SCIENTIFIC NOTE

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Genetic differentiation between 'Araucana' creole and 'Hampshire Down' sheeps in Chile

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Genetic diversity studies in domestic animals allow evaluating genetic variation within and among breeds mainly for conservation purposes. In Chile exist isolated recovery programs, conservation and characterization of animal genetic resources, a consequence of which the vast majority of them have not been characterized, poorly used, and some of them have become extinct. The aim of this research was to determine genetic diversity and relationship between 'Araucana' creole and 'Hampshire Down' sheeps based on microsatellite markers; sheep breeds with similar phenotypic characteristics, raised in the south of Chile. A total of 64 'Araucana' sheep ('Araucana' from Freire, AF: 27, 'Araucana' from Padre Las Casas, AP: 10, 'Araucana' from Chol Chol, AC: 15, 'Araucana' from Villarrica, AV: 12) and 43 'Hampshire Down' sheep ('Hampshire' from Marchigue, HM: 18, 'Hampshire' from Valdivia, HV: 11, 'Hampshire' from San José, HS: 14) were analyzed using 17 microsatellite markers for determine the genetic diversity and relationship between breeds. A total of 284 alleles were observed with average polymorphic information content equal to 0.76, showing that the microsatellites panel used was highly informative. Estimated heterozygosity ranged from 0.73 in 'Hampshire Down' to 0.85 in 'Araucana'. The low inbreeding or endogamy coefficient (F_{IS}, 0.022) and total inbreeding estimate (F_{IT}, 0.070) indicated low level of inbreeding within and among breeds. The phylogenetic tree showed a separation between HS and HV, and the other sheep populations. The results indicated high genetic variability, low inbreeding, and low genetic differentiation, except for HV and HS, and were in according with geographical location and breeding practices.

Key words: Breed assignment, creole sheep, genetic variability, microsatellites.

INTRODUCTION

Local or creole breeds are an important genetic resource for the small-scale agriculture in many countries. However, the genetic characterization of this population is scarce and is necessary to aid in the developing of strategies for conservation and utilization of these breeds (Kunene et al., 2009; Soma et al., 2012).

With the arrival of the Spaniards to the American continent in the 16th century, several domestic animal species were introduced, including domestic sheep (*Ovis aries* L.) In Chile, there were only Iberian sheep until the 19th century. In 1837 the first import of 'Merino' sheep arrived from Australia and in 1840 'Dishley', 'Southdown', 'Hampshire Down', 'Leicester', 'Rambouillet' and 'Negretti' breeds came from England (Correa, 1938).

Currently there are 38 breeds in Chile, the majority introduced in the last 15 yr; nevertheless, there are domestic creole populations derived from crossbreed or adaptations of the Spanish flock during the Conquest, as is the case of the 'Chilota' and the 'Araucana' creole (De la Barra et al., 2011).

The 'Araucana' sheep is a creole zoogenetic resource of Chile, which distributes primarily into the La Araucanía Region (38°54' S, 72°40' W), managed by small producers, majority of the Mapuche ethnic group. It is probable that its origin comes from Iberian and British sheep flock (Mujica, 2009), maintaining external appearance these British sheep.

This creole sheep is adapted to the environmental conditions of southern Chile and has great maternal abilities, high prolificacy and a shorter anestrus compared to others breeds (Sepúlveda, 1999; Quiñones et al., 2012). It has a adult body weight of 57.8 ± 8.1 kg, a height at withers of 58.8 ± 2.9 cm and a height to the rump of 59.8 ± 3.0 cm (Bravo and Sepúlveda, 2010) and commercial yield of the carcass is greater than 50% with a 59% of the regional composition pieces being the first category (Bravo et al., 2010). The farmers greatly value this creole sheep for his rusticity, prolificacy, resistance to foot diseases and lambs with early maturing for meat production. One of the most important characteristics of

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the creole sheep is outstanding food conversion which enables it to survive well in marginal grass conditions.

The aim of this research was to determine the genetic diversity and relationship between the 'Araucana' creole and the 'Hampshire Down' sheep based on microsatellite markers. This investigation will provide the first record of genetic diversity of the 'Araucana' creole population, contributing to the characterization of this sheep, which is a vital zoogenetic resource for biodiversity and part of the Chilean cultural property.

MATERIALS AND METHODS

Sampling and molecular analysis

A total of 107 blood samples were collected from jugular vein into EDTA containing tubes (Table 1), 64 of which belonging to the three of 'Hampshire Down' flocks and 43 belonging to four 'Araucana' flocks located in southern Chile (34°10'02" S, 70°43'37" W and 39°48'30" S, 73°14'30" W). All samples were collected from randomly chosen unrelated individuals in the seven flocks, in order to get samples as representative as possible.

Genomic DNA was extracted using the Blood DNA Miniprep Kit (Axygen Biosciences, Union City, California, USA). A total of 17 microsatellite markers (Table 2) based on the guidelines of International Society for Animal Genetics (ISAG) and the Food Agricultural Organization's, Domestic Animal Diversity Information System (FAO DAD-IS) program was used to generate allelic data. The markers were: OarFCB11, DS5S2, McM527, OarAE129, INRA063, MAF65, SPS115, MAF214, OarFCB304, HSC, INRA023, INRA005, OarCP49, BM1258, INRA132, CSSM31, and SPS113. Forward primers were labeled at the 5` region with fluorophores (FAM, VIC, NED, and PET). Four multiplex were amplified by polymerase chain reaction (PCR). The primer sequences, size ranges, multiplex information and PCR thermal cycles can be found on the ISAG (http://www.isag.us/Docs/ISAG2006_AppliedGeneticsSheepGoats.pdf) and FAO (2004). The size amplified by each primer set was determined via capillary electrophoresis in an automatic ABI Prism 3130 DNA Sequencer (Applied Biosystems, Foster City, California, USA). The results of the capillary electrophoresis were read using the GeneMapper software, version 4.0 (Applied Biosystems, Foster City, California, USA).

Table 1. Description of the flocks analyzed.

Flock	Breed	Location	Flock size	Number of sheep analyzed
AF	Araucana	Freire	120	27
AP	Araucana	Padre Las Casas	63	10
AC	Araucana	Chol Chol	47	15
AV	Araucana	Villarrica	88	12
HM	Hampshire Down	Marchihue	82	18
HV	Hampshire Down	Valdivia	108	11
HS	Hampshire Down	San José	152	14

AF: 'Araucana' from Freire; AP: 'Araucana' from Padre Las Casas; AC: 'Araucana' from Chol Chol; AV: 'Araucana' from Villarrica; HV: 'Hampshire Down' from Valdivia; HS: 'Hampshire Down' from San José; HM: 'Hampshire Down' from Marchihue.

Statistical analysis

F-stat software was used to determine population variables: number of alleles per locus and population, allelic frequency per population, genetic diversity and F_{IS} per locus and population (Goudet, 2002). The degree of differentiation among studied populations was determined using Nei's statistics for population and Wright's fixation index (F_{ST}). Hardy-Weinberg equilibrium, the observed heterozygosity and expected heterozygosity with its standard errors, were calculated for each population and loci using the Genetix software v. 4.02 (Belkhir et al., 2000). Polymorphism information content (PIC) per locus was determined by means of the algorithm described by Bolstein et al. (1980). The PHYLIP software v. 3.62 (Felsenstein, 2004) was used to construct the phylogenetic tree based on Nei's genetic distances using

Table 2. Microsatellite makers, multiplex reaction (Plex), associated chromosome (Chr.), size range per locus (SR), Nei's F-statistics for each microsatellite on all the populations evaluated and polymorphism information content (PIC) per locus.

Locus	Plex	Chr.	SR (bp)	H_o	H_e	H_S	H_T	G_{ST}	G_{ST}'	PIC
D5S2	1	8	179-197	0.74	0.70	0.74	0.80	0.08	0.09	0.70
INRA005		10	124-154	0.92	0.83	0.87	0.90	0.04	0.05	0.82
INRA023		3	102-258	0.72	0.82	0.89	0.94	0.06	0.07	0.82
MAF65		15	113-151	0.88	0.80	0.84	0.85	0.02	0.02	0.80
McM0527		5	163-177	0.78	0.77	0.81	0.82	0.01	0.01	0.77
SPS113		10	218-248	0.27	0.63	0.88	0.92	0.05	0.06	0.63
HSC	2	20	247-301	0.78	0.75	0.80	0.87	0.08	0.09	0.75
INRA063		14	163-191	0.80	0.68	0.72	0.75	0.04	0.04	0.68
OarFCB11		2	122-156	0.87	0.71	0.74	0.76	0.04	0.05	0.72
OarFCB304		19	148-182	0.91	0.79	0.83	0.83	0.01	0.01	0.79
MAF214		16	143-193	0.91	0.79	0.82	0.91	0.09	0.11	0.79
OarAE129		5	135-165	0.50	0.71	0.79	0.83	0.03	0.04	0.71
OarCP49		17	66-108	0.74	0.80	0.84	0.90	0.07	0.08	0.80
BM1258	3	20	98-128	0.85	0.75	0.79	0.82	0.04	0.05	0.75
INRA132		20	142-174	0.91	0.83	0.87	0.88	0.02	0.02	0.83
CSSM31	4	23	126-162	0.75	0.76	0.81	0.83	0.02	0.03	0.76
SPS115		15	167-305	0.98	0.86	0.90	0.94	0.04	0.05	0.86

H_o: Observed heterozygosity, *H_e*: expected heterozygosity, *H_S*: genetic diversity within populations, *H_T*: total genetic diversity, *G_{ST}*: proportion of genetic variation found among populations.

the Neighbor-joining (NJ) methodology (Saitou and Nei, 1987). The robustness of the tree was evaluated using 10000 bootstrap resampling. The genetic structure, the true number of populations (clusters) and the allocation of individuals from each cluster were determined using the STRUCTURE software v. 2.3.3 (Pritchard et al., 2000). The Ln Pr (G|K) was calculated for *K* ranging from 1 to 10, without prior information on the breed of origin, to estimate the most likely number of clusters in the dataset with 50 independent runs for each K (Evanno et al., 2005).

RESULTS AND DISCUSSION

Microsatellite analysis

All the microsatellite markers were successfully amplified in the two breeds. A total of 284 alleles were identified with the 17 loci. Table 2 shows genetic variability measurements corresponding to the 17 loci. The average H_e was 0.76 ± 0.06 and the average H_o was 0.78 ± 0.17. The highest H_e was observed in the loci INRA005 and INRA132 and the lowest was observed in the locus SPS113, whereas the highest H_o was observed in the locus SPS115 and the lowest in the locus SPS113. The PIC considering all loci was equal to 0.76 with ranges from 0.63 (SPS113) to 0.86 (SPS115), showing that the microsatellites panel used was highly informative (PIC > 0.50) and generally in Hardy-Weinberg equilibrium except for marker SPS113 that showed the largest difference between observed and expected heterozygosity. The significant deviation observed for the SPS113 may be explained by unobserved null alleles.

The number of alleles (TNA) per locus was 8 (D5S2 and McM527) to 37 (SPS115) with an average of 16.71 ± 7.53 alleles per locus (Table 3).

The number of alleles observed for each breed and per locus ranged between 1 and 16. AC and HM sheep presented the lowest number of alleles observed per locus, whereas AF sheep presented the maximum number of alleles observed per locus.

The genetic diversity for locus and population were calculated with F-stat software, in each population evaluated had the following ranges: AF, 0.65-0.96; AP, 0.74-0.97; AC, 0.00-0.98; AV, 0.58-0.95; HV, 0.58-1.00; HS, 0.65-1.00 and HM, 0.50-0.95. HS presented the lowest value of genetic diversity and AP the highest.

Table 4 illustrates the average ranges of expected heterozygosity as an indicator of genetic variability among the populations evaluated, which varied between 0.71 for HM and 0.82 for AF. The observed heterozygosity ranges for the populations varied between 0.73 for HM and 0.85 for AC. The results of average observed heterozygosities and expected in the breeds evaluated were greater than those provided by De la Barra et al. (2010) in four sheep breeds in Chile ('Chilota', 'Suffolk Down', 'Corriedale', 'Romney Marsh'). The high values of the heterozygosities obtained in this study are within the ranges that appeared in Alpine sheep breeds from Germany, Italy and Slovenia (Rendo et al., 2004; Dalvit et al., 2008) and they indicate that the populations evaluated display a high degree of crossbreeding, which is corroborated by the low endogamy coefficients (F_{IS}) obtained.

Table 3. Total number of alleles (TNA) per locus, number of alleles observed per locus and breed.

Locus	TNA	AF	AP	AC	AV	HV	HS	HM
D5S2	8	7	4	5	4	5	5	5
INRA005	16	11	5	1	4	3	3	6
INRA023	30	12	8	5	8	7	7	6
MAF65	16	22	12	4	12	12	8	8
McM527	8	11	7	9	9	6	9	10
SPS113	14	6	6	4	7	7	6	6
HSC	20	8	10	6	8	7	8	5
INRA063	12	12	11	6	8	13	11	5
OarFCB11	14	6	8	6	4	7	7	1
OarFCB304	14	8	6	4	6	6	6	4
MAF214	23	10	6	7	8	5	3	4
OarAE129	10	14	9	7	9	7	6	3
OarCP49	19	12	8	7	10	6	6	7
BM1258	13	8	10	6	9	8	9	5
INRA132	16	14	10	9	11	7	8	7
CSSM31	14	11	10	6	9	6	5	8
SPS115	37	16	12	7	13	7	7	10
Mean	16.71	11.06	8.35	5.82	8.17	7.00	6.71	5.94
SD	7.53	4.04	2.42	1.94	2.63	2.37	2.09	2.22

AF: 'Araucana' from Freire; AP: 'Araucana' from Padre Las Casas; AC: 'Araucana' from Chol Chol; AV: 'Araucana' from Villarrica; HV: 'Hampshire Down' from Valdivia; HS: 'Hampshire Down' from San José; HM: 'Hampshire Down' from Marchihue.

Table 4. Breeds studied, sample size of each breed, average number of alleles per breed, allelic richness per breed, genetic diversity per breed, average heterozygosities observed and expected, and F_{IS} per breed.

Breed	Sample sizes	Number of alleles per breed	Allelic richness	Genetic diversity	Average heterozygosity		F_{IS}[1]
					Observed	Expected	
A	59	14.6	9.429	0.847	0.80	0.84	0.050
H	43	10.4	7.577	0.806	0.78	0.79	0.031
Flocks per breeds							
AF	27	11.06	1.835	0.835	0.81	0.82	0.023
AP	10	8.35	1.859	0.866	0.76	0.81	0.113
AC	15	5.82	1.799	0.793	0.85	0.73	-0.077
AV	12	8.18	1.842	0.849	0.77	0.80	0.083
HV	11	7.00	1.770	0.779	0.80	0.74	-0.058
HS	14	6.71	1.771	0.782	0.75	0.74	0.017
HM	18	5.94	1.781	0.787	0.73	0.71	0.083

AF: 'Araucana' from Freire; AP: 'Araucana' from Padre Las Casas; AC: 'Araucana' from Chol Chol; AV: 'Araucana' from Villarrica; HV: 'Hampshire Down' from Valdivia; HS: 'Hampshire Down' from San José; HM: 'Hampshire Down' from Marchihue; F_{IS}: inbreeding coefficient.
[1]Nonsignificant differences (P > 0.05).

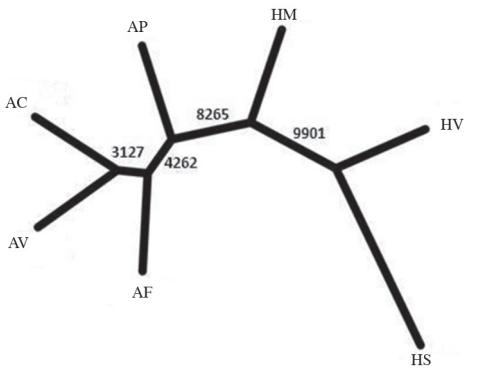
The test of Hardy-Weinberg showed that all the tested populations are in equilibrium (P > 0.05) (Table 4). The inbreeding coefficients (F_{IS}) estimates for each population evaluated appear in Table 4. The values differed between each population with ranges from -0.22 to 0.52 in AF; -0.24 to 0.65 in AP; -0.41 to 0.41 in AC; -0.25 to 0.77 in AV; -0.37 to 1 in HV; -0.514 to 1 in HS, and -0.23 to 0.548 in HM. Considering the average F_{IS} value in the seven populations evaluated, there is a 2.6% heterozygote deficit. A heterozygote deficit of 1.7% is observed for HS and 11.3% for AP. Consanguinity as a parameter determining the structure of a population is a result of the mating of related individuals, and this usually causes a loss of heterozygotes in a population. According to the results obtained, only the AP population presented a certain degree of consanguinity, since most loci showed a heterozygote deficit, thereby confirming that measures must be taken to avoid the loss of genetic diversity in this population (Avellanet et al., 2007).

According to the heterozygosities in sheep populations evaluated, it was detected that there is no loss of genetic diversity in the AF, AP and AV populations, since He values were slightly higher than the Ho values, results that demonstrate that these sheep populations have a highly genetic diversity. By contrast, an increase in the loss of diversity in the populations of AC, HV, HS and HM could be anticipated over time as a result of inbreeding, a phenomenon that is occurring in the 'Chilota' and 'Romney Marsh' breeds present in Chile (De la Barra et al., 2010).

Table 5. Matrix of Nei's standard genetic distances between breeds.

Breed	AF	AP	AC	AV	HV	HS	HM
AF	-	-	-	-	-	-	-
AP	0.212	-	-	-	-	-	-
AC	0.375	0.516	-	-	-	-	-
AV	0.174	0.291	0.514	-	-	-	-
HV	0.545	0.697	0.690	0.579	-	-	-
HS	0.545	0.690	0.759	0.572	0.254	-	-
HM	0.444	0.576	0.948	0.559	0.634	0.691	-

AF: 'Araucana' from Freire; AP: 'Araucana' from Padre Las Casas; AC: 'Araucana' from Chol Chol; AV: 'Araucana' from Villarrica; HV: 'Hampshire Down' from Valdivia; HS: 'Hampshire Down' from San José; HM: 'Hampshire Down' from Marchihue.



AF: 'Araucana' from Freire; AV: 'Araucana' from Villarrica; AC: 'Araucana' from Chol Chol; AP: 'Araucana' from Padre Las Casas; HM: 'Hampshire Down' from Marchihue; HV: 'Hampshire Down' from Valdivia; HS: 'Hampshire Down' from San José.

Figure 1. Phylogenetic tree based on Nei's standard distances (1972). The numbers indicate the proportion of bootstrap resampling.

Genetic differentiation

Nei's F-statistics were calculated for each microsatellite (Table 2). The ranges of the observed heterozygosity (H_o), expected heterozygosity (H_e), genetic diversity within populations (H_S), total genetic diversity (H_T), proportion of genetic variation found among populations (G_{ST}) and G_{ST}' values were: H_o: 0.266-0.982; H_e: 0.634-0.860; H_S: 0.72-0.9; H_T: 0.75-0.94; G_{ST}: 0.01-0.09 and G_{ST}': 0.01-0.11. The G_{ST} differentiation coefficient is generally low in the two breeds and loci (0.0435). In addition, the high genetic diversity among breeds can be explained by the high heterozygosities observed (Ligda et al., 2009).

The phylogenetic relationships based on neighbor-joining tree constructed on the basis Nei's genetic distances are in good agreement (Table 5 and Figure 1); it was observed that the genetically closest populations are AF and AV (0.174), which can be justified by the existing geographical proximity between the two flocks, which allows a constant flow of animals. By contrast, the most distant populations are AC and HM (0.948), populations that are in different regions of the country and that differ with respect to the environmental conditions in which they are handled. Furthermore, it is observed that the HV and HS populations are highly differentiated with respect to the other populations evaluated. They present genetic differences to AF, AP, AC, AV, with distances between 0.545 and 0.697. This genetic distance is due mainly to the populations of HV and HS having taken more than 10 yr using artificial insemination with frozen 'Hampshire Down' semen imported from New Zealand, which is also reflected in the divergences from HM (0.634 and 0.691). In more graphic form, the existing genetic distances between the populations evaluated can be seen in Figure 1. Two clusters were identified: the first was comprised of AF, AV, AC, AP, and HM, and the second of HV and HS, and it can be seen that the HS population is the one that presents the greatest degree of differentiation among the seven sheep populations evaluated.

The population assignment analysis performed with the STRUCTURE software with the number of expected population (K) ranging from 1 to 10. The Ln Pr (G|K) increased from $K = 2$ to $K = 7$, reached a plateau at $K = 2$. Therefore it was assumed that $K = 2$ is the most likely number of clusters. For $K = 2$, the populations group identified included: the AF, AV. AC, AP, and HM in the cluster I; and HV and HS in the clusters II (Figure 2). To determine the plateau was used of the method Evanno et al. (2005), which identifies the most accurate value of K. Also the ΔK profile showed a clear peak. The presence of a common ancestor in the evaluated populations

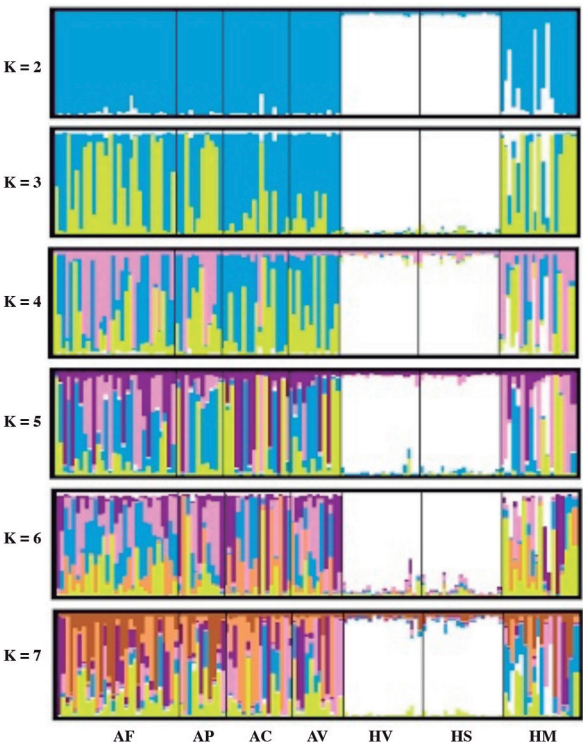


Figure 2. Genetic structures of seven sheep populations for *K* ranging from 2 to 7: 'Araucana' from Freire (AF), 'Araucana' from Padre Las Casas (AP), 'Araucana' from Chol Chol (AC), 'Araucana' from Villarrica (AV), 'Hampshire Down' from Valdivia (HV), 'Hampshire Down' from San José (HS), and 'Hampshire Down' from Marchihue (HM). Where K is the number of clusters assumed and the length of the colored segment represent the individuals estimated proportion of membership to a particular cluster.

can be validated with historical information of sheep production in Chile, which mentions the 'Hampshire Down' as the breed that well suited to the conditions of the central and southern regions of the country (Correa, 1938). Therefore, the populations of 'Araucana' present in La Araucanía Region exhibit external appearance of the 'Hampshire Down'; however, according to processes of natural selection, adaptation and crossbreed their genetic composition was modified, and they currently differ in their genetic structure.

These characterization studies make it possible to develop and implement strategies to preserve and use these local genetic resources, which are very important for the small-scale production sector in southern Chile.

CONCLUSIONS

This study provides the first record of genetic structure and state of genetic diversity for the 'Araucana' creole and 'Hampshire Down' sheep present in Chile, demonstrating that there are sufficient levels of genetic diversity in the populations analyzed and the 'Araucana' differs genetically from the 'Hampshire Down' population. Moreover, the reported population parameters may serve as reference for the establishment of conservation policies evaluated breeds.

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