RESOLVING FOLLICLE POPULATIONS WITHOUT BIOPSY – ENHANCING OPTICAL FIBRE DIAMETER ANALYSIS OF WOOL AND FUR QUALITY

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Abstract. *The coats of mammals contain fibres from three different types of follicles in varying proportions. The quality and performance of wool and fur products is critically dependent on exactly how these three classes of fibre interact with each other, especially in the various Northern European Short-Tail sheep breeds. Follicle populations are normally determined by making microscopic studies on skin biopsy samples. However, modern, computerised optical equipment and methods give quick and easy statistics on fibre diameter (FD) distributions. The aim of this study is to determine if the distribution of follicle types can be extracted directly from these statistics, with particular reference to the needs of the Swedish Northern European Short-tail (NEST) sheep industry. Our work shows that FD data on fleece samples from three different Swedish NEST breeds can indeed be processed to yield their S/P ratio directly without resorting to skin biopsy, and suggests that this may in many cases also be possible even with single-coated breeds such as Merino. During this work we have made surprising discoveries especially concerning the Gotland pelt sheep, where we see that each follicle type produces its own specific proportion of black and white fibres. We also see that the ratio of secondaries to primaries (S/P ratio) that is typically about 2 in most NEST breeds, has been reduced to only 0,06 during subjective Gotland breeding for good curl structure. Our continued research involves a nation-wide optical FD inventory of the fleece of over a thousand Swedish Finewool sheep, and continued exploration of the possibilities and limitations of optical FD analysis for Northern European Short-Tail sheep and other fur animals in which the balance between primary and secondary fibres is important.*

Key words: *OFDA, distribution, primaries, secondaries, pigmentation.*

INTRODUCTION

Optical fibre diameter (FD) analysis was originally developed to meet the needs of the Merino and Mohair industries. The OFDA 100, is unique in its capacity to include measurements on kemps and pigmented fibres. However, since either of these fibre types is a sufficient indication for Merino or Mohair individuals to be culled, the programmers have not endeavoured to distinguish them in more detail.

Northern European Short-tail (NEST) is a group of hardy sheep breeds spread through the Scandinavian countries. A comprehensive (but incomplete) list of NEST breeds is available on Wikipedia [1]. Some NEST breed descriptions include both kemps and pigmented fibres, so that breeders need to measure them separately. Moreover, NEST breeds are basically double-coated; knowledge of the ratio of secondary to primary follicles (S/P ratio) is desirable but has not been available from optical FD analysis. S/P is normally determined by skin biopsy which demands considerably more time and resources than procuring a fibre sample [2].

The aim of this study is to investigate if it is possible to extend the processing of optical FD data, originally developed for Merino and Mohair, to meet these specific needs of the Swedish NEST industry.

Swedish short-tail breeds are often collectively termed as Swedish Landraces to distinguish them from imported non-NEST breeds. They are grown between north latitudes 55° N and 67° N, mostly on natural grazing, some of it in forestry. They are housed and fed hay and supplements during the winter, when grass growth has ceased. Fertility is often high with twins and triplets being the norm. Together with early sexual maturity, this means that skilled and determined breeding can make fast progress. Each breed is customarily coupled to specific fibre products and textile craft techniques. Breeders who can fulfil these special demands on fleece properties gain extra income from their flocks. This entails determined selective breeding, traditionally based on subjective judgement skills. This paper seeks to demonstrate that objective optical FD analysis can simplify landrace breeding selection in the three breeds selected below.

Gotland (Swedish Pelt Sheep) are bred to produce short-cut lustrous silvery lambskins with a specific style of distinctive curls. In sheep, curl or fibre crimp is produced in a periodic rhythm ranging from seven days

in Merinos to over three weeks in Gotlands. Crimp size depends on the length of fibre produced during each rhythm period[3][4]. Growth rate typically correlates with fibre diameter, so coarser fibres have longer crimp waves with larger curl radius. In the Gotland with its double-coated NEST origin, secondary fibres are finer and therefore have smaller crimp radius than primary fibres. If the curvature spread within each follicle type is too wide, the fibres do not match each other and they will not find their way back into the same neat curl structure after being disturbed - such skins will not retain their typical Gotland appearance. Breeders must also aim for a primary FD around 45 µm to give robustly springy fibres, while seeking to maximise lustre and to match a specific shade of grey. Breeders need to master the simultaneous evaluation of all these traits, together with low hide weight and high meat production. [5].

Swedish Finewool or Finull is closely related to Finnsheep and shares its extreme fertility, even in meat breed crosses. However, while Finnsheep breeding has been striving to achieve both softness and robust pelts simultaneously, in Sweden the successful Gotland breed specialisation in pelts has left the Finewool free for over eighty years to concentrate on breeding specifically for lustrous softness. [6].

Rya is a Swedish long-primary NEST resembling the Spelsau, Icelandic and others. However, the Rya differs from these in that it has been specifically bred to eliminate kemps in accordance with the demands of Swedish textile handicraft traditions. [7].

MATERIALS AND METHODS

Optical fibre diameter analysis with an OFDA 100 became available in Sweden in the province of Jämtland in 2007 as part of the regional EU project Ull-Rika, a feasibility study for the re-introduction of Merino sheep to Sweden to supply the local cold-climate underwear industry. Analysis services are now available nation-wide by post (UllFORuM, Torsta AB, Ösavägen 20, SE 836 94 Ås, Sweden), with discounts to breed societies.

Wool samples. Since this study is intended to explore possibilities rather than generate statistics, the wool samples used in this study are not random samples, but have been deliberately chosen to explore the variations present in the Swedish short-tail population. Samples are from UllFORuM's routine production runs, from breed societies [5]-[7] and from an innovative sheep cooperative seeking to re-introduce grazing of mountain pasture [8].

Wool fibre measurements. The wool samples were analysed using the optical FD analyser (OFDA100; BSC Electronics Pty Ltd, Western Australia, Australia). The OFDA 100 is the only optical fibre diameter (FD) analyser that can also measure opacity (in %), fibre curvature (in °/mm) and surface roughness (in three size categories). All samples were manually aqueous scoured in accordance with the procedure specified in 2008 by OFDA's European distributor [9]-[12]. UllFORuM has rationalised this procedure by combining 35 plastic mesh cells into a rack that fits into a plastic container with 30 l of water with 50 ml of detergent (Power Scour from unicornfibre.com). The dried and conditioned fleece is then subsampled using OFDA's guillotine device designed to provide 2mm snippets of wool which are then distributed across a microscope slide by the OFDA rotary sample spreader.

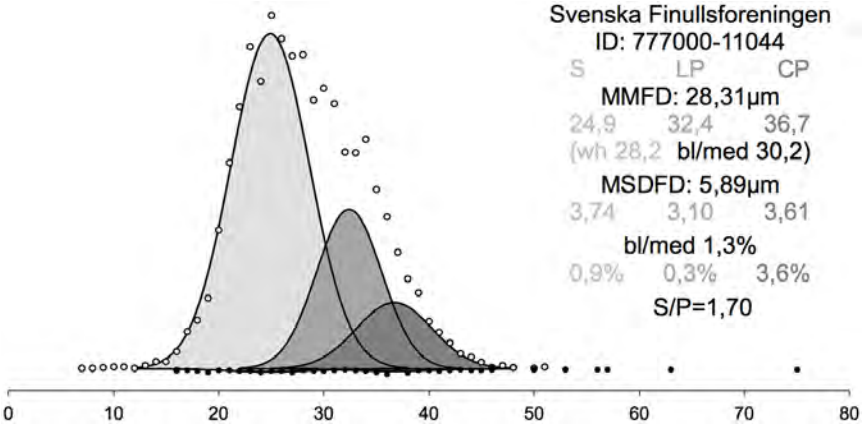
OFDA file types. The OFDA software can generate many types of files with various selections of key parameters. The most complete standard file is the MESFILE (.MES) which sorts fibres into 150 bins from 1 µm to cut-off at 150 µm. OFDA measures several thousand snippets in two minutes, which is about the time required for the operator to prepare the next slide. Bins around the mode may contain several hundred fibres, while bins in the tails of the histogram contain only a few fibres. Here, the shape of the distribution is concealed by stochastic variations which cause problems during analysis. To some extent this can be alleviated by using the High Resolution (.HRS) file format together with the MESFILE. HRS contains a list of 0,1 µm bins from 1 µm with cut-off at 25 µm. If this information is not saved, it is lost as soon as the next sample is scanned, and it cannot be recovered. We strongly advise that OFDA 100 is always set to record both MES and HRS files.

Mathematical analysis. Our first OFDA histograms of NEST fleece samples from Gotland and Rya showed obvious diameter zones that seemed to correspond to secondaries, lateral primaries and central primaries respectively. It seemed likely that the FD distribution could be modelled by superimposing three Normal distributions – if their parameters could be deduced from the data. Most spread-sheet programs deduce the parameters of the single Normal distribution that best models a given data set, but resolving superimposed Normal distributions is more difficult. Not finding any available software to suit our needs, we have developed

our own algorithms in the form of an Excel workbook, as a tool to resolve the necessary parameters from OFDA data on samples from Swedish NEST breeds.
We regret that we are unable to publish our algorithms at this time. However, we can assist other researchers by processing small numbers of their submitted histograms tables (with real or synthesised data) and returning our results.

RESULTS AND DISCUSSION

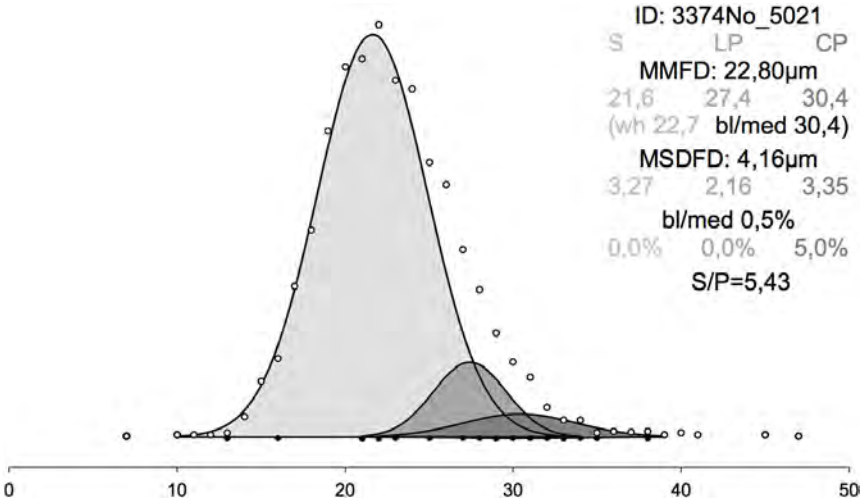
Using our algorithms, we have been able to resolve OFDA histogram data into three superimposed normal distributions, one for each of the three follicle types.
In order to make the following histograms with three follicle types more legible, we have chosen to depict the white fibre distribution upwards (white dots) and black fibre distribution downwards (black dots) rather than superimposing them on each other in the conventional way.
In the following Figures, the populations of secondary, lateral primary and central primary fibres are shown in three darkening shades of grey.



S = secondary, L = Lateral, C = Central, P = primary, MM = measured mean, FD = fibre diameter, MSD = measured standard deviation, bl/med = percentage of black and/or medullated fibres

Figure 1. **A Swedish Finewool histogram resolved into its follicle types**

The ***Swedish Finewool*** breed society [6] is currently running a Ministry of Agriculture financed OFDA inventory of Swedish Finewool fleece samples from 1000 ewes and 100 rams. We plan to do follicle-type analysis on all these samples during 2015, of the type shown in Figure 1.
Experimentally, we have also been able to resolve ***Merino*** samples into follicle types, as shown in Figure 2.

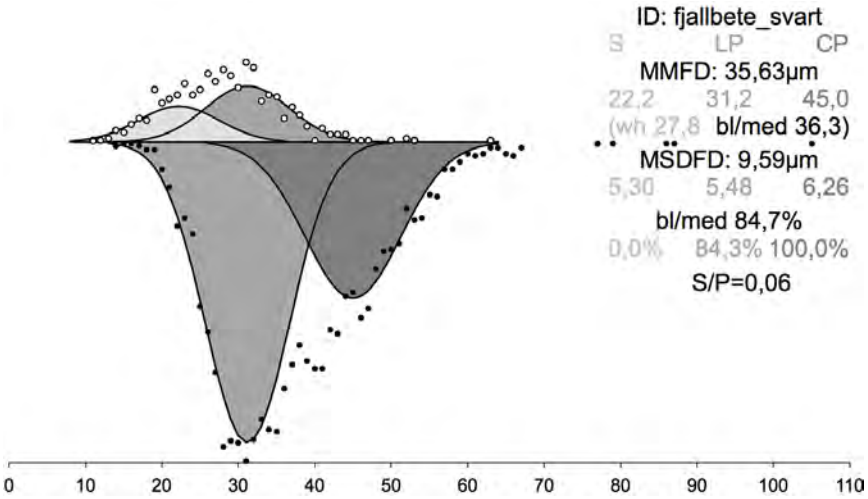


S = secondary, L = Lateral, C = Central, P = primary, MM = measured mean, FD = fibre diameter, MSD = measured standard deviation, bl/med = percentage of black and/or medullated fibres

Figure 2. **A Merino histogram resolved into its follicle types**

More work is needed to establish the exact conditions under which this is or is not possible, including the amount of skewness required, and to establish confidence levels for the parameters obtained. Some of this work needs to be done on animals on which skin biopsy follicle analysis has been performed.

Gotland histograms have been amenable to revealing the proportions between their follicle types as shown here in Figure 3.

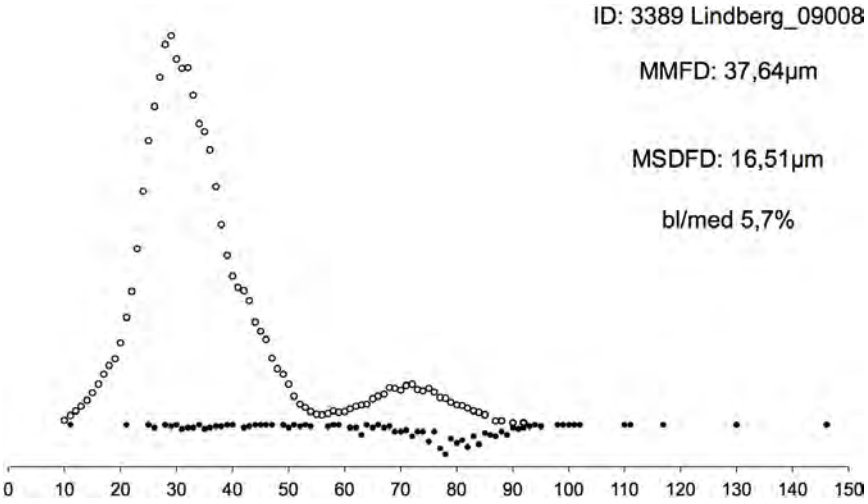


S = secondary, L = Lateral, C = Central, P = primary, MM = measured mean, FD = fibre diameter, MSD = measured standard deviation, bl/med = percentage of black and/or medullated fibres

Figure 3. **A Gotland histogram revealing an exceptionally low S/P ratio of only 0,06**

Gotland skins are grey, but contain no grey fibres; under magnification it is easily seen that they are a mix of black fibres and white fibres. Our histograms reveal them as mixture of three different greys, as in Figure 3. Here, 84,3% of the lateral primaries LP are black throughout their diameter range, while the secondaries S are 0% black and the central primaries CP 100% black. Many other variants occur in the population; another of our Gotland samples shows 0% black LP, 12,6% black S, and 93,7% black CP. If we mate a grey ewe with a ram that has the same apparent shade but quite different distributions, what shade will the lambs have? Follicle resolution may assist grey sheep breeders in selecting parents that are grey for similar reasons, while also making it easier to plan Gotland breeding to continue to eliminate the secondaries.

Samples of classical ***Rya*** fleece are trimodal and easy to analyse into three follicle types.



MM = measured mean, FD = fibre diameter, MSD = measured standard deviation, bl/med = percentage of black and/or medullated fibres, S = secondary, P = primary, L = Lateral, C = Central

Figure 4. **Example of a Rya histogram that refuses to resolve into S, LP and CP**

However, we see many Rya histograms with features that do not match the concept of three superimposed normal distributions, as shown in Figure 4. We see modes with higher or lower kurtosis than normal. We see thicker tails and thinner tails. We see unexpected "swellings" between secondaries and primaries that make us wonder whether suggestions about a fourth primary in camels might also apply to Ryas [2]. However, in the sample material we have at present these effects, although big enough to disturb our algorithms, are not large enough to rise above the stochastic noise level with confidence. There is something going on here, but we do not know what it is. Here, more work is required.
More work is also needed to see if the OFDA 100 can be made to distinguish between fibres that are opaque because they are ***kemps*** (medullated) and fibres that are opaque because they are ***pigmented*** [13].
There are several ***issues*** with the OFDA 100 MESFILE format, and how it causes problems in Excel – contact us to share our experiences and workarounds.

CONCLUSIONS

S/P ratios can be deduced from OFDA data without resorting to skin biopsy techniques. More work will be needed to determine the exact conditions under which this is or is not possible.

Modelling diameter distributions. Fleece fibre population can be modelled by three Normal distributions specified by seven parameters:

Table 1

Fibre Population expressed as three overlapping Normal Distributions

Follicle Type	Mean Fibre Diameter FD	Standard Deviation of FD	Population as function of Secondary/Primary ratio S/P
Secondaries S	FD_S	SD_S	
Lateral Primaries LP	FD_{LP}	SD_{LP}	
Central Primaries CP	FD_{CP}	SD_{CP}	

We suggest that these seven parameters will be more useful for genetic selection work for fleece and fur quality than earlier methods using generic FD and SD augmented by parameters expressing kurtosis and skewness. More work is required to determine the exact conditions necessary to guarantee convergence in the calculations involved, but our algorithms work on all the NEST samples that we have looked at and we have seen that they can also work with Merino samples of sufficient skewness.

Gotland S/P ratios. The subjective selection procedures that have been used to improve curl structure have not led to a better curvature match between primaries and secondaries; instead, they have led to a drastic reduction in the number of secondary fibres, so that modern Gotland pelts have S/P ratios as low as 0,06.

Pigmentation. In Gotland, each follicle type produces its own proportion between white and pigmented fibres. We see similar tendencies in other pigmented NEST breeds such as Spelsau which we have not yet measured. This could imply that genes for grey colour may regulate different follicle types in different ways [13].

Diameter of pigmented fibres. The pigmentation proportion does not change between the thinnest and the thickest fibres within the same follicle type; to a first approximation it appears to be constant. We conclude that, despite the fact that black fibres are black because they have received grains of melanin that are absent in white fibres, it is not to a first approximation possible to see any diameter difference between the white and the black fibres within a single follicle type.

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Gotland Silver I have ever analysed; and to Sonja Thyen for many kitchen-table conversations based on her decades of work as official optical microscopist and wool reviewer to both Ryaklubben and Svenska Finullsföreningen.

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