Is collagen quantity and properties involved in wrinkle formation and/or in follicle development?

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Contents

1 Introduction

We refer to the Introduction section of the Jackson and Watts(2017) [10] document, where we review the place of wrinkle or skin folds in Merino breeding, and where we specifically refer to the suggestion that all skin wrinkle must be culled from a sheep flock, before they can be successfully selected for the SRS-Merino breeding criteria. This statement leads to the questions of when and how wrinkle development interferes with follicle development to such an extent that breeding of superior Merinos using SRS methodology should be impossible in the presence of wrinkle. An attempt was made , in Jackson and Watts(2017) [10] to put forward the hypothesis that wrinkles form in the foetus at the same stage as secondary follicle development so would be likely to affect secondary follicle density or S/P ratio or secondary fibre diameter. This was based on a somewhat obsure reference (Bogolyubsky (1940) [1]) which asserts that wrinkles were observed forming in foetal skin of Karakul and Merino lambs at around 100 days of gestation. There are no other studies of wrinkle development, but there is a considerable literature on follicle development (see Fraser and Short(1960) [3] and Maddocks and Jackson(1988) [12] and Ryder and Stevenson(1968) [18] for reviews). There is some literature on collagen development in sheep skin, and we will look at that below.

What is proposed here is that the amount and type (and maybe timing and arrangement in the skin) of collagen development might be a common factor involved with both wrinkle development and follicle development. So what is known about collagen? Well, it is already there in the foetal skin at the time follicles develop (Knight et al (1993) [11]). These authors distinguish two collagen types (Type III or 'soft' collagen, and Type I or 'hard' collagen) and note that Type III is highest at 75 days of gestation, and falls progressively as the foetus develops, while Type I is low at day 75 and rises to over 50 percent by birth. Collagen fibres are formed from cells called *fibroblasts*. At 75-80 days the fibroblasts appear as plump, immature cells surrounded by reticular collagen fibres which are composed of Type III collagen. By birth the fibroblasts have matured and the collagen fibres may be intermeshed to varying degrees. If the fine reticular fibre pattern remains, it is soft collagen, if the fibres intermesh the collagen tissue is hardened to various degrees.

The arrangement (location) of collagen in the skin is also important. In adult skin, Mitchell(1984) [13] distinguishes 5 layers in a vertical section of sheep skin

Layer1 epidermis is mainly keratinised protein

Layer2 contains wool follicles and accessory glands, and is part of the dermis. Sometimes called *papillary layer*.

Layer3 layers 2 and 3 together called 'dermis' . Contains fibrous proteins, collagen, and elastin. Sometimes called *reticular layer* although the structure is not always reticular, but may be interwoven.

Layer4 contains volunntary muscle, collagen and elastin

Layer5 fat tissue

Mitchell notes that Layer2 is much weaker than Layer 3 (collagen not as hard). When wrinkles or folds occur in the skin, Layers 1,2, and 3 buckle up into a fold, while Layers 4-5 are straight. It appears as if wrinkles are formed either by an overgrowth of Layers 1-3, or by a shrinkage or tightening of Layer 4. Mitchell has demonstrated this conclusively by showing that if Layer4 (and Layer 5) are dissected away from a skin specimen with wrinkles, the folds in Layers 1-3 flatten out. So in a wrinkled sheep, Layer 4 is holding the skin under some tension, which relaxes when Layer 4 is removed.

So there is some link between collagen development in the skin, and wrinkle formation. If the collagen grows unevenly across the boundary between Layers 3 and 4, the top 3 layers form a fold to accomodate. Since wrinkles mostly form in rows, this unevenness of collagen development must be in one dimension - if it were 2 dimensional the upper 3 layers of skin would form a lump, rather than a fold. There is no reported observation of directional unevenness of collagen across Layers 3/4, but it must be so, or we would get lumps rather than folds.

We need to also ask if there are links between collagen development and follicle development.

2 The collagen-link hypothesis

Given the facts reviewed above, we propose two mechanisms by which the presence of skin wrinkles could affect follicle development and function. Both of these involve collagen properties.

2.1 The wrinkle - hard collagen - fibroblast numbers - pre-papilla cell numbers - follicle density - fibre diameter link

In skin with wrinkle development, there is more Type I (hard) collagen in the dermal layer (and probably in layer4 too). Hard collagen reguires more mature fibroblasts (because it has more fibres), and hence more fibroblasts must diffeentiate from the mesenchyme cells. This leaves fewer mesenchyme cells to differentiate into pre-papilla cells. Fewre pre-papilla cells leads to less S follicle initiation, less branched follicles, lower S/P ratio, and higher S fibre diameters.

The reverse situation is where the collagen stays soft (Type III) annd in a reticular pattern in the dermis. Wrinkles do not form (because the soft collagen is more elastic and therefore Layers 3 and 4 can stay flat. Soft collagen requires fewer fibroblast cells to differentiate (because it has fewer fibres), leaving more capacity for pre-papilla cell differentiation, leading to more S follicles initiated, more branching follicles, higher S/P ratio, and finer S fibres.

So it is a question of balance between collagen development and follicle development. We have no idea what controls the balance. It is undoubtedly genetic. The work of our colleague Dr Philip Moore and collaborators (Xavier

et al (2013) [24] and Gordon-Thomson et al (2007) [4]) suggests that the Notch pathway genes are implicated.

If this proposed mechanism is correct we expect

- wrinkled sheep will have either more collagen or harder collagen or both in the dermal layer

- sheep with more or harder collagen will have lower pp-cell numbers,with consequent lower S/P ratio and higher S fibre diameters.

These expectations can be tested. We do that by measuring collagen amount and softness in the dermal layer of skin specimens, and relating it to wrinkle, S/P ratio, and diameter of secondary fibres.

2.2 The wrinkle - hard collagen - curved follicles - curved fibres link

In skin with wrinkle development, there is more hard collagen in the dermis. The downgrowths of epithelial tissue which form developing follicles have to penetrate and displace the dermal tissue. If the dermis is soft collagen with a reticular pattern, the follicle downgrowths can readily penetrate dermal tissue and are straight. If the dermis has hard, intermeshed collagen, the follicle downgrowths can not readily penetrate the dermis to fuill depth, so they grow in a curved shape and the bulbs are closer to the epidermis. The orientation of the plane of curvature would depend on spatial considerations. Curved follicles grow curved fibres, with a greater proportion of para cortex, and a more highly keratinised paracortex.

So it is a question of the way the bulb cells differentiate to form the fibre cortex in straight versus curved follicles. In curved follicles there is more bilateral asymmetry in the fibre cortex. If there is more keratinization it means bulb cell differentiation into fibre proceeds for longer, so the fibre length growth rate would be slower. There may also be differences in blood vessel supply to follicles between skins with curved and straight follicles (Nay(1966) [16]). Collagen development and blood vessel development may therefore interact.

If this proposition is correct we expect

- wrinkled sheep to have more or harder collagen in the dermal layer (as in proposition 1)

- sheep with more or harder collagen to have curved follicles

- sheep with curved follicles to grow curved fibres, with a more radical ortho/para segmentation

- sheep with curved follicles to have a lower fibre length growth rate

These expectations can also be tested. We already know that the overall genetic correlation of wrinkle score with crimp frequency is 0.46 (95 percent confidence limits 0.40-0.51) , and the genetic correlation of wrinkle score with

follicle curvature score is 0.69 (95 percent confidence limits 0.65-0.74) (Jackson (2017) [8]). These strong correlations are an overall corfirmation, but do not prove that collagen is the linking factor. In the present study we have collagen measurements, but we do not have follicle curvature scores, so testing the above expectations will have to be completed elsewhere.

3 Materials and Methods

3.1 Sheep studied

This is a small study. A total of 106 sheep, representing fine, medium, and strong wool strains of Merino, were sampled from 5 flocks over the period 1988 to 2003. The flocks and sheep were chosen to ensure that all four grades of SkinType (as defined by Watts et al (2017) [23]) were well represented. The four SkinType grades contain information on skin wrinkle, but are not wrinkle alone.

3.2 Measurements

The following measurements and scores were available

SkinType visual scores for sheep skin type. Four grades SRS, semi-SRS, flat, and tight, as defined by Watts et al (2017) [23].

TST total skin thickness in mm. Measured with a ruler graduate in 0.1 mm divisions at 3x magnification on the midside skin sample trimmed of wool stuble and subdermal fat. It consists of epidermis, papillary layer, and reticular layer (layers 1 to 3).

CST compressed skin thickness in mm. Measured on the trimmed sample with a Mitutoyo ballpoint depth guage (graduated in 0.1 mm divisions) at four sites. Analyses are of the mean CST over 4 sites.

CMP compressibility as a percentage. Calculated from CST and TST as $CMP = 100(TST - CST)/TST$. Measures the reduction in thickness under compression as a percentage of the uncompressed thickness.

SkinSoft skin softness score or ease with which the skin bends or buckles. Five grades (1=hard, unable to bend), to (5=supple, bends easily). Assessed by manually bending the trimmed skin sample in two directions (north-south = across the rows of follicle groups) and (east-west = along the rows of follicle groups).

S/P ratio of secondary to primary follicle numbers. This ratio is normally used as a measure of secondary follicle density which is independent of skin expansion during growth. Measured on skin sections.

Fn follicle number per unit area in follicles per mm^2. Measured on skin sections with a correction for shrinkage during processing

Dp mean fibre diameter of secondary fibres in μm. Measured on skin sections.

DpSD standard deviation of secondary fibre diameters in μm. Measured on skin sections.

3.3   Interpretation of measurements

Some care is needed in defining what these measurements actually represent. The collagen measurements should be seen as follows

TST amount of collagen in the dermis (ie layers 2 and 3)

CST would reflect both the amount of collagen (as in TST) and its softness

CMP compressibility should reflect collagen softness only, since it is relative to TST.

SkinSoft bending a sheet of material compresses one side and extends the other. So this score should also reflect compressional softness of collagen. There may however be some effect of collagen thickness, since a thick sheet does not bend as easily as a thin sheet.

The SkinType scores do not only reflect degree of skin wrinkle. We repeat the score descriptions here for clarification

tight sheep with fleeces consisting of thick and stiff staples. The fleece is often excessively greasy, short in length and forms closed backs. The skin is very thick and forms wrinkles to varying degrees over the body. No attempt was made to subdivide this class on degree of wrinkle .

flat relatively plain bodied sheep with fleeces consisting of lightweight staples. The wool is non-lustrous and has low crimp amplitude. There are two subtypes: a soft and marginally loose skin with widely spaced fibre bundles and thin staples; and a thick and taut skin with flat, wide staples that are not soft.

semi-SRS plain bodied sheep with fleeces consisting of long, thin staples. Fibre bundles are not present. The wool is soft and semi-lustrous. The crimp amplitude is not as pronounced as for SRS animals. The skin is loose.

SRS plain bodied sheep with fleeces consisting of very long, and closely packed fibre bundles and thin staples. The wool is very soft, lustrous and has high crimp amplitude (deep crimp) and low crimp frequency (bold crimp). In long wool, the fleece parts along the backline. The skin is very loose.

So only the *tight* SkinType sheep are wrinkled. The other three grades are distinguished on skin looseness and thickness. Looseness means the opposite of taut skin and able to me moved laterally on the sheep. Thickness is obvious and would be expected to relate to amount of collagen. Looseness is not so obvious.

It means the skin is larger than the underlying tissues so the skin surface area exceeds the smooth body surface area, but not by forming folds or wrinkles. It is not clear where the looseness is in relation to the 5 skin layers defined above. Wool properties are also involved in assessing the higher grades.

3.4 Statistical Methods

Data were imported into the R statistical program [17] and analysed using the *lm()* function for regressions, and the *aov()* function for analysis of variance.

4 Results

4.1 Collagen data summary

The collagen measurements are new traits not previously studied. We report their means and standard deviatins in Table 1

Table 1: Means and standard deviations for collagen measurements separately for each flock

Flock	Strain	TST		CSTmean		CMP	
		Mean	SD	Mean	SD	Mean	SD
1	medium	2.51	0.342	0.95	0.246	61.9	8.24
2	medium	2.24	0.363	0.85	0.381	62.4	12.65
3	fine	2.63	0.337	0.62	0.121	76.2	5.27
4	strong	2.46	0.384	0.77	0.176	68.5	5.81
5	medium	2.52	0.316	0.87	0.203	65.6	6.10

The fine Merino flock has the largest TST, the smallest CST, and therefore the largest compressibility(CMP).

We need to look at the four individual CST readings from two points of view - are they repeatable, and is there any trend such as might be caused by the first compression influencing subsequent readings. First simply look at a plot of Flock means for CST for each of the four repeat readings (Figure 1)

We see that there is no obvious trend with time. One compression does not influence the results from subsequent compressions. It is therefore satisfactory to use CSTmean.

The correlations between successive readings are shown in Table 4.1.

We see that the readings are higly repeatable, and that the repeatability does not decline with time gap.

4.2 SkinType and collagen

The collagen measurements for the four SkinTypes will tell us whether wrinkle and skin looseness are associated with amount and type of collagen. Figure 2 shows the TST of the four SkinTypes as a boxplot.
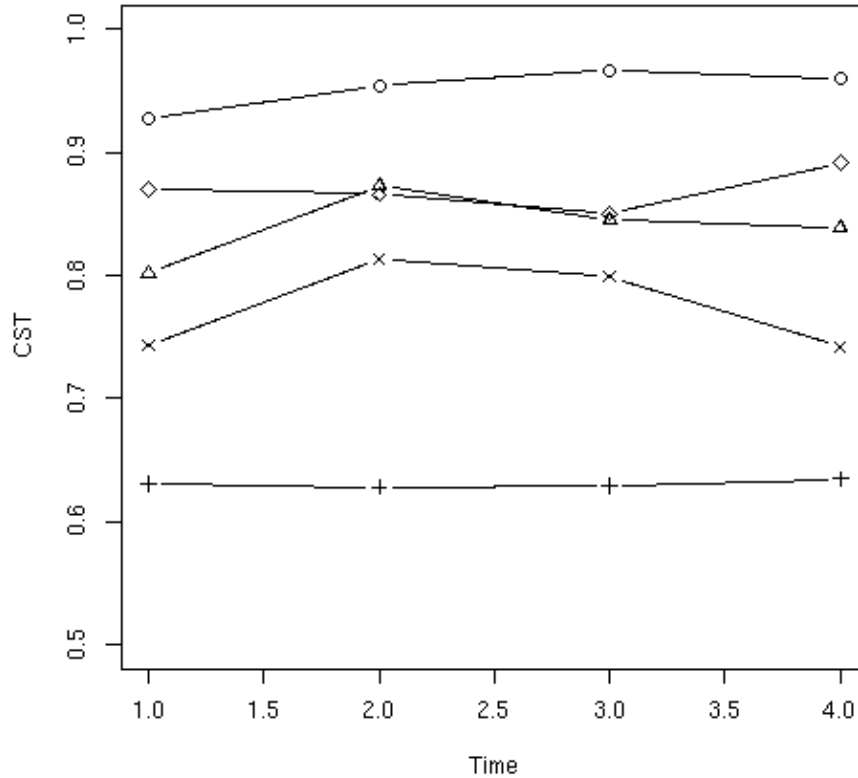
Figure 1: Plot of Flock means for each of the four repeat CST readings in time series order.

Only the SRS SkinType seems to differ, but we need to test the significance. WE do this with an analysis of variance. Table 3 shows that there are significant Flock differences, but no significant differences between SkinTypes, and no interaction.

So we conclude that the total thickness of collagen in the dermis is not related to either wrinkle (the *tight* grade) or to looseness (the *SRS and senmi-SRS* grades).

Figure 3 shows the CST of the four SkinTypes as a boxplot.

Here we see that there substantial differences, and in the order expected. The *tight* SkinType has the greates compressed skin thickness, followed by the *flat* SkinType, followed by *semi-SRS*, and *SRS* SkinType has the smallest CST. We check that this is significant with another analysis of variance in Table 4

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Table 2: Correlations between the four repeat CST measurements

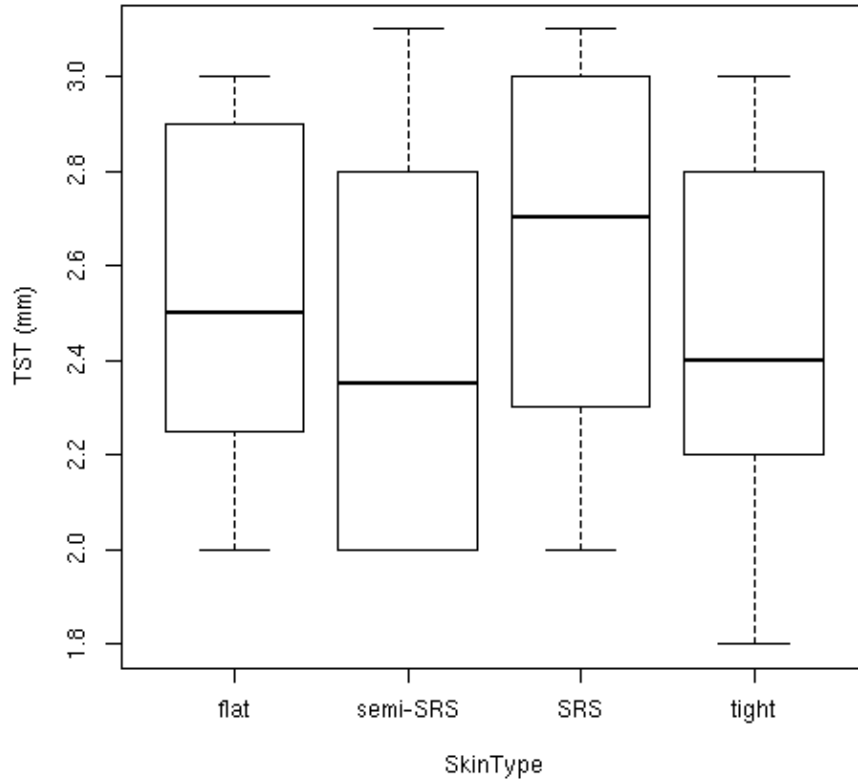|	CST1	CST2	CST3	CST4
CST1	1.00	0.85	0.84	0.85
CST2	0.85	1.00	0.86	0.87
CST3	0.84	0.86	1.00	0.85
CST4	0.85	0.87	0.85	1.00

Figure 2: Boxplot of TST values for all sheep in each of the four SkinTypes

In this case both the Flock and SkinType effects are significant, but not the interaction. So we conclude that thickness of collagen in the dermis after compression is greater in wrinkled sheep (*tight* grade), and is reduced in sheep with loose skin (*SRS* grade).

We suspect that because the SkinType differences were in CST but not in

Table 3: Analysis of variance of TST measurements

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Flock	4	1.53	0.38	3.13	0.0182
SkinType	3	0.30	0.10	0.83	0.4826
Flock:SkinType	5	0.68	0.14	1.11	0.3617
Residuals	93	11.36	0.12		
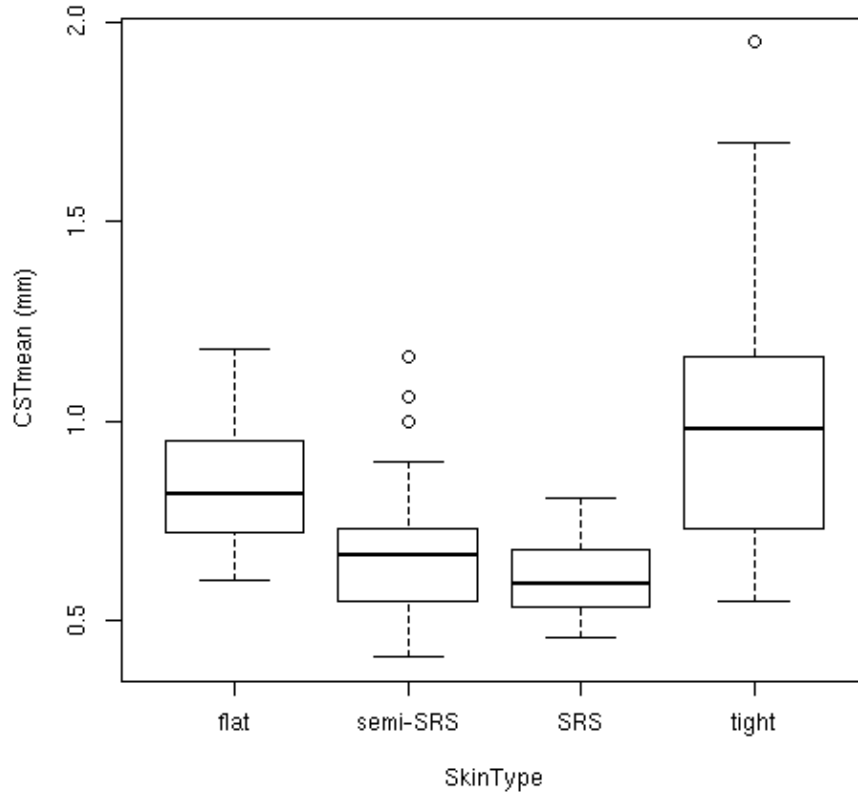
Figure 3: Boxplot of CST values for all sheep in each of the four SkinTypes

TST that what we are looking at is differences between hard and soft collagen, the latter being more readily compressed. We can express this more succinctly by analysing compressibility (CMP).

Figure 4 shows the CMP measurement of the four SkinTypes as a boxplot. Here we see a similar picture to the CST graphs, there being substantial

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Table 4: Analysis of variance of CST measurements

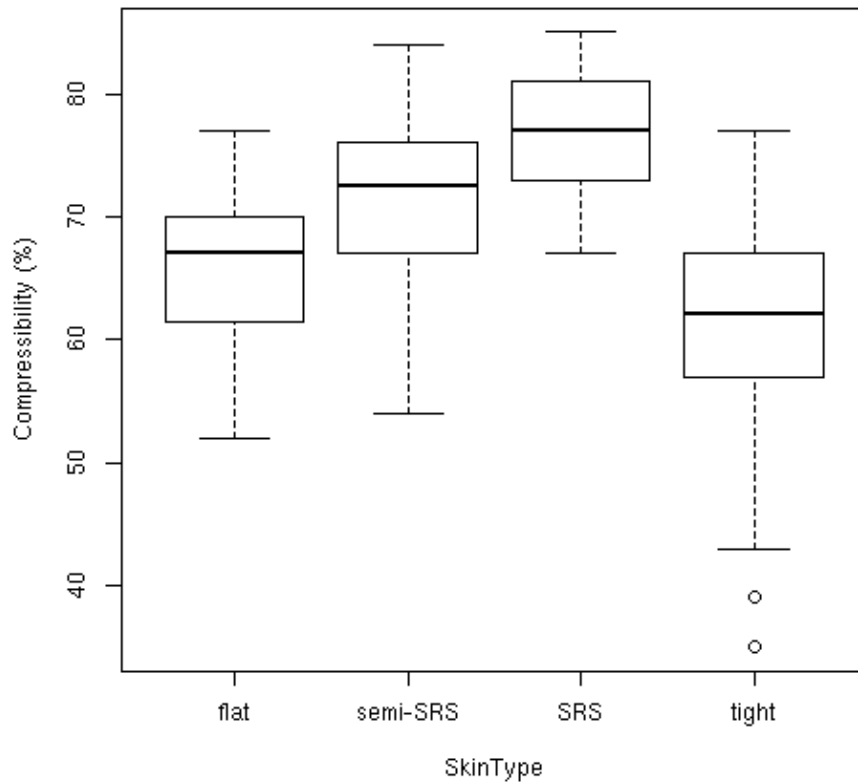|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Flock	4	1.42	0.36	7.83	0.0000
SkinType	3	0.87	0.29	6.36	0.0006
Flock:SkinType	5	0.26	0.05	1.14	0.3434
Residuals	93	4.22	0.05		

Figure 4: Boxplot of CMP values for all sheep in each of the four SkinTypes

differences and in the order expected. The *tight* grade, which is wrinkles, is only 60 percent compressible, and the others range up to 80 percent in order. We check the significvance in another analysis of variance in Table 5 tex table generated in R 3.4.2 by xtable 1.8-2 package

As for CST the Flock and SkinType effects are significant, but not the

Table 5: Analysis of variance of skin compressibility (CMP) measurements

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Flock	4	2986.36	746.59	15.72	0.0000
SkinType	3	1172.83	390.94	8.23	0.0001
Flock:SkinType	5	436.47	87.29	1.84	0.1131
Residuals	93	4417.96	47.50		

interaction. So we conclude that compressibility is least in wrinkled sheep (*tight* grade), and is greatest in sheep with loose skin (*SRS* grade). So the collagen is harder in wrinkled sheep, and softer in sheep with loose skin.

4.3   Collagen Flock differences

A feature of the analyses of the previous section is that there were large and significant Flock differences in all three traits (TST, CSTmean, and CMP). We documented these in Table 1. It should be noted that not all SkinTypes were present in each Flock, so there could be some bias in these Flock means. We can circumvent this to some extent by looking at the two-way table of means Flock x SkinType. We do this for CMP in Table 4.3

Table 6: Means of CST for all combinations of Flock and SkinType. The NA entries are cells with no data available.

Flock	tight	flat	semi-SRS	SRS
1	55.2	64.32	NA	NA
2	53.0	NA	66.3	72.3
3	NA	NA	75.0	78.1
4	67.3	69.7	69.0	NA
5	63.0	NA	65.8	75.0

We see that the SkinType differences are consistent across Flocks. We also see that the Flock differences are consistent across SkinTypes. That is what the absence of interaction in Table 5 shows.

So we need to reach an understanding that SkinType is not the only thing correlated with collagen measurements. Flocks difffer in TST, CSTmean and CMP in ways that are not explained by Flock differences in the proportions of sheep with each SkinType (look for example at the semi-SRS column of Table4.3). These SkinType independent Flock differences may be genetic or non-genetic. Something else affects the amount and hardness of collagen without affecting SkinType. We are sure of one thing, it is not a shift in classing standards for SkinType between Flocks.

4.4 Collagen and skin softness appraisal

We have another way of checking on whether the CSTmean and CMP measurements actually represeent collagen softness. We can compare them with the SkinSoft scores. The correlations between SkinSoft score and the 3 collagen measurements are given in Table 7

Table 7: Correlations among TST, CSTmean, CMP, and SkinSoft score

	TST	CSTmean	CMP	SkinSoftScore
TST	1.00	0.33	0.14	-0.29
CSTmean	0.33	1.00	-0.88	-0.72
CMP	0.14	-0.88	1.00	0.61
SkinSoftScore	-0.29	-0.72	0.61	1.00

The correlation of SkinSoft score with CSTmean is slightly higher than that with CMP. Dividing by TST may be introducing errors. There is only a small correlation with TST, and that is expected. Bending softness is more a matter of softness of the material than of minor variations in material thickness. Compare sheet aluminium with sheet steel, minor thickness variations will not make sheet steel bend like aluminium.

4.5 Collagen and follicle characteristics

Figure 5 shows the correlation beetween S/P ratio and TST measurements across all Flocks.

The correlation of 0.11 is not significant.

Figure 6 shows the correlation between S/P ratio and CSTmean measurements across all Flocks.

The correlation 0f -0.38 is significant ($P < .01$). There is one serious outlier point in Figure 6 - the green point from Flock 2 at the right of the Figure. It was a sheep with an extremely thick tight skin, but it did not have an unusually low S/P ratio. If we omit this one outlier the correlation becomes -0.44 which is also significant ($P < .01$).

The other thing to note in Figure 6 is that the Flocks tend to form a series of groups along the curve. That is there is also a negative relationship between the Flock means, in addition to that among individual values. If we plot the same points but label each point with its SkinType, we get Figure 7

This shows that the four SkinTypes also form a series of groups so there is also a negative relationship between SkinType means. The correlation is of course the same as in Figure 6.

We noted in section 4.3 that SkinType differences in CSTmean and Flock differences in CSTmean seemed to be independent. However now we observe that so far as the correlation of CSTmean with S/P ratio is concerned, SkinType differences, and Flock differences and individual differences all seem to part of the same continuous negative relationship. This is something of a mystery. What it says is that it doesnt matter whether differences in CSTmean are
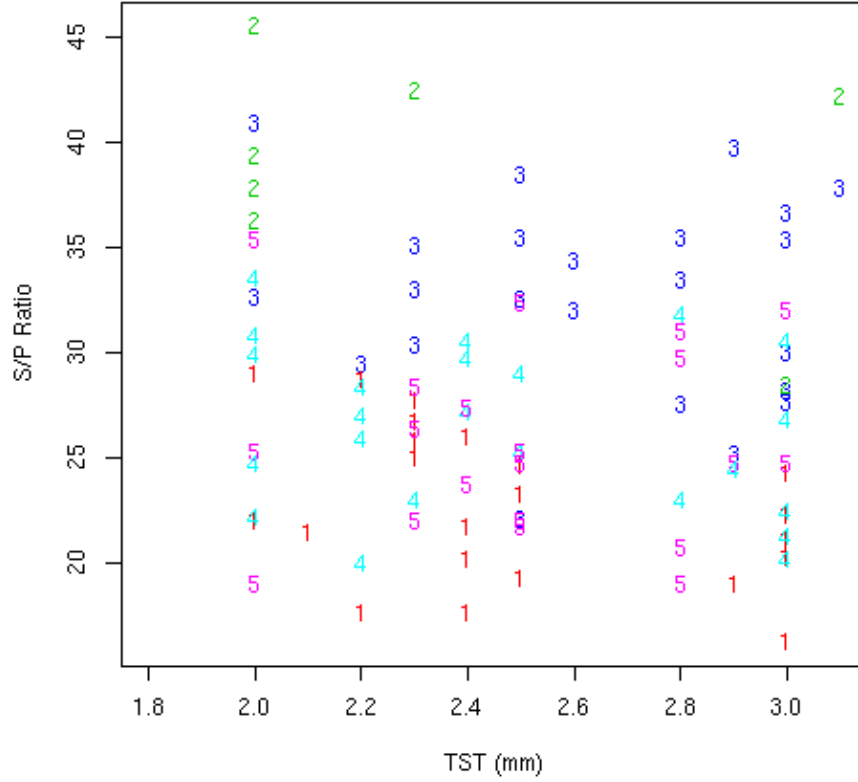
Figure 5: Plot of TST measurements against S/P ratio. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is 0.11 which is not significant for 106 observations

observable as SkinType or not, they are all correlated with S/P ratio. Perhaps the SkinType classing grades are not observing some of the collagen differences.

The main point is that there is a significant negative relationship of CST-mean with S/P ratio. This is what the collagen-link hypothesis predicts, so it is an important confirmation.

Figure 8 shows the correlation between S/P ratio and CMP measurements across all Flocks.

For compressibility versus S/P ratio there are two outlier points, and the correlation (0.36) is similar to that for CSTmean, but of course is now positive - soft compressible skins have a high S/P ratio. Again the remarks about Flocks grouping along the relationship line apply. We do not bother to look at a graph
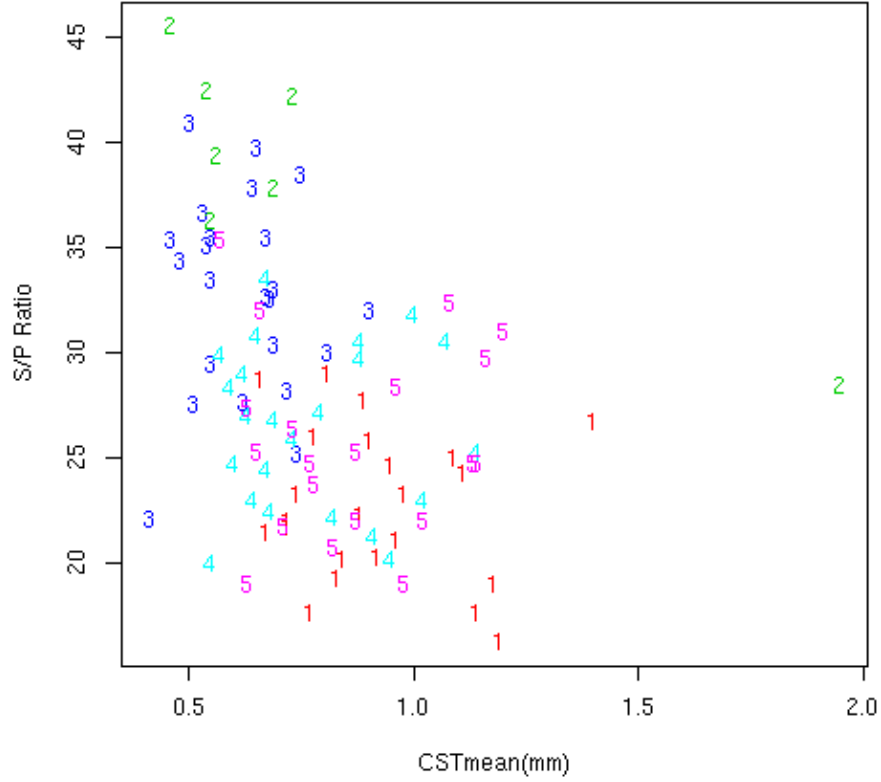
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Figure 6: Plot of CSTmean measurements against S/P ratio. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is -0.38 which is significant at the 1 percent level for 106 observations

showing SkinType instead of Flock for each point, because the same should apply as for CSTmean, above.

We have chosen to look at S/P ratio rather than follicle number per unit area. There are two reasons

- it is the secondary follicle number which the collagen-link hypothesis predicts would be affected by excess collagen formation. Follicle number per unit area would of course reflect this, but it is a more indirect consequence

- S/P ratio is basically a way of expressing secondary follicle density independent of any effects of varying body growth on skin expansion. So we would expect S/P ratio to be free of some other confounding factors
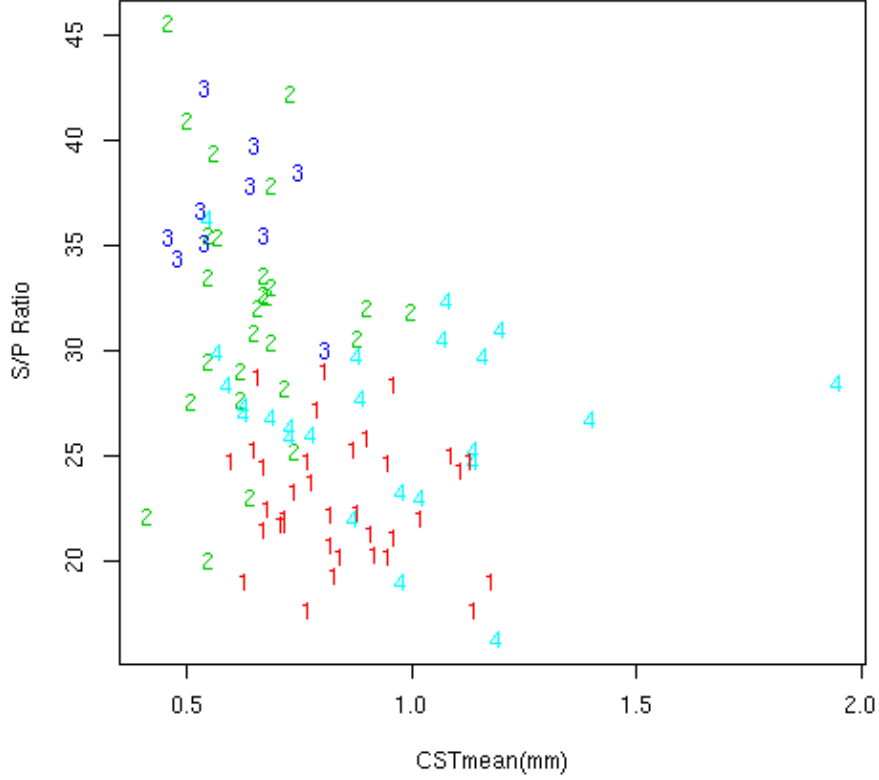
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Figure 7: Plot of CSTmean measurements against S/P ratio. The numbered points reveal the SkinType to which each data point belongs (1="flat", 2="semi-SRS", 3="SRS", 4="tight"). The correlation of these points is -0.38 which is significant at the 1 percent level for 106 observations

What we shall do is just have a quick look at follicle number by computing correlations. These are shown in Table 8. We also include the SkinSoft score here, as it gives similar results to the collagen softness measurements.

As expected, follicle number has the same relationship with CSTmean and CMP as does S/P ratio. It has a different correlation with TST, but these correlations (-0.11 and 0.19) are small and the 0.19 is only just significant at the $(P, .05)$ level. The surprise is that the correlation of SkinSoft score with S/P ratio is as strong as the correlations of the CSTmean and CMP measrements with S/P ratio. The scoring is clearly just as accurate as the measurements. It does however have a different lower correlation with Fn.
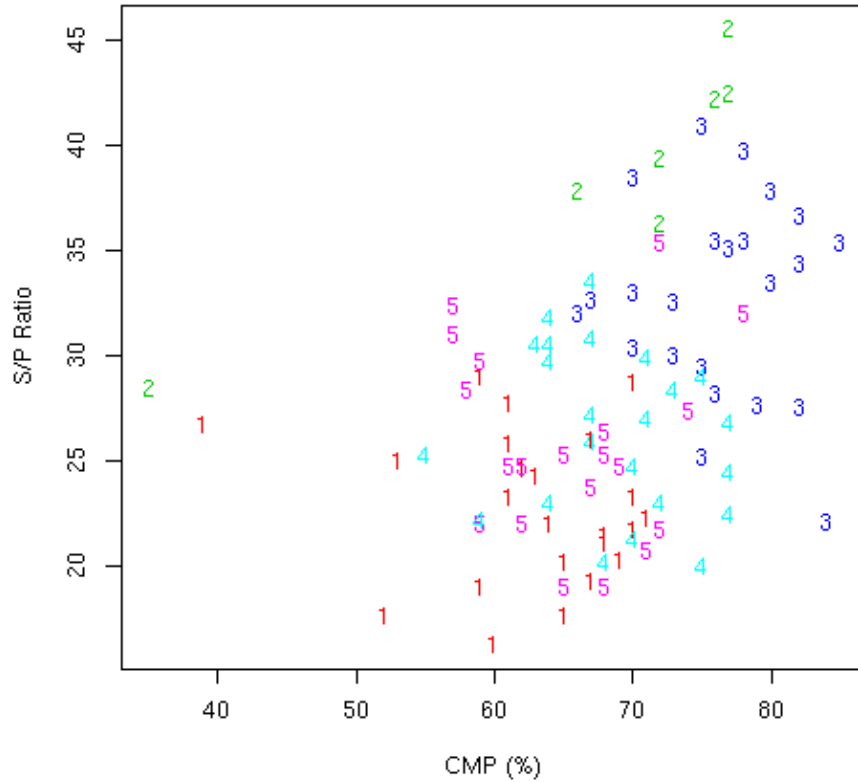
16
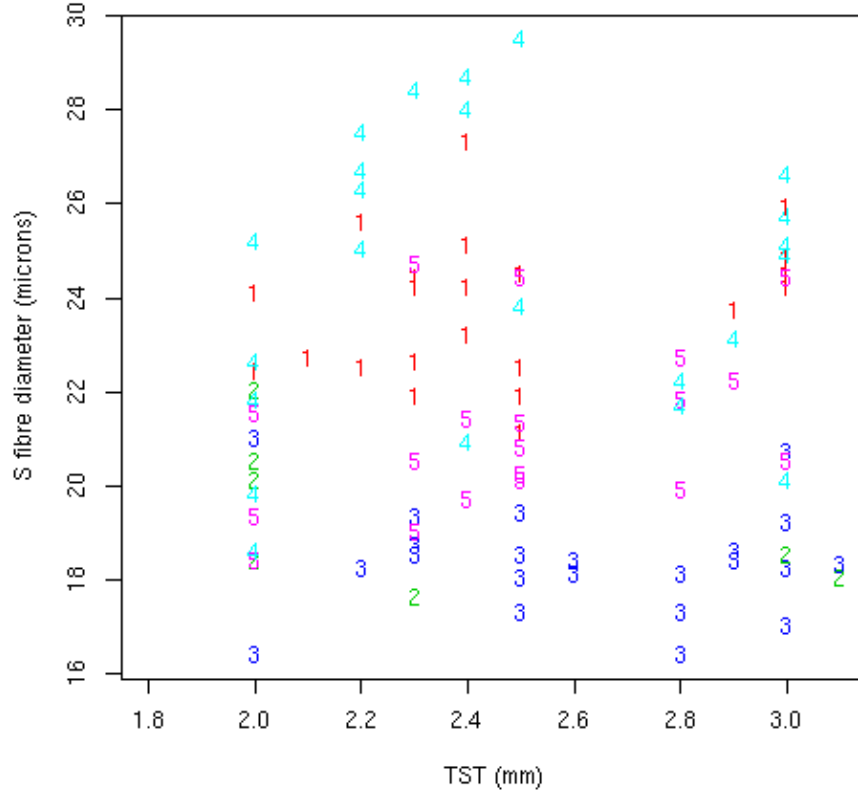
Figure 8: Plot of CMP measurements against S/P ratio. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is 0.36 which is significant at the 1 percent level for 106 observations

This proves that we can put follicle number aside and just concentrate on the relationships of collagen with S/P ratio.

4.6   Collagen and Fibre growth

The collagen-link hypothesis postulates that sheep with high and/or hard collagen skins will have higher average diameter of secondary fibres, more curved fibres, and a lower fibre length growth rate. It does not postulate anything about the variability of fibre diameter. The effect on mean secondary fibre diameter is not direct, it is secondary to the effect on S/P ratio.

Figure 9 shows the correlation of TST measurement with meandiameter of

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Table 8: Correlations among TST, CSTmean, CMP, SkinSoft, S/P, and Fn

	TST	CSTmean	CMP	SkinSoft	S/P	Fn
TST	1.00	0.34	0.13	-0.26	-0.11	0.19
CSTmean	0.34	1.00	-0.88	-0.73	-0.37	-0.33
CMP	0.13	-0.88	1.00	0.64	0.36	0.43
SkinSoft	-0.26	-0.73	0.64	1.00	0.36	0.26
S/P	-0.11	-0.37	0.36	0.36	1.00	0.38
Fn	0.19	-0.33	0.43	0.26	0.38	1.00

secondary fibres.

Figure 9: Plot of TST measurements against mean secondary fibre diameter. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is -0.04 which is not significant for 106 observations

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The correlation is close to zero and not significant.

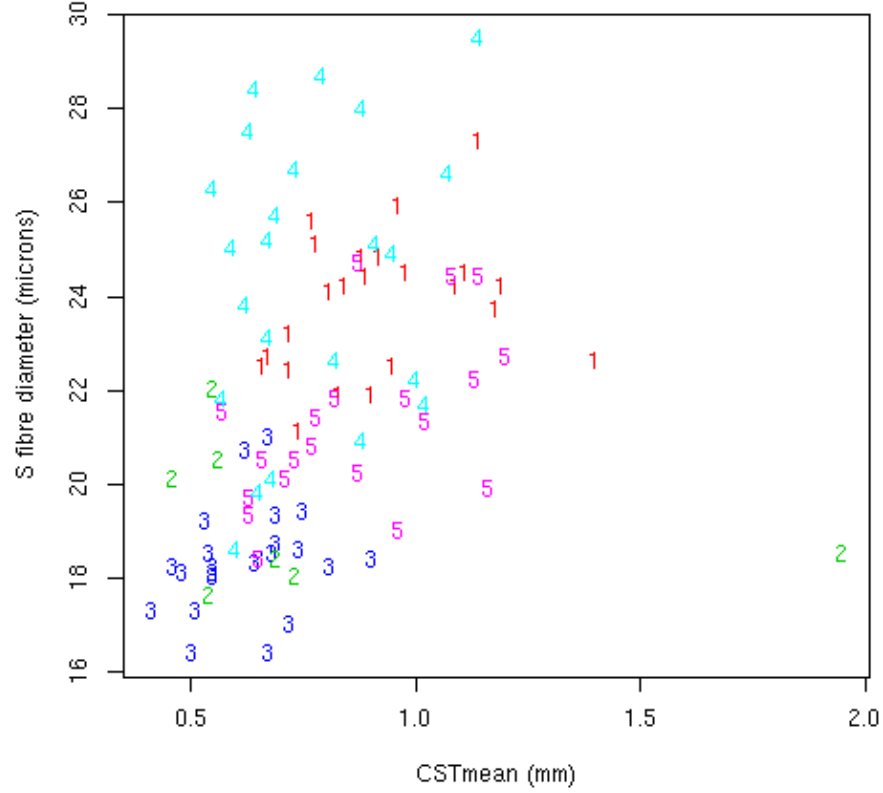Figure 10 shows the correlation of CSTmean measurement with mean diameter of secondary fibres.

Figure 10: Plot of CSTmean measurements against mean secondary fibre diameter. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is 0.34 which is significant at the 1 percent level for 106 observations

This correlation is 0.34 and is significant ($P < 0.01$). Flock 4 seems to have a higher mean S diameter than the other flocks, but this difference is not associated with a correlated difference in CSTmean. Hence Flock 4 appears above the other points on the graph. We know there things other than CSTmean which affect mean S fibre diameter - for example the numbers of pre-poapilla cells which initiate the secondary follicle. There is one outlier point.

Figure 11 shows the correlation of CMP measurement with mean diameter
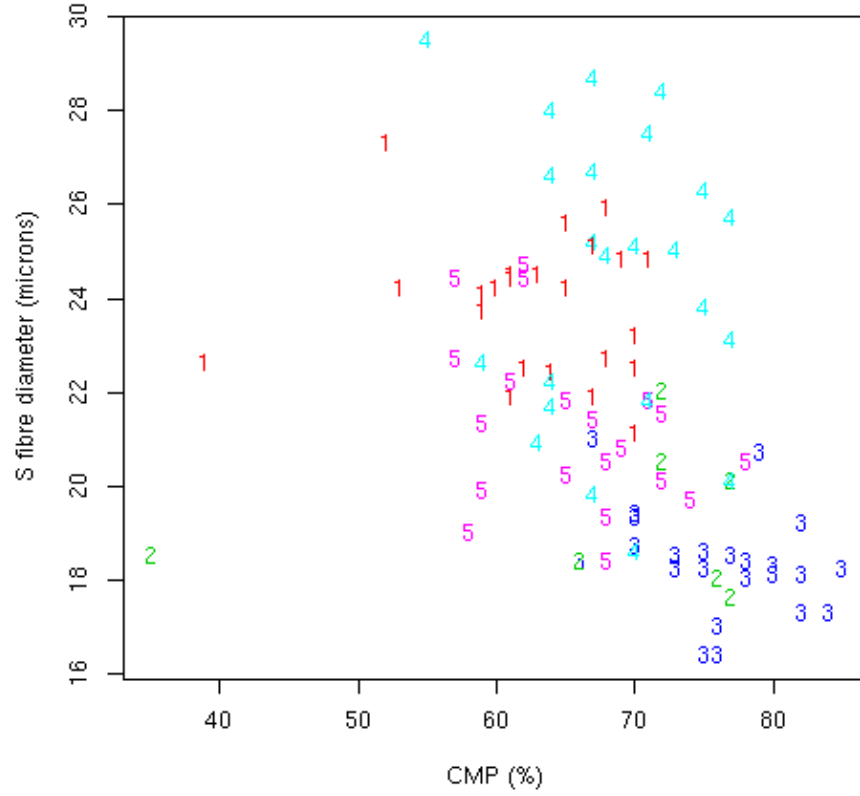
of secondary fibres.

Figure 11: Plot of CMP measurements against mean secondary fibre diameter. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is -0.40 which is significant at the 1 percent level for 106 observations

This correlation is -0.40 and is significant ($P < 0.01$). Again Flock 4 seems to have a higher mean S diameter than the other flocks, and appears above the other points on the graph. There are two outlier points.

Figure 12 shows the correlation of TST measurement with standard deviation of diameter of secondary fibres.

The correlation is close to zero and not significant.

Figure 13 shows the correlation of CSTmean measurement with standard deviation of diameter of secondary fibres.

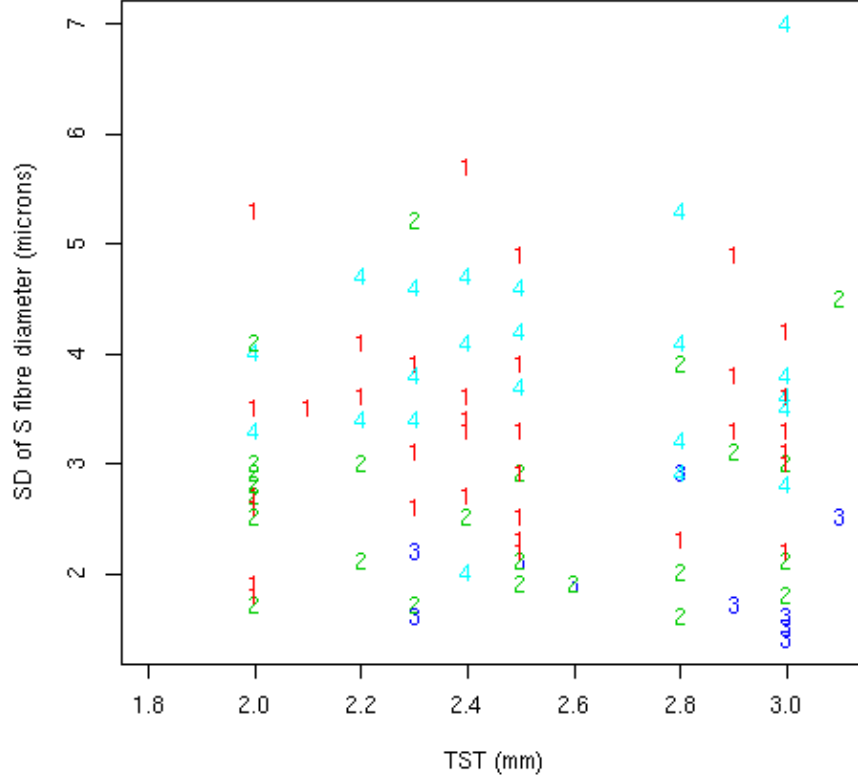The correlation is 0.39 and is significant ($P < 0.01$). In this case there little

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Figure 12: Plot of TST measurements against standard deviation of secondary fibre diameter. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is -0.01 which is not significant for 106 observations

suggestion of Flock 4 being higher in DsSD than expected from CSTmean. So in this case other factors are not operating. It is likely that the effect of collagen on DsSD is not via mean Ds, but is a conseuence of the *wrinkle - hard collagen - curved follicles - curved fibres link*. It is known that skins with high follicle curvature score also have high follicle unevenness score (genetic correlation is 0.82±0.02 Jackson(2017) [8]), and therefore grow fibres of less uniform diameter.

Figure 14 shows the correlation of CMP measurement with standard deviation of diameter of secondary fibres.

The correlation is -0.43 and is significant ($P < 0.01$). Again Flock 4 does not stand out, so the remarks above apply. Hard collagen makes curved follicles,
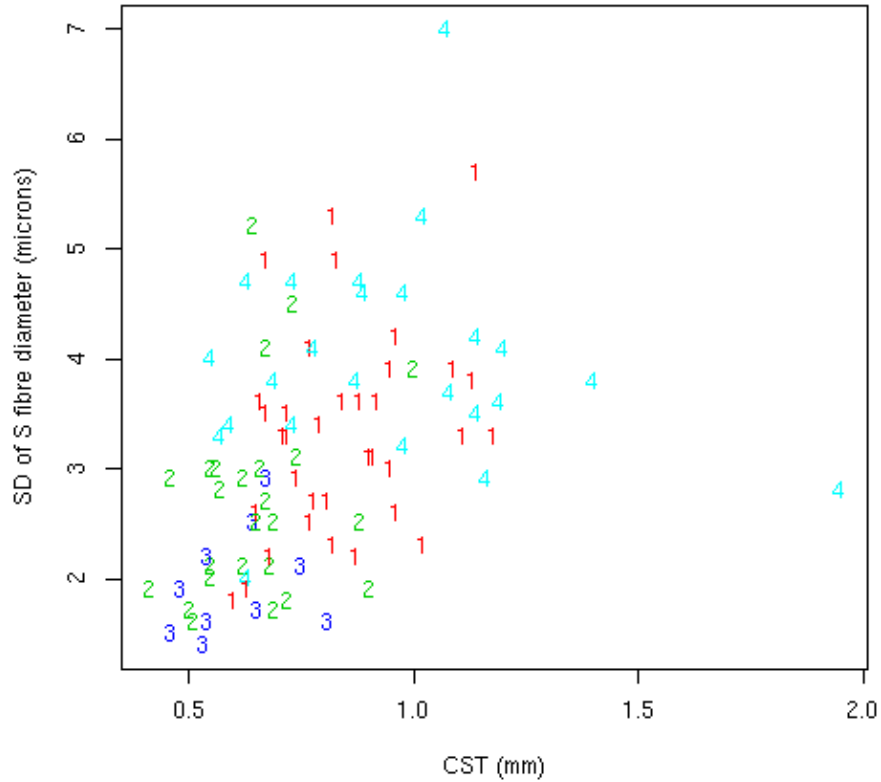
Figure 13: Plot of CST measurements against standard deviation of secondary fibre diameter. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is 0.39 which is significant at the 1 percent level for 106 observations

which are uneven in their anatomy and in their fibre growth.

What should be looked at here is the relations of collagen measurements to follicle curvature and follicle unevenness scores. We do not have the data.

[Jim, I went looking for follicle curvature and length growth rate data - there is lots in the staple structure file, but on these sheep we have CrimpType but no SkinType. I am sure you have this info. We could use it here. It would at least confirm the collagen-curved follicles - curved fibres link.]

[An alternative would be to get follicle curvature and unevenness (and probably depth) on the current data, where we have the actual collagen measurements. Is that possible?]
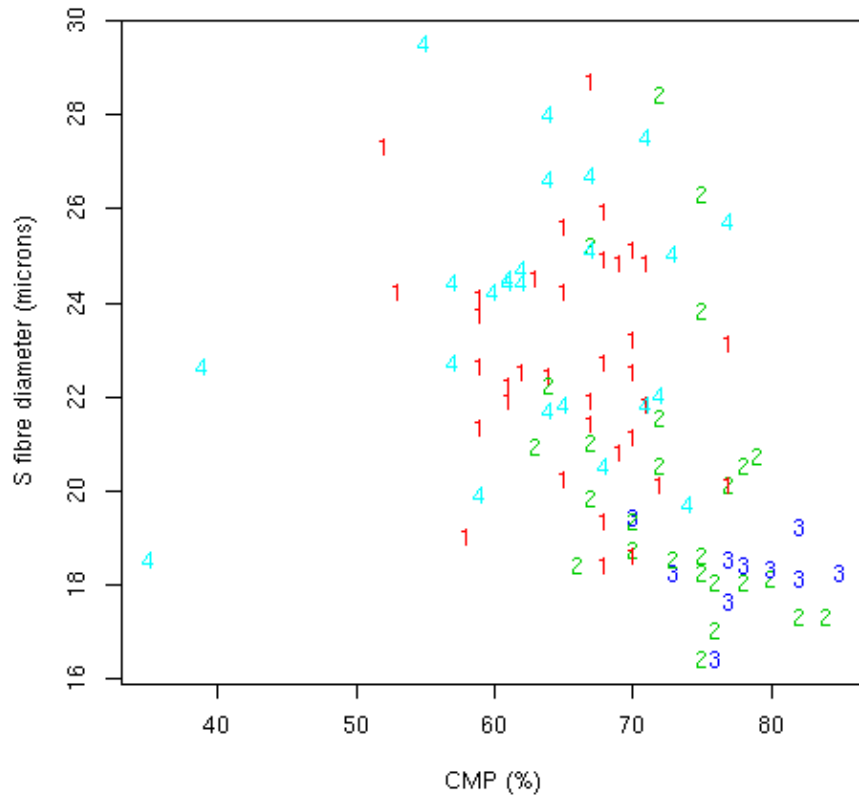
Figure 14: Plot of CMP measurements against standard deviation of secondary fibre diameter. The numbered points reveal the Flock to which each data point belongs. The correlation of these points is -0.43 which is significant at the 1 percent level for 106 observations

5   Discussion

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