A working understanding of coat colors and genetics in Soay Sheep

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I contend that gaining a working understanding of the genetics of coat colors in Soay sheep is quite readily obtained by anyone who has the time to do some reading. There is little need to synthesize the available literature into one complete, comprehensible document because all the pieces are out there to be read. They are, admittedly, somewhat complicated by extraneous material and inconsistent terminology.

I thought what might be helpful is a 'roadmap'—a defined sequence of documents to be read, which taken together should give the novice a firm grasp of the key important concepts, and thereby lead to better decisions about sheep breeding. It won't give you a PhD in genetics, but I'll bet it will make you a better Soay sheep breeder!

Accordingly, these are my suggested reading materials and sequence for this topic.

1. If one is uncertain about basic Mendelian inheritance genetic concepts, I would first read about the simple pea experiments done by Mendel. Here is a link to such a site:
   http://www.ndsu.nodak.edu/instruct/mcclean/plsc431/mendel/mendel1.htm
   While the examples are all from plants, to apply it to Soay, substitute on this page "mouflon pattern" for Tall, and "self-colored" for Short. Replace the symbols D with A+ (Agouti Wild), and d with Aa (Non-agouti, or self).
2. The discussion above covers only one trait. There are two coat color gene loci of considerable interest to Soay breeders, and if they are independent, they behave as described in the following page:
   http://www.ndsu.nodak.edu/instruct/mcclean/plsc431/mendel/mendel3.htm
   In this page, substitute A+ (Mouflon) for G (green seeds); Aa (self) for g (yellow seeds), and for the second trait in Soay – Brown or Black eumelanin, substitute BB (Black, or dark phase) for W (round seeds) and Bb (brown, or light phase) for w (wrinkled seeds).
   It may be noted that this site is a good overview for other terms that may pop up in your reading, notably penetrance, co-dominance, and epistasis. *Personally, I don't think you need to understand any of those topics (at least yet) to make good progress in understanding Soay sheep coat color genetics. Just ignore them for now.*
3. I would next go to a site that applies these concepts specifically to sheep, although it is Shetlands rather than Soay. I think the Schaltz Farm site is particularly good—it is at:
   http://www.shaltzfarm.com/shcolor.html
   Start at the top level and read all the genetics pages. Note that they use At to mean wild type agouti pattern. This is the same as what I call A+ (mouflon, or wild pattern). C-B&P[1] use the A+ wild. Shaltz Farm calls Aa 'solid', which is logical, but it is more commonly called 'self' (C-B&P) or more correctly, 'self-colored' or sometimes 'non-agouti'.
4. Note, when you get to the Schaltz Farm "Primer" page,
   http://www.shaltzfarm.com/shcolprim.html
   I would suggest that for now you accept my contention that:
   a. There are more agouti (**pattern**) locus alleles in Shetlands than Soay. I think there are **only two** in Soay (A+ mouflon, and Aa self). I do not think Awt white, Ag grey, or Agt grey mouflon, or Ab badgerface exist in Soay, so ignore references to them!
   b. I don't believe the **Extension**, nor the **Dilution** genes exist (or at least are active) in Soay, so references to them may also be ignored. Keep it simple!

[1] C-B&P means Clutton-Brock and Pemberton, 2004, *Soay Sheep, Dynamics and Selection in an Island Population.*

5. At this point one should be familiar with these terms:
   genes, locus (plural loci), alleles, dominant, recessive, homozygous, heterozygous, Agouti locus (A), Brown locus (B), White spotting locus (S).
   In contrast, I don't think it's important to fully understand (yet):
   co-dominance, epistasis, incomplete penetrance, extension, dilution, autosomal, eumelanin, phaeomelanin[2]. Just skim right over those for now.
6. The reader may want to take a short diversionary side trip here and read a good overview summary by Dr. Phil Sponenberg. It is written for goat coat colors, but the introductory portions all apply and it is a highly authoritative summary, and I think quite current. Reading this now will reinforce the basics. You should also note in this article the definition of the terms *epistasis, eumelanin, phaeomelanin.* Once he gets to the specific choices for the various loci, I would stop reading as goats seem to have far more complexity (options) than Soay sheep.
   http://www.cagba.org/Goat_Color_Explained_copy1.pdf
7. These two coat color traits (pattern and body color light or dark phase) in Soay are covered in significant detail in Appendix 2 of *C-B&P,* ***Soay Sheep***. If you don't have a copy of this book you should obtain one, as it is readily available. In particular, read pages 321-324, and study Tables A2.1, A2.2, and A2.3. These data are particularly significant because:
   a. They represent a tally of all the Soay sheep in one existing, unaltered (presumably) equilibrium breeding population.
   b. There are sufficient numbers of sheep measured to give good statistical significance to even the very infrequent color morphs (i.e., light phase self)
   c. The agreement between a very simple underlying genetic model and the actual observed results is, in my experience, astounding (for biological data).

   From Table A2.1, note the relative equilibrium frequencies (on Hirta) of the 4 color morphs:

Dark wild	67.5%	A+/A_[3], BB/B_	aka dark mouflon
Light wild	27.4%	A+/A_, Bb/Bb	aka moorit mouflon
Dark self	3.6%	Aa/Aa, BB/B_	aka solid black
Light self	1.5%	Aa/Aa, Bb/Bb	aka self-colored moorit

   Note the low frequency of light self on Hirta. As far as I know there has never been a light self Soay born in North America.

   In interpreting Tables A2.2 and A2.3, note that it can be somewhat confusing that they report two sections in each table, (a) and (b). This labeling can easily be confused with the gene loci labels for Agouti and Brown, that is A and B. In my opinion the title of Table A2.2 would have been clearer were it written thus:

   Observed and expected outcome for all combinations of matings at the ***colour locus*** *(Brown, or B) for 1132 offspring with paternity inferred at 80% confidence (table section a), and for 556 offspring with paternity inferred at 95% confidence (table section b).*

   Analogous changes should be made in the title of Table A2.3.

[2] Also commonly spelled pheomelanin (a Google search turns up about equal usage of both spellings…)

[3] When 'underscore' __is used it means that the phenotype (appearance) is the same regardless of whether the individual has the dominant or recessive allele. The underscore means either allele could be present.

Then, the really important thing one should observe in the table is the *very good agreement*, when paternity is quite confident, between expected frequencies from a simple underlying model and what actually occurred.. Note particularly that the disagreement between simple model and actual results in the 80% paternity confidence sections is attributed by the authors to uncertainty about who the real sire was, *not* uncertainty about how the genes will interact and express themselves. For the Soay breeder, this means as a practical matter that when you know for sure the paternity (because you control who mates), you can be VERY CONFIDENT in the coat color morph outcomes.

8. The next topic on a logical path to understanding the practical genetics of Soay sheep coat colors is the relationship between the actual biochemical pigments that cause the coat colors that we observe, and the relationship of those pigments to the pattern and color genes. Read about the two pigments- eumelinin (two forms, black and brown), and phaeomelinin (red browns if heavy pigment, down through tans, beige, and almost white if sparsely pigmented.). The best explanation of this I have found starts with this MISDR[4] site:
   http://www.angis.org.au/bin/Databases/BIRX/birx_doc?mis+4
   and the key section to study is shown below:

Alleles at the Agouti locus determine the arrangement of eumelanic and phaeomelanic areas over the body and within individual fibres. The resulting patterns are typically symmetrical.

The distinction between eumelanins (black, brown) and phaeomelanins (red, yellow) may not be precisely determined from visual or light microscope study and there is often an overlap of pigment types even within the same melanocyte (pigment producing cell). Distinction of pigment type can be determined from biochemical analysis, electron spin resonance or electron microscopy.

The distinction between the melanins is a very important one since the hair of wild sheep has been much changed to produce woolled types. As a result, colours caused by phaeomelanins will vary from brown or red in haired types to yellow or nearly white in most woolled types. Eumelanins will likewise vary in colour and shade.

Depending upon which form of eumelanin is present (Black or Brown, according to the B allele), and secondly the level of pigmentation, heavily pigmented black eumelinin mouflon pattern Soay look almost like solid blacks. Soay with much more lightly pigmented black eumelinin have only black areas, typically a mane, a ruff, kneecaps, face, etc. Then depending upon the phaeomelanin level, a reddish brown may also show up in the body coat.

9. The contention that the observed light and dark phases of Soay observed on Hirta are directly due to the Brown (B) locus, and that the underlying mechanism is dominant black at the brown locus (BB) arises due to the black form of eumelanin, and recessive brown (Bb) arises due to brown form of eumelanin is given on page 12 of the MISDR:
   http://www.angis.org.au/bin/Databases/BIRX/birx_doc?mis+12
   and the specific comment most relevant to how these pigments exhibit themselves is (red text are my added (red) comments to better interpret the statements):

   At the Brown locus the wild allele (BB) allows the expression of eumelanin as black. (That is, black pigmented hairs somewhere on the upper body – not entirely (self-colored) black.) The brown allele (Bb), which is recessive, causes the expression of eumelanins as brown.

10. Key concepts at this point for two color morphs[5] in Soay sheep are that:

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[4] MISDR:, **M**endelian **I**nheritance in **S**heep **D**ocument **R**eader, hosted by Australian National Genomic Information Service (ANGIS).

[5] From Lowder paper: "Polymorphism is…defined as…the coexistence in one interbreeding population often two or more sharply distinct and genetically occurring forms."

a. There are only two choices for main body eumelanin pigment (upper body in mouflon pattern, entire body in self pattern), and only one can occur in any given Soay. They must have either Black (BB) or Brown (Bb) eumelanin. The actual appearance of these morphs can, however, vary considerably depending upon the pigmentation concentration.

b. Phaeomelinin (very light tan, yellowish on through coppery, reddish-browns, depending on the pigmentation density), is what determines the appearance of the light areas of mouflon pattern (belly, etc.).

c. Phaeomelanin also occurs to highly variable levels within the same hairs that contain eumelanin on the upper body, and thus the overall upper body color of dark phase (BB) mouflon pattern can range from nearly black (with heavy eumelanin masking any phaeomelanin that might be present).

d. The best way to differentiate the brown of eumelanin from the brown of phaeomelanin is to think of eumelanin brown as "chocolate brown" – like that found in Chocolate Labrador retrievers, and the phaeomelanin brown as "red-brown", like an Irish Setter[6].

The concepts developed to this point are pulled together most succinctly in the recent (July 2005) Master's thesis work done by Bethan Lowder. The summary paper can be found at:
http://www.shef.ac.uk/aps/mbiolsci/lowder-bethan/bethan-lowder-project-report.pdf
This paper pulls together the full gamut of evidence supporting the simple dominant/recessive genetic model for coat pattern and colors; that it is directly caused by specific genes at identified loci, which control via enzymes the biochemical pathways leading to production of eumelanin and phaeomelanin pigments. Thank goodness for us that the work was done on Soay sheep!

11. At this point it is worthwhile to take a little side trip to consider the work reported in the classic work ***Island Survivors*** (Jewell et. al. 1974) ("IS"). Many might wonder why this was not cited earlier as a key resource. There are two factors that, in my opinion, significantly impair the usefulness of the work in IS for this discussion:

    a. The work, while a milestone in many respects, is now over 30 years old. Significant work has been done in the whole area of coat color genetics in all breeds of sheep and goats, and considerable effort put toward standardization of terminology, identification of specific gene locations, etc. This makes comprehension of the relevant material in IS on coat color (pages 88-97) difficult when using current nomenclature (as laid out above). That being said, the terminology for light and dark phase, and wild and self patterns should be readily grasped, as that is consistent with C-B&P terminology.
    The biggest difference is their notation for alleles. They use A_4 as mouflon (or wild) pattern (now A+), and A_5 for self (now Aa). They use B_1 for Black eumelanin (now BB) and B_2 for Brown eumelanin (now Bb). If one makes all the replacements above in the tables presented on pages 96 and 97 of IS, the notation and results would be consistent with C-B&P[7] and most current Shetland and Icelandic breeder sites where genotype codes are listed.

    b. The example data presented on pages 96 and 97 from the ABRO flock test matings is too few observations to either develop or affirm a genetic model. There simply are not enough matings and offspring. The data CAN be used to test whether exceptions can be found, and one can examine each mating result and see if it is consistent with the current theory, but the data is only illustrative, not definitive. The authors explicitly state the key result of the ABRO flock matings: *"Numerous test matings with the ABRO Soay sheep have produced no evidence that*

[6] In fact, these two pigment (eumelanin and phaeomelanin) are the pigments controlling hair color in all mammals. Online references can be found for mice, rats, rabbits, cats, dogs, horses, cattle, sheep, goats, and even humans.

[7] Although C-B&P use superscripted second characters, thus B^b instead of Bb, but the same characters.

would oppose the above interpretation".
Even if one takes into consideration that a lot of additional work has been done since 1974 and the coat color theories may have changed, I have studied every single offspring phenotype reported on those two pages and there is not a single case that does not follow the model as described in the most recent Soay works cited above (C-B&P ***Soay Sheep***).
The most telling comment is: "So far no dark, self-colored offspring have appeared in the ABRO flock". This is a result, I believe, of too few offspring to detect a trait which occurs at a very low rate. Note that the populations reported in the ABRO flock were 8 rams and ~25 ewes. There were no self-colored black rams, and no self-colored black ewes. This is not *required* to produce self-colored blacks, but the odds are greatly reduced if you don't have them in the matings. Thus it likely takes many more crosses before they "pop up unexpectedly".
In fact, it is my contention that the data in pages 96 and 97 are an excellent example of why Soay breeders with flocks of significant number (15 to 100 Soay) in the PNW have a difficult time observing, strictly from their own Soay breeding results, the underlying genetic mechanisms of say, self-colored blacks. They are just too few and far between to deduce the underlying causes without careful study.

12. Ryder, 1983, in ***Sheep and Man***, also briefly discusses coat color genetics, based upon his work in 1974. He reports (pages 536-537) on the 4 patterns in Shetland and Orkney (now known as N. Ronaldsay) and compares them to Soay. He also has an interesting speculative chart on pattern and horn type evolution for various breeds on page 549.

13. Sunbleaching – Some self-colored black Soay sheep turn quite medium brown after exposure for a few months. Some sunbleach very little, and some only sunbleach on some areas (like the body), and not on other areas (face, legs, etc.). I have a theory to explain why this occurs…. but will save that fairly long theory for another session. For now, only consider the "new coat" colors—freshly grown hairs, or the color of the roots down underneath that do not get sunbleached.

14. The third key, genetic coat color trait of interest to many Soay sheep enthusiasts is white spotting. It should be considered a separate locus and independent of pattern (agouti locus) and color (brown or black). The white spotting allele is noted as SS for no white spotting (dominant), and Ss, presence of white spotting (recessive). One should understand that nearly all white on the underparts of the mouflon pattern, which is shown in some Soay, is NOT due to white spotting gene, but is instead very lightly pigmented phaeomelanic pigment areas. White spotting almost always shows up first as a poll spot, then forehead spot, then a blaze, then tail tip, 'socks', and usually last on the upper body. White spots are completely lacking pigment, and usually do not have mixed colored hairs within the spot "body", unlike nearly-white mouflon pattern underparts which even at the whitest will have some slight tan cast. White spotting is best described at the ANGIS site, web page 17:
http://www.angis.org.au/bin/Databases/BIRX/birx_doc?mis+17

15. With a grasp of these 3 coat color traits (agouti pattern, black/brown color, and white spotting) one can study the listed genotypes for photographs of example sheep on sites for Shetlands and Icelandics and begin to associate the genotype codes to phenotype appearances. Ignore agouti patterns other than At or A+ (mouflon) and Aa (self). Ignore other loci like M. Good examples can be found for Shetlands at Sheltering Pines:
http://shelteringpines.homestead.com/shelteringpines.html
and for Icelandics at Hawks Mountain Ranch:
http://www.hawksmountainranch.com/colorpatterngenetics.html

Figure 1. The four possible coat color patterns in Soay sheep. Note that any of these four can also have white spotting, yielding 2^3=8 'color types'.

Color Morphs of Soay Sheep from Hirta

Terms and notation used by 4 significant Soay sheep references.

1. Doney et. al. ***Island Survivors***, (1974) Chapter 4, pp 88-125.
2. ***Mendellian Inheritance in Sheep***, (2003?), online document reader pages 2, 6, & 12
3. Clutton-Brock & Pemberton, ***Soay Sheep***, (2004), Cambridge Univ. Press, Figure 2.7 and Appendix 2, pp 321-327.
4. Lowder, Bethan (2005) ***The genetic basis of a coat colour polymorphism in Soay sheep***, on-line Masters Thesis. (Does not address nor use terms for pattern).

Dark Phase Wild pattern (DPWP)

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1.	'dark' B_1	'mouflon pattern' A_4
2.	'black' BB	'black and tan' At,
3.	'dark" BB	'wild' A+
4.	'dark' G	'wild' (NA)

Light Phase Wild pattern (LPWP)

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1.	'light' B_2	'mouflon pattern' A_4
2.	'brown' Bb	'black and tan' At,
3.	'light" Bb	'wild' A+
4.	'light' g	'wild' (NA)

Dark Phase Solid pattern (DPSP)

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1.	'dark' B_1	'self-colour' A_5
2.	'black' BB	'non-agouti' Aa
3.	'dark" BB	'self' Aa
4.	'dark' G	'self' (NA)

Light Phase Solid pattern (LPSP)

So uncommon that no Soay photo could be found. This is a Shetland.
(Sheltering Pines)

1.	'light' B_2	'self-colour' A_5
2.	'brown' Bb	'non-agouti' Aa,
3.	'light" Bb	'self' Aa
4.	'light' g	'self' (NA)