Population Structure of Coyote (*Canis latrans*) in the Urban Landscape of the Cleveland, Ohio Area

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Abstract. To obtain information on the population structure of coyote (*Canis latrans*) in an urban setting, a non-invasive genetic sampling technique was applied that consisted of DNA isolation from scat collected around the Cleveland metropolitan area. Muscle tissue was provided from two other coyotes. Amplifying the mitochondrial D-loop or control region produced 33 haplotypes from just 57 coyote sequences and two additional dog sequences from putative coyote-dog hybrids. The mitochondrial DNA genetic diversity in the Cleveland area was high, π 0.02, and composed of six distinct haplotype lineages. In addition, F_{st} values ranged from 0.07 between collections east and west of the Cuyahoga River to 0.17 for samples separated between the Cuyahoga River valley and the adjacent Rocky River valley to the west. Interstate highways may restrict gene flow between populations that are based around the numerous large parks while channeling migrants towards the urbanized center. The low number of haplotypes sampled may attest to a poor reproductive rate after arrival, and the two dog-like haplotypes, which were confirmed using a nuclear gene, may be a secondary consequence of low mating opportunities for surviving males.

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INTRODUCTION

The coyote is considered a relative newcomer to eastern cities, like Cleveland, Ohio. The first official sighting of a coyote in Ohio may have been 1919, in Logan County, as documented in a popular sportsman's magazine of the time (Weeks and others 1990). From the late 1970's to the present, urban sprawl increased (Clapham 2003), and this growth around Cleveland created extensive hilly farmlands mixed with wooded areas, including open forests, forest-edge habitat, agricultural and urban areas. Such a habitat mix is often preferred by coyotes, especially where human-associated food supplements natural prey (Morey and others 2007).

Surrounding the urbanized areas are rings of parks. Howling surveys of coyote populations began in the Cuyahoga Valley National Park (CVNP) in 1993, from which estimates of populations ranged from 40 animals in 1996 to 88 in 2000 (Cepek 2000). Because direct observations of coyotes are uncommon, virtually nothing is known about their social structure and organization, group sizes, or how populations vary along an urban-rural gradient. Regionally, most of the coyote habitat is surrounded by human development, increasing the potential for interactions between coyotes and the public. Therefore, studies that assess the genetic variation of the population are necessary to help address several questions related to the movement of coyotes: 1) has the coyote population derived from one or several separate origins as coyotes migrated into the region, 2) has any population subdivision occurred due to habitat urbanization and fragmentation, and 3) have any hybridization events occurred with other canines, i. e. domestic dogs.

Non-invasive genetic sampling (NGS), in which the source of DNA is left behind in an animal's feces (Taberlet and others 1999; Waits 2004; Waits and Paetkau 2005), allowed us to collect DNA without the capture or disturbance of coyotes. Coyotes tend to deposit feces along established routes, such as roads or territorial boundaries (Kohn and Wayne 1997). For the present genetic analysis, part of the control region or D-loop of the mitochondrial DNA was used as a marker, as both mutations and substitutions may occur most rapidly in this sequence (Brown and others 1979; Saccone and others 1987; Ballard and Whitlock 2004). Canine mtDNA has a typical mammalian type mtDNA, although Kim and others (1998) have identified a heteroplasmy in the control region of domestic dogs (Canis familiaris) that causes its length to fluctuate depending on the number of tandem repeats present. Therefore, gene sequencing clearly separates coyotes from dogs. Furthermore, hybridization events between coyote and dog are detectable where a coyote type scat may occur with a dog type mitochondrial sequence. This can be verified by applying a nuclear marker gene.

MATERIALS AND METHODS

Sample Preparation

Scat samples (N=63) were collected in 2005 and 2006 from the two largest continuous areas of parkland in the Cleveland area. These lands lie within the Cuyahoga Valley National Park, which abuts the southern suburbs, and several Cleveland Metroparks along the Rocky River that form the western edge of a ring of parks that surround the city (Fig.1). Most scat samples were found on trails and utility easements within the selected sites. Scats were screened

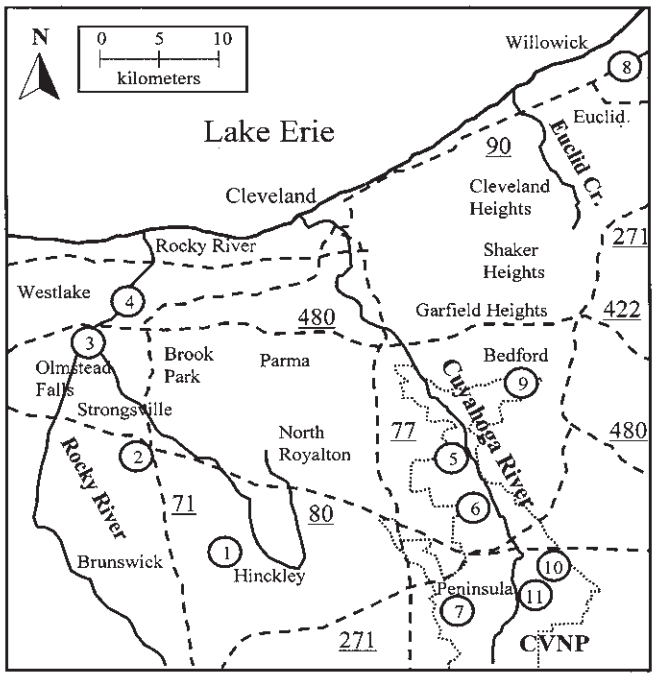

Figure 1. Eleven collection localities (circles) of coyote scat samples obtained in the Cleveland Metropolitan area. Numbers correspond with Table 1. Major streams are indicated in solid lines and interstate highways in broken lines.

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in the field based on size, morphology, and content. Selected areas were surveyed every two weeks in order to locate fresh samples. UTM and latitude/longitude coordinates were taken for each scat sample found using a handheld GPS unit (Magellan). Scat was collected using disposable gloves and transported back to the laboratory in individual Ziploc™ bags. In the laboratory, several small samples (180-220 mg each) from the outside of each scat were placed in microcentrifuge tubes and frozen at -20°C until extractions were performed.

DNA was extracted from the scat samples using the Qiagen™ QIAmp DNA stool mini-kit following the manufacturer's instructions (Qiagen, La Jolla, CA) yielding 200 µl of DNA extract. The quality of the extracted DNA was checked using 1% agarose gels; 10% of the total volume of extract (20µl) was size fractionated to verify the presence of high molecular weight genomic DNA and to estimate its concentration.

A canine-specific primer set was designed for amplification of a 533 base pair (bp) segment of the mitochondrial DNA control region. The primer set was designed using the complete mitochondrial genome sequence of *Canis familiaris* available in GenBank (accession no. U96639). Primer sequences were, sense: 5' TATATTGAATCACCCCTACTGTG; antisense: 5' GGCATATYCCCTGAGAGCAG. Amplification of DNA was performed in 20 µl reaction volumes containing: 2µl DNA, 1µl each of sense and antisense primer (0.12 mg/ml), 0.1 µl Taq polymerase (Fermentas, Hanover, MD), 12.5 µl 2 x buffer B (Epicentre, Madison, WI)), 1.5 µl 10 x enhancer (Epicentre), and 2 µl H_2O. The thermocycler (Techne Genius) settings for PCR were as follows: initial denaturation at 95°C for 2.5 min; 5 cycles of 30 sec at 95°C, 30 sec at 65°C, and 1 min at 72°C; followed by 25 cycles of 30 sec at 95°C, 30 sec at 60°C, and 1 min at 72°C, with a final extension of 72°C for 7 min.

The PCR products were separated on 1.2% agarose gels using a 1 kb marker ladder (Fermentas) as a size marker and were subsequently stained with ethidium bromide and visualized using UV light. The amplified D-loop DNA fragments were excised from the gels, purified using a freeze-phenol extraction process, and concentrated to a final volume of 12 µl using ethanol precipitation. These purified PCR products were cloned by ligation into the plasmid pGEM-T Easy (Promega, Madison, WI), which was then transformed into chemically competent E. coli cells (SURE strain, Stratagene, La Jolla, CA) using the TransForm Aid method (Fermentas). Cells were plated on agar plates containing ampicillin and 5-Bromo 4-Chloro 3-Indolyl-β- D-Galactopyranoside (X-Gal).White colonies were selected and grown overnight for purification of plasmid DNA. The plasmid DNA was purified using Qiagen columns following the manufacturer's instructions (Qiagen).

DNA Sequencing and Statistical Analysis

Recombinant DNAs were sequenced on a Beckman CEQ 8000 sequencer using T7 and SP6 primers. Fragment sequences were then read from the chromatograms using the computer program Sequencher™ (Gene codes 1999). In most cases both reads were identical or of enough quality to adjust discrepancies; in a few cases additional clones were sequenced for verification. Coyote scat samples were pooled into three distinct populations to assess geographic divergence.

Haplotype relationships were assessed both by phenetic and character based methods. A haplotype network was constructed using median joining algorithms (Bandelt and others 1999) in Network v. 4.2.0.1 (Rohl 2004). A gene tree was constructed using MrBayes 3.1.2 after determining that the HKY+G model was the most applicable to the data, based on MrModel test 2.2 in PAUP (Swofford 1998).

Mitochondrial DNA variation was described based on haplotype diversity (H), and nucleotide diversity (π). Population structure between western, central and eastern areas (Fig. 1) was tested using statistical estimates of divergence (Wright 1978). All calculations were performed with Arlequin 3.11 (Excoffier and others 2006).

After sequencing, two scat samples that were determined to have a domestic dog mtDNA haplotype were set aside to be analyzed with a bi-parentally inherited nuclear marker. A small region (335 bp) of exon 2 of the canine MHC class II gene DLA-DRB1 (Kennedy and others 1998) was selected for amplification as it showed the highest degree of polymorphism (17 alleles) in a study that compared European wolves with dogs (Seddon and Ellegren 2002). Primers were designed from published DLA-DRB1 gene sequences found in GenBank (accession no. AY126646 - AY126665): Primer sequences, sense: 5'TGACCGGATCCCCCCGTCCCCACAG; antisense: 5' TGTGTCACACACCTCAGCACCAGGCGCCC. Procedures for amplification and sequencing were the same as those used for mtDNA amplification. Obtained nuclear sequences were then compared to known coyote and dog sequences using DNA isolated from a blood sample a local veterinarian provided and from muscle samples derived from a male and a female coyote killed by a hunter.

RESULTS

From 63 scat samples and two muscle tissue samples, 57 D-loop region DNA sequences were obtained, which provided 33 different coyote-type haplotypes (accession numbers EU400544-EU400576). The coyote type sequence was a 531 bp segment with some variation in size among haplotypes caused by several small indels that were identified at bp position 40, 53 and 455. A consensus sequence derived from the two dog-type sequences (accession numbers EU400577-EU400578) was 539 bp in length, indicating that the D-loop region of the coyote is slightly shorter than that for dogs.

In 57 samples just five haplotypes were present in multiple copies; 28 were recovered only once (Table 1). The most common haplotype (N=12) occurred only in samples collected in the Cuyahoga Valley National Park, a second present in just eight copies occurred in each region, while none of the three present in smaller numbers (one each with N= two, three or four copies) occurred in the eastern region.

Corresponding with high levels of gene diversity is a measure of nucleotide diversity, π or the average proportion of differences between any two samples drawn at random. That value approached 0.02 or about 9-10 differences between each haplotype. This variation is easily visualized within a haplotype network (Fig. 2), which highlighted at least six potential haplotype groups based on the large number of mutations separating each cluster. A Bayesian assessment of the posterior probabilities using a control region sequence from dog as an outgroup confirmed the presence of each of these clusters, and it added support for a seventh group by separating sequence 11 from the cluster 16,18,27 (Fig 3). The Bayesian analysis also differed by placing outlying haplotype 33 as a sister taxon to a cluster that includes haplotype 1 rather than one with haplotype 2, although both clusters only include eastern samples.

Plotting geographical distribution on the arrangement of haplotypes indicated that the coyote populations in the Cleveland area do not freely intermix. Samples partitioned among three

regions, (1) east of the Cuyahoga River, (2) west of the Cuyahoga River but still in the CVNP, and (3) in the Rocky River watershed, possessed a fixation index (F_{st}) of 0.077. Pair wise F_{st} analyses indicated differences progressively increasing with distance, as the Metropark populations along the Rocky River differed from the central population, as determined from samples collected between a large highway (I77) and the Cuyahoga River at 0.070, and this western group of samples differed from samples collected east of the Cuyahoga River at an F_{st} of 0.17. The two CVNP groups, which were physically separated only by the Cuyahoga River, were not significantly different from each other.

Possible Hybrids

Two scat samples found at site 5 in the CVNP had the same characteristic morphology as coyote scat, but the mitochondrial DNA obtained had a dog-specific signature sequence. Of the exon 2 sequences of DLA obtained for each sample, several were sequenced and two sequence types for each mitochondrial haplotype were found, one-coyote like and one dog-like. The dog and coyote types differed in 19 (first haplotype) – 23 (second haplotype) positions in the 335 bp fragment, and each pair of dog-like and coyote-like sequences were different (accession numbers EU400579-EU400582).

DISCUSSION

High levels of variation among haplotypes obtained from the eastern/central and western localities suggest that coyotes within the Cleveland Metropolitan area are abundant and partially isolated. Although sample sizes are not large, almost half of all scats analyzed produced a unique haplotype, and only five haplotypes were shared among individuals. The mean number of multiple occurrences among haplotypes (1.7 ± 0.4) is about half that found by Kohn and others (1999) who applied multilocus genotyping to coyote scat from the Santa Monica Mountains near Los Angeles. To summarize, these 33 haplotypes correspond to the minimum number of maternal family groups required to be present in the region, and even if every haplotype in common at each site is assumed to come from the same individual, at least 43 different coyotes must have produced scat sampled here.

This high level of diversity fits theoretical predictions for population composed of recent colonists to an area (Hedrick 2000). Sightings of coyote were rare before the 1970s (Weeks and others

Table 1

Numbers and location of mitochondrial D-loop hapoltypes.

Coyote Haplotype Data	01	02	03	04	05	06-33	Totals	gene divrsity, H	SD of H	π x 100 (nucleotide diversity)	SD of π x100
"Western"		**4**	**2**	**1**		**13**	**20**	0.963	0.03	1.78	0.953
1. Parker Rd Bridal Trail, Hinkley						1	1				
2. Albion Rd. Mill Stream Run						2	2				
3. Lewis Rd Riding Range				1		1	2				
4. Rocky River Stables		4	2			9	15				
"Central"	**6**	**1**	**2**	**2**	**2**	**13**	**26**	0.945	0.03	1.77	0.036
5. Sanitation Rd, CVNP	6	1	2	1		11	21				
6. Riverview Pipeline, CVNP					2	1	3				
7. Everett Rd, CVNP				1		1	2				
"Eastern"	**6**	**3**				**2**	**11**	0.673	0.12	1.47	0.836
8. Willowick	1					1	2				
9. Circle Emerald	2						2				
10. CVNP Fieldstaion	2	2					4				
11. Truxell Rd	1	1				1	3				
Overall N &	12	8	4	3	2	28	57	0.932	0.02	1.79	0.924
Frequencies	0.211	0.140	0.070	0.053	0.035	0.018					

1990), but these canines are now common in the region. Park personnel estimated in the late 1990s that at least 40-70 live within the 13,770 hectare Cuyahoga Valley National Park (Cepek 2000), which is contiguous to a large county park system that runs along most of the area's larger streams. Therefore, the coyote populations are likely large and increasing due to abundant prey (Cepek 2004). The annual home range of urban coyotes has been estimated at an average of 10.8 km^2 (Atkinson and Shakleton 1991), decreasing as urbanization levels increase (Grinder and Krausman 2001). Such a pattern, which suggests that the CVNP alone could support at least 100 coyotes, is applicable to the other parks around Cleveland (Bollin-Booth 2007).

The patchy nature of Cleveland's Parks (Fig. 1) combined with high levels of urbanization closer to Lake Erie produces a landscape where coyote populations are predicted to be isolated, and possibly ephemeral to the north. North-south migratory routes are provided by the riparian corridors along the Cuyahoga River in the center, Rocky River to the west, and Euclid Creek and the Chagrin River to the east [collections in the Chagrin Valley were restricted during our study period due to a rabies incident in one coyote (Frischkorn 2005)]. The Cuyahoga River, however, did not isolate populations within the CVNP, but the Cuyahoga Valley populations were genetically different from populations along the Rocky River. The major barriers between these localities are downtown Cleveland at Lake Erie's coast, and two major interstate highways farther south, I71 and I77 (Fig. 1). Even large coyote populations outside of Los Angeles are impacted by a combination of natural and man-made features to the environment. Riley and others (2006) tracked coyotes with radio collars and applied molecular markers to assess diversity, and they found that while individuals occasionally will cross even large highways, these structures form the territorial boundaries, and gene flow across them is greatly reduced. Previously, effects of interstate highways to structure populations of small and less mobile species like voles (Gerlach and Musolf 2000) and amphibians (Reh and Seiz 1990; Lesbarreres and others 2006) were well documented, but with high traffic levels even at night and barrier fences to keep deer off the roads, movement of large carnivores may be restricted to bridges from secondary roads and underpasses.

The second impact coyotes face from contact with humans, is interactions with domestic dogs. The combination of a coyote type scat, a dog-like mitochondrial sequence, and a mixed nuclear DNA marker, confirmed that two scat samples derived from coyote-dog hybrids. That the dog-type control region haplotypes also differed suggested that the putative hybridization events were separate

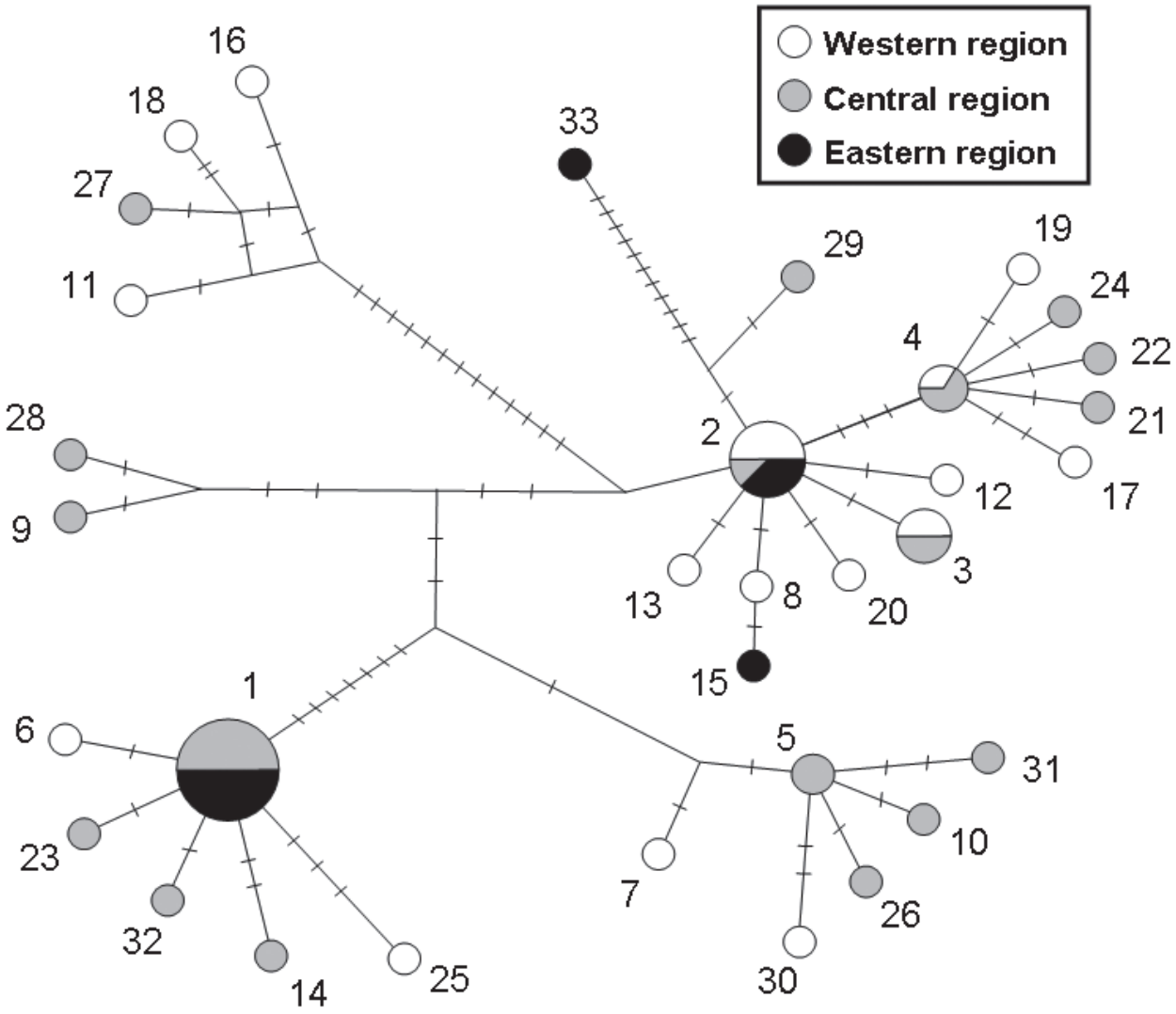

FIGURE 2. A network of the 33 coyote haplotypes for the control region of the mitochondrion. Regions correspond with Fig. 1: the western region is sites 1-4; the central region is sites 5-7; the eastern region is sites 8-11. Hatch marks indicate the number of DNA base differences between connecting haplotypes.

even though both were collected in the same region. While scat of dogs and coyote can be sufficiently variable as to overlap in appearance (Kohn and others 1999), care was taken to sample only coyote scat. Canines readily hybridize (Schmutz and others 2007), although coydogs are expected to derive from a female dog and male coyote, as coyotes provide parental care, which is believed to be nearly an obligate requirement for successful reproduction of canines in nature (Boitani and Ciucci 1995; Pal 2005). The cause of hybridization is not known, but appears to occur where the patchy and potentially ephemeral nature of the populations reduce mating opportunities for coyote males. As these hybrids integrate into the coyote populations, introgression of dog genes, previously identified in the western United States, could impact wild canine populations widely (Adams and others 2003).

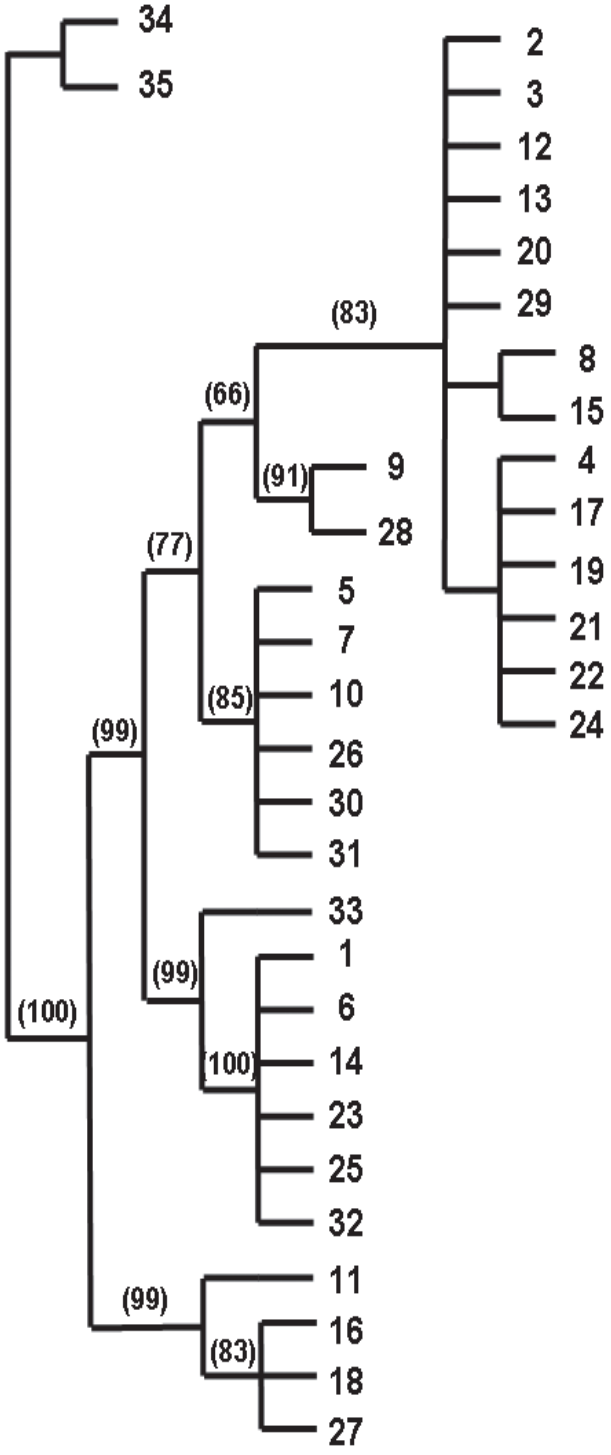

Figure 3. A gene tree for the control region of coyotes (haplotypes 1-33) was constructed using MrBayes 3.1.2, based on an HKY+G model algorithm for base changes in the sequence. Numbers at the end nodes correspond to the haplotype designation (Fig. 2) and the parenthetical notations within the tree correspond to the % of the time that arrangement was retained in the tree during randomization. Haplotypes labeled 34 and 35 are the putative hybrids that match to domestic dogs.

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