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DIVERSIDADE GENÉTICA E HISTÓRIA EVOLUTIVA DO LOBO-GUARÁ (*CHRYSOCYON BRACHYURUS*)

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SUMÁRIO

DEDICATÓRIA E AGRADECIMENTOS

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RESUMO

O lobo-guará (*Chrysocyon* brachyurus) é o maior canídeo sul-americano, que possui uma ampla distribuição geográfica, porém atualmente é considerada uma espécie vulnerável. As principais ameaças são a perda de habitat, atropelamentos, doenças originárias do cachorro doméstico e perseguição. Nesse estudo foram analisadas seqüências de três fragmentos de DNA mitocondrial e seis genes nucleares, de indivíduos representando a maior parte da distribuição da espécie, com o objetivo de entender sua história evolutiva. O lobo-guará apresentou a menor diversidade nucleotídica ($\pi = 0.0013$) registrada para as espécies da ordem Carnivora. Usando a abordagem de coalescência, encontramos evidências significativas de *bottleneck* anterior ao último máximo glacial seguido de uma grande expansão populacional durante este período. Além disso, pudemos recuperar a distribuição atual com um modelo de predição de nicho, além da distribuição durante o último máximo glacial e também durante o último período interglacial. A área apropriada de ocorrência da espécie decresceu e foi deslocada para o sul do continente durante o último período interglacial, porém cresceu consideravelmente, além da distribuição atual, durante o último máximo glacial. Baseados nestes resultados nós associamos as mudanças na temperatura e umidade durante o Quaternário recente com as mudanças na distribuição da vegetação, as quais devem ter moldado a presente variabilidade genética do lobo-guará.

ABSTRACT

The maned wolf (*Chrysocyon brachyurus*) is the largest South American canid, and has been classified as endangered or near endangered by international conservation agencies and authorities from several countries throughout its distribution. Habitat loss, road killing, domestic dog’s diseases and persecution are the principal threats related to anthropogenic impact. We studied nucleotide substitution from three fragments of the mitochondrial DNA and six nuclear loci from samples obtained from most of the maned wolf distribution to uncover the evolutionary history of the species. We found that the maned wolf has the smallest genetic diversity ($\pi = 0.0013$) reported so far for a species of the order Carnivora, presenting no geographic structure. Using coalescent approaches we found clear signals of a bottleneck before the Last Glacial Maximum followed by a huge population expansion dated to the Last Glacial Maximum. Moreover, we predicted the current modeling distribution for maned wolf and projected its distribution at the Last Glacial Maximum and at the Last Interglacial period, showing that the putative suitable area for this species decreased and was displaced to southern South America during the Last Interglacial period and increased considerably at the Last Glacial Maximum. Based on these results we associated the changes in temperature and humidity during the Late Quaternary to the changes in vegetation distribution which would have shaped the genetic landscape of the maned wolf.

Capítulo 1. APRESENTAÇÃO

1.1. Canídeos

A família Canidae, pertencente à ordem Carnivora (subordem Caniformia, superfamília Canoidea) (Flyn & Nedbal, 1998) é representada atualmente por 16 gêneros e 36 espécies, que divergiram nos últimos 10 milhões de anos e incluem os cachorros, raposas, lobos e coiotes (Nowak, 1999). Essas espécies são amplamente distribuídas, ocorrendo nos mais diversos habitats em todos os continentes, exceto nas ilhas do Caribe, Madagascar, Taiwan, Filipinas, Borneo, Nova Guiné e Antártica (Eisenberg, 1989; Wayne, 1996; Einsenberg & Redford, 1999; Savolainen *et al*. 2002; Macdonald & Silero-Zubiri, 2004).

Os canídeos originaram-se em torno de 50 milhões de anos, no final do Eoceno, na América do Norte, de um grupo de carnívoros da família Miacidae (Macdonald & Silero-Zubiri, 2004). Tedford (1978) propõe três grandes radiações dos canídeos: as subfamílias Hesperocyoninae, Borophaginae e Caninae, sendo as duas primeiras já extintas. No final do Mioceno, os Caninae atravessaram o Estreito de Bering e chegaram à Europa, iniciando uma explosiva radiação que originou as espécies modernas do Velho Mundo (Wayne *et al.* 1989; Wang *et al.* 2004). No fim do Plioceno e início do Pleistoceno, em torno de três milhões de anos atrás, os canídeos chegaram a América do Sul, através da formação do istmo do Panamá e rapidamente adaptaram-se ao continente (Wang *et al.* 2004).

A distribuição de um canídeo pode ser altamente restrita, como no exemplo de *Lycalopex fulvipes* que é endêmico da ilha de Chiloe, no Chile, enquanto que outras espécies habitam vários continentes como no caso da raposa vermelha (*Vulpes vulpes*). A ação antrópica alterando diversos ambientes naturais é um dos principais fatores que afetam amplamente a distribuição atual de várias espécies da família: pelo menos sete delas aumentaram e nove diminuíram sua distribuição no último século (Macdonald & Silero-Zubiri, 2004).

Os canídeos variam quanto ao peso, desde algumas raposas (*Vulpes cana*, *V. zerda*) em que os indivíduos adultos podem pesar menos de 1 Kg, até mais de 60 kg, como no lobo cinza (*Canis lupus*) (Macdonalds & Silero-Zubiri, 2004). Os canídeos divergem dos demais carnívoros por possuírem os membros alongados e semi-rígidos terminando em patas digitígradas. As patas anteriores usualmente possuem cinco dígitos (sendo um deles reduzido) e as posteriores quatro, com garras bem desenvolvidas e não retráteis (Stains, 1975; Emmons & Feer, 1990). A fórmula dentária dos canídeos é 3142/3143 - incisivos, caninos, pré-molares, molares - com incisivos não especializados, fortes caninos, pré-molares afiados e molares preensores, num total de geralmente 42 dentes. Essa fórmula dental é a mais próxima dos grupos ancestrais de Carnivora, já que nas outras famílias desse grupo, essa fórmula é reduzida (Stains, 1975; Macdonalds & Silero-Zubiri, 2004).

O amadurecimento sexual da maioria dos canídeos ocorre com apenas um ano de idade. As fêmeas têm, em geral, uma gestação por ano que dura em média 63 dias, havendo cuidado parental e muitas vezes de outros membros do grupo (Stains, 1975; Emmons & Feer, 1990; Nowak, 1999).

1.2. ***Chrysocyon brachyurus*** **(Illiger, 1815)**

Lobo-guará, lobo-de-juba, aguará-guazú ou simplesmente guará, são as denominações atribuídas à espécie *Chrysocyon brachyurus* que etimologicamente significa cão-dourado-de-cauda-curta (Breyer, 1977).

O lobo-guará é o maior canídeo da América do Sul, alcançando 125 cm de comprimento e pesando de 20-25 kg em estado selvagem e 30 kg em cativeiro. Possui uma altura de 75-90 cm. O comprimento da cabeça e corpo é de 100-130 cm e a cauda em torno de 30-45 cm. Tem porte esguio, destacado pelas pernas compridas. Os pêlos são espessos, com tonalidades castanhas e brilhantes, levemente avermelhados. Na parte superior da cabeça, possui uma área de longos pêlos negros (crina), assim como no focinho e nas partes médias de suas pernas. O interior das orelhas, peito e ponta da cauda, possui uma área branca (Dietz, 1985).

Segundo Breyer (1979), o guará é um animal de hábitos noturnos, muito tímido e solitário. Ocupa áreas que alternam savanas, pastagens altas (charcos, pantanal, pampas e caatinga), montes e também cerrados brasileiros, áreas abertas e de transição e planícies onduladas. Demarca um território estimado em 27 km^2 por indivíduo e que pode ser compartilhado com a parceira (Dietz, 1984).

O período reprodutivo da espécie se estende de fevereiro a abril e a gestação é de 63-65 dias. Seu comportamento é geralmente monogâmico, convivendo por longos períodos com a mesma parceira. A fêmea é monoestérica anual. Os nascimentos se concentram entre os meses de julho e agosto com uma prole média de dois filhotes (Beccaceci, 1985).

Os machos são mais ativos do que as fêmeas. Procuram alimento do entardecer até os primeiros momentos da manhã (Carvalho & Vasconcelos, 1995). O lobo-guará tem hábito alimentar onívoro, consumindo pequenos mamíferos (maioria roedores), aves, répteis, peixes de água doce, insetos, frutas e gramíneas. No Brasil, a fruta do lobo (*Solanum lycocarpum*) ou lobeira, é um arbusto que constitui a sua alimentação natural por longos períodos em que faltam outros alimentos (Dietz, 1984). Anuros, tais como *Leptodactylus ocellatus* e *L. gracilis*, são facilmente caçados (Carvalho & Vasconcelos, 1995). Bestelmeyer & Westbrook (1998) observaram o lobo-guará alimentando-se do veado-campeiro (*Ozotoceros bezoarticus*) no cerrado brasileiro. Frutas da lobeira, roedores e aves somam 61,5% da sua dieta (Motta-Junior *et al.* 1996).

No princípio do século, a espécie se estendia do Mato Grosso até o norte da Patagônia e parte do Uruguai (Dietz, 1984). Atualmente a espécie se distribui no centro, nordeste e sul do Brasil, chaco paraguaio e boliviano, Peru e nordeste da Argentina (Rodden et al. 2004) (Ver figura 1, capítulo 2).

Chrysocyon brachyurus é considerada uma espécie vulnerável e ameaçada (Coimbra-Filho, 1972; Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA); International Union for Conservation of Nature and Natural Resources (IUCN, 2003); Endangered Species Act (US ESA); Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)).

O único estudo populacional genético de lobo-guará analisou 10 loci de proteínas de oito indivíduos da Estação de Águas Emendadas, Distrito Federal e detectou apenas um polimorfismo com a enzima fosfoglicoisomerase (PGI) com apenas um indivíduo heterozigoto. A análise de quatro loci de microssatélites resultou em todos os loci polimórficos (Rodrigues, 2002).

Estudos de filogeografia e diversidade genética de populações com canídeos foram realizados com poucas espécies até o momento, em especial com lobo, coiote (Vilà *et al*. 1999), cachorro selvagem africano (Girman *et al*. 2001) e graxaim-do-mato (Tchaika *et al.* 2006), sendo essa última, a única espécie estudadada América do Sul.

1.3. Marcadores Moleculares

O uso de técnicas moleculares para estudos filogenéticos e intra-específicos tem proliferado nos últimos anos. Diferentes metodologias têm sido desenvolvidas e utilizadas em conjunto com dados morfológicos e ecológicos entre outros. A filogeografia, área em recente expansão, que tem como princípios estudar a estrutura genética e a história evolutiva de populações naturais usando marcadores moleculares (Johnson *et.al.* 2006).

O DNA mitocondrial (mtDNA), embora seja apenas um locus, é uma importante ferramenta no estudo da história das espécies, devido a ter herança materna e a alta taxa de substituição (Avise, 2000; Jennings & Edwards, 2005). O mtDNA nos animais consiste de um genoma haplóide, circular, de pequeno tamanho, que está presente em centenas a milhares de cópias por célula. Nos vertebrados, está organizado em um segmento circular num total de 16 a 18 kb (Avise, 2000). Sua seqüência tem 13 genes codificadores de proteínas, dois genes para rRNA, 22 genes para tRNA e uma região controladora que contém seqüências regulatórias para duplicação e início de transcrição (Graur & Li, 2000). A região controladora é frequentemente usada em estudos de genética de populações dada sua alta variabilidade (segmentos hipervariáveis HVS1 e HVS2). Os genes codificadores de proteínas, mais conservados, como o ATPase-8 e o Citocromo Oxidase I (COX I), são utilizados para análise de filogenia acima do nível específico (Graur & Li, 2000). Em mamíferos, seqüências de mtDNA tem uma taxa de mutação de cinco a dez vezes maior do que os genes nucleares (Wang *et al*. 2004).

Entretanto, apesar destas vantagens, pode haver certa limitação no emprego deste marcador, dado que sua análise representa apenas a história evolutiva das linhagens maternas das populações (Wayne, 1996). Nos eucariotos, os polimorfismos nucleares são amplamente distribuídos pelo genoma e as regiões não codificantes ou intergênicas do DNA apresentam-se mais variáveis do que as regiões codificantes e podem ter assim um amplo emprego como marcadores moleculares. Porém, marcadores nucleares são ainda pouco usados em estudos de genética de populações devido às taxas de mutação mais baixas do que o mtDNA. Sua análise, além disso, deve levar em conta fatores como recombinação, seleção e a diploidia (Hare, 2001; Bardeleben *et al*. 2005). A combinação de marcadores de mtDNA com marcadores de locos nucleares começa a ser uma importante ferramenta para aumentar a qualidade dos estudos filogenéticos e filogeográficos.

Tendo em vista a carência de estudos genéticos e o status de conservação do lobo-guará, o objetivo da presente tese é contribuir significativamente para a compreensão da história evolutiva de *C. brachyurus*.

Os resultados desta tese estão apresentados em dois artigos científicos: um sobre a baixa diversidade genética da espécie no mtDNA e outro, sobre a filogeografia e história evolutiva do lobo-guará, analisando loci nucleares e mtDNA, finalizando com as conclusões gerais.

Capítulo 2 - Extremely low genetic diversity in mtDNA in the largest South American Canid (*Chrysocyon brachyurus*)

Extremely low genetic diversity in the largest South American Canid (*Chrysocyon brachyurus*)

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Abstract

The maned wolf is the largest South American canid, and has been classified as endangered or near endangered by international conservation agencies and authorities from several countries throughout its distribution. Habitat loss, road killing, domestic dog's diseases and persecution are the principal threats related to anthropogenic impact. In this study we show using control region sequences of the mitochondrial DNA from most of the species distribution that the maned wolf has the smallest genetic diversity ($\pi = 0.0013$) reported so far for a species of the order Carnivora, presenting no geographic structure. This species shows clear signal of a historical bottleneck followed by population expansion. We infered that the expansion occurred during or at the end of the last glacial maximum and the bottleneck likely occurred before that during the glacial period.

INTRODUCTION

Species belonging to the order Carnivora commonly represent the highest trophic level in the ecosystems, seemingly living without the pressure of predation, although they are very susceptible to the environmental changes and can suffer from enormous threat in response to small alterations on the proportion of preys and habitat fragmentation (Muñoz-Durán, 2002). In the South America (SA) there are several species of the order Carnivora, which have been considered as threatened species by IUCN (International Union for Conservation of Nature; IUCN, 2008). Among these we can find almost all SA felines. On the other hand, in the family Canidae the great majority of SA canids are classified as Least Concern, and only *Lycalopex fulvipes* is considered threatened. The Maned wolf (*Chrysocyon brachyurus*), the largest SA canid (95-115 cm of length and weighting 20-30 kg), is classified as near threatened by IUCN, although it is considered as endangered species by Brazilian and Argentinean authorities (Rodden *et al*. 2008). Maned wolf is endemic to central SA, inhabiting the grasslands and scrub forests (*cerrado*) from the north-eastern Brazil to south, through the chaco of Paraguay, Uruguay, Bolivia and Argentina (Fig 1; Rodden *et al*. 2008).

Currently, the most significant threat to maned wolf populations is the drastic reduction of habitat; the cerrado has been reduced to about 20% of its original area (Myers *et al*. 2000). Moreover, maned wolves are killed on roads and by domestic dogs that are also an important source of disease (Rodden *et al*. 2008).

These threats represent the antropogenic impact on the maned wolf populations, which is recent and alarming. However, all the SA fauna suffered dramatic changes in composition and structure during the Quaternary period (de Vivo & Carmignotto, 2004; Koch & Barnosky, 2006). The most radical change was perhaps the megafauna extinction between about 200,000 and 8,500 years ago. There are several hypotheses about the SA megafauna extinction, the most debated are the climatic changes and the human overkill. The canid fauna was severely affected by this extinction process, as attested by the fossil records in the SA (Berta, 1987; Prevosti & Vizcaíno, 2006). The survival of the maned wolf (and the others smaller South America canids) has been related with its generalist diet (Berta, 1987; Muñoz-Durán, 2002). Therefore, an important point without clarification is: how the maned wolf, as a large mammal, was affected by the events that caused the megafauna extinction in SA?

Herein we use the polymorphisms in the mitochondrial control region to analyze the genetic diversity in the maned wolf. Our goal in this study was to assess the degree of genetic diversity available throughout the whole distribution; and evaluate the past evolutionary history of maned wolf. Based on these results, we aimed to produce information for the conservation programs focusing the maned wolf.

MATERIALS AND METHODS

Population sampling and molecular methods

A total of 87 specimens from 54 localities was sampled in Brazil and Argentina, covering most of the species range (Fig. 1). We obtained samples from the department of Corrientes, northern Argentina, and from the following Brazilian states: Santa Catarina (SC), São Paulo (SP), Rio de Janeiro (RJ), Minas Gerais (MG), Espírito Santo (ES), Mato Grosso do Sul (MS), Mato Grosso (MT), Goiás (GO) and Distrito Federal (DF).

DNA was extracted from tissues and blood following Sambrook *et al*. (1989), the first hypervariable segment (HVS-I) of the mitochondrial DNA (mtDNA) control region (CR) was amplified by the Polymerase Chain Reaction (PCR). Primers, PCR and sequencing protocols were used as described in Tchaicka *et al.* (2006). Sequences were manually edited using BioEdit 6.0.7 (Hall, 1999), aligned using the ClustalX 1.83 program (Thompson *et al.* 1997), and deposited in GenBank (the accession numbers will be avalaible for the final manuscript).

Diversity indices and coalescence approaches

The ARLEQUIN 3.1 program (Schneider *et al.* 2000) was used to estimate population diversity statistics such as: nucleotide (π) and haplotype diversity (Hd), Watterson's theta (θw), Tajima's (Tajima, 1983) and Fu's (Fu, 1997) neutrality tests and their statistical significance; and mismatch distribution analyses (Rogers & Harpending, 1992).

Fluctuation in the population size (G) and the demographic parameter θ were inferred using the coalescent approach implemented in the package LAMARC 2.1.2

(Kuhner, 2006). We used the substitution model selected by the minimum theoretical information criterion test (AIC) in MODELTEST 3.6 (Posada & Crandall, 1998) and set the initial θ based on the results of θw calculated in ARLEQUIN. Our search strategy was composed by three replicates of 10 initial chains with 500 samples and two final chains with 10,000 samples. The sampling interval was set in 20, and the burn-in was set to 1,000 samples for each chain. The confidence interval for θ and G was calculated using the percentile approach.

Female effective population size (N_{ef}) was estimated using $N_{ef} = \theta/2\mu$ (μ = substitutions/site/generation). Generation time for maned wolf was estimated as reported in Grazziotin *et al.* (2006) and was based on the life time information of 7.3 years described in Maia & Gouveia (2002) and Rodden *et al.* (2008). For the parameter μ we assumed a rate of sequence evolution of 3.68×10^{-8} substitutions/site/year, which was estimated for *Cerdocyon thous* (Tchaicka *et al.* 2006).

Population structure

We used the NETWORK 4.5.0.0 (www.fluxus-engineering.com) program to infer a haplotype network employing the median-joining network (MJN) method (Bandelt *et al.* 1999).

To investigate the correlation between genetic and geographical variation we used Mantel correlation analysis implemented in the AIS 1.0 program (Alleles-In-the-Space; Miller, 2005). We conducted 1,000 permutations to access the statistical significance, and the distribution of geographical and genetic distances was normalized using logarithmic transformation ($\log_{10}$). The latitudinal and longitudinal positioning of the samples were used when available; otherwise, we used the geographical center of the country where the individuals were collected as an approximation of the geographic location (based on IBGE databank, Brazilian Institute of Geography and Statistics).

We explored the geographic pattern of the genetic diversity conducting an "Interpolate genetic landscape shape" analysis (IGLS) implemented in AIS 1.0. This approach constructs a connectivity network of sampled areas using the Delaunay triangulation method. After that, the average nucleotide difference between individuals from each linked sampling area is assigned to the midpoint of the vectors that link the vertices. Finally, the inverse distance-weighted interpolation algorithm is applied to estimate the genetic distances on a uniformly space grid (Miller, 2005). Analyses were performed using two grid sizes (50 × 50, 100 × 100) and two distance weighting parameters (0.5 and 1.0), as well as, the analyses conducted using the raw genetic distances and the residual genetic distances (residuals generated by the linear regression of genetics *vs.* geographical distances).

RESULTS AND DISCUSSION

Genetic variability

The control region alignment resulted in 584 base pairs (bp), and only six variable sites were found among the 87 samples. Five single base-pair substitutions (five transitions) and one *indel* were observed, which defined seven haplotypes (Table 1). The diversity indices showed a very low diversity, the maned wolf has the lowest nucleotide diversity ($\pi = 0.0013$) for the control region among all carnivores studied until the present (see Table 2). Commonly, the genetic diversity has been used in conservationist approaches as an estimator for the potential fitness of a species (DeSalle & Amato, 2004). The African cheetah, *Acynonyx jubatus,* has been the exemplary case (O'Brien *et al.* 1985; Merola, 1994) of shallow genetic diversity, since several studies with different genetic markers (Marker *et al*. 2008), have shown it to be one of the least genetic diverse wild mammal (Freeman *et al*. 2001). Taking this into account, the results for genetic diversity in maned wolf are intriguing and in some aspects disturbing. The control region in maned wolf is more than 10 times less diverse than in cheetah, and even if we compare it with a subgroup of one of the cheetah subspecies, maned wolf nucleotide diversity is still more than twice minor (Table 2). Maned wolf haplotypes diversity is on the same way unusually very small (0.0669). These results together with the negative although not significant neutrality tests (Tajima's D = - 0.1147 and Fu's Fs = - 0.9323) suggest some kind of recent bottleneck followed by population expansion (see below).

Haplotypes relationship and population structure

The median-joining network (Fig. 1a) shows two rare haplotypes (H01 and H03), which are found only in Argentina (Fig 1c), one very common haplotype (H02) found throughout almost the whole distribution, and four other haplotypes with intermediate frequencies that are not geographically structured. This network pattern, in which several low frequency haplotypes are connected by short branches (a single mutation) to the most common, widespread haplotypes, can be interpreted as a signal of a relatively recent expansion from a least diverse population of maned wolf.

A significant, although very small positive correlation between the genetic and geographic diversity ($r = 0.184$, $P = 0.0069$) was found by the Mantel test. However, the IGLS analysis (Fig. 2) agrees clearly with the pattern found on the haplotype distribution. Most of the genetic diversity across the landscape is concentrated among GO and DF samples, and within SP samples, and the greatest diversity was found between Argentina versus Brazilian samples. This pattern agrees well with the general result of low diversity (flat area in Fig. 2) with regions of concentrated genetic diversity (peaks in Fig. 2), but without geographic structure, as showed by the haplotype distribution.

This lack of a strong genetic structure probably can be interpreted as a direct result of the low genetic diversity for DNA sequences found in maned wolf and not as a signal of high level of recent gene flow. Some studies using short tandem repeats regions (STR) described some localized genetic structure, but not geographical structure, in this species (de Mattos *et al*. 2004; Salim *et al*. 2007), and a normal level of heterozygosity when compared with other canids. Therefore, two different patterns arise from these markers: an early scenario produced by the mtDNA sequences and a more recent depicted by the STRs.

Demographic history

The substitution model indicated by MODELTEST program was HKY. Using this model the coalescent analysis of genetic variability implemented in LAMARC showed a significant population growth with a G of 369 (confidence interval, CI: 106-881) and a θ of 0.0015. Assuming a generation time of six years, we estimated a historical N_{ef} of approximately 3,340 individuals (CI: 1,260-3,760) for the maned wolf. This estimate is consistently smaller than the population census, approximately 23,600 animals are estimated as the total population being almost 13,000 mature individuals (Rodden *et al*. 2008)

The mismatch distribution analysis showed a unimodal distribution (Fig. 3) that agrees with a model of a bottleneck followed by a population expansion (Rogers & Harpending, 1992) and corroborates the results of LAMARC. Based on these results we used tau (τ) to calculate the expansion time, where $\tau = 2\mu.t$, (t = expansion time) and we found an age of 19,000 years ago (7,570-32,600) for the population expansion. The point estimate matches with the end of the last glacial period (the Last Glacial Maximum), and that also corresponds to the final period of the megafauna extinction. Therefore, the bottleneck period that reduced the genetic diversity in the maned wolf should have happened before this time, during the glacial period.

Conservation

Some studies have showed that the geographic distribution of maned wolf is increasing (Santos *et al*. 2003). As an open area dweller it apparently took advantage of the deforestation of previous close ecosystems to expand its distribution, although in a patchy way. However, at the same time the accelerated declining of cerrado should reduce the availability of alimentary resources (Cortenay, 1994) and likely produced severe barriers to current gene flow in this species. Furthermore, we show this species presents an extremely low mtDNA genetic diversity probably as a consequence of a strong and very recent population bottleneck, One important question to be studied next is to better understand the genomic extent of this low mtDNA diversity in maned wolf using other, autosomal loci.

References

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Berta A (1987) Origin, diversification, and zoogeography of the large South American Canidae. *Fieldiana Zoology*, **39**, 455-471.

Coutenay O (1994) Conservation of the maned wolf: fruitful relations in a changing environment. *Canid News*, **2**, 41-43.

De Mattos PSR, Del Lama MA, Toppa RH, Arno RS (2004) Populational genetic structure of free-living maned wolves (*Chrysocyon brachyurus*) determined by proteic markers. *Brazilian Journal Biology*, **64**(3B), 639-644.

De Salle R, Amato G (2004) The expansion of conservation genetics. *Nature Reviews Genetics*, **5**, 702-712.

De Vivo M, Carmignotto AP (2004) Holocene vegetation change and the mamamals fauna of South American and Africa. *Journal of Biogeography*, **31**, 943–957.

Freeman AR; Machugh DE; McKeown S; Walzer C; McConnell DJ.; Bradley DG (2001) Sequence variation in the mitochondrial DNA control region of wild African cheetahs (Acinonyx jubatus). *Heredity*, **86**, 355–362.

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693–709.

Grazziottin FG, Monzel M, Echeverrigaray S, Bonatto, S (2006) Phylogeography of the *Bothrops jararaca* complex (Serpentes: Viperidae): past fragmentation and island colonization in the Brazilian Atlantic Forest. *Molecular Ecology*, **15**, 3969–3982.

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

IUCN (World Conservation Union) (2008) *IUCN Red List of Threatened Species*. <www.iucnredlist.org>. World Conservation Union, Gland, Suíça.

Koch PL, Barnosky AD (2006) Late Quaternary extinctions: state of the debate. Annual *Review of Ecology Evolution and Systematics*, **37**, 215-250.

Kuhner MK (2006) Lamarc 2.0: maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics*, **22**, 768–770.

Maia OB and Gouveia AMG (2002) Birth and mortality of maned wolves *Chrysocyon brachyurus* (Illiger, 1811) in captivity. *Brazilian Journal of Biology*, **62**, 25-32.

Marker LL, Wilkerson AJP, Sarno RJ, Martenson J, Breitenmoser-Wursten C, O’Brien SJ, Johnson WE (2008) Molecular Genetic Insights on Cheetah (Acinonyx jubatus) Ecology and Conservation in Namibia. *Journal of Heredity*, **99**(1), 2–13.

Merola M (1994) A Reassessment of Homozygosity and the Case for Inbreeding Depression in the Cheetah, Acinonyx jubatus: Implications for Conservation. *Conservation Biology*, **8**, 961-971.

Miller MP (2005) Alleles in space: computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity*, **96**, 722–724.

Muñoz-Durán J (2002) Correlates of speciation and extinction rates in the Carnivora. *Evolutionary Ecology Research*, **4**, 963–991.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

O'Brien SJ, Roelke ME, Marker L, Newman A, Winkler CA, Meltzer D, Colly L, Evermann JF, Bush M, Wildt DE (1985) Genetic Basis for Species Vulnerability in the Cheetah. *Science*, **227**, 1428-1434.

Posada D, Crandall KA (1998) modeltest: testing the model of DNA substitution. *Bioinformatics Applications Note*, **14**, 817–818.

Prevosti FJ, Vizcaíno SF (2006) Paleoecology of the large carnivore guild from the late Pleistocene of Argentina. *Acta Palaeontologica Polonica*, **51**(3), 407–422.

Rodden M, Rodrigues F, Bestelmeyer S (2008) *Chrysocyon brachyurus*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 24 November 2008.

Rogers AR, Harpending HC (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Salim DC, Akimoto AA, Carvalho CB, Oliveira SF, Grisolia CK, Moreira JR, Klautau-Guimarães MN (2007) Genetic variability in maned wolf based on heterologous short-tandem repeat markers from domestic dog. *Genetics and Molecular Research*, **6**(2), 348-357.

Sambrook J, Fritch EF, Maniatis T. Molecular cloning - A laboratory Manual. 2 ed. New York: Cold Spring Harbor Laboratory Press. 1989.

Santos EF, Setz ZEF; Gobbi, N (2003) Diet of the maned wolf (Chrysocyon brachyurus) and its role in seed dispersal on a catle ranch in Brazil. *Journal of Zoology*, **260**, 203-208.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN: A Software for Population Genetic Data Analysis, Version 2.0. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland.

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetic*, **105**, 437–460.

Tchaicka L, Eizirik E, Oliveira TG, Cândido-Jr. JF, Freitas TRO (2006) Phylogeography and population history of the crab-eating fox (*Cerdocyon thous*). *Molecular Ecology*, **16**, 819-838.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F (1997) The clustal windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.

Weber D, Stewart B, Garza J, Lehman N (2000) An empirical genetic assessment of the severity of the northern elephant seal population bottleneck. *Current Biology*, **10**, 1287-1290.

Table 1. Mitochondrial DNA haplotypes identified from maned wolf samples refer to the aligned position in our 871 bp data set.

Haplotypes	Variable sites	#
	911114	
	60121	
	3949	
H 1	GTCCA-	46
H 2	GTTCA-	18
H 3	GTTTA-	6
H 4	GTCCAG	6
H 5	ATTTA-	6
H 6	GTCCG-	2
H 7	GCCCAG	3

Table 2. Nucleotide diversity within some species of the order Carnivora**.**

Common name	**Species**	***π***	**Reference**
coyote	*Canis latrans*	0.0460	Vilà *et al.*, 1999
African wild dog	*Lycaon pictus*	0.0309	Girman *et al.*, 2001
gray wolf	*Canis lupus*	0.0260	Vilà *et al.*, 1999
red fox	*Vulpes vulpes*	0.0168	Mercure *et al.*, 1993
arctic fox	*Alopex lagopus*	0.0090	Dalen *et al.*, 2004
kit-fox	*Vulpes macrotis*	0.0069	Mercure *et al.*, 1993
Northern elephant seal	*Mirounga angustirostris*	0.0065	Weber *et al*., 2000
crab-eating fox	*Cerdocyon thous*	0.0190	Tchaicka et al., 2006
	C. thous Southern Clade	0.0080	Tchaicka et al., 2006
	C.thous Northern Clade	0.0210	Tchaicka et al., 2006
Cheetah	*Acynonyx jubatus*	0.0131	Freeman *et al.*, 2001
	A.j. jubatus	0.0077	based on Freeman *et al.*, 2001
	a subgroup of *A.j. jubatus*	0.0029	based on Freeman *et al.*, 2001
maned wolf	*Chrysocyon brachyurus*	0.0013	this study

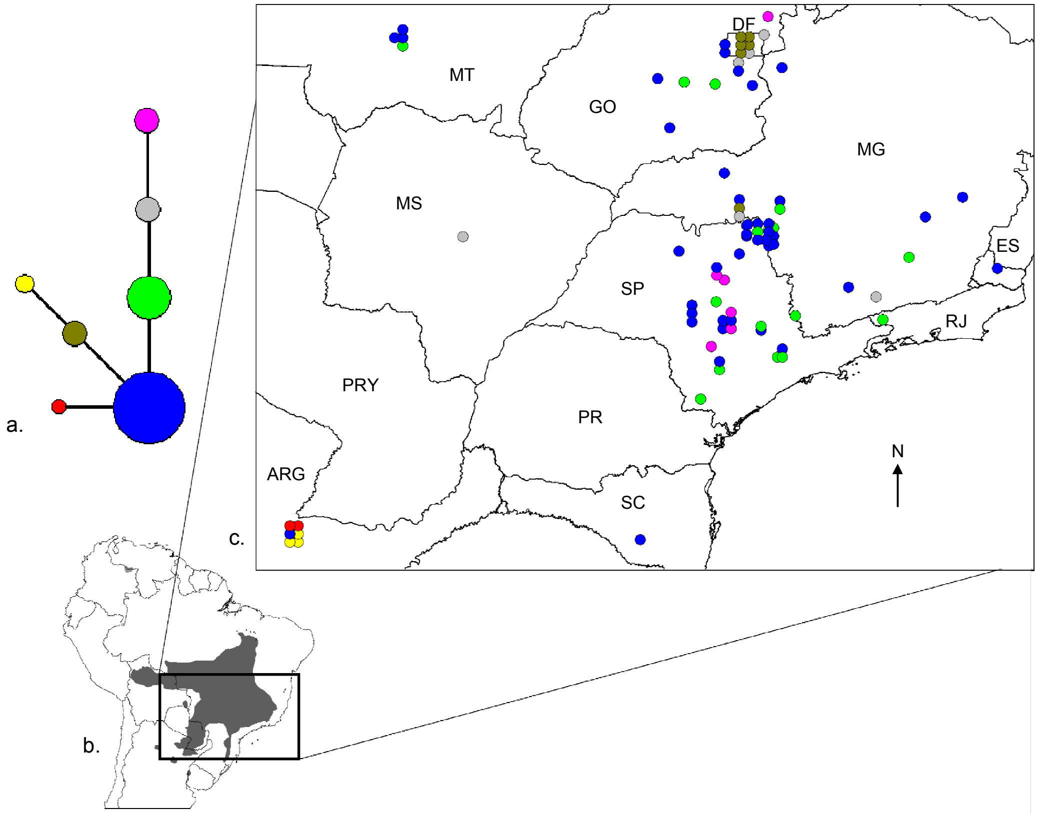

Fig. 1. Geographic distribution of the maned wolf, collected samples and haplotypes: a. Median-Joining haplotype network; each circle represents a different haplotype (red, H01; blue, H02; yellow, H03; green, H04; grey, H05; brown, H06 and pink, H07) with size proportional to its relative frequency; b. geographical distribution of maned wolf in South America (grey area in the map); c. expanded map showing the sampled localities and the haplotypes distribution.

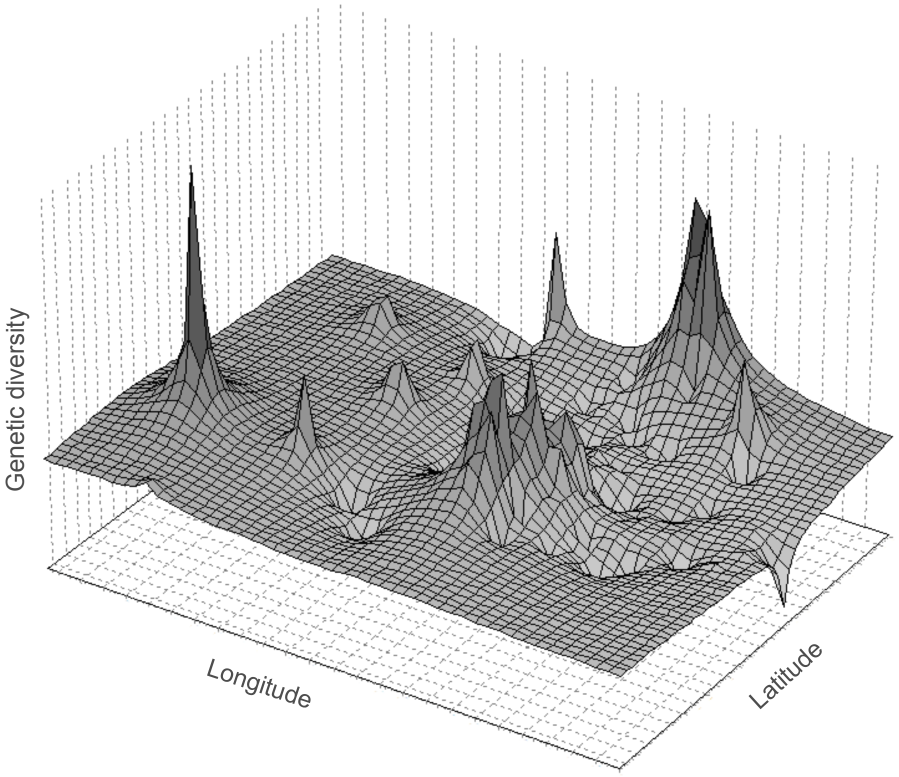

Fig. 2. Landscape Interpolate Shape analysis; genetic landscape representation using a 50 × 50 grid with the distance weighting parameter of 0.5.

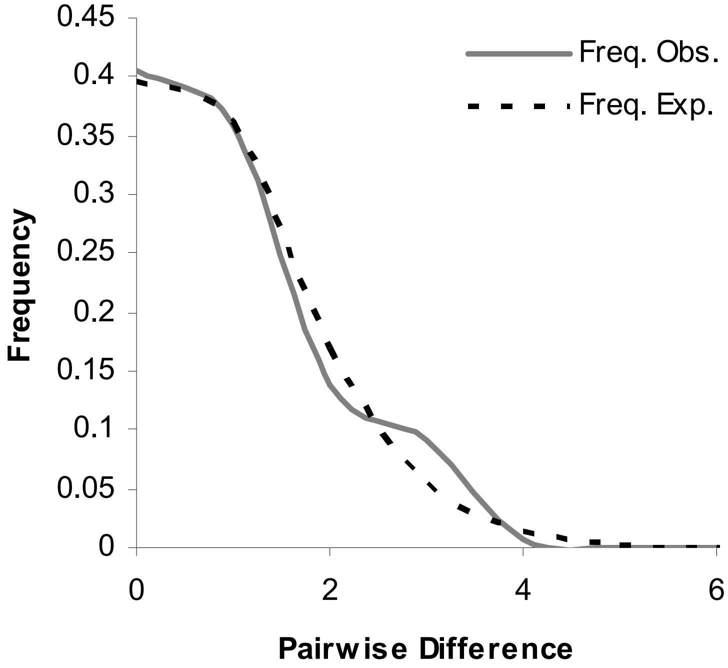

Fig. 3 Mismatch distribution for the genetic diversity of mtDNA control region sequences in Maned wolves.

APPENDIX

List of samples and samples sites for Maned wolf.

Code	H #	Nickname	Sex	Locality*	State/Province	Country	Lat	Long
1_arg	H01		F		Corrientes	Argentina	-27.483	-58.817
2_arg	H02		F		Corrientes	Argentina	-27.483	-58.817
3_arg	H03		F		Corrientes	Argentina	-27.483	-58.817
4_arg	H01		F		Corrientes	Argentina	-27.483	-58.817
5_arg	H03		F		Corrientes	Argentina	-27.483	-58.817
8_arg	H03		F		Corrientes	Argentina	-27.483	-58.817
96_001	H02	Bonita	F	Tres Corações	Minas Gerais	Brazil	-21.683	-45.267
96_002	H02	Linda	F	Tres Corações	Minas Gerais	Brazil	-21.683	-45.267
96_003	H04	Rabo torto	M	Pouso Alegre	Minas Gerais	Brazil	-22.367	-46.550
96_004	H05	Magrela	F	Aiuruoca	Minas Gerais	Brazil	-21.917	-44.600
96_012	H02	Tio Nego	M	Bauru	São Paulo	Brazil	-22.317	-49.067
96_014	H04	Monica	F	Angatuba	São Paulo	Brazil	-23.483	-48.400
96_017	H02	Bauru	F	Brotas	São Paulo	Brazil	-22.283	-48.117
96_020	H05	Nica	F	Campo Grande	Mato Grosso do Sul	Brazil	-20.450	-54.617
96_021	H02	Nova (MW-88)	F	Uberaba	Minas Gerais	Brazil	-19.750	-47.917
96_023	H02	Barrinha	M	Castilho	São Paulo	Brazil	-20.867	-47.917
96_024	H06	Magrela / Alfa	F	Uberaba	Minas Gerais	Brazil	-19.750	-47.917
96_025	H05	Brava	F	Uberaba	Minas Gerais	Brazil	-19.750	-47.917
96_026	H02	Vereda	F	Araxá	Minas Gerais	Brazil	-19.583	-46.933
96_028	H04	Lobao 1	M	Araxá	Minas Gerais	Brazil	-19.583	-46.933
96_031	H02	Preta- MW	F	Jundiai	São Paulo	Brazil	-23.183	-46.867
96_032	H04	Lobao 2	M	Jundiai	São Paulo	Brazil	-23.183	-46.867
96_034	H04	Ragua	M	Araçaíba	São Paulo	Brazil	-24.400	-48.850
96_035	H02	Pati	F	Brotas	São Paulo	Brazil	-22.283	-48.117
96_036	H07	Paul	M	Brotas	São Paulo	Brazil	-22.283	-48.117
96_040	H02	Aaran	M	Brotas	São Paulo	Brazil	-22.283	-48.117
96_041	H07	Fafa	F	Brotas	São Paulo	Brazil	-22.283	-48.117
96_043	H07	Pastel	M	Itatinga	São Paulo	Brazil	-23.117	-48.600
96_046	H05	Velhao	M	Luziania	Goiás	Brazil	-16.217	-47.933
96_047	H02	Sarita	F	Cristalina	Goiás	Brazil	-16.767	-47.600
96_049	H02	Guapo	M	Sto Ant. Descoberto	Goiás	Brazil	-15.967	-48.267
96_051	H05	Fujao	M	Formosa	Goiás	Brazil	-15.533	-47.317
96_052	H07	Flavia	F	Santa Rosa	Goiás	Brazil	-15.083	-47.217
AE_01	H05	Miguelao	M	Aguas Emendadas	Distrito Federal	Brazil	-15.783	-47.883
AE_Helga	H06	Helga	F	Aguas Emendadas	Distrito Federal	Brazil	-15.783	-47.883
AE_Id_Fix	H06	Idea Fix	M	Aguas Emendadas	Distrito Federal	Brazil	-15.783	-47.883
AE_75	H06	Cleo	F	Aguas Emendadas	Distrito Federal	Brazil	-15.783	-47.883
AE_LG82	H06	Clementina	F	Aguas Emendadas	Distrito Federal	Brazil	-15.783	-47.883
AE_LG92	H06	Clements	M	Aguas Emendadas	Distrito Federal	Brazil	-15.783	-47.883
bCbr301	H04	Paquito	M	Serra da Canastra	Minas Gerais	Brazil	-20.233	-47.083
bCbr302	H02	Pierre	M	Serra da Canastra	Minas Gerais	Brazil	-20.233	-47.083
bCbr303	H02	Mama	F	Serra da Canastra	Minas Gerais	Brazil	-20.233	-47.083
bCbr304	H02	brinco 93	M	Serra da Canastra	Minas Gerais	Brazil	-20.133	-47.200
bCbr305	H02	brinco 91	M	Serra da Canastra	Minas Gerais	Brazil	-20.133	-47.200
bCbr306	H02	Jim	M	Serra da Canastra	Minas Gerais	Brazil	-20.133	-47.267
bCbr307	H04	Mel	F	Serra da Canastra	Minas Gerais	Brazil	-20.267	-47.200
bCbr308	H02			Serra da Canastra	Minas Gerais	Brazil	-20.367	-47.167
bCbr309	H04			Serra da Canastra	Minas Gerais	Brazil	-20.367	-47.167

bCbr312	H02		F	SP 334, Km 362	São Paulo	Brazil	-20.183	-47.733
bCbr313	H02		F	SP 334	São Paulo	Brazil	-20.183	-47.733
bCbr314	H07		F	SP 330, Km 246	São Paulo	Brazil	-21.383	-48.467
bCbr315	H02		F	SP 330, Km 261	São Paulo	Brazil	-21.200	-48.467
bCbr317	H02		F	SP 334, Km 261	São Paulo	Brazil	-20.433	-47.733
bCbr318	H04		M	SP 318, Km 248	São Paulo	Brazil	-22.033	-48.483
bCbr319	H02		F	SP 334, Km 387	São Paulo	Brazil	-20.150	-47.717
bCbr320	H07		M	SP 255, Km 31	São Paulo	Brazil	-21.500	-48.283
bCbr325	H02		F	Itapira	São Paulo	Brazil	-22.717	-47.383
bCbr327	H04			São João da Boa Vista	São Paulo	Brazil	-22.633	-47.383
bCbr364	H04		F	BR381 Km 30	São Paulo	Brazil	-23.383	-46.983
bCbr371	H02		F	Serra da Canastra	Minas Gerais	Brazil	-20.133	-47.267
bCbr372	H04		F	Serra da Canastra	Minas Gerais	Brazil	-20.133	-47.267
bCbr373	H02	brinco 24	F	Serra da Canastra	Minas Gerais	Brazil	-20.133	-47.267
bCbr376	H04	brinco 376	F	Serra da Canastra	Minas Gerais	Brazil	-20.267	-47.200
bCbr377	H02			Serra da Canastra	Minas Gerais	Brazil	-20.267	-47.200
Cbr02	H02			Cuiabá	Mato Grosso	Brazil	-15.600	-56.083
Cbr03	H04			Resende	Rio de Janeiro	Brazil	-22.467	-44.433
Cbr04	H02			Cuiabá	Mato Grosso	Brazil	-15.600	-56.083
Cbr05	H04			Cuiabá	Mato Grosso	Brazil	-15.600	-56.083
Cbr06	H02			Cuiabá	Mato Grosso	Brazil	-15.600	-56.083
Cbr07	H02			Cuiabá	Mato Grosso	Brazil	-15.600	-56.083
extr2SC	H02		F	Lages	Santa Catarina	Brazil	-27.817	-50.317
LG002	H02	Velhao	M	Luziania	Goiás	Brazil	-16.217	-47.933
LG003	H02	Guapo	M	Sto Ant. Descoberto	Goiás	Brazil	-15.967	-48.267
LG006	H02	Dunga	M	Unaí	Minas Gerais	Brazil	-16.333	-46.883
LG007	H02	Lobo	M	Uberlandia	Minas Gerais	Brazil	-18.900	-48.283
LG009	H04	2080	M	Goiania	Goiás	Brazil	-16.683	-49.250
LG013	H02	2081	F	Joviania	Goiás	Brazil	-17.800	-49.600
LG015	H02	1964	F	Nazario	Goiás	Brazil	-16.600	-49.900
LG017	H04	1614	M	Alexania	Goiás	Brazil	-16.733	-48.500
LG027	H02	Capixaba	M	São Jose do Calçado	Espírito Santo	Brazil	-21.217	-41.650
LG028	H04	Caramandai	M	Carandaí	Minas Gerais	Brazil	-20.950	-43.800
LG031	H02	Camila	F	Ipatinga	Minas Gerais	Brazil	-19.483	-42.500
LG032	H02	Chorao	M	Aguas de Sta Barbara	São Paulo	Brazil	-19.967	-43.400
LG033	H02	Bauru	F	Bauru	São Paulo	Brazil	-22.317	-49.067
LG034	H02	Monica	F	Angatuba	São Paulo	Brazil	-23.483	-48.400
LG036	H02	Tio Nego	M	Bauru	São Paulo	Brazil	-22.317	-49.067
LG040	H02	Mona	F	São Jose do Rio Preto	São Paulo	Brazil	-20.800	-49.383

*Our sampling scheme was based on the availability of biological material with known geographical origin handled in zoos and breeding conservation programs, and carcasses of road killed maned wolf. H#, Haplotype code, see text and Fig. 1.

Capítulo 3 - Falling with forest rise? The megafauna extinction and the evolutionary history of the largest South American canid (*Chrysocyon brachyurus*)

Falling with forest rise? The megafauna extinction and the evolutionary history of the largest South American canid (*Chrysocyon brachyurus*)

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Running title: The Quaternary influence in the maned wolf evolutionary history.

Keywords: Genetic diversity – demographic history – Carnivora – niche modeling.

Abstract

The South American fauna suffered huge changes in its composition throughout the late Quaternary period. Almost all very large mammals vanished from this continent during the worldwide extinction event known as Last Quaternary Extinction. There are several hypotheses to explain this event, the two most debated ones are the impact caused by climatic changes and the impact caused by the Quaternary colonization of the continent by human populations. For the climatic impact theory, the association between climatic and vegetation changes is one of the principal assumptions used to explain the extinction of megafauna. Some studies associated the increment in humidity and temperature occurred in some periods with the expansion of forest and retraction of grassland, that reduced the area for the large mammals and inducing the extinction. Herein, we studied the evolutionary history of maned wolf, the largest extant canid from South America and a survivor of the Last Quaternary Extinction period. We analyzed six nuclear loci and three mitochondrial fragments using coalescent approaches, and found an extremely shallow genetic variability with clear signals of a bottleneck before the Last Glacial Maximum followed by a huge population expansion dated to the Last Glacial Maximum. Moreover, we predicted the current modeling distribution for maned wolf and projected its distribution at the Last Glacial Maximum and at the Last Interglacial period, showing that the putative suitable area for this species decreased and was displaced to southern South America during the Last Interglacial period and increased considerably at the Last Glacial Maximum. Based on these results we associated the changes in temperature and humidity during the Late Quaternary to the changes in vegetation distribution which would have shaped the genetic landscape of the maned wolf.

INTRODUCTION

The Late Quaternary Extinction (LQE) of the megafauna has been a recurrent issue in scientific debates (Koch & Barnosky, 2006). There are several and very different hypotheses to explain the worldwide extinction that happened between 100 and 8.5 thousands years ago (the exact period is unclear), when more than 90 genera of very large mammals, and some entire orders vanished completely (Barnosky *et al*., 2004). These hypotheses range from: catastrophic effects caused by an extraterrestrial body impact (Firestone *et al.* 2007); epidemic hiperdiseases caused by the contact of faunas from different continents, which did not have defenses against the pathogenic foreigners (MacPhee & Marx, 1997); and changes in the intrinsic dynamics of the ecosystem that produced instability and the complete collapse of megafauna (Forster, 2004). However, within this vast diversity of hypothesis, the scientific debate has been focused in two principal and broad kinds of explanations: the human impact and the climatic change impact (see pros and cons for other hypotheses in Koch & Barnosky, 2006). These hypotheses are applied to all continents, even when there are huge historical differences between them (Koch & Barnosky, 2006).

The human impact hypothesis is principally based on the overkill effect, which assumes that the extinctions in the quaternary period were caused by high levels of Pleistocene humans hunting in their expansion around the continents, in a "blitzkrieg" way (Martin, 1973). Based on this hypothesis the hunting rate was higher than the birth rate for the large mammals, inducing a population bottleneck towards the complete extinction of megafauna. On the other hand, some studies point to an indirect impact caused by human presence, like diseases dissemination (MacPhee & Marx, 1997) and habitat alteration (Burney *et al*. 2004; Edwards & Macdonald 1991).

In the opposite side, the supporters of climatic hypothesis argue that the climate changes in quaternary were strong enough to produce the megafauna extinction (Barnosky 1986; Graham & Lundelius, 1984). These changes were caused by the earth glacial cycles and would affect the flora and fauna distribution in a magnitude larger than the fitness capability of the extinct mammals.

Among all continents, probably the South American fauna suffered the most dramatic changes in composition and structure during the Quaternary period (de Vivo & Carmignotto, 2004; Koch & Barnosky, 2006). Orders like Notoungulata, Litopterna and Proboscidae, families like Gliptodontidae, Megatheriidae, and Equidae, as well as, genus and species form several groups were completely extinct from South America (SA). Unfortunately, these extinctions represent the least known among all extinctions happened in Quaternary period (Koch & Barnosky, 2006). Therefore, there is no clear favored hypothesis for the megafauna extinction in SA, as the human impact for Australia and the climatic impact for Europe (Barnosky *et al*. 2004).

Concerning the canid fauna, there are several fossil records in the South America (Langguth, 1975; Berta, 1987; Wayne *et al*. 1997; Wang *et al*. 2004, Macdonald & Silero-Zubiri, 2004) showing that the extant species represent only a shadow of the diversity found before the Pleistocene-Holocene period

We can argue that if the impacts on megafauna in South America during the Quaternary were caused by human hunting we should expect that extant mammals that was not a target for humans and not feed exclusively on megafauna would not show signal of Quaternary demographic impact. On the other hand if environmental changes were the main causes of population reduction, we should expect that the survivors that have the same biological characteristics than the extinct megafauna would probably show signals of population size reduction for that exact period.

We can think that the Maned wolf (*Chrysocyon brachyurus*) was a likely candidate to the LQE, based on the follows arguments: a) Since *Chrysocyon* and its sister group, genus *Speothos*, has a large divergence time (see Prates *et al*., in press) and the former fossil records were only found in South America (~30 000 ybp; Berta, 1987), it is likely the manef wolf was present in SA throughout LQE period; b) An important point in the LQE is that its impact over the fauna is very selective. It has been argued that only large mammals were severely affected, as a hunting preference effect or as an indirect effect of slow breeding and small offspring, which could also reduce the fitness to environmental changes (Johnson, 2002). The Maned wolf has an average of one or two offsprings per year (Rodden *et al*. 2004), so it is included in the slow breeding category; c) The extinctions were concentrated in open area dwellers (Johnson, 2002), and it has been argued that the impact of fast changes to a close habitat in some periods of Quaternary could be responsible for the megafauna extinction (de Vivo & Carmignotto, 2004). The Cerrado is the principal habitat of maned wolf and it is a savanna-like formation, characterized by very strong dry seasons on the winters, that is usually covered by dense grassland with sparse shrubs and small trees (Ratter *et al*., 1997). Therefore, the maned wolf matches almost all the principal features to be considered a candidate to megafauna extinction, sharing several features with the great majority of species that vanished in Quaternary. The Maned wolf is omnivorous, specialized in small to medium-sized vertebrates and large fraction of its diet is composed by fruits (Rodden *et al*. 2004), then it has been argued that maned wolf (and the others smaller South America canids, like the species from genus *Lycalopex*, *Cerdocyon*, and *Atelocynus*) could have survived based on its broad range of alimentary items, contrasting with the other extinct large canids that were specialized in carnivory, feeding frequently on mega herbivorous (Berta, 1987).

In a recent paper Prates *et al*. (in press) showed that the maned wolf presents the least diverse mitochondrial DNA (mtDNA) control region in all studied species of Carnivora. Here, we suggest that the genetic diversity of maned wolf in South America was strongly affected (directly or indirectly) by the Quaternary climatic changes. We based our hypothesis on evidences of displacements in the potential distribution area to more suitable areas in southern South America in the interglacial periods, and on genetic estimates based on mtDNA and six nuclear loci that a huge population expansion occurred during the Last Glacial Maximum (LGM). These results reinforce the hypothesis that climatic and vegetation changes strongly shaped the extant SA mammalian fauna.

MATERIALS AND METHODS

Population sampling and molecular methods

A total of 54 localities was sampled in Brazil and Argentina, covering most of the species range (Fig. 1), totalizing 87 specimens. We obtained samples from the department of Corrientes, northern of Argentina, and from the following Brazilian states: Santa Catarina (SC), São Paulo (SP), Rio de Janeiro (RJ), Minas Gerais (MG), Espírito Santo (ES), Mato Grosso do Sul (MS), Mato Grosso (MT), Goiás (GO) and Distrito Federal (DF). Our sampling scheme was based on the availability of biological material from blood samples of individuals with known geographical origin handled in zoos and breeding conservation programs, and carcasses of road killed maned wolf.

DNA was extracted from tissues and blood using phenol-chloroform method (Sambrook *et al*. 1989) and nine different fragments were amplified by the Polymerase

Chain Reaction (PCR): (I) the first hypervariable segment (HVS-I) of the mitochondrial DNA (mtDNA) control region (CR) (II) the complete atp8 gene and (III) partial atp6 gene using primers ATP8-DF1 (5'-TCTCACATGGAATTTAACCATGA-3') and ATP8-DR1 (5'– GAATTTCAGCTTTGGGTGCT-3'); (IV) the second intron of the X-linked *Proteolipid Protein 1* (*PLP1*) gene using primers described by Murphy *et al.* (1999); (V) intron 14 of *Feline Sarcoma Protooncogene* (*FES*) using primers described by Venta *et al.* (1996); (VI) intron 8 of *Precursor 1 of Cholinergic Receptor Nicotinic Alpha Polypeptide* (*CHRNA1*) using primers described in Lyons *et al.* (1997); and using the primers described in Johnson *et al*. (2006): (VII) the partial *Silver Protein* gene (SILV); (VIII) the partial RASA gene; and (IX) the partial *T-complex protein 1* gene (TCP1).

PCR reactions follow the references for each marker, products were examined on a 1% agarose gel, purified with Shrimp Alkaline Phosphatase and Exonuclease I, and sequenced using MEGABACE 1000 automated sequencer as described in Tchaicka *et al.* (2006). Chromatograms were checked with the Chromas 1.45 software (Technelysium), sequences were manually edited using BioEdit 6.0.7 (Hall, 1999) and aligned using the ClustalX 1.83 program (Thompson *et al.* 1997). Sequences were deposited in GenBank (the accession numbers will be available for the final manuscript).

Dealing with different datasets

To deal with differences in our genetic dataset (see below) our analyses were divided in three approaches. The inference of phylogenetic relationships among haplotypes, geographical distribution of variability and diversity indexes were based only on the mitochondrial DNA. As the three fragments of mitochondrial sequences (ATP8, ATP6 and CR) are a single locus we concatenated them in a single haplotypes for these analyses.

For the other analyses of population parameters using coalescent based methods we used the whole dataset, three mitochondrial and six nuclear genes, structured in seven loci (one mitochondrial and six nuclear) totalizing almost 3,000 base pairs.

Inferences of phylogenetic relationships of the lineages

We used NETWORK 4.5.0.0 (www.fluxus-engineering.com) program to construct a haplotype network employing the median-joining network (MJN) method

(Bandelt *et al.*, 1999). To infer a Maximum Likelihood phylogenetic tree among canids we utilized the PAUP* 4.0 program (Swofford, 2002). The search method was conducted with 100 replicates of TBR (tree bisection reconnection) algorithm using a Neighbor-Joining phylogram as starting tree. To determine the appropriate model of nucleotide sequence evolution we employed the MODELTEST 3.6 (Posada & Crandall, 1998) program and we used the minimum theoretical information criterion test (AIC) as suggested by Posada & Buckley (2004).

Diversity indices and coalescence approaches

The ARLEQUIN 3.1 program (Schneider *et al.*, 2000) was used to estimate population diversity statistics such as nucleotide (π) and haplotype diversity (HD), Watterson's theta (θw) Tajima's (Tajima, 1983) and Fu & Li's (Fu & Li 1993) neutrality tests and their statistical significance, F-statistics (F_{ST}; Hudson *et al.* 1992) and mismatch distribution analyses (Rogers & Harpending 1992).

Parameters such population growth (*G*) and theta (θ) were inferred using the MCMC method implemented in the package LAMARC 2.1.2 (Kuhner, 2006). We used the substitution model selected in MODELTEST for each fragment and set the initial parameters of θ based on the results of θw calculated in ARLEQUIN. Our search strategy was composed by three replicates of 10 initial chains and two long final chains. The initial chains were performed with 500 samples and a sampling interval of 20 (10,000 steps), using a burn-in of 1,000 samples for each chain. The two final chains were carried out with the same burn-in and interval sampling, but with 10,000 samples (200,000 steps). The confidence interval for theta and growth rate was calculated using the percentile approach.

We estimated the effective population size (N_e) using $N_e = \theta/4\mu$ (μ = evolutionary rate per generation, θ = theta, adjusting for diploid or haploid sequence). Generation time was calculated as a mean between the youngest reported age at maturity and the average reported life span minus 10% as a compensation for probability of survival until old ages. There are no information about longevity of maned wolfs in the field (Rodden *et al.* 2004), in captivity the maximum age recorded is 16 years and the average age of death is 7.3 years for those animals that were born in captivity and overcome the first year of age (Maia & Gouveia, 2002), so we used a mean between both values. Juveniles attain the sexual maturity at one year, but usually reproduce only at the second year (Rodden *et al.* 2004). To estimate the rate of sequence evolution for each fragment we used a relaxed molecular clock method based on a calibration point under the Bayesian approach implemented in Beast 1.4.8 (Drummond & Rambaut, 2003). Not all evolutionary rates could be estimated, because some fragments did not have enough information (number of sequences in canids or polymorphisms) to achieve the convergence of posterior probability distribution. Therefore, we estimated the substitution rate only to CR, ATP8/ATP6, CHRNA and SILV fragments. We used the Yule Speciation process as the Tree Prior, and set the priors for all molecular parameters based on the MODELTEST results. The calibration point represents the likely divergence between *Canis lupus* and *C. latrans*, based on the fossil record from *C. lupus* (700,000 years ago; Kurtén, 1968) and *C. latrans* (1 million years ago; Kúrten & Anderson, 1980), the prior was set as a normal distribution with mean 1.0 and standard deviation of 0.05, with the initial value set in 1 million. For the other introns (PLP, RAS, and TCP1) we used a mean value between the two rates estimated for intronic regions (CHRNA and SIL). The parameters for MCMC search were set in 3,000,000 chains sampling to log file each 1000 trees. The posterior distributions were checked and adjustments in the operators were done when suggested by the results.

As LAMARC indicated population growth (see results) we used the exponential model for population growth implemented in BEAST v1.4.8 to estimate the time of the most recent common ancestor (T_{MRCA}) for all sequences of maned wolves. This Bayesian approach incorporates the uncertainty in the genealogy by using MCMC integration under a coalescent model and use the exponential model for population growth as a prior for the time for each node. We used the evolutionary models suggested by MODELTEST and a length chain of 10,000,000 sampling to log file each 1000 trees.

Niche modeling

To estimate an ecological niche model for the current distribution of maned wolf we took our 87 sample points and 20 more sample points which were not used in our genetic analysis to increase our geographic sampling. Other three points were collected from literature. The data was checked in the DIVA-GIS software (Hijmans *et al*. 2002) for distribution bias or errors. We used 19 bioclimatic layers as predictors and the bioclimatic variables at a spatial resolution of 2.5 arc-min (Hijmans *et al*. 2005). The realized ecological niche was modeled using the MAXENT software for species habitat modeling (MAXENT; Phillips *et al.* 2006). The MAXENT algorithm estimates geographic distributions of species from locality point data by finding the maximum entropy distribution (Phillips *et al.*, 2006). MAXENT was ranked among the most effective methods for species distribution modeling from presence-only data in a recent comprehensive model comparison study (Elith *et al.*, 2006).

We divided the occurrence data into 75% of training data, the data used for model prediction, and 25% of test data, the data used for model validation. The resulting model was evaluated using the Receiver Operating Characteristics Curve (ROC) calculating the area under the curve (AUC). To generate a binary (presence/absence) prediction map we used accumulative threshold value that balances training omission, predicted area, and threshold value (Phillips *et al.*, 2006). After evaluating that the model predictions under current climate were satisfactory based on ROC, we used palaeoclimatic surfaces generated with the Paleoclimatic Modelling Intercomparison ProjectECHAM3 atmospheric general circulation model (DeutschesKlimarechenzentrum Modellbetreuungsgruppe, 1992).

Based on the results of Prates *et al.* (*in press*) we developed the hypotheses of historical distributions using projections for the LGM (app. 21 000 ybp) and the period before LGM, called the Last Interglacial period (LIG; app. 120 000 ybp). Data files were downloaded at their original resolution (www.worldclim.org).

RESULTS

Sequence variation

The alignment of nine fragments resulted in 2,974 base pairs (bp), 871 bp from mitochondrial DNA and 2,103 bp from nuclear DNA (Table 1). From all loci analyzed only six variable sites were found among the samples, and all were found in the first hypervariable segment of the mtDNA control region (Prates *et al.*, *in press*). Therefore, no new polymorphisms for the maned wolf were found in the 2390 bp analyzed here (Table 1).

The previously reported nucleotide diversity for maned wolf mtDNA was 0.0013. As reported by Prates *et al.* (*in press*) all the observed polymorphisms were single base-pair substitutions, which defined seven mtDNA haplotypes and the statistical parameters based on haplotype frequency, pairwise differences and number of polymorphic sites (as HD, Tajima's D and Fu's Fs) remain the same (Table 1), reinforcing the previously found signals.

Phylogeographic pattern

Among the seven haplotypes found in the 87 maned wolves, the ML phylogenetic tree (not showed) positioned the H6 haplotype (Table 1) from Argentina as the sister group of all others, although this is not significant. The median-joining network (Figure 2) corroborates the low differentiation found by phylogenetic methods. The network pattern can be interpreted as a signal of a relatively recent population expansion for the maned wolf, in which several localized lineages are connected by short branches to the most common, widespread haplotype.

Evolutionary model

The substitution model indicated by AIC for each loci within canids can be seen in Table 2, as well as the coalescent estimations for the substitution rates. The HKY model was selected for almost all loci, whereas for CHRNA the K80 model was selected, which differs from the former in assuming equal proportion for each nucleotide. Because only transitions were found, the estimated ti/tv rate was very high (tending to infinity), so to set this parameter (as a starting point or a fixed parameter) in further analyses we assumed a ti/tv rate of twice the exact number of nucleotides for each loci.

The estimated substitution rate for canids ranged from 1.44E-09 for SIL to 6.69E-08 for CR. For the CR this was very similar to that found by Savolainen *et al.*, (2002), which estimated a rate of 7.10E-08 using the genetic divergence in a phylogenetic tree including sequences from dogs, coyotes and wolves and based on the same calibration point of 1.0 My for coyote/wolf divergence. On the other hand, our estimated rate was almost twice faster than the evolutionary rate estimated for *Cerdocyon thous*, wolves, and coyotes using a different approach (3.68E-08; Tchaicka *et al*. 2007).

The coalescent analysis of genetic variability conducted in LAMARC for all loci produced signals similar to the results showed by Prates *et al*. (*submited*). However, the strength for these signals was much stronger. The parameter G showed a huge population growth with a value 11.6 times higher than that estimated using the CR fragment only (Table 3). On the same way, the N_e estimated using all loci was 25%

lower than previously estimated (Table 3). Therefore, the quality of the demographic estimates obtained with more loci and more nucleotides increased considerably, providing stronger support for the maned wolf small N_e, as well as, for a huge population size expansion. The distribution density for the log likelihood obtained for each locus can be seen in Figures S1 to S6 in the supplementary information.

The coalescent T_{MRCA} for all sequences within the maned wolf was a little higher than the population expansion estimated by Prates *et al.* (*in press*). The median value for the coalescent time was 22,800 ybp (CI 18,300-211,000) indicating the LGM period as the origin of all current genetic diversity in maned wolf (Figure 3).

Models prediction of potential distribution

The modeling predictions of current and past distribution for maned wolf are showed in Figure 4. Under current climatic condition the model prediction resulted in an AUC of 0.976 suggesting the high predictive power of the model. The predicted current distribution shows high similarity with the field estimated distribution of maned wolf (Rodden *et al.* 2004). The main discordance was the distribution in southern Brazil and Uruguay, which was showed as a suitable area for maned wolves, although not represent a current area for their distribution. However, the distribution showed in Fig. 1 does not represent a natural distribution, but the contemporary distribution, and there are records from the beginning of the last century that extend the maned wolf distribution to Uruguay and southern states of Brazil (Rodden *et al.*, 2004).

Projections of the current distribution on the past layers (Figure 4) showed large changes throughout time in the predicted area for potential niche. In relation to the current distribution the results show that during the LIG the suitable area for maned wolf decreased and was displaced to the South, spreading well into northeast Argentina. On the other hand, the projection for the LGM shows a potential distribution much larger than the current, spreading mainly to the North, to the central South America and to parts of the area occupied today by Amazon Forest.

DISCUSSION

Shallow genetic diversity

Prates *et al.* (*in press*) suggested that the very small diversity found in maned wolf mtDNA control region, although contrasting with the very preliminary findings that reported normal diversity for microsatellites loci (de Mattos *et al*., 2004; Salim *et al*., 2007), could be a signal of a historical population reduction. They argued that the mtDNA may reflect an earlier population bottleneck dating back to late Pleistocene whereas the microsatellites may tell a more recent history after the population expansion. Our extended results showing absence of polymorphism in five introns and two mtDNA coding regions, indicate that the mtDNA shallow diversity is spread in the maned wolf genome and should therefore reflects a demographic event, not an idiosyncratic event on the mitochondrial genome, such as a selective sweep.

The alternative hypothesis that this absence of diversity in these nuclear loci is due to a very slow substitution rate is not supported, since three of the analyzed introns were also studied in a close canid species, *Cerdocyon thous*, and all showed considerable levels of diversity. Based on a similar sampling scheme, Tchaicka *et al.* (2007) found four, six and four haplotypes, for the introns FES, CHRNA and PLP respectively, and the nucleotide diversity ranged from 0.0004 to 0.0009. Besides, with these data they inferred that *C. thous* demographic history went back >400,000 years ago. These results suggest that, although the evolutionary rates for the introns are several times slower than the mtDNA regions, combined they present enough information to distinguish between species that underwent a very recent bottleneck from one with a relatively long history. Similarly, we should expect more genetic diversity for maned wolf only if its N_e had been much larger than the estimated here. The N_e for *C. thous* was estimated as 400,000 individual, almost 400 times more than for maned wolf.

Consequently, the extremely low genetic diversity found in maned wolf could only be completely explained assuming a very small and recent ancestral population size for this species. This ancestral N_e should have been small enough to imprint a very strong signal of bottleneck even in the nuclear loci, which is four times larger than the N_e for mtDNA, and thereby much less susceptible to bottlenecks.

Population expansion and coalescent time

As we can see in Table 3 the estimated fluctuation in population size for the CR fragment showed a significant signal of population growth, although the neutrality tests (Table 1) were not significant. However, the power of these tests to infer population expansions is limited, since when the expansion is very recent and the number of segregating sites is very small, the difference between the two estimators of θ, could rarely be large enough to produce a significant signal. The coalescent approach presents much more power to identify fluctuations in population size, in special with the addition of more loci in the analysis (Felsenstein, 2005). Our T_{MRCA} estimate agrees with the above scenario since all diversity of maned wolf coalesced very recently at approximately 23,000 ybp. Therefore, the present result are a refinement of our previous values (Prates *et al.*, *in press*), and the huge value for *G* and the very small historical N_e estimated using all loci agree with the genetic signal of the current population size estimated in almost 13,000 mature individuals on field (Rodden *et al.* 2004).

Distribution modeling and evolutionary scenario

The predicted current distribution for maned wolf is concordant with an open dweller species. Even showing a putative distribution in some areas where the there are established costal Atlantic forest, the principal area prediction was clearly inside the current Cerrado region. The physiognomy of some Brazilian states could explain this prediction of maned wolf on some forest regions. As we used only climatic layers and some Brazilian regions, as São Paulo state, present a mosaic between forest and Cerrado, the threshold used probably homogenized the more finely organized mosaic areas.

Projection of the modeled distribution on the past layers agrees with the idea of Cerrado expansion in the LGM (Haffer, 1969). Carnaval & Moritz (2008) using modeling predictions for past distribution of Atlantic forest showed that the forest was contracted in this period in agreement with several paleopalinological studies (Ledru, 1993, Ledru *et al.*, 1996, Behling & Negrelle 2001), which have showed that, when not completely replaced by open areas the forest in LGM reduced significantly. Therefore, this scenario agrees completely with a maned wolf expansion during the LGM. Moreover, all the genetic diversity for maned wolf coalesces in this very moment in the past in agreement with this hypothesis.

On the other hand, for the LIG period we could expect the opposite in relation of open areas distribution. It has been showed (Otto-Bliener *et al.* 2006) that the LIG period (about 120,000ybp) was one of the warmest and wettest recent periods in the world history. The predicted distribution for maned wolf in LIG are smaller than in other periods, but the predominant signal is a displacement to southern regions where in current times maned wolf cannot habit, because these regions are too dryer to be suitable the high diversity of fruits that maned wolf feeds (Prevosti *et al*. 2004). There is no fossil record for maned wolves in those regions for LIG, however we could compare this period with a similar period of increased humidity and temperature. The Holocene Thermal Maximum or Holocene Climate Optimum (HCO) was a short period in which an increase in temperature and humidity has been recorded for several regions in the world (Ciais *et al*. 1992; Kaufman *et al*. 2004). The period recorded for HCO ranges from 12,000 to 7,500 ybp varying with the region studied, but all these studies agree that this period was warmer and wetter than the current climate, so we can expect a similar pattern of forest expansion and open area reduction, as well as a displacement of open dwellers to southern areas. Prevosti *et al*. (2004) presented fossil records of maned wolf in Argentina for an area 1,000 km farther south from the current distribution for maned wolf (Figure 5). They dated these fossils about 8,500 ypb, coinciding with the HCO period, supporting maned wolf southern displacement during HCO and by analogy supporting our modeling prediction of a southern distribution for maned wolf in warmer and wetter periods.

Our evolutionary scenario for the maned wolf is depicted in Figure 5. The maned wolf suffered a strong population reduction before LGM and based in our projection of distribution modeling this period could be in LIG, when the climate was very favorable for forest expansions and Cerrado retractions. During this period the distribution of maned wolf would be displaced to the southern regions of South America.

With the advance of climatic changes and the rising of cooler condition in LGM the area suitable for Cerrado biome and consequently for the maned wolves increased, and the species underwent a population expansion that imprint a clear genetic signal. After that period in late Holocene the change for a wetter and warmer climate in HCO displaced the vegetation distribution again, and maned wolf could be found in southern areas as evidenced by fossil records. We argue that HCO did not produced any significant effect in population size for open area dwellers as maned wolf since it had a much smaller impact on vegetation than LIG (Otto-Bliener *et al*. 2006; Kaufman *et al*. 2004).

Megafauna extinction and maned wolf survival

This scenario has several similarities with the hypothesis of historical vegetation changes influencing the megafauna extinction (de Vivo & Carmignotto, 2004).

As pointed in Introduction, there are several hypotheses to explain the megafauna extinction, and most are applied to all continents, and sometimes without concern about their differences. On the other hand, there are some hypotheses that are based on specific characteristics from each continent such as that proposed by de Vivo & Carmignotto (2004), related to differences between Africa and South America mammal fauna composition.

The similarities between South America and African continent in their landscapes, as the presence of extensive rain forests, savannas, steppes, and deserts are based on the similar global position of both continents, but this similarity contrasts with the huge difference in the extant mammal fauna (Vrba, 1993). This contrast is somewhat intriguing since some amount of faunistic convergence is expected under similar landscape evolution. The principal differences lie in the composition and behavior of faunistic elements: in Africa there are many large mammals, whereas South America is characterized by a great diversity of small mammals. However, during the Miocene both faunas showed high levels of convergence, with several ecologically analogous large mammals in South America (Cooke, 1972; Patterson & Pascual, 1972). This scenario changed abruptly during the Quaternary, when the South American megafauna was almost completely extinct, but this fauna from Africa almost not changed its composition. The climatic changes that occurred in both continents were similar during this period as in the whole globe (Petit *et al.* 1999; Jimin *et al.* 1998; Yokoyama *et al.* 2000), although the vegetational changes were probably very different. Some authors (Vrba, 1993; de Vivo & Carmignotto, 2004) suggested that this difference in vegetation distribution is the principal cause for the distinct present composition between South America and Africa. They argued that the geographic position of South America and Africa allowed differences in the distribution of similar vegetation cover. In Africa there is a tropical forest localized in the middle of the continent, bordered northern and southern by savanna and deserts, in South America the tropical forest is localized in the middle to north position and the open and dry areas are spread to southern regions. This difference could have a crucial role in the evolution of faunistic elements. During the wetter and warmer climates the forest increased in area and the open area dwellers should have to move accordingly. Based on this the authors suggested that while in Africa the open dweller fauna always had both borders to migrate to open areas, in South America the single path to migrate may have impacted negatively the survival of its megafauna (de Vivo & Carmignotto, 2004).

There are several criticisms concerning some points in this hypothesis (Araujo *et al*. 2004). The main objection is that it assumes a simplistic view, in which a dryer global climate increased open vegetation formations in Africa and South America and a wetter climate produced dense forest areas. This view did not take into account several local characteristics, such as the Tropical Convergence Zone and El-niño Southern Oscillations. Other criticisms are focused in some estimated dates for organic layers and the lack of consensus on the effects of HCO in South America (Araujo *et al*. 2004).

We concede that the de Vivo & Carmignotto´s hypothesis is not a complete explanation for the extinction of megafauna in South America and that other elements should be involved in a complex mixed model of human impact and climatic impact as claimed by Koch & Barnosky (2006). However, we showed that in the maned wolf, a likely survivor of the megafauna extinction in South America, the climatic and vegetation changes during the late Quaternary were the prime events that shaped the genetic diversity of this species.

References

Araujo AGM, Neves WA, Piló LB (2004) Vegetation changes and megafaunal extinction in South America: comments on de Vivo and Carmignotto (2004). *Journal of Biogeography*, **31**, 2039-2040.

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Barnosky AD (1986) "Big Game" extinction caused by late Pleistocene climatic change: Irish Elk (*Megaloceros giganteus*) in Ireland. *Quaternary Research*, **25**, 128–35.

Barnosky AD, Koch PL, Feranec RS, Wing SL, Shabel AB (2004) Assessing the Causes of Late Pleistocene Extinctions on the Continents. *Science*, **306**, 70-75.

Behling H, Negrelle RRB (2001) Late Quaternary tropical rain forest and climate dynamics from the Atlantic lowland in southern Brazil. *Quaternary Research*, **56**, 87-101.

Berta A (1987) Origin, diversification, and zoogeography of the large South American Canidae. *Fieldiana Zoology*, **39**, 455-471.

Burney DA, Burney LP, Godfrey LR, Jungers WL, Goodman SM, Wright HT, Jull AJT (2004) A chronology of late prehistoric Madagascar. *Journal of Human Evolution*, **47**, 25-63.

Burney DA, Flannery TF (2005) Fifty millennia of catastrophic extinctions after human contact. *Tree*, **20**, 395–401.

Carnaval AC, Moritz C (2008) Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. *Journal of Biogeography*, **35**, 1187-1201.

Ciais P, Petit JR, Jouzel1 J, Lorius C, Barkov NI, Lipenkov V, Nicolaïev V (1992) Evidence for an early Holocene climatic optimum in the Antarctic deep ice-core record. *Climate Dynamics*, **6**, 169-177.

Cooke HBS (1972) The fossil mammal fauna of Africa. Evolution, Mammals, and Southern Continents. In: Keast A, Erk FC, Glass, B, State University of New York Press, Albany, pp. 89-139.

De Mattos PSR, Del Lama MA, Toppa RH, Schwantes AR (2004) Populational genetic structure of free-living maned Wolf (*Chrysocyon brachyurus*) determined by proteic markers. *Brazilian Journal of Biology*, **64**, 639-644.

De Vivo M, Carmignotto AP (2004) Holocene vegetation change and the mammal faunas of South America and Africa. *Journal of Biogeography*, **31**, 943-957.

Deutsches Klimarechenzentrum(DKRZ)Modellbetreuungsgruppe (1992) *The ECHAM3 atmospheric general circulation model*. DKRZ Tech. Report No. 6, Deutsches Klimarechenzentrum, Hamburg, Germany, 184 pp.

Drummond AJ, Rambaut A (2003) BEAST version 1.0, Available from http://evolve.zoo.ox.ac.uk/beast/.

Edwards KJ, MacDonald GM (1991) Holocene palynology. II. Human influence and vegetation change. *Progress in Physical Geographic*, **15**, 364–91.

Elith J, Graham CH, Anderson RP, Dudik M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, Overton JM, Peterson AT, Phillips SJ, Richardson KS, Scachetti-Pereira R, Schapire RE, Soberón J, Williams S, Wisz MS, Zimmermann NE (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129-151.

Felsestein J. (2005) "*PHYLIP version 3.6*" Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Firestone RB, Westc A, Kennettd JP, Beckere L, Bunchf TE, Revayg ZS, Schultzh PH, Belgyag T, Kennetti DJ, Erlandsoni JM, Dickensonj OJ, Goodyeark AC, Harrish RS, Howard GA, Kloostermanm JB, Lechlern P, Mayewskio PA, Montgomery J, Poreda R, Darrah T, Que Hee SS, Smith AR, Stich A, Toppings W, Wittke JH, Wolbach WS, (2007) Evidence for an extraterrestrial impact 12,900 years ago that contributed to the megafaunal extinctions and the Younger Dryas cooling. *PNAS*, **104**, 16016–16021.

Forster M, (2004) Self-organised Instability and the extinction of the Australian megafauna. *Oikos*, **103**, 235-239.

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693–709.

Graham RW, Lundelius ELJ (1984) Coevolutionary disequilibrium and Pleistocene extinction. See Martin & Klein 1984, pp. 223–49.

Haffer J (1969) Speciation in Amazonian Forest Birds. *Science*, **165**, 131-137.

Hall TA (1999) bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hijmans RJ, Guarino L, Rojas E (2002) *DIVA-GIS*. A geographic information system for the analysis of biodiversity data. Manual. Lima, International Potato Center, 73 p.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965-1978.

Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, **132**, 583–589.

Jimin S, Zhongli D, Tungsheng L (1998) Desert distributions during the glacial maximum and climatic optimum: Example of China. *Episodes*, **21**, 28-31.

Johnson CN (2002) Determinants of loss of mammal species during the Late Quaternary ‘megafauna’ extinctions: life history and ecology, but not body size. *Proceedings of the Royal Society Biology*, **269**, 2221–2227.

Johnson WE, Eizirik E, Pecon-Slattery J, Murphy WJ, Antunes A, Teeling E, O’Brien SJ (2006) The Late Miocene Radiation of Modern Felidae: A Genetic Assessment. *Science*, **311**, 73-77.

Kaufman DS, Ager TA, Anderson NJ, Anderson PM, Andrews JT, Bartlein PJ, Brubaker LB, Coats LL, Cwynari LC, Duvallj ML, Dykek AS, Edwards ME, Eisner WR, Gajewskin K, Geirs AO, Hup FS, Jennings AE, Kaplan MR, Kerwin MW, Lozhkin AV, MacDonald GM, Miller GH, Mock CJ, Oswald WW, Otto-Bliesner BL, Porinchu DF,. R.uhland K,. Smol JP, Steig EJ, Wolfe BB (2004) Holocene thermal maximum in the western Arctic (0–180W). *Quaternary Science Reviews*, **23**, 529–560.

Koch PL, Barnosky AD (2006) Late Quaternary extinctions: state of the debate. *Annual Review of Ecology Evolution and Systematics*, **37**, 215-250.

Kuhner MK (2006) lamarc 2.0: maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics*, **22**, 768–770.

Kurtén B, Anderson E. (1980) *Pleistocene mammals of North America*. New York: Columbia University Press.

Kurtén B (1968) *Pleistocene mammals of Europe*. Aldine Publishing Company, Chicago. 317 pp.

Langguth A (1975) *Ecology and Evolution in the South American Canids*. In: The Wild Canids (ed Fox MW), Litton Educational Publishing, New York, p. 192-206.

Ledru MP (1993) Late Quaternary environmental and climatic changes in Central Brazil. *Quaternary Research*, **39**, 90 -98

Ledru MP, Braga PIS, Soubies F (1996) The last 50 000 years in the Neotropics (Southern Brazil): evolution of vegetation and climate. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **123**, 239–257.

Lyons LA, Laughlin TF, Copeland NG, Jenkins NA, Womack JE, O'Brien SJ (1997) Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. *Nature Genetics*, **15**, 47-56.

Macdonald DW, Sillero-Zubiri C (2004) *Introduction*. In: Macdonald DW, Sillero-Zubiri C, Hoffmann M (eds) Canids: foxes, wolves, jackals and dogs: status survey and conservation action plan. Gland, Switzerland and Cambridge, UK, IUCN Canid Specialist Group.

Maia OB, Gouveia AMG (2002) Birth and mortality of maned wolves *Chrysocyon brachyurus* (Illiger, 1811) in captivity. *Brazilian Journal Biology*, **62**, 25-32.

Martin PS (1973) The discovery of America. *Science*, **179**, 969-974.

MacPhee RDE, Marx PA (1997) The 40,000 year plague: humans, hyperdisease, and first contact extinctions. In Natural Change and Human Impact in Madagascar. Eds Goodman SM, Parmason BD, Smithsonian Institution Press, Washington, 168-217.

Murphy WJ, Sun S, Chen ZQ, Pecon-Slattery J, O'Brien SJ (1999) Extensive conservation of sex chromosome organization between cat and human revealed by parallel radiation hybrid mapping. *Genome Research*, **9**, 1223-1230.

Otto-Bliesner BL, Marshall SJ, Overpeck JT, Miller GH, Hu A, CAPE Last Interglacial Project members (2006) Simulating Arctic Climate Warmth and Icefield Retreat in the Last Interglaciation. *Science*, **311**, 1751-1753.

Patterson B, Pascual R (1972) *The fossil mammal fauna of South America*. In: Keast A, Erk FC, Glass B (Eds). Evolution, mammals and southern continents. Albany, State University New York Press, 543p.

Petit JR, Jouzel J, Raynaud D, Barkov NI, Barnola JM, Basile I, Bender M, Chappellaz J, Davis M, Delaygue G, Delmotte M, Kotlyakov VM, Legrand M, Lipenkov VY, Lorius C, P´epin L, Ritz, C, Saltzman E, Stievenard M (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature*, **399**, 429–436.

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231-259.

Posada D, Crandall KA (1998) modeltest: testing the model of DNA substitution. *Bioinformatics Applications Note*, **14**, 817–818.

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, **53**, 793–808.

Prevosti FJ, Bonomo M, Tonny EP (2004) La distribución de Chrysocyon brachyurus (Illiger, 1811) (mammalia: carnívora: canidae) durante el holoceno en la Argentina: implicancias paleoambientales. *Mastozoologia Neotropical*, **11**, 27-43.

Ratter JA, Ribeiro JF, Bridgewater S (1997) The Brazilian Cerrado Vegetation and Threats to its Biodiversity. *Annals of Botany*, **80**, 223-230.

Rodden M, Rodrigues FHG, Bestelmeyer S (2004) *Maned wolf (Chrysocyon brachyurus)*. 38-44, In: Sillero-Zubiri, C., Hoffmann, M. and Macdonald, D.W. (eds). 2004. Canids: Foxes, Wolves, Jackals and Dogs. Status Survey and Conservation Action Plan. IUCN/SSC Canid Specialist Group. Gland, Switzerland and Cambridge, UK.

Rodden M, Rodrigues F, Bestelmeyer S (2008) *Chrysocyon brachyurus*. In: IUCN 2008. 2008 IUCN Red List of Threatened Species. <www.iucnredlist.org>.

Rogers AR, Harpending HC (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Salim DC, Akimoto AA, Carvalho CB, Oliveira SF, Grisolia CK, Moreira JR, Klautau-Guimarães MN (2007) Genetic variability in maned wolf based on heterologous short-tandem repeat markers from domestic dog. *Genetics and Molecular Research*, **6**(2), 348-357.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual* 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Savolainen P, Zhang Y, Luo J, Lundenberg J, Leitner T (2002) Genetic Evidence for an East Asian Origin of Domestic Dogs. *Science*, **298**, 1610-1613.

Schneider S, Roessli D, Excoffier L, 2000. *ARLEQUIN, version 2.000*. A Software for Population Genetics Data Analysis. Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.

Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tajima F (1983) Evolutionary Relationship of Dna Sequences in Finite Populations Center far Demographic and Population Genetics. Texas, *Genet*ics, **105**, 437-460.

Tchaicka L, Eizirik E, Oliveira TG, Cândido-Jr. JF, Freitas TRO (2007) Phylogeography and population history of the crab-eating fox (*Cerdocyon thous*). *Molecular Ecology*, **16**, 819-838.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F (1997) The clustal windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.

Venta PJ, Brouillette JA, Yuzbasiyan-Gurkan V, Brewer GJ (1996) Gene-specific universal mammalian sequence-tagged sites: application to the canine genome. *Biochemichal Genetics*, **34**, 321-341.

Vrba ES (1993) Turnover-pulses, the Red Queen, and related topics. *American Journal of Science*, **293**, 418-452.

Yokoyama Y, Lambeck K, Deckker PD, Johnston P, Fifield LK (2000) Timing of the Last Glacial Maximum from observed sea-level minima. *Nature*, **406**, 713-716.

Wang X, Tedford, RH, Van Valkenburgh B, Wayne RK (2004) *Ancestry.* In: Macdonald DW, Sillero-Zubiri C, Hoffmann M (eds) Canids: foxes, wolves, jackals and dogs: status survey and conservation action plan. Gland, Switzerland and Cambridge, UK, IUCN Canid Specialist Group.

Wayne RK, Geffen E, Girman DJ, Koepfli KP, Lau LM, Marshall CR. Molecular systematics of the Canidae. *Systematics Biology* 1997; **46 (4):** 622-653.

Table 1. Gene diversity and population parameters for the whole distribution of *Chrysocyon brachyurus*.

Fragment		n	bp	S	h	HT	π	Neutrality tests	
								FF	TD
mtDNA	Control region (CR)	87	584	6	7	0.0669	0.0019	- 0.9323	- 0.1147
						(0.0450)	(0.0014)		
	Atp8/atp6	87	287	0	1	-	-	-	-
	Total mtDNA	87	871	6	7	0.0669	0.0013	- 0.9323	- 0.1147
						(0.0450)	(0.0009)		
ncDNA	PLP1	40	382	0	1	-	-	-	-
	FES	40	327	0	1	-	-	-	-
	CHRNA1	40	284	0	1	-	-	-	-
	SILV	40	350	0	1	-	-	-	-
	TCP1	8	350	0	1	-	-	-	-
	RASA2	8	410	0	1	-	-	-	-
	Total ncDNA	-	2103	0	1	-	-	-	-
Total mtDNA and ncDNA		87	2974	6	1	-	-	-	-

n (sample size), bp (base pairs), S (polymorphic sites), h (number of haplotypes), HT (haplotype diversity), π (nucleotide diversity), FF (Fu´s Fs) and TD (Tajima's D).

Table 2. Estimations of substitution model and substitution rate for each fragment within Canid.

loci	sub. Model	sub. Rate	95%L	95%U
CR	HKY	6.69E-08	2.30E-08	1.95E-07
ATP8	HKY	3.08E-08	2.16E-09	4.00E-08
CHRNA	K80	1.81E-09	5.75E-10	3.00E-09
SIL	HKY	1.44E-09	3.00E-11	2.84E-09
PLP*	HKY	1.62E-09	-	-
RASA*	HKY	1.62E-09	-	-
TCP1*	HKY	1.62E-09	-	-

* substitution rate as an average between SIL and CHRNA estimations

Table 3. Comparison among estimations of population size and exponential growth using a coalescent approach based only on mtDNA control region and on all 6 loci.

	Θ	N_{ef}	G
CR*	0.0015	1,840	369
	(0.0005-0.0017)	(694-2,070)	(106-881)
All loci	0.0009	1,130	4,286
	(0.0007-0.0011)	(951-1,430)	(1,231-10,221)

*Data from Prates *et al*. (in press); (Θ) theta; (N_{ef}) female effective sample size; (G) exponential growth parameter; values in parentheses are the confidence interval.

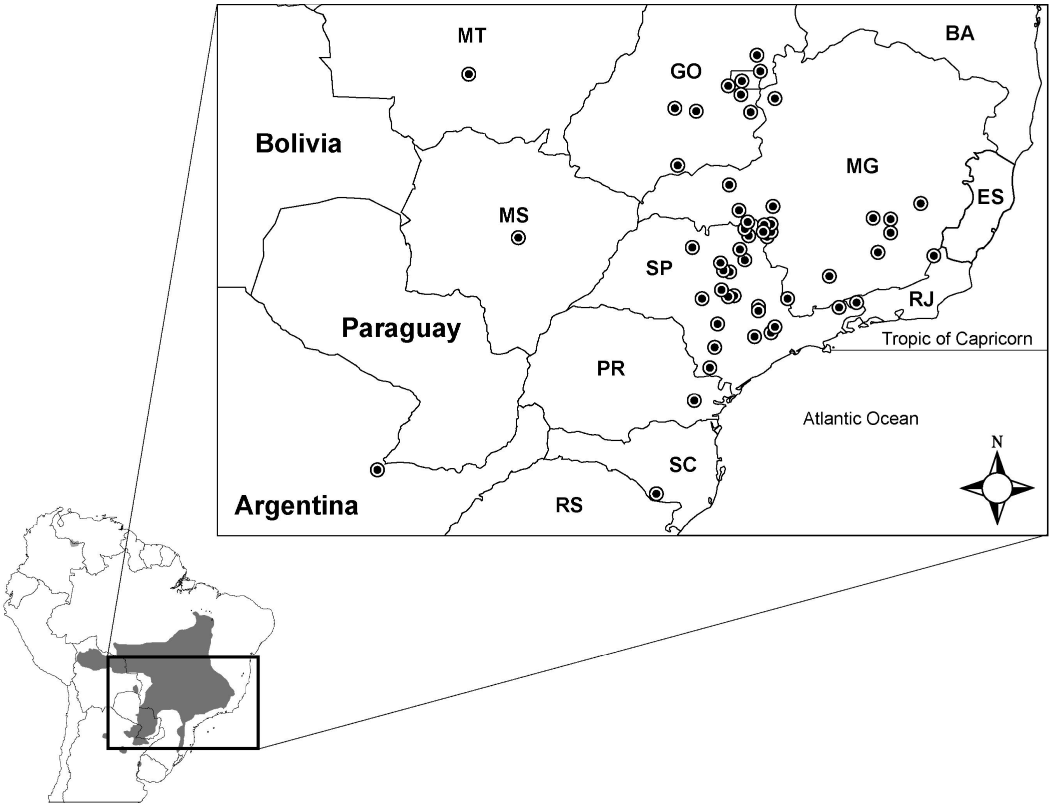

Figure 1 Geographical distribution of Maned Wolf (*Chrysocyon brachyurus)* showing the localities sampled.

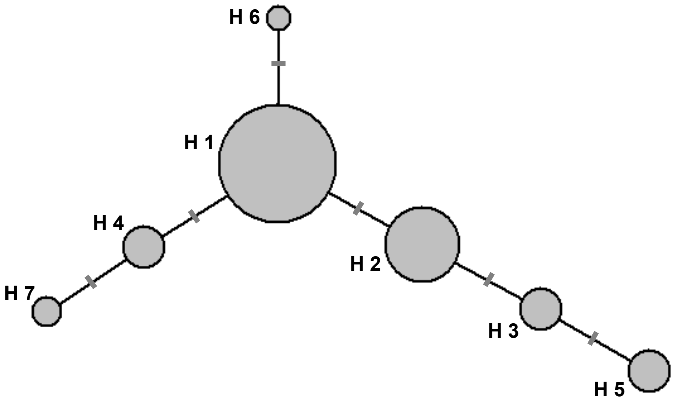

Figure 2. Median-Joining Haplotype Network of mitochondrial locus. Each circle represents a different haplotype (label as in Table S1 - Supp. Mat.) with size proportional to its relative frequency. The gray bars represent the number nucleotide substitutions inferred in that branch.

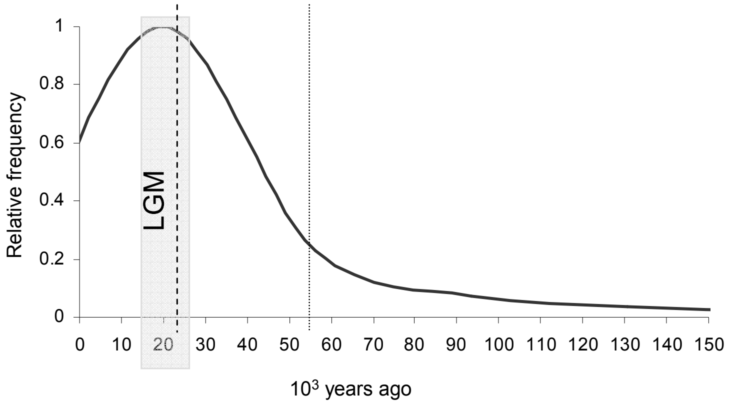

Figure 3. Bayesian estimation of T_{MRCA} density from maned wolf sequences. Traced line, median value (22,800 ybp); doted line, mean value (56,300 ybp).

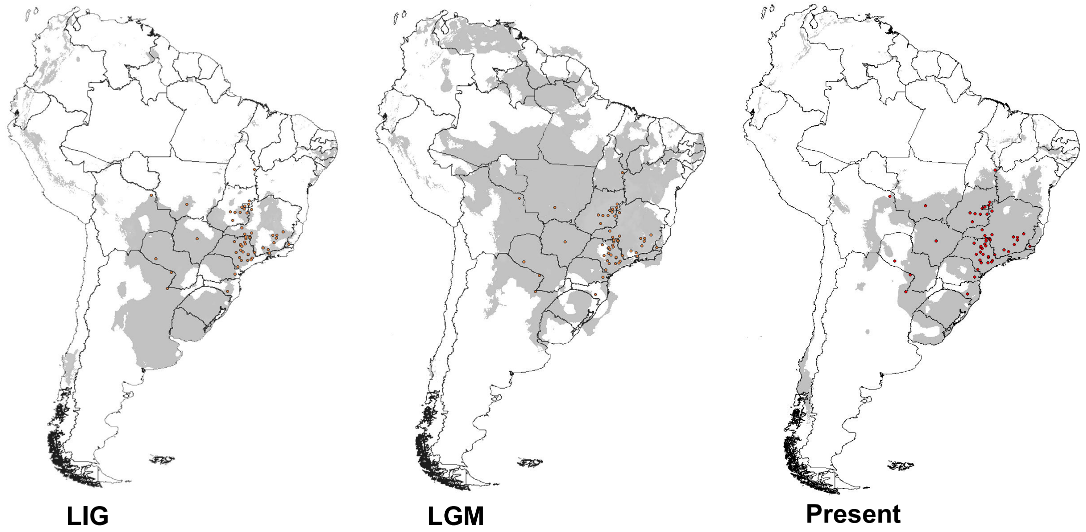

Figure 4. Potential distribution models predicted for maned wolf under current conditions (Present), Last Glacial Maximum (LGM) and Last Interglacial period (LIG). Areas predicted to be suitable conditions are shaded in grey. Samples used in prediction are shown with red circles.

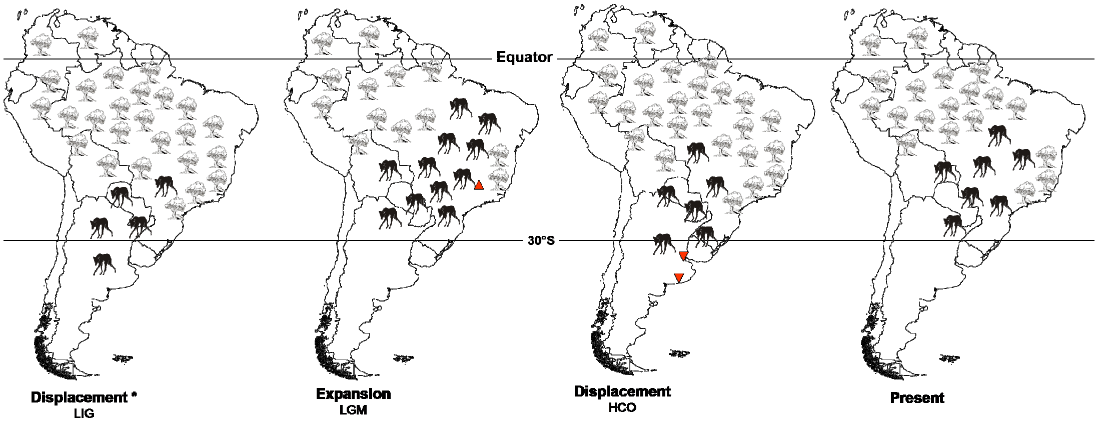

Figure 5. Hypothetical scenario concerning fluctuations in distribution and population size for maned wolf. Red triangle, fossil record form Pleistocene (30,000 years ago; Berta, 1984); red inverted triangles, fossil record from Holocene (8,500 years ago; Prevosti *et al*., 2004); * Displacement in LIG could be followed by reduction in population size.

Supplementary information

Table S1. List of localities for maned wolf included in predictions of potential distribution models.

Code_point	Localities	State	Lat	Long
1) 96/001	Tres Corações	MG	-21.683	-45.267
2) 96/002	Tres Corações	MG	-21.683	-45.267
3) 96/003	Pouso Alegre	MG	-22.367	-46.550
4) 96/004	Aiuruoca	MG	-21.917	-44.600
5) 96/012	Bauru	SP	-22.317	-49.067
6) 96/014	Angatuba	SP	-23.483	-48.400
7) 96/017	Brotas	SP	-22.283	-48.117
8) 96/020	Campo Grande	MS	-20.450	-54.617
9) 96/021	Uberaba	MG	-19.750	-47.917
10) 96/023	Castilho	SP	-20.867	-47.917
11) 96/024	Uberaba	MG	-19.750	-47.917
12) 96/025	Uberaba	MG	-19.750	-47.917
13) 96/026	Araxá	MG	-19.583	-46.933
14) 96/028	Araxá	MG	-19.583	-46.933
15) 96/031	Jundiai	SP	-23.183	-46.867
16) 96/032	Jundiai	SP	-23.183	-46.867
17) 96/034	Araçaíba	SP	-24.400	-48.850
18) 96/035	Brotas	SP	-22.283	-48.117
19) 96/036	Brotas	SP	-22.283	-48.117
20) 96/040	Brotas	SP	-22.283	-48.117
21) 96/041	Brotas	SP	-22.283	-48.117
22) 96/043	Itatinga	SP	-23.117	-48.600
23) 96/046	Luziania	GO	-16.217	-47.933
24) 96/047	Cristalina	GO	-16.767	-47.600
25) 96/049	Sto Ant. Descoberto	GO	-15.967	-48.267
26) 96/051	Formosa	GO	-15.533	-47.317
27) 96/052	Santa Rosa	GO	-15.083	-47.217
28) AE/ 75	Aguas Emendadas	DF	-15.783	-47.883
29) AE/01	Aguas Emendadas	DF	-15.783	-47.883
30) AE/Helga	Aguas Emendadas	DF	-15.783	-47.883
31) AE/Idea Fix	Aguas Emendadas	DF	-15.783	-47.883
32) AE/LG 82	Aguas Emendadas	DF	-15.783	-47.883
33) AE/LG92	Aguas Emendadas	DF	-15.783	-47.883
34) LG002	Luziania	GO	-16.217	-47.933
35) LG003	Sto Ant. Descoberto	GO	-15.967	-48.267
36) LG006	Unaí	MG	-16.333	-46.883
37) LG007	Uberlandia	MG	-18.900	-48.283
38) LG009	Goiania	GO	-16.683	-49.250
39) LG013	Joviania	GO	-17.800	-49.600
40) LG015	Nazario	GO	-16.600	-49.900
41) LG017	Alexania	GO	-16.733	-48.500
42) LG027	São Jose do Calçado	ES	-21.217	-41.650
43) LG028	Carandaí	MG	-20.950	-43.800
44) LG031	Ipatinga	MG	-19.483	-42.500
45) LG032	Aguas de Sta Barbara	SP	-19.967	-43.400
46) LG033	Bauru	SP	-22.317	-49.067
47) LG034	Angatuba	SP	-23.483	-48.400
48) LG036	Bauru	SP	-22.317	-49.067

49) LG040	São Jose do Rio Preto	SP	-20.800	-49.383
50) extr2SC	Lages	SC	-27.817	-50.317
51) Cbr02	Cuiabá	MT	-15.600	-56.083
52) Cbr03	Resende	RJ	-22.467	-44.433
53) Cbr04	Cuiabá	MT	-15.600	-56.083
54) Cbr05	Cuiabá	MT	-15.600	-56.083
55) Cbr06	Cuiabá	MT	-15.600	-56.083
56) Cbr07	Cuiabá	MT	-15.600	-56.083
57) 1arg	Corrientes	ARG	-27.483	-58.817
58) 3 arg	Corrientes	ARG	-27.483	-58.817
59) 4 arg	Corrientes	ARG	-27.483	-58.817
60) bCbr301	Serra da Canastra	MG	-20.233	-47.083
61) bCbr302	Serra da Canastra	MG	-20.233	-47.083
62) bCbr303	Serra da Canastra	MG	-20.233	-47.083
63) bCbr304	Serra da Canastra	MG	-20.133	-47.200
64) bCbr305	Serra da Canastra	MG	-20.133	-47.200
65) bCbr306	Serra da Canastra	MG	-20.133	-47.267
66) bCbr307	Serra da Canastra	MG	-20.267	-47.200
67) bCbr308	Serra da Canastra	MG	-20.367	-47.167
68) bCbr309	Serra da Canastra	MG	-20.367	-47.167
69) bCbr312	SP 334 Km 362	SP	-20.183	-47.733
70) bCbr313	SP 334	SP	-20.183	-47.733
71) bCbr314	SP 330 Km 246	SP	-21.383	-48.467
72) bCbr315	SP 330 Km 261	SP	-21.200	-48.467
73) bCbr317	Rod. Cândido Portinari Km 261	SP	-20.433	-47.733
74) bCbr318	SP 318 Km 248	SP	-22.033	-48.483
75) bCbr319	SP 334 Km 387	SP	-20.150	-47.717
76) bCbr320	SP 255 Km 31	SP	-21.500	-48.283
77) bCbr325	Itapira	SP	-22.717	-47.383
78) bCbr327	São João da Boa Vista	SP	-22.633	-47.383
79) bCbr364	Rod. Fernão Dias Km 30 BR381	SP	-23.383	-46.983
80) bCbr371	Serra da Canastra	MG	-20.133	-47.267
81) bCbr372	Serra da Canastra	MG	-20.133	-47.267
82) bCbr373	Serra da Canastra	MG	-20.133	-47.267
83) bCbr376	Serra da Canastra	MG	-20.267	-47.200
84) bCbr377	Serra da Canastra	MG	-20.267	-47.200
85) LG019	Goiânia	GO	-16.683	-49.250
86) LG026	Mariana	MG	-20.367	-43.417
87) LG029	Ribeirão Preto	SP	-21.167	-47.783
88) LG030	Belo Horizonte	MG	-19.917	-43.933
89) LG035	Curitiba	PR	-25.433	-49.283
90) LG039	Sorocaba	SP	-23.500	-47.483
91) LG043	Sorocaba	SP	-23.500	-47.483
92) LG044	Uberlândia	MG	-18.900	-48.283
93) LG046	Uberlândia	MG	-18.900	-48.283
94) 96006	Bauru	SP	-22.317	-49.067
95) 96007	Bauru	SP	-22.317	-49.067
96) 96011	Araxá	MG	-19.583	-46.933
97) 96018	Brotas	SP	-22.283	-48.117
98) 96029	Araxá	MG	-19.583	-46.933
99) 96030	Uberlândia	MG	-18.900	-48.283
100) 96037	Brotas	SP	-22.283	-48.117
101) 96038	Brotas	SP	-22.283	-48.117
102) 96039	Brotas	SP	-22.283	-48.117

103) 96042	Brotas	SP	-22.283	-48.117
104) 96048	Cristalina	GO	-16.767	-47.600
105) 8 arg	Corrientes	ARG	-27.483	-58.817
106) LG042	Sorocaba	SP	-23.500	-47.483
107) 5 arg	Corrientes	ARG	-27.483	-58.817
108) Deem & Emmons, 2005	Noel Kempff Mercado National Park	BOL	-14.298	-61.024
109) Redford & Eisenberg, 1992		ARG	-25.174	-58.227
110) Redford & Eisenberg, 1992		PAR	-23.241	-60.388
111) APA Jalapão - EIA/RIMA	Mateiros	TO	-10.626	-46.499

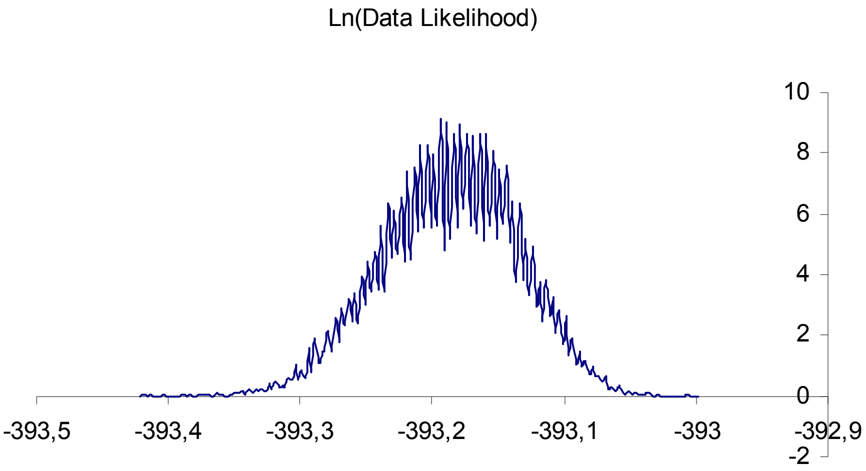

Figure S1. Ln likelihood density for LAMARC search based on "CHRNA1" sequences for maned wolf.

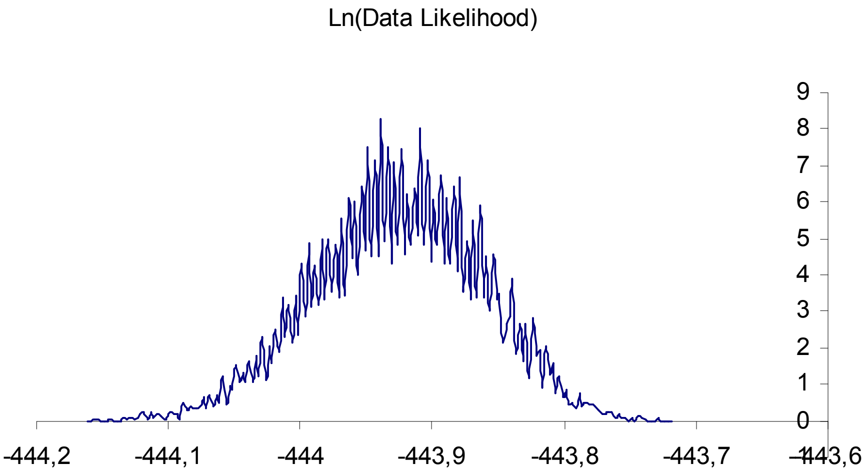

Figure S2. Ln likelihood density for LAMARC search based on "FES" sequences for maned wolf.

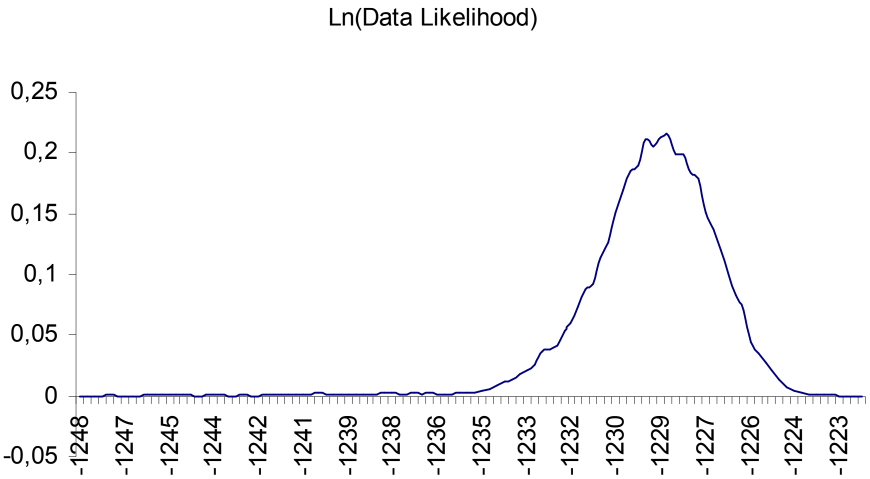

Figure S2. Ln likelihood density for LAMARC search based on "CCRATP8" sequences for maned wolf.

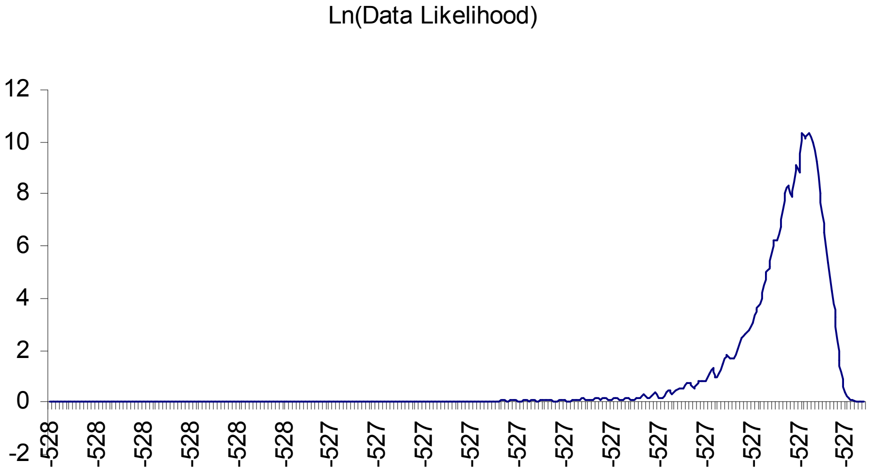

Figure S3. Ln likelihood density for LAMARC search based on "PLP" sequences for maned wolf.

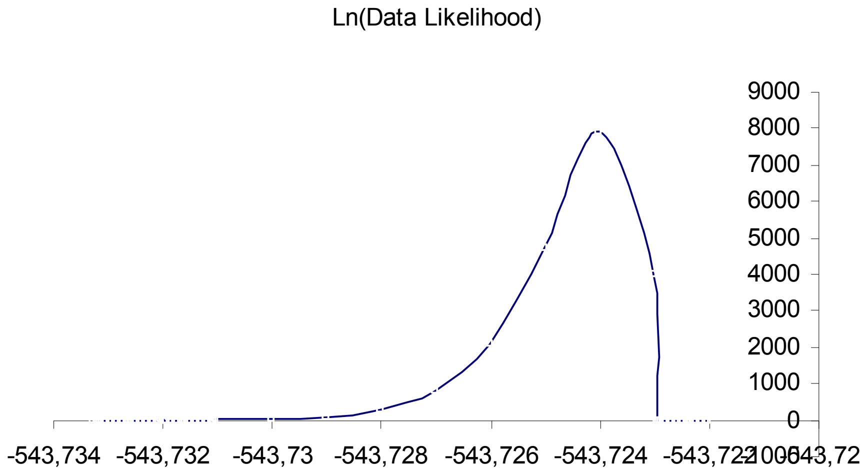

Figure S4. Ln likelihood density for LAMARC search based on "RASA2" sequences for maned wolf.

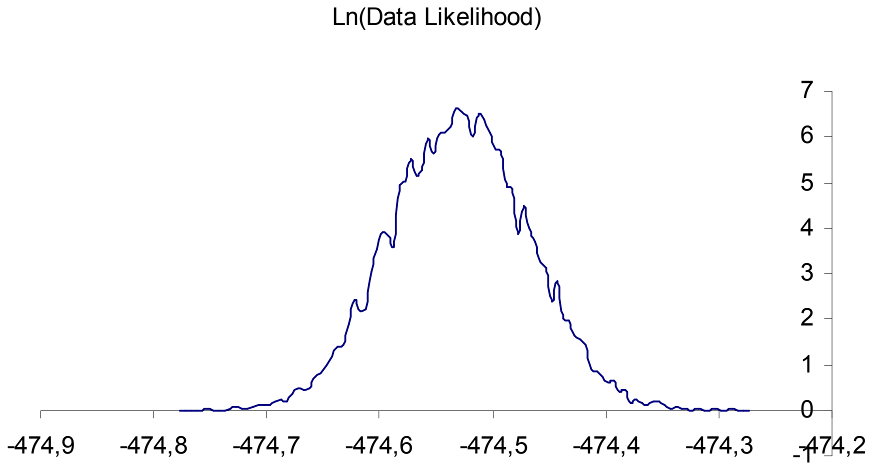

Figure S5. Ln likelihood density for LAMARC search based on "SILV" sequences for maned wolf.

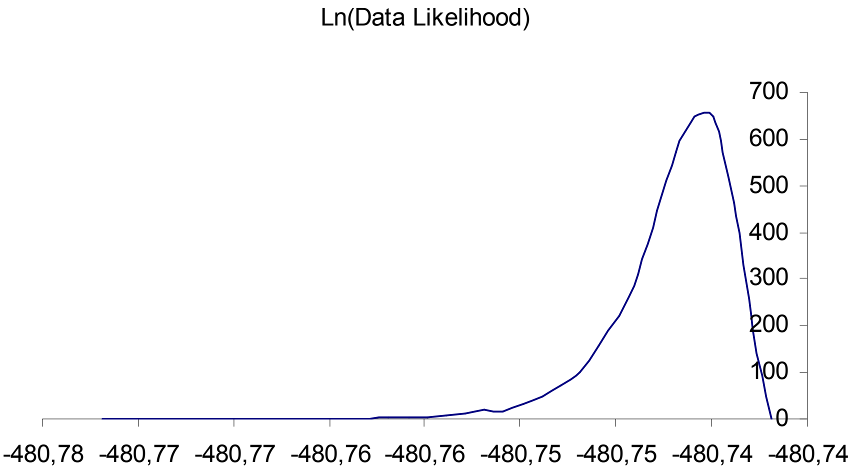

Figure S6. Ln likelihood density for LAMARC search based on "TCP" sequences for maned wolf.

Conclusões gerais

Os resultados obtidos no presente estudo contribuem para o preenchimento de lacunas relevantes da historia evolutiva e dinâmica populacional do lobo-guará (*Chrysocyon brachyurus*).

O lobo-guará tem a menor diversidade nucleotídica (0,0013) para a região controladora do mtDNA de todos os carnívoros já estudados. A amostragem utilizada neste estudo cobre praticamente toda a área de distribuição da espécie. A região controladora no lobo-guará é mais de 10 vezes menos diversa do que nos guepardos como um todo, sendo esta diversidade ainda menor do que a de um subgrupo de uma das subespécies de guepardos. A diversidade haplotípica do lobo-guará também é muito pequena (0,0669), não sendo geograficamente estruturadas. A maior diversidade genética, entretanto, está concentrada entre as amostras de Goiás, do Distrito Federal e São Paulo.

Nós analisamos outros fragmentos mitocondriais, como os genes ATP8 e ATP6, e seis *loci* nucleares, onde não encontramos nenhum sítio polimórfico nas amostras. Assumindo um tempo de geração de seis anos, nós estimamos um tamanho efetivo histórico de aproximadamente 3.340 indivíduos (IC: 1.260 - 3.760) para as seqüências da região controladora do lobo-guará. Essa estimativa é consistentemente menor do que o censo populacional de aproximadamente 23.600 animais e com uma população de quase 13.000 indivíduos maduros (Rodden *et al*. 2004). Essa baixa diversidade genética encontrada no lobo-guará pode ser explicada assumindo um pequeno tamanho ancestral das populações dessa espécie.

O padrão da rede de haplótipos, o "*mismatch distribution*" unimodal e os resultados do LAMARC podem ser interpretados como um sinal de um *bottleneck* populacional recente seguido de expansão populacional. Nós inferimos que a redução populacional ocorreu antes do último máximo glacial enquanto a grande expansão populacional teria ocorrido durante este período.

Os resultados sugerem que as mudanças climáticas ocorridas no Quaternário recente afetaram (direta ou indiretamente) a diversidade genética do lobo-guará na América do Sul e que há evidências de deslocamento na sua área de distribuição em direção ao sul do continente pelo menos durante o ótimo climático do Holoceno ocorrido entre 12 e 7,5 mil anos atrás.

Referências bibliográficas

Avise LC. *Phylogeography: the History and Formation of Species*. Massachusetts: Harvard University Press, Cambridge. 2000.

Bardeleben C, Moore RL, Wayne RK. A molecular phylogeny of the Canidae based on six nuclear loci. *Molecular Phylogenetics and Evolution*. 2005; 37 (3): 815-831.

Beccaceci MD. *Plan aguará guazú. Status poblacional del aguará guazú (Chrysocyon brachyurus) en la provincia de Formosa.* Buenos Aires: La Fundación Vida Silvestre Argentina,. 1985. 12 p.

Bestelmeyer SV, Westbrook C. Maned wolf (*Chrysocyon brachyurus*) predation on pampas deer (*Ozotoceros bezoarticus*) in central Brazil. *Mammalia*. 1998; 62: 591-595.

Breyer FRS. O guará. *Natureza em Revista.* 1977; 2: 50-53.

Breyer FRS. *O guará no Rio Grande do Sul (Mammalia, Canidae).* Brasília: IBDF/FBCN. 1979. 44p.

Carvalho CT, Vasconcellos LEM. Disease, food and reproduction of the maned wolf - *Chrysocyon brachyurus* (Illiger) (Carnivora, Canidae) in southeast Brazil. *Revista Brasileira de Zoologia*. 1995; 12(3): 627-640.

Coimbra-Filho AF. *Mamíferos ameaçados de Extinção no Brasil*. In: Espécies da Fauna Brasileira em Extinção. Rio de Janeiro: Academia Brasileira de Ciências.1972. p 13-98.

Dietz JM. Ecology and social organization of the Maned wolf (*Chrysocyon brachyurus*). *Smithsonian Contribution to Zoology.* Washington: Smithsonian Institution Press. 1984; 392: 1-51.

Dietz JM. *Chrysocyon brachyurus*. *Mammalian Species*. 1985; 234: 1-4.

Eisenberg JF. *Mammals of the neotropics*: *The Northern Neotropics*. Chicago: The University Chicago Press; 1989. p. 263-265.

Eisenberg JF, Redford KH. *Mammals of the Neotropics*: *The Central Neotropics*. Chicago: The University of Chicago Press; 1999.

Emmons LH, Feer F. *Neotropical rainforest mammals*: A field guide. Chicago: The University of Chicago Press; 1990. p.134-136.

Flynn JJ, Nedbal MA. Phylogeny of the Carnivora (Mammalia): Congruence vs Incompatibility among Multiple Data Sets. *Molecular Phylogenetics and Evolution*. 1998; 9 (3): 414–426.

Girman DJ, Vilá C, Geffen E. Patterns of population subdivision, gene flow and genetic variability in the African Wild Dog (*Lycaon pictus*). *Molecular Ecology*. 2001; 10 (7): 1703-1723.

Graur D, Li WH. *Fundamentals of molecular evolution*. 2. ed.. Sunderland: Sinauer Associates, Inc. 2000. 284 p.

Hare MP. Prospects for nuclear gene phylogeography. *Trends in Ecology and Evolution.* 2001; 16 (12): 700–706.

IBAMA. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. Brasília. *On line*. Disponível em: http://www.ibama.gov.br/

IUCN (World Conservation Union). IUCN red list of threatened species. IUCN – World Conservation Union, Gland, Suíça. 2003.

Jennings WB, Edwards SV. Speciational history of Australian grass finches (Poephila) inferred from thirty gene trees. *Evolution*. 2005; 59 (9) 2033–2047.

Johnson WE, Eizirik E, Pecon-Slattery J, Murphy WJ, Antunes A, Teeling E, O'Brien SJ. The late Miocene radiation of modern Felidae: a genetic assessment. *Science.* 2006; 311 (5757): 73-77.

Macdonald DW, Sillero-Zubiri C. *Introduction*. In: Canids: foxes, wolves, jackals and dogs: status survey and conservation action plan (eds Macdonald DW, Sillero-Zubiri C, Hoffmann M). Gland, Switzerland and Cambridge, UK, IUCN Canid Specialist Group, 2004.

Motta Júnior JC, Talamoni SA, Lombardi JA, Simokomaki K. Diet of the maned wolf, *Chrysocyon brachyurus*, in central Brazil. *Journal of Zoology*. 1996; 240: 277-284.

Nowak RM. *Walker's Mammals of the World*. New York: Ed Johns Hopkins University Press; 1999.

Rodden M, Rodrigues FHG, Bestelmeyer S. *Maned wolf (Chrysocyon brachyurus)*. In: Canids: foxes, wolves, jackals and dogs: status survey and conservation action plan (eds Macdonald DW, Sillero-Zubiri C, Hoffmann M). Gland, Switzerland and Cambridge, UK, IUCN Canid Specialist Group. 2004.

Rodrigues FHG. *Biologia e conservação do lobo-guará na Estação Ecológica de Águas Emendadas*. Distrito Federal. Universidade Estadual de Campinas, Campinas. 2002

Savolainen P, Zhang Y, Luo J, Lundenberg J, Leitner T. Genetic Evidence for an East Asian Origin of Domestic Dogs. *Science*. 2002; 298: 1610-1613.

Stains HJ. *Distribution and Taxonomy of the Canidae*. In: The wild canids: their systematics behavioral ecology and evolution (ed. Fox MW). New York: Van Nostrand Reinhold Company; 1975. p. 3-26.

Tchaicka L, Eizirik E, Oliveira TG, Cândido-Jr. JF, Freitas TRO. Phylogeography and population history of the crab-eating fox (*Cerdocyon thous*). *Molecular Ecology.* 2006; 16: 819-838.

Tedford RH *History of dogs and cats: a view from the fossil record*. In Nutrition and management of dogs and cats. St. Louis: Ralston Purina Co. 1978. p 1-10.

Vilà C, Amorim IR, Leonard JA. Mitochondrial DNA Phylogeography and Population History of the Grey Wolf (*Canis lupus*). *Molecular Ecology*. 1999; 8 (12): 2089–2103.

Wang X, Tedford, RH, Van Valkenburgh B, Wayne RK. *Chapter 2 Ancestry*. In: Canids: foxes, wolves, jackals and dogs: status survey and conservation action plan (eds Macdonald DW, Sillero-Zubiri C, Hoffmann M). Gland, Switzerland and Cambridge, UK, IUCN Canid Specialist Group. 2004.

Wayne RK, Valkenburgh, BV, Kat PW, Fuller TK, Johnson WE, O'Brien, SJ Genetic and morphological divergence among sympatric canids. *Journal of Heredity*. 1989; 80: 447- 454.

Wayne RK. *Conservation Genetics in the Canidae*. In: Conservation Genetics: case histories from nature (eds Avise JC, Hamrick JL). New York: Chapman & Hall; 1996.