Paternity testing using microsatellite DNA in alpacas (*Vicugna pacos*)

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INTRODUCTION

Accurate methods for parentage determination are very important for domestic animal breeders, as well as for the development of breed registries. Over the past decade, many DNA based techniques have been developed and put into practice utilizing polymerase chain reaction (PCR). Microsatellites, or short tandem repeats (STRs), are repeat sequences of up to six base pairs (Hancock, 1991). When amplified by PCR and observed through gel electrophoresis, microsatellite alleles can be distinguished by differences in the number of the repeats.

In most countries, including the U.S.A, Canada, United Kingdom and Australia, verification of parentage using DNA analysis, normally microsatellites, is required to place an animal in a breed registry. In Peru, however, registries are based entirely on observation and controlled breeding. In the present study, use of DNA analysis to certify the accuracy of one such registry has revealed shortcomings.

MATERIALS AND METHODS

Genomic DNA from 47 alpacas (*Vicugna pacos*)(11 fathers, 18 mothers, 18 offspring)registered at IVITA´s Marangani Research station in Canchis Province, Cusco, Peru, was extracted from blood using the ULTRACLEAN DNA Blood Spin Kit ® (Mo Bio Inc). Ten microsatellite loci (LCA19, LCA22, LCA5, LCA23, YWLL08, YWLL29, YWLL36, YWLL40, YWLL43 and YWLL46) (Lang *et al.*, 1996; Penedo *et al.*, 1998) were amplified in three multiplex PCR reactions with the QIAGEN Multiplex PCR Kit® (QIAGEN) and 10 µl made up of: 25 ng genomic DNA, 2mM of each primer (one labelled at the 5' end with a fluorochrophor) and 5 µl QIAGEN Multiplex Kit Master Mix.

PCR followed using the protocol: 1 cycle of 95°C for 15 minutes; 25 cycles of 94°C for 30 seconds, 61°C (for the first multiplex) and 59°C (for the second and third multiplexes) for 90 seconds and 72°C for 60 seconds; with a final extension of 60°C for 30 minutes in a thermocycler model 9700 GeneAmp® (Perkin Elmer).

PCR products were separated in a 4.25% polyacrylamide gel (Gene Page® - AMRESCO) at 1000 V in TBE 1X using ABI 377 DNA sequencers® (Applied Biosystems). Allele sizes were determined using ABI Genotyper® v.2.5 (Applied Biosystems). Probability exclusion (individual and accumulated), heterozygosity and polymorphic information contents (PIC) were calculated using Cervus 2.0 Parentage analysis©.

RESULTS AND DISCUSSION

The ten microsatellites amplified and were polymorphic. Allele number varied between 4 for loci LCA22 and YWLL46, and 20 for locus YWLL08, with an average of 9.1 per locus, similar to Lang *et al.* (1996) and Penedo *et al.* (1998), but with slight variations in loci YWLL08 and YWLL36 likely due to the distinct origin of the animals. The exclusion probability for each locus varied between 0.174 (YWLL46) and 0.844 (YWLL08) with a accumulated exclusion probability of 0.9998 for ten microsatellites (Table 1). Furthermore, four of the microsatellites were found to be highly informative based on their higher individual exclusion probabilities (YWLL08, YWLL36, LCA23 and YWLL29).

Table 1. Allele number, individual and accumulated exclusion probability (EP and EPa), heterozygosity and polymorphic information content (PIC) for the different microsatellites

Multiplex	Microsatellite	EP	EPa	$H_{(o)}$	PIC
M 1	LCA 19	0.548		0.702	0.709
	YWLL 29	0.634		0.787	0.786
	YWLL 40	0.408		0.660	0.604
	YWLL 46	0.174	0.9191	0.383	0.307
M 2	LCA 23	0.621		0.638	0.779
	LCA 22	0.294		0.511	0.477
	YWLL 36	0.733	0.9286	0.894	0.852
M 3	YWLL 43	0.452		0.304	0.639
	LCA 5	0.575		0.809	0.744
	YWLL 08	0.844	0.9637	0.915	0.918
M1 + M2 + M3	10 Microsatellites		0.9998		

All 18 paternity cases were resolved (Figure 1). Four errors (22%) in parentage assignment were documented relative to the breeding records of the research station. In one case it was not possible to determine the true father because he was not among the samples provided. These results clearly demonstrate the necessity of DNA testing to ensure accurate recordkeeping and gurantee the parentage of registered animals.

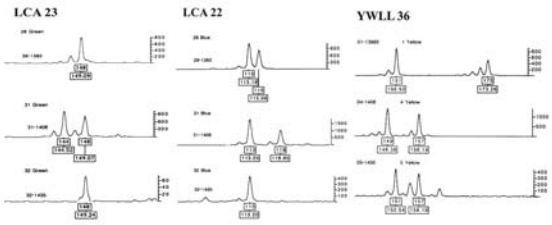

Figure 1. Electropherograms (in Genotyper v.2.5) (ABI PRISM)
(a) Locus LCA23 labeled with fluorochrophor TET. The samples correspond to: 1383 (Father), 1408 (Mother) and 1435 (Offspring). (b) Locus LCA22 labeled with fluorochrophor FAM. (c) Locus YWLL36 labeled with fluorochrophor HEX. Y axis indicates amplification intensity in fluorescence units.

CONCLUSIONS

Analysis of ten microsatellites (LCA19, LCA22, LCA5, LCA23, YWLL08, YWLL29, YWLL36, YWLL40, YWLL43 and YWLL46) permits paternity determination with an exclusion probability of 0.9998 in alpacas.
Analysis of six microsatellites (YWLL43, LCA5, YWLL08. LCA 22, LCA 23 and YWLL 36) in two multiplex PCR reactions determines paternity with an exclusion probability of 0.9637.

ACKNOWLEDGMENT

Special thanks to Juan Olazábal (IVITA, Marangani), Rosa Dávalos (IVITA, Huancayo), Matilde Fernandez and the authorities and workers at the IVITA, Marangani for help with sample collection and logistics. Support for this research was supplied by CONOPA (Peru), Cardiff University (UK) and the Darwin Initiative for the Survival at Species (UK).

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