Are dermal papilla cell counts in individual follicles Poisson distributed?

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Contents

1 Introduction

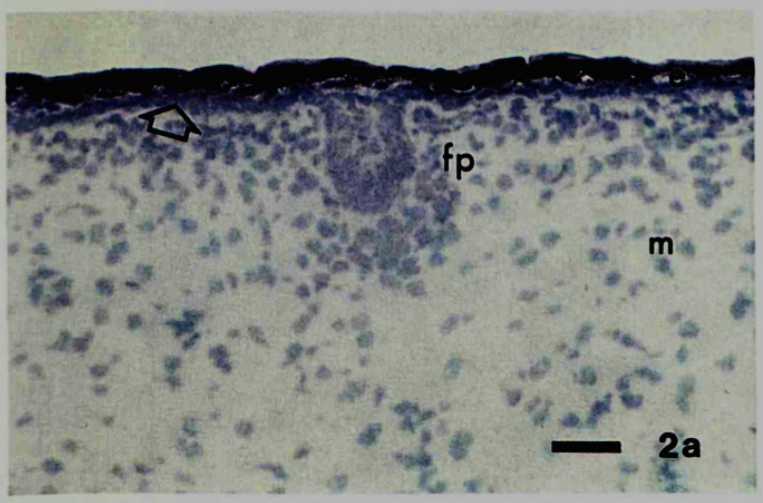
It has been shown that there exists in the dermis of the the developing sheep foetus a population of cells, known as pre-papilla cells, which migrate to the sites of follicle formation and differentiate, ending up in the papilla of the follicle bulb (Moore, etal (1989) [21], Moore etal (1998) [22]). 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.

Pre-papilla cells form cell aggregates at the sites of follicle formation. Figure 1 (courtesy of Philip Moore) shows cells aggregating at sites. Pre-papilla cells are specialized fibroblasts and actually migrate to the follicle development sites to form aggregates.

In Figure 1 one can see aggregates of pre-papilla cells positioned below the bulb of the developing follicle downgrowth. In a fully developed folllicle these aggregated cells end up inside the papilla.

There may be one further cell division of the pre-papilla cells after they form an aggregate, but each aggregate is essentially a sample from a population of unaggregated pre-papilla cells. As such there is possibly sampling variation in the number of cells which form each aggregate. Such sampling variation in numbers of cells per aggregate should follow a Poisson distribution.

Given that the number of cells in each aggregate determines the base diameter of each follicle, and given that we have an empirical equation relating dermal papilla cell number to checked fibre diameter, we can investigate whether a poisson sampling of dermal papilla cell number generates a reasonable looking fibre diameter distribution.



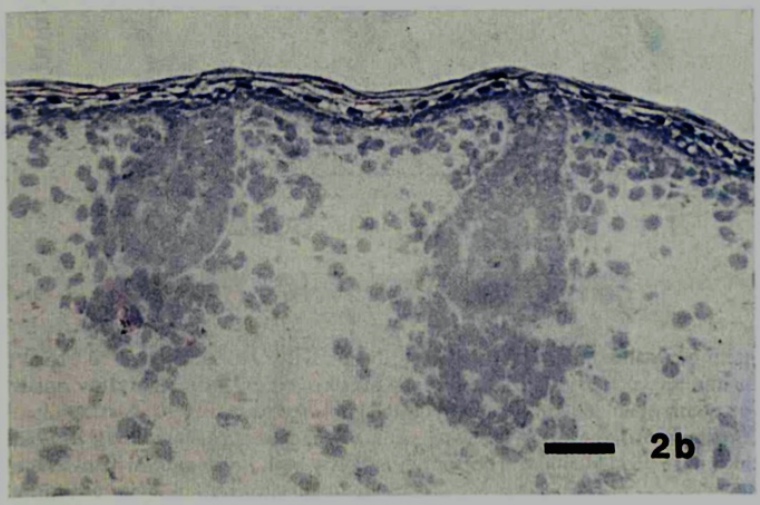


Figure 2. EGF immunoreactivity in skin of 76-d sheep fetus. Cryostat sections were incubated with affinity-purified EGF antiserum *(a)* or pre-immune serum *(b)* and counterstained with Mayers hematoxylin. *a)* The reaction product is present in the periderm and intermediate layer of the epidermis; follicle primordia are unstained. *b)* Control section. *Bar,* 30 μm. e, epidermis; fp, follicle plug; m, mesenchyme.

Figure 1: Developing follicles with associated dermal papilla cell aggregates

2 Materials and Methods

The statistical language *R* [23] has a function *rpois(n,λ)* which generates n random values sampled from a Poisson distribution with parameter λ. Parameter λ is both the mean and variance. One can therefore generate a moderately large number of random poisson variates and inspect the distribution.

What we actually want is the distribution of diameter, given the Poisson simulated distribution of dermal papilla cell numbers. To get diameter distribution we transform the simulates papilla cell numbers (C) to diameters (D) using

$$A = 72.2312 + 3.9270C$$

where (A) is fibre cross sectional area, and then

$$D = \sqrt{\frac{4A}{\pi}}$$

giving a set of diameters which can be displayed as a distribution.

3 Results

3.1 Sampling considerations for pre-papilla cell aggregations

3.1.1 Poisson samples

Start with two Poisson samplings of 5000 papilla cell numbers from populations with means of 50 and 100 papilla cells per follicle.

```
> samp.5000.50 <- rpois(5000,50)
> samp.5000.100 <- rpois(5000,100)
> mean(samp.5000.50)
[1] 50.0694
> var(samp.5000.50)
[1] 49.84735
> mean(samp.5000.100)
[1] 99.9076
> var(samp.5000.100)
[1] 100.7876
```

So 5000 would seem to be a large enough sample, the sample means are very close to the population means of 50 and 100.

The frequency histograms for these samples are shown in Figure 2

We can see that the sample with mean 100 has a larger spread (expected because the variance is the same as the mean in Poisson populations). There is nothing else remarkable, both distributions look symmetric . The sample with mean 50 has a slight right skew Poisson samples with such high mean counts are expected to look symmetric like normal distributions. Poisson samples with mean less than about 10 are markedly skewed.

3.1.2 Poisson sample distributions converted to diameter distributiuons

The samples of papilla cell counts with means of 50 and 100 were converted to fibre diameter. The fibre diameter histograms are shown in Figure 3

The means and variances for the two histograms in Figure 3 are 50 cells - mean=18.4, var=0.911, 100cells - mean=24.2, var=1.066. So the variance of diameter increases with the mean. The two diameter histograms are close to symmetric.

Note the difference in the scale for density (the Y axis) in Figures 2 and 3. This is because the widths of each frequency class are different in the two Figures. The densities sum to 1.0 when multiplied by the class width, ie it is the area under the class intervals that sums to 1.0.

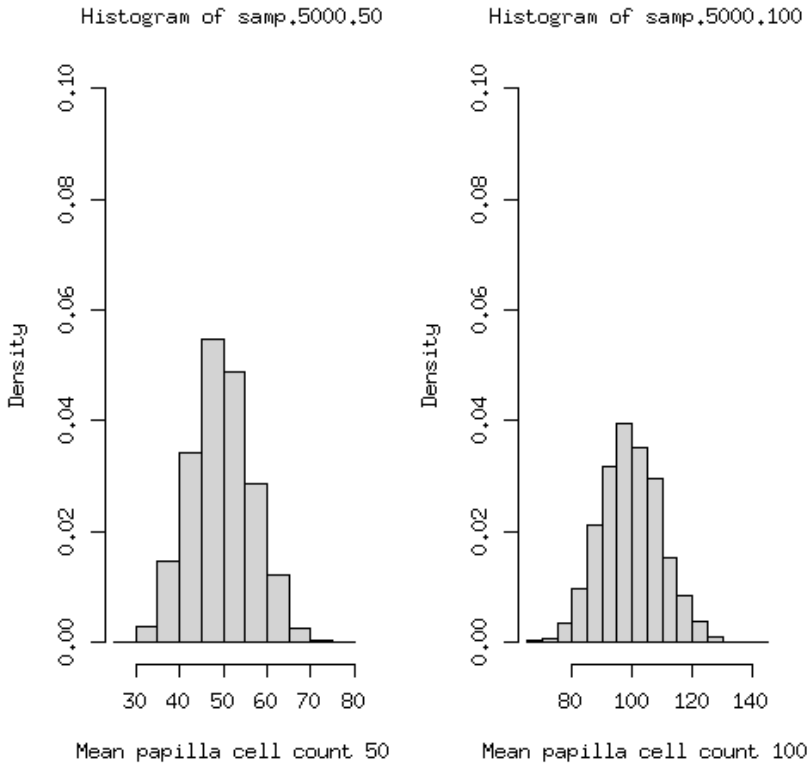


Figure 2: Histograms of papilla cell count samples from Poisson populations with means of 50 and 100 cells

3.1.3 Allowing for differences in papilla cell counts between follicle types

From the above, it is clear that there is no way that a simple Poisson sampling for papilla cell numbers can generate a skewed fibre diameter distribution. Skewed diameter distributuons are common. We have to look elsewhere for the source of skewed diameter distributions.

One possibility is differences in the mean papilla cell count between the various types of follicle . Pc, Pl, So, and Sd follicles tend to form a series of decreasing sizes, so there may well be larger papilla cell counts in the Pc, Pl, and So follicles, and fewer in the Sd follicles.

We can simulate this by using the trio group modelling software (Jackson and Moore (2021) [15]). This software generates a trio group of follicles and calculates their fibre diameters, starting with the following parameters

sositeno number of So sites in a trio group (default 20). Pc sites are fixed at

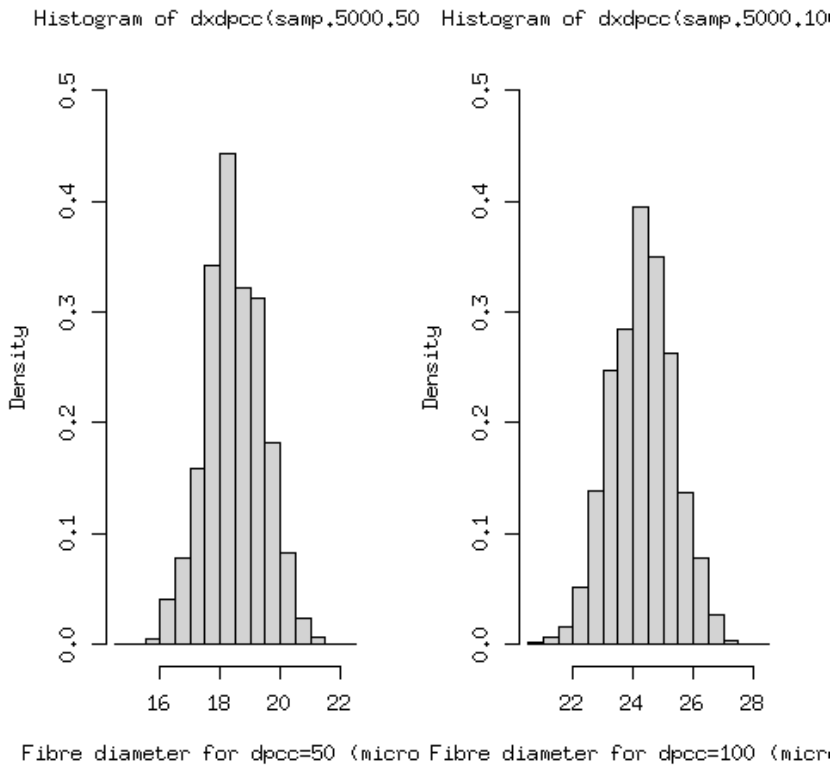


Figure 3: Histograms of fibre diameter for samples of papilla cell counts from Poisson populations with means of 50 and 100 cells

1 and Pl sites are fixed at 2.

follinitrate follicle initiation rate for Pc, Pl, and So follicles - ie for follicles with sites (default 4 per day per trio group)

sdfollinitrate follicle initiation rate for Sd follicles (default 8 per day). Follicles without sites initiate more rapidly.

pcstarttime day number of start of Pc initiation (default 64).

plstarttime day number of start of Pl initiation (default 70).

sostarttime day number of start of So initiation (default 86).

dayzerocellno number of pre-papilla cells per trio group at day zero (default 160, day zero default is day 60).

pcavecellno mean number of papilla cells aggregating at a Pc follicle site (default 65).

plavecellno mean number of papilla cells aggregating at a Pl follicle site (default 64).

soavecellno mean number of papilla cells aggregating at an So follicle site (default 59).

sdavecellno mean number of papila cells aggregating at an Sd follicle (default 53).

cellbirthprob cell birth probability for pre-papilla cell population (default 0.12)

ztime zero time for pre-papilla cell population (default day 60).

There are also some parameters setting foetal growth curves. These are only used to get densities. The model runs on counts of cells and follicles. Densities are an aftercalculation.

There are a lot of parameters. Some of them can simply be left at the default values. We might begin by looking at the result obtained by modelling one trio group with all parameters set to the above default. The model is simply a giant bookkeeping exercise tabulating each follicle as it is initiated and keeping track of differentiated and undifferentiated papilla cells. A list of all the follicles initiated in one such run is given in Table 3.1.3

Table 1: Follicle initiation table for one run of trio group simulation software . Parameters sositeno=10, follinitrate 4 per day, sdfollinitrate 6 per day, pcs tarttime=64, plstarttime=70, sostarttime=86, dayzerocellno=340, pcavecellno=90, plavecellno=89, soavecellno=62, sdavecellno=60, ztime=60

follno	time	index	folltype	diffcellno	Diam	ppcellno
1.00	64.00	5.00	Pc	83.00	22.52	488
2.00	70.00	11.00	Pl	97.00	24.02	711
3.00	70.00	11.00	Pl	107.00	25.04	711
4.00	86.00	27.00	So	39.00	16.94	2252
5.00	86.00	27.00	So	62.00	20.05	2252
6.00	86.00	27.00	So	59.00	19.67	2252
7.00	86.00	27.00	So	54.00	19.03	2252
8.00	87.00	28.00	So	62.00	20.05	2252
9.00	87.00	28.00	So	61.00	19.92	2252
10.00	87.00	28.00	So	60.00	19.80	2252
11.00	87.00	28.00	So	50.00	18.49	2252
12.00	88.00	29.00	So	61.00	19.92	2233
13.00	88.00	29.00	So	56.00	19.29	2233

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Table 1 – *Continued from previous page*

follno	time	index	folltype	diffcellno	Diam	ppcellno
14.00	89.00	30.00	Sd	61.00	19.92	2329
15.00	89.00	30.00	Sd	85.00	22.74	2329
16.00	89.00	30.00	Sd	67.00	20.66	2329
17.00	89.00	30.00	Sd	66.00	20.54	2329
18.00	89.00	30.00	Sd	65.00	20.42	2329
19.00	89.00	30.00	Sd	64.00	20.30	2329
20.00	90.00	31.00	Sd	64.00	20.30	2143
21.00	90.00	31.00	Sd	49.00	18.36	2143
22.00	90.00	31.00	Sd	62.00	20.05	2143
23.00	90.00	31.00	Sd	69.00	20.90	2143
24.00	90.00	31.00	Sd	53.00	18.89	2143
25.00	90.00	31.00	Sd	65.00	20.42	2143
26.00	91.00	32.00	Sd	65.00	20.42	1985
27.00	91.00	32.00	Sd	63.00	20.17	1985
28.00	91.00	32.00	Sd	74.00	21.49	1985
29.00	91.00	32.00	Sd	62.00	20.05	1985
30.00	91.00	32.00	Sd	60.00	19.80	1985
31.00	91.00	32.00	Sd	64.00	20.30	1985
32.00	92.00	33.00	Sd	49.00	18.36	1786
33.00	92.00	33.00	Sd	81.00	22.29	1786
34.00	92.00	33.00	Sd	56.00	19.29	1786
35.00	92.00	33.00	Sd	75.00	21.61	1786
36.00	92.00	33.00	Sd	59.00	19.67	1786
37.00	92.00	33.00	Sd	66.00	20.54	1786
38.00	93.00	34.00	Sd	49.00	18.36	1570
39.00	93.00	34.00	Sd	59.00	19.67	1570
40.00	93.00	34.00	Sd	67.00	20.66	1570
41.00	93.00	34.00	Sd	61.00	19.92	1570
42.00	93.00	34.00	Sd	59.00	19.67	1570
43.00	93.00	34.00	Sd	71.00	21.14	1570
44.00	94.00	35.00	Sd	58.00	19.54	1353
45.00	94.00	35.00	Sd	54.00	19.03	1353
46.00	94.00	35.00	Sd	60.00	19.80	1353
47.00	94.00	35.00	Sd	54.00	19.03	1353
48.00	94.00	35.00	Sd	49.00	18.36	1353
49.00	94.00	35.00	Sd	66.00	20.54	1353
50.00	95.00	36.00	Sd	64.00	20.30	1141
51.00	95.00	36.00	Sd	72.00	21.26	1141
52.00	95.00	36.00	Sd	71.00	21.14	1141
53.00	95.00	36.00	Sd	67.00	20.66	1141
54.00	95.00	36.00	Sd	63.00	20.17	1141
55.00	95.00	36.00	Sd	55.00	19.16	1141
56.00	96.00	37.00	Sd	59.00	19.67	858

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Table 1 – *Continued from previous page*

follno	time	index	folltype	diffcellno	Diam	ppcellno
57.00	96.00	37.00	Sd	74.00	21.49	858
58.00	96.00	37.00	Sd	52.00	18.76	858
59.00	96.00	37.00	Sd	73.00	21.38	858
60.00	96.00	37.00	Sd	66.00	20.54	858
61.00	96.00	37.00	Sd	61.00	19.92	858
62.00	97.00	38.00	Sd	65.00	20.42	555
63.00	97.00	38.00	Sd	56.00	19.29	555
64.00	97.00	38.00	Sd	68.00	20.78	555
65.00	97.00	38.00	Sd	59.00	19.67	555
66.00	97.00	38.00	Sd	51.00	18.63	555
67.00	97.00	38.00	Sd	48.00	18.22	555
68.00	98.00	39.00	Sd	67.00	20.66	261
69.00	98.00	39.00	Sd	50.00	18.49	261
70.00	98.00	39.00	Sd	56.00	19.29	261
71.00	98.00	39.00	Sd	49.00	18.36	261
72.00	98.00	39.00	Sd	54.00	19.03	261
73.00	98.00	39.00	Sd	61.00	19.92	261

We see 73 follicles forming one trio group listed in order of initiation. The column *diffcellno* is the number of papilla cells aggregated into each follicle. This has been converted to diameter in the *Diam* column. It is a fine wooled sheep with n S/P ratio of 70/3 = 23.3. The whole process takes 35 days, from day 64 to day 98, ie initiation is completed long before birth, in this case. Pre-papilla cell numbers per trio group are shown in the column headed *ppcellno*. What stops the process is using up of all available pre-papilla cells.

The diameters in Table 3.1.3 are shown as a histogram in Figure 4

This distribution has some skew due to the coarseer diameters of primary fibres. However a trio group is only about 100 follicles (in the above case 73). We need to run the model about 50 times - ie generate 50 trio groups , and pool the resultant follicle papilla cell numbers, and fibre diameters.

3.1.4 More than one trio group

We ran the *trio()* modelling software 50 times and pooled the diameters of all fibres from all follicles initiated in all trio groups. The histogram of these diameters is shown in Figure 5

This now looks very much like a typical Merino fibre diameter distribution for a single sheep. A sample from a bale might look different, because it would include variation between fleeces.

We ran the *trio()* modelling software again, this time setting the numbers of papilla cells per follicle to Pc=160, Pl=150, So=40, Sd=35, to simulate a

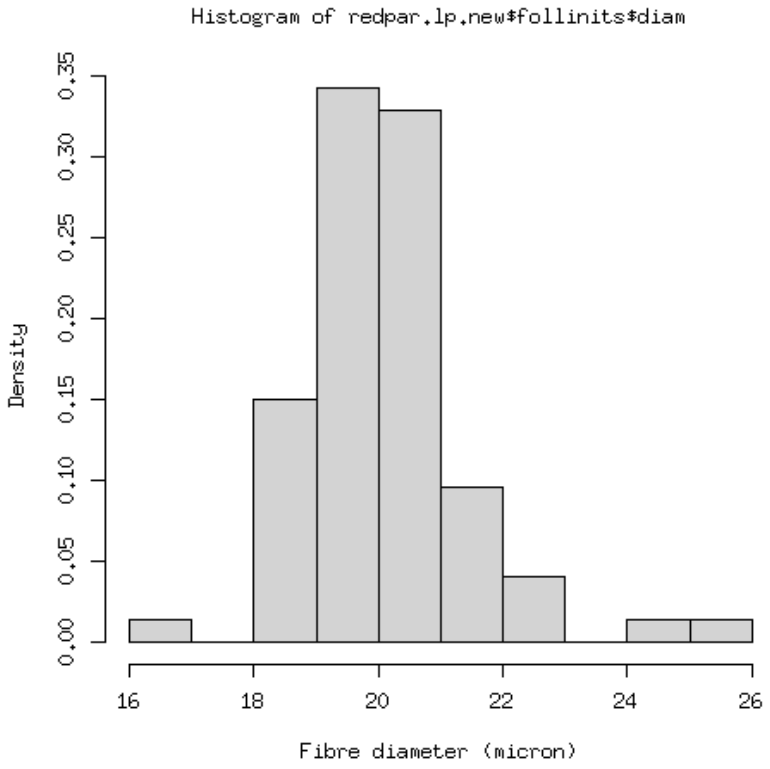

Figure 4: Histogram of fibre diameter for the one trio group in Table **??**

primitive 2-coated fleece. The resultin histogram, from 50 trio groups is shown in Figure 6.

This diameter distribution is rather like that of a primitive sheep, for example those shown by Ryder(1984) [24] The diameters of primary fibres might have been set larger, ie more papilla cells per primary follicle.

3.2 Comparing simulated with actual fibre diameter distributions

Some data from CSIRO genetic research flocks is available with fibre diameter distribution measured on skin sections and an associated follicle density and S/P ratio measured on the same section. The animals were Merino of medium to fine diameter sampled at 18 months of age. We chose a number of sheep representing the range of variation in these flocks, and attempted to simulate the fibre diameter distribution of each starting with values for Dp, Ds, converted to Pc,Pl,So,Sd papilla cell numbers, and with the other starting parameters

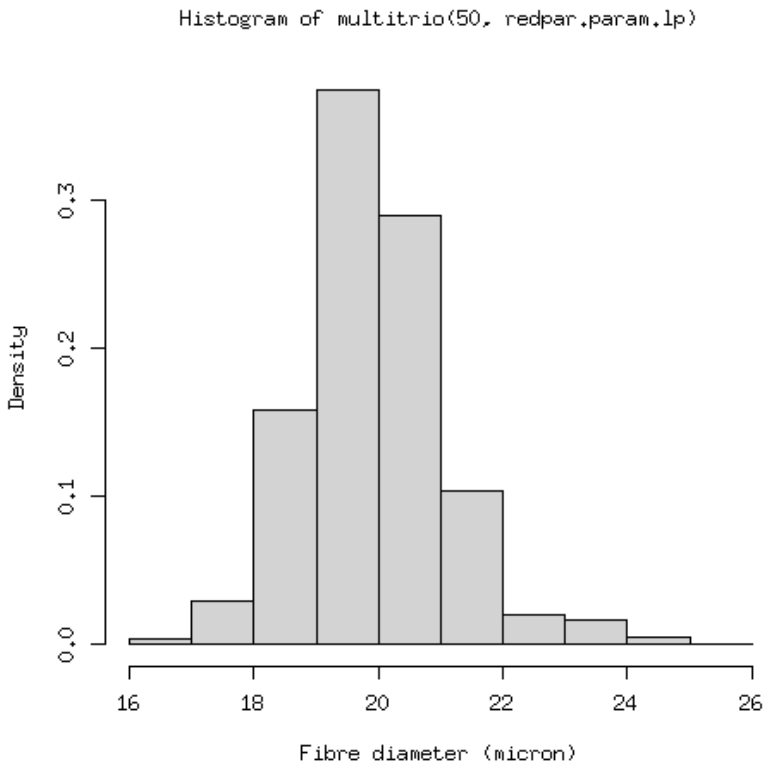

Figure 5: Histogram of fibre diameter for 50 trio groups modelled with the *trio()* software with the same parameters as Table 3.1.3

permuted to attempt to get a matching distribution , and a matching S/P ratio.

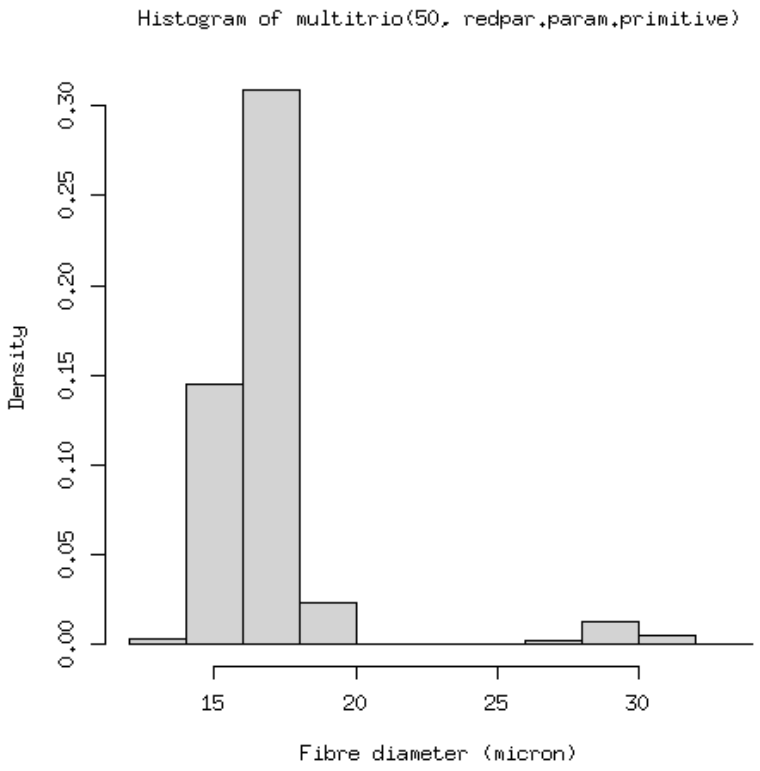

Figure 6: Histogram of fibre diameter for 50 trio groups modelled with the *trio()* software with the same parameters as Table 3.1.3 except papilla cells per follicle altered to Pc=160, Pl=150, So=40, Sd=35 to simulate a primitive 2-coated fleece

4 Discussion

Our thesis is that most of the variation in diameter between fibres within a sheep is controlled by a random sampling of dermal papilla cell numbers forming aggregates. That is , it is not controlled at all, it is chance variation. We emulate this with sampling from a Poisson distribution.

However there is a small amount of fibre diameter variation that is due to systematic differences in dermal papilla cell number between follicle types (Pc, Pl, So, Sd) within a sheep. This variation is under genetic control, and is what is responsible for skewed within sheep fibre diameter distributions. We have ben able to show that one can simulate fibre diameter distributions with skewness or bimodality, simply by allocating more dermal papilla cells to the earliwer forming follicle types (Pc, Pl, So).

It may be that instead of making follicle types, the most realistic strategy may be to allocate dermal papilla cell numbers to follicles according to their time of initiation. The effect on diameter distribution would be similar. We have not tried this approach, but it may be more biologically meaningful.

We are trying to cope with the issue of while we are sure that dermal papilla cell number controls maximim or potential or base diameter (actually cross sectional area) , all our data are of *checked* diameter, ie what the follicles grow when they have to share a limited nutrient supply among all the follicles in a trio group.

Some data on changes in diameter along staples (staple diameter profiles) give a view of how much the *check* effect can vary over time, at least in so far as mean diameter is concerned. What we really need to know is how *check* effects affect diameter distribution.

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A Appendix

The raw data are listed here for completeness