Questions regarding developmental control of fibre diameter and fibre length growth rate in sheep

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1 Introduction

This is not a research paper or a review. What we are trying to do is set down some ideas relevant to control of fibre diameter and fibre length growth rate in sheep.

The focus is on fibre diameter. We know that the number of papilla cells in a follicle bulb partly determines the diameter of the fibre grown. We want to underatand what else is involved so that we can understand how variation in fibre diameter between and along fibres arises.

Fibre length growth rate is included, because it is felt that understanding the relationship between diameter and length will help to unravel the diameter control issue.

2 The papilla cell model

The fleece of a Merino sheep consists of something of the order of 100 million wool follicles each growing a fibre of approximate circular cross section whose diameter may be anything from 10 to 50 microns, and may vary along the length of the fibre. The length growth rate of individual fibres may be between 100 and 1000 microns per day.

The determination of follicle number is understood. The work of Moore and Jackson(1984) [15], Moore, Jackson and Lax(1989) [16], and Moore, Jackson, Isaacs, and Brown (1998) [17] suggests that a population of cells known as pre-papilla cells is responsible for follicle initiation at sites at which these cells aggregate. These pre-papilla cells end up in the papilla cavity of the follicle bulb, and the number of papilla cells in a bulb tend to relate to the size of the follicle and the diameter of the fibre it grows.

We can calculate the consequences for follicle number from assuming various combinations of parameters of the papilla cell population and its dynamics and differentiation. There is a writeup and software for this calculation (Jackson and Moore (2018) [12]). There is also a writeup (Swan(1999) [28]) which tackles the algebra of pre-papilla cell population numbers. If we assume a relation between papila cell number and fibre diameter, we can even calculate mean fibre diameters.

We do have an empirical relationship between average no of papilla cells per follicle and mean fibre diameter. It is in Figure 4 of Jackson and Moore (2018) [12]. The correlation in Figure 4 is 0.81 - so only 67 percent of the variance in papilla cell number is explained by fibre diameter. There is room for some other effects to operate. Figure 4 is a mix of sheep from four selection lines. There is no evidence that any selection line deviates from the linear regression line.

However there is more to fibre diameter variation than the number of papilla cells can explain. Mean fibre diameter is a character with an enormous amount of phenotypic plasticity - it varies with seasonal conditions resulting in variation in diameter along the fibre. Papilla cells may be involved in controlling this

variation. In species which undergo a regular hair growth cycle, papilla cells direct the growth and decline of follicles as they go thru the various stages of the hair growth cycle, and fibre diameter varies with these stages. The physiological and hormonal signalling which affects follicles and fibre growth seems to operate via the papilla cells. In species like sheep in which follicles are almost permanently in Anagen phase, this effect is suppressed, but may not be entirely absent. One of the major modifications made to sheep in domestication is the shift from seasonal shedding to continuous fibre growth. There is also variation in fibre diameter (and in papilla cell number) between follicles on the one sheep. This variance of fibre diameter between fibres is unexplained by the papilla cell model, and it is not known to what extent it depends on the number of papilla cells which aggregate in the bulb of each follicle, or to what extent it depends on other factors such as variation in the density or sizes of follicles. It is known that the type of follicle has an effect on fibre diameter in some sheep - primary follicles sometimes grow fibres of a larger diameter than those grown by secondary follicles.

3 Do follicles compete or share?

The classic paper on this is Fraser and Short(1951) [5]. In Fraser and Short(1952) [6] evidence is presented in the form of

> "..a negative correlation occurred between the size of a fibre and the number, size, and distance of fibres adjacent to it. "

It is suggested that there is a maximum distance over which this negative correlatin occurs. There is also an extensive discussion in Fraser and Short(1960) [8].

We focus here on the possibility of competition between adult follicles for some resource which is required to grow fibre. The issue of competition during follicle initiation and development is left aside.

3.1 The maths of competition and sharing

There are two possibilities. Organs can actively compete for a resource, of they can passiively share in it. Most organs seem to operate by passive sharing. In mammals, the circulation system is massively good at sharing nutritional resources around. The notable exceptions are the foetus, and cancer cells. These actively corner more than their share of everything. So, the big question, are follicles like most organs, or are they like cancer cells? We think the answer is obvious, the skin is like most other organs, it shares, both between the skin and other tissues, and between organelles within the skin.

To understand the subtle difference look at the maths. Sharing is the easiest. We all learnt about sharing in primary school - it is called division. If there are 12 lollies and 4 kids we all learnt to do $12/4 = 3$, and work out that they each get 3 lollies. Division is equal sharing.

Now, what if the sharing is not equal? There has to be some reason for a recipient receiving an unequal share. It may be that the recipient is more actively competitive, or it may be that some external agent is supervising the sharing and imposes a rule. In either case the maths behind it is something we all learnt a little later in primary school - fractions or ratios. A fraction or proportion specifies a share. A ratio specifies a rule for sharing - one for me, two for you, etc. Fractions and ratios dont identify the reasons behind the unequal sharing, the just say it exists.

Conclusion. We can not distinguish between competition and unequal sharing for other reasons. Not by looking at the maths anyway - we might get somewhere by knowing about the biology. We can probably compute each follicle's share of resources, simply by calculating each follicle's fibre output as a fraction of the whole fibre output of the sheep. It would be a rather small fraction, of the order of 10^{-8}.

3.2 Follicle function

This is a huge topic. There are good reviews in .... What we are looking for here are things that might affect sharing. There are at least three levels

between follicles within a sheep some follicles might differ in a way which leads them to receive a greater or lesser share of nutritional resources than their neighbours. We know this is true of primary follicles. It may also operate within follicle groups or across the whole skin.

between different parts of the fleece bearing surface we know lots about this. Diameter and length growth rate variation over the body of the sheep has been extensively studied. A key reference is Chapman and Young(1958) [**?**]. The issue of how this site variation relates to follicle structure and function is not well researched.

between sheep all the follicles on a sheep might be different in some way which leads the whole skin to receive a greater or a lesser share of nutritional resources

So lets make our tentative list of things that might affect sharing

follicle attributes this is actually quite difficult. Size apart, we can not imagine anything within a follicle that might cause it to consume more resources. So lets concentrate on size - if there are more bulb cells dividing to make fibre then we have a larger organ which might use more resources if they were available. It is another matter to say that if resources were limited a larger follicle might 'rob' its neighbours. If a follicle is simply 'more hungry' for resources, then, unless something actually supplies extra resources, it remains hungry. It can not go out and forage for extra on its own.

skin attributes outside the follicle the obvious issue here is blood supply to follicles. Studies of Ryder(1955) [23] show that follicle bulbs with a larger papilla cavity have more blood vessels inside the papilla. The work of Nay(1966) [18] shows that the arrangement of blood vessels in the papillary layer of sheep skin is different in sheep with tangled and straight follicles. This is a much more likely source of modifications to sharing. If follicles develop with different blood vessel supplies, then they may have different resource consuming abilities. This may be related to the number of papilla cells or it may be an independent issue.

the trio group local environment we refer to this as the *locale* effect. Most of the variation in fibre diameter is between follicles within a trio group. So the anatomy and physiology of the trio group is responsible for any unequal sharing of resources between follicles within the group.

whole sheep attributes some sheep simply make more resources available to all their tissues, wool follicles included. This would contribute to between sheep variation, but not to other levels within a sheep.

Tenatative conclusion. There is likely to be an unequal sharing of resources between follicles. The follicle attributes that seem to be correlated with growth of larger fibres (and therefore consumption of more resources) are likely to be developed in synchrony with skin atttributes such as blood supply so that enhance supply of resources. Biology works like that. So while it might seem that certain follicle types grab a larger share of resources, the truth is they are actually given a larger share. Follicle biology seems to be an 'old school tie' system, not a 'rat race'.

3.3 Closer look at Fraser and Short(1952) [6]

If we are going to deviate from Fraser and Short(1952) [6] we need to go back over that paper, and see exactly where it is we differ. Fraser and Short(1952) [6] is an amazing piece of work. It is only three sheep, a Ryeland, a Lincoln, and a medium strong Merino. The samples came from Dr Carter's collection. The measurements were as follows - choose a fibre at random, draw a circle around it of radius 147 micron and measure the distance to every follicle within that circle, and its fibre diameter. Repeat for 173 and 293 micron. Repeat for around 70 randomly chosen fibres.

The analyses consisted of regressions of the diameter of the central randomly chosen fibre on a measure of the total cross sectional area of growing fibre in the surrounding circle. The measure was intended to describe the total fibre output of the surrounding follicles. The regressions were done with and without weighting according to the distance of each follicle from the centre of the circle. We reproduce their Figure 1 in Figure 1, so we can see how good a fit the regressions were.

There is substantial variation around the regression line. This is not unexpected. Biological data are always noisy. This is the 'best' result - the Lincoln

An infinite range of inverse functions of distance of separation could be analysed, and it is intended in a later paper to detail the determination of the function relating intensity of competition to distance of separation.

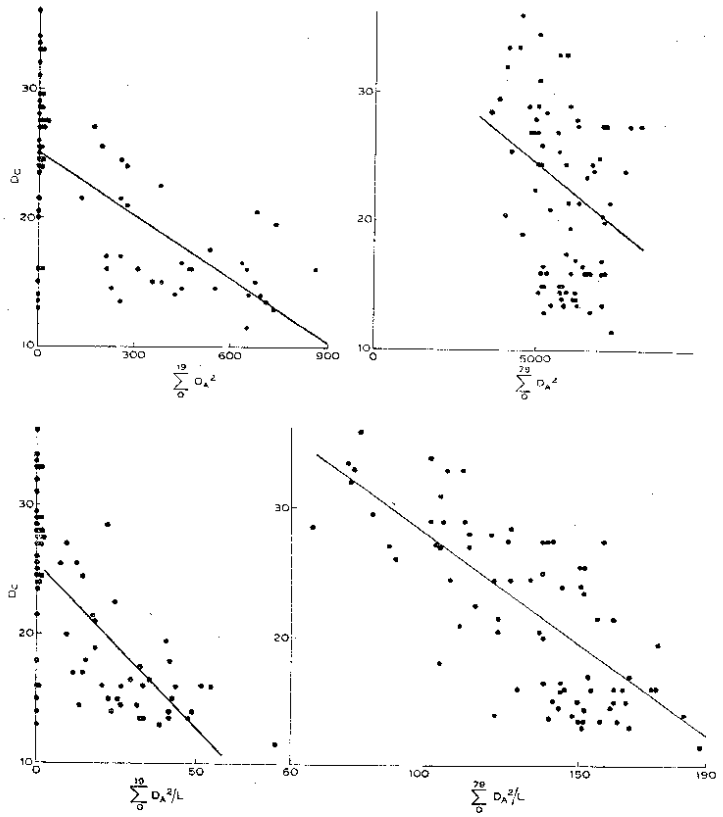

Fig. 1.—Scatter diagrams for the Lincoln sample of the diameters of the central fibres plotted against the sum of the diameters squared of the adjacent fibres. This summation is given from 0 to 79 and from 0 to 19. The two top diagrams are for summation without correction for distance. The two bottom diagrams are for summation after correction for distance.

Figure 1: Figure 1 from Fraser and Short(1952) [6]. These data are for the Lincoln sheep only. The left side graphs are for the 147 micron circle, the right graphs for the 173 micron circle. The lower graphs are weighted by distance between follicles, the upper graphs are not weighted.

sample had the most significant regressions. We comment on this below. The weighting by distance did make some improvement. The regressions were better

for the smaller diameter circle. That all makes sense.

What we now have to do is delineate our areas of concern. These are as follows

follicle positions these data came from horizontal sections of skin at sebaceous gland level. What is sectioned at that level is the follicle shaft, not the bulb. Bulbs deviate substantially from the position of the shaft, especially in sheep with high follicle curvature. This is why the Lincoln data have a better fit - the positional information reflects the bulb position more accurately in the Lincoln because they have straight follicles. The Ryland and Merino specimens would have some degree of follicle curvature. It is the position of the bulb which may matter in relation to competition, because the bulb is the active tissue involved in growing fibre.

length growth rate As a measure of fibre growth activity of the surrounding circle of skin, Fraser and Short used the sum of the squares of the diameters of all the follicles in the circle. This accounts for number of follicles, and fibre cross sectional area, but not fibre length growth rate. We do not know if diameter and length are correlated in this scenario. So one part of their measure of follicle output of fibre is missing.

confounding fibre growth and development we are going to take a punt and assert that most of the regression significance comes from follicle number, rather than from diameter. Follicle number is purely a development factor. It does not change as the follicles grow fibre in the adult. So the contribution of follicle number to the regression is not at all an indicator of competition between fibres while they are growing fibre. It may indicate competition during development, but more recent work (Moore etal (1998) [17]) indicates that it reflects variation in numbers of sites occupied by papilla cells, or, in the case of Merino sheep, follicles formed by branching from other follicles.

Diameter, on the other hand, could reflect either factors operating during development (such as the number of papilla cells that aggregate at sites) or factors that operate during fibre growth (such as the expansion and shrinkage of follicles that occurs over the hair growth cycle, or with nutritional and photoperiodic variations). So diameter mixes up developmental factors with adult growth factors. We suggest that the diameter effect on the regressions is likely to have been minimal - ie it is all about follicle number, which means it is all about development, not current growth.

trio groups the measurement method consisted of measuring all fibres within a circle drawn around a randomly chosen fibre. Thus the sampling circle could span trio group boundaries. Basically Fraser and Short have ignored the trio group structure of the skin. We consider this to be a major deficiency. We take steps to remedy this by definig a *locale* effect which embraces all effects on follicles within a trio group.

Is there any way we can address these issues. Yes. The paper says

> "The data from which the analysis detailed below was made are very bulky and have been lodged, with the accompanying statistical condensations and copes of the original drawings, with CSIRO Head Office Library, Melbourne, where they can be consulted. "

Maybe we should request access to these data, and see if we can redo the analyses separately for follicle number and fibre diameter. That would at least address the third point above.

We have also taken steps to introduce trio groups into the conceptual framework, and to this end the following section defines what we mean by a *locale* effect.

3.4 Defining a *locale* effect for trio groups

The word *locale* means *a place where something happens.* In our context the place where things happen to developing or mature woolgrowing follicles is inside the trio group.

3.5 Birthcoats and fibre diameter

This is a separate document (Jackson, Moore, Swan, and Watts (2018) [13]). The document deals with a wider range if issues than fibre diameter. We summarizehere the conclusions which are relevant to fibre diameter and length growth rate.

- we identify Dry's "base" with papilla cell number
- we identify Dry's "prenatal check" with the *locale* effect developed in this current document
- we propose diameter of Pc fibre sickle tips in the birthcoat as a measure of "base" unaffected by "check"
- we assert that adult Dp is a useful measure of "base" but can also be affected by "check"

The other relevant item is our general birthcoat hypothesis

The processes which control fibre diameter and length growth rate and fibre curvature in the foetus are the same as those which control them in the adult fleece

That is important because we have identified a papilla cell number effect and a *locale* effect in birthcoats, so birthcoats are an ideal place to study the operation of these effects. In birthcoats the *locale* effect varies with time as the follicles develop and the animal grows. So the time variations will tell us something of what the *locale* effect is and how it operates.

3.6 Follicle trio groups

One of the messages from the classic papers of H.B. Carter (Carter(1943) [1] and Carter and Hardy (1947) [2]) is that the *trio group* of three primary follicles and numbers of associated secondary follicles is the *biological unit* of sheep skin. What happens inside one trio group is repeated all over the woolgrowing surface, with some variations according to body sites. When you switch to a different sheep, you may get a slightly differnet trio group, but it is again repeated all over the body.

So it has to be said that a large part of variation in fibre diameter between fibres within a fleece is due to variations in follicle and skin structure within the trio group. So studying trio groups is an important approach to trying to understand what controls fibre diameter variation.

Unfortunately data on trio groups, particularly with fibre diameters measured, is rather limited. We are going to try and quantify the *locale* effect within follicle groups with analyses of two small special datasets.

3.6.1 Compound follicles

We have a small dataset which counts of the number of fibres emerging from a common outer root sheath of a follicle were obtained . This is a measure of degree of follicle branching. The data are analysed in a separate document (Jackson, Swan, and Watts (2018) [14]). THere are two things to note from these data

- the number of branches in a branching follicle varies widely. Primary follicles varied from 1 to 7 branches, and secondary follicles varied from 1 to 25 branches. Thus if branching affects diameter it is likely to produce a lot of follicle to follicle variation locally within a trio group.
- the mean diameter for a sheep was shown to depend on both the overall follicle density and the mean number of branches per follicle

In the document Jackson, Swan, and Watts (2018) [14] it was concluded that the density effect was global and the mean bundle size effect was a local density effect within trio groups.

We want to make a slightly different inference here. Density is indeed a global parameter, but it will be very similar to within trio group density because trio groups do not vary much and we can simply note that there will be a slight difference because overall density includes connective tissue areas outside of trio groups. So we might be able to conclude that diameter depends on within trio group density, and on local variations in and around compound follicles.

This is getting very close to what we would like to define as the *locale* effect on diameter.

3.6.2 Trio group areas and follicle numbers

We have a data set in which counts of follicles within a trio group and trio group areas were measured. This allow a direct investigation of trio group parameters

and how they affect mean fibre diameter and variance of fibre diameter. He data are analysed in a separate document (Swan, Watts, and Jackson (2018) [29]. We note the conclusions

3.7 Where to ?

The mere idea of unequal sharing of some resource among follicles does not help much sort out why the shares are unequal. That is what we need to know - who or what is dealing out the resource unequally, and on what basis.

One thing is certain. We need to get quantitative. That is what the next section is about. We try to get some real data on length and diameter, and to write some equations.

4 Fibre length growth rate and fibre diameter

This is about quantifying individual fibre length growth rates and fibre diameters and trying to write some equations defining how length growth rate and diameter are related to each other and to other things. So we are putting explanations on hold and trying to establish some facts.

4.1 Understanding how one follicle functions

We need to tackle this before looking at variations between follicles. For the present purpose, one fibre grown over a nominal short time interval can be described as a cylinder of keratin with a length (L) and diameter(D). We are neglecting internal structure and shape (curvature and cross section) and are not allowing diameter to vary along the length of the cylinder.

The amount of keratin material in the cylinder can be described by its volume (V), which is related to length and diameter by an identity $V = \frac{\pi}{4}D^2L$. So we have 3 variables, V, D, and L. What the identity relationship says is that only 2 of the three could be under independent biological control. The third is automatically fixed by the other two.

So we already have a problem. Which 2 of the 3 are under biological control? There are three possibilites summarized in Figure 2.

We think the correct causal model is Figure 2(b). This corresponds to transforming the volume equation to

$$L = \frac{V}{\frac{\pi}{4}d^2}$$

So we are saying that D and V are what is biologically determined, and L is simply a consequence of setting D and V.

4.1.1 Glass beads in a cylinder model

How do we justify saying that L is not determined? Lets start with an analogy. Take a beaker of glass beads and pour them into a measuring cylinder. The

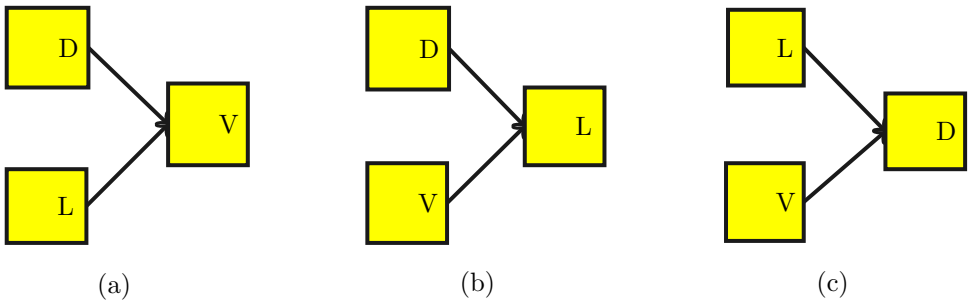

Figure 2: Path diagrams showing three possible causal relationships between fibre volume (V), fibre diameter (D) and fibre length (L).

beads form a stack of diameter equal to the diameter of the mesuring cylinder, and the height of the stack corresponds to L. Put a larger beaker of beads into the cylinder and the diameter stays the same, but L increases. Use a smaller cylinder with the same volume of beads and diameter of the stack will be less, but L will be greater.

So, to what extent is the growth of a wool fibre like making a cylindrical stack of glass beads? We suggest it is exactly like it mathematically, but not , of course, physically. To justify that we need to identify the biological factors which correspond to the volume of beads and the setting of the diameter of the cylinder. Here is our best guess

V volume is an easy guess. Each glass bead corresponds to a cortical cell. So V is the total volume of cortical cells (per unit time). That in turn depends on the number of cells (per unit time) and the average cell volume. That in turn depends on the number of basal cells in the bulb (the cells which divide to form the cortical cells) and their mitotic rate, and certain cortical cell differentiation processes which determine their average volume. V is also at least proportional to the amount of resources used to grow the fibre. We come to that later.

D diameter is more difficult. Some of the basal bulb cells divide to form cortical cells which migrate up to form fibre. Some of the basal cells divide to form cells which migrate up to form the inner root sheath, which is not part of the fibre, and is eventually resorbed. It would seem that follicles which produce larger fibres have larger bulbs, larger stems, larger papilla cavities, and more papilla cells in the papilla cavity. It is also known that larger papilla cavities have more blood vessels inside the cavity. We might guess that the papilla cells influence the size of the array of basal cells which become fibre, (they probably influence more than that - the whole follicle seems to get bigger). The only thing we know is that size can vary with time (eg nutritional changes, photoperiod effects, hair growth cycle effects) in an adult follicle and that the diameter of the fibre varies with it. There

are also genetic differences in average follicle size between animals. There are also developmental differences in average size between P, So, and Sd follicles. There is also variation in size within each of these follicle types.

We also need to identify any biological factors which correspond to differences in fibre length growth rate, bacause if these exist our 'glas bead' model is inadequate. Here is our best efffort to understand length growth rate ofa fibre.

L length growth rate of a fibre is affected by one factor apart from V and D. As the bulb cells migrate upwards from the bulb into the follicle shaft they continue to differentiate. Some become inner root sheath cells. Some become fibre cortex, and as this occurs the cells lengthen and grow microfibrils of protein which eventually grow out of the cell ends and join up to form a fibril matrix which is the fibre cortex. Because the differentiating cortical cells elongate they contribute to fibre length growth rate.

So, we have identified two biological factors which invalidate the 'glass beads in a cylinder' model.

inner root sheath bulb cells are partitined into inner root sheath and fibre. Estimates of the proportion which become fibre (....) are around 50 percent. So not all of the bulb cells contribute to fibre diameter, or to fibre volume.

cortical cell differentiation those cells which do become cortical cells continue to differentiate, and this involves increase in length. So L is under some biological control because the amount of cell lengthening could be varied independently of D or V.

We need to make another attempt to find an adequate abstraction.

4.1.2 Modified glass beads in a cylinder model

Lets stick with our beaker of glass beads, and measuring cylinder, but let us now have some very special beads which behave as follows

- after the beads enter the cylinder, a proportion (γ) stick to the wall of the cylinder and are not further involved in forming the fibre, except that they reduce the diameter of the fibre below that of the cylinder, and they reduce the volume of bulb cells which become fibre.
- those beads which become part of the fibre expand lengthwise by an amount (λ), but do not change in width. This lengthwise expansion contributes to fibre length growth rate.

Under these modifications, our original $V = \frac{\pi}{4}D^2L$ identity no longer applies. We will rewrite it

$$V_0 = \frac{\pi}{4}D_0^2L_0$$

where V_0 and L_0 refer to the volume and length of a *virtual* fibre which would contain all the bulb cells and have no cell length expansion.

If only a fraction γ of bulb cells enter the fibre, we have $V_f = \gamma V_0$, and the diameter will be reduced to $D_f^2 = \gamma D_0^2$ or $D_f = \sqrt{\gamma D_0}$. So we have another *virtual* fibre which contain a fraction γ of the bulb cells, but with no cell expansion. For this *virtual* fibre we have $V_f = \frac{\pi}{4} D_f^2 L_0$. The subscript f refers to this *virtual* fibre.

If we now expand the length of the cortical cells by λ, we have $L_d = \lambda L_0$, and diameter will stay the same at $D_{fd} = D_f$, so for the real fibre we have

$$V_{fd} = \frac{\pi}{4} D_{fd}^2 L_d$$

The subscript d refers to this *differentiated* fibre, that is to the actual fibre. So this is just the equation for the volume of the real fibre, as a cylinder. We can substitute for L_d and D_{fd} and get

$$V_{fd} = \frac{\pi}{4} \gamma D_0^2 \lambda L_0$$

This ties together 5 parameters $(V_{fd}, D_0^2, \gamma, L_0, and \lambda)$, but it is not quite the form we want. V_{fd} is a result, L_0 is an intermediate, and only $D_0^2, \gamma, and \lambda$ are biological variables. We need to get the right hand side of the equations purely in terms of biological variables, and we need equations for 3 results, V_{fd}, D_{fd}, and L_d. Getting that is a fairly simple transformation which leads to

$$V_{fd} = \gamma \lambda V_0 \quad (1)$$

$$D_{fd}^2 = \gamma D_0^2 \quad (2)$$

$$L_d = \lambda \left[\frac{V_0}{\frac{\pi}{4} D_0^2} \right] \quad (3)$$

Equations 1, 2, and 3 are our equations of fibre growth, given the modified glass beads in a cylinder model. They predict the volume growth rate, diameter, and length growth rate of a fibre given the biological parameters D_0, γ, λ, and V_0. We summarize these 4 biological parameters and their meaning in Table 1

We see in Table 1 that parameter V_0 is complex. It is meant to sum up all the output of the follicle bulb, that is the bulb produces a certain volume of cells per unit time. If we delve into how the bulb does this we can break V_0 up into a number of subparameters. We are going to try and get away with just having one parameter for bulb function.

We also see something important in equation 2. Fibre diameter depends only on two things - the diameter of the follicle wall (D_0) and the proportion of bulb cells that go into fibre (γ). In particular it does not depend on the bulb activity. We interpret D_0 as a general follicle size parameter - large follicles are large all over - ie in diameter and length and bulb size and papilla size. There are only two ways a follicle can alter the diameter of its fibre - get bigger overall, or alter the proportion of cells going into fibre.

Table 1: Definition and interpretation of the 4 biologically determined parametrs used in equations 1, 2, and 3

Parameter	Model Description	Biological Interpretation
D_0	Diameter of the cylinder	Internal diameter of the follicle wall or outer root sheath. Also diameter of fibre plus inner root sheath.
γ	Proportion of the glass beads which do not adhere to the wall of the cylinder	Proportion of the bulb cells which form fibre, rather than inner root sheath
λ	Degree to which glass beads expand lengthwise	degree to which cortical cells which enter the fibre cortex expand in length during differentiation
V_0	Volume of glass beads poured into the cylinder	Volume of cells produced by mitoses in the follicle bulb per unit time. Involves the number of basal cells in the bulb, the mitotic rate, and the average cell size before differentiation.

In contrast, length growth rate is far more complicated. It depends on bulb cell volume per unit time, it depends inversely on how much of that volume is required to form the diameter (of the fibre plus inner root sheath ie D_0), and it depends on cortical cell length expansion during differentiation. So a follicle can alter its length growth rate output by churning out more bulb cells, by changing (fibre plus inner root sheath) diameter, or by changing the cortex structure formed during differentiation so that length expansion is changed.

Volume growth rate (equation 1), which is the same thing as fibre weight if there is no medullation and specific gravity is not varying, does not depend on (D_0). It cancels out in doing the substitutions to get equation 1. That is a little strange. What it is saying is that it doesnt matter whether you spread a certain volume of cells over a large or a small diameter cylinder, the volume stays the same. It is all about the volume of material the bulb produces in the first place (modified by γ and λ). Wool production is basically follicle bulb output. Diameter is something else.

We need to look at some observations to see if our 3 equations stand up to scrutiny.

4.2 Sources of variation in fibre diameter

We have the classic study of Dunlop and McMahon(1974) [4] which used data from a range of sources to come up with a partitioning of variance of fibre diameter into along fibre, between fibre, between sites over the body of the sheep, and between sheep. The aim was to see how these sources contributed to the overall variance of diameter which one would obtain by measuring a

core sample froma bale. The result obtained was 20 percent between sheep, 60 percent between fibres, 10 percent between sites, and 10 percent along the fibre. The most important source of variation in fibre diameter is therefore between fibres within one body site on a sheep, suggesting that variation within the follicle group is the major factor.

We need to expand that a bit for our purposes, because we want to know about follicle function in relation to variation of fibre diameter. So we make the following categories and attempt to review what is known about follicle function in relation to each

Genetic lines Steinhagen, etal (1985) [25] measured the inner and outer diameters of the follicle wall, separately for primary and secondary follicles, on ten breeds of wool sheep. These measurements were made at sebaceous gland level, that is after the inner root sheath has been resorbed. It might be assumed that the excess of the inner diameter of the follicle wall over the diameter of the fibre represents the space that was occupied lower down the follicle tube by the inner root sheath. We will attempt to interpret their results in that way. Their ratio of fibre diameter to inner diameter of the follicle wall represents the square root of our γ parameter. Taking square roots we get estimates of gamma for various breeds as shown in Table 4.2

Table 2: Table of data from Steinhagen etal (1985) [25] with two columns added for our γ parameter calculated for primary and secondary follicles assuming that the inner follicle wall diameter is the diameter of the inner root sheath

Breed	Dp	Ds	IDp	IDs	ODp	ODs	DpovIDp	DsovIDs	GammaP	GammaS
Merino	23.50	24.80	26.70	28.60	47.60	53.00	87.90	86.70	0.77	0.75
MerinoLandrace	27.50	27.00	31.30	28.30	72.30	69.80	87.80	95.20	0.77	0.91
FinnishLandrace	31.20	27.40	36.20	30.50	69.20	68.40	86.30	89.70	0.74	0.80
IleDeFrance	28.90	28.60	31.80	30.00	78.30	79.00	91.10	95.40	0.83	0.91
DorsetHorn	30.20	29.60	33.90	31.90	70.30	74.70	89.00	92.90	0.79	0.86
Cheviot	31.60	30.00	36.20	33.60	77.20	82.20	87.10	89.50	0.76	0.80
Texel	31.70	32.90	35.80	36.50	80.40	80.10	88.70	90.10	0.79	0.81
BorderLeicester	38.50	39.00	42.80	43.00	81.00	83.70	90.00	90.80	0.81	0.82
BleuDeMaine	31.80	32.00	36.00	34.30	82.80	82.90	88.30	93.50	0.78	0.87
GermanWhitehead	39.60	37.60	47.00	42.50	89.20	86.30	84.30	88.60	0.71	0.78

The mean γ for primary follicles is 0.775, and the mean γ for secondary follicles is 0.833. The standard error of these means over 10 breeds is 0.0033. So the difference is significant. There is not a large variation betwen breeds (0.0011 for γ for primary follicles, and 0.0334 for γ for secondary follicles). We cannot test the signoficance of the breed differences, because we do not have the data for individual sheep within a breed.

Sheep within a flock Henderson(1965) [9] developed a technique for digesting skin so that single follicles could be dissected out with the fibre intact, allowing measurement of follicle dimensions and fibre diameter and length. He found strong correlations of follicle length , papila volume and bulb diameter with fibre diameter when he studied variation between follicles

within a sheep, but could not find these correlations at a between sheep level. He also found strong between sheep correlations of follicle length with fibre length, but correlations of papilla volume with fibre length were lower. He also noted that nutrition and photoperiodicity affected the amount of germinative tissue in the follicle bulb. Henderson's study is a mixed bag.

Body sites Fibre diameter varies in a systematic way ove rth body of the sheep (Chapman and Young (1958) [3]). Fibre diameter is finest at the shoulder and coarsest at the britch. So there is a front to rear gradient, but there is no back to belly gradient. The interesting thing is that density (fibres per cm^2) has both a front to rear gradient and a back to belly gradient. So there are density changes from back to belly which do not cause diameter changes. That begs an explanation.

Nutrition There have been many studies of experimentally applied nutritional effects on wool growth. The book Reis and Black (19..) [] is a good summary. Poor nutrition leads to smaller fibre diameters and to smaller fibre length growth rates, and the two tend to change in proportion so that the ratio L/D stays constant for one sheep under changing levels of nutrition (ref). The ratio L/D is not constant from sheep to sheep (Jackson and Downes(19) [**?**]. What we do not know is how the follicle implements these nutritional changes in diameter. No-one has bothered to look at how the follicle responds in the two critical aspects, general size and/or bulb size, and the parameter γ which is the proportion of bulb cell output whic form the fibre rather than the inner root sheath. Nutritional effects are systemic. All follicles and fibres on the animal are affected. Within a trio group, the various types of follicle do not necessarily respond equally, but they are all affected. All follicles get a share, but the sharing is not equal. Inequalities in sharing are due to local effects within a follicle group.

Photoperiod Priestley and Rudall (1965) [21]noted that in winter thinning of the fleece in Herdwick sheep, the inner root sheath becomes thicker on one side in some primary follicles which continue to grow fibres in the winter months. Photoperiod effects are systemic, like nutritional effects, but the follicles do not necessarily respond in the same way. It is suspected that photoperiod effects are related to the hair growth cycle, and that they are therfore likely to affect diameter by modification of the γ parameter. Although the effect is systemic, follicles on different parts of the sheep respond differently and the fleece growing area is markedly different to the hair growing areas on face and legs. Different types of follicle within a group also respond differently - primary follicles shed at a different time to secondary follicles in sheep which shed seasonally, and in sheep with continuous growth, usually only the secondary follicles jhave continuous growth, primary follicles still shed but asynchronouslyand not seasonally.

Hair cycle Straile(1965) [26] observed in rabbits that when hairs change in diameter as they go thru stages of the hair growth cycle, the follicle outer

root sheath does not change its size, but instead the inner root sheath changes in a way which complements the changes in fibre diameter. This complementary change in the inner root sheath occurs in Huxleys layer, which is the central of three sublayers found in the inner root sheath. Priestley and Rudall (1965) [21] found the same for zigzag fibres in rats, and also noted that the proportion of bulb output going to the fibre (rather than the inner root sheath) coud be less than 50 percent.

Follicle types within a group Schinckel(1961) [24] noted that types of hair follicles that grow large hairs have large bulbs and dermal papillae, whereas types that grow small hairs have small bulbs and dermal papillae. Straile (1965) [26] notes this, but also argues that bulb size is more strongly correlated with size of the hair plus inner root sheath, than with size of the hair alone. It would seem that primary follicles have a different γ to secondary follicles (see the above reference to Steinhagen, etal (1985) [25] in the *Genetic lines* section. Primaries generally have larger fibres, and a smaller γ. Does that make sense?

4.3 Causes of variation in fibre diameter

This is different from the previous section. Sources are not causes. What we try to do here is to draw together all of the factors that we have identified that can cause fibre diameter to vary. Here is our list of 'levers' or 'pathways' through which th sjheep is able to alter fibre diameter

- the number of pre-papilla cells that agglomerate at a follicle site and which eventually become the papilla cells
- the locale effect within a trio group
- outside influences such as nutrition or stress which apply uniformly to all trio groups
- hair growth cycle activity such as that activated by photoperiod

In addition to that we have identified two means by which an individual follicle can alter the diameter of its fibre

- the apportioning of follicle bulb cells between inner root sheath and developing fibre cortex
- the overall size of the follicle and all its components including the bulb, the papilla, the root sheath and all follicle dimensions.

It is not clear which of the above two follicle effects applies to each of the above four pathways. There is evidence that hair growth cycle activity operates via the inner root sheath/cortex partitioning method. There is evidence that the *local* effect operates via the follicle size method.

4.4 Data on length growth rate and diameter for individual fibres

We have some data on Merino sheep with diameter and length growth rate measured several times along the fibre, using fibre autoradiograph techniques to label fibres at specific times. The analyses of these data are in a separate document (Jackson() []).

[documnet not completed yet]

5 Quantitative genetic study of fibre diameter

A number of quantitative genetic studies have been made in which variation in fibre diameter has been partitioned into various categories of genetic variance. These analyses are reported in a separate document (Jackson() []).

[document not completed yet]

One result from these analyses, which is of interest in relation to the current investigation of the control of fibre diameter, is the finding that gentic variance in fibre diameter is not all additive. There is a considerable proportion ofadditive x additive epistatic variation. This is interesting because it implies that there is more than one set of genes controlling fibre diameter, and that at least two of these different sets of genes interact. That is one set of genes enhances the effect of the other set.

This is what one would expect if diameter were controlled by more than one mechanism, for example by papilla cell numbers and by locale effects. The genes controlling papilla cell numbers would obviously interact with the genes controlling locale.

6 Discussion - can we enhance the papilla cell model to account for fibre diameter?

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