

Compound follicles in Merino sheep and fibre diameter

Neville Jackson , Paul Swan, and Jim Watts

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1 Introduction

We are interested in knowing how the various types of follicles found in a trio group vary in fibre diameter. There is ample data in diameter of fibres from primary follicles and secondary follicles, for example Carter(1968) [2] documents mean Dp and Ds for a wide range of sheep breeds. For the Australian Merino strains in Carter's survey, Dp was larger than Ds by amounts varying from -0.6 to 3 microns for fine-wool strains, 4 to 7 microns for medium wool strains, and 7 to 8 microns for strong wool strains. Another way of expressing this is to say that the ratio Dp/Ds varied from 0.9 up to 1.4. Carter's survey did not include data on modern SRS Merinos. A full comparison, including modern SRS data can be found in Jackson(2017) [13]

What is much less available is data on the diameters of the various types of secondary follicles and their fibres. Merino sheep have an S/P ratio greater than 10, and up to 40, the Merino-derived breeds are around 10, and all other breeds have S/P ratio of 3 to 7. This very substantial difference has been achieved by forming compound follicles (Hardy and Lyne(1956) [4]). The cell biology mechanism behind formation of compound follicles has been explained by Moore et al (1998) [18]. It involves a population of pre-papilla cells which aggregate at follicle sites in the developing foetal skin. The numbers of papilla cells which aggregate at each site determine the size of the follicle that develops and the diameter of the fibre that it grows. If there are pre-papilla cells left over after all sites have been used, they form extra follicles by branching from existing follicles. Merino sheep have lots of pre-papilla cells and form many branching follicles, mostly secondary follicles, but branching primary follicles have also been observed. There is a small amount of data linking papilla cell number to fibre diameter collected by Dr Philip Moore. These have been re-presented and re-analysed in Jackson and Moore(2018) [15] where Figure 4 on page 10 shows quite clearly that fibre diameter is related to number of papilla cells per follicle.

Apart from that, we have just one small study of the number of branches in compound follicles. We are going to attempt here to see if the number of branches in a compound follicle influences fibre diameter.

2 Sheep population studied

Data from CSIRO sheep breeding experiments (AB16a and AB17 in CSIRO jargon) are utilised. These are all medium and fine wool Merino sheep. These experiments were started by Dr Alex Fraser in 1954. They consisted of 2 lines of medium Merino sheep selected for high and low S/P ratio, and two lines of fine Merino sheep selected for high and low primary follicle density. There is a writeup by Rendel and Nay(1978) [21]

From the 1971 drop in the above 4 selection lines six male and six female sheep were chosen at random, for detailed study of compound follicles. So 12 sheep from each of 4 selected lines , making a total of 48 sheep.

3 Traits measured

Biopsy samples were serial sectioned down to sebaceous gland level. Sections were stained with Haematoxylin, Eosin and picric acid. A section was chosen, as near to the skin surface as possible, so that fibres could be seen emerging from a common root sheath. Counts were made of the numbers of fibres emerging from a follicle, for a sample of both primary and secondary follicles. It was not possible to determine which fibre was emerging from the secondary original follicle.

Observations were also available, on the same 48 sheep, of follicle number per unit area (Fn), S/P ratio (Fr), and primary follicle number per unit area (Fp). Average fibre diameter (Diam) by the airflow technique was also available.

4 Statistical techniques

Fibre counts were summarized as the relative frequency of follicles with various numbers of emerging fibres, and as the relative frequency of fibres emerging from follicles of various bundle sizes.

The mean size (ie number of branches) of secondary follicles was calculated in the usual manner summing the products of class frequencies and class values.

The regression of average fibre diameter on mean size of secondary follicles was calculated with and without additional predictor variables Fn, Fr, and Fp, using the R function *lm()*. The R statistical software [22] is free and open source software and is available for Windows, Mac, and Linux systems. Using additional predictor variables enables us to study the effect of bundle size on fibre diameter as a partial regression holding other variables constant.

5 Results

The difference between the four selected lines in the usual skin measurements (Fn, Fr, and Fp) are shown in Table 1

One can see that there has been a substantial direct response to selection in all four lines, and that there have been correlated changes in the unselected traits.

The counts of fibres emerging from common follicle root sheaths are summarized in Table 2

Differences between the selected lines are not immediately obvious. There is a strain difference, the Fp+ and Fp- sheep are finewool Merinos and they have a higher proportion of single fibre follicles, both primary and secondary. Also the Fr+ selected line tend to have a higher proportion of very highly branched follicles than the Fr- line.

To see the differences more clearly we compute the mean secondary follicle size for each of the selected line x sex groups. These are given in Table 3, along with the mean fibre diameter for each group.

Table 1: Means of follicle number per unit area(Fn), S/P ratio (Fr) and primary follicle number per unit area (Fp),for 6 sheep of each sex, representing the four selection lines Fr+, Fr-, Fp+, Fp-.

Selection Line	Sex	Mean Fn	Mean Fr	Mean Fp
Fr+	ram	109.4	37.3	2.87
Fr+	ewe	105.6	40.2	2.63
Fr-	ram	54.5	12.5	4.07
Fr-	ewe	45.9	11.8	3.57
Fp+	ram	69.5	14.0	4.63
Fp+	ewe	65.2	12.9	4.65
Fp-	ram	58.8	28.3	2.05
Fp-	ewe	49.8	25.1	1.9

To see the relationship we need to plot the data of Table 3. We do this in Figure 1

So we have a suggestion of a relationship, but it is not significant.

It is possible that some other variables are involved in determining diameter. To look into this we will add other variables to the regression. We will look at overall density (Fn), S/P ratio (Fr), and primary density (Fp).

We added, in turn, Fn, Fr, and Fp to the regression fit of diameter on mean secondary follicle bundle size. The result is shown in Table 4

Table 4 shows that the regression of diameter on Fn is significant, both on its own (last section of table) and in the presence of mean secondary bundle size. It also shows that regression of diameter on mean secondary bundle size is not significant on its own (first section of table) but is marginally significant (8 percent probability) in the presence of Fn. The othe variables had no significant relationship with diameter.

We can illustrate this multiple regression of diameter on secondary bundle size and Fn by plotting the fitted value (ie the diameter estimated from the multiple regression equation) against the actual diameter, for out eight data points. This is shown in Figure 2

That is a much better fit than Figure 1. The R^2 value has increased from 0.27 to 0.89.

To prove that the improvement is not all due to adding Fn, we look at the regression of diameter on Fn alone. This is the last section in Table 4. The R^2 value here is 0.79. So it is not quite as good without secondary follicle bundle size. Secondary follicle bundle size contributes something when added on top of Fn, but it is only just significant and it only lifts the R^2 value from 0.79 to 0.89.

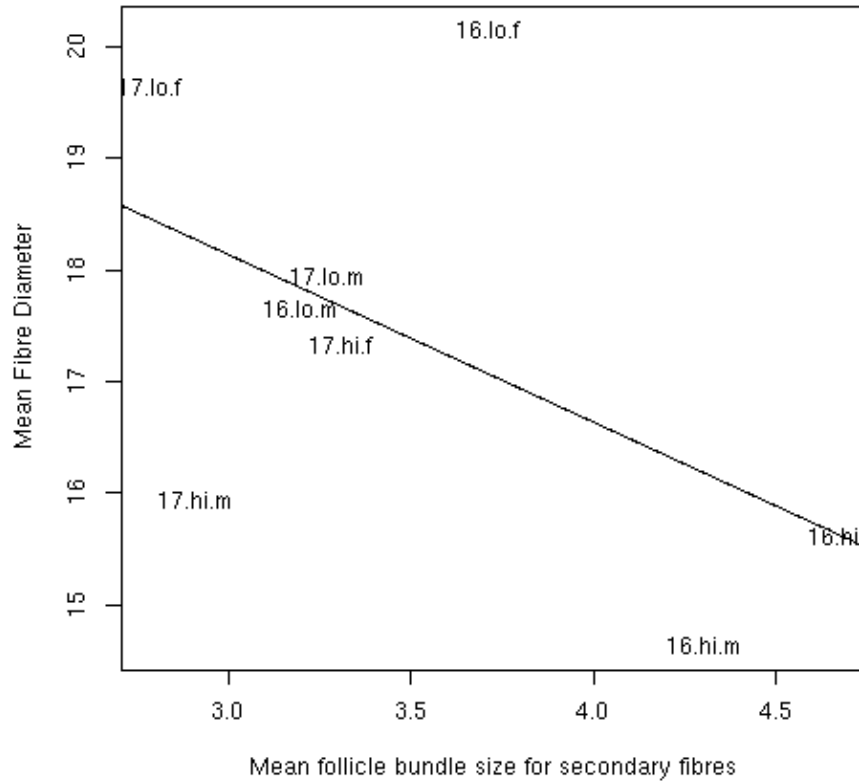


Figure 1: Plot of mean follicle bundle size for secondary fibres against mean fibre diameter for six sheep of each sex representing the four selection lines Fr+, Fr-, Fp+, Fp-. The solid line is a regression of diameter on bundle size ($\text{Diam} = 22.62 - 1.497 * \text{Size}$). The multiple correlation squared is 0.27. The regression is not significant.

Table 2: Frequency of primary and secondary compound follicles of various sizes for six sheep of each sex representind the four selected lines Fr+, Fr-, Fp+, and Fp-

Follicle size	Fr+ ram	Fr+ ewe	Fr- ram	Fr- ewe	Fp+ ram	Fp+ ewe	Fp- ram	Fp- ewe
Primary follicles:								
1	93.44	95.08	97.67	98.10	99.321	98.19	97.38	99.07
2	4.73	2.36	0.31	0.0	0.79	0.33	1.26	0.0
3	1.19	0.0	0.72	1.41	0.0	0.36	0.67	0.0
4	0.0	1.28	1.29	0.0	0.0	0.0	0.7	0.0
5	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.93
6	0.64	0.0	0.0	0.49	0.0	0.36	0.0	0.0
7	0.0	1.28	0.0	0.0	0.0	0.36	0.0	0.0
Secondary follicles :								
1	36.1	39.95	30.21	30.97	42.67	36.68	49.00	62.63
2	23.81	20.15	24.34	23.52	23.55	23.80	21.43	18.49
3	15.28	15.00	24.16	19.42	19.65	17.90	14.04	7.85
4	10.84	11.03	15.51	14.41	9.09	12.38	7.91	6.59
5	5.34	2.94	3.24	6.92	3.62	6.13	3.29	2.14
6	3.66	4.31	1.41	2.09	0.87	1.93	2.11	0.82
7	1.83	1.93	0.83	1.25	0.31	0.53	0.81	0.68
8	0.92	0.88	0.25	0.45	0.082	0.37	0.64	0.37
9	0.70	1.14	0.00	0.58	0.08	0.15	0.55	0.23
10	0.41	1.16	0.06	0.14	0.00	0.15	0.19	0.21
11	0.58	0.50	0.00	0.00	0.00	0.00	0.057	0.00
12	0.24	0.42	0.00	0.32	0.082	0.00	0.00	0.00
13	0.043	0.41	0.0	0.0	0.0	0.0	0.0	0.0
14	0.14	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16	0.0	0.12	0.0	0.0	0.0	0.0	0.0	0.0
17	0.087	0.11	0.0	0.0	0.0	0.0	0.0	0.0
25	0.043	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3: Mean size (number of branches) of secondary follicles and mean fibre diameter, for six sheep of each sex representing the four selection lines Fr+, Fr-, Fp+, and Fp-.

Selection Line	Sex	Mean Bundle size	Mean Fibre Diameter
Fr+	ram	4.30	14.63
Fr+	ewe	4.67	15.60
Fr-	ram	3.19	17.65
Fr-	ewe	3.71	20.15
Fp+	ram	2.91	15.92
Fp+	ewe	3.30	17.32
Fp-	ram	3.27	23.00
Fp-	ewe	2.78	19.63

Table 4: Estimates of partial regression coefficients for regression of fibre diameter on mean secondary follicle bundle size and on additional variables Fn, Fr, and Fp

Regression of Fibre Diameter on Secondary bundle size (MeanSc)				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	22.6220	3.6060	6.27	0.0008
MeanSc	-1.4973	1.0091	-1.48	0.1884
Regression of Diameter on MeanSc and MeanFn				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	19.1615	1.6553	11.58	0.0001
MeanSc	1.5368	0.7096	2.17	0.0826
MeanFn	-0.1033	0.0193	-5.35	0.0031
Regression of Diameter on MeanSc and MeanFr				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	21.6063	4.1197	5.24	0.0033
MeanSc	-0.8661	1.4530	-0.60	0.5771
MeanFr	-0.0529	0.0831	-0.64	0.5520
Regression of Diameter on MeanSc and MeanFp				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	24.9256	4.7291	5.27	0.0033
MeanSc	-1.6723	1.0655	-1.57	0.1773
MeanFp	-0.5120	0.6478	-0.79	0.4651
Regression of Diameter on MeanFn				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	22.2336	1.0840	20.51	0.0000
MeanFn	-0.0699	0.0147	-4.74	0.0032

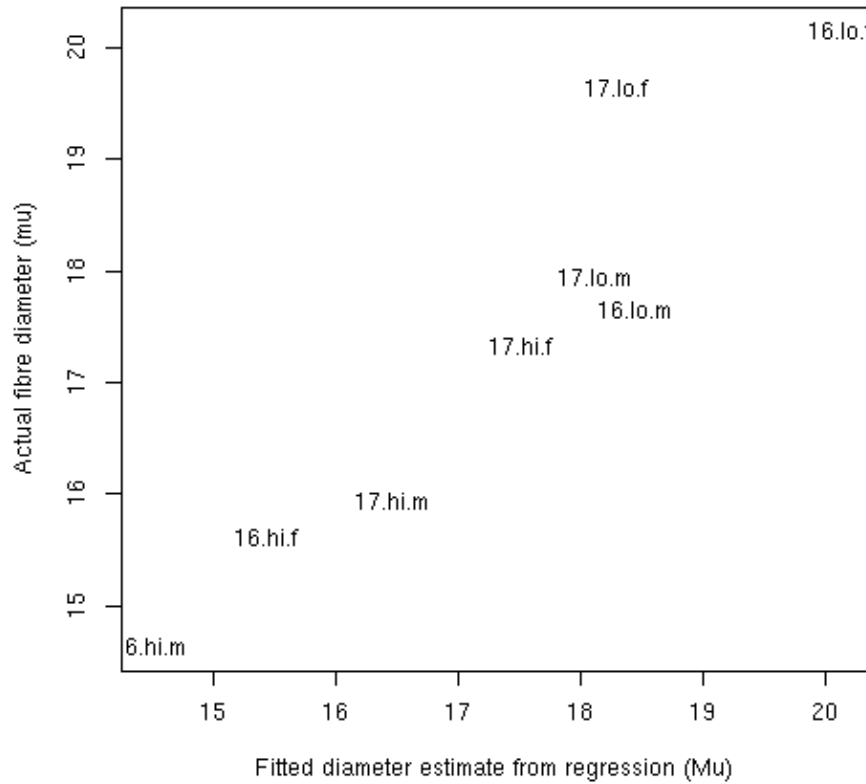


Figure 2: Plot of estimated diameter against actual diameter for the multiple regression of diameter in smean secondary follicle bundle size and Fn. The points are means of six sheep of each sex representing the four selection lines Fr+, Fr-, Fp+, Fp-. The regression equation is $\text{Diam} = 19.16 - 0.103 * \text{Fn} + 1.536 * \text{Size}$. The multiple correlation squared is 0.89. The regression is significant.

6 Discussion

We have shown that mean fibre diameter is affected by follicle number per unit area (Fn) and mean secondary follicle bundle size. What does this mean? If we interpret Fn as an indicator of *global follicle density* and secondary follicle bundle size as an indicator of *local follicle density*, then we can draw the diagram shown in Figure 3 which is a path diagram suggesting a causal model for fibre diameter.

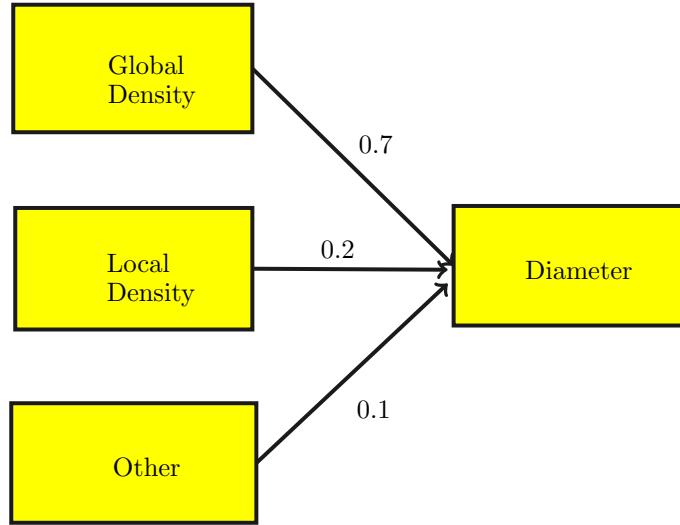


Figure 3: Path diagram showing possible causes of fibre diameter variation. The numbers on each path show the proportion of variance explained

So, if we are willing to assume that density variation causes diameter variation, Figure 3 says that 70 percent of diameter variation is globally determined, and another 20 percent is locally determined, and the final 10 percent is unexplained.

Given what we know about the role of papilla cell numbers in determining fibre diameter (Moore et al (1998) [18]), the above interpretation is probably incorrect. What it means is that if the papilla cells are shared around over more follicles, then there tend to be less papilla cells per follicle, and therefore finer fibres. The sharing is during follicle development, not while the fibre is growing in an adult functioning follicle. What is being shared is the papilla cells, and there is a global and a local component to that. We can be quite sure that this is not evidence of competition between follicles for nutrients during fibre growth.

A few words of caution. Is secondary follicle bundle size really an indicator of local density? Well it is a rough one, when compound follicles have lots of branches the follicles tend to be locally dense. It would be preferable to have some actual counts of local density.

We need to be aware too, that this study is a very small amount of data, and that the counts of secondary follicle bundle size were subject to a large sampling error. We were only able to analyse means for the line x sex groups, not individual sheep, because we needed to attenuate some of the errors by averaging.

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