

Trio follicle groups in Merino sheep and fibre diameter

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Contents

1	Introduction	2
2	Sheep population studied	2
3	Traits measured	2
4	Statistical techniques	4
5	Results	4
5.1	All covariates included	5
5.2	Components of follicle density (Fn)	9
5.2.1	Fn alone	9
5.2.2	Groups per unit area and follicles per group	9
5.2.3	Intra group density and trio group area ratio	9
5.3	Things other than density	9
6	Discussion	10

1 Introduction

We are interested in knowing how important is the local environment within a trio group (which we call *locale*) in control of fibre diameter.

2 Sheep population studied

Data were collected from 12 Merino flocks sampled at various times over the years 2001 to 2016. The sheep sampled ranged in age from 13 to 24 months, and there were some rams and some ewes. Numbers of sheep per flock varied from 11 to 82. There were 82 ewes and 257 rams, a total of 339 sheep. The flocks were mostly bred towards SRS Merion type, but there were two which were normal Merinos.

3 Traits measured

Biopsy samples were serially sectioned down to sebaceous gland level. Sections were stained with Nile Blue sulphate. Under the microscope follicle trio groups were identified and the area of the groups and its follicle count obtained. Several trio groups were measured per sheep and the measurements averaged.

In addition follicles were chosen at random within a group and their distance from their nearest neighbour measured.

Observations were also available, on the same 339 sheep, of follicle number per unit area (Fn), S/P ratio (Fr), and primary follicle number per unit area (Fp). Average diameter of primary fibre (Dp) and of secondary fibres (Ds), and of all fibres (Dskin). Diameters were measured on skin sections.

All the measured traits are summarised below

Dp mean diameter of primary fibres (μm^2).

SDDp standard deviation of diameter of primary fibres (μm^2).

Ds mean diameter of secondary fibres (μm^2).

SDDs standard deviation of diameter of secondary fibres (μm^2).

SovP ratio of number of secondary follicles to number of primary follicles. No units

Fn follicle number per mm^2 . Determined by counting follicles in skin sections viewed under a projection microscope at 50x magnification.

IGNorth inter-group distance in the north direction (μm). Distance between adjacent follicle groups measured from the outer edge of the sebaceous glands of the primary central follicle to the lateral margin of the follicle group above it. The 'North' direction on a skin section is defined as the 'top' of the image when the rows of follicle groups are from side to side and the primary fibres are on the 'top' margin of the groups.

IGSouth inter-group distance in the South direction (μm). Measured from the lateral margin of the same follicle group as used for IGNorth to the outer edge of the sebaceous glands of the central primary follicle of the follicle group below it.

IGEeast inter-group distance in the East direction (μm).

IGWest inter-group distance in the West direction (μm).

FollGpArea mean area of a follicle group (mm^2).

AreaPerFoll mean area per follicle calculated as FollGpArea/FollperGp (mm^2)

IntGpDens density of follicles within a follicle group ($nopermm^2$)

FollperGp mean number of follicles per group

IFDist mean inter-follicle distance (μm). Measured as the distance between a follicle and its nearest neighbour.

IGNorth inter-group distance in the north direction (μm). Distance between adjacent follicle groups measured from the outer edge of the sebaceous glands of the primary central follicle to the lateral margin of the follicle group above it. The 'North' direction on a skin section is defined as the 'top' of the image when the rows of follicle groups are from side to side and the primary fibres are on the 'top' margin of the groups.

IGSouth inter-group distance in the South direction (μm). Measured from the lateral margin of the same follicle group as used for IGNorth to the outer edge of the sebaceous glands of the central primary follicle of the follicle group below it.

IGEeast inter-group distance in the East direction (μm).

IGWest inter-group distance in the West direction (μm).

FollCurv follicle curvature score. 1 = straight, 7 = curved.

FollDep follicle depth vertically from skin surface to bulb (mm).

In addition we calculate the following from the above basic traits

Fnp number of primary follicles per mm^2 . Calculated as $Fnp = Fn/(Sovp+1)$.

Fns number of secondary follicles per mm^2 . Calculated as $Fns = Fnp * Sovp$.

Dskin mean fibre diameter (μm). Obtained from fibre measurements on skin sections.

SDDskin standard deviation of fibre diameter (μm). Obtained from fibre measurements on skin sections.

In addition the following fibre length measurements were made

FibLen mean fibre length per unit time (mm/day). Obtained by withdrawing 50 fibres from n staples and measuring the stretched length.

SDFiblen standard deviation of fibre length per unit time (mm/day).

CVFiblen coefficient of variation of fibre length, as a percentage.

4 Statistical techniques

We use the R Statistical Language [21] to analyse the data. The techniques used involve fitting a number of linear models, with fixed effects for Flock, Age, and Sex taken out, and with fibre diameter predicted from regressions on various skin measurements. This allowed us to assess, at the between sheep level, how much the trio group specific parameters contributed to diameter, compared with the overall parameters such as follicle density (Fn).

We analyse mean diameter of primary and secondary fibres separately. These should not differ - the *locale* effect should affect primary and secondary follicles equally. This is not to say the primary and secondary follicles can not differ in mean diameter. They clearly can, but that is mediated via other factors, not the *locale* environment, which should be equal for all follicles at a given time, although it can vary with time, as seen in the birthcoat.

We also analyse standard deviation of diameter of primary and secondary fibres. The *locale* might influence variability between follicles simply by determining whether the follicles are under stress or free to vary.

5 Results

We start with a linear model which will remove fixed effects of Flock, Sex, and Age, so that what we are studying is variation between sheep within a flock. The model is of the form

$$D = \mu + Flock + Sex + b_{Age} + \sum_{i=1}^{i=n} b_i X_i \quad (1)$$

where

D is fibre diameter

$Flock$ is a fixed effect for one of 11 flocks

Sex is a fixed effect for one of two sexes

b_{Age} is a regression on age in months

$\sum_{i=1}^{i=n} b_i X_i$ are regressions on a number of covariates X_i

Fitting this model leads to an analysis of variance of diameter and to estimates of the various regression coefficients and fixed effects.

5.1 All covariates included

We start with a model including all possible covariates. This is just to set a baseline. It is not a good way to achieve an intelligent interpretation. It just singles out all the covariates that might be part of locale and sums up how much sheep to sheep variation in diameter they might explain.

We start with analyses of variance from fitting this model to Dp and Ds. These are shown in Table 1 For primary fibres, the effects that are both large and significant are IntGpDens, Fn, FollCurv, and FollDep. The R^2 value is 0.385 ($R = 0.621$).

For secondary fibres the effects that are both large and significant are IntGpDens, Fn, and FollCurv. There are also smaller and more marginally significant effects of IGSouth, Np, and AreaPerFoll. FollDep is close to significant (13 percent) . The R^2 value is 0.439 ($R = 0.662$)

There is clearly a lot of sheep to sheep variation in Dp and Ds which is not explained by the *locale* effects. That is to be expected. Variation in papilla cell number per follicle also affects diameter, and is not part of the jem locale effect.

The actual partail regression coefficients for the fit of Table 1 are given in Table 2.

We have omitted the Flock and Sex effects. The magnitudes of these partial regressions are difficult to interpret alone as they are relative to the mean of each covariate. That is why the covariate means are included in Table 2. If we just lookat the signs there are some interesting things - the Fn coefficients are negative so high density iplies low diameter. But the coefficients for IntGpDens are positive? That opens up a whole lot of questions which tend to undermine the usefullness of this full model fit. These are *partial* regressions - so the coefficient for IntGpDens is adjusted so that all other covariates are equal - in particular it is the effect of IntGpDens when Fn is help constant. We need to think about what that means. Not here. The following sections resolve this issue.

The other large and significant effects (FollCurv and FollDep) seem to have coefficients with an appropriate sign.

We endthis section with a note about multiple regression. If covariates are correlated, their effects can be difficult to separate. That is the case here. Table 3 presents the correlations between all of the covariates.

Table 1: Analyses of variance from fitting the model specified in equation 1 with all possible covariates

Mean diameter of primary fibres (Dp)					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Flock	10	439.31	43.93	9.16	0.0000
Sex	1	0.00	0.00	0.00	0.9863
Agenum	1	0.28	0.28	0.06	0.8080
IGNorth	1	0.06	0.06	0.01	0.9101
IGSouth	1	1.71	1.71	0.36	0.5513
IGEast	1	1.39	1.39	0.29	0.5913
IGWest	1	4.90	4.90	1.02	0.3135
IntGpDens	1	54.35	54.35	11.33	0.0009
Fn	1	22.06	22.06	4.60	0.0333
Np	1	3.77	3.77	0.79	0.3763
FollCurv	1	20.26	20.26	4.22	0.0412
FollDep	1	32.84	32.84	6.84	0.0096
IFDistMean	1	2.15	2.15	0.45	0.5041
FollGpArea	1	2.74	2.74	0.57	0.4508
FollperGp	1	0.45	0.45	0.09	0.7607
AreaPerFoll	1	1.46	1.46	0.30	0.5819
Residuals	195	935.66	4.80		
Mean diameter of secondary fibres (Ds)					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Flock	10	142.24	14.22	6.89	0.0000
Sex	1	9.47	9.47	4.59	0.0334
Agenum	1	17.13	17.13	8.30	0.0044
IGNorth	1	7.22	7.22	3.50	0.0630
IGSouth	1	8.41	8.41	4.07	0.0449
IGEast	1	1.75	1.75	0.85	0.3577
IGWest	1	0.31	0.31	0.15	0.7001
IntGpDens	1	58.46	58.46	28.32	0.0000
Fn	1	35.39	35.39	17.14	0.0001
Np	1	9.45	9.45	4.58	0.0337
FollCurv	1	11.23	11.23	5.44	0.0207
FollDep	1	4.66	4.66	2.26	0.1346
IFDistMean	1	0.90	0.90	0.43	0.5109
FollGpArea	1	0.32	0.32	0.16	0.6930
FollperGp	1	1.42	1.42	0.69	0.4079
AreaPerFoll	1	6.27	6.27	3.04	0.0830
Residuals	195	402.60	2.06		

Table 2: Fitted partial regression coefficients for the model of equation 1

Effect	Dp coefficient	Ds coefficient	Covariate Mean
Intercept	11.4	14.4	-
Age	-0.0307	0.0353	-
IGNorth	0.00238	0.0000407	139.6
IGSouth	0.00142	-0.00131	128.6
IGEast	0.00118	0.00220	79.1
IGWest	0.00175	-0.00138	74.1
IntGpDens	0.00891	0.0145	86.6
Fn	-0.000453	-0.0000594	72.0
Np	0.655	0.802	2.64
FollCurv	0.257	0.236	2.66
FollDep	1.88	0.785	1.90
IFDistMean	0.0118	-0.0123	22.63
FollGpArea	0.868	0.245	0.964
FollperGp	-0.00186	-0.00156	80.1
AreaPerFoll	0.0000501	0.0001039	12064.8
Mean Diameter	16.63	18.73	-

Table 3: Correlations among the covariates used in the full model of equation 1

	Fn	IGNorth	IGSouth	IGEast	IGWest	IFDistMean	FollGpArea	FollperGp	IntGpDens	AreaPerFoll	FollCurv	FollDep
Fn	1.00	0.13	0.13	0.01	-0.09	-0.18	-0.43	0.39	0.76	-0.65	-0.15	-0.07
IGNorth	0.13	1.00	0.58	0.04	-0.04	-0.09	0.01	0.14	0.15	-0.09	-0.15	-0.11
IGSouth	0.13	0.58	1.00	0.00	-0.08	-0.15	0.08	0.29	0.18	-0.17	-0.17	-0.14
IGEast	0.01	0.04	0.00	1.00	0.29	0.07	0.03	0.09	0.02	-0.06	-0.00	-0.08
IGWest	-0.09	-0.04	-0.08	0.29	1.00	0.21	-0.02	-0.01	-0.00	0.03	0.14	-0.14
IFDistMean	-0.18	-0.09	-0.15	0.07	0.21	1.00	0.13	-0.06	-0.16	0.20	0.33	-0.04
FollGpArea	-0.43	0.01	0.08	0.03	-0.02	0.13	1.00	0.37	-0.57	0.50	0.14	0.03
FollperGp	0.39	0.14	0.29	0.09	-0.01	-0.06	0.37	1.00	0.48	-0.43	-0.00	-0.03
IntGpDens	0.76	0.15	0.18	0.02	-0.00	-0.16	-0.57	0.48	1.00	-0.81	-0.15	-0.06
AreaPerFoll	-0.65	-0.09	-0.17	-0.06	0.03	0.20	0.50	-0.43	-0.81	1.00	0.15	0.07
FollCurv	-0.15	-0.15	-0.17	-0.00	0.14	0.33	0.14	-0.00	-0.15	0.15	1.00	-0.06
FollDep	-0.07	-0.11	-0.14	-0.08	-0.14	-0.04	0.03	-0.03	-0.06	0.07	-0.06	1.00

We see that F_n is highly correlated with $IntGpDens$ and $AreaPerFoll$ and that these latter 2 are also highly correlated with each other. The remaining correlations are manageable. We attack this issue with a different approach on the following sections.

5.2 Components of follicle density (F_n)

5.2.1 F_n alone

5.2.2 Groups per unit area and follicles per group

5.2.3 Intra group density and trio group area ratio

5.3 Things other than density

6 Discussion

We have shown that mean fibre diameter is affected by follicle number per unit area (F_n) and mean secondary follicle bundle size.

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

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