gene.

In 2004 we did artificial insemination with some of our ewes (using imported semen from Iceland). One black mouflon ewe (A4A5) “Ember,” was bred using semen from Iceland ram “Abel.” Abel is described by Southram (the ram station in Iceland that exports frozen semen to the United States) as a “homozygous white ram.” This means he has two pattern genes for white, hence he can only throw a white gene to his offspring. Since white is a dominant pattern gene, all of his offspring will be white. Indeed as of November 2007, there have been 14 lambs out of Abel (born via artificial insemination) registered with the Canadian Livestock Records Corporation. All of those lambs have been white. They will of course carry a hidden recessive pattern inherited from the dam.

When “Ember” delivered her AI twins on our farm in the spring of 2005, she had (as expected) a white ewe and a white ram. Her ewe lamb (LF96R) remained on the farm and we named her “Alexa.”
I believe I have a good understanding of how the colors and patterns are inherited. So, armed with my knowledge of color genetics, the lambs out of Ember would have the possible hidden recessive pattern of either mouflon or solid, as her dam (Ember) is a black mouflon (A4A5). A5.

In the fall of 2005 we bred Alexa (shown at right) to a black/grey ram, LF104R (A2A5) (shown below).

The following spring she delivered a set of twins, a white ram and a black grey/mouflon (A2A4) ewe. Because the lamb was a grey/mouflon, I knew that Alexa’s recessive pattern (under the white dominant pattern) was the mouflon gene.

(Incidentally, Alexa had a twin, a ram who is also white. So far he has sired lambs that are solid, so his hidden recessive is A5, solid).
In the fall of 2006 Alexa was bred to a white ram, LF167S (who proved to carry the grey gene, as he sired several grey lambs the spring of 2007). I noticed Alexa as she was in labor with these lambs. As she began to deliver the first lamb, I was delighted to see a black nose and black toes emerging. Knowing she’d had a grey/mouflon ewe the previous year, I expected this lamb to also be a grey/mouflon (since I knew the sire carried grey and Alexa carried mouflon). Imagine my surprise to see that the lamb (a ram) was not mouflon! After he was cleaned off and had nursed, I dipped his navel and looked all over for the tell-tale mouflon markings. There were none! He was all black with just a dusting of white hairs on his muzzle and in his ears, indicating the grey gene (from his sire). Alexa also delivered a 2nd lamb, a white ewe. As I sat there and watched this set of twins, I knew that I had a very unusual case here. The ram lamb was supposed to be mouflon pattern. I already knew Alexa carried mouflon from her own dam - which was demonstrated with her 2006 ewe lamb. So what happened to the mouflon pattern? I did a mental check of all the rams on the farm, the location of the breeding groups, etc. from the previous fall. There had been no breech of fences and in fact, based on the rams that were on the farm, it still wouldn’t make any sense for this lamb to not be mouflon - because if the solid pattern came from another ram, then where would the grey have come from? - we should have still seen mouflon pattern!

In his book on the inheritance of color in Icelandic sheep, Dr. Adalsteinsson described cases of mutated color/pattern genes, and I wondered if this was the case with Alexa. In fact, I decided this had to be the only explanation for the pattern of this ram lamb.

From page 116 in the book the author states:
“Colour description was available on 3452 lambs from these matings. Of these, 23 lambs or 0.67 per cent, showed colours other than expected. The exceptions are so few that the main hypothesis about the allelic system at the A locus is confirmed.

The unexpected cases are described in detail. One case can be ascribed to mutation of A1 to A5. Another case can be ascribed to mutation of A5 to A2 in a brown ram leading to colour mosaicism and mosaicism in the gonads of the ram as well.”

“A case of colour mosaicism in a phenotypically grey ram is described. Through a breeding test the ram
was shown to have had the zygotic genotype A4A5. The allele A4 is shown to have mutated to A1 at the 4-cell or 8-cell stage of the zygote."

Dr. Phil Sponenberg, who has also done extensive research into sheep color genetics also mentions gene mutations that may cause unexpected colors. From an article he wrote he says, “To slightly confuse the issue even further, on some occasions colored x colored matings give white lambs. This happens in Romney and Coopworth flocks more commonly than in Border Leicesters, and the most likely culprit is the Extension locus with two choices: dominant black, and “normal” or “wild” type. This genetic variant is not usually associated with Longwools, and it could have arrived in them either by mutation or by some crossing in the past. Regardless of how it came into the breeds, it is in them, and the animals with the variant appear to be typical purebreds with no obvious outside breeding.” (from “Color Genetics in Border Leicester Sheep”)

I decided the only way to “prove” that there was indeed a color/pattern mutation (and not faulty parentage) was to do DNA testing and verify the parentage of the four lambs that Alexa has produced so far. The DNA parentage testing was done at the UC Davis Veterinary Genetics Laboratory. This involved collecting hairs from just above and between the toes of the hooves of each sheep involved: LF 96R (Alexa, the dam), LF104R (sire of the 2006 lambs), LF167S (sire of the 2007 lambs) and each of the lambs born in these pairings: LF177S, LF178S, LF235T and LF1236T. (A big thank you to my fellow breeders who collected hairs from the sheep involved for the study!)

UC Davis was very prompt; I received the report back within a week of mailing the samples. I was not surprised to have the parentage verified. I have copied the summary report here. As you can see, each of the lambs was indeed the offspring of LF96R (Alexa) and the rams she was bred to.

Parentage and Genetic Marker Report for LF 235T(SE2054):
LF 235T 613289 qualifies as an offspring of LF96R 593843 and LF167S 603958.

Parentage and Genetic Marker Report for LF 236T(SE2052):
LF 236T 613290 qualifies as an offspring of LF96R 593843 and LF167S 603958.
Parentage and Genetic Marker Report for LF 177S (SE2058):
LF 177S 604328 qualifies as an offspring of LF96R 593843 and LF104R 595011.

Parentage and Genetic Marker Report for LF 1785 (SE2056):
LF 1785 604329 qualifies as an offspring of LF96R 593843 and LF104R 595011.

When the original color inheritance studies were done in Iceland, it was not possible for the researchers to prove gene mutation or verify parentage. Exceptions to lamb color expectations were ascribed to faulty parentage (including possible mis-mothering) or faulty color description on the part of the farmer. When these explanations failed, then it was thought that mutations of A locus allele had occurred.

I was able to correspond with Dr. Sponenberg via email, and I explained the unusual phenomenon that had occurred with Alexa’s lambs. He was kind enough to take time to write me back. He noted: “It is... a general pattern in wool sheep that the patterns vary little within a single allele. This is not the case in goats or hair sheep, where some individual alleles are extremely variable as to final phenotype. So, the only question would be whether this is what you are seeing, rather than a new mutation. At this stage, though, mutation is a likely explanation.”

Dr. Sponenberg pointed me to the work of Roger S. Lundie and I reread the article “The Genetics of Colour in Sheep - Some Basics” (pages 112-113). There I found a more expansive explanation of color or pattern mutations.

“Genes are capable of reproducing themselves exactly for many cell generations. Occasionally, however, the chemical arrangement of a gene may change, resulting in a different structure and action of the gene, and hence, under certain circumstances, in a new character. Such a change is called a mutation and is a rare event. The mutated gene now reproduces itself in its changed form just as faithfully as it previously copied its original form. This mutation may be inherited.”

He also writes about “multiple alleles,” which would seem to be an explanation for the variance in pattern of Alexa’s lambs.

“So far only pairs of alleles have been considered. A gene locus may mutate to produce a new allele on more than one occasion. Each separate event could give rise to the same mutant but this need not be so. Although each mutant would only arise very rarely, if each one produces a distinctive phenotype, it would be possible for a succession of alleles at one locus to be brought together over a period of time. Where this occurs they are known as multiple alleles. No matter how many members a set of alleles may contain, only two of them may normally occur within a

Color Genetics of Icelandic Sheep

The study of inheritance of colors in Icelandic sheep was originally initiated in 1957. The motive was financial in nature; dark grey pelts were bringing higher prices than pelts of other colors. The breeders wanted to understand the genetics for inheritance of the grey color in order to produce more dark grey lambs. As the study commenced, it was soon discovered that inheritance of the grey color was only part of a larger equation. In 1959 the Department of Agriculture of the University Research Institute released funds to establish an experimental sheep flock to study color inheritance. Several other studies were carried out on other, private farms and data was collected from those flock books.

Genes affecting color in Icelandic sheep are found on three loci: A, B and S. The A locus controls pattern; the B locus controls color and the S locus controls spotting (or non-spotted).

All Icelandic sheep are either black or brown (moorit) genetically. White sheep are still black or moorit underneath that white fleece. White is in fact a pattern (A1 locus). The other patterns are identified as A2 (grey), A3 (badgerface), A4 (mouflon), A5 (solid - or absence of pattern) and A6 (grey mouflon).

To add to the variety, sheep can also be spotted or not spotted. To be a spotted sheep, they must have inherited a spotting gene from each parent. They can also carry a hidden spotting gene if only one parent is spotted (or throws a hidden recessive spotting gene). The spots are so varied in how they can be exhibited that in Iceland they have actually named and classified 92 spotting patterns that include descriptions such as: dark eyerings; dark cheeks; Jacob's markings; hood and blaze; coat with blaze, stockings, etc.
somatic cell and only one in a gamete.” (page 114)

On our farm we have had 272 lambs born over eight lambing seasons. In all of the lambs - except with Alexa and her lambs - all of the colors & patterns of the lambs were explainable and predictable based on the colors and patterns (dominants and recessives) of their parents.

Without an understanding of how the colors & patterns are inherited, one would not be able to recognize when there was a variant - or a mutation - of a gene, as I observed the season that produced lamb LF235T. I plan to breed Alexa to a colored ram next, so that the sire’s contribution to the lambs’ patterns is understood. I am very interested to see if she produces another lamb as a result of the mutated pattern gene.

References:


Sponenberg, D. Phillip, DVM, PhD, “Color Genetics in Border Leicester Sheep” (from Internet reprint of article).