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Small Ruminant Research 71 (2007) 123–129
Small Ruminant Research
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Mitochondrial heteroplasmy in Control Region DNA of South American camelids

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Received 11 January 2006; received in revised form 19 April 2006; accepted 27 April 2006
Available online 25 July 2006

Abstract

In the present work, polymerase chain reaction-single strand conformation polymorphism and sequencing were used to investigate the length and nucleotide variability in the Control Region mitochondrial DNA of the four South American camelid species from Argentina. To asses these the complete Control Region of 20 animals, 5 each of guanaco, llama, alpaca and vicuña species were cloned. Seventy-three clones corresponding to the 20 animals were screened and 7 different SSCP patterns were identified. Sequencing of all clones showed 9 different haplotypes contained in the 350 bp hypervariable segment of the Control Region. Interestingly, 3 guanacos, 3 vicuñas, 3 alpacas and 1 llama were heteroplasmic for different nucleotide positions. The screening of the Control Region mitochondrial DNA in blood samples from about 200 wild guanacos from Argentine Patagonia supported the above results. After comparison with other vertebrate species, we concluded that nucleotide substitutions are the main cause of heteroplasmy found in Control Region mitochondrial DNA of these taxa.

Keywords: Control Region mtDNA; Single nucleotide heteroplasmy; SSCP-DNA sequencing; South American camelids

1. Introduction

Mammalian mitochondrial DNA (mtDNA) is a 16–18 kb circular molecule located within the mitochondria. As part of this cytoplasmic DNA, there is a large non-coding region named Control Region (CR) containing the origin of replication of the heavy strand and the H-strand and L-strand promoters. The CR shows a high sequence variation even among related species and is currently used as molecular marker to track geographic patterns of genetic diversity and evolution, gene flow, demographic expansion and hybridization among genetically closed related individuals (Bruford et al., 2003). However, for a more comprehensive assessment of the usefulness of this molecular marker, rates and patterns of CR variability need to be critically examined in a wide range of taxa (Moum and Bakke, 2001).

Population studies on many species revealed variation in the size of the mitochondrial genome. Three types of polymorphisms have been identified: (1) duplication of large sequences including structural genes in bird species (Abbott et al., 2005), (2) nucleotide number variation at the homopolymer tract in human and cattle species (Bendall and Sykes, 1995; Hauswirth et al., 1984), (3) mtDNA length polymorphisms caused by different copy number of tandemly repeated sequences. The occurrence of repeated motifs in the CR, with extensive length variability among individuals (Nagata et al.,

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doi:10.1016/j.smallrumres.2006.04.016

1999) or different number of repeats in one individual (heteroplasmy), has been reported for various species (Xu and Arnason, 1994; Hiendleder et al., 1998).

Recently, we reported the structure and organization of the Control Region mitochondrial DNA (CR mtDNA) in the four species of South American camelid (SAC) (Maté et al., 2004). Within this region and close to the flanking $tRNA^{phe}$ gene, we described a 350 bp hypervariable segment that includes 10 polymorphic sites and a 78 nucleotide repeated motif located between the conserved sequence blocks CSBI and CSBII. This motif comprises three repeats of 26 bp each, differing in sequence but not in length when compared between each other and among individual camelids. We defined seven different haplotypes, taking the sequence of a wild guanaco as reference.

For many years, most mitochondrial genomes in one individual have been assumed to be identical, even when different tissues are compared. A bottleneck mechanism during oogenesis was generally believed to be responsible for maintaining homoplasmy (Bendall and Sykes, 1995). However, the notion of predominant homoplasmy has been questioned in recent reports on mammals, some of which describe extremely high levels of heteroplasmy. Based on these findings of heteroplasmy caused by mitochondrial length or single nucleotide variability, we proposed to further investigate the genetic diversity of the CR mitochondrial DNA in the four species of South American camelids.

Accordingly, we report here results obtained for 73 CR mtDNA clones from 20 SAC individuals by combining PCR-SSCP and DNA sequencing. Findings showed extensive heteroplasmy caused by single nucleotide substitutions in the CR of SAC. SSCP and sequencing analyses of the mitochondrial hypervariable region from wild guanaco populations of Argentine Patagonia supported these findings.

2. Materials and methods

The complete Control Region mitochondrial DNA of 20 South American camelids (SAC), five unrelated individuals each of guanaco, llama, alpaca and vicuña species was amplified by PCR using the conditions and primers (set 1) previously described (Maté et al., 2004). The amplified product was run in 1% agarose gel and the 1.2 kb band cut. DNA was purified and ligated into the p-GemT-easy vector (Promega Corp., Madison, WI, USA). The ligation mix was used to transform JM109 cells (Promega Corp.) from which recombinant plasmids were purified by the QIAprep Spin Miniprep kit (QIAgen, Valencia, CA, USA). The 350 bp CR hypervariable segments from 73 clones were amplified in a 15 μl volume containing 1× PCR buffer (10× buffer is 500 mM KCl, 200 mM Tris–HCl pH 8.4), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 U of Taq polymerase (Invitrogen, Carlsbad, CA, USA), 10 ng of cloned target DNA and 1 μM of each of the following primers: Fw 5′ TACAATACGACTGTGTTGCC 3′ and Rv 5′ TGAATATCATTTACCCGCAT 3′ (set 2). These primers were designed based on the complete CR mtDNA sequence from SAC reported in Maté et al. (2004). Cycling profile included an initial denaturation step of 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 56 °C, 1 min at 73 °C and a final extension at 73 °C during 10 min. The PCR product was analysed by SSCP technique for which 30 μl Low Ionic Strength (LIS) loading buffer (10% sucrose, 0.01% xylene cyanol FF and 0.01% bromophenol blue) was added to each 15 μl of the product. After heating denaturation and fast cooling, 10 μl sample was loaded to a 10% neutral polyacrylamide gel. Electrophoresis was carried out at 350 V, in 0.5% TBE buffer for 20 h at 5 °C. Afterwards, gels were fixed in 5% ethanol, stained with 0.1% silver nitrate and developed with 3% sodium carbonate. In order to match SSCP patterns with the corresponding mitochondrial haplotypes, 3 to 7 clones per animal were automatically sequenced on both strands by using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were analyzed using an Automated 3730 DNA analyzer (Applied Biosystems). Results from the DNA sequencing were validated using alternatively two set of primers (sets 1 and 2) for amplification of the CR hypervariable segment. The relationship among the obtained mitochondrial haplotypes was displayed by generating a Median Joining Network using the Network 4.1.1.2 software (Bandelt et al., 1999).

The 350 bp hypervariable CR mtDNA segments from about 200 wild guanacos from six localities from Argentine Patagonia were investigated by SSCP for which DNA from blood samples was amplified by PCR using the primer set 2. Individuals exhibiting SSCP patterns not clearly defined respect to those previously identified were sequenced. Multiple alignment of the sequences was obtained by using the GeneDoc v 2.6.002 software (Nicholas and Nicholas, 1997).

3. Results

3.1. Analysis of the clones

The primer set 1 flanking the complete CR mtDNA was used to amplify genomic DNA isolated from 20 individuals representing the four SAC species. PCR products

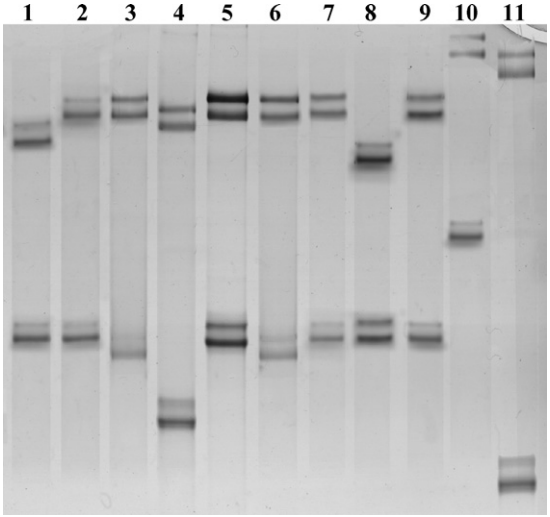

Fig. 1. SSCP patterns from hypervariable Control Region mitochondrial DNA clones in the four species of SAC. 1, 2 patterns of two clones from one guanaco individual; 3, 4, 5 patterns from one vicuña; 6, 7, 8 patterns from one llama and 9, 10 SSCP patterns from one alpaca. 11 SSCP profile from one vicuña with a 26 bp motif deletion.

Table 1
Control Region mitochondrial DNA haplotypes defined from 11 polymorphic sites

Haplotype	1 6 5	2 2 4	2 5 9	2 9 1	3 2 8	3 4 0	3 7 2	4 0 1	4 2 1	4 5 4	5 0 2
H1	A	A	A	G	T	A	C	T	T	A	T
H2[a]	G	–	–	–	–	G	–	–	–	–	–
H3[b]	G	T	–	–	A	G	–	–	–	–	–
H4[c]	G	T	–	T	A	G	–	–	–	–	–
H5	G	T	–	–	A	G	–	C	–	–	–
H6	G	T	–	T	A	G	–	–	–	G	–
H7	G	T	G	–	A	G	–	–	C	–	C
H8[d]	G	T	–	–	A	G	del	–	–	–	–
H9	G	T	–	–	A	G	T	–	–	–	–

Vertical numbers in the upper box indicate nucleotide positions, relative to the clone C sequence of reference guanaco (a); Below, nucleotide changes and the resulting haplotypes are shown. Dots represent identity with the reference sequence.
[a]H2, [b]H3 and [c]H4 haplotypes are indistinguishable by SSCP analysis.
[d]H8 presents a 26 bp motif deletion that includes the polymorphic site 373.

were cloned in a plasmid vector and recombinants (three to seven per individual) were checked by EcoRI digestion and 1% agarose gel electrophoresis. All cloned animals showed an expected band about 1200 bp long comprising the CR mtDNA. Amplification of the hypervariable fragment with primer set 2 described above showed a 350 bp long insert in 68 clones from 19 out of 20 individuals. The exception was a vicuña individual in which five clones showed a 324 bp insert (Fig. 1).

SSCP analysis of all 73 clones resulted in seven different patterns; however sequencing of these patterns revealed nine different haplotypes. Seven of these coincided with those previously described by Maté et al. (2004), while the other two (haplotypes 8 and 9), corresponded to newly described haplotypes (GenBank data base accession number DQ270409–DQ270408, respectively) (Table 1). The sequence analysis of haplotype 8 with striking high electrophoretic mobility showed that this vicuña individual underwent a 26 bp deletion affecting one of the three repeated motifs of the CR mtDNA.

The haplotype distribution in the whole South American Camelid sample analysed here is shown in the Table 2. As shown H3 besides to be the most frequent haplotype observed it is shared by the four species while H1 being the second one in frequency it is absent from alpacas.

The median joining network generated with the mtDNA haplotypes allowed inferring the relationships among the nine CR mitochondrial haplotypes. Accordingly, the networks constructed suggest H3 as the haplotype from which the rest of the haplotypes described here, derived (Fig. 2).

The most remarkable finding of this study is the presence of heteroplasmic individuals. SSCP analysis of 39 clones from 10 animals (3 guanacos, 3 vicuñas, 3 alpacas and 1 llama) showed more than one different profile for the same individual (Fig. 1). In order to further investigate these findings each clone was sequenced on both strands with primer sets 1 and 2 and more than one haplotype per animal was observed. On the other hand, heteroplasmic sites showed to correspond mostly with the CR mtDNA polymorphic sites previously described (Maté et al., 2004). Table 3 shows the mitochondrial haplotype distribution in the 10 heteroplasmic animals.

As can be seen in the table each heteroplasmic individual shows between 2 and 3 different haplotypes. H3

Table 2
Haplotype distribution in 20 cloned animals

Species	Haplotypes									
	H1	H2	H3	H4	H5	H6	H7	H8	H9	Total
Llama	14	–	3	–	1	–	1	–	–	19
Alpaca	–	–	9	1	–	1	5	–	–	16
Guanaco	6	1	9	–	–	–	1	–	–	17
Vicuña	4	–	6	–	2	3	–	5	1	21
Total	24	1	27	1	3	4	7	5	1	73

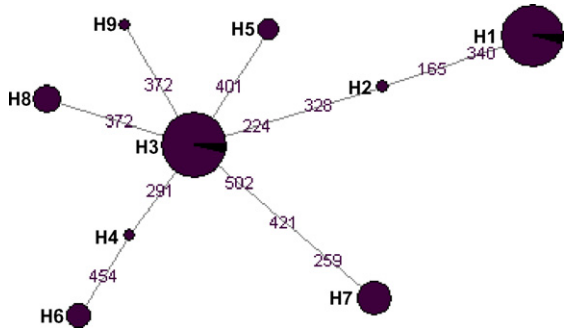

Fig. 2. Median joining network showing the phylogenetic relationships between the nine observed mitochondrial control region haplotypes. Numbers along branches indicate the nucleotide position differentiating each haplotype. The number of each haplotype appears in the respective circle. The circle area is proportional to the frequencies of each of the haplotypes.

haplotype is the only one present in the 10 heteroplasmic individuals and also it is the most frequent in the four species. While H7 haplotype is shared by guanaco, llama and alpaca H6 is found in alpaca and vicuña, H5 in llama and vicuña and H1 and H4 are private haplotypes of guanaco and alpaca, respectively.

Table 3
Haplotype frequency in 10 heteroplasmic animals from the four SAC species

Animal	No. of clones	Haplotype–%	Haplotype/species–%
Guanaco a	4	H1–25	
		H3–75	
Guanaco b	4	H1–75	H1–33
		H3–25	H3–58
			H7–8
Guanaco c	4	H3–75	
		H7–25	
Vicuña a	3	H3–33	
		H6–67	
Vicuña b	5	H3–40	H3–54
		H5–40	H5–15
		H8–20	H6–23
			H8–8
Vicuña c	5	H3–80	
		H6–20	
Llama a	3	H3–34	H3–34
		H5–33	H5–33
		H7–33	H7–33
Alpaca a	3	H3–67	
		H7–33	
Alpaca b	4	H3–75	H3–73
		H6–25	H4–9
			H6–9
Alpaca c	4	H3–75	H7–9
		H4–25	

Table 4
Mitochondrial haplotype distribution in a Patagonian guanaco population

Localities	No.	Haplotypes							
		H1	H2	H3	H4	H5	H6	H7	H8
Pilcaniyeu	28	2	–	26	–	–	–	–	–
Río Mayo[a]	39	30	–	37	–	2	–	–	–
Las Heras[b]	28	–	–	25	–	5	–	–	–
La Esperanza	24	1	–	23	–	–	–	–	–
Camarones[c]	34	–	–	34	–	2	–	–	–
Cabeza de vaca	46	1	–	45	–	–	–	–	–

N Analysed individuals per locality.
[a] 30 animals having heteroplasmy due to coexistence of H1 and H3 patterns.
[b] 2 animals having heteroplasmy due to coexistence of H3 and H5 patterns.
[c] 2 animals having heteroplasmy due to coexistence of H3 and H5 patterns.

3.2. Population screening

The population study included genomic DNA from 198 wild guanacos in which the CR mtDNA hypervariable segment was amplified using primer set 2. All animals showed a single agarose electrophoresis band corresponding to 350 bp long segment. Table 4 shows the mitochondrial haplotype distribution as shown by PCR-SSCP in all guanacos here analysed.

In 34 out of the 198 animals studied the SSCP analysis of the PCR products showed the simultaneous presence of two electrophoretic patterns per individual suggesting heteroplasmy (Fig. 3, Table 4).

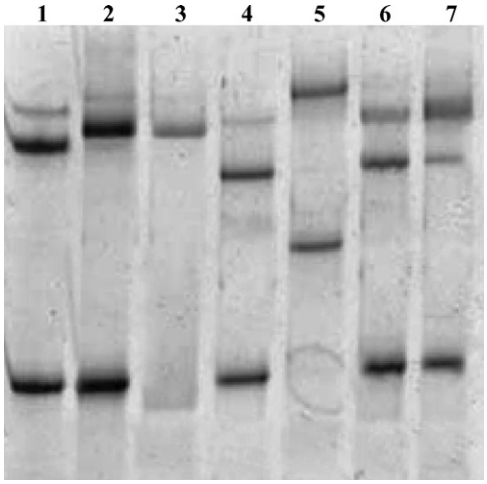

Fig. 3. SSCP patterns corresponding to mtDNA hypervariable segment amplified by PCR in genomic DNA from two wild guanacos. 1–5 SSCP patterns P1, P2, P3, P4 and P5; 6–7 P2 and P4 SSCP overlapping patterns in mtDNA from two different animals.

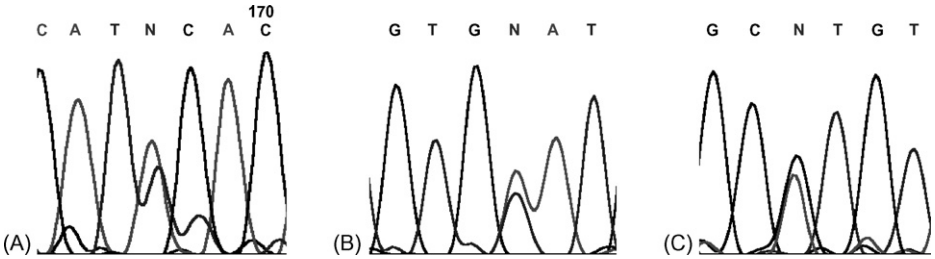

Fig. 4. Electropherograms illustrating mtDNA heteroplasmy in genomic DNA from the same guanaco individual. (A) Forward sequence of A/T transversion at 328 position; (B) reverse sequence of A/T transversion at the same nucleotide position; (C) forward sequence of A/G transition at 340 polymorhic site.

In order to discard coamplification of CR mtDNA and nuclear copies of this region, we generated templates for sequencing both strands using two sets of primers in separate amplification reactions. Direct sequencing of these fragments showed identical sequences, thus discarding false heteroplasmy due to nuclear copies of the CR mtDNA. Fig. 4 shows electropherograms from sequencing of PCR amplified hypervariable segments in one guanaco individual. Pictures A and B depict forward and reverse reactions for the A/T heteroplasmy of the 328 polymorphic site and C shows forward sequence of A/G heteroplasmy at 340 nucleotide position.

4. Discussion

In the present study, length polymorphisms and heteroplasmy of cloned CR mtDNA from four SAC species were analysed by PCR-SSCP techniques combined with DNA sequencing. Very low level of length polymorphism was detected since only one vicuña out of the 20 animals cloned showed deletion of a 26 bp long repeated motif.

On the other hand, the whole sample showed nine mitochondrial haplotypes from which haplotype 3 (H3) besides to be the most frequent it is the only one shared by the four species. The median joining network constructed points to H3 as the ancient haplotype owing to its presence in the four SAC species and the fact that it is the haplotype from which the other haplotypes derived by 1–4 mutational steps. On the other hand, the presence of H6 in vicuñas and alpacas gives support to the current hypothesis that the alpaca is a domesticated vicuña (Wheeler, 1995). In addition, the presence of H7 in guanacos, llamas and alpacas would agree with Kadwell et al. (2001) proposal of guanaco-llama mitochondrial introgression in the alpaca.

The finding of single nucleotide heteroplasmy in the four SAC species is worth to be mentioned. This feature was confirmed by repetitive forward and reverse sequencing. Moreover, this type of heteroplasmy was proved highly frequent in blood samples from a Patagonian guanaco population of about 200 individuals. Accordingly, PCR amplification of the CR hypervariable segment followed by SSCP analysis showed several animals with more than one SSCP profile. Sequencing of each profile confirmed the presence of at least two distinct mitochondrial haplotypes per individual, consistent with the different SSCP profiles observed. Nuclear copies of CR mtDNA in SAC have not yet been described. However, we discarded this possibility as the origin of false heteroplasmy by sequencing mitochondrial hypervariable segments amplified with two sets of primers. We observed that nucleotide positions causing heteroplasmy coincided with several nucleotide sites accountable for polymorphisms among individuals (Maté et al., 2004). This finding suggests that the same mutational mechanism is responsible for both the generation of single nucleotide heteroplasmy and polymorphism (Moum and Bakke, 2001). On the other hand, according to Grzybowski (2000) the existence of positive correlation between heteroplasmy and mutation rate at particular nucleotide positions suggests that heteroplasmy might be an intermediate stage between two homoplasmic conditions.

A bottleneck mechanism during oogenesis was generally believed to be responsible for maintaining homoplasmy. However, the argument of predominant homoplasmy has been questioned in recent reports on mammals, some of which described extremely high levels of heteroplasmy. Mitochondrial heteroplasmy has been reported in a wide range of species, including insects (Kondo et al., 1990), birds (Crochet and Desmarais, 2000), fish (Nesbø et al., 1998) and mammals (Gyllensten et al., 1991; Schwartz and Vissing, 2002). The heteroplasmic condition mostly involves length variation caused by a variable number of tandem repeats in the Control Region. This variation is thought to be caused by slipped strand misspairing during

replication (Densmore et al., 1985). Heteroplasmic single nucleotide polymorphisms have been also reported in several species (Hauswirth et al., 1984; Moum and Bakke, 2001). All of these forms of heteroplasmy can be generated by de novo mutations in an individual or by oocyte heteroplasmy.

Animal mitochondrial DNA has been generally believed to be maternally inherited. However, failure in finding the paternal mtDNA might be due to a technical matter: the extremely small contribution of sperm mtDNA compared to oocyte mtDNA. Thus, paternal leakage, which occurs when the paternal mitochondria are not completely eliminated during the egg fertilization, could be another possible mechanism producing heteroplasmy. Paternal leakage has been observed in several species through experimental crosses and backcrosses among different species. Thus, Kondo et al. (1990) reported that in 0.2% *Drosophila* lines studied, the mitochondrial maternal type was completely or partially replaced by the paternal type generating heteroplasmy in the latter case. In *Mus*, Gyllensten et al. (1991) proposed the persistent transmission of the leaked paternal mtDNA to subsequent generations; contrarily Shitara et al. (1998) observed that the paternal mtDNA leakage is limited to the first generation of an interspecific *M. musculus* × *M. spretus* cross and does not occur in progeny from subsequent backcrosses. These observations suggest that species-specific exclusion of sperm mtDNA in mammalian fertilized eggs is extremely stringent, indicating strictly maternal inheritance of mtDNA.

The social organization of wild South American camelids comprises three groups: family groups with an adult male leader and up to seven females with their 1-year-old offspring, young males and single adult males (Puig and Videla, 1995). Due to the polyginic character of the familial group, it is likely that the male leader backcrosses with F1 females. According to Gyllensten et al. (1991) report, this could favor paternal mtDNA leakage giving rise to heteroplasmic offspring. However, it is not possible to determine whether mitochondrial DNA has leaked recently or if it took place in previous generations and was followed by maternal transmission of the heteroplasmy. From the present results we may conclude that single nucleotide heteroplasmy is frequently found in SAC. De novo mutation might be discarded because differences between haplotypes from heteroplasmic animals involved more than one mutation step. However, further studies are necessary in order to determine whether oocyte heteroplasmy or paternal mtDNA leakage, combined or individually, may be responsible for the single nucleotide heteroplasmy shown here.

5. Conclusion

Mitochondrial heteroplasmy has been reported in species ranging from insects to mammals. The heteroplasmic condition mostly involves tandem repeats length variation caused by slipped strand misspairing during replication. However, heteroplasmic single nucleotide polymorphisms due to the novo mutations, oocyte heteroplasmy and paternal leakage have been also seen in several species. In this report, we present single nucleotide polymorphisms as the main cause of heteroplasmy in the four South American camelid species. The repeated motif contained in the Control Region was shown involved in a length polymorphism not heteroplasmic. Further studies will be necessary to investigate the mechanism responsible for the single nucleotide heteroplasmy reported here.

Acknowledgements

The authors thank Dr. D. Delamo (CENPAT, Puerto Madryn), Dr. J. von Thüngen (INTA-Bariloche), Dr B. Carpinetti (Estación ECAS, Buenos Aires), Dr. V. Roig (Mendoza Zoo) and Ing. Hugo Lamas (INBIAL, Jujuy) for providing the Argentinean camelid blood samples used in this investigation. Special thanks go to S. Diaz and G. Giovambattista from CIGEBA, UNLP, for the help during the SSCP analysis, to E. Fernandez for technical assistance and to B. Tosti for the English revision. This study was supported by grants from ANPCyT, CONICET and CICPBA of Argentina.

References

Abbott, C., Double, M., Trueman, J., Robinson, A., Cockburn, A., 2005. An unusual source of apparent mitochondrial heteroplasmy: duplicate mitochondrial Control Region in *Thalassarche albatrosses*. Mol. Ecol. 14, 3605–3613.

Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48, b.

Bendall, K., Sykes, B., 1995. Length heteroplasmy un the first hipervariable segment of the human mtDNA control region. Am. J. Hum. Genet. 57, 248–256.

Bruford, M., Bradley, D., Luikart, G., 2003. DNA markers reveal the complexity of livestock domestication. Nat. Rev. Genet. 4, 900–910.

Crochet, P.A., Desmarais, E., 2000. Slow rate of evolution in the mitochondrial Control Region of gulls (Aves: Laridae). Mol. Biol. Evol. 17, 1797–1806.

Densmore, L.D., Wright, J.W., Brown, W.M., 1985. Length variation and heteroplasmy are frequent in mitochondrial DNA from parthenogenetic and bisexual lizards (genus Cnemidophorus). Genetics 110, 689–707.

Grzybowski, T., 2000. Extremely high levels of human mitochondrial DNA heteroplasmy in single hair roots. Electrophoresis 21, 548–553.

Gyllensten, U., Wharton, D., Josefson, A., Wilson, A., 1991. Paternal inheritance of mitochondrial DNA in mice. Nature 352, 255–257.

Hauswirth, W., Van De Walle, M., Laipis, P., Olivo, D., 1984. Heterogeneous mitochondrial DNA D-Loop sequences in bovine tissue. Cell 37, 1001–1007.

Hiendleder, S., Lewalski, H., Wassmuth, R., Janke, A., 1998. The Complete mitochondrial DNA Sequence of the domestic sheep (*Ovis aries*) and comparison with the other major ovine haplotype. J. Mol. Evol. 47, 441–448.

Kadwel, M., Fernandez, M., Stanley, H., Baldi, R., Wheeler, J., Rosadio, R.Y., Bruford, M., 2001. Genetic analysis reveals the wild ancestors of the llama and the alpaca. Proc. R. Soc. Lon. B Biol. Sci. 268, 2575–2584.

Kondo, R., Satta, Y., Matsuura, E.T., Ishiwa, H., Takahata, N., Chigusa, S.I., 1990. Incomplete maternal transmission of mitochondrial DNA in *Drosophila*. Genetics 126, 657–663.

Maté, M.L., Di Rocco, F., Zambelli, A., Vidal-Rioja, L., 2004. Mitochondrial DNA Structure and Organization of the Control Region of South American Camelids. Mol. Ecol. Notes 4, 765–767.

Moum, T., Bakke, I., 2001. Mitochondrial Control Region structure and single heteroplasmy in the razorbill (*Alca torda*; *Aves*). Curr. Genet. 39, 198–203.

Nagata, J., Masuda, R., Tamate, H., Hamasaki, S., Ochiai, K., Asada, M., Tatsuzawa, S., Suda, K., Tado, H., Yoshida, M.C., 1999. Two Genetically Distinct Lineages of the Sika Deer, *Cervus nippon*, in Japanese Islands: Comparison of Mitochondrial D-Loop Region Sequences. Mol. Phylogenet. Evol. 13 (3), 511–519.

Nesbø, C.L., Arab, M.O., Jakobsen, K.S., 1998. Heteroplasmy, length and sequence variation in the Control Regions of three percid fish species (*Perca fluviatilis*, *Acerina cernua*, *Stizostedion lucioperca*). Genetics 148, 1907–1919.

Nicholas, K., Nicholas, H., 1997. GeneDoc: A Tool For Editing And Annotating Multiple Sequense Alignments. Distributed by the author. http://www.psc.edu/biomed/genedoc.

Puig, S., Videla, F., 1995. Comportamiento y organización social del guanaco. In: Puig, S. (Ed.), Técnicas Para el Manejo del Guanaco. UICN, Gland, Switzerland, pp. 97–118.

Schwartz, M., Vissing, J., 2002. Paternal inheritance of mitochondrial DNA. N. Engl. J. Med. 34, 576–580.

Shitara, H., Hayashi, J., Takahama, S., Kaneda, H., Yonekawa, H., 1998. Maternal inheritance of mouse mtDNA in Interspecific hybrids: segregation of the leaked paternal mtDNA Followed by the prevention of subsequent paternal leakage. Genetics 148, 851–858.

Wheeler, J.C., 1995. Evolution and present situation of the South American camelidae. Biol. J. Linnean Soc. 54, 271–295.

Xu, X., Arnason, U., 1994. The complete mitochondrial DNA sequence of the horse, Equus caballus: extensive heteroplasmy of the Control Region. Gene 148, 357–362.