CHAPTER 23

Genetic Analysis of the Origins of Domestic South American Camelids

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Introduction

Ancestors of the family Camelidae originated in North America during the Eocene, 40–45 MYA, with the division between Lamini and Camelini (the tribes of New and Old World camelids, respectively) dating to 11 MYA (Webb 1974; Harrison 1979). Their subsequent migration to South America and Asia occurred 3 MYA (Webb 1974), with representatives of the extant New World genera *Lama* and *Vicugna* appearing 2 MYA (Hoffstetter 1986) in South America.

Two branches of the Lamini evolved from the ancestral North American *Pliauchenia* (11–9 MYA). The first exclusively North American branch contains *Alforjas* (10–4.5 MYA) and *Camelops* (4.5–0.1 MYA), while the second includes *Hemiauchenia* (10–0.1 MYA), *Palaeolama* (2–0.1 MYA), *Lama* (2 MYA–present), and *Vicugna* (2 MYA–present), all of which are found in South America. Although a recent article suggests that *Hemiauchenia* should be classified within *Palaeolama* (Guerin and Faure 1999), it remains clear that *Lama* and *Vicugna* evolved from *Hemiauchenia*. By the end of the Pleistocene, the only surviving members of the *Lamini* were the South American *Lama* and *Vicugna*.

The Lamini are classified within the order Artiodactyla, suborder Tylopoda and family Camelidae. Some taxonomists have favored classification into two genera and four species: *Lama guanicoe* (guanaco), *L. pacos* (alpaca), *L. glama* (llama) and *Vicugna pacos* (vicuña) (Cabrera and Yepes 1960), while many others have favored placing the four species within the genus *Lama*. Recent genetic studies, however, have shown that the Camelidae are most likely composed of two genera, each containing two species: *L. guanicoe* (guanaco) and *L. glama* (llama), and *V. vicugna* (vicuña) and *V. pacos* (alpaca) (Kadwell et al. 2001).

Four subspecies of guanaco (*L. guanicoe guanicoe* in Patagonia, Tierra del Fuego, and Argentina south of 35° S; *L.g. huanacus* in Chile; *L.g. cacsilensis* in the high Andes of Peru, Bolivia, and northeast Chile; and *L.g. voglii* on the eastern slope of the Andes in Argentina between 21–32° S) and two of vicuña (*V. vicugna mensalis* from 9°30'–18° S and *V.v. vicugna* from 18–29° S) have been described. In the case of the guanaco, virtually nothing is known about the northernmost *L.g. cacsilensis*, whose relict populations, perhaps 3,500 animals in total, are highly endangered. Research has been carried out on the behavior and ecology of *L.g. guanicoe* and *V.v. mensalis* (Franklin 1983), and recently information on the genetic diversity of extant wild populations has become available (Palma et al. 2001; Wheeler et al. 2001). These studies indicate that the two northernmost forms, *L.g. cacsilensis* and *V. vicugna mensalis*, are the ancestors of the domestic llama and alpaca respectively.

The alpaca has variously been described as descending from the guanaco, the vicuña, and as a llama? vicuña hybrid, while the llama is thought to originate from the guanaco. These contradictory hypotheses have been developed primarily from the study of morphological and behavioral variations among living animals, while archaeozoological evidence has pointed toward domestication of the alpaca from the vicuña in the wet puna of Peru's central Andes 6,000–7,000 years ago and toward possible multiple domestications of the llama from the guanaco in the dry punas of southern Peru, Chile, and Argentina (Wheeler 1995; see Mengoni Goñalons and Yacobaccio, Chapter 16, this volume).

In 1775, Frisch (1775) attributed the origin of the llama to the guanaco and the alpaca to the vicuña, an opinion subsequently supported by Ledger (1860), Darwin (1868), Antonius (1922), Faige (1929), Krumbiegel (1944, 1952), Steinbacher (1953), Frechkop (1955), Capurro and Silva (1960), Akimushkin (1971), and Semorile et al. (1994). Other authors have concluded that both domestic camelids descend from the guanaco, and that the vicuña was never domesticated (Thomas 1891; Peterson 1904; Hilzheimer 1913; Lönnberg 1913; Brehm 1916; Cook 1925; Weber 1928; Herre 1952, 1953, 1976, 1982; Röhrs 1957; Fallet 1961; Zeuner 1963; Herre and Thiede 1965; Herre and Röhrs 1973; Bates 1975; Pires-Ferreira 1981/82; Kleinschmidt et al. 1986; Kruska 1982; Jürgens et al. 1988; and Piccinini et al. 1990). In the 1930s, López Aranguren (1930) and Cabrera (1932) suggested that the llama and the alpaca evolved from presently extinct wild precursors, based on the discovery of 2 Myr Plio-Pleistocene *L. glama*, *L. pacos*, *L. guanicoe* and *V. vicugna* fossils in Argentina, and that the guanaco and vicuña were never domesticated. This position is no longer considered a possible alternative. Finally, Hemmer (1975, 1983, 1990) attributes llama ancestry to the guanaco, but has deduced on the basis of shared morphological and behavioral traits that the alpaca originated from hybridization between the llama and vicuña.

Conclusions about llama and alpaca ancestry have, in large part, been based upon morphological changes produced by the domestication process. During the 1950s, Herre and Röhrs (Herre 1952, 1953, 1976; Röhrs 1957; Herre and Röhrs 1973)

examined alterations in the mesotympanal area of the skull related to a decrease in llama and alpaca hearing acuity and reported an overall reduction in cranial capacity of both domestic species relative to the guanaco. In contrast, they found the vicuña cranium to be the smallest of all living Lamini, and, based on the premise that domestic animals are smaller than their ancestors, concluded that this species was never brought under human control. Herre and Röhrs consider the llama and alpaca to be "races of the same domestic species bred for different purposes" (Herre 1976: 26). Research on the relationship of brain size relative to body size by Kruska (1982) also found the vicuña to be smaller than the alpaca and the llama, which in turn were smaller than the guanaco, suggesting that the latter is the only ancestral form. Nonetheless, papers by Jerison (1971) and Hemmer (1990) report the ratio of alpaca brain size to body size to be smaller than in the vicuña, permitting a different conclusion about origins of the domestic forms. These contradictory data on size reduction are almost certainly a product of sampling as neither subspecific variation in the wild forms nor the possibility of hybridization between the domestic animals were considered in any of the studies (see Mengoni and Yacobaccio, Chapter 16, this volume).

Based on the study of pelage characteristics (skin thickness, follicle structure, secondary/primary ratio, fiber length and diameter, coloration) in living camelids, Fallet (1961) found the llama to be an intermediate evolutionary stage between the wild guanaco and the specialized, fiber-producing alpaca. Fallett concluded that the absence of transitional characteristics between vicuña and alpaca fleeces eliminates the former from consideration as an ancestral form. This deduction is, in part, based on the assumption that llamas have been selected exclusively for use as pack animals, whereas alpacas have been bred for fiber production. Nonetheless, new data on preconquest llama and alpaca breeds in Peru have revealed the prior existence of a fine-fiber-producing llama, as well as an extra-fine-fiber alpaca that is transitional between the vicuña and a second, prehispanic, fine-fiber alpaca breed (Wheeler, Russel, and Redden 1995).

Research on camelid behavior has produced contradictory hypotheses concerning llama and alpaca origins. Krumbiegel (1944, 1952) and Steinbacher (1953) argue that the alpaca is the domestic vicuña based on unique, shared behavioral traits that are said to differ from those observed in the guanaco and llama. Hemmer, on the other hand, concludes that although some alpaca behavior patterns match those of the vicuña, others are intermediate between those of vicuña and guanaco, suggesting that "the alpaca is a mixture of both lines, [produced] by crossbreeding of captured vicunas with the only initially available domestic animal, the llama" (1990: 63). It has also been suggested that the vicuña was never domesticated because it is more territorial than the guanaco (Franklin 1974). Nonetheless, this assumption is open to question because it is based on a study of guanacos located at the southernmost extreme of their range where seasonal migration in response to severe climatic changes is essential for survival (Franklin 1982, 1983). Further to the north, where vicuña and guanaco ranges overlap and where llama and alpaca domestication occurred (Wheeler 1995), a more benign climate and a constant food supply permit the characteristic sedentary social organization of the vicuña (Franklin 1982, 1983). Although data concerning behavior of the guanaco in this region are lacking, it is possible that the limited sedentary territorial organization observed in some Patagonian groups plays a more important role in these less-extreme climatic conditions.

Analysis of hemoglobin amino acid sequences in vicuña, alpaca, llama, and guanaco from Hannover Zoo, Germany, led Kleinschmidt et al. (1986), Jürgens et al. (1988), and Piccinini et al. (1990) to the conclusion that the vicuña was never domesticated. However, earlier research on blood and muscle samples with descending bidimensional chromatography (circular and descending) for hydrolyzed muscle samples and horizontal electrophoresis for blood serum samples from llama, alpaca, vicuña, guanaco, and alpaca × vicuña hybrids at Santiago Zoo (Cappuro and Silva 1960) indicated a llama-guanaco and alpaca-vicuña subdivision, as have more recent data from ribosomal genes (Semorile et al. 1994). Other researchers using immunological, electrophoretic analysis and protein sequencing have found it impossible to draw conclusions about llama and alpaca ancestry (Miller et al. 1985; Penedo et al. 1988). Cytogenetic studies (Capanna and Civitelli 1965; Taylor et al. 1968; Larramendy et al. 1984; Gentz and Yates 1986) indicate that all four species of the South American Camelidae (SAC) have the same 2n = 74 karyotype. Analysis of satellite DNA, mitochondrial cytochrome *b* gene, and nuclear microsatellites in large sample sets has documented extensive hybridization among the domestic SAC (Vidal Rioja et al. 1987; Saluda-Gorgul et al. 1990; Stanley et al. 1994; Kadwell et al. 2001). Recent studies of the fiber from mummified ninth- and tenth-century llamas and alpacas provide additional evidence that postconquest hybridization has modified the genetic makeup of living populations (Wheeler et al. 1995), a fact that may well explain the diversity of conclusions about their ancestry.

In an attempt to solve the question once and for all, the first South American camelid mitochondrial DNA sequences were analyzed by Stanley, Kadwell, and Wheeler in 1994. In this study, sequence data from a short (158 bp) but highly informative region of the cytochrome *b* gene were used to examine the phylogenetic affiliations of alpaca and llama. Unfortunately, although the results confirmed that *Lama* and *Vicugna* are valid genera, which separated 3–2 MYA, the origin of the domestic forms remained unclear since there was evidence for considerable bidirectional hybridization.

From the mid–late 1990s, nuclear microsatellite DNA markers began to be isolated from a number of South American camelids (Lang et al. 1996; McPartlan et al. 1998; Penedo et al. 1998; Obreque et al. 1998; Obreque et al. 1999; Penedo et al. 1999a, 1999b; Sarno et al. 2000), and now in excess of 70 such markers are available. Because the strict maternal inheritance of mitochondrial DNA in most mammals restricts its use in studies of hybridization, especially in domestic livestock (e.g., MacHugh et al. 1997), the most recent work on SAC domestication has also included analysis

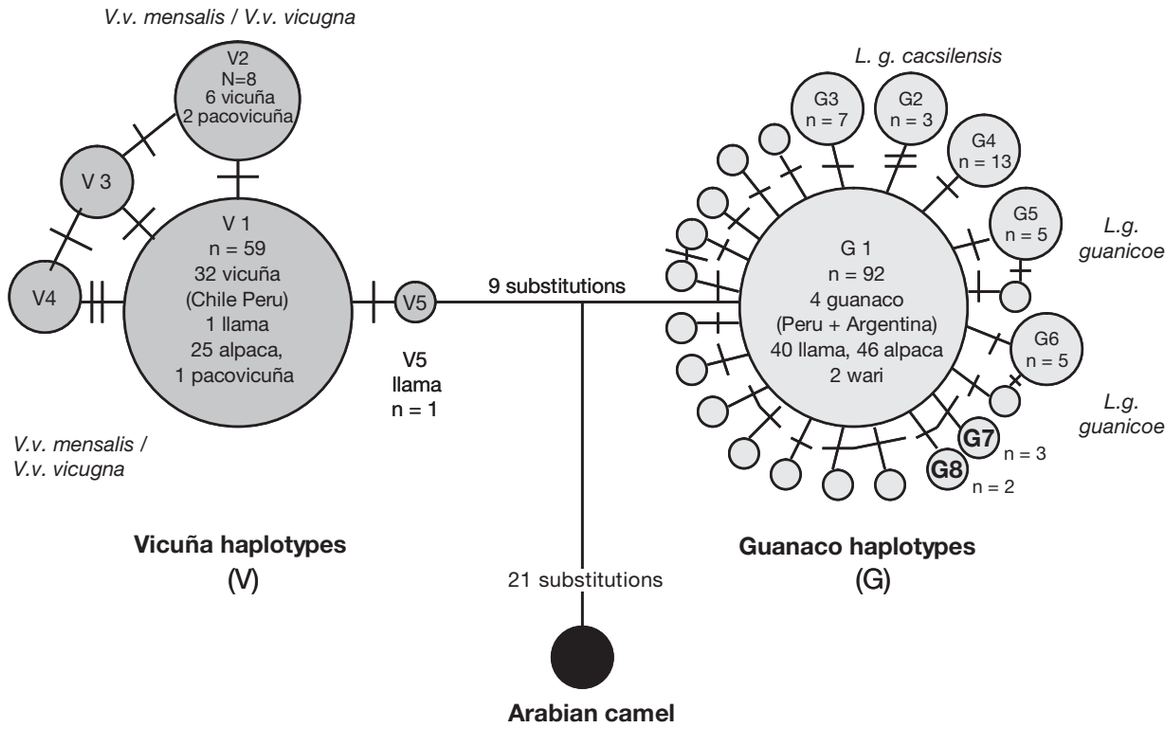

FIGURE 23.1 Minimum spanning network representing the relationships among cytochrome *b* mitochondrial haplotypes. Halpotypes are shown as circular nodes, and the number of substitutions connecting each sequence is represented by numbers or dashes on each connecting line. The relative frequency of each haplotype is represented by the area of each circle. Dark gray circles indicate vicuña haplotypes, and light gray circles represent guanaco haplotypes. Wild samples are specifically referred to where present. Phylogenetic analysis (maximum parsimony and neighbor joining based on uncorrected p, JC, and K2P sequence distances) recovered an equivalent pattern to the network, and high (>90%) bootstrap support was always found for the major split between V and G haplotypes (not shown).

of microsatellites. Recently, Kadwell et al. (2001) used a large sample set collected throughout the geographic range of the four species (771 samples) and analyzed it for four microsatellites. Cytochrome *b* sequences were also analyzed from a subset comprising 211 samples. The results of this study with some subsequent statistical analysis of the results and their implications for camelid evolution form the basis of the material in the remainder of this chapter.

The Present Study

As far as possible, sample collection sites spanned the geographic range of both wild species and included alpaca samples (including "suri" and "huacaya" fleece types) from Peru, Argentina, Chile, and Bolivia (n ≤ 141); llama samples (a range of morphological types) from the same countries (n ≤ 60); guanaco *(L. g. guanicoe* and *L. g. cacsiliensis)* from Peru and Argentina (n ≤ 122); and vicuña *(V. v. vicugna* and *V. v. mensalis)* from Peru, Argentina, and Chile (n ≤ 440). Samples were taken only from those individuals whose phenotype conformed to accepted morphological criteria for domestic forms.

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The phylogenetic relationships of the llama and alpaca were first established by sequencing the same region of the cytochrome *b* gene as in Stanley et al. (1994) (deposited in Genbank accessions U06425–30). Two hundred and eleven individuals were sequenced (21 guanaco, 42 vicuña, 54 llama, 84 alpaca, and 10 hybrids including alpaca/vicuña and llama/alpaca crosses). DNA was extracted from blood or skin using standard Proteinase K digestion followed by organic extraction using phenol and phenol/chloroform, and total DNA was precipitated in 100% ethanol (Stanley et al. 1994; Bruford et al. 1998). DNA samples were stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The cytochrome *b* primers L14724 and H14900 were used for PCR, and amplifications were carried as in Stanley et al. (1994). PCR products were purified and DNA sequencing was carried out as in Stanley et al. 1994. Sequences were aligned and the unique sequences (from here on called "haplotypes") were also deposited in Genbank under accession numbers AF373809–373833. Among-haplotype divergence and haplotype frequencies were calculated for the guanaco, vicuña, llama, and alpaca samples (Stanley et al. 1994), and a minimum spanning network (Kruskal 1965; Bandelt et al. 1999) was generated using the program MINSPNET (Excoffier 1993; Figure 23.1). The distribution patterns of domestic SAC haplotypes were then compared with those of the wild SAC sample.

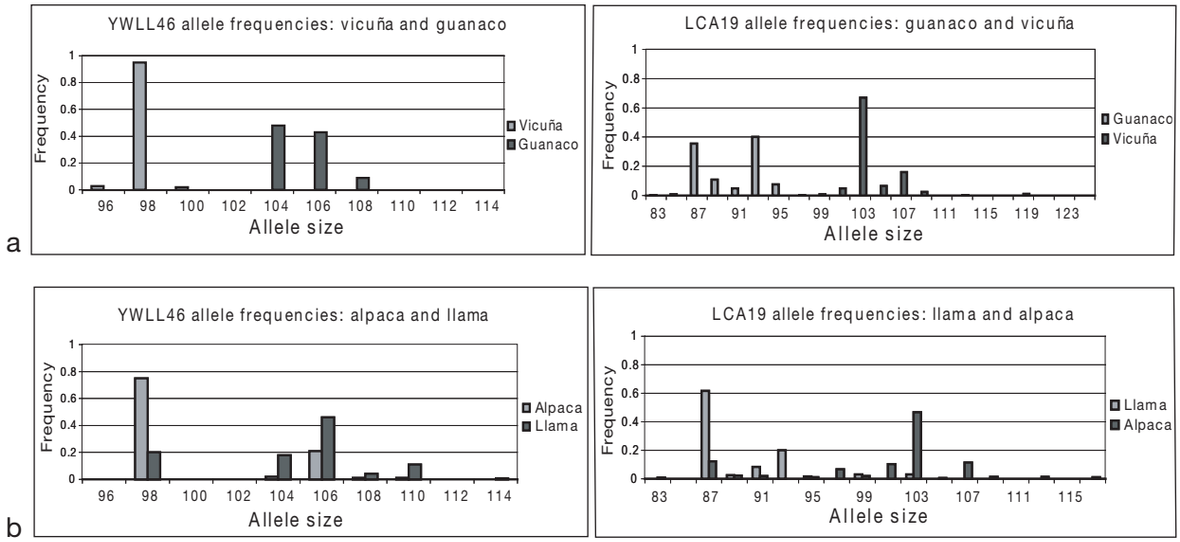

FIGURE 23.2 Allele size and frequency histograms for LCA 19 and YWLL 46, respectively: (a) vicuña and guanaco; (b) llama and alpaca. The histograms represent the frequencies of each allele for both loci in the four SAC "species." The domestic species (alpaca and llama) are plotted together, as are the wild species (vicuña and guanaco). Distributions were generated using guanaco n = 104 [LCA 19] and 177 [YWLL 46]; llama, n = 56 [LCA 19] and 58 [YWLL 46]; alpaca, n = 80 [LCA19] and 82 [YWLL 46]; vicuña, n = 227 [LCA19] and 231 [YWLL 46].

The maternal inheritance of mitochondrial DNA means that hybridization studies will only inform us on female introgression, and this can be very misleading, especially in domestic livestock (e.g., MacHugh et al. 1997). Here, we also applied nuclear DNA markers that are biparentally inherited and thus equally represent female and male lineages. Four microsatellite loci (YWLL 38, YWLL 43, YWLL 46, and LCA 19; Lang et al. 1996; Penedo et al. 1998) were typed for 669–771 individuals, including the 211 individuals sequenced above (Kadwell et al. 2001; Figure 23.2). Three measures of genetic distances were used. First, an allele-sharing distance was estimated as 1 p(s) (where p(s) is the proportion of shared alleles between two individuals). This measure is very useful as it allows calculation of genetic distances between individuals when a number of loci are available. The second measure of genetic distance used was Reynolds' distance (Reynolds et al. 1983), a measure commonly used in livestock analysis where genetic drift has a major impact on allele frequencies. The third measure used in this study, $(\text{delta mu})^2$ (Goldstein et al. 1995), estimates population differences using allele size differences under a stepwise mutation model. This distance was developed especially to analyze microsatellite data, as they are thought to evolve under a similar mutational model where the gain, or loss, of repeated DNA units is commonly observed. All genetic distances were estimated using the program MICROSAT v1.5d (Eric Minch, Stanford University 1999).

A form of ordination, known as factorial correspondence analysis, was then performed on allele frequency data. Here, the genetic diversity among populations (in this case vicuña, guanaco, llama, and alpaca) is expressed as factors that explain the inertia of the set of points (representing an individual) in a multidimensional space defined by the presence and absence of alleles within samples. In Figure 23.3, we show the dimensions that explain the highest proportion of the inertia (Benzécri 1973). This ordination can be thought of as analogous to displaying the two principle components in a Principle Components Analysis (PCA), although the approach is slightly different. The relationships between populations can be judged by examining how individuals from the sample cluster in two, three, or more dimensions. Because of the large number of alleles, we commonly find that the axis can very clearly identify clusters while providing relatively low proportions of the total inertia. Correspondence analysis is used as an exploratory tool. The analysis was performed using the Genetix software (Belkhir 1999).

Combined Analysis

To further assess introgression in llama and alpaca populations, two approaches were used. First, we applied methods that allow us to estimate admixture between two so-called parental populations in a hybrid or admixed population. This means that, in the three cases, we assumed for simplicity that llama and alpaca were the result of an admixture event between vicuña and guanaco some time in the past and that gene flow was limited afterwards. To estimate the proportion of vicuña (or guanaco) genes within llama and alpaca, we used three methods. The first two estimators, mC and mY, were developed by Chakraborty et al. 1992 (based on a previous method developed by Long 1991), Bertorelle (1998), and Bertorelle and Excoffier (1998). The first estimator uses only information on allele frequencies while the

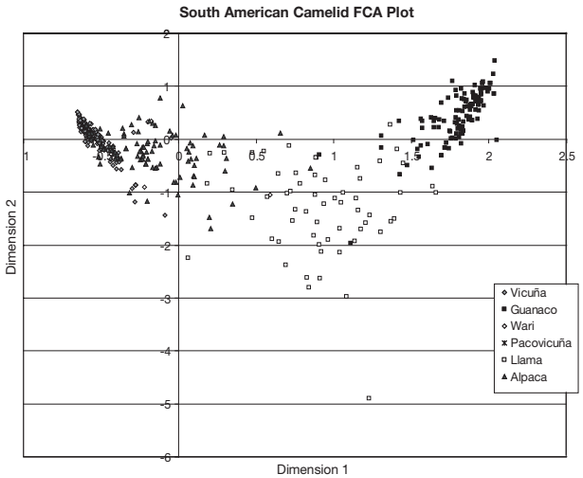

FIGURE 23.3 Two-dimensional factorial correspondence plot for allele frequencies at four microsatellite loci in all SACs. Almost half of the explained correspondence (15%) is found in factors 1 and 2, represented on the horizontal and vertical axes respectively. Clusters in two-dimensional FCA plots represent samples with similar allele frequencies. As expected, vicuña and guanaco clusters are far apart in the plot and form tight groupings. In contrast, alpaca (which group most closely to vicuña) are more disperse and llama are the most dispersed group (and are found in the same half of factor 1 in the plot as guanaco).

second also uses molecular information such as the number of substitutions for mtDNA or the allele size differences for microsatellites. These two estimators are implemented in the program ADMIX1_0 (Bertorelle 1998). The third method allows us to estimate (and account for) both drift and admixture (Chikhi et al. 2001) and produces posterior distributions for the parameters of interest, namely, p1, the contribution of one parental population (it was arbitrarily chosen to be vicuña) and three parameters measuring drift in the two parental and the hybrid population. For details, see Chikhi et al. (2001) and Langella et al (2001) for descriptions of the corresponding software. All three methods are model-dependent and assume that there has been a major admixture event that was relatively limited in time. There is good reason to hypothesize that the admixture between llama and alpaca occurred primarily on two occasions—at the time of the Spanish conquest and during the past 25 years. These methods are therefore expected to work best when the model is a reasonable approximation of reality. As should be clear from previous sections, the admixture processes that have taken place in llama and alpaca are more complex than assumed by any of the three methods. However, previous work has shown that even if admixture is more complex, such methods are expected to estimate overall levels of admixture, even when they are not as discrete as assumed by the models (see review by Chakraborty 1986).

The second approach was made possible by the fact that for mtDNA and for two of the four microsatellites, differences between vicuña and guanaco were extremely clear. As we were able to identify "typical" guanaco and vicuña alleles, concordance between mitochondrial and microsatellite data in terms of introgression patterns could be assessed in more detail. We thus re-analyzed the 211 individuals typed for both mtDNA and microsatellites. Genotypes were coded "V" (vicuña) or "G" (guanaco) for mtDNA and "V," "G," or "H" (hybrid) for YWLL 46 and LCA 19 depending on their allele sizes with reference to the guanaco and vicuña ranges, and we examined the data for each locus separately and combined. This type of approach is not always possible as the "typical" status of an allele, in practice, is impossible to state with certainty (drift might have eliminated an allele from one population, making it look typical of another one). This type of approach, therefore, cannot be used to make quantitative statements but can be powerful as a qualitative or exploratory approach (see Goldstein and Chikhi 2002).

Results

Mitochondrial DNA

Twenty-six unique haplotypes found in the 211 SACs were analyzed. Uncorrected distances within SACs ranged between 0.006 (one substitution) and 0.089 (14 substitutions). The minimum spanning network (Figure 23.1) revealed two major groups, which represent the same reciprocally monophyletic clades found previously by Stanley et al. (1994). The first group contained all vicuña ("V") and the second contained all guanaco ("G"). The branch leading to the Arabian camel exhibited 21 substitutions. The domestic camelids were found within both groups, but 81% (including 73% of alpaca) were found within the "G" (guanaco) group. A minority (19%) comprising alpaca, pacovicuña, and just two llamas were found within the "V" group. These results add support to the findings of Stanley et al. (1994), who found no evidence for consistent segregation of mtDNA alleles with taxa defined in the analysis. Wild vicuña and guanaco mtDNA were reciprocally monophyletic with 5.8–8.9% sequence divergence being found between the two lineages, recapitulating the suggestion in Stanley et al. (1994) that these species diverged from a common ancestor 2–3 MYA. Furthermore, Stanley's finding that nearly all modern llamas possess a "guanaco" haplotype was also supported in this analysis (Table 23.1), where all except 2 llamas from a sample of 54 individuals possessed guanaco mtDNA. However, the much-expanded alpaca data (n = 84) revealed a different picture, with only 27% of individuals possessing vicuña mtDNA (Table 23.1), in contrast to the 50% described previously.

Microsatellites

Unusually in such studies, the microsatellite markers feature a large number of alleles exclusive to either the vicuña or the guanaco. These alleles, which range in frequency between 33% and 100% at different loci, also occupy predominantly different allele size ranges. The upper graphs in Figure 23.2

Please confirm or clarify.

TABLE 23.1
Three Locus Genotypes for Samples Where All Three Types of Data Are Available

	Vicuña n = 42	*Alpaca* n = 84	*Guanaco* n = 21	*Llama* n = 84	*Wari* n = 7	*Alpaca/ Vicuña* n = 3
GGG	—	—	21	32	—	—
GGH	—	—	—	15	1	—
GGV	—	1	—	—	—	—
GHH/GHX/GXH	—	2/1/1	—	2	1	—
GXV	—	1	—	—	—	—
GVH/GHV	—	18	—	2	1	—
GVG	—	2	—	1	—	—
GVV/GXV	—	34/1	—	—	4	—
VVV	42	17	—	—	—	3
VVH/VHV	—	3	—	—	—	—
VGG	—	—	—	2	—	—
VGV	—	1	—	—	—	—
VHH	—	2	—	—	—	—

Loci are ordered mtDNA, LCA 19, and YWLL 46; for example, GVH indicates guanaco mtDNA, vicuña genotype at LCA 19, and a hybrid genotype at YWLL 46 (X signifies that the sample could not be typed).

show allele frequency histograms for locus YWLL 46 for wild vicuña and guanaco: the allele sizes do not overlap. Such loci provide powerful tools for the discrimination of ancestral genomes in modern domestic stock. The lower graphs in Figure 23.2 show equivalent histograms for the llama and alpaca, which displayed similar patterns. However, the patterns of genetic similarity were in direct contrast to those revealed by mtDNA. Strong similarities between the allele size distributions of vicuña and alpaca and between guanaco and llama were observed. For YWLL 46, the 98-bp allele had a frequency of 0.95 in the vicuña and 0.75 in alpaca, while the 104- and 106-bp alleles had a combined frequency of 0.91 in the guanaco and 0.64 in llama. Analysis of the four microsatellites showed that the genetic distances between vicuña and alpaca and between guanaco and llama (Table 23.2) were almost always much lower than those between vicuña and guanaco, vicuña and llama, or guanaco and alpaca. Distances between alpaca and llama were mostly intermediate.

Please confirm or clarify.

However, a second feature of microsatellite frequencies was the presence, at a low frequency, of "vicuña" alleles in the llama sample and of "guanaco" alleles in the alpaca sample, suggesting bidirectional introgression in both domestic forms. Notwithstanding, a striking pattern emerged from the factorial correspondence analysis (Figure 23.3), where guanaco and vicuña formed two tightly clustered and distinct groups. Additionally, alpaca formed a cohesive group, clustering strongly with the vicuña. In contrast, the llamas and hybrids formed a much more diffuse group. The llama samples, although tending to cluster with guanaco on axis 1, were more intermediate with respect to the wild species when compared with the alpaca sample and were also the most genetically diffuse group on axis 2. The most likely explanation for the separation between llama and guanaco in our sample is that the guanaco samples are from the austral form, *L.g. guanicoe*, and not the highland *L.g. cacsilensis*. The two samples of *L.g. cacsilensis*, the most likely ancestral subspecies of the llama, fall in the middle of the llama samples.

Combined Data and Admixture Analysis

The admixture proportions were nonconcordant between the mitochondrial and microsatellite analyses in the alpaca, where the estimated microsatellite proportion of vicuña genome was much higher than the mtDNA estimate (0.310 mtDNA; 0.903 ± 0.108 mC and 0.823 mY for microsatellites). In the llama, although both estimates were low, the microsatellite admixture proportions were an order of magnitude higher. It is nonetheless evident using all markers and estimates that the proportion of vicuña DNA is much lower in llama than in alpaca.

In order to investigate the reason for these results, we applied a computer-intensive method that we had developed (Chikhi et al. 2001). Apparent differences of admixture estimates between loci can be the result of both admixture and drift (or even selection). As simulations have shown, if drift has been important, for instance, different loci may appear to indicate very different admixture levels, even though they have been submitted to the same process (Chikhi et al. 2001). This is crucial as the two previous methods do not allow a separation of the effects of admixture and drift. Figure 23.4 shows the results of the admixture analysis for the four microsatellite loci. For comparison, we have also plotted the intervals as vertical bars (estimate ± one SD) obtained with mC (admixture estimator based on allele frequencies in the parental and hybrid populations, shown as solid lines) and

TABLE 23.2
Pairwise Genetic Distances between the Four SACs

	Vicuña	*Guanaco*	*Alpaca*	*Llama*
(delta mu)^2				
Vicuña	—			
Guanaco	28.928	—		
Alpaca	1.089	19.781	—	
Llama	17.162	10.784	9.892	—
Reynolds' distance				
Vicuña	—			
Guanaco	0.729	—		
Alpaca	0.173	0.433	—	
Llama	0.627	0.174	0.267	—
1 p(s)				
Vicuña	—			
Guanaco	0.963	—		
Alpaca	0.337	0.841	—	
Llama	0.825	0.522	0.616	—

(delta mu)^2 is the genetic distance based on mean-squared difference in allele size; Reynolds' distance is a genetic distance based on the difference in allele frequencies, assuming an Infinite Alleles Models; 1 p(s) is a proportion of shared alleles.

mY (admixture estimator based on allele size differences in the parental and hybrid populations, as dashed lines). This figure clearly shows that (1) the four loci exhibit much less variability among themselves in the alpaca than they do in the llama, suggesting a very strong and consistent admixture signal in the alpaca in contrast to the llama; (2) the alpaca and llama distributions appear very different, especially highlighting the very limited introgression of vicuña into llama; and (3) the same pattern is observed for the three different estimators, even though they can be rather different in some cases (in particular for locus YWLL 43 in llama). Note also that both mC and mY can give estimates of admixture that are outside the expected [0,1] range (twice in llama for mY and once for mC in alpaca). When all four loci are used together, the difference in admixture levels between alpaca (Figure 23.5, bottom) and llama (Figure 23.5, top) becomes even more obvious, with the posterior distribution becoming much thinner (and therefore more precise) and with the modal (i.e., the most probable) value for the vicuña contribution being 84.1% for alpaca and 14.4% for llama.

Please confirm or clarify.

The combined three-locus analysis of the 211 individuals produced striking results (Table 23.1). Of the 54 llamas, 96% possessed a "G" mtDNA haplotype, with 90% and 61% possessing a pure "G" genotype for LCA19 and YWLL 46, respectively. Of the 84 alpacas, only 27% possessed a "V" mtDNA haplotype, while 70% and 79% possessed pure "V" LCA19 and YWLL 46 genotype respectively. Of the llamas tested in this study, 60% exhibited a "GGG" type, but only 20% of alpacas exhibited a "VVV" type. Extensive nuclear introgression was detected in the llama, with 37% showing one or more "vicuña" alleles at LCA19 and/or YWLL46. In contrast, much of the introgression in the alpaca was mitochondrial, with 40% of samples showing a "GVV" type.

Discussion and Future Research

In isolation, the finding that a large proportion of modern-day alpacas possess guanaco mtDNA is in accordance with hypotheses that alpacas, in common with llamas, descend from the guanaco. However, as in Stanley et al. (1994), the presence of substantial numbers of alpaca possessing vicuña mtDNA also raises the possibility that the alpaca is of mixed origin or has undergone substantial hybridization. The limitations of mtDNA in the context of gene flow and evolution in livestock are obvious since historical and modern-day agricultural practice has often used desirable males to sire large numbers of females.

The microsatellites provided a stark contrast to the mtDNA, and the existence of two loci with nonoverlapping allele size ranges in the wild ancestors allowed us an unusual opportunity to compare patterns of divergence in relatively large numbers of domestic animals. Inspection of allele frequency distributions, genetic distances, and factorial correspondence all revealed a striking similarity between the alpaca and the vicuña. Each genetic distance estimate was lowest for the

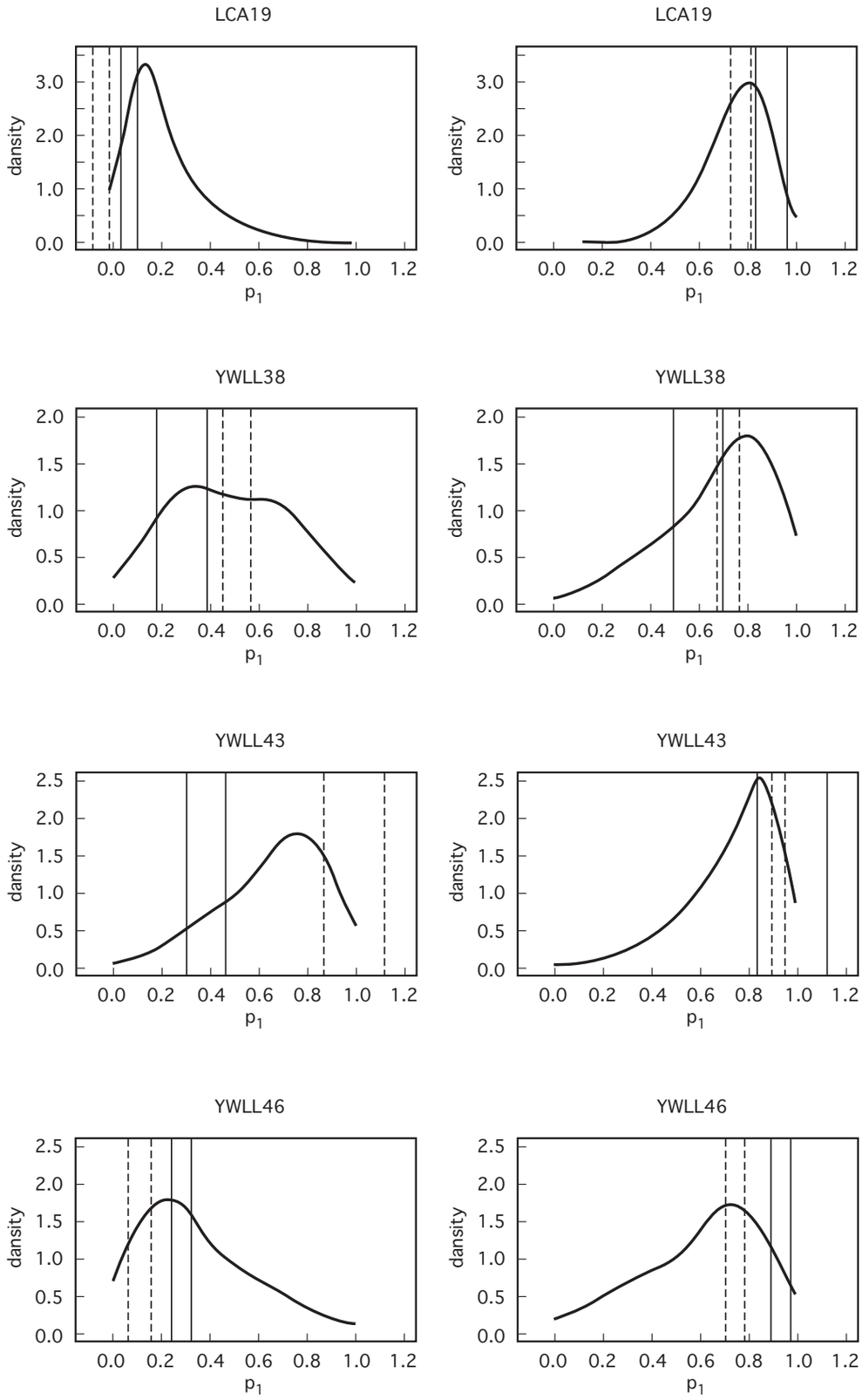

FIGURE 23.4 Admixture analysis using four microsatellite loci. Posterior probability density distributions for vicuña contribution in llama (p_1, left-hand graphs) and llama contribution in vicuña (p_1, right-hand graphs) are given for each locus. For comparison, mC and mY estimates (± one SD) are given as solid and dashed lines, respectively.

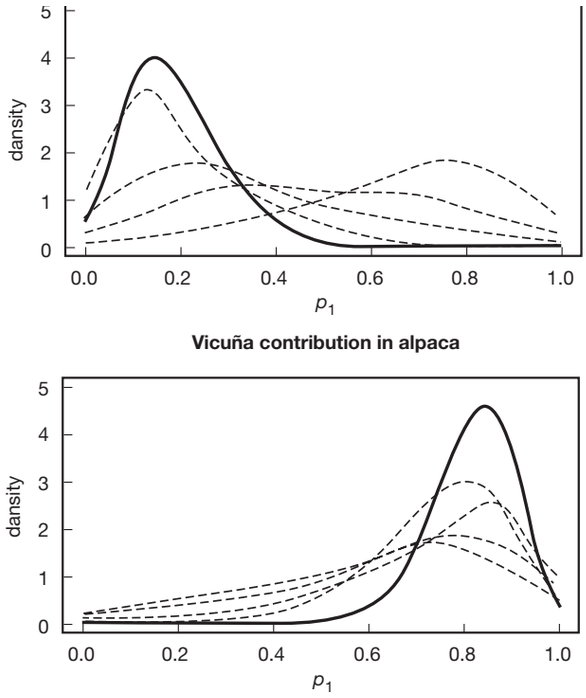

FIGURE 23.5 Posterior density distributions for admixture contributions (p_1) calculated from a combined analysis of four loci (solid line). For comparison, dashed lines show distributions for the individual loci.

alpaca-vicuña comparison, and factorial correspondence showed that alpaca and vicuña overlap almost completely. These data point toward a very close genetic affinity between alpaca and vicuña, a finding in direct conflict with the mtDNA data.

The microsatellites also supported a close relationship between llama and guanaco. Of the genetic distance estimates, both Reynolds's and the allele-sharing distances were second lowest for the guanaco-llama comparison, and the Reynolds's distance estimate was almost identical to that between alpaca and vicuña. Other data, however, were more equivocal, with factorial correspondence revealing a dispersed pattern for the llama intermediate between vicuña and guanaco and with (delta mu)^2 distances slightly lower for the llama-alpaca comparison than for llama-guanaco. Although none of the above is indicative of a close relationship between llama and vicuña, they suggest nuclear gene-flow between llama and vicuña, or, more likely, between llama and alpaca. Another possibility is that the ancestral llama was extremely genetically diverse. The guanaco and llama have much greater geographic ranges than do the vicuña and alpaca, which may have led to greater intraspecific differentiation historically, reflected in the greater diversity in nuclear and also mitochondrial DNA (21 guanaco haplotypes as opposed to 5 in vicuña). In fact, the most likely explanation lies in the geographic distribution of our samples. It was very difficult to obtain samples of *L.g. cacsilensis*, the northern, high-altitude guanaco subspecies, and the few samples available fall in with the llama distribution. Archaeozoological data suggest that the llama could have derived from this subspecies, and recent mtDNA data published by Palma et al. (2001) indicate an ancestral relationship between *L.g. cacsilensis* and *L. glama*. Additionally, the archaeozoological data point to the possibility of at least three independent domestication events, one each in Peru, Chile, and Argentina (Wheeler 1995; see also Mengoni Goñalons and Yacobaccio, Chapter 16, this volume).

The results presented here show significant differences between mitochondrial and microsatellite levels of introgression. These differences can occur for a number of reasons. The most obvious is that mtDNA is transmitted by females: the results presented here are simply the reflection of differential contributions from males and females of vicuña and guanaco origin. This seems to be a reasonable explanation, as we have noted above, given practices used in domesticated camelids. It may be worth noting that this hypothesis could be tested if data were available on the Y chromosome, as we would then expect a higher vicuña contribution in both llama and alpaca than observed here. Another possibility is suggested by our previous work on the estimation of admixture on simulated data sets (Kadwell et al. 2001). We found that loci simulated under the same conditions of drift and admixture could generate different posterior distributions, pointing sometimes to very different point estimates. This had led us to suggest that point estimates should be used with care, and the whole distribution should always be checked. Here, we found that the four loci generated very similar posterior distributions for alpaca, whereas for llama significant differences could be observed. For instance, the posterior distribution is rather flat for YWLL 38, whereas for locus YWLL43 the modal value is 0.76 (i.e., 76% of vicuña genes instead of the 14.4% figure obtained for the four loci together). This pattern could be the result of a much larger level of genetic drift in llama as compared to alpaca, at least in the samples considered here. As our method allows us to estimate drift, we checked what the estimated drift was for alpaca and for llama. We do find, indeed, that drift appears larger for llama than for alpaca with modal values being 0.07 vs. 0.041 (measured in units of generations/population size), respectively. One has to be cautious here, as we have already stated that the admixture methods used assume very simple models, which are unlikely to be correct. For instance, the vicuña contribution in llama might actually be the result of introgression with alpaca rather than with vicuña. The effect of drift, which we have found is the compound effect of drift and of sampling. We wish to note that the increased drift observed in llama could be the result of different factors, which include sampling, and could be investigated in the future.

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The suggestion of substantial mitochondrial introgression in the alpaca and nuclear introgression in the llama was reinforced when admixture was measured for both markers. The low estimated admixture proportion of vicuña mtDNA present in alpaca (0.31) was in contrast with the high proportion estimated for the microsatellites (0.82–0.90). Further, the

extremely low admixture proportion of vicuña mtDNA in the llama (0.02) contrasted equally strongly with microsatellite estimates (0.22–0.39), which also suggest substantial nuclear admixture in the llama.

Inspection of the three-locus genotypes confirmed many of the above findings. Only 27% of alpacas were mitochondrially "vicuña," although 40% of alpaca possessed vicuña microsatellite alleles with guanaco mtDNA haplotypes. Such a pattern suggests that introgression of guanaco (or more likely llama) mtDNA may have occurred recurrently within alpaca populations but may have been accompanied more recently by a reversion to line or stock breeding within local alpaca populations.

The lack of written records, both preconquest documents and present-day breed registries, in the Andean region means that any such inference is speculative. Table 23.1 suggests that mitochondrial introgression has occurred much less frequently in the llama. However, although nuclear introgression is similar in alpaca and llama, in the expanded microsatellite dataset it seems to have occurred at a higher level in llamas (two to three times higher than in alpaca from the admixture analysis), which may partly account for the more dispersed factorial correspondence pattern, but this warrants further investigation.

The implications of these data are potentially important for the way in which these genetic resources are managed in the future. In our sample, only 35% of domestic animals have not undergone any detectable hybridization. In particular, there are very large numbers of detectable hybrids in the alpaca (80%)—accentuated when using mitochondrial DNA. Forty percent of llama show detectable signs of hybridization, with mitochondrial introgression virtually absent.

During the last 20–25 years, large-scale hybridization between llamas and alpacas has been carried out in the Andes (Bustinza 1989). Specifically, male alpacas have been bred to female llamas to increase the population of animals producing higher-priced "alpaca" fiber, and male llamas have been bred to female alpacas to obtain greater fleece weights and, thus, increased income. With sale price traditionally determined by weight, and no consideration given to fineness, the quality of alpaca fiber has decreased markedly over the past 25 years. Indigenous Quechua- and Aymara-speaking herders subdivide the hybrids into llamawari or waritu (llama-like) and pacowari or wayki (alpaca-like) respectively, depending upon physical appearance (Flores Ochoa 1977; Dransart 1991). The F_1 offspring are fertile, tend to be intermediate in size, and can be back-crossed to either parental type. Further, recent intensive selection for white fleece in modern alpaca may also have involved bidirectional hybridization. A combination of these practices and our results could explain the taxonomic confusion surrounding the domestic forms in the recent past, as it is likely that many specimens used in previous taxonomic studies were hybrids.

Given the extreme hybridization in present-day alpacas, DNA analysis has been critical in resolving the origin of this domestic form. Since our results suggest the vicuña as the ancestor of the alpaca, we propose that the classification of the alpaca should be changed from *Lama pacos* L. to *Vicugna pacos* L. The degeneration of quality and value in fleece of present-day alpacas and llamas has therefore probably been the result of extensive hybridization, probably beginning with the conquest and continuing to the present day. While it was believed that these crosses were between different forms of a single domestic animal descended from the guanaco, there was little concern about the economic impact of such introgression. However, given that the alpaca is likely to be descended from the vicuña, the negative impact on fleece quality of such crosses is now evident. The use of DNA analysis to identify and eliminate hybrid animals from the breeding pool is essential, since the antiquity of the ongoing hybridization process makes it impossible to accurately identify all hybrids on the basis of phenotypic characteristics. Additionally, the knowledge that the alpaca descends from the vicuña opens new routes for the improvement of alpaca fiber production, not only through the identification of hybrids and their elimination from purebred elite herds, but through the back crossing of purebred alpacas to their vicuña ancestor in order to possibly improve fiber fineness.

Although 90% of the alpaca fiber produced in Peru today has a diameter greater than 25 µm and fetches low prices on the world market ($3–$30/kg, 1980–1995), preconquest animals produced fiber of 17–22 µm (Wheeler et al. 1995), similar to cashmere (15–17 µm: $60–$120/kg, 1980–1995). It is possible, therefore, that identification of the remaining pure alpacas may aid in recovery of the fine-fiber characteristics of preconquest animals. In 2002, CONOPA, Coordinadora de Investigación y Desarrollo de Camélidos Sudamericanos, began a survey of alpaca populations in the central Peruvian Andes, designed to identify genetically pure alpacas and to determine the relationship between fiber fineness and purity. In the future, a core herd will be established and accelerated reproductive technology will be applied to rapidly increase selected purebred animals in order to ensure survival of the species and promote repopulation programs in the Andes.

The knowledge that the alpaca is the domestic vicuña also necessitates a reevaluation of vicuña conservation policy. Although the vicuña has been listed as endangered under the Convention on International Trade in Endangered Species of Wild Fauna and Flora CITES (Appendix I) since its inception in 1975, all Peruvian vicuñas, and large segments of the Chilean, Argentine, and Bolivian populations, have been reclassified as threatened (Appendix II), permitting controlled commercialization of live shorn fiber. With unprocessed fiber currently valued at approximately $500/kg, vicuña fleece is the most expensive natural fiber in the world and represents an important potential source of income for the extremely poor rural populations on whose lands the animals live. To date, Peru's rational use policy has produced an important increase in vicuña numbers, but demands for greater control over the species through construction of fences, intensive rearing, and selection are growing. Judging by the impact of such measures on the alpaca, such interventions in the long

run will lead to a deterioration of fiber quality and fineness (which, at 12–14 mm, is the basis of its value), and increased limitation on movement, especially of the nonterritorial male bachelor bands, represents a significant new threat to this species.

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