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Short communication

Spatial structure of skin follicles in Suri and Huacaya alpacas

Gabriela Molina[a,b,*], Ingrid Teich[a,c], Marco Antonini[d], Carlo Renieri[e], Antonietta La Terza[e], Monica Balzarini[a,c]

[a] Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba Ing Agr, Felix Aldo Marrone 746 Ciudad Universitaria, 5000, Córdoba, Argentina
[b] Facultad de Ciencias Agropecuarias, Universidad Católica de Córdoba Avenida Armada Argentina 3555, X5016DHK Córdoba, Argentina
[c] CONICET. Argentina
[d] ENEA C.R. Casaccia UTAGRI Inn, S.M. di Galeria, 00060 Roma, Italy
[e] School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III da Varano, 62032, Camerino (MC), Italy

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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-

* Corresponding author at: Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba Ing Agr, Felix Aldo Marrone 746 Ciudad Universitaria, 5000, Córdoba, Argentina.
E-mail addresses: gabmolin@agro.unc.edu.ar, gabmolin@gmail.com (G. Molina), ingridteich@gmail.com (I. Teich), marco.antonini@enea.it (M. Antonini), carlo.renieri@unicam.it (C. Renieri), antonietta.laterza@unicam.it (A. La Terza), mbalzari@agro.unc.edu.ar (M. Balzarini).

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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