



High-resolution spatial phenotyping of fibre diameter and staple length over Corriedale sheep fleeces



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ABSTRACT

The variability of raw wool traits over fleeces is still poorly known. We conducted a 2-year field study on 10 Corriedale ewes aimed at characterizing the spatial distribution of fibre diameter (FD) and staple length (SL) over fleeces. After ~340 d of growth in two consecutive years we determined FD (μm ; OFDA 2000) and SL (cm; ruler) on 128 staples (~1.5 cm basal diameter) per animal collected over a regularly spaced grid. FD and SL varied between animals (FD: $P < 0.001$; SL: $P < 0.05$) and years (FD: $P < 0.006$; SL: $P < 0.001$). Variation between staples approached (FD, 80%) or surpassed (SL, 146%) variation between animals. Quadratic (FD, longitudinal) and quartic trends (SL, longitudinal; FD and SL transverse) best described the systematic variation along the axes of the fleece. Repeatabilities between years, from data pooled up to the animal \times year level, were 0.88 ± 0.08 (estimate \pm s.e., $P < 0.001$) for FD and 0.52 ± 0.24 ($P < 0.05$) for SL. Repeatabilities at individual sampling points over the fleece varied between 0.47 ± 0.26 and 0.94 ± 0.04 for FD, and from 0.0 ± 0.33 to 0.84 ± 0.10 for SL. Spatial ranges (distances required to consider two samples uncorrelated; estimated by variogram analyses) varied among animals ($P < 0.04$). They were higher ($P < 0.03$) for FD than for SL (5.2 vs. 3.7, s.e.d. = 0.46) and repeatable between years for both variables (FD: 0.87 ± 0.08 , $P < 0.001$; SL: 0.72 ± 0.15 , $P < 0.01$). Maps of traits produced by ordinary kriging exposed non-linear longitudinal and transverse trends of FD and SL over the fleeces, revealed a limited number of spatial features shared by most animals, and showed that some features of the spatial patterns uncovered are conserved over time. Spatial patterns and properties differed between FD and SL possibly reflecting both prenatally determined follicle populations and regionalized effects of environmental variables preferentially affecting SL. Maps of variance components and repeatabilities were bilaterally asymmetric suggesting the occurrence of waves of wool growth cycling over the fleece. Findings could be relevant for the design of experiments, fleece classing, selection of sampling points, and breeding. Alternative selection criteria and phenotyping methods would have to be developed for progressing from a fleece-oriented approach based on a single universal sampling point towards methods embracing 2D variability. Associated costs would limit applications to specialty wools.

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1. Introduction

Wool is a heterogeneous product. The economic efficiency of its industrial processing depends on a number of raw wool traits of which mean fibre diameter (FD), yield, and staple length (SL) are the most important ones (Atkins, 1997). Fine, high yielding wools with uniformly long fibres

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usually trade at top prices. Both FD and SL have moderate to high heritabilities (Atkins, 1997; Safari et al., 2005) and vary between breeds, flocks, individuals within flocks, regions and staples within individual fleeces, and between individual fibres within staples (Turner and Young, 1969); FD also varies along individual fibres, and according to orientation due to the elliptical cross section of the fibres.

Variation between flocks and individuals has been widely exploited in the past through purchasing of superior sires and within-flock selection. However, and at least for FD, the most important component, accounting for over 50% of total variation in both Australian Merino (Dunlop and McMahon, 1974) and New Zealand Romney (Sumner and Revfeim, 1973), is the variation between fibres within staples. But exploiting this source of variability, originating in the diversity and spatial arrangement of wool follicles (Ryder and Stephenson, 1968) is beyond reach for current wool processing technology.

Variation in FD between individual points or regions within fleeces seems to be more important in some breeds than others and ranges from about half (Merino: Dunlop and McMahon, 1974) to three times (Romney: Sumner and Revfeim, 1973) the variation observed between individuals.

The phenotypic variation of fleece traits over the body has received attention for almost a century. Turner (1956) reviewed early literature on a variety of breeds and concluded that FD increased following an antero-posterior gradient and that the evidence for a similar dorsoventral gradient was weak. Staple length, in turn, decreased along an antero-posterior gradient and there was indication that it also decreased from the mid-side line towards both the backline and the belly. Later work (Young and Chapman, 1958; Doney, 1959; Sumner and Revfeim, 1973; Fish et al., 2002) confirmed those general trends and contributed to support the widespread use of a single mid-side sample as representative of the individual phenotype for a variety of raw wool traits. However, in a few of the early studies the number of sites sampled over the body was considerable (up to 40), and the spatial distribution of FD and SL values suggests that more elaborate patterns than just mere gradients could be uncovered by increasing sampling resolution. Higher resolution would also allow for exploring the possibility of spatial patterns varying systematically among individuals, sexes, age groups, and lines or breeds. Current availability of advanced portable equipment for on-farm wool trait assessment, such as the Optical Fibre Diameter Analyser (OFDA; Baxter et al., 1992), opens the field for such kind of intensive over-the-fleece wool sampling.

Recent work in other domestic fibre producing species suggests a renewed interest in the study of over-the-fleece variation of fibre traits (Angora goats: Newman and Paterson, 1999; Taddeo et al., 2000; McGregor and Butler, 2008, 2009; Alpaca: McGregor et al., 2012).

In this study we aimed at producing a high-resolution characterization of the spatial distribution of FD and SL over the fleece of adult sheep. The information was then applied to produce spatially referenced displays of phenotypes, variance components for the traits, and repeatabilities over time. Preliminary results have been already reported elsewhere (Rodríguez Iglesias et al., 2007, 2009a,b).

2. Materials and methods

2.1. Location, animals, and general management

The study was conducted at the Argerich Experiment Station (latitude: 38°44'S) using 10 ewes (3- to 4-year-old at the onset of the study) picked at random from an experimental Corriedale flock. Adult Corriedales produce fleeces in the approximate range of 26–32 μm (www.nzsheep.co.nz/Corriedale, www.corriedale.org.au) with finer females below that minimum, and coarser males above the maximum. Staple length, in turn, varies from 8 to 15 cm approximately, mostly depending, like FD, upon breeding, sex and nutrition. The breed is moderately seasonal for both reproduction (Rodríguez Iglesias et al., 1993) and fibre production (Nagorcka, 1979).

Experimental ewes were not exposed to rams during the study and were run with the rest of the flock except during breeding and lambing periods. In order to mimic regular grazing schedules, experimental ewes had access to winter crops during the periods corresponding to late gestation and early lactation of mated ewes, and grazed native pastures for the rest of the year. General health, liveweight, and body condition score (Jefferies, 1961) on a 1 (thin) to 5 (obese) scale were monitored once a month to anticipate wellbeing issues. Management, health care and experimental procedures were consistent with standards suggested in the Consortium guide (*Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, 1999).

2.2. Wool sampling and determinations

Ewes were shorn in November 2006, November 2007, and October 2008. In the first two occasions, wool was closely clipped after shearing to ensure a uniform remnant of wool fibres of approximately 0.5 cm throughout that set the baseline for SL assessment performed shortly before the next shearing.

Wool staples of approximately 1.5 cm of basal diameter (always collected by the same person) were removed, using surgical scissors and as close to the skin surface as possible, in October of 2007 and 2008 after 339 and 342 days of post-clipping growth, respectively. Samples were collected from 120 points determined by the intersections of a regular square grid of 10 longitudinal by 12 transverse color lines drawn on the surface of the fleeces with the help of a cotton string impregnated with coloring powder. Two additional samples, aligned with the grid, were collected from the upper part of each leg thus totaling 128 samples per individual. First and last longitudinal lines were drawn approximately 5 cm above the contact line between fleece and belly on both sides of the animal; the eight remaining longitudinal lines were then uniformly spaced from one side to the other. The first transverse line was aligned to the base of the neck and extended down to both sides of the chest; the last transverse line was aligned to the insertion of the tail and followed the shape of the breech.

Grid design was aimed at achieving even coverage of the skin surface under the fleece with squares of approximately 1×1 map units with the exact dimension of a map unit varying from one individual to another (6.8 ± 0.25 cm by 7.5 ± 0.20 cm across subjects; mean \pm s.d. at skin level). However, as wool grows so does the distance separating longitudinal lines (but not transverse ones) on the surface of the fleece relative to their projections at skin level and this effect varies among animals. Utmost care was exercised to minimize this potential cylindrical projection bias during sampling. Alternative procedures (e.g. using elastic grids instead, or unwarping of digital photographs to assign spatial coordinates after sampling; Rodríguez Iglesias et al., 2007) have similar drawbacks.

The unstretched length of the staple (SL) was determined to the nearest 0.5 cm using a metric ruler graduated in mm. Each sample was then numbered and stored in polyethylene bags for later FD determination. Samples ($n = 1280$ per year) were processed within four months of collection using OFDA 2000 (Optical Fibre Diameter Analyser; Baxter et al., 1992). Samples were scoured before analysis (sonication in isopropanol-hexane 20:80) in six batches per animal with the scouring solution changed every three batches. A possible effect of batch order on FD determinations was statistically explored and found not significant (results not reported). Only OFDA results for mean fibre diameter (i.e. OFDA's *Average Micron*) and for SL determined as described above were used in the study. Fibre diameter readings by OFDA cover the entire length of the staple at 0.5 cm intervals; slightly over four million readings of individual fibres

contributed to each year's dataset. All data were expressed as deviations from relevant means before analyses.

2.3. Spatial analyses and mapping

Three statistical procedures were applied to characterize spatial variability: *surface regression fitting* to identify systematic surface trends (composed themselves of longitudinal, transverse, and interaction terms of wool traits over fleeces), *experimental variogram fitting* to model how the spatial dependence between samples within fleeces varies as a function of the distance between those samples, and *ordinary kriging* to compose maps of predicted spatial patterns of wool traits over fleeces by means of predicting a dense cover of points on the basis of the trait values determined for effectively sampled points ($n=128$) and the spatial arrangement and dependence among them according to distance (as assessed from variogram fitting).

Polynomial surfaces of up to fourth degree were considered for describing systematic trends over the fleece; a quartic trend would be the minimum degree required to represent putative transverse gradients for SL (e.g. Turner, 1956). Starting with quartic trends for both coordinates, reduced models were selected following a *backward elimination* procedure in which non-significant terms were successively removed. Only significant ($P < 0.01$) regression coefficients were retained in reduced model equations. Each studied variable was subjected to systematic trend removal before variogram fitting but only trends for FD and SL for individual subjects across years and for overall averages across subjects and years, are reported.

For any attribute varying in space, the expectation is that samples closer to each other will be more similar than if farther apart. Computation of *experimental variograms* is widely applied (e.g. Wackernagel, 2003) to quantify shape and strength of the spatial dependence between samples as a function of distance between sampling points. Spatial dependence is assessed as the *semivariance*, a variance-like statistic calculated as the weighted average of squared differences between pairs of points located at increasing distances across the surface. Of special interest is the *spatial range* resulting from variogram fitting, i.e. the distance between sampling points at which the semivariance reaches an upper limit. Pairs of sampling points separated by distances larger than the spatial range would be expected to generate trait values independent of each other. Among the licit models available for variogram fitting we selected *spherical models*, extensively used for that purpose in a variety of applications. If h is the distance between samples and a is the spatial range, in a spherical model the semivariance takes the value $C_0 + C_1(1 - \rho)$, where C_0 and C_1 are parameters and ρ is equal to $1 - (1.5h/a) + (h/a)^3/2$ when $h \leq a$, and 0 otherwise. Variograms were fitted to original (FD and SL) and derived variables to estimate spatial ranges, and as a required input for spatial interpolation through kriging (see below). Variogram fitting was performed on the residuals from surface regression fitting, i.e. after removing all significant systematic trends detected for each data set as described above.

Ordinary kriging is an exact spatial interpolator with BLUP properties meaning that, under correct use, it will produce minimum variance (Best), (U)nbaised predictions for unobserved locations on the basis of some (L)inear predictor which is a function of data collected from effectively sampled locations. In the same way that mixed model equation methods are applied to predicting breeding values of animals after accounting for (co-)variances between traits and genetic relationships between relatives (e.g. Mrode, 2005), kriging accounts for the spatial covariance (as assessed in the variograms) and relative orientation and distances between data points when applied for predicting values at unobserved locations. Maps of both recorded and derived variables were then composed on the basis of a regularly spaced grid of 20,293 kriging predictions spreading within a boundary shaped after a sheep pelt and drawn 0.5 map units beyond peripheral sampled data points. The VESPER software (Minasny et al., 2005) was used for variogram and kriging calculations. Akaike's information criterion (Akaike, 1974) was applied for assessing model adequacy when fitting experimental variograms. A neighborhood of a minimum of 12 and a maximum of 24 data points were allowed to influence predictions; i.e. it was assumed that sampled points located beyond the boundaries of such neighborhood would have negligible influence on predictions. Variograms and maps of both FD and SL were produced for each individual in each year, for an 'average fleece' in each year (by using as data the averages across animals calculated at each sampling point), for an 'average fleece' across animals and years, and for the variance between animals, the residual variance, and the repeatabilities between years

estimated for each sampling point over the fleece (see below). Spatial ranges were estimated from variogram fitting to those data sets.

2.4. Statistical analyses

The effects of year (2007 and 2008, fixed), sampling site (1–128, fixed), side (left vs. right, fixed), sampling site within side (1–64), side within animal, site within side within animal, and animal (A to J, random), were tested for inclusion in parsimonious models of the variation of both FD and SL. Year and animal effects were tested at the year \times animal level of aggregation (i.e. samples were averaged over sampling sites and sides for each of 10 subjects sampled in 2 years); side effects were tested on data aggregated up to the side level.

We estimated *variance components*, i.e. contributions of random effects to the total variability, for the animal and residual terms included in the reduced models for both traits (FD and SL) and from those we calculated *repeatabilities* (Becker, 1992) as proxys of the consistency of individual measurements from one year to the next. In order to assess the consistency across years of the spatial variability observed over fleeces we also estimated variance components and repeatabilities for each individual sampling point ($n = 128$) using the 10 data pairs available (10 subjects measured in two consecutive years) per sampling point. We then mapped the spatial variability over fleeces contributed by the phenotypic differences between animals (the between-animal variability), that contributed by other random sources (the residual variance), and the repeatabilities of both traits over the fleece.

Spatial ranges for individual animals within years, estimated from the fit of spherical models to the experimental variograms, were submitted to analysis of variance to test for differences between variables, animals, and years, and to extract corresponding variance components for estimating repeatabilities of the spatial ranges between years. S-Plus 2000 (MathSoft, 2000) was used for both statistical analyses and mapping.

3. Results

All subjects maintained good health during the study. Liveweights and body condition scores (Table 1) varied over time (data not shown) following expected seasonal patterns with minima in late winter and maxima in early summer. Similar mean liveweights (53 ± 5.6 , 56 ± 5.9 and 55 ± 5.0 ; $P > 0.39$) and body condition scores (3.1 ± 0.84 , 2.8 ± 0.59 and 2.9 ± 0.37 ; $P > 0.57$) were recorded around shearings; those means, in turn, were close to yearly averages (Table 1).

3.1. Mean values of raw wool traits

As shown in Table 2, when averaged across sampling points both FD and SL varied between animals (FD: $P < 0.001$; SL: $P < 0.05$) and years (FD: $P < 0.006$; SL: $P < 0.001$). Year effects were associated with mean differences of about $1 \mu\text{m}$ for FD and 1 cm for SL. Maximum differences between animals were close to $4.5 \mu\text{m}$ for both growing seasons (individual data not shown). Corresponding figures for SL were about 2 and 1 cm for 2007 and 2008, respectively.

Side was not a significant source of variation for any of the two variables neither as a main effect across animals ($P = 0.51$ and $P = 0.99$ for FD and SL, respectively) or when nested within animal ($P = 0.90$ and $P = 0.99$, respectively). In contrast, sampling site was a consistent source of variation either within animal (i.e. as a factor with 128 levels, $P < 0.001$ for both variables) or within side within animal (i.e. as a 64-level factor, $P < 0.001$ for both variables).

Maximum differences in FD between individual staples within animals varied from 6 to $11 \mu\text{m}$ in both years; SL

Table 1
Mean liveweights (kg) and body condition scores (scale 1–5) of 10 Corriedale ewes measured at monthly intervals in two consecutive years (n = 24).

Variable	Animal										Year	
	A	B	C	D	E	F	G	H	I	J	2007	2008
Liveweight (kg) (\pm SD)	53.1 (3.2)	49.3 (3.1)	51.8 (2.8)	55.9 (3.4)	54.9 (3.5)	49.6 (3.1)	59.4 (2.7)	58.7 (2.7)	67.0 (3.1)	49.4 (3.6)	55.4 (5.7)	54.2 (5.5)
Body condition score (\pm SD)	2.2 (0.43)	3.0 (0.29)	2.9 (0.53)	2.7 (0.50)	2.9 (0.54)	2.8 (0.47)	2.9 (0.40)	3.0 (0.65)	3.2 (0.55)	2.9 (0.68)	2.9 (0.28)	2.8 (0.30)

differences ranged from 3 to 5.5 cm in 2007 and from 3 to 4.5 cm in 2008.

3.2. Longitudinal and transverse trends

Surface trends varied among subjects except for SL transverse trends which uniformly required a fourth degree polynomial for description (Table 3). Variation of FD along longitudinal axes was best described by quadratic trends in 7 out of 10 cases. Also in 7 out of 10 subjects accounting for transverse FD variation required a fourth degree polynomial. Longitudinal variation of SL was the most heterogeneous among subjects with instances of linear to quartic trends uncovered. When averaged across subjects, a quadratic trend was enough to describe longitudinal FD variation whereas quartic trends were required for the other three axis-trait combinations. Surface regression fitting explained considerable individual spatial variation (0.54–0.85 for FD; 0.33–0.77 for SL) and around 80% of the spatial variation of average trends across subjects for both traits (Table 3).

3.3. Variance components and repeatabilities

Repeatabilities between years, estimated from data pooled up to the animal \times year level, were 0.88 ± 0.08 (estimate \pm s.e., $P < 0.001$) for FD and 0.52 ± 0.24 ($P < 0.05$) for SL. The variance between sampling points within animals (FD: 1.81 ± 0.0132 ; SL: 0.328 ± 0.0006 ; estimated variance component \pm approximate s.e.) represented 80% (1.81/2.25) and 146% (0.328/0.225) of the variation between animals (FD: 2.25 ± 1.160 ; SL: 0.225 ± 0.0120) for FD and SL, respectively.

The between-animal component for FD varied from 1.430 to 6.358; its residual variation from 0.320 to 2.737. Corresponding ranges for SL were 0–1.389 and 0.117–1.040, respectively.

Repeatabilities calculated at individual sampling points varied between 0.47 ± 0.26 and 0.94 ± 0.04 for FD with an average (\pm s.d.) of 0.76 ± 0.10 . For SL repeatabilities averaged 0.36 ± 0.23 varying from 0.0 to 0.33 to 0.84 ± 0.10 and including 15% (19/128) of estimates of zero. Sampling point estimates of variance components and repeatabilities were applied for mapping those attributes over the fleece (see below).

3.4. Variograms and ranges

Spatial ranges, assessed in mapping units, were estimated for individual animals, for an 'average fleece' across individuals for each year, and for the between-animal and residual variance components and the repeatabilities (Table 4). Spatial ranges of individual animals were not affected by year of sampling ($P > 0.6$), varied between animals ($P < 0.04$), and were higher ($P < 0.03$) for FD than for SL (5.2 vs. 3.7; s.e.d. = 0.46). A trait \times year interaction was found not significant ($P > 0.9$). For the 'average fleeces' in each year, spatial ranges followed the trend of higher distances for FD (6.3 and 5.1) than for SL (3.8 and 3.7) and reasonably matched the averages of the spatial ranges estimated from individual animal data. Spatial ranges for

Table 2

Mean fibre diameters (μm) and staple lengths (cm) from 10 Corriedale ewes sampled in two consecutive years. Means are averages of 128 sampling points evenly distributed over the fleece.

Variable	Animal										s.e.d.	P	Year		s.e.d.	P
	A	B	C	D	E	F	G	H	I	J			2007	2008		
	Fibre diameter (μm)	30.4	31.5	28.3	30.6	29.1	31.8	32.8	28.4	31.6			30.3	0.55		
Staple length (cm)	9.1	8.5	9.3	9.1	8.6	9.5	8.5	9.2	7.9	8.9	0.38	0.050	9.3	8.4	0.17	0.001

Table 3

Polynomial degree of longitudinal and transverse gradients as assessed from surface fitting to individual data averaged over years (2007 and 2008) and to Overall, and corresponding R^2 for the reduced surface regression models.

Animal	Fibre diameter			Staple length		
	Longitudinal	Transverse	R^2	Longitudinal	Transverse	R^2
A	2	4	0.65	4	4	0.77
B	3	4	0.68	4	4	0.69
C	2	2	0.77	2	4	0.66
D	3	4	0.84	3	4	0.33
E	2	2	0.55	1	4	0.41
F	2	4	0.84	4	4	0.41
G	2	4	0.85	3	4	0.50
H	2	4	0.68	3	4	0.64
I	2	4	0.54	3	4	0.56
J	4	2	0.77	4	4	0.66
Overall	2	4	0.80	4	4	0.75

variance components and repeatabilities showed a similar trend (Table 4).

Spatial ranges for individual animals were found highly repeatable between years for both FD (0.87 ± 0.08 ; $P < 0.001$) and SL (0.72 ± 0.15 ; $P < 0.01$).

3.5. Mapping of phenotypes, variance components and repeatabilities

Two-dimensional images in Fig. 1 through 4 illustrate the variability revealed by the high-resolution sampling. Spatial patterns of data averaged across animals and years for both variables (upper left in Figs. 1 and 2) showed clear bilateral symmetry which was also present, though to a less

Table 4

Estimated spatial ranges (in mapping units; 1 mapping unit ~ 7 cm) for original and derived regionalized variables. For point values within animals, spatial range estimates are averaged (\pm SE) within year; the rest are single estimates.

Variable	Year	
	2007	2008
Point values within animals (\pm SE)		
Fibre diameter	5.1 ± 0.23	5.3 ± 0.23
Staple length	3.5 ± 0.22	3.8 ± 0.22
Point averages across animals		
Fibre diameter	6.3	5.1
Staple length	3.8	3.7
Between animals variance		
Fibre diameter		6.9
Staple length		4.1
Residual variance		
Fibre diameter		7.7
Staple length		3.6
Repeatability		
Fibre diameter		6.7
Staple length		4.2

noticeable degree, in the accompanying individual animal examples. Patterns of standard errors of prediction (lower left in Figs. 1 and 2, omitted for the rest of the maps) are shown as typical instances of the spatial distribution of the uncertainty associated with point predictions. As expected, prediction error was either nil or minimal for sampled points.

Lowest values for FD in the 'average fleece' (upper left, Fig. 1) clustered in the upper part of the front legs; highest values were observed in the area adjacent to the breech with noticeable twin extensions pointing from that area towards the rear flank. Twin spots of higher-than-average FD were also noticeable on or close to the shoulder blades. However, only the pattern of finer wool on the upper part of the front legs was consistently present in all individuals. Example F in Fig. 1 was subjectively selected as the closest one to the average pattern; conversely, examples G and J are mutually contrasting cases of departure from such a pattern.

The average spatial distribution of SL (upper left, Fig. 2) showed lowest values clustering into two distinct areas: the upper part of the extremities, and along the backline. Two areas with the longest staples ran from the base of the neck down to both sides of the chest (uppermost part of the maps). On both laterals, starting just behind the shoulder blades and extending towards the rear along the flanks, there were two other rear-pointing triangle-shaped areas of longer than average staples; these two sets of areas of long staples merged in some animals. There were individual exceptions to each of those patterns although shorter staples on the upper part of the extremities and longer ones around the area of the neck were fairly consistent findings across individuals. As in Fig. 1, individual H in Fig. 2 is an example of closeness to the average pattern across animals; the other two illustrate mutually contrasting departures from it. Example A is an extreme case of spread of the area

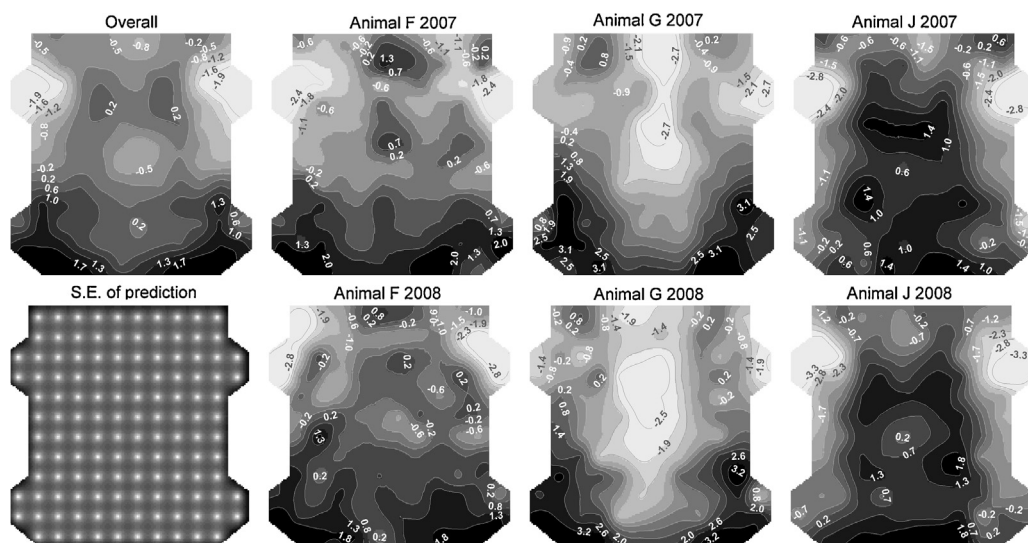


Fig. 1. Spatial distribution of fibre diameter (FD) over the fleece (head is up, tail is down). Maps are for FD deviations from overall means ($n = 128$) for sampling points averaged across individuals and years ($n = 20$, upper left) with corresponding s.e. of prediction (lower left) varying from 0.096 (lightest) to 0.414 (darkest), and for 3 individual animals (F, G and J from left to right) mapped in both 2007 (upper row) and 2008 (lower row).

of short staples along the back line, particularly on the lumbar area. Example F is an extreme case of staple length along the back line shorter than on the upper part of the extremities. In both Figs. 1 and 2 it is clearly noticeable that the individual spatial patterns recorded in 2007 (upper three rightmost maps) were essentially preserved in 2008 (lower three rightmost maps).

To illustrate the areal extent of sampling points potentially representative of mean values over the fleece, contour isolines in Fig. 3 were adjusted to show the slack around mean values for pre-set tolerances of either $1 \mu\text{m}$ (FD) or 0.5 cm (SL) above or below mean values across animals recorded in each year. Points inside those boundaries

would be within given tolerances. Points within given tolerance boundaries for both FD and SL would yield samples simultaneously representative of fleece averages for both variables. Fig. 3 also highlights the similarity of spatial patterns across years for both variables.

The variance between animals (upper and lower left in Fig. 4) increased more or less centripetally from a core area of minimum variance restricted to the dorsolumbar area in FD (upper left) and extending down the flanks in SL (lower left). Estimates fluctuated over the fleece with a reasonably bilateral, symmetric pattern for SL but noticeably shifted towards the right for FD. Residual variances and repeatabilities (center and right in Fig. 4) showed even more

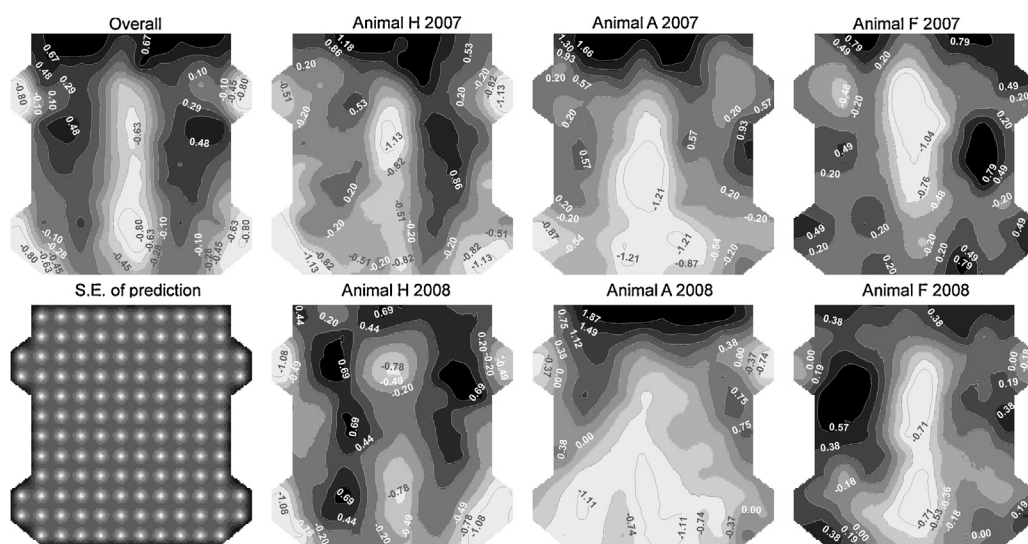


Fig. 2. Spatial distribution of staple length (SL) over the fleece (head is up, tail is down). Maps are for SL deviations from overall means ($n = 128$) for sampling points averaged across individuals and years ($n = 20$, upper left) with corresponding s.e. of prediction (lower left) varying from 0.069 (lightest) to 0.297 (darkest), and for 3 individual animals (H, A and F from left to right) mapped in both 2007 (upper row) and 2008 (lower row).

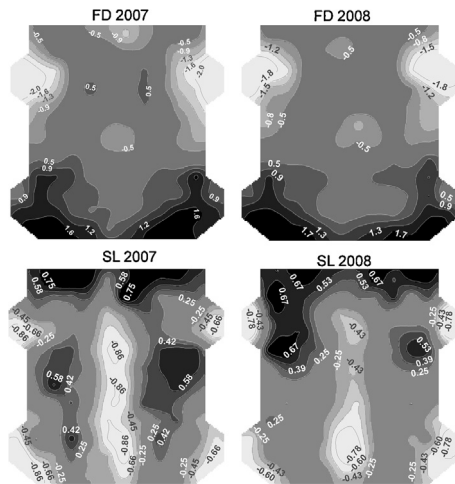


Fig. 3. Spatial distribution of fibre diameter (FD, upper row) and staple length (SL, lower row) over 'average' fleeces (head is up, tail is down) grown in two consecutive years (2007 left, 2008 right). Maps show deviations from overall means ($n = 128$) for points averaged across individuals within year ($n = 10$). Scales were adjusted to highlight pre-set tolerances of either $1 \mu\text{m}$ (FD) or 0.5 cm (SL) above or below overall means.

dissimilar spatial distributions for the two variables. Lowest estimates for the residual variance of FD (upper center) clustered in an elongated core area surrounded by higher values, shifted in the opposite direction than the between animals variance and noticeably asymmetric relative to the back line. The corresponding pattern for SL (lower center) also included a core area of low values increasing towards the borders but still retaining some bilateral symmetry.

Low and high repeatability estimates of FD at individual sampling points clustered into two distinct areas on the front right and left sides of the body, respectively (upper right, Fig. 4). Highest sampling point estimates of the repeatability of SL clustered in a well delimited area right behind the neck whereas lowest estimates were recorded around two merging spots on the left lateral of the body (lower right, Fig. 4).

4. Discussion

The 2-year field study was conducted over a period of increasingly dry weather that most probably explains the finer, shorter wool samples collected in 2008 than in 2007 (Table 2). Liveweights and body condition scores did not reveal any telling differences or trends that could suggest exposure to extreme drought-induced nutritional conditions. As no attempts were made to mitigate occasional forage shortages beyond usual grazing practices, most naturally occurring variability (excluding that induced by pregnancy and lactation) probably contributed towards the variance between animals and between years. In spite of that contribution and the limited number of animals sampled, the analyses detected differences between animals and between years for both FD and SL. The evenness and intensity of sampling within individuals, allowing for precise estimations of individual means in each year, and the considerable genetic and permanent environmental components for both traits (Atkins, 1997; Safari et al., 2005) explain the detection of animal and year effects for both traits in spite of the limited number of animals sampled.

Absence of sampling side effects was much anticipated considering the highly conserved bilateral plan of

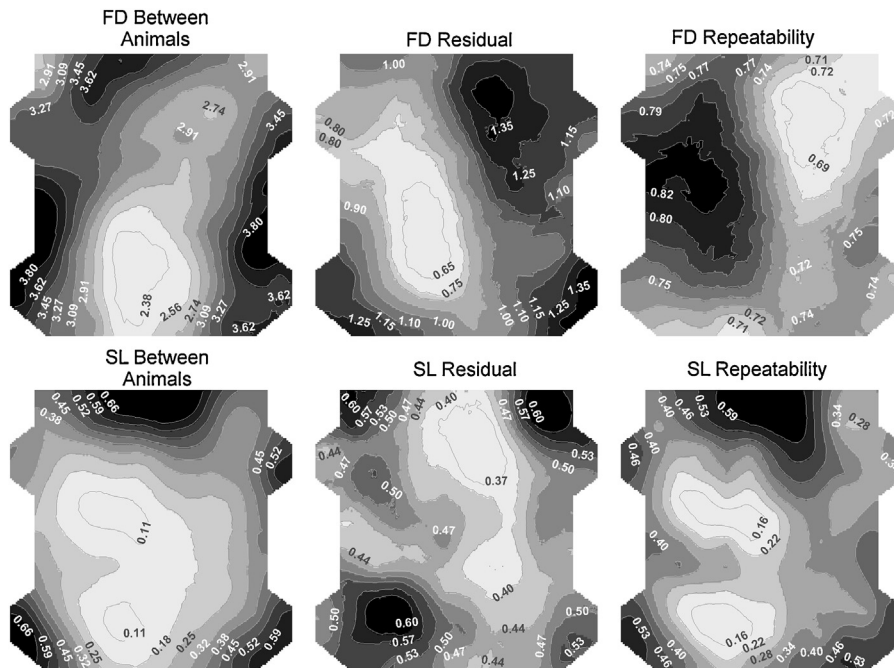


Fig. 4. Spatial distributions of fibre diameter (FD, upper row) and staple length (SL, lower row) between animal (left) and residual (center) variance components, and of repeatability between years (right).

vertebrates. However, as bilateral symmetry would be a key, readily identifiable feature of any spatial pattern, testing for side effects was warranted.

Surface trend analyses revealed heterogeneity among subjects, and more complex individual and average trends than previously described for both traits. With at least a second degree polynomial required to account for longitudinal variation, the concept of antero-posterior gradients suggested for both traits in classical (e.g. Turner, 1956) and current literature (e.g. Fish et al., 2002) should be questioned. 'Gradient' implies monotonic change and our analyses showed that variation changed direction at least once along the axis. Transverse variation on each side of the body fitted the expected quadratic trend for SL but did not support the back-to-belly gradient pattern expected for FD. Thus, variation along both axes exceeds linearity for the two traits and it is markedly regionalized as discussed below.

The estimated repeatability of pooled FD data was high relative to single-sample estimates in the literature (e.g. Young et al., 1960; Turner and Young, 1969; Atkins, 1997) that set a high end boundary of about 0.6–0.7. The repeatability of SL was similar to that reported for Merino ewes and rams (Young et al., 1960). Repeatability can be expected to be higher for an average ($n = 128$ in our case) than for single samples due to a reduction in variance of temporary environmental origin; that probably explains the relatively high FD estimate. However, apart from environmental factors systemically affecting both traits (e.g. nutrition, photoperiod), SL is also prone to be affected by other factors (e.g. rubbing against abrasive surfaces, UV radiation exposure, preferential particulate deposition on the back region, wool damage from ectoparasites) capable of inducing *regionalized* spatial variation over a fleece. This source of variation may have offset the benefit of dense sampling thus explaining the comparatively lower repeatability estimate for SL. Averages of repeatability estimates at sampling points over the fleece were more similar between variables (FD: 0.47, SL: 0.36) than point estimates, but still much more variable (including a number of zero estimates) for SL than for FD, a result to be expected from regionalized environmental effects inflating SL variation over the fleece.

The variation in FD between sampling points within animals in our Corriedale dataset was 80% of the estimated between-animal component. This figure is higher than the 50% contribution estimated in Merinos (Dunlop and McMahon, 1974), and suggests that classing some fleece areas to different clip lines according to FD could be justified depending on cost of labor, price premiums for finer wools, and consistency of spatial patterns across animals. We are not aware of comparable figures for SL. A much higher estimate of relative regional variation for SL than for FD is also in line with regionalized environmental effects inflating SL spatial variation, as discussed above.

The pattern emerging from the variogram analyses strongly suggests that most of the spatial covariances studied have systematically shorter spatial ranges for SL than for FD (Table 3). This was unexpected considering the conditional dependence of both traits on local follicle types and densities (Ryder and Stephenson, 1968). Wool follicle populations are established in intrauterine life (Hardy and

Lyne, 1956) apparently through a mechanism involving a latent pre-pattern of initiation sites (Moore et al., 1998; Rogers, 2006). Mature follicles would then produce fibre under systemic biochemical regulation within the constraints imposed by their types and densities (Galbraith, 2010). Shorter ranges for SL than for FD could be intrinsic to prenatal patterns, consequence of spatially differential physiological regulation of fibre growth in diameter and length, or proneness of SL to environmental factors with regionalized effects, among other possibilities. Sorting out possible contributions of those sources is beyond reach of our data set. Nonetheless, the high repeatability of the spatial ranges suggests that not only point estimates of FD and SL are repeatable, as was already known, but also some of the properties of the spatial patterns, and possibly the spatial patterns themselves. This latter issue is currently under investigation (Rodríguez Iglesias et al., unpublished results). Associating spatial ranges to wool traits is also relevant for the design of experiments, e.g. when multiple sampling points are used to estimate phenotypic or genetic parameters in fibre-producing species (e.g. Taddeo et al., 2000 and McGregor and Butler, 2008 in mohair goats).

Expanding the surface trend analyses, maps of FD and SL spatial variation showed complex non-linear patterns with zones of steep transitions at both ends of the distribution of both variables (i.e. finest and coarsest, longest and shortest); the degree and extent of non-linearity varied across individuals. Other key findings exposed were a richness of patterns that clearly exceeded what polynomial surfaces could describe, marked heterogeneity among individuals also including contrasting features, consistency of sequential patterns within the time scale of the study, and occurrence of a limited set of features shared by most individuals. The spatial heterogeneity found among individuals may explain why certain oriented trends (e.g. mid-side line towards backline and belly trend in SL) were detected in some of the classical studies but not in others (see Turner, 1956). Apart from the richer-than-expected longitudinal and transverse variation found, the high-resolution mapping revealed features missed in classical studies. Examples are the twin projections of coarser wool extending from breech to rear flank (noticeable to the naked eye in some animals), the twin spots of higher-than-average FD up from the shoulder blades, and the longer-than-average staples around the base of the neck and down to the chest. This latter trait, possibly a relic of the neck ruff of primitive sheep (Maijala, 1997) still present in some contemporary breeds (e.g. Djallonké), was probably missed in classical studies because the area itself was rarely sampled.

Focusing on average patterns across individuals, it seems that fleece areas simultaneously representative of mean values for both FD and SL superimpose to a very limited extent (Fig. 3). Widening the comparison to individual patterns (Figs. 1 and 2) suggests that the use of single, universal sampling sites across traits, and possibly across individuals within traits, should be questioned. The temporally consistent spatial variability revealed in the study casts doubts on the significance of both 'average' patterns across individuals and over-the-fleece means. Our preliminary 'average' patterns could well be mosaic constructs of alternative, essentially different spatial patterns

rather than truly quantitative syntheses of some continuous underlying variation with ontogenetic relevance. On the other hand, and for any raw wool trait, closeness to fleece average (Fig. 3) could be in conflict with variation between animals (Fig. 4). Both attributes may contribute towards selection response in breeding plans. Identifying an area representative of the whole fleece is critical to control measurement error when breeding for over-the-fleece averages assessed in a representative area. On the other hand, basing selection on measurements taken from areas of high between-animal variability could be a superior breeding strategy because it will produce larger selection differentials and, consequently, larger selection responses (depending on the variability of relevant parameters over the fleece; see e.g. McGregor and Butler, 2008 for an example of variation of heritability over goat fleeces). In other words, breeding to produce finer wool in coarse areas of the fleece, for example, could be a better strategy than selecting individuals on the basis of FD determined in a representative area. In this sense, closeness to fleece average and maximization of selection differentials may be in conflict if those two attributes reach maxima in non-overlapping areas of the fleece.

In spite of those caveats, findings do highlight the scope available for improving the precision of phenotype assessment. Other things being equal, more precise phenotyping would accelerate genetic change through increased heritabilities. For specialty wools in particular (e.g. superfine and ultrafine Merinos) precise phenotyping may also involve determining fleece areas with desired levels of traits or combinations thereof. Depending on their genetic bases, the inclusion of fleece patterns as selection criteria is another possibility. Breeding for fleeces with limited regionalized variation may contribute towards fibre uniformity, a key requirement for wool processing. It remains to be determined if those genotypes exhibit defined spatial patterns as seems to be the case for Corriedales in this study.

Departures from bilateral symmetry, like those detected for variance components and repeatabilities were unexpected. Given the absence of side effects in the original data, the cause of the asymmetry remains obscure. Residual variances in our design have temporal origin. They represent variation within animals between years after adjustment for the effects of year of sampling thus implying differential variation between years in different parts of the body. The simplest explanation for such a pattern would be the occurrence of waves of wool growth variation diffusing over follicle populations with circannual or lesser rhythmicity. The well-known hair follicle cycle (see Galbraith, 2010 for a recent review), a highly conserved feature in mammals, is a natural candidate for explaining the finding. However, we are not aware of any wool follicle cycling study exploring variability over body regions. Although the asymmetry of the patterns is noticeable (particularly for FD), a major caveat is the possibility that the spatial distributions of variances may just reflect randomness. A controlled experiment would be required to explore this issue.

The rich spatial heterogeneity, contrasting features, and consistency over time revealed in the study suggests potential for moving from a single-sample 1D paradigm into

a two-dimensional area of research with significance for experimental design, sampling and breeding. Identifying genetic and prenatal permanent environmental sources of variation (i.e. fetal programming) affecting spatial distributions would be critical for valuing the role of fleece spatial patterns in wool breeding programs.

5. Conclusions

Variation of FD and SL over sheep fleeces was characterized by non-linear longitudinal and transverse trends, a limited number of spatial features common to most subjects, and marked and temporally stable spatial heterogeneity between animals. Spatial patterns and properties (e.g. spatial range, over-the-fleece repeatabilities) differed between FD and SL possibly reflecting both prenatally defined follicle population patterns and regionalized effects of environmental variables preferentially affecting SL. If confirmed, bilaterally asymmetric spatial patterns of variance components could suggest the occurrence of waves of wool growth cycling over the fleece.

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