Dermal papilla cell counts and fibre diameter

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1 Introduction

It has been shown that there exists in the dermis of the the developing sheep foetus a population of cells, known as pre-papilla cells, which migrate to the site s of follicle formation and differentiate, ending up in the papilla of the follicle bulb (Moore, etal (1989) [18], Moore etal (1998) [19]). These cells are of mesenchymal origin, in contrast to all other tissue in the follicle, which arises from the epidermis. It is thought that these cells control follicle development, and, in particular, that the number of papilla cells which end up in a follicle bulb at least partly determines follicle size and fibre diameter, and perhaps other follicle and fibre characteristics.

2 Materials and Methods

The sheep observed were from CSIRO single character selection lines described by Turner, Brooker and Dolling (1970) [23]. Four lines were studied - high staple length, low staple length, high fibre diameter, amd low fibre diameter selection. The sheep measured were born in 1971 to 1978 and were a random sample of the available animals. Measurements were on biopsy specimens and wool samples taken at 2 years of age.

Fibre diameter data were with the airflow technique (ref?) Phil will have to do a methodology here !!

The observations made were

- dermal papilla cell count per follicle
- dermal papilla cell count per mm^2
- surface area of sheep (estimated from bodyweight) m^2
- body weight (2 yrs) Kg
- Np no of primaries per mm^2
- Nps no of follicles per mm^2
- Cww clean wool weight Kg
- Cwwperua clean wool weight per unit area
- Stal staple length mm
- Staladj staple length adjusted to 365 days mm
- Diam fibre diameter μm
- Cww2 another clean wool weight Kg
- Cww2adj clean wool weight adjusted to 365 days

- SorT coding for single=1, twin=2
- Hcap coding for handicap SPA=6, SPM=7, TPA=8, TPM=9
- Line coded L+=1, L-=2, D+=5, D-=6

2.1 Statistical Methods

Data were imported into the R statistical program [20] .

The regression relationship between dermal papilla cell count and diameter was estimated assuming nboth variables were subject to measurement error. Total least squares or orthogoinal regression was used.

The effects of Line and SorT (single or twin) in dermal papilla cell count per follicle was examined using analysis of variance and tables of means.

3 Results

The average number of pre-papilla cells per follicle is something for which we actually have some limited data. A sample of 42 sheep, from a CSIRO selection experiment with 4 lines selected for high and low fibre diameter and high and low staple length, were skin sampled and counts of dermal papilla cells per follicle bulb made. For 34 of these 42 sheep, an average fibre diameter measurement was available.

3.1 Dermal papilla cell number and fibre diameter

The relationship obtained between average number of dermal papilla cells per follicle and average fibre diameter is shown in Figure 1

In Figure 1 the black dashed regression line is obtained by regressing dermal papilla cell count on diameter. Its formula is

$$C = -48.2981 + 5.678D$$

where

C is dermal papilla cell count per follicle

D is mean fibre diameter

The red dashed line is obtained by regressing diameter on dermal papilla cell count. Its formula is

$$D = 12.5031 + 0.1184C$$

which when reversed to put C on the Y-axis becomes

$$C = -105.6005 + 8.4459D$$

The issue with these regressions is that both dermal papilla cell count and diameter are variables with measurement and sampling errors. Classical regression assumes the variate on the X-axis is known without error. So neither of

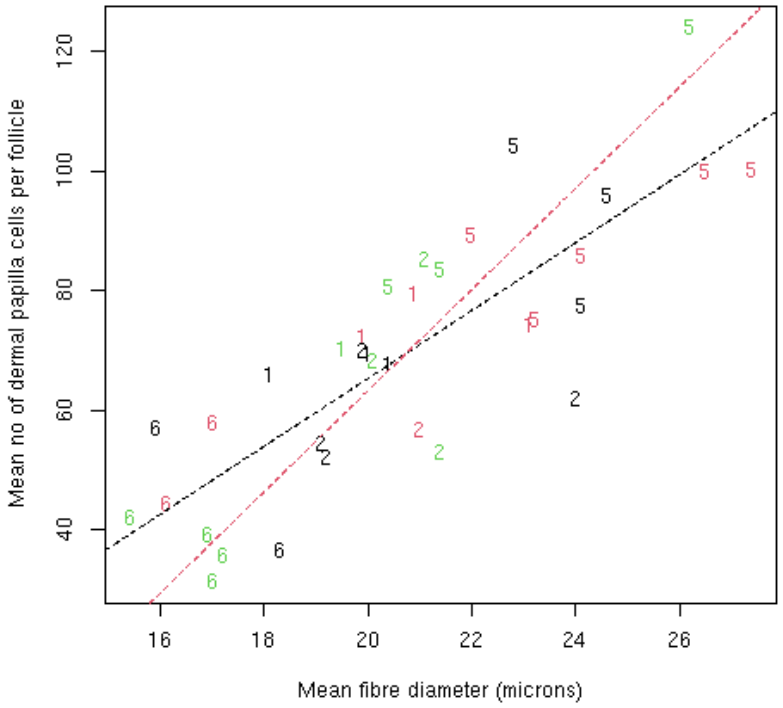

Figure 1: Plot of mean fibre diameter against mean number of dermal papilla cells per follicle for 34 sheep from CSIRO selection experiments. The coloured numbers representing each point indicate the selection line (1 = high staple length, 2 = low staple length, 5 = high fibre diameter, 6 = low fibre diameter). The two dashed lines are linear regressions of papilla cell count on diameter (black) and of diameter on papilla cell count (red).

the above regressions is appropriate as a calibration to predict diameter from dermal papilla cell count.

The correct approach is to use total least squares regression (also known as orthogonal regression) . When we do orthogonal regression we get

$$C = -104.3949 + 8.386858D$$

which is so close to the red dashed line that it plots on top of it. So this is our formula for diameter prediction, we just have to reverse transform it to get

$$D = 12.44744 + 0.119234C$$

Why is the orthogonal regression closer to one of the simple regressions?

Because one variable has more error than the other. The orthogonal regression bisects the two simple regressions in proportion to the relative size of their error variances.

3.2 Dermal papilla cell number and cross sectional area

Relating dermal papilla cell number to diameter as in the previous section is a kludge. Theoretically dermal papilla cell number should relate to cross sectional area of fibres, so using diameter makes a scale mismatch. We estimate average fibre cross sectional area as

$$A = \frac{\pi}{4} D^2$$

This ignores the contribution which variance of diameter makes to mean cross sectional area. We can not do anything to allow for variance because the data does not include variance of diameter. The relationship between dermal papilla cell number and cross sectional area is shown in Figure 2

In Figure 2 The black dashed regression line is obtained by regressing dermal papilla cell number on cross sectional area. Its formula is

$$C = 10.82950 + 0.16980A$$

where

C is dermal papilla cell count per follicle

A is estimated mean fibre cross sectional area

The red dashed regression line is obtained by regressing cross sectional area on dermal papilla cell count. Its formula is

$$A = 72.2312 + 3.9270C$$

which when transposed to put C on the Y-axis becomes

$$C = -18.39348 + 0.2546473A$$

The correct approach is to use total least squares regression (also known as orthogonal regression) . When we do orthogonal regression we get

$$C = 9.99927 + 0.172209A$$

which is so close to the black dashed line that it plots on top of it. So this is our best formula for cross sectional area prediction, we just have to reverse transform it to get

$$A = -58.06468 + 5.80689C$$

Why is the orthogonal regression on cross sectional area similar to the blask dashed line but orthogonal regression on diameter close the red dashed line? Because changing from diameter to cross sectional area has altered the balance of errors between the two variates by changing the scaling. Cross sectional area has a larger mean and variance than diameter.

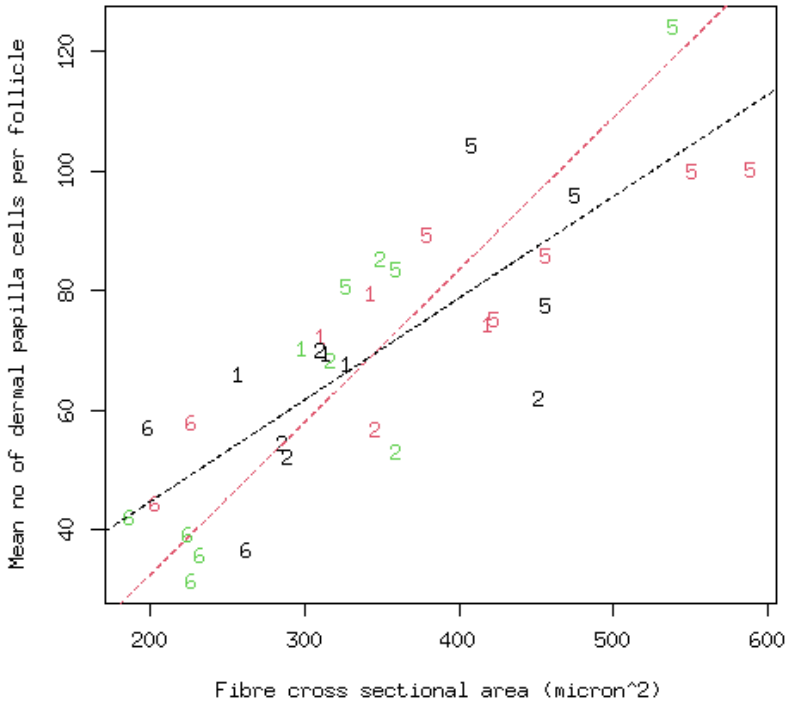

Figure 2: Plot of mean fibre cross sectional area against mean number of dermal papilla cells per follicle for 34 sheep from CSIRO selection experiments. The coloured numbers representing each point indicate the selection line (1 = high staple length, 2 = low staple length, 5 = high fibre diameter, 6 = low fibre diameter). The two dashed lines are linear regressions of papilla cell count on cross sectional area (black) and of cross sectional area on papilla cell count (red).

3.3 Line and SorT effects on dermal papilla cell number

A model was fitted as follows

```
> aov2.dpcc <- aov(Dpccperfoll ~ SorT + Line + 1, data = dpcc.df)
> summary(aov2.dpcc)
            Df Sum Sq Mean Sq F value   Pr(>F)
SorT         1    624     624    4.74   0.0381 *
Line         3  11094    3698   28.10 1.36e-08 ***
Residuals   28   3684     132
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
9 observations deleted due to missingness
```

This shows that Line differences were highly significant and SorT effects were marginally significant. The effects (deviations from the overall mean) for Line and SorT were

```
> model.tables(aov2.dpcc)
Tables of effects

 SorT
         1      2
     3.505 -5.392
rep 20.000 13.000

 Line
       1      2     5      6
    1.78 -6.006 21.53 -25.91
rep 7.00  7.000 11.00   8.00
```

The big difference is between D+ and D- Lines (coded 5 and 6). If diameter is related to dermal papilla cell count as we assert, one would expect selection for diameter to alter dermal cell papilla count. It has.

Twins (coded 2 for SorT) have a lower dermal papilla cell count. Twins also have a lower diameter. This is a bit more problematic. Twins have lower growth and therefore probably have a smaller population of pre-papilla cells for aggregates to form from (bad english). Why Twins would form aggregates with fewer cells is a mystery, unless one assumes aggregate formation is affected by the size of the population of unaggregated cells. That is something we need to think about.

4 Discussion

There is other published data on dermal papilla cell numbers in follicles of other species. In mice, Chi, Wu, and Morgan(2013) [5] observe that dermal papilla cell numbers vary between 20 and 100 per follicle, with guard hairs having more than other fibre types, and Zigzag fibres least. This agrees with our adult sheep estimates. The papilla cell numbers fluctuated with the mouse hair cycle, and their variation correlated with hair length and diameter. So the number of dermal papilla cells is not fixed for the life of a follicle, it can fluctuate with the hair cylcle, and fibre dimensionss fluctuate with it. Sheep are different from mice in that most of their follicles are in Anagen for long periods. What we need to note here is a caution that adult dermal papilla cell numbers per follicle are not necessarily the numbers of pre-papilla cells used to initially form the follicle.

Things other than dermal papilla cell count affect observed mean fibre diameter. We need to refer to Dry's concepts of *base* and *check* in birthcoats. *Base* is

the diameter a follicle would grow if there were no other follicles around it sharing resources. *Check* is the diameter actually observed from follicles suffering some degree of *check* due to the surrounding follicles in the trio group.

Dermal cell papilla count controls base diameter (ie maximum potential diameter). It does this by controlling the overall potential size of the follicle, the follicle bulb, and the fibre, not by controlling the partitioning of differentiating bulb cells between the inner root sheath and the fibre. See Jackson, Swan, and Watts (2018) [12] full presentation of the maths of how a follicle controls fibre length growth rate and fibre diameter.

Our regression equation estimates *checked diameter* because the diameter data used to fit the regressions are checked diameters. We do not know how much *check* there is in these data. We may just assume it is some percentage of the unknown *base diameter*.

The only place where *base diameter* can be directly observed is in the birth-coat. The coarse curved sickle tips on Pc fibres are the unchecked diameter of fibres growing in utero from Pc follicles, with no other follicles around them to provide a *check* and probably an ample nutrient supply. They are indeed coarse, except in SRS Merinos (and in Wensleydales), and from this we suggest that SRS Merinos have small numbers of papilla cells in primary follicles.

As far as predicting diameter goes, our equation gives an estimate of *checked diameter* at the average degree of check existing in the sheep used to fit the equation. We may be able to apply some estimate of 'percentage of check' which would presumably come from considerations of intra-trio-group-density and nutrition.

There is a well known across breed relationship between diameter and density. See Figure 11 on page 26 of Jackson (2017) [11] which shows data from Carter(1968) [4] with breed means for Dp plotted against breed means for S/P ratio. The dramatic fall in Dp as S/P ratio increases is evidence of the *check* effect, Dp is reduced in the presence of increasing numbers of secondary follicles in the trio group. Modern Merino sheep are fine because of the *check* effect of increasing numbers of secondary follicles, with the possible exception of SRS Merinos, which also have a lower *base* ie a lower number of dermal papilla cells per follicle.

There is also variation between follicles within an individual sheep. That is all dpcc, ie all *base*. The density and the nutrition are same for all follicles within a sheep except for minor body variations. So diameter distribution within a fleece is all *base*. That gives us some hope that we can understand diameter distribution within a fleece purely by studying random variation in the numbers of dermal papilla cells in follicles. Another document (dpcpoisson.pdf) looks at using sampling from a Poisson distribution to emulate the distribution of dermal papilla cell numbers across follicles.

Everything we have said in Discussion about diameter applies equally to cross sectional area. Our regression equation predicts checked cross sectional area, at the average level of check applying in our data set. We do not at this stage know how to predict maximum or potential diameter or cross sectional area, even though we know that *base or maximim or potential* is what number

of papilla cells actually determines.

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A Appendix

The raw data are listed here for completeness

	Tagprospect	Slideno	Tagarmid	Dpccperfoll	Dpccpermm2	Surfarea	Bwt	Np	Nps
1	A8000	431/80	71E4012	51.85	NA	0.8593	79.5	NA	NA
2	B0660	006/85	71E4533	85.70	2802	0.7066	22.0	2.1	32.7
3	A7994	424/10/1	72E4030	124.05	NA	0.9667	35.2	NA	NA
4	A8010	428/80	72E4078	36.40	NA	0.9703	35.4	NA	NA
5	B0661	729/87	73E4037	89.25	4177	0.7509	24.1	3.9	46.8
6	B0663	007/85	73E4083	83.50	NA	0.8805	30.6	NA	NA
7	-8581	003/85	73E4132	57.00	4617	0.8417	28.6	3.1	81.0
8	B0651	974/83	73E4289	100.15	3585	0.7878	25.9	1.6	35.8
9	A8003	425/80/1	73E4327	41.85	NA	0.8140	27.2	NA	NA
10	A8007	427/	73E4437	67.60	NA	0.8239	27.7	NA	NA
11	A8009	423/80	73E4591	75.15	NA	0.8100	27.0	NA	NA
12	B0718	004/85	74E4015	35.50	NA	0.8319	28.1	NA	NA
13	A8008	433/80	74E4069	61.70	2178	0.8766	30.4	2.4	35.3
14	B0719	976/83	74E4073	57.60	2742	0.8319	28.1	2.5	47.6
15	A8002	434/	74E4074	31.30	2513	0.8515	29.1	3.7	80.3
16	B0655	973/83	74E4096	95.80	3621	0.7878	25.9	2.3	37.8
17	B0662	972/83	74E4216	72.10	2992	0.9903	36.5	2.9	41.5
18	A7997	435/80	74E4338	52.95	NA	0.9408	33.8	NA	NA
19	A8006	426/80	74E4466	104.20	NA	0.7777	25.4	NA	NA
20	B0653	971/83	74E4467	99.80	2565	0.8100	27.0	2.8	25.7
21	B0717	979/83	74E4501	70.05	3390	0.8901	31.1	3.3	48.4
22	-8586	002/85	74E4506	77.20	3682	0.8417	28.6	4.9	47.7
23	A7995	430/80	74E4552	74.10	3409	0.9703	35.4	3.7	46.0
24	A8005	429/80	74E4595	39.05	2866	0.8100	27.0	3.2	73.4
25	B0654	005/85	75E4008	69.80	NA	0.9334	33.4	NA	NA
26	-8598	975/83	75E4093	79.35	3103	0.8766	30.4	2.7	39.1
27	B8590	008/85	75E4274	80.60	2636	0.9334	33.4	2.4	32.7
28	-8584	977/83	75E4298	54.20	2607	0.8319	28.1	2.5	48.1
29	B0652	980/83	75E4299	56.50	2689	0.7592	24.5	2.8	47.6
30	B0658	978/83	75E4389	68.15	2051	0.7838	25.7	1.2	30.1

31	-8583	981/83	75E4421	69.15	2282	0.8200	27.5	2.8	33.0
32	-8593	001/85	75E4490	44.10	3303	0.8805	30.6	2.8	74.9
33	-8603	012/85	75E4522	85.05	4295	0.9903	36.5	4.4	50.5
34	-8600	009/85	75E4533	65.85	2647	0.9371	33.6	3.0	40.2
35	-8602	744/87	76E4172	43.00	4055	0.6719	20.4	3.6	94.3
36	-8589	970/83	76E4328	47.15	3267	0.6318	18.6	2.7	69.3
37	-8604	982/83	77-5226	61.15	2941	0.6719	20.4	2.7	48.1
38	-7996	422/84/3	77-5253	59.90	NA	0.6829	20.9	NA	NA
39	A7998	436/80	78L5006	70.25	NA	0.8805	30.6	NA	NA
40	-8596	010/85	78L5015	60.10	3245	0.7215	22.7	4.2	54.0
41	A7999	439/80	78L5024	64.60	NA	0.8631	29.7	NA	NA
42	-8580	011/85	78L5073	51.00	3320	0.8060	26.8	3.5	65.1

	Cww	Cwwperua	Stal	Staladj	Diam	Cww2	Cww2adj	SorT	Hcap	Line
1	1.77	NA	63	65	19.2	1.77	1.81	1	6	2
2	2.18	3.09	80	83	24.1	2.18	2.20	1	6	5
3	2.54	NA	92	95	26.2	2.59	2.57	1	6	5
4	2.09	NA	90	93	18.3	2.09	2.10	2	8	6
5	2.12	2.82	72	89	22.0	1.73	2.12	1	6	5
6	2.49	NA	90	111	21.4	2.04	2.49	1	6	5
7	2.33	2.77	80	99	15.9	1.90	2.33	1	6	6
8	2.24	2.84	70	87	27.4	1.83	2.24	1	6	5
9	1.73	NA	70	87	15.4	1.41	1.73	1	6	6
10	2.17	NA	85	105	20.4	1.78	2.17	1	6	1
11	1.80	NA	73	90	23.2	1.47	1.80	2	8	5
12	2.03	NA	77	94	17.2	1.71	2.03	1	7	6
13	2.17	2.48	58	71	24.0	1.83	2.17	2	8	2
14	1.59	1.91	78	95	17.0	1.33	1.59	2	8	6
15	2.08	2.44	92	112	17.0	1.75	2.08	2	8	6
16	1.77	2.25	88	107	24.6	1.49	1.77	1	6	5
17	2.63	2.66	98	119	19.9	2.21	2.63	1	6	1
18	1.75	NA	50	61	21.4	1.48	1.75	1	6	2
19	2.01	NA	83	101	22.8	1.69	2.01	1	7	5
20	1.70	2.10	85	103	26.5	1.43	1.70	2	8	5
21	2.63	2.95	95	116	19.5	2.21	2.63	2	8	1
22	1.44	1.71	63	77	24.1	1.21	1.44	2	9	5
23	2.42	2.49	100	122	23.1	2.04	2.42	1	6	1
24	1.56	1.93	68	83	16.9	1.32	1.56	2	8	6
25	1.59	NA	53	79	19.9	1.16	1.59	1	6	2
26	2.34	2.67	82	123	20.9	1.71	2.34	2	8	1
27	1.76	1.77	55	82	20.4	1.29	1.76	1	6	5
28	1.52	1.83	48	72	19.1	1.11	1.52	2	8	2
29	1.34	1.76	48	NA	21.0	0.98	NA	NA	NA	2
30	1.18	1.52	37	55	20.1	0.86	1.18	1	6	2
31	1.70	2.07	73	109	20.0	1.25	1.70	2	8	1
32	1.76	2.00	55	82	16.1	1.29	1.76	1	6	6
33	1.83	1.85	47	70	21.1	1.34	1.83	2	8	2

```
34 2.48     2.65   73      109 18.1 1.81    2.48    1    6    1
35 2.24     3.33   84       NA   NA 2.06      NA   NA   NA    6
36 2.04     3.23   84       NA   NA 1.88      NA   NA   NA    6
37 2.00     2.98   72       NA   NA 2.00      NA   NA   NA    2
38 1.78       NA   90       NA   NA 1.78      NA   NA   NA    6
39 2.52       NA  115       NA   NA 2.55      NA   NA   NA    1
40 2.05     2.84  113       NA   NA 2.07      NA   NA   NA    1
41 2.15       NA   70       NA   NA 1.37      NA   NA   NA    1
42 1.22     1.51   57       NA   NA 1.57      NA   NA   NA    2
>
```