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Genetic Differentiation among Andean Camelid Populations Measured by Blood Protein Markers.

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ABSTRACT

Genetic differentiation of populations of guanacos (n=2), vicuñas (n=1), llamas (n=3) and alpacas (n=5) in the Andes (Argentina, Bolivia and Peru) was measured using three blood protein markers (phosphogluconate dehydrogenase, esterase D and diaphorase). Variability was generally lower within populations of the wild species (guanacos and vicuñas). Both principal component analysis and clustering analysis with a distance measure demonstrated the genetic proximity between alpaca and vicuña and between llama and guanaco. The protein markers successfully resolved this genetic relationship and supported the findings of a preliminary study by KAWAMOTO *et al.* (2004). Distribution of specific protein genes and differences in the frequency are discussed in relation to phylogeny, domestication and hybridization of camelids in the Andes.

INTRODUCTION

In considerations of the origin and domestication of South American camelids, the phylogenetic relationships among wild and domestic species remain controversial. One main area of research is the contribution of each wild species and the role of hybridization in the domestication and formation of extant populations of South American camelids (WHEELER, 1995). These include the wild guanaco (*Lama guanicoe*) and vicuña (*Vicugna vicugna*) as well as the domestic llama (*L. glama*) and alpaca (*L. pacos*). The llama is generally believed to be a descendant of the guanaco. However, multiple hypotheses on the domestication process of the alpaca have been proposed: first, domestication from the guanaco (for example, HERRE, 1952);

second, domestication of a crossbred such as a domestic llama and vicuña cross (HEMMER, 1990) or a guanaco and vicuña cross (VIDAL-RIOJA *et al.*, 1994); and third, domestication from the vicuña (WHEELER, 1995).

Recent studies using mitochondrial DNA (mtDNA) and microsatellite DNA revealed close relationships between guanaco and llama and between vicuña and alpaca (STANLEY *et al.*, 1994; KADWELL *et al.*, 2001). These data support the domestication of the alpaca from the vicuña hypothesis, which was first suggested by archeological evidence (WHEELER, 1991). Further, mtDNA and microsatellite variant heterogeneity in the llama and the alpaca were interpreted as being the result of bi-directional hybridization, which was also inferred from phenotypic changes observed in ancient domestic animals (WHEELER *et al.*, 1995).

In a previous study, we examined blood protein polymorphisms of South American camelids in order to develop new genetic markers for use in assessing the relationships and origins of the domestic animals. The electrophoretic variations in an erythrocyte esterase (known as esterase D (EsD) in humans) and NADH-dependent diaphorase (Dia) proved useful as genetic markers (KAWAMOTO *et al.*, 2004). Preliminary analysis showed close relationships between guanaco and llama and between vicuña and alpaca, suggesting independent domestication processes from different wild species. In this study, we applied several protein markers to various llama and alpaca populations from Argentina, Bolivia and Peru. Using analysis of genetic polymorphisms, we compared genetic diversity within and among camelid species in the Andes.

MATERIALS AND METHODS

Samples

Blood samples were collected from 13 guanacos, 19 vicuñas, 95 alpacas and 69 llamas (Table 1). Additionally, blood samples from 7 wari, a llama and alpaca crossbred, were collected and examined in this study. Guanaco samples were obtained in November 2002 courtesy of INTA (Instituto Nacional de Tecnología Agropecuaria), Buenos Aires, Argentina, and Santa Cruz Zoo, Bolivia (Fig. 1). Vicuña samples were collected at Pampa Geléras National Conservation Area, Peru in June 2002 (KAWAMOTO *et al.*, 2004). Llama, alpaca and wari samples were collected from the following sources and sites in Bolivia and Peru: 10 llama and 10 alpaca samples, courtesy of Catolica Boliviana University, Tiwanaku, Bolivia; 10 alpaca samples collected from a farm at Altiplano University, Puno, Peru; and 15 llama and 10 alpaca samples collected at the camelid festival held in November 2002, Puno, Peru. Many of the samples from Peru were collected during a field expedition around Puica, Arequipa Province in October 2003 (Figs. 1, 2 and 3). Samples from a total of 65 alpacas, 44 llamas and 7 waris were collected at Capilla and Ccachu, Peru.

Heparinized bloods were separated into plasma and cells by centrifugation. Cells were washed two times with 0.9% saline, then stored in a freezer until protein polymorphism

Table 1. Samples examined in this study.

Name of animal	Sampling locality	Sample no.	Abbreviation	Sampling time	Source
Guanaco (*Lama guanicoe*)	Beunos Aires, Argentina	10	GAR	2002.11.	INTA*
	Santa Cruz, Bolivia	3	GBL	2002.11.	Santa Cruz Zoo
Vicuña (*Vicugna vicugna*)	Pampa Galéras, Peru	19	VPG	2002. 6.	National Conservation Area
Alpaca (*Lama pacos*)	Tiwanaku, Bolivia	10	ABL	2002.11.	Catolica Boliviana University
	Capilla, Peru	16	ACP	2003.10.	
	Ccachu, Peru	49	ACC	2003.10.	
	Puno, Peru	10	APA	2002.11.	Altiplano University
	Puno, Peru	10	APF	2002.11.	Camelid Festival
Llama (*Lama glama*)	Tiwanaku, Bolivia	10	LBL	2002.11.	Catolica Boliviana University
	Ccachu, Peru	44	LCC	2003.10.	
	Puno, Peru	15	LPF	2002.11.	Camelid Festival
Wari (Alpaca × Llama)	Ccachu, Peru	7	WCC	2003.10.	

*INTA = Instituto Nacional de Tecnología Agropecuaria

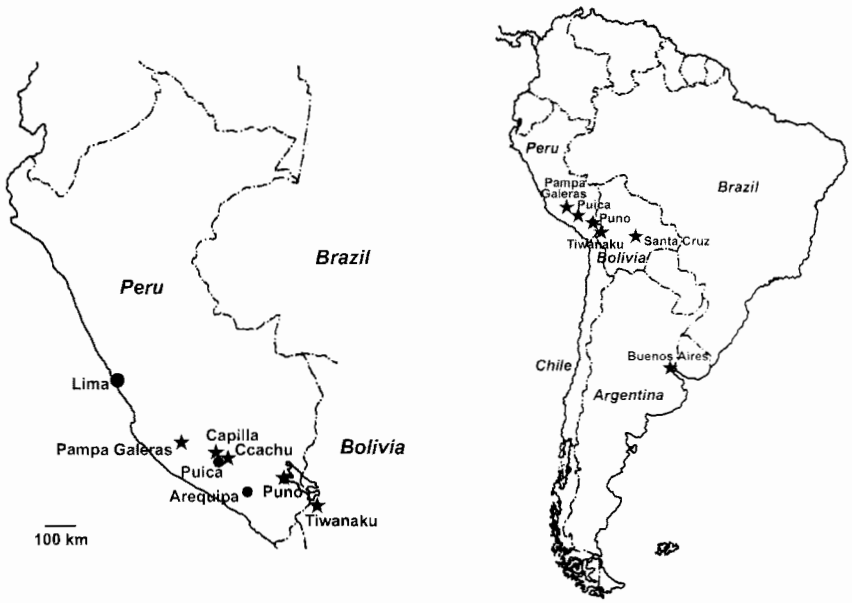

Fig. 1. Map showing study sites in Argentina, Bolivia and Peru. Stars indicate the collection places of blood samples.

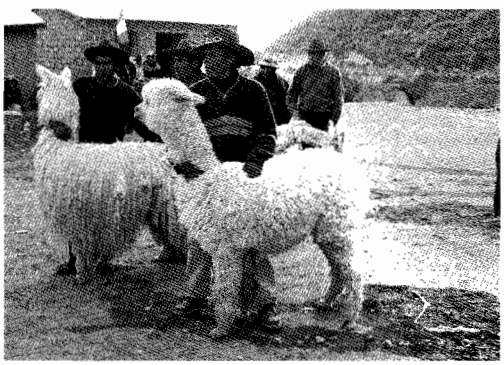

Fig. 2. Alpacas at a festival, Puica, Arequipa, Peru.

Fig. 3. A llama at Ccachu, Arequipa, Peru.

determination.

Protein polymorphism determination

All blood proteins were analysed at the Amano Museum, Lima, Peru. Polymorphic analysis of three erythrocyte enzymes, phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), esterase D (EsD, EC 3.1.1.–) and diaphorase (Dia, EC 1.6.–.–) was conducted by conventional starch gel electrophoresis. The following buffers were used: Tris-citrate buffer (System I in SHAW and PRASAD, 1970) for PGD and EsD, and Tris-EDTA-borate buffer (HARRIS and HOPKINSON, 1978) for Dia. Histochemical staining was carried out following the methods of HARRIS and HOPKINSON

(1976, 1978).

Data analysis

Allele frequencies were estimated by the simple counting method assuming autosomal codominance. Genetic variability within populations was quantified using calculations of expected mean heterozygosity. The relationship among populations was examined using principal component analysis of the correlation coefficient matrix of gene frequencies following angular transformation ($\theta=\sin^{-1}\sqrt{p}$, where p is an allele frequency). A rootless tree was constructed by the neighbor-joining method (SAITOU and NEI, 1987) using the Euclidean distances in multidimensional space.

RESULTS

Distribution of alleles

Protein marker genes were distributed ubiquitously in the four species. For EsD, extensive polymorphism with two alleles was observed; however, vicuña had only allele *1* (Table 2). EsD allele *3* was restricted to llama from Puno and guanaco from Buenos Aires and was present with low frequency. For PGD, allele *2* was observed only at low frequencies in alpaca and vicuña. For Dia, allele *2* was widely observed, but was absent in vicuña. The other three alleles of Dia showed varied distributions: Dia allele *1* was found with high frequency both in vicuña (0.95) and alpaca (0.50–0.84), but not at all in guanaco; Dia allele *3* was found in alpaca from Tiwanaku with extremely high frequency (0.90) but in a few alpaca samples from Ccachu and Puno, in llama from Tiwanaku, and in vicuña from Pampa Geléras; and a variant of Dia, named allele *4*, was observed in only one alpaca sample from Ccachu.

Genetic variability of populations

Expected mean heterozygosity is summarized in Table 2. In general, wild guanaco and vicuña showed lower variability than domestic alpaca and llama. The simple means of heterozygosity estimates were 0.218 and 0.206 for alpaca (5 populations) and llama (3 populations), respectively. The heterozygosity estimate was 0–0.132 with a mean of 0.066 for the two guanaco populations and 0.092 for a vicuña population. The wari, a llama and alpaca crossbred, from Ccachu showed an intermediate level of variability compared to the parental populations in the same locality. Among the domestic populations, alpaca from Capilla and Ccachu as well as llama from Puno showed relatively lower variability. However, alpaca from the farm of Altiplano University showed higher variability.

Genetic differentiation among populations

Genetic similarity among camelid populations is shown in the two-dimensional projections

Table 2. Estimates of allele frequency and expected mean heterozygosity.

Animal	Population*/ Locality	Sample no.	*EsD*			*PGD*		*Dia*				Expected mean heterozygosity
			1	*2*	*3*	*1*	*2*	*1*	*2*	*3*	*4*	
Guanaco	GAR / Beunos Aires	10	0.75	0.20	0.05	1.00	–	–	1.00	–	–	0.132
	GBL / Santa Cruz	3	–	1.00	–	1.00	–	–	1.00	–	–	0.000
Vicuña	VPG / Pampa Galéras	19	1.00	–	–	0.90	0.10	0.95	–	0.05	–	0.092
Alpaca	ABL / Tiwanaku	10	0.75	0.25	–	0.95	0.05	–	0.10	0.90	–	0.217
	ACP / Capilla	16	0.97	0.03	–	0.97	0.03	0.84	0.16	–	–	0.128
	ACC / Ccachu	49	0.90	0.10	–	0.99	0.01	0.82	0.16	0.01	0.01	0.167
	APA / Puno	10	0.65	0.35	–	1.00	–	0.50	0.45	0.05	–	0.333
	APF / Puno	10	0.80	0.20	–	0.95	0.05	0.80	0.20	–	–	0.245
Llama	LBL / Tiwanaku	10	0.60	0.40	–	1.00	–	–	0.90	0.10	–	0.220
	LCC / Ccachu	44	0.40	0.60	–	1.00	–	0.15	0.85	–	–	0.245
	LPF / Puno	15	0.67	0.30	0.03	1.00	–	–	1.00	–	–	0.153
Wari	WCC / Ccachu	7	0.79	0.21	–	1.00	–	0.21	0.79	–	–	0.221

*For abbreviation of population name, see Table 1.

onto the first two principal components in a scattergram shown in Fig. 4. Using multivariate scaling, 54.0 percent of the total diversity was attributed to the first component and 17.1 percent to the second component. The results showed the close relationship between alpaca and vicuña and between llama and guanaco. Differences in the two guanaco populations, particularly in the

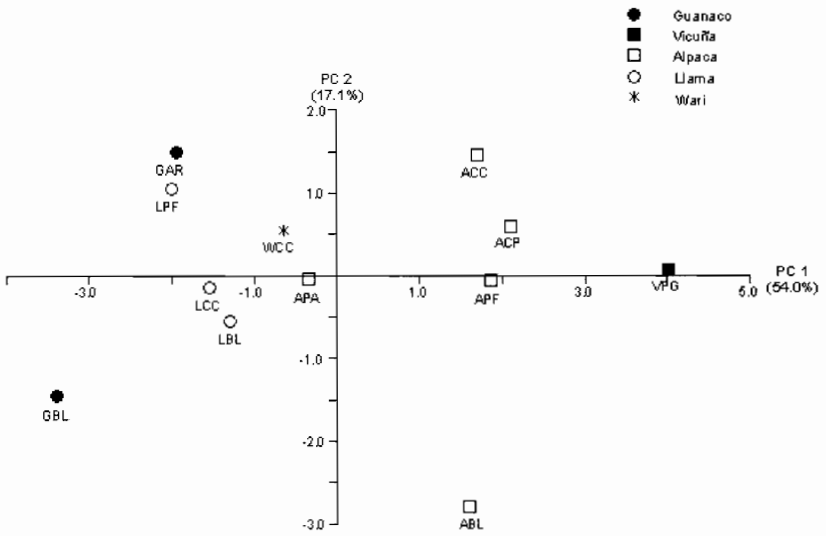

Fig. 4. Two dimensional projection of Andean camelid populations onto the first two principal components. For abbreviation of population names, see Tables 1 and 2.

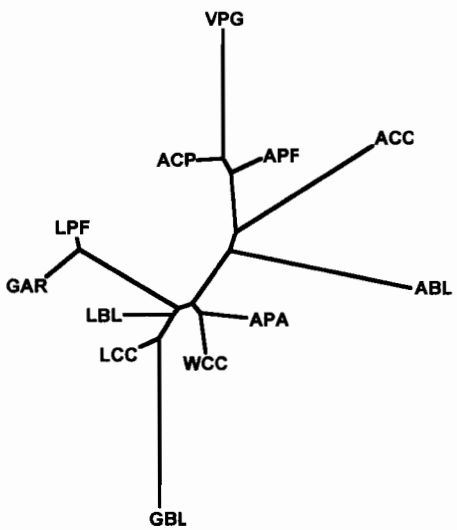

Fig. 5. An unrooted tree constructed by the neighbor-joining method. The distance measure was the Euclidean distance which was obtained from the component scores in the principal component analysis. For abbreviation of population names, see Tables 1 and 2.

second component, suggest local genetic differentiation of this wild species. Among the five alpaca populations, the samples from Altiplano University at Puno and those from Catolica Boliviana University at Tiwanaku were greatly diverged from other populations. By contrast, differentiation among llama populations was relatively low. In the projection given in Fig. 4, the wari population from Ccachu was positioned directly between alpaca and llama raised at the same study site.

The genetic relationships of the populations based on the clustering distance method shown in Fig. 5 show similar relationships among camelids in the Andes. Though the two wild forms are distantly related, in general, domestic forms exhibit stronger pairings with each of the wild forms: guanaco-llama and vicuña-alpaca.

DISCUSSION

The protein markers developed in the previous study (KAWAMOTO *et al.* 2004) proved to be effective for the assessment of genetic relationships among camelids in the Andes. Allele types differed greatly between the two wild species. Particularly, differences in allele segregation at the Dia locus were apparent between guanaco and vicuña. Mutations producing diagnostic markers EsD allele *2* and PGD allele *2* likely arose after the speciation of guanaco and vicuña. Thus, distribution of these alleles could provide significant information about the genetic relationships and domestication of these species. However, the significance of the present results requires further assessment. It is noteworthy that in this study, guanaco populations from different localities show great difference at EsD. This may be important for future studies investigating local differences in gene diversity in wild species.

The present results support previous findings of the genetic relationships of the four camelid species. These results also correspond to the results obtained from molecular phylogenetic studies of mtDNA and microsatellite DNA (STANLEY *et al.*, 1994; KADWELL *et al.*, 2001). Among the study populations, the degree of genetic variability was higher in domestic forms than in wild forms, and Dia allele *4* was present only in domestic animals (Table 2). The latter finding could result from insufficient sampling of wild forms, but it could also indicate the accumulation of a new mutant gene after domestication.

An alternative interpretation of the present results could be made to explain the origin of alpaca and llama forms. A simple and the most plausible interpretation is the independent domestication from each wild species, from guanaco to llama and from vicuña to alpaca. This hypothesis is supported by other molecular markers (KADWELL *et al.*, 2001). However, the paucity of sample populations in this study makes it difficult to decisively reject the other possible routes of domestication. The distribution of Dia *1* and *3* alleles, which are present in vicuña but not in guanaco, in llama populations requires explanation. Identification of these protein genes in presently unstudied guanaco populations would lend support for domestication

occurring only from the guanaco lineage. An alternative hypothesis considers that the domestication occurred through hybridization. The EsD *2* allele was present in alpaca, guanaco and llama, but absent in vicuña. This distribution pattern is not completely explained by the independent domestication hypothesis. Thus, the possibility of alpaca being founded by the crossing of vicuña with llama (HEMMER, 1990) or with guanaco (Vidal-Rioja *et al.*, 1994) could not be entirely rejected based on this study.

Diversity in genetic variability was apparent among the populations of domestic forms (Table 2). Interestingly, alpaca (APF) and llama (LPF) population samples collected at a camelid festival at Puno had lower variability than alpaca population samples from a farm at Altiplano University, Puno (APA). The variability was also low for population samples (ACP, ACC, LCC) taken from fields around Puica, Arequipa, Peru, where traditional animal rearing and breeding practices seemed to be followed. Differences in genetic variability may reflect recent changes in utilization and breeding of domestic camelids. Llama had been used as pack animals in local transportation, but this demand has been decreased in recent years by the development of a roadway network. Alpaca numbers have increased due to fiber production demands, and also crosses with llama have been made in recent years in efforts to increase the yield of fiber (WHEELER, 1995; KADWELL *et al.*, 2001).

These changes in social and economic valuation of domestic camelids may correlate with the genetic variability introduced by the hybridization of alpaca with llama. Thus, it is of interest to further investigate populations of domestic forms to elucidate the correlation between social change and genetic variability both in developed and undeveloped areas of the Andes. From such a comparative study, it may be possible to further assess the influence of hybridization on local differentiation, as well as its role on the domestication process.

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Genetic Differentiation among Andean Camelid Populations Measured by Blood Protein Markers.

血液タンパク質標識で測ったアンデス地方のラクダ科動物集団の遺伝的分化

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南米アンデス高地に生息するラクダ科動物の系統関係，家畜化，交雑を研究するために開発した血液タンパク質変異の遺伝標識を用いて，アルゼンチン，ボリビア，ペルーで集団調査をおこなった（Fig. 1）。野生種のグアナコ（2集団）とビクーニャ（1集団），家畜種のアルパカ（5集団）（Fig. 2）とリャマ（3集団）（Fig. 3）およびアルパカとリャマの交雑家畜ワリ（1集団）から合計203個体の血液試料を集め（Table 1），3種類の赤血球酵素（Dia：ディアフォラーゼ，EsD：エステラーゼD，PGD：グルコン酸－6－リン酸脱水素酵素）の多型を電気泳動法で分析した。

採用したタンパク質の遺伝標識はラクダ科動物の遺伝子構成のちがいを把握するのに有効であり，各遺伝子座において対立遺伝子の頻度分布にちがいがみられた。また，種間のちがいだけでなく，種内集団間でも遺伝子構成のちがいが認められた（Table 2）。遺伝的変異性については，家畜集団のほうが野生種の集団より高い傾向が観察された。遺伝子頻度の推定値にもとづく主成分分析（Fig. 4）とクラスター分析（Fig. 5）の結果は，リャマがグアナコに，アルパカがビクーニャに近縁であることを示唆し，予報（KAWAMOTO *et al.*, 2004）の結果を支持した。この結果は，アルパカとリャマがいずれもグアナコを家畜化したとする考えに反するものである。アンデス地方における家畜と人間の関係は変化しており，家畜集団に観察された遺伝子構成や変異性のちがいには，道路網の整備や毛の需要などの社会的変化にともなう交雑の影響も考えられる。