

Does follicle development affect the spatial layout
of sheep skin?

Neville Jackson and Jim Watts

27 Jan 2018

Contents

1	Introduction	2
2	The follicle development - dermal expansion hypothesis	2
3	Materials and Methods	4
3.1	Sheep studied	4
3.2	Measurements	4
3.3	Statistical Methods	5
4	Results	5
4.1	Breed comparisons of amount of follicle tissue per unit area . . .	5
4.2	Using N_p as a measure of dermal expansion	9
4.3	Data on variation within a wrinkled Merino flock	12
4.4	Rex mutation	17
5	Discussion	20

1 Introduction

An attempt was made , in Jackson and Watts(2017) [13] 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 obscure 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) [5] and Maddocks and Jackson(1988) [15] and Ryder and Stevenson(1968) [22] for reviews). There is some literature on collagen development in sheep skin, and we will look at that below.

In Watts and Jackson(2017) and attempt was made to measure collagen type and content, and to relate it to follicle development and to wrinkle development. The study showed that soft, pliable skins with high compressibility had little or no wrinkle development , high follicle densities, fine secondary fibres, less curved follicles, less uneven follicles, and less uneven secondary fibres It was suggested taht there were two mechanisms involved

- a tradeoff relationship between fibroblast development and follicle development
- a mechanical interference of collagen fibrils with follicle shape and arrangement.

. It was not clear exactly what the mechanism was behind the relationship of compressibility to wrinkle. It was noted that dissection of layer 4 away from the dermis resulted in the wrinkles flattening out - so wrinkle is clearly an excess growth of dermis over that of layer 4, combined with an attachment of the dermis to layer 4.

What we investigate here is the suggestion that it is the follicle development which causes the dermis to expand at a greater rate than layer4

2 The follicle development - dermal expansion hypothesis

We noted above that wrinkle development and follicle development occur at the same time in the developing foetal lamb - at about 100days. Wrinkles are certainly visually obvious at birth, and so are follicles because we can see the growing fibres in the birthcoat.

It is one thing to say that because they occur together, wrinkles might affect follicle development. It is another thing to say, as we do here, that it is the other way around - that the growth and development of follicle tissue in the dermis is what causes the dermis to expand at a greater rate than the adjoining layer 4. That extra dermal expansion will not necessarily result in wrinkle - if

layer 4 is tightly bound to the lower dermis (presumably by excessive collagen formation) then the dermis will have to fold as it expands, but if the dermis is only loosely bound to layer 4, it can expand without folding.

So if that is how it works, why do only Merino (and Merino derived) sheep have wrinkles? Because only Merino sheep have the vastly greater development of secondary follicles with consequent higher S/P ratio and follicle branching. In all non-Merino breeds the amount of follicle tissue laid down during secondary follicle development is not sufficient to expand the dermis enough to cause it to fold.

So if SRS-Merino has an even higher S/P ratio than normal Merinos, how is it that they do not develop even more skin folds? Because they also have 'loose' skin - that is skin in which the dermis is not tightly bound to layer 4, but is free to detach and move. The involvement of collagen amount and type in this 'looseness' is conjecture at this stage, but is supported by Watts and Jackson(2017) [28]

There is another clue in the fact that skin folds occur in a pattern over the body of a sheep. On the side of the sheep the folds run in a dorso-ventral direction. This corresponds to the direction in which rows of follicle groups run. What we call the E-W direction on an horizontal skin section corresponds to the dorso-ventral direction on the sheep. The rows of follicle groups run parallel to the folds of skin in a sheep with folds. The rows of follicle groups run parallel to the 'laneways' in a sheep without folds. It is clear what is happening. The downgrowths of follicle tissue into the dermis occur in rows (because the follicle groups are in rows), so they push the dermis aside in rows as they make space to grow. If the dermis is going to expand and fold, it will fold in rows. If the dermis is going to just expand, because it is not bound to the subdermal tissue, there will occur rows of dermis pushed aside by the growing rows of follicles, and we will get 'laneways'. This is not proof, but it is very suggestive that secondary follicle development is involved in wrinkle formation, especially the branching secondary follicles as these are confined to close proximity to the follicle groups. One might object that skin folds are on a larger scale than rows of follicle groups. The answer is that scale does not matter - any sideways expansion will cumulate up to the larger scale of a fold.

There is one contrary piece of evidence. Carter(1943) [3] states that follicle groups start out in an orderly pattern in the lamb, but the pattern tends to disrupt as the sheep matures. Carter attributes this disruption to collagen growth affecting the follicle arrangement. We are saying here that follicle growth moves the collagen around, at least while the follicles are initiating. Perhaps both occur. Carter links excessive connective tissue growth with formation of wrinkles and folds. That is not necessarily incompatible with what we are proposing here, if connective tissue growth binds the dermis to the subdermal layers.

3 Materials and Methods

To investigate the 'follicles cause dermal expansion' hypothesis, we need a measure of the amount of follicle tissue in skin. We start by noting that the diameter of a follicle stem at sebaceous gland level is close to 3 times the diameter of the fibre it contains. So the area of follicle tissue per mm^2 at that level is

$$F_a = 10^{-6} F_n \pi \left[\frac{3D}{2} \right]^2$$

F_a will vary between 0 and 1 and will represent the follicle tissue area in mm^2 per mm^2 , so it is unitless.

We next note that the average length of follicles is represented by follicle depth (Fd) in mm . So the volume of follicle tissue per mm^2 of cross section is

$$F_v = F_a F_d$$

F_v will be mm^3 of follicle tissue per mm^2 of cross section, so it will be in mm .

The most relevant parameter to area expansion of the dermis is F_a , so we will be concentrating on F_a . We note that it is at sebaceous gland level, so it is just the follicle stems. Accessory glands are not counted in this measure, only follicle walls and the contained fibre.

We note that it is possible to define F_a for secondary follicles only, and that this may be the more relevant parameter. We also note that F_a is relative to a 1 mm^2 area of adult skin, that is after the expansion of dermal tissue has occurred.

3.1 Sheep studied

We use the Carter(1968) [4] to look at F_a over a range of breeds.

3.2 Measurements

The following measurements and scores were available for the Carter(1968) [4] data

The following measurements and scores were available for the Jackson(2017) [10] ab32 and ab20 CSIRO data

The following measurements and scores were available for the collagen dtts and Jackson(2017) [28] dataset

SkinType visual scores for sheep skin type. Four grades SRS, semi-SRS, flat, and tight, as defined by Watts et al (2017) [27].

- 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 Statistical Methods

Data were imported into the R statistical program [21] and analysed using the *lm()* function for regressions, and the *aoV()* function for analysis of variance.

4 Results

4.1 Breed comparisons of amount of follicle tissue per unit area

We look at the range of values for F_{ap} and F_{as} over all breeds sampled by Carter(1968) [4]. These are shown as histograms in Figures 1 and 2.

We see a very considerable range in values for both F_{ap} and F_{as} . A very small number of breeds have F_{ap} larger than 0.10, whereas many breeds have F_{as} larger than 0.10.

We need to identify the breeds. This is done in Table 1

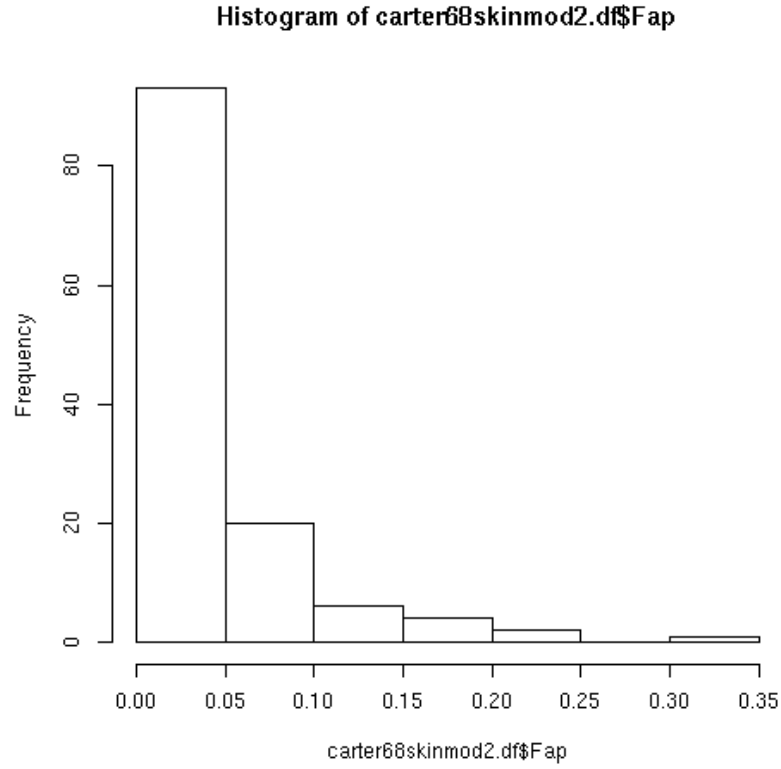


Figure 1: Histogram of breed means for area of primary follicle tissue per mm^2 of skin at sebaceous gland level from data of Carter(1968) [4]

Table 1: Means of Fap and Fas for the Breeds of sheep sampled by Carter(1968) [4]

	Breed	Fap	Fas
1	Ak-karaman	0.02	0.03
2	American Merino	0.01	0.09
3	American Rambouillet	0.01	0.13
4	Arabi	0.04	0.04
5	Awassi	0.04	0.04
6	Bellari White	0.19	0.02
7	Bikaneri	0.07	0.05
8	Blackhead Persian	0.17	0.02
9	Black Kurumbai Adu	0.10	0.03
10	Border Leicester	0.04	0.10

Continued on next page

Table 1 – *Continued from previous page*

	Breed	Fap	Fas
11	Cheviot	0.03	0.08
12	Chokla	0.03	0.03
13	Columbia	0.01	0.11
14	Corriedale	0.02	0.15
15	Daglic	0.05	0.06
16	Debouillet	0.01	0.08
17	Dorset Horn	0.02	0.13
18	Early Fine Merino	0.01	0.12
19	Early Fine Merino (Rambouillet)	0.01	0.13
20	English Leicester	0.03	0.11
21	German Merinofleischaf	0.01	0.09
22	German Merinolandschaf	0.02	0.12
23	Icelandic	0.05	0.06
24	Ile-de-France	0.01	0.08
25	Improved Apulian	0.01	0.08
26	Imroz	0.08	0.08
27	Ivesi	0.06	0.05
28	Jaisalmeri	0.04	0.03
29	Kali	0.04	0.03
30	Karakaya	0.07	0.05
31	Karakul	0.06	0.05
32	Kerradi	0.04	0.03
33	Kivircik	0.04	0.06
34	Limousin	0.06	0.07
35	Lincoln	0.05	0.12
36	Magra	0.04	0.03
37	Malpura	0.05	0.02
38	Mandya	0.30	0.04
39	Marwari	0.04	0.02
40	Merino Tor Mancina	0.01	0.09
41	Navajo	0.02	0.08
42	Nellore	0.22	0.01
43	Nilgiri	0.03	0.07
44	Non-Peppin Fine-Medium Merino	0.01	0.15
45	Non-Peppin Medium Merino	0.01	0.19
46	NSW Fine Merino	0.01	0.17
47	Ossimi	0.05	0.13
48	Ouda	0.16	0.02
49	Peppin Medium Merino	0.01	0.18
50	Polwarth	0.01	0.14
51	Portuguese Merino	0.01	0.08
52	Prealpes du Sud	0.03	0.09

Continued on next page

Table 1 – *Continued from previous page*

	Breed	Fap	Fas
53	Rahmani	0.04	0.12
54	Romney Marsh	0.03	0.12
55	Ryeland	0.01	0.07
56	Sakiz	0.07	0.06
57	SA Strong Merino	0.02	0.21
58	Scottish Blackface	0.11	0.04
59	Soay	0.05	0.03
60	Sonadi	0.05	0.01
61	Sopravissano Rossi	0.01	0.08
62	Southdown	0.02	0.12
63	Suffolk	0.02	0.06
64	Swaledale	0.12	0.04
65	Swedish Landrace (Carpet)	0.08	0.10
66	Swedish Landrace (Fine)	0.04	0.12
67	Tanganyika Long-tailed	0.13	0.01
68	Targhee	0.01	0.12
69	Tas. Fine Merino	0.01	0.15
70	Turkish Merino	0.01	0.08
71	Vic. Fine Merino	0.01	0.14
72	Welsh Mountain	0.09	0.07
73	Wiltshire Horn	0.07	0.08
74	Yankasa	0.21	0.03

and we collapse these breeds into Types in Table 2

Only the African and Indian hair sheep and the Carpetwools have F_{ap} exceeding 0.10. The breeds with F_{as} exceeding 0.10 are the Merino and Merino derived breeds plus the Egyptian Ossimi and Rahmani. Neither of these Egyptian breeds seem to be wrinkled, but the Merino and its derivatives certainly can be wrinkled.

From these data Fas between 10 and 15 percent seems to be the cutoff point above which dermal expansion is too great and can lead to wrinkle. If the the follicles in a wrinkled sheep are 15-20 percent of the skin area, that means that the amount of dermal expansion caused by the follicle formation can only be 15-20 percent. That seems a bit small to account for large folds. However, remember that F_{as} is relative to a 1 mm^2 area after expansion has occurred. To check, we need a measure of dermal expansion. We attempt to develop that in the next section

Before we leave Fas and Fap, we should look at the relation between them. Figure 3 shows the relationship between means of all 126 flocks sampled by Carter(1968) [4].

It would seem that sheep either have a lot of primary follicle tissue, or a lot of secondary follicle tissue, but not both. It would seem that the limit is

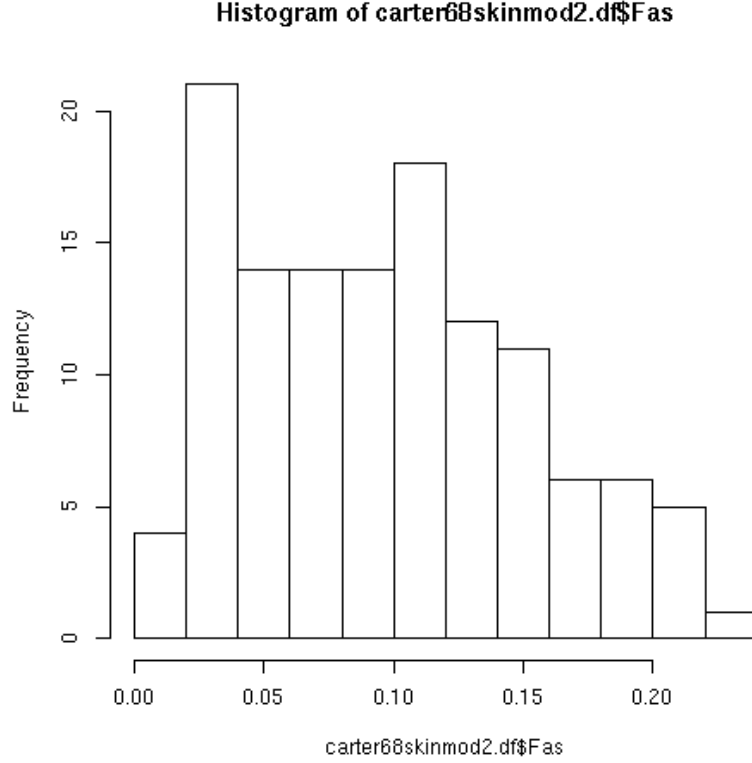


Figure 2: Histogram of breed means for area of secondary follicle tissue per mm^2 of skin at sebaceous gland level from data of Carter(1968) [4]

about 30 percent of skin area consisting of follicle tissue, and this can be all primaries, nearly all secondaries, or a mixture. The numbers in the body of the graph (representing F_a for each point as a percentage) show that sheep with a high F_a occur along a curve towards the centre of the graph, while sheep with a low F_a occur in the bottom left corner. This is a classic demonstration of the pre-papilla cell hypothesis (Moore et al (1998) [19]).

4.2 Using N_p as a measure of dermal expansion

It is often assumed that all sheep have the same density of primary follicles at the time when they initiate, and that subsequent differences in N_p per unit area reflect differences in skin growth. Skin growth would include expansion of the dermis during secondary follicle initiation, as proposed here.

It is therefore of interest to have a look at N_p variation in the Carte(1968) [4] data, and in particular to see if it is lower in breeds which exhibit wrinkle.

Table 2: Means of Fap and Fas for Types for sheep sampled by Carter(1968) [4]

	Type	Fap	Fas
1	African	0.17	0.02
2	Asian	0.05	0.05
3	Carpetwool	0.11	0.05
4	Cheviot	0.03	0.08
5	Corriedale	0.02	0.15
6	Downswool	0.02	0.10
7	Egyptian	0.04	0.13
8	European	0.04	0.09
9	Icelandic	0.05	0.06
10	Indian	0.09	0.03
11	Longwool	0.04	0.11
12	Merino	0.01	0.14
13	Polwarth	0.01	0.14
14	Soay	0.05	0.03
15	USA	0.02	0.10
16	Wiltshire	0.07	0.08

Table 3 shown the means of N_p for the sheep type groupings of Carter(1968) [4] and Table 4 shows the breed means.

tex table generated in R 3.4.2 by xtable 1.8-2 package

Table 4: Means of N_p for the Breeds of sheep sampled by Carter(1968) jtecart:68

	Breed	N_p
1	Ak-karaman	1.80
2	American Merino	2.80
3	American Rambouillet	2.40
4	Arabi	2.10
5	Awassi	1.90
6	Bellari White	3.00
7	Bikaneri	6.60
8	Blackhead Persian	3.70
9	Black Kurumbai Adu	3.60
10	Border Leicester	3.00
11	Cheviot	2.87
12	Chokla	3.80
13	Columbia	1.80
14	Corriedale	2.13
15	Daglic	2.90
16	Debouillet	3.00

Continued on next page

Table 4 – *Continued from previous page*

	Breed	Np
17	Dorset Horn	2.90
18	Early Fine Merino	4.00
19	Early Fine Merino (Rambouillet)	3.45
20	English Leicester	2.50
21	German Merinofleischaf	2.10
22	German Merinolandschaf	2.70
23	Icelandic	1.50
24	Ile-de-France	2.10
25	Improved Apulian	2.30
26	Imroz	3.20
27	Ivesi	2.50
28	Jaisalmeri	3.20
29	Kali	3.10
30	Karakaya	2.10
31	Karakul	3.30
32	Kerradi	1.50
33	Kivircik	2.50
34	Limousin	2.00
35	Lincoln	2.53
36	Magra	3.60
37	Malpura	3.10
38	Mandya	4.70
39	Marwari	3.40
40	Merino Tor Mancina	2.60
41	Navajo	1.80
42	Nellore	2.30
43	Nilgiri	4.10
44	Non-Peppin Fine-Medium Merino	2.25
45	Non-Peppin Medium Merino	2.40
46	NSW Fine Merino	3.40
47	Ossimi	3.00
48	Ouda	3.20
49	Peppin Medium Merino	3.10
50	Polwarth	3.52
51	Portuguese Merino	3.10
52	Prealpes du Sud	2.40
53	Rahmani	3.00
54	Romney Marsh	2.95
55	Ryeland	2.10
56	Sakiz	2.80
57	SA Strong Merino	3.27
58	Scottish Blackface	2.17

Continued on next page

Table 4 – *Continued from previous page*

	Breed	Np
59	Soay	3.60
60	Sonadi	2.80
61	Sopravissano Rossi	2.40
62	Southdown	3.80
63	Suffolk	3.50
64	Swaledale	2.30
65	Swedish Landrace (Carpet)	2.10
66	Swedish Landrace (Fine)	1.80
67	Tanganyika Long-tailed	3.30
68	Targhee	2.00
69	Tas. Fine Merino	2.96
70	Turkish Merino	2.40
71	Vic. Fine Merino	3.62
72	Welsh Mountain	3.13
73	Wiltshire Horn	2.70
74	Yankasa	3.40

There is no clear result. The Merino means are intermediate. Clearly growth differences are interfering with our attempt to use N_p as an indicator of dermal expansion.

4.3 Data on variation within a wrinkled Merino flock

We have a set of data in which degree of wrinkle is actually recorded for each sheep as a scores for neck wrinkle and body wrinkle according to the photographic standards of Turner, etal (1953) [25]. We also have skin histology measurements for these sheep, so we can calculate F_{ap} and F_{as} . This flock is the CSIRO experimant known as AB32, for which genetic parameters are reported by Jackson(2017) [10]. There is also a summary of the genetic parameter estimates relevant to wrinkle score in Jackson and Watts(2017) [13].

The first thing we note is that if we look at the relationship of individual sheep Fas estimates and wrinkle scores we see nothing interesting. The correlation is near-zero. There is too much noise. The reason we see something with the Carter data above is that we looked at flock means, which were means of around 20 animals. So what we propose to do here is to look at relationships using sire group means. We actually list all 78 sire group means in Table 5.

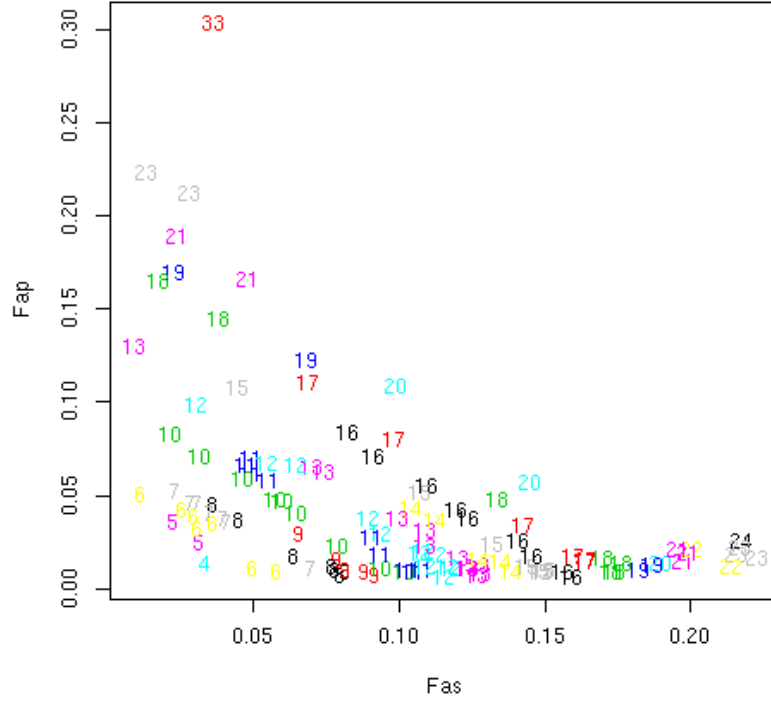


Figure 3: Plot of flock means for area of secondary follicle tissue per mm^2 of skin against area of primary follicle tissue per mm^2 of skin for the 126 flocks sampled by Carter(1968) [4]. The numbers representing each point are the total amount of follicle tissue per mm^2 of skin as a percentage.

Table 5: Sire family means for 8 traits for CSIRO dataset reported by Jackson(2017) [13].

SId	Fap	Fas	Fa	WrN	WrB	WrT	Sarea	Fnpua
SId	Fap	Fas	Fa	WrN	WrB	WrT	Sarea	Fnpua
80L1014	0.01	0.20	0.21	2.90	2.40	5.30	1.05	2.84
80L1041	0.01	0.23	0.24	2.47	1.79	4.26	1.09	2.72
80L1048	0.01	0.21	0.22	2.93	2.21	5.14	1.13	2.72
80L1061	0.01	0.19	0.20	1.67	1.33	3.00	1.12	3.10
80L1088	0.02	0.20	0.21	2.39	1.93	4.32	1.08	3.44
80L1102	0.01	0.19	0.21	2.33	1.48	3.81	1.08	3.14
80L1109	0.01	0.19	0.21	1.96	1.46	3.42	1.07	3.30

Continued on next page

Table 5 – *Continued from previous page*

SIId	Fap	Fas	Fa	WrN	WrB	WrT	Sarea	Fnpua
80L1133	0.01	0.19	0.20	2.18	1.55	3.73	1.07	3.72
80L1139	0.01	0.16	0.17	2.17	1.33	3.50	1.07	3.29
80L1144	0.01	0.18	0.19	2.56	1.92	4.48	1.08	2.84
80L1157	0.02	0.20	0.22	2.50	1.67	4.17	1.01	3.31
80L1158	0.01	0.21	0.22	1.86	1.33	3.19	1.13	3.93
80L1190	0.01	0.20	0.21	2.00	1.38	3.38	1.09	3.13
80L1212	0.01	0.17	0.18	2.29	1.65	3.94	1.05	2.87
80L1213	0.01	0.19	0.20	2.48	1.74	4.22	1.06	2.83
80L1259	0.01	0.21	0.22	2.67	1.56	4.22	1.01	2.75
80L1264	0.01	0.18	0.19	2.33	1.67	4.00	1.02	3.16
80L3002	0.02	0.21	0.23	2.75	2.00	4.75	1.01	3.07
80L3013	0.01	0.22	0.24	4.50	3.00	7.50	1.07	2.76
80L3034	0.01	0.19	0.21	2.50	2.00	4.50	1.03	2.62
80L3035	0.01	0.20	0.21	2.71	1.86	4.57	1.06	3.19
80L3063	0.01	0.19	0.20	3.00	2.00	5.00	1.02	2.79
80L3130	0.01	0.21	0.22	2.75	1.75	4.50	1.08	3.09
80L3138	0.01	0.18	0.19	2.00	1.50	3.50	1.05	2.82
80L3187	0.02	0.21	0.23	2.80	2.20	5.00	0.97	3.17
80L3215	0.01	0.21	0.22	2.00	2.00	4.00	1.01	2.50
80L3238	0.02	0.24	0.26	3.00	2.00	5.00	0.92	3.80
80L3245	0.02	0.22	0.23	2.75	1.75	4.50	1.10	3.57
80L3251	0.02	0.22	0.24	2.00	2.00	4.00	0.98	3.79
80L3268	0.01	0.20	0.21	2.75	1.25	4.00	1.05	3.59
80L3352	0.02	0.22	0.23	2.00	1.67	3.67	1.04	3.54
80L3372	0.01	0.21	0.22	2.67	2.00	4.67	0.96	3.23
80L3394	0.02	0.23	0.25	2.50	1.50	4.00	1.04	3.96
82L2006	0.01	0.22	0.23	3.60	3.20	6.80	1.07	2.26
82L2036	0.01	0.21	0.22	1.86	0.86	2.71	1.04	2.97
82L2068	0.01	0.21	0.23	2.57	2.14	4.71	1.07	2.70
82L2143	0.01	0.23	0.24	2.05	1.37	3.42	1.00	3.13
82L2148	0.01	0.22	0.22	2.40	1.60	4.00	1.05	2.66
82L2170	0.01	0.26	0.27	2.75	1.88	4.62	0.85	4.33
82L2172	0.02	0.25	0.27	2.00	1.21	3.21	0.98	3.10
82L2248	0.01	0.22	0.23	2.00	1.50	3.50	1.03	2.62
82L2250	0.01	0.27	0.28	3.00	1.75	4.75	1.06	2.38
82L2267	0.01	0.21	0.22	2.45	1.45	3.91	1.06	2.46
82L2288	0.01	0.25	0.26	1.62	1.12	2.75	1.10	3.03
82L2296	0.01	0.22	0.23	2.19	1.31	3.50	1.02	2.88
82L4028	0.01	0.22	0.23	1.60	1.00	2.60	1.12	2.50
83L1014	0.01	0.22	0.24	2.17	1.39	3.56	0.93	3.52
83L1055	0.01	0.23	0.25	2.17	1.61	3.78	0.94	3.53
83L1058	0.02	0.23	0.24	2.25	2.05	4.30	0.91	3.62
83L1087	0.02	0.20	0.22	2.83	2.33	5.17	0.87	3.43

Continued on next page

Table 5 – *Continued from previous page*

SIId	Fap	Fas	Fa	WrN	WrB	WrT	Sarea	Fnpua
83L1118	0.02	0.26	0.27	2.33	1.87	4.20	0.89	3.89
83L1119	0.02	0.22	0.23	2.73	2.00	4.73	0.88	3.61
83L1143	0.02	0.20	0.22	3.10	2.30	5.40	0.90	3.51
83L1144	0.02	0.22	0.24	2.47	2.24	4.71	0.87	3.30
83L1159	0.01	0.20	0.21	2.05	1.45	3.50	0.89	3.73
83L1199	0.02	0.23	0.25	2.43	2.14	4.57	0.91	3.53
83L1233	0.01	0.22	0.23	1.83	1.17	3.00	0.87	3.99
83L1237	0.02	0.22	0.24	2.71	2.21	4.93	0.85	3.27
83L1253	0.01	0.20	0.22	2.55	2.00	4.55	0.91	3.53
83L1265	0.02	0.25	0.26	3.09	2.64	5.73	0.83	3.67
83L3022	0.01	0.19	0.21	2.67	2.00	4.67	0.86	3.81
83L3026	0.01	0.22	0.23	3.50	2.00	5.50	0.81	3.87
83L3096	0.01	0.23	0.24	4.00	3.00	7.00	0.90	3.09
83L3102	0.02	0.25	0.27	2.50	2.00	4.50	0.86	3.32
83L3144	0.02	0.22	0.24	3.75	3.00	6.75	0.86	4.13
83L3171	0.02	0.25	0.27	3.67	2.67	6.33	0.89	3.72
83L3214	0.02	0.19	0.21	3.00	2.00	5.00	0.92	3.96
83L3222	0.02	0.22	0.24	3.43	2.71	6.14	0.90	3.81
83L3275	0.01	0.23	0.24	2.50	2.00	4.50	0.92	3.56
83L3278	0.02	0.26	0.27	3.00	3.00	6.00	0.82	4.29
83L3294	0.03	0.16	0.19	2.00	0.00	2.00	0.84	4.03
83L3296	0.02	0.28	0.29	3.00	2.50	5.50	0.88	4.12
83L3309	0.02	0.22	0.23	2.60	2.20	4.80	0.92	3.73
83L3346	0.02	0.24	0.25	2.00	1.50	3.50	0.84	4.02
83L3371	0.02	0.22	0.23	2.50	2.00	4.50	0.89	3.17
83L3409	0.02	0.22	0.24	2.25	1.25	3.50	0.90	3.64
83L3458	0.01	0.23	0.24	3.00	2.00	5.00	0.79	3.95
83L3467	0.02	0.21	0.23	2.00	1.00	3.00	0.99	3.85

Relationships among sire group means are a rough way of looking at genetic correlations. We use this approach, rather than a full genetic analysis, because we do not really know what traits we are interested in yet.

We start by looking at the sire group means correlation of F_{as} with total wrinkle score (WrT). This is shown in Figure 4

We see a positive correlation of 0.29, which is significant at $P < 0.05$ for 74 sire groups. The numbers labelling each point show that N_p does not vary in any consistent way across this graph. We interpret this as follows. F_{as} indicates likely dermal expansion due to secondary follicle development, so we would expect higher dermal expansion to be associated with higher wrinkle scores, and it comes out that way. We do not expect a very strong correlation, because wrinkle formation depends on collagen structure as well as on follicle induced dermal expansion. N_p indicates overall skin expansion, due to overall growth,

Table 3: Means of N_p for Types of sheep sampled by Carter(1969) [4]

	Type	Npua
1	African	3.40
2	Asian	2.37
3	Carpetwool	2.52
4	Cheviot	2.87
5	Corriedale	2.13
6	Downswool	3.14
7	Egyptian	3.00
8	European	2.13
9	Icelandic	1.50
10	Indian	3.64
11	Longwool	2.75
12	Merino	2.99
13	Polwarth	3.52
14	Soay	3.60
15	USA	1.87
16	Wiltshire	2.70

as well as extra dermal expansion induced by follicle development. We do not expect a strong relationship of N_p with wrinkle.

We next note that the scoring of wrinkle seems to be affected by size of the animal. Figure 5 shows that sheep with large surface area tend to get a lower wrinkle score.

The correlation of $Sarea$ with WrT is -0.30, significant at $P < 0.05$. The number labels on the points again repress N_p , and this time there is a pattern. The low N_p sheep are high $Sarea$ and low wrinkle. So it is reasonable to conclude that animals with high growth (of the whole body, and the skin) stretch out their wrinkles which were formed as a lamb, and reduce the wrinkle score. This is probably what happens with British Longwool sheep, which were shown in the previous section to have quite a high F_{as} value, but do not show wrinkle as an adult.

So we need to try and take surface area into account. We note again that N_p reflects both overall growth and extra skin expansion due to secondary follicle development, while $Sarea$ reflects only overall growth. We therefore take the ratio $N_p/Sarea$ as an indicator of skin expansion 'corrected for' growth. Figure 6 shows that $N_p/Sarea$ ratio is indeed correlated with F_{as} .

The correlation is 0.33 which is significant at $P < 0.01$. The point labels show a tendency for high wrinkle scores to occur at high F_{as} as observed in Figure 4. There is no obvious relation of wrinkle score to $N_p/Sarea$ ratio.

The end point for this dataset is that we think we can measure the extra dermal expansion due to secondary follicle development, but it does not necessarily relate strongly to wrinkle scores, because wrinkle also depends on collagen development. There is no measure of collagen in this dataset, so that is about

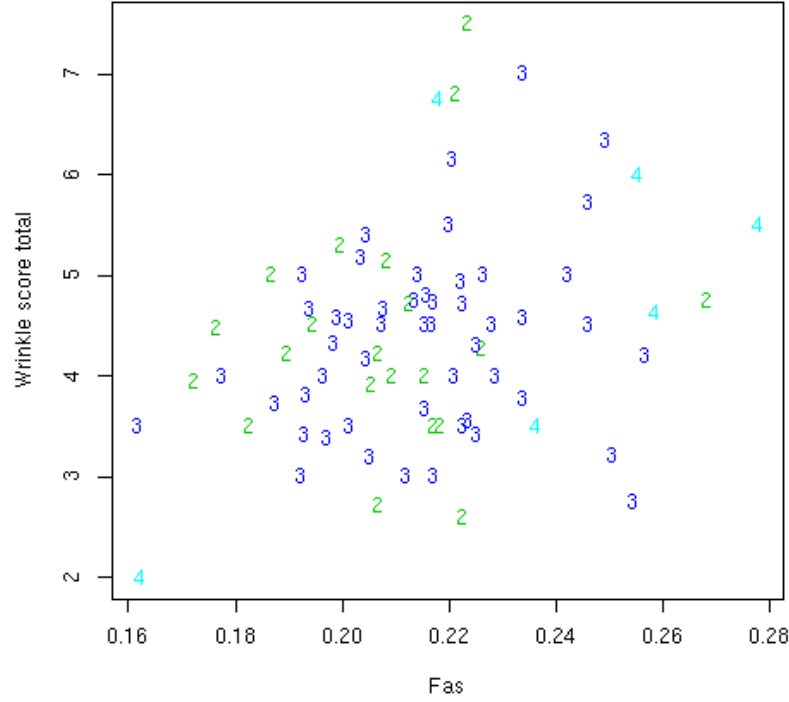


Figure 4: Plot of sire group means of secondary follicle tissue per mm^2 against total wrinkle score (WrT). The numbers representing each point are Np .

as far as we can proceed.

4.4 Rex mutation

It was noted in Jackson(2017) [11] that there was a substantial jump in S/P ratio during evolution of the Finewool Merino, and that this jump may have been produced by mutation at the Rex locus. In other species (cats, rodents) Rex mutation produces a high S/P ratio and a fine coat with no guard hairs and crimp waves in the fibres.

The other substantial difference between Australian Merinos and other breeds is wrinkle. It is not known when wrinkle appeared in Merino evolution. It is not documented whether Rex mutation in other species produces wrinkle. We thought we would have a look. By googling pictures of Rex mutant cats we uncovered the images shown in Figure ?? of a Cornish Rex cat, and the image shown in Figure ?? of a Devon Rex cat. We think the wrinkles are obvious.

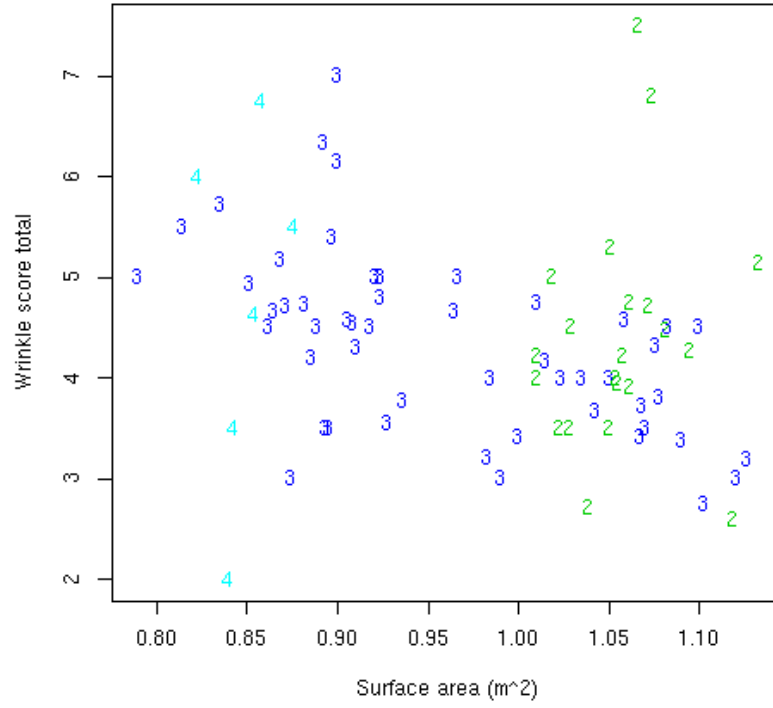


Figure 5: Plot of sire group means of urface area (m^2) against total wrinkle score (WrT). The numbers representing each point are Np .

The Devon Rex also shows the wavy fibres clearly.

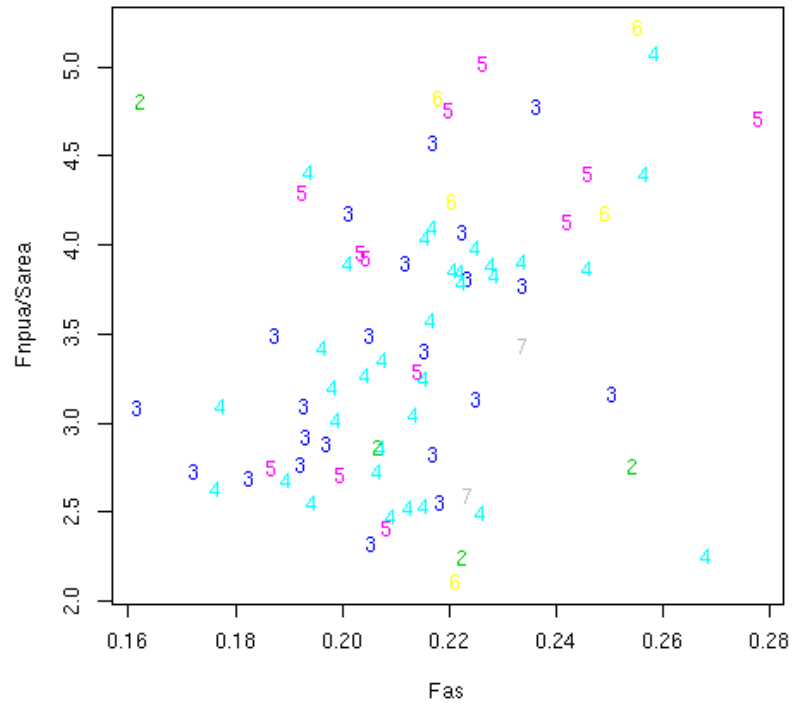


Figure 6: Plot of sire group means of secondary follicle tissue per mm^2 against the ratio N_p/S_{area} . The numbers representing each point are WrT .

5 Discussion

References

- [1] Bogolyubsky S.N. (1940) cited by Fraser A.S and Short B.F. (1960) The Biology of the Fleece. Animal Research Laboratories Technical Paper No 3. CSIRO Melbourne 1960.
- [2] Brown, G.H., and Turner, Helen Newton. (1968) Response to selection in Australian Merino sheep. II. Estimates of phenotypic and genetic parameters for some production traits in Merino ewes and an analysis of the possible effects of selection on them. Aust. J. Agric. Res. 19:303-22
- [3] Carter H.B. (1943) Studies in the biology of the skin and fleece of sheep. 1. The development and general histology of the follicle group in the skin of the Merino. 2. The use of tanned sheepskin in the study of follicle population density. 3. Notes on the arrangement, nomenclature, and variation of skin folds and wrinkles in the Merino. C.S.I.R. Bulletin No 164, Melbourne, 1943
- [4] Carter,H.B. (1968) Comparative Fleece Analysis Data for Domestic Sheep. The Principal Fleece Staple Values of Some Recognised Breeds. Agricultural Research Council, 1968
- [5] Fraser A.S and Short B.F. (1960) The Biology of the Fleece. Animal Research Laboratories Technical Paper No 3. CSIRO Melbourne 1960.
- [6] Gordon-Thompson, C., Botto, S.A., Cam, G.R., and Moore, G.P.H. (2008) Notch pathway gene expression and wool follicle cell fates. Aust. J. Exp. Agric. 48(5) 648-656
- [7] Jackson, N., Nay, T, and Turner, Helen Newton (1975) Response to selection in Australian Merino sheep. VII Phenotypic and genetic parameters for some wool follicle characteristics and their correlation with wool and body traits. Aust. J. Agric. Res. 26:937-57
- [8] Jackson, N. (2015) Genetic relationship between skin and wool traits in Merino sheep. Incomplete manuscript.
- [9] Jackson, N. (2017) Genetics of primary and secondary fibre diameters and densities in Merino sheep. URL <https://github.com/nevillejackson/atavistic-sheep/mev-rewrite/supplementary/genetic-parameters/psparam.pdf>
- [10] Jackson, N. (2017) Genetic relationship between skin and wool traits in Merino sheep. Part I Responses to selection and estimates of genetic parameters. URL <https://github.com/nevillejackson/Fleece-genetics/tree/master/skinandfleeceparameters/ab3220/skinwool1.pdf>
- [11] Jackson, N. (2017) What are the defining characteristics of a primitive sheep relative to a modern Merino sheep?

- URL <https://github.com/nevillejackson/atavistic-sheep/mev-rewrite/supplementary/primitive/primitive.pdf>
- [12] Jackson, N., Maddocks, I.G., Lax, J., Moore, G.P.M. and Watts, J.E. (1990) Merino Evolution, Skin Characteristics, and Fleece Quality. URL <https://github.com/nevillejackson/atavistic-sheep/mev/evol.pdf>
 - [13] Jackson, N. and Watts, J.E. (2017) What is known about the genetics of wrinkle score in Merino sheep? URL <https://github.com/nevillejackson/Fleece-genetics/wrinkle/wrinkle.pdf>
 - [14] Knight, K.R., Lepore, D.A., Horne, R.S., Ritz, M., Kumta, S. and O'Brian, B.M. (1993) Collagen content of uninjured skin and scar tissue in foetal and adult sheep. *Int. J. Exp. Pathol.* 74(6):583-591
 - [15] Maddocks, I.G. and Jackson, N. (1988) Structural studies of sheep, cattle, and goat skin. CSIRO, Division of Animal Production, Sydney.
 - [16] Menton, D.N. and Hess, R.A. (1980) The ultrastructure of collagen in the dermis of tight-skin (Tsk) mutant mice. *The Journal of Investigative Dermatology* 74:139-147
 - [17] Mitchell, T.W. et al (1984) *Wool Technology and Sheep Breeding*, No IV, 200-206
 - [18] Moore G.P.M., Jackson, N., and Lax, J. (1989) Evidence of a unique developmental mechanism specifying both wool follicle density and fibre size in sheep selected for single skin and fleece characters. *Genet. Res. Camb.* 53:57-62
 - [19] Moore, G.P.M., Jackson, N., Isaacs, K., and Brown, G (1998) *J. Theoretical Biology* 191:87-94
 - [20] Nay, T. (1966) Wool follicle arrangement and vascular pattern in the Australian Merino. *Aust. J. Agric. Res.* 17:797-805
 - [21] R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
 - [22] Ryder, M.L. and Stevenson, S.K.(1968) *Wool Growth*. Academic Press, London.
 - [23] Turner, Helen Newton (1956) *Anim. Breed. Abstr.* 24:87-118
 - [24] Turner, Helen Newton(1958) *Aust. J. Agric. Res.* 9:521-52
 - [25] Turner, Helen Newton, Hayman, R.H., Riches, J.H., Roberts, N.F., and Wilson, L.T. (1953) Physical definition of sheep and their fleece for breeding and husbandry studies: with particular reference to Merino sheep. CSIRO Div. Anim. Hlth. Prod. Div. Rept. No. 4 (Ser SW-2 mimeo)

- [26] Turner, Helen Newton, Brooker M.G. and Dolling, C.H.S (1970) Response to selection in Australian Merino sheep. III Single character selection for high and low values of wool weight and its components. *Aust.J.Agric.Res.* 21:955-84
- [27] Watts, J.E., Jackson, N., and Ferguson, K.A. (2017) Improvements in fleece weight weight and wool quality of Merino sheep selected visually for high fibre density and length. URL https://github.com/nevillejackson/SRS-Merino/Paper_2_Revised_10_November_2017.docx
- [28] Watts, J.E. and Jackson, N. (2017) Is collagen quantity and properties involved in wrinkle formation and/or in follicle development? URL <https://github.com/nevillejackson/SRS-Merino/tree/master/supplementary/coplagen/collagen.pdf>
- [29] Xavier, S.P., Gordon-Thomson, C. Wynn, P.C., McCullagh, P., Thomson, P.C., Tomkins, L., Mason, R.S., and Moore, G.P.M.(2003) Evidence that Notch and Delta expressions have a role in dermal condensate aggregation during wool follicle initiation. *Experimental Dermatology*, 22:656-681