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Short communication

Spatial structure of skin follicles in Suri and Huacaya alpacas

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ABSTRACT

The present study aimed at characterizing the type and arrangement of skin follicles of Suri and Huacaya alpaca. Samples (11 Suri and 10 Huacaya) were collected by punch skin biopsy from the midside of alpaca and processed for histological study. Each biopsy was examined using projection microscope. Follicular groups were identified and the position of each secondary and primary follicle was recorded. The ratio of secondary to primary follicles (S/P ratio) was compared between breeds using the Wilcoxon test. The spatial structure of the follicles was analyzed with Ripley's K function and the L function. To detect deviations from Complete Spatial Randomness at different spatial scales simulated confidence envelopes were calculated. The S/P ratio did not differ between Huacaya (7.1 ± 0.52) and Suri (7.21 ± 0.62). There is evidence of statistically significant spatial structure of the follicles in both breeds at small spatial scales. However, at a higher spatial scale, the proportion of samples with a clustered spatial structure of follicles was significantly higher in Huacaya. The study of skin follicles spatial pattern opens up new possibilities for improving knowledge of the potential role of skin follicle in alpaca fibre production.

1. Introduction

Alpaca (*Vicugna pacos*) is a species most famous for its good quality fibre production. Its homogeneously fine, long and soft fleeces make it highly demanded by the textile industry (Antonini et al., 2004). Two types of fleece, Huacaya and Suri, are described in alpaca. Huacaya represents the most common phenotype, which involves a single coated fleece characterized by compact, soft and highly crimped fibres with blunt-tipped locks; all these features closely resemble those of Merino sheep. Huacaya is often reported as the wild type, whereas Suri is thought to be derived from Huacaya through gene mutation, possibly with reduction of fitness (Presciuttini et al., 2010). Suri has straight, less-crimped, lustrous silky fibres, which are very similar to mohair from Angora goat, but not as bright (Renieri et al., 2009).

Each fibre is produced from an individual follicle. Two distinct types of follicles, primary and secondary, are formed within the dermis during gestation. These follicles are determined in order of initiation and distinguished histologically by their associated accessory structures (Hocking et al., 1996). Primary and secondary follicles differ in the presence of a sweat gland and erector pili muscle in the former type. Skin follicular structure represents one of the most important characters for the selection of improved fibre production (Charry, 1998). Skin follicle productive potential is measured via the ratio of secondary to primary follicles (S/P) .This ratio has been extensively used to compare sheep breeds (Carter and Clarke, 1957; Barton et al., 2001; Hynd et al., 2009; Ferguson et al., 2012) as well as Suri and Huacaya alpacas (Calle-Escobar, 1984; Ferguson et al., 2000; Antonini et al., 2001, 2004; Antonini, 2010; McGregor, 2006).

In addition to the S/P ratio, the spatial distribution of follicles could be used to characterize breeds. Point pattern analysis may help to study skin follicular structures and find parameters suitable for classification and identification (Illian et al., 2007). One of the main goals of this paper is to understand the characteristics of follicular spatial distribution of two types of fleece in alpaca: Hua-

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Table 1
Compared parameters of skin follicular pattern between Suri and Huacaya and *p*-value of the statistical comparison (Wilcoxon test for S/P ratio and Chi square to test homogeneity of proportions). S/P: secondary to primary follicles ratio, PF: primary follicles, FG: follicular group.

Compared Parameters	SURI (n = 11)	HUACAYA (n = 10)	p-value
Samples with 3 as a maximum number of PF per FG	9.1%	60%	0.02
Samples with 2 as a maximum number of PF per FG	54.5%	30%	0.39
Samples with 1 as a maximum number of PF per FG	36.4%	10%	0.31
Expected S/P ratio ± standard error	7.21 ± 0.62	7.10 ± 0.52	0.94
Samples with a non random pattern of follicles at distances <150 μm	100%	100%	–
Samples with a non random pattern of follicles at distances >150 μm	36%	90%	0.02

caya and Suri. To analyze the follicular characteristics, besides the study of the S/P ratio, we also studied the spatial pattern of primary and secondary follicles and compared that pattern between Suri and Huacaya alpaca breeds. The results of the present study may contribute to the understanding of the mechanisms of fibre distribution pattern and provide information for genetic improvement programmes focused on fine fibre-producing animals.

2. Materials and methods

2.1. Samples

Experimental field work was performed at the experimental station of INIA (the Peruvian National Institute for Agronomic Innovation) located in Santa Lucia District, Puno Department, Quimsachata, Peru. The Station covers an area of 6282 ha and is at approximately 4400 m a.s.l. The typical climate in the study area is that of the high Andean Puna ecosystem. The experimental station is focused on alpaca and lama breeding, conservation and genetic improvement. The animals used in the present study were chosen from the pedigree registry of the Station to ensure they were effectively unrelated.

Skin biopsies were taken with a suitable punch with 0.8 cm of diameter, from the right mid-side of each animal, i.e. approximately above the 10th rib, about halfway down the body. This body area has been found to be more representative of fleece characters than other fleece regions. Sampling was performed in 1998 and involved young animals (Antonini et al., 2004). Skin samples were fixed in Bouin solution and stored in 80% alcohol for shipment to Italy.

2.2. Laboratory analysis

Stored skin samples were dehydrated in a graded ethanol series and embedded in paraffin. Transverse sections of 7 μm were cut with a rotary microtome and stained using the Sacpic staining procedure, modified by Nixon (Nixon, 1993). Each section was examined under an Olympus TH4-200 projection microscope (10×) and digitalized with AnaLysis® software. In order to obtain the best-quality and largest digital images, 10 samples from Huacaya and 11 from Suri were selected for histological sections. The level immediately below the sebaceous gland was defined as the most suitable depth containing with the maximum number of detectable primary follicles for microscope observations, thereby increasing the possibility of identifying the follicular groups (McCloghry et al., 1997a; Antonini, 2010; García and Pezo, 2012).

The results were adjusted for sample shrinkage that occurs when fixing the skin biopsy in relation to the diameter of the trephine (McCloghry et al., 1997b). A correction factor (area of mounted skin section/area of the trephine) was calculated from this measurement (McCloghry et al., 1997b; Steinhagen and Bredenhann, 1987). An adjustment factor of 53.3% was applied to alpaca biopsies In each analyzed sample we determined follicular groups and identified the primary and secondary follicles in each group. For each follicle its spatial coordinates x and y were recorded.

2.3. Data analysis

Follicular groups (FG) were characterized in all samples. The maximum number of primary follicles (PF) per FG was recorded for each sample and the proportion of samples with each maximum was compared between Suri and Huacaya with a Chi square to test homogeneity of proportions (Marascuilo, 1977). The ratio of secondary to primary follicles (S/P) was also calculated for each sample. The mean S/P ratio was compared between Huacaya and Suri using the Wilcoxon test (Wilcoxon, 1945), a non-parametric statistical test, with Info-Stat software (Di Rienzo et al., 2013).

To study the spatial pattern of the follicles, we calculated Ripley's K-function. The simplest use of Ripley's function is to test Complete Spatial Randomness (CSR), i.e. test whether the observed events are consistent with a homogeneous Poisson process. In exploratory analyses, the K estimate is a useful statistic that summarizes aspects of inter-point "dependence" and "clustering". For inferential purposes, the estimate of K is usually compared to the true value of K for a completely random point process (Poisson). Deviations between the empirical and theoretical K curves may suggest spatial clustering or spatial regularity. As the estimation of K is hampered by edge effects arising from the unobservability of points of the random pattern outside the studied window, an edge correction is needed to reduce bias (Baddeley et al., 2015). The correction we implemented is the border method or "reduced sample" estimator (Ripley, 1981). A more powerful test is obtained if the variance is stabilised, by using the L function in place of K. Therefore we calculated both the K and L functions and simulated confidence envelopes under CSR for both of them (Cressie, 1991; Diggle, 2003; Ripley, 1977, 1981). The confidence envelope is constructed by distributing points randomly in the study sample and calculating K or L for that distribution. Each random distribution of the points is called a "permutation". After distributing the points several times, the upper and lower envelopes of the simulated functions are shown in a plot. The plots obtained show the functions for a continuous interval of distances and the envelopes obtained by simulation. If the calculated K or L functions deviate from the simulated envelopes, the null hypothesis of CSR is rejected. For each sample we first recorded if CSR was rejected at any distance. Then, to characterize the spatial patterns, we divided the distance interval in two (<150 and >150 μm) and recorded for each sample if CSR could be rejected in each of them. The proportion of samples with spatial structure of skin follicles was compared between breeds with a Chi square to test homogeneity of proportions (Marascuilo, 1977). Spatial analyses were performed in the R environment (R Development Core Team, 2011) using Spatstat library (Baddeley and Turner, 2005).

3. Results

3.1. Follicular groups

In total, 887 follicular groups were found, 471 in Suri and 416 in Huacaya. The average number of follicular groups in Suri samples was 42.8, ranging from 19 to 58, whereas in Huacaya the number of

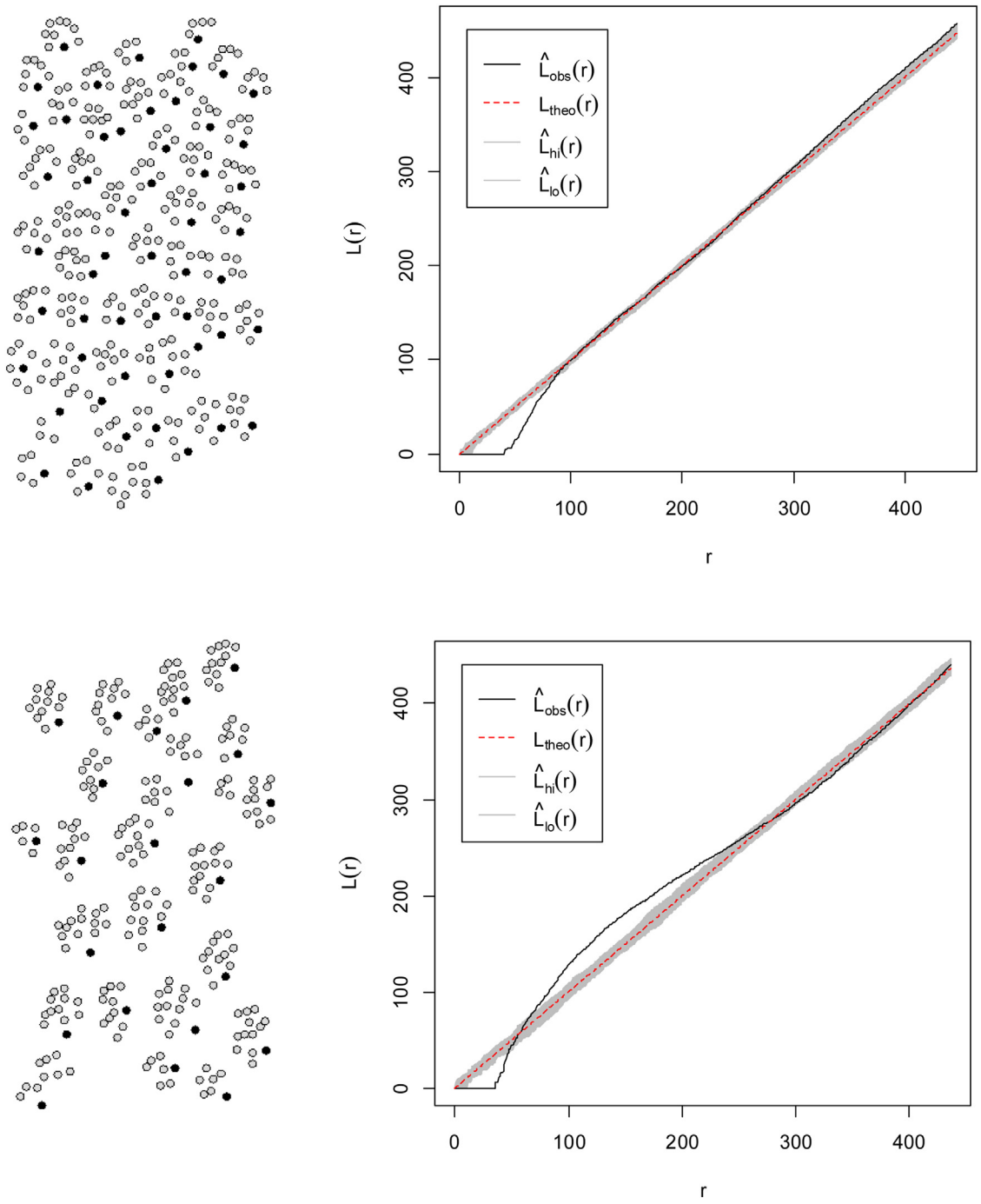

Fig. 1. Spatial structure of skin follicles in a Suri (above) and Huacaya (below) sample. Left: schematic point patterns of skin follicles, black and grey circles correspond to primary and secondary follicles, respectively. Right: L-function versus distance in μm (r). The black solid line corresponds to the observed L function (L_{obs}), the dotted line to the theoretical L-function (L_{theo}) and the grey area to the envelope obtained through simulation.

follicular groups per sample varied from 23 to 56, with an average of 41.6. Follicular groups were formed by one, two or three primary follicles and a variable number of secondary follicles (ranging from 0 to 33). Most follicular groups (851) had only one primary follicle, 26 FG had two PF and 10 FG had three PF. From the 11 Suri samples, only one (9.1%) had FG with three PF, whereas in Huacaya six samples (60%) showed FG with three PF. This difference was statistically significant ($p = 0.02$), indicating that the probability of finding follicular groups with three PF in Huacaya is higher (Table 1). On the contrary, the difference in the proportion of FG with two PF and with one PF was not statistically significant ($p = 0.39$ and $p = 0.31$, respectively) (Table 1).

Regarding the relation between secondary and primary follicles in the sample, which is used to measure productive potential, the mean S/P follicular ratio ± the standard error was 7.1 ± 0.52 for Huacaya samples and 7.21 ± 0.62 for Suri samples. The Wilcoxon test, used to compare the S/P ratio between Suri and Huacaya, showed that the there were no significant differences when all follicular groups are considered ($p = 0.94$).

3.2. Follicular spatial pattern

The analysis of Ripley's K functions and their confidence envelopes showed that in all samples the null hypothesis of CSR is rejected, indicating that there is evidence of statistically significant spatial structure of the follicles in both breeds. The empirical K functions calculated in each sample deviated from the simulated envelopes in all cases, and therefore the null hypothesis of a homogeneous Poisson process was rejected in every sample of both Suri and Huacaya. These results show that there is inter dependence among the location of the follicles and that they are not randomly distributed in the skin. As in both breeds all samples showed a significant spatial structure of the skin follicles, no comparison was performed between Suri and Huacaya regarding the proportion of samples with spatial structure.

A more detailed characterization of the spatial structure was obtained analyzing the L function (Fig. 1 and Table 1). With this function, we analyzed the spatial structure of follicles at two spatial scales, at distances lower and higher than 150 μm. All samples showed lower values of the L function than expected by a random process at distances lower than 150 μm, indicating a uniform spatial pattern at a small spatial scale. Therefore, at this spatial scale, skin follicles are evenly distributed, maximizing the space among them. As in all samples a uniform spatial structure of skin follicles was found at the smaller spatial scale in both breeds, no comparison was made between Suri and Huacaya.

On the contrary, at higher distances, the L function was higher than the simulated envelope in 4 Suri samples and in 9 Huacaya samples, indicating a statistical significant clustering of follicles at distances higher than 150 μm in both breeds. As opposed to the uniform distribution found at lower distances, in the clustered distribution, follicles are found in patches, minimizing the distance among neighbors. In this case, the proportion of samples with a clustered distribution of follicles at distances higher than 150 μm was compared between breeds. The proportion of samples with a spatial structure at this spatial scale was significantly higher in Huacaya ($p = 0.02$).

4. Discussion

Similarly to other reports, we found that follicular groups were formed by one or more primary follicles and a variable number of secondary follicles (Calle-Escobar, 1984; McGregor, 1995; Badajoz et al., 2009; Ferguson et al., 2012). The S/P ratio has been extensively used to compare sheep breeds (Carter and Clarke, 1957) and its relationship with the quality and quantity of wool are important to improve fibre production (Williams and Winston, 1987). Previous studies of Australian alpacas indicate a range of secondary to primary follicle ratios (S/P ratio) of 4:1 to 9:1 (McGregor, 1995). Antonini et al. (2004) reported mean S/P ratios ranging from 6.9 to 9.4, with a mature ratio being obtained at ~4 months of age. The S/P ratios found in our study fall within the limits of the reported intervals. However, Ferguson et al. (2012) reported that there were statistical differences between the average S/P ratios of the two breeds involved in our study. He reported that in 12 Huacaya alpacas and 24 Suri alpacas the mean ± S.E. ratio of secondary to primary follicles (S/P ratio) was 5.1 ± 0.5 for Huacaya and 7.3 ± 0.2 for Suri alpacas. The difference between the S/P ratios found in our study are not consistent with this previous study; indeed, the results of Wilcoxon test showed no significant statistical differences between Huacaya (7.1 ± 0.5) and Suri (7.2 ± 0.6). Even with a higher standard error, associated to the sample size used for Suri alpacas, the average S/P ratio in our study was similar to the previously reported. Meanwhile differences in S/P ratio were higher for Huacaya. Such differences could be due to several factors, including methodological issues such as the number of follicles counted per sample. Ferguson et al. (2012) counted 100 follicles for each sample and in this study, we measured approximately 339 follicles per sample. Nevertheless, we did find that in Huacaya it is more probable to find follicular groups with 3 primary follicles. In Suri alpaca, Badajoz et al. (2009) observed groups of three primary follicles, one large and two smaller ones on each side. We also found follicular groups with 3 primary follicles in Suri samples, but less frequently than in Huacaya.

The relationship between skin follicle characteristics and fibre characteristics has been established in sheep (Rendel and Nay, 1978; Williams and Winston, 1987; Maddocks and Jackson, 1988) and total follicle density (TDF) has been found to be negatively related to mean follicle density (MFD) (Moore et al., 1996). In Suri and Huacaya Alpacas, this relationship was also found, and given the large number of secondary follicles in alpacas, secondary follicle density was found to be the main determinant of TFD and MFD (Ferguson et al., 2012). As in sheep, the number, type and arrangement of follicles in the skin of alpacas can explain some of the differences in fibre characteristics. The difference in the skin of Suri and Huacaya breeds accounts for the variation in growth characteristics of the hair fibres. High crimp levels increase inter-fibre cohesion after spinning. As a result, fibres can be spun into fine yarns of great length for a given weight of wool, with the consequent increased market values. Conversely, the absence of crimp, such as in the Suri type, results in a fibre that has a high sheen and felts fast (Plowman et al., 2009). In this study we did not measure fibre characteristics, but did find differences in the spatial structure of skin follicles between Alpacas breeds. It would be interesting to simultaneously analyze fibre characteristics and spatial arrangement of skin follicles to test if spatial structure explains productive fibre traits. Our results showed that follicles have a spatial structure in both breeds. At small spatial scales, which can be interpreted as a within follicular group scale, follicles have a regular or uniform structure. This means that there is repulsion among follicles regarding their spatial position. At higher spatial scales, a clustered spatial structure was found in many samples, particularly in Huacaya, indicating a stronger spatial structure in this breed. Even with a small sample size, a higher level of clustering in the skin follicles could be related to a finer crimp in Huacaya. This finding may not be representative of the breeds, and a larger sample size should be studied. However, we found a spatial structure of skin follicles in all samples, indicating that in alpacas, follicles are not randomly distributed.

Further experimentation and enhanced knowledge will facilitate the inclusion of molecular techniques for targeted selection in order to improve the pattern of skin follicles. This has important applications for accurately understanding the underlying factors controlling follicle distribution and determining the relationship with fibre quality traits.

5. Conclusion

This study revealed that skin follicles are not randomly distributed in two Alpaca breeds and a higher level of clustering was found in Huacaya samples. The difference between Suri and Huacaya types of fleece could involve differences in the spatial structure of skin follicles.

Conflict of interest

The authors declared there is no conflict of interests.

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