Quaternary International 317 (2013) 14–18

Contents lists available at ScienceDirect

Quaternary International

journal homepage: www.elsevier.com/locate/quaint

Molecular identification of a *Fuegian dog* belonging to the Fagnano Regional Museum ethnographic collection, Tierra del Fuego

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ARTICLE INFO

Article history:
Available online 9 August 2013

ABSTRACT

Native-European contact in Tierra del Fuego was a rapid process, for which little ethnographic information has been produced. Although the *Fuegian dog* seemed to have been important to Selk'nam people's life, the taxonomic status of this extinct animal is still uncertain. The aim of the present work was to determine the zoological identity of a taxidermized canid belonging to a Fagnano Regional Museum collection, Río Grande, Tierra del Fuego. For this purpose, DNA from *Fuegian dog* and patagonian wild canids (*Lycalopex culpaeus*, *Lycalopex griseus* and *Lycalopex gymnocercus*) hairs was extracted. An mtDNA Region Control fragment was amplified by PCR and sequenced. Sequence alignment was performed among the sequences that were obtained in this research and the *Canis lupus familiaris* sequence from GenBank. Pairwise analysis showed a higher identity between the *Fuegian dog* and the culpeo fox (97.57%), with greater divergence with the current domestic dog (88.93%). These results were supported by the molecular phylogenetic analysis, suggesting atypical fox domestication by hunter-gatherers.

1. Introduction

The recent history of Aboriginal people of Tierra del Fuego has been influenced by European contact, which represented a dramatic impact on their culture and biology. The ethnographic information is scarce because the population reduction process lasted for only a few decades (García Moro, 1992).

Fuegian hunter–gatherers have been distinguished mainly by their provisioning of marine or terrestrial resources. During the historical period, Selk'nam people occupied much of the hinterland of the Isla Grande de Tierra del Fuego. The ethnographic reports note the importance of dogs to these groups and their closeness to them (Gallardo, 1910; Gusinde, 1982). In addition, maritime hunter–gatherers also possessed dogs at the time of colonization (Orquera and Piana, 1999). The *Fuegian dog* taxonomic status is uncertain, but ethnographic accounts speculate about its zoological identity. In this sense, there are two hypotheses about the *Fuegian dog* origin: One suggests that it originated through domestication of fox (Gallardo, 1910) or through a dog and fox hybridization (Señoret, 1896), which is unlikely. The other proposes that dogs were brought by Europeans (Emperaire, 1946; Gusinde, 1982; among others).

The initial stage (1520-*ca.* 1850) of the European contact with the inhabitants of Tierra del Fuego which was dominated by indirect contact (Borrero, 1992) is little understood. As the spread of dogs occurred early in Patagonia, they could have arrived to Isla Grande de Tierra del Fuego by crossing the Estrecho de Magallanes through exchanges between Aborigines from the mainland and the archipelago.

The recent past reconstruction of native populations of Tierra del Fuego includes examination of biological material and written remains from museum collections. In the present work, a taxidermized canid was studied. This specimen has been identified as a *Fuegian dog*, which was the pet of a Selk'nam. The aim of this work was to establish the identity of this canid by a comparative genetic analysis of mitochondrial DNA (mtDNA) Control Region fragments of *Fuegian dog* with other patagonian wild canids, and the domestic dog.

2. Materials and methods

During the 2008 campaign, hairs from a domesticated canid belonging to a Selk'nam were obtained from the Fagnano Regional Museum (Salesian Congregation of Río Grande, Tierra del Fuego) where it has been preserved. The canid was taxidermized before the mid-twentieth century at a nearby ranch, in Río Grande, and was donated to the Fagnano Regional Museum. Salesian and

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http://dx.doi.org/10.1016/j.quaint.2013.07.030

ranchers asserted that this canid was a native-bred dog (Apolinaire pers. comm., 2013; Ticó pers. comm., 2007) that belonged to Aborigines who inhabited the area.

La Candelaria was a Salesian Mission from 1893 to 1942, and since then an Agrotechnical School (De Agostini, 1956). However, some Selk'nam that had spent their childhood in the Salesian Mission continued to visit it until the second half of the twentieth century (Fancioni pers. comm., 2013). The Fuegian aboriginal extinction was rapid. In 1919, only 279 pure Selk'nam were counted (Gusinde, 1951) and in 1966, 13 mestizos were registered (Champan, 2002). Persecution by ranchers led Selk'nam to hide out in the more inhospitable area of the island (Bridges, 1886; Dabbene, 1904) while others were integrated as farm labor (Borrero, 2001).

This canid, with a short light ocher coat, has a height of about 40 cm, a lean, small head, and is reminiscent of a greyhound (Fig. 1). We decided to examine only hairs because minimal destruction was expected in this type of sample. Scales and medulla hair were observed by light microscopy for zoological determination according to Chehebar and Martin (1989). For comparative genetic analysis, hair samples from Patagonian wild canids were obtained. Hairs of gray fox (*Lycalopex griseus*) and culpeo fox (*Lycalopex culpaeus*) were obtained from museum collections (Fagnano Regional Museum of Rio Grande and Scaglia Museum of Mar del Plata, respectively), while the pampas fox (*Lycalopex gymnocercus*) hairs came from a specimen found dead on a route of the Province of Buenos Aires. The DNA extraction was conducted following the Authenticity Criteria to Determine Ancient DNA Sequences (Hofreiter et al., 2001; Willerslev and Cooper, 2005; Fulton, 2012). The hair lysis was performed according to Allen et al. (1998) with some modifications. Briefly, 1, 5 and 10 hair fragments were incubated in 50 μl of PCR-compatible buffer at 56 °C for 3 h, and proteinase k was deactivated at 95 °C for 15 min. A negative extraction control was added. PCR amplifications of each DNA specimen were performed by triplicates using 1 μl of DNA in a final volume of 12.5 μl, using 0.5 units of Taq Platinum (Invitrogen), 5% of DMSO (Finnzymes), 2 mM of $MgCl_2$ (Invitrogen), 200 μm of each dNTP (Finnzymes) and 0.2 mM of each primer to amplify mtDNA Control Region fragments of canids: Pex1F and H3R pair (~350 bp), Pex2bF and Pex2bR pair (~150 bp); and Pex3R and H3R pair (~140 bp) (Nyström et al., 2006). PCR conditions were as follows: an initial denaturing step (94 °C for 3 min) followed by 40 cycles at 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 45 s, and a final extension step (72 °C for 10 min). Negative PCR controls were added. The specificity and size of amplification products were assessed by electrophoresis in 2% (w/v) Tris–borate/EDTA (TBE) agarose gels and stained with SYBR® Gold Nucleic Acid Gel Stain (Invitrogen). Triplicates of each specific gene fragments were sequenced and the obtained sequences and chromatograms were analyzed. The obtained consensus sequences and the *Canis lupus familiaris* sequence from NCBI GenBank (HQ452419.1) were aligned using the multiple alignment tool Clustal W2.0.9 (Larkin et al., 2007). Pairwise analysis was performed between sequences, and Identity percentage was determined.

Molecular Phylogenetic analyses were conducted in MEGA5 (Tamura et al., 2011). Thus, sequences alignment was performed with the mtDNA Control Region sequences of canids produced in the present work along with domestic dog (*C. lupus familiaris*) sequence (HQ452419.1) and other South American canid sequences: crab-eating fox (*Dusicyon thous*) (EF107031.1) and the hoary fox (*Lycalopex vetulus*) (EF107033.1). The evolutionary history was inferred by using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). The bootstrap consensus tree was inferred from 1000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The mtDNA Control Region sequence of South American procyonid *Nasua nasua* (NC020647.1) was used as an outgroup.

3. Results and discussion

In this work, we performed a DNA extraction from hair samples of museum collections. Although ancient DNA studies have been traditionally performed from bone parts, in recent years the hair has been recognized as an important source of genetic evidence. Mitochondrial DNA is of particular interest mainly because keratinized hair cuticle can be easily decontaminated. DNA purified from all samples was detected by one-step PCR showing DNA free of contaminants and inhibitors of the PCR reaction.

The DNA extraction of each hair sample was evaluated by PCR amplification. In this regard, we were able to amplify mtDNA Control Region fragments of 150 bp, 140 bp and 350 bp from *L. gymnocercus* and *L. culpaeus* single hair. In addition, 150 bp and 140 bp fragments from *L. griseus* and *Fuegian dog* were amplified. However, only 10 hairs from the *Fuegian dog* were adequate for PCR analysis due to the nature of the sample. The passage of time and inadequate preservation of the specimen could explain the amplification of only the shortest fragment. The different results among samples were consistent with the antiquity of all samples.

Fig. 1. Taxidermized canid exhibited at the Fagnano Regional Museum. Photo: Prof. Julio Tutak.

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