Genetics and Molecular Biology, 25, 1, 37-41 (2002)

www.sbg.org.br

Evaluation of polymorphism in ten microsatellite loci in Uruguayan sheep flocks

Ivanna Tomasco, G. Wlasiuk and E.P. Lessa

Laboratorio de Evolución, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Abstract

The allele frequencies of 10 microsatellite loci previously described for sheep as BM1314, BM6526, OarFCB128, OarHH64, OarCP20, OarHH47, OarFCB48, OarHH35, OarHH72 and BM2508 were estimated for the Uruguayan flocks. A representative sample of 101 individuals composed by the two predominant breeds (76% Corriedale and 24% Australian Merino) was used. The sample did not show a significant tendency towards substructuring, in spite of presenting some significantly different allele frequencies between races. The Corriedale sample presents three loci in which the presence of null alleles is possible. The markers were highly variable, showing between 7 and 15 alleles each. The Polymorphism Information Content Index ranged from 0.63 to 0.87 and the Exclusion Probability from 0.39 to 0.75 for a cumulative Exclusion Probability of 99.98%. These results suggest the effectiveness of this set of loci for testing genetic relatedness. This is the first report of microsatellite variation in Corriedale.

Key words: sheep, polymorphism, microsatellites.

Received: March 14, 2002; accepted: March 25, 2002.

Introduction

For every program of breeding and selection, it is desirable to rely on a precise assessment of parentage to assist, for example, in the elimination of undesirable recessive alleles, in early selection for future breeding, or to ensure accurate pedigrees and registration. Microsatellites, segments of the nuclear genome composed of tandem repeat of short-sequence motifs, have become excellent candidate markers for this kind of studies (Queller *et al.*, 1993), since they are numerous, highly variable and easy to score.

A large number of highly polymorphic microsatellites have been characterized and mapped in domestic animals, including sheep, cattle and other ruminants (de Gortari *et al.*, 1997, 1998; Hayes *et al.*, 1996; Jenkings *et al.*, 1997), facilitating the use of these markers in parentage testing. For this kind of test to be acceptable, it must provide a high degree of certainty in the assignment of parentage, which can be evaluated with the Exclusion Probability (Weir, 1996). This requires a previous knowledge of allele frequencies in the population of interest and certainty about the assumptions of the model, like independence of the markers utilized and Hardy-Weinberg equilibrium.

The goals of this study were to analyze the polymorphism of a set of microsatellites previously described as polymorphic in sheep and to evaluate their usefulness for relatedness testing in Uruguayan sheep flocks.

Send correspondence to Ivanna Tomasco. Facultad de Ciencias, Universidad de la República, Iguá 4225, 11400 Montevideo, Uruguay. E-mail: ivanna@fcien.edu.uy.

Material and Methods

We analyzed ten microsatellite loci in a sample of the Uruguayan flocks considering only pure-breed individuals. The analysis were carried out separately for two subsamples: one of 77 Corriedale individuals from a fair, and another of 24 Australian Merinos from an experimental field (all of them are expected to be unrelated ewes), as well as on the combined dataset. Corriedale, the predominant race in the country, has not been previously characterized using microsatellite markers.

Total DNA extractions were made with SDS/proteinase K/NaCl/alcohol precipitation (modified from Miller *et al.*, 1988) from skin preserved in 95% ethyl-alcohol. The microsatellite loci OarHH35, OarHH64, OarHH47 (Henry *et al.*, 1993), OarVH72 (Pierson *et al.*, 1993), OarFCB128 (Buchanan and Crawford 1993), OarFCB48 (Buchanan *et al.*, 1994), OarCP20, BM1314, BM6526, (Crawford *et al.*, 1995), and BM2508 (Mulsant *et al.*, 1998) were amplified by PCR (Polymerase Chain Reaction) for all animals; excluding OarHH35 and OarHH64 all of them map on different chromosomes. Amplification was carried out in a total volume of 12.5 μL containing the following constituents: 6.25 μL of DNA (0.4 μg/mL) used as a template, 1X Taq Polymerase Buffer, 240 μM of each dNTP, 240 nM of

each primer, 0.5 units of Taq Polymerase and variable concentration of $MgCl_2$ depending on the locus (from 1.5 to 3.0 mM). PCR amplifications were performed in a Rapidcycler™ (Idaho Technology) by an initial denaturation of 3 min at 94 °C, followed by 35 cycles of 45 s of denaturation at 94 °C, 45 s of annealing at 50 °C and 45 s of extension at 72 °C, and a final extension of 7 min at 72 °C.

The amplified products were electrophoresed in 7%, 0.80 mm thick denaturing polyacrylamide gels (1000-1200 V, 1-2 h), and the DNA bands were visualized by silver staining (Sanguinetti *et al.*, 1994). Genotypes were scored by at least two persons independently and the absolute sizes of the alleles were determined in relation to a 10bp DNA size standard (GIBCO BRL).

On the basis of allele and genotype frequencies, Hardy-Weinberg equilibrium and linkage disequilibrium were tested using the software *GenePop*, version 3.1b (Raymond and Rousset, 1995). A Bonferroni correction was used to adjust significance levels in all tests to an overall $\alpha = 0.05$. Observed heterozygosity (H_{obs}), Polymorphism Information Content (PIC, Botstein *et al.*, 1980) and Exclusion Probability assuming that the genotype of the lamb and one of its parents is known (Q, according to Weir, 1996) were calculated.

The software *Structure* (Pritchard *et al.*, 2000) was used in order to assess levels of subdivision between two races (10,000 Burn-in period, 10,000 MCMC repetitions, and default parameters).

Results

Allele length in base pairs, allele frequencies, H_{obs}, PIC, Q and the number of individuals analyzed per locus are given in Table I. All loci were highly polymorphic, showing between 7 and 15 alleles, PICs between 0.63 and 0.87 and H_{obs} in the range of 0.62 to 0.84 for the whole sample. The two loci that map on the same chromosome (OarHH35 and OarHH64) did not show a significant linkage ($p > 0.373$, SE = 0.052).

The loci OarHH35, OarHH64 and OarFCB128 showed significant departures from Hardy-Weinberg equilibrium in the whole population and in the Corriedale sample (Table II). With the exception of OarFCB128, OarVH72 and BM6526, all loci presented significant differences in allele frequencies between Merino and Corriedale samples. Some alleles were much more frequent in one race than in the other (*e.g.*, allele 123 in OarHH35 and 146 in OarHH47, which are 14 and 8 times more frequent, respectively in Merino than in Corriedale, or allele 75 in

Table I - Loci, absolute allele sizes in base pairs, allele frequencies, observed heterozygosity (H_{0bs}), polymorphism information content (PIC), exclusion probability (Q) and number of individuals analyzed per locus and sample. The whole population combines all the Merinos and Corriedales; p-values refer to the comparison of allele frequency distributions between Merino and Corriedale samples: *and NS means significant and non-significant difference, respectively. Markers are followed by ♦ if their absolute allele sizes are not concordant with those reported in the literature.

Locus	Allele size (Bp)	Allele frequencies		
		Whole pop	Merino	Corriedale
Sample size (n)		101	24	77
OarHH35	117	0.010	0	0.013
	121	0.094	0.229	0.052
	123	0.079	0.271	0.019
	125	0.347	0.167	0.403
	127	0.109	0.229	0.071
	129	0.322	0.104	0.390
	131	0.010	0	0.013
	133	0.005	0	0.006
	135	0.015	0	0.019
	137	0.010	0	0.013
		p-value	0.00001*	
	H_{obs}	0.72	0.77	0.70
	PIC	0.71	0.75	0.62
	Q	0.53	0.57	0.43
	130	0.025	0	0.032

Table I (cont.)

Locus	Allele size (Bp)	Allele frequencies		
		Whole pop	Merino	Corriedale
Sample size (n)		101	24	77
OarHH64	132	0.060	0.043	0.065
	136	0.015	0	0.019
	138	0.299	0.191	0.331
	140	0.055	0	0.071
	142	0.109	0.277	0.058
	144	0.199	0.191	0.201
	146	0.239	0.298	0.221
		p-value	0.00260*	
	H_{obs}	0.69	0.83	0.65
	PIC	0.77	0.72	0.76
	Q	0.59	0.44	0.54
OarHH47	126	0.020	0	0.026
	128	0.005	0.021	0
	132	0.005	0	0.007
	134	0.085	0	0.112
	136	0.095	0.063	0.105
	138	0.055	0.104	0.039
	140	0.485	0.583	0.454
	142	0.110	0	0.145
	144	0.025	0	0.033
	146	0.035	0.104	0.013

Table I (cont.)

Locus	Allele size (Bp)	Allele frequencies		
		Whole pop	Merino	Corriedale
Sample size (n)		101	24	77
	148	0.060	0.083	0.053
	150	0.020	0.042	0.013
		p-value	0.00002*	
	H_{obs}	0.66	0.54	0.70
	PIC	0.72	0.60	0.72
	Q	0.55	0.43	0.56
	142	0.010	0	0.013
OarFCB48	144	0.120	0.261	0.078
♦	146	0.025	0.022	0.026
	148	0.370	0.217	0.416
	150	0.275	0.109	0.325
	152	0.015	0.043	0.006
	154	0.040	0.109	0.019
	156	0.070	0.152	0.045
	158	0.005	0	0.006
	160	0.010	0	0.013
	162	0.015	0.065	0
	164	0.010	0	0.013
	166	0.005	0	0.006
	168	0.025	0.022	0.026
	170	0.005	0	0.006
		p-value	0.0001*	
	H_{obs}	0.73	0.96	0.66
	PIC	0.73	0.79	0.67
	Q	0.55	0.67	0.46
	100	0.122	0	0.149
OarFCB128	112	0.058	0.114	0.045
♦	114	0.243	0.286	0.234
	120	0.005	0.029	0
	122	0.048	0	0.058
	124	0.196	0.143	0.208
	126	0.259	0.400	0.227
	128	0.069	0.029	0.078
		p-value	0.00732 NS	
	H_{obs}	0.68	0.59	0.70
	PIC	0.78	0.68	0.79
	Q	0.63	0.45	0.63
	127	0.051	0.068	0.046
OarVH72	129	0.454	0.318	0.493
	131	0.036	0.068	0.026
	133	0.276	0.409	0.237
	137	0.036	0	0.046
	139	0.102	0.114	0.099
	141	0.026	0	0.033

Table I (cont.)

Locus	Allele size (Bp)	Allele frequencies		
		Whole pop	Merino	Corriedale
Sample size (n)		101	24	77
	143	0.020	0.023	0.020
		p-value	0.09948 NS	
	H_{obs}	0.62	0.64	0.62
	PIC	0.66	0.66	0.65
	Q	0.48	0.47	0.47
	71	0.005	0	0.006
OarCP20	73	0.337	0.313	0.344
	75	0.119	0.021	0.149
	77	0.114	0.125	0.110
	79	0.129	0.104	0.136
	81	0.015	0.021	0.013
	83	0.238	0.271	0.227
	85	0.035	0.146	0
	87	0.005	0	0.006
	89	0.005	0	0.006
		p-value	0.00070*	
	H_{obs}	0.72	0.79	0.70
	PIC	0.76	0.72	0.75
	Q	0.59	0.58	0.57
	140	0.213	0.159	0.321
BM1314	150	0.075	0	0.100
	155	0.086	0.045	0.100
	157	0.144	0.182	0.131
	159	0.149	0.364	0.077
	161	0.057	0.045	0.062
	163	0.034	0	0.046
	165	0.023	0.023	0.023
	197	0.023	0	0.031
	169	0.006	0	0.008
	171	0.069	0.045	0.077
	173	0.098	0.114	0.092
	175	0.023	0.023	0.023
		p-value	0.00444*	
	H_{obs}	0.84	0.77	0.86
	PIC	0.87	0.76	0.81
	Q	0.76	0.61	0.77
	124	0.016	0.027	0.013
BM6526	126	0.026	0.054	0.019
	128	0.005	0	0.006
	130	0.016	0.027	0.013
	132	0.068	0	0.084
	134	0.204	0.162	0.214
	136	0.079	0	0.097
	138	0.183	0.108	0.201

Table I (cont.)

Locus	Allele size (Bp)	Allele frequencies		
		Whole pop	Merino	Corriedale
Sample size (n)		101	24	77
	140	0.079	0.162	0.058
	142	0.298	0.459	0.260
	144	0.010	0	0.013
	146	0.005	0	0.006
	148	0.010	0	0.013
		p-value	0.02158 NS	
	H_{obs}	0.75	0.67	0.77
	PIC	0.80	0.70	0.80
	Q	0.64	0.51	0.65
	88	0.005	0	0.007
BM2508	92	0.351	0.125	0.412
	100	0.138	0.150	0.135
	104	0.016	0.075	0
	112	0.410	0.650	0.345
	114	0.043	0	0.054
	116	0.037	0	0.047
		p-value	0.00001*	
	H_{obs}	0.65	0.50	0.69
	PIC	0.63	0.50	0.63
	Q	0.39	0.32	0.44

OarCP20 which is seven times more frequent in Corriedale than in Merino). Other alleles were found only in one sample, and sometimes at high frequency (*e.g.*, alleles 134 and 142 of OarHH47 in Corriedale, and allele 85 of OarCP20 in Merino). In several cases, these frequencies differed from those reported in the literature (see Henry *et al.*, 1993, for OarHH47, OarHH35, OarHH64; Buchanan and Crawford 1993, for OarFCB128; Ede *et al.*, 1995, for OarCP20; and Buchanan *et al.*, 1994, for OarFCB48). There were also differences in one base pair between absolute allele sizes reported for OarFCB48 and OarFCB128 and our observations but it was easy to resolve the correspondences on the basis of allele frequencies. Direct comparisons would be needed to assess the reason for this discrepancy.

Analyses of subdivision using the software *Structure* (Pritchard *et al.*, 2000) did not find a significant tendency towards substructuring in the sample (data not shown), in spite of the presence of two breeds.

Discussion

The loci used in the analysis are effectively independent, since the only two that map on the same chromosome (OarHH35 and OarHH64) did not show significant linkage disequilibrium. The departures from Hardy-Weinberg equilibrium shown by OarHH35, OarHH64 and OarFCB128 in the Corriedale sample were probably the cause of the departure in the whole population. This could be due to the presence of null alleles in these loci in Corriedale or be the consequence of several years of intensive selection on these breeds (Usha *et al.*, 1995). To avoid this problem, these loci can be excluded from parentage analysis and the cumulative Q would vary from 99.99% to 99.77% and from 99.98% to 99.75% for the whole population and the Corriedale stock, respectively. For Merinos, the cumulative Q with ten loci was 99.93%.

In spite of the presence of several differences in allele frequencies between races, these were insufficient to result in substantial separation between these breeds. These two races are known to be related, since Corriedale was originated from crosses between pure Merino and Lincoln sheep (Majala, 1997), so this result is not entirely surprising. Coupled with the fact that most of Uruguayan sheep are not completely pure, these observations suggest that allele

Table II - Observed p-values and standard errors (SE) of Hardy-Weinberg exact tests for heterozygote deficit.

	p - value and SE					
Locus	Whole pop		Merino		Corriedale	
OarHH35	0.0022*	0.0015	0.4380	0.0110	0.0011*	0.0008
OarHH64	0.0000*	0.0000	0.7105	0.0102	0.0000*	0.0000
OarHH47	0.0243	0.0091	0.0319	0.0076	0.0855	0.0180
OarFCB48	0.5571	0.0555	0.9664	0.0075	0.2182	0.0422
OarFCB128	0.0003*	0.0003	0.0478	0.0052	0.0016*	0.0010
OarVH72	0.2808	0.0219	0.3717	0.0161	0.2687	0.0212
OarCP20	0.0917	0.0177	0.5716	0.0236	0.0319	0.0111
BM1314	0.2119	0.0294	0.1718	0.0233	0.3823	0.0386
BM6526	0.0658	0.0210	0.0835	0.0115	0.1427	0.0287
BM2508	0.2454	0.0217	0.1807	-	0.6460	0.0252

*Means significant departures from equilibrium, after a Bonferroni correction (corrected $\alpha = 0.005$). See text.

frequency estimations derived from the whole sample may be sufficient for future applications in relatedness tests. Also, such tests may exclude the loci suspected to have null alleles without a marked reduction in the probability of exclusion.

The fact that estimated frequencies differed from those reported in the literature in most loci analyzed, stresses the importance of independent estimates for different regions and races.

In sum, these results indicate that the selected system of markers is highly effective for relatedness studies, with a Probability of Exclusion greater than 99.9% considering all loci. The significance of this report, the first of this kind in the country, is that it offers interesting perspectives for the incorporation of molecular genetic techniques to animal breeding in Uruguay. In addition, our results represent an original contribution of allele frequencies for regional Corriedale and Australian Merino races.

Acknowledgments

We are grateful to Jaime Mendoza, Roberto Cardellino and Mario Azzarini, from SUL (Secretariado Uruguayo de la Lana) for encouragement and collaboration in the field work, and to CSIC (Comisión Sectorial de Investigación Científica de la Universidad de la República) for financial support of this work.

References

Botstein D, White RL, Skolnick M and David R (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314-331.

Buchanan F and Crawford AM (1993) Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci. Anim. Genet. 24:145.

Buchanan FC, Galloway SM and Crawford AM (1994) Ovine microsatellites at the OarFCB5, OarFCB19, OarFCB20, OarFCB48, OarFCB129 and OarFCB226 loci. Anim. Genet. 25:60.

Crawford AM, Dodds KG, Ede AJ, Pierson CA, Montgomery GW, Garmonsway HG, Beattie AE, Davies K, Maddox JF, Kappes SW, et al. (1995). An autosomal genetic linkage map of the sheep genome. Genetics. 140:703-724.

de Gortari MJ, Freking BA, Kappes SM, Leymaster KA, Crawford AM, Stone RT and Beattie CW (1997) Extensive genomic conservation of cattle microsatellite heterozygosity in sheep. Animal. Genetics. 28:274-290.

de Gortari MJ, Freking BA, Cuthbertson RP, Kappes SM, Keele JW, Stone RT, Leymaster KA, Dodds KG, Crawford AM and Beattie CW (1998) A second-generation linkage map of the sheep genome. Mamm. Genome. 9:204-209.

Ede AJ, Pierson CA and Crawford AM (1995) Ovine microsatellites at the OarCP9, OarCP16, OarCP20, OarCP21, OarCP23 and OarCP26 loci. Anim. Genet. 26:129-130.

Henry HM, Penty JM, Pierson CA and Crawford AM (1993) Ovine microsatellites at the OarHH35, OarHH41, OarHH44, OarHH47and OarHH64 loci. Anim. Genet. 24:222.

Hayes H, Le Chalony C, Goubin G, Mercier D, Payen E, Bignon C and Kohno K (1996) Localization of ZNF164, ZNF146, GGTA1, SOX2, PRLR and EEF2 on homologous cattle, sheep and goat chromosomes by fluorescent in situ hybridization and comparison with the human gene map. Cytogenet. Cell Genet. 72:342-346.

Jenkins ZA, Henry HM, Galloway SM, Dodds KG and Montgomery GW (1997) Comparative linkage mapping of genes on sheep chromosome 3 provides evidence of chromosomal rearrangements in the evolution of Bovidae. Cytogenet. Cell Genet. 78:272-274.

Majala K (1997) Genetics aspects of domestication, common breeds and their origin. In: Piper L and Rubinsky A (eds) The genetics of sheep. CAB International, U.K , pp. 13-50.

Miller SA, Dikes DD and Polesky HH (1988) A simple salting procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16:215.

Mulsant P, Schibler L, Lecerf F, Riquet J, Chitour N, Eggen A, Cribiu EP, Lanneluc I and Elsen JM (1998) Regional mapping of the FecB (Booroola) region of sheep chromosome 6. Anim. Genet. 29:36-37.

Pierson CA, Hanrahan V, Ede AJ and Crawford AM (1993) Ovine microsatellites at the OarVH34, OarVH58, OarVH61 and OarVH72 loci. Anim. Genet. 24:224.

Pritchard JK, Stephens M and Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics. 155:945-959.

Queller DC, Strassmann JE and Hughes CR (1993) Microsatellites and Kinship. Tree. 8:285-288.

Raymond M and Rousset F (1995) GenePop (version 1.2). Population genetics software for exact test and ecumenisism. J. Hered. 86:248-249.

Sanguinetti CJ, Neto ED and Simpson AJG (1994) Rapid silver staining and recovery of PCR products separated on polyacrilamide gels. Biotechniques. 17:915-918.

Usha AP, Simpson SO and Williams JL (1995) Probability of random sire exclusion using microsatellite markers for parentage verification. Anim. Genet. 26:155-161.

Weir BS (1996) Genetic data analysis II. Sinauer Associates, Inc. Publishers. Sunderland, Massachusetts, 445 pp.