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Y-chromosome and mtDNA variation confirms independent domestications and directional hybridization in South American camelids

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Summary

Investigations of genetic diversity and domestication in South American camelids (SAC) have relied on autosomal microsatellite and maternally-inherited mitochondrial data. We present the first integrated analysis of domestic and wild SAC combining male and female sex-specific markers (male specific Y-chromosome and female-specific mtDNA sequence variation) to assess: (i) hypotheses about the origin of domestic camelids, (ii) directionality of introgression among domestic and/or wild taxa as evidence of hybridization and (iii) currently recognized subspecies patterns. Three male-specific Y-chromosome markers and control region sequences of mitochondrial DNA are studied here. Although no sequence variation was found in *SRY* and *ZFY*, there were seven variable sites in *DBY* generating five haplotypes on the Y-chromosome. The haplotype network showed clear separation between haplogroups of guanaco–llama and vicuña–alpaca, indicating two genetically distinct patrilineages with near absence of shared haplotypes between guanacos and vicuñas. Although we document some examples of directional hybridization, the patterns strongly support the hypothesis that llama (*Lama glama*) is derived from guanaco (*Lama guanicoe*) and the alpaca (*Vicugna pacos*) from vicuña (*Vicugna vicugna*). Within male guanacos we identified a haplogroup formed by three haplotypes with different geographical distributions, the northernmost of which (Peru and northern Chile) was also observed in llamas, supporting the commonly held hypothesis that llamas were domesticated from the northernmost populations of guanacos (*L. g. cacilensis*). Southern guanacos shared the other two haplotypes. A second haplogroup, consisting of two haplotypes, was mostly present in vicuñas and alpacas. However, Y-chromosome variation did not distinguish the two subspecies of vicuñas.

Keywords *DBY*, d-loop, introgression, llama, patrilineage, vicuña

Studies of the evolutionary history of domestication have relied predominantly on the genetic variation of autosomal microsatellites or the mitochondrial genome. Relatively few studies have used markers from the male-specific region of the Y chromosome. Although less variable, the Y-chromosome is paternally inherited, and most of this chromosome

does not undergo homologous recombination at meiosis. Genetic data from the non-recombining portion of Y-chromosome loci are good candidates for extracting evolutionary information of the patrilineal history for mammals, and analyses based on the Y-chromosome in combination with mtDNA often reveal different patterns between female and male lineages (Pidancier *et al.* 2006). Additionally, Y-chromosome patterns are of particular interest in livestock species because the most common breeding strategies rely on a relatively small number of males each generation (Lindgren *et al.* 2004). Indeed, very low rates of nucleotide diversity have been reported within the Y-chromosome of horse (Lindgren *et al.* 2004), cattle (Hellborg & Ellegren

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2004; Götherström *et al.* 2005), pig (Ramirez *et al.* 2009), goat (Cinar Kul *et al.* 2015) and sheep (Meadows *et al.* 2006). Despite that, Y-chromosome variation has been useful in understanding the domestication of horses (Lindgren *et al.* 2004) and in confirming the independent domestications of swamp and river buffalo (Yindee *et al.* 2009).

The origins of South America's domestic alpaca (*Vicugna pacos*) and llama (*Lama glama*), despite new autosomal microsatellites and mitochondrial genome evidence, have been difficult to resolve in part because of hybridization and their near extirpation during the Spanish conquest. Guanaco (*Lama guanicoe*), vicuña (*Vicugna vicugna*) and domestic forms have the same number of chromosomes, $2n = 72$ (Taylor *et al.* 1968) and very similar C and G banding patterns (Bunch *et al.* 1985). However, fine-scale differences on the short arms of chromosome 1 separate camels, guanacos and llamas from vicuñas and alpacas (Marín *et al.* 2007a). At the molecular level, The *cytochrome b* gene and control region sequences of mitochondrial DNA (mtDNA) provide relatively weak support for the phylogenetic relationships among wild and domestic forms of camelids (Stanley *et al.* 1994; Kadwell *et al.* 2001; Marín *et al.* 2007a) but have confirmed the occurrence of extensive hybridization between llamas and alpacas, probably since the arrival of Europeans and facilitated by the fact that interspecies crossbreeding generated fertile hybrids (Gray 1954; Skidmore *et al.* 2001). Genetic studies suggesting that there are only two subspecies of guanaco, instead of four—*L. g. cacsilensis* and *L. g. guanicoe* (Marín *et al.* 2013), and *L. g. voglii* and *L. g. huancacus* (for morphological details, see Wheeler 1995; González *et al.* 2006)—were not supported. In contrast, similar analyses confirmed the two morphologically described vicuña subspecies *V. v. mensalis* and *V. v. vicugna* (Wheeler 1995; Marín *et al.* 2007b). Here we present the first Y-chromosome sequence data combined with mtDNA haplotype data from the same individuals in the four species of South American camelids (SAC). Our objectives were: (i) to test hypotheses about the origin of domestic camelids, (ii) to identify directionality of introgression among domestic and/or wild taxa as evidence of hybridization using sex-linked and (iii) to assess currently recognized subspecies patterns.

Our analyses included 99 guanacos and vicuñas from Peru, Bolivia, Argentina and Chile as well as 86 llamas and alpacas (Table S1). We sequenced two regions of SRY and one region of both ZFY and DBY of the Y chromosome. In addition, we sequenced the hypervariable I domain of the control region of mtDNA (Table S2). DBY and hypervariable I domain sequences were deposited in GenBank (accession nos. KY420200–KY420384 and KY420385–KY420569 respectively).

There was no sequence variation observed in ~2500 bp of ZFY and SRY fragments. However, in 684 bp of DBY (a Y-chromosomal single-copy sequence) there were seven polymorphic sites (Table S3) that defined five haplotypes.

Three of these were relatively common (DBY1, DBY2 and DBY4) compared to two minor haplotypes (DBY3 and DBY5), which were observed in only two patrilineages in SAC, suggesting that the major haplotypes originated prior to domestication (Fig. 1a). However, mitochondrial DNA variation for the same set of individuals had a high degree of polymorphism, with 64 polymorphic sites and 81 haplotypes (Table S4) that revealed four well-defined clusters with Bayesian analysis of population structure (BAPS) analysis (Appendix S1) that we call d-loop 1, d-loop 2, d-loop 3 and d-loop 4 (Fig. 1b).

The DBY network reflects a clear divergence between guanaco–llama and vicuña–alpaca haplogroups, which are separated by five mutational steps (Fig. 1a). Specifically, the near total absence of shared haplotypes between guanacos and vicuñas indicates that there is a reproductive barrier that maintained genetic integrity without hybridization between wild forms despite past bottlenecks and low population size some decades ago (Marín *et al.* 2007b, 2013) and in spite of the observation that crossbreeding among species produces fertile hybrids in captivity (Gray 1954), as was observed in our alpaca–northern guanaco hybrid sampled in captivity. Reproductive barriers may also impede extensive hybridization (Ward *et al.* 2001). In contrast, alpacas and llama shared haplotypes, supporting the idea that hybridization occurred among domestic camelids during and after domestication and/or after the Spanish conquest (Wheeler 1995).

The guanaco–llama haplogroup was formed by DBY1, DBY2 and DBY3. Haplotype DBY1 was observed in one northern guanaco and 92% of llamas, whereas haplotypes DBY2 and DBY3 were largely restricted to southern guanacos (with the exception of one alpaca that had haplotype DBY2). The occurrence of three haplotypes in the guanaco and a frequency of 100% of haplotype DBY1 in the northern guanacos and most of llamas strongly suggest that llamas are derived from northern populations of guanacos. Moreover, our results confirm the subspecific differentiation of guanacos into *L. g. cacsilensis* and *L. g. guanicoe* (Marín *et al.* 2013). Therefore, based on parental lineage patterns, we propose that the border between *L. g. cacsilensis* and *L. g. guanicoe* occurs around 31° S in Chile. Y-chromosome patterns may partly reflect the reproductive behaviour of males, which often dominate a territory for several years (Young & Franklin 2004), and of females, which largely have higher mobility across landscape without male control (Franklin 1983). This behaviour could lead to disparate patterns between genetic markers (mitochondrial and Y-chromosome results).

A vicuña–alpaca haplogroup was formed by DBY4 and DBY5. DBY4 was found in 95% of the vicuñas, 92% of the alpacas, four llamas and one northern guanaco, whereas haplotype DBY 5 was restricted to only two alpacas (Fig. 1a). In contrast with guanaco, there was no evidence of subspecific differentiation among vicuñas (*V. v. mensalis*

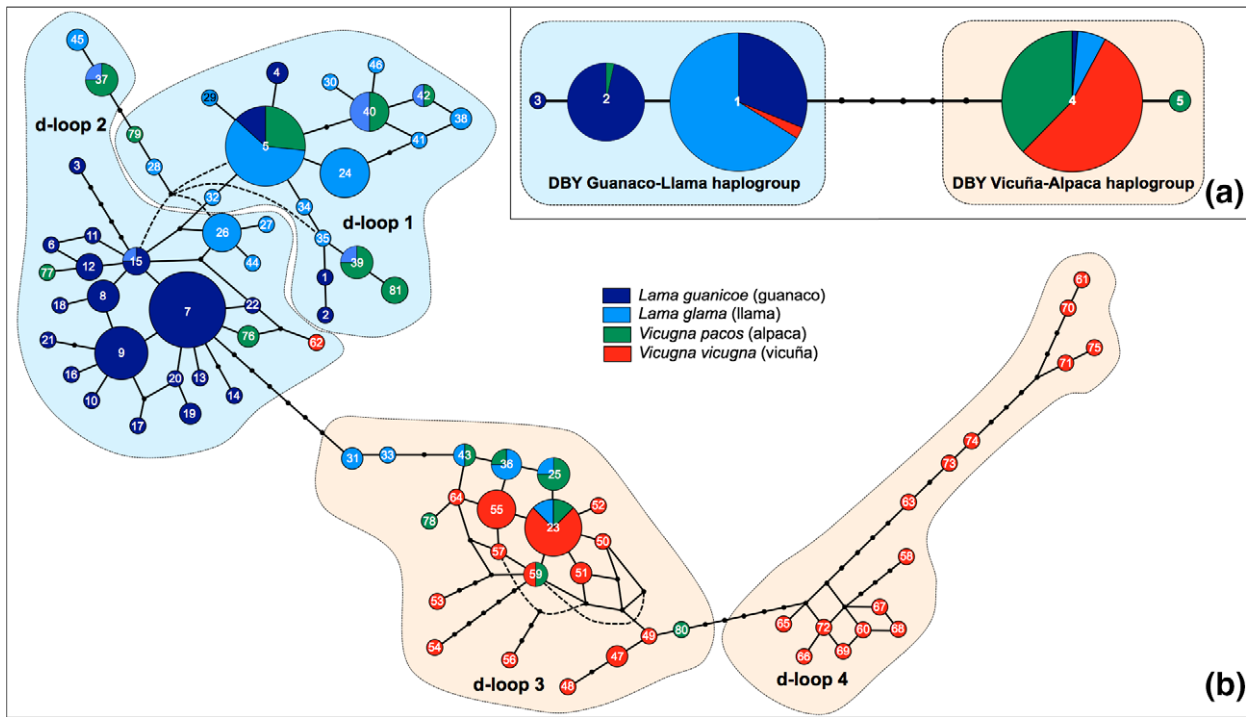


Figure 1 Minimum spanning network representing the relationships among five *DBY* haplotypes (a) and 81 d-loop haplotypes (b) grouped into four clusters detected by BAPS (Appendix S1). Each haplotype is represented by a circle and number, with its size proportional to haplotype frequencies. Colours indicate phenotypic species: dark blue = guanacos; light blue = llamas; green = alpacas; red = vicuñas.

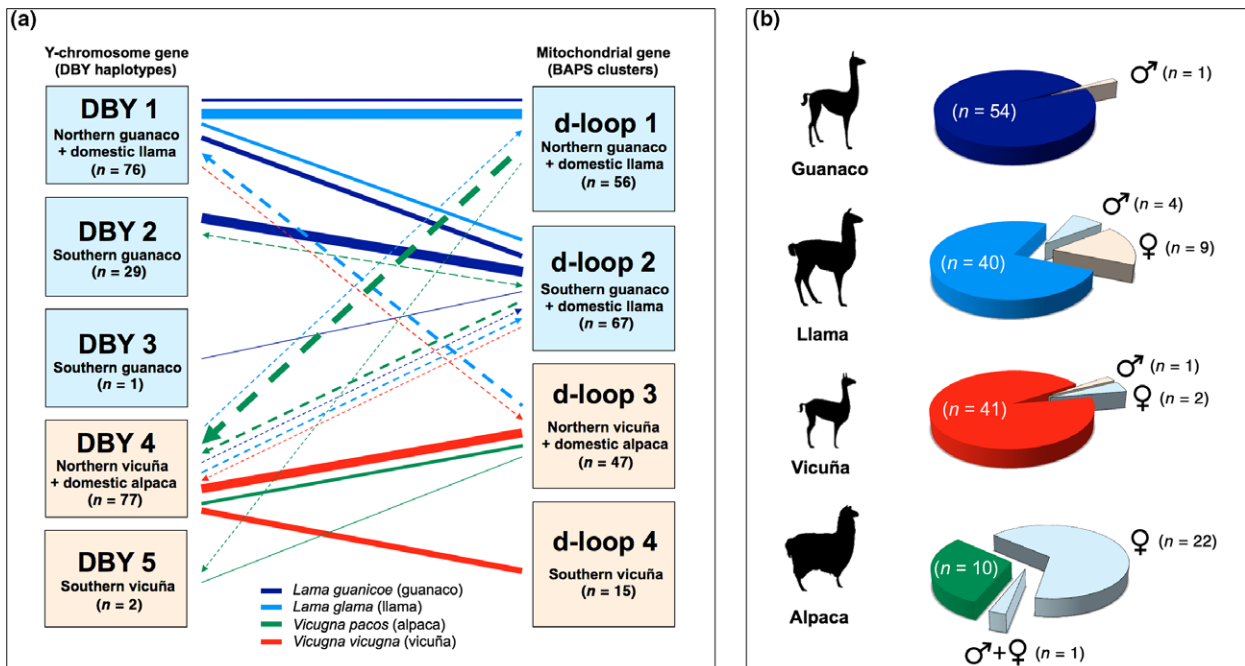


Figure 2 (a) Relationships among the five *DBY* haplotypes and four BAPS clusters of d-loop sequences observed in four species of South American Camelids. *DBY1*, *DBY2* and *DBY3* were observed predominantly in guanacos and llamas and *DBY4* and *DBY5* in vicuñas and alpacas. The thicknesses of the lines are proportional to frequency (Table S5). Discontinuous lines depict hybrid individuals, and arrows indicate the direction of introgression. (b) Proportion and number of pure (following colour scheme of Fig. 1 for phenotypic species) and hybrid individuals. Hybrids are noted with ♂ to represent the proportion with paternal introgression, ♀ for maternal introgression and ♂ + ♀ to show the proportion (only one individual) with evidence of paternal and maternal introgression.

and *V. v. vicugna*), as clearly shown by mtDNA data (Marín *et al.* 2007b) and here. Mitochondrial and Y-chromosome patterns probably reflect a more rigid reproductive strategy by which females are confined to territory actively controlled by the male (Franklin 1983). Absence of differentiation using Y-genes is perhaps a function of sample size, but a rapid exchange of territorial males and high male mobility may also be a factor.

The mitochondrial phylogenetic network (Fig. 1b) suggests that the divergence among vicuña–alpaca maternal lineages is larger than that observed between the guanaco and llama. Although relatively few hybrids were observed among the wild individuals, hybridization was much more common in domestic individuals, among which 67% of alpacas had evidence of maternal introgression and 25% of llamas had patterns consistent with maternal and paternal introgression (Fig. 2, Table S5).

The SAC represent a unique opportunity for further study because, unlike what has occurred in many other domestic livestock, the two wild ancestors of both domestic forms are still common and co-occur through much of their range (Wheeler 1995; Gentry *et al.* 2004). Additional sequence data from a larger sample of individuals is needed to better understand the chronology of domestication, hybridization and geographic patterns of Y-chromosomal variation, especially from domestic and wild populations near Peru, Argentina and Bolivia where domestication is presumed to have happened. These data will likely be relevant for the genetic management of SAC populations, especially for alpacas, which have experienced significant declines in fibre quality that have been linked to increased hybridization (Wheeler *et al.* 1995). The differences between maternal and paternal genetic introgression patterns reported here may be the consequence of deliberate hybridization by breeders of alpaca males with llama females, probably to increase body size and fleece weight, followed by subsequent backcrossing with alpaca males to improve the fibre fineness (Kadwell *et al.* 2001). The patterns observed here should be confirmed with additional individuals of known origin and additional genetic markers. Given the extreme maternal introgression observed in present-day alpacas, the pattern of Y-chromosomal variation has been important to confirm independent domestications of llama from guanaco and alpaca from vicuña. Our results provide strong support for the hypotheses that domestic camelids descended from wild camelids and not an extinct form (López-Aranguren 1930; Cabrera 1932) or that hybridization between llama and vicuña produced the alpaca (Hemmer 1990). Finally, our findings highlight the importance of analysing both maternally and paternally inherited loci to obtain a comprehensive understanding of geographic structure in wild camelids and the domestication of these species, which have been essential to the lifestyle and economy of the Andean peoples.

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Supporting information

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Appendix S1 Materials and methods.

Table S1 List of male South American camelids analysed.

Table S2 List of primers used for polymerase chain reaction (PCR) and sequencing.

Table S3 Variation at seven polymorphic sites among five haplotypes of South American camelids of *DBY* fragment (Y chromosome).

Table S4 Variation at 64 polymorphic sites among 81 haplotypes of South American camelids of hypervariable I domain sequences (d-loop, mtDNA).

Table S5 Distribution of the five *DBY* haplotypes of four clusters of d-loop sequences observed in four species of SAC.