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## Genetic Variability in Six Mexican Gray Wolf (*Canis lupus baileyi*) Populations Determined by Microsatellite Markers

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### Abstract

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A study was conducted to evaluate genetic diversity in six Mexican gray wolf populations based on six microsatellite loci. Allelic frequencies, heterozygosity and genetic distances were determined with a pairwise analysis of the genetic distance means to demonstrate that the six wolf populations are genetically very close (0.17-0.41), with marked grouping between populations. Notable differences were observed between allelic frequency profiles for the six microsatellites in the six studied Mexican gray wolf populations. Further studies using Mexican gray wolf populations and other related canid populations are recommended.

Key words: Microsatellite, DNA, polymorphism, genetic diversity, Mexican gray wolf.

### Introduction

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The Mexican gray wolf (*Canis lupus baileyi*) is at the greatest risk of extinction. The current challenge is to maintain this subspecies' genetic diversity by preventing

the expression of deleterious or damaging genes, without changing the original germ plasm, in a population of 128 certified pure individuals. Molecular-level genetic studies have lead to substantial progress in determining genetic variability within species, subspecies and populations. Genetic diversity is responsible for significant biological phenomena such as species evolution, intra-species evolution and the presence of unique, non-repeatable differential characteristics in each individual. Variations in the genome sequence are the cause of variability and thus, in a broader sense, the concept of diversity is synonomous with genetic polymorphism (Luque and Herráez, 2001).

To measure polymorphism, the proportion of heterozygotic individuals in a population is calculated. Using this method, wolves in captivity have been shown to lose genetic diversity, which can negatively impact reproductive function and affect subspecies survival time (O'Brien *et al.*, 1985). García-Moreno *et al.* (1996) studied DNA in three Mexican gray wolf lineages and suggested that a cross between the three lineages would preserve allelic diversity and alleviate the risk of endogamic depression. The present study was aimed to identify the degree of existing polymorphism in the current certified population of Mexican gray wolf in Mexico through evaluation of six microsatellite markers.

### **Materials and Methods**

A total of 33 Mexican gray wolves (*Canis lupus baileyi*) from six populations maintained in captivity in Mexico were used following strict principles of animal care. Blood samples were collected from these gray wolves. The DNA was extracted using the protocol suggested in the Wizard™ Genomic DNA Purification Kit (Promega Corporation, Madison, WI).

Genetic diversity at six microsatellite loci was determined using CPH4, CPH5,

CPH7, CPH12, CPH18 and CPH20 primers, which are known to be useful in amplifying several Canidae species (Fredholm and Wintero, 1995). The PCR conditions for each locus were individually optimized, varying MgCl<sub>2</sub> concentrations and using 50 ng of total DNA, 1.25 U of Taq DNA polymerase (Promega Co, Madison, Wi. USA) and 0.5 mM dNTPs in a 10 ml final volume. For PCR product analysis in a LICOR automated sequencer, 5'-primers were labeled with IRD800. Amplification was done using the Touchdown method (Hecker and Roux, 1996). The PCR products were denatured at 95C for 5 min and then submitted to electrophoresis on a 6.5% denaturing polyacrylamide-bisacrylamide gel for 2 h. Allele size data for each locus were obtained using SAGA GTTM software.

Genetic differences were calculated according to Molina (1992) and Falconer (1966) with the SAS® program (SAS, 1992). A pairwise analysis of the genetic distance means was performed to estimate the proportions of allelic substitutions that have occurred per genetic locus during independent evolution of the studied populations (Rohlf, 1993; POPGENE, 1999). The genetic distance matrices needed to build the dendrogram were generated using the genetic frequencies of the six loci in a UPGMA with the NTSYS-PC program (Rohlf, 1993). All data were analyzed following a Chi square test ( $\chi^2$ ). Differences were considered statistically significant if P = 0.05.

### **Results and Discussion**

All the six microsatellite loci exhibited a high degree of polymorphism in the six analyzed populations, with total alleles per locus ranging from 1 to 5 (Table 1). The number of effective alleles varied from 1.69 to 2.22. It is important to remark the differences in the number of alleles per locus between populations, locus CPH7 showed the lowest number.

Table 1  
Number of alleles, expected and observed heterozygosity of the 6 microsatellite loci

Loci	Population**	Number of alleles	He	Ho
CPH4	W1	3	0.4835	0.4286
	W2	2	0.3333	0.3333
	W3	5	0.8444	1.0000
	W4	4	0.7229	0.4545*
	W5	3	0.8383	1.0000
	W6	2	0.3556	0.4000
CPH5	W1	4	0.7143	0.8571
	W2	4	1.0000	1.0000
	W3	3	0.6000	0.3333
	W4	4	0.5758	0.5455*
	W5	2	0.5000	0.5000
	W6	3	0.6444	1.0000
CPH7	W1	1	0.0000	0.0000
	W2	1	0.0000	0.0000
	W3	3	0.3778	0.2000*
	W4	2	0.1732	0.1818
	W5	2	0.5000	0.5000
	W6	1	0.0000	0.0000
CPH12	W1	4	0.6923	0.5714
	W2	4	0.8667	1.0000
	W3	4	0.6444	0.4000
	W4	3	0.3846	0.8571*
	W5	3	0.8333	1.0000
	W6	3	0.6444	1.0000
CPH18	W1	2	0.3626	0.4286
	W2	2	0.6000	0.3333
	W3	2	0.3556	0.0000*
	W4	2	0.4545	0.2727
	W5	2	0.5000	0.5000
	W6	2	0.3556	0.4000
CPH20	W1	3	0.4725	0.1429*
	W2	2	0.3333	0.3333
	W3	2	0.2500	0.2500
	W4	2	0.0909	0.0909
	W5	1	0.0000	0.0000
	W6	2	0.3556	0.4000

\*Loci deviated from Hardy-Weinberg equilibrium ( $P<0.05$ )

\*\*W1-7 samples from Puebla, W2-3 samples from Tamaulipas, W3-5 samples from Edo. México, W4-11 samples from Cd. México, W5-2 samples from Guanajuato, W6-5 samples from Chihuahua.

Different populations also had quite different deviations from the Hardy-Weinberg equilibrium. Population W4 had significant deviations ( $P<0.05$ ) in loci CPH4, CPH5 and CPH12, population W3 showed deviations in loci CPH7 and CPH18, and W1 appeared only in one (CPH20) of the six studied loci. Deviations in the Hardy-Weinberg equilibrium can result from a wide variety of causes. These can lead to high consanguinity levels in a population from mating between related individuals, or from the presence of a certain reproductive substructure in the population (Aranguren-Méndez *et al.*, 2005). Also, the sample size (33 individuals) could be influencing these deviations.

Heterozygosity varied between the loci. Loci CPH4, CPH5 and CPH12 had the highest heterozygosity versus CPH7, which had the lowest (Table 1). The CPH20 and CPH18 loci had a moderate degree of heterozygosity.

The frequency histograms showing the 6 loci from the six Mexican gray wolf populations (Fig. 1) demonstrated that the alleles in loci CPH4, CPH5 and CPH12 had the highest frequencies and were present in all six populations. This contrasts with locus CPH7, which only had three alleles, two of which were of minimum frequency (160 and 162) in the W3, W4 and W5 populations.

In a study using seven microsatellites, including three used in the present study, Dolf *et al.* (2000) reported that CPH4 matches allele lengths 140, 142, 144, 146 and 148. However, these lengths are more frequent in domestic dogs. For locus CPH20, allele 105 is present in all six populations with a frequency ranging from 0.8 to 1.0.

Using the UPGMA method (Rohlf, 1993), mean genetic distances in the six populations were shown to range from 0.17 to 0.41. These vary slightly from those of García-Moreno *et al.* (1996), who reported values from 0.091 between the Aragón and Ghost Ranch

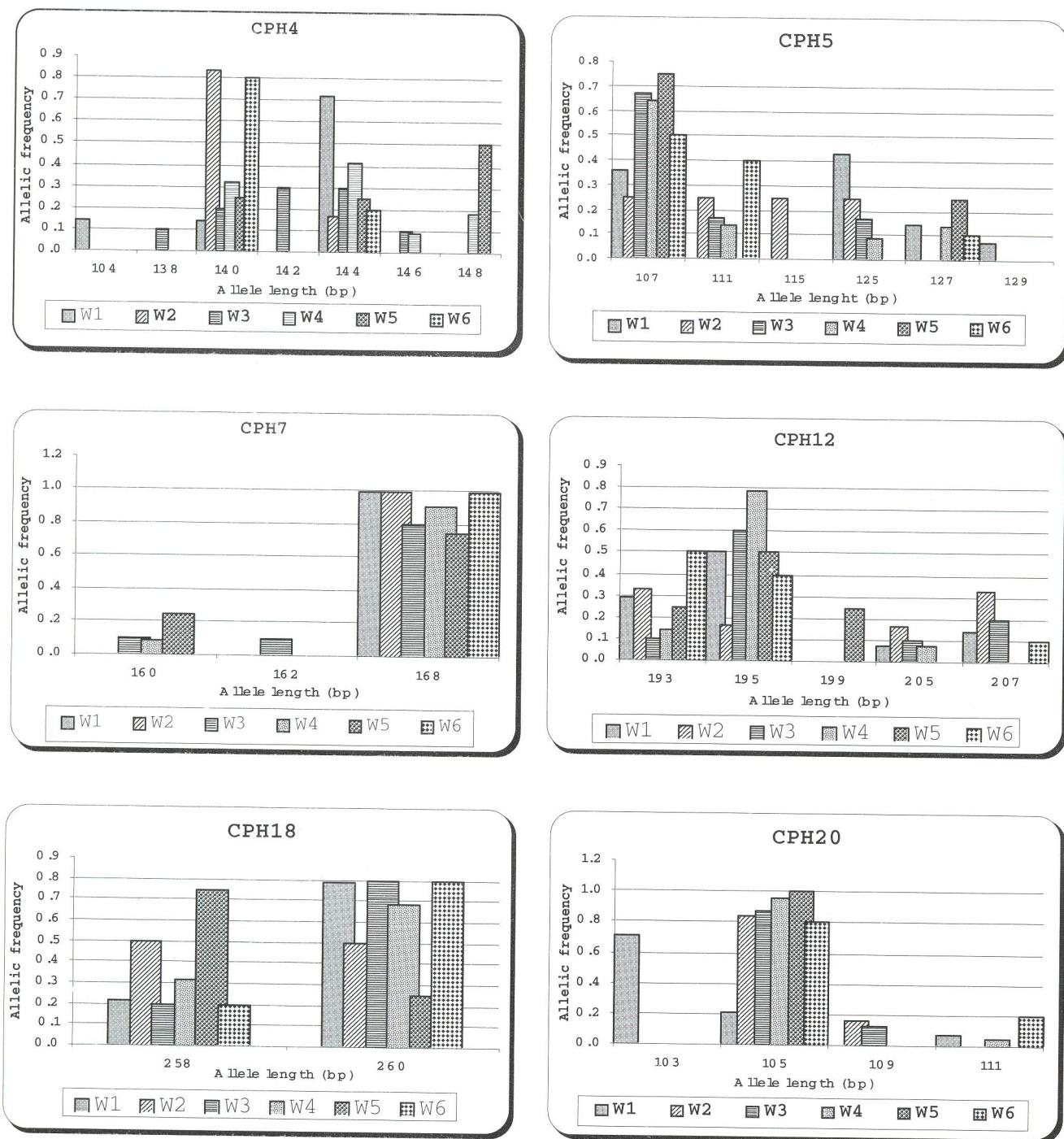


Fig. 1. Showing allelic frequencies histogram of six microsatellite loci in six Mexican grey wolf populations

lineages and 0.318 between the Certified and Ghost Ranch lineages, these data were supported by the Nei genetic distances. The authors also concluded that Mexican wolf populations generally exhibited short distances between themselves in comparison with other canid populations.

Phylogenetically, the W3, W4 and W5 populations had short genetic distances, meaning there is likely a high degree of kinship between them (Fig. 2). Populations W2 and W6 were also very close to each other, though with greater distances. Population W1, in contrast, was clearly

different from the other groups, which is also supported by its high degrees of heterozygosity for five of the six evaluated markers (Table 1).

Overall, in this study, average heterozygosity varied from 35 to 45%, which falls within the 10 to 44% range reported by García-Moreno *et al.* (1996) for three Mexican gray wolf lineages. These data need to be interpreted cautiously when determining to what extent this degree of heterozygosity has remained intact. Many species experience a decrease in reproduction in low-density populations for reasons unrelated to genetics. However, the polymorphism exhibited by the six studied populations in the present study (Fig. 2) is unexpected considering that all six derive from the single Certified lineage formed over a decade ago and protected by the Mexican Gray Wolf Recovery Program, which includes their reproduction in captivity.

Notable differences were observed among allelic frequency profiles for the six microsatellites in the six Mexican gray wolf populations studied. However, more studies are recommended involving Mexican gray wolf and closely related canid populations for conclusions.

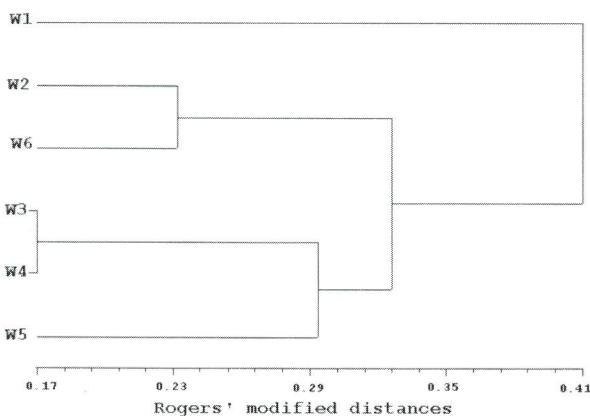


Fig. 2. Dendrogram for six Mexican gray wolf populations grouped using UPGMA, based on genetic frequencies of six microsatellite loci (Genetic distances, Rohlf, 1993).

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मार्टिनेज-ट्रिप। छुद्र उपग्रह मार्करों द्वारा छः मेक्सिकन धूसर भेड़ियों (कैनिस लुपस बाइलेयी) में ज्ञात आनुवंशिक परिवर्तिता।

छः भेड़िआ समुदायों में आनुवंशिक समीपता (0.17–0.41) दिखाने के लिये एलेलिक बारंबारता, विषमयुग्मजता और आनुवंशिक दूरियों को आनुवंशिक दूरी के औसत के जोड़े में विश्लेषण से समुदायों में स्पष्ट वर्गीकरण किया गया। छः मेक्सिकन धूसर भेड़िया समुदायों के छः छुद्र उपग्रहों के लिए एलेक्ट्रिक बारंबारता प्रोफाइल में स्पष्ट अंतर पाया गया। मेक्सिकन धूसर भेड़िए के समुदायों एवं अन्य संबंधित श्वरदंतियों के समुदायों के साथ और अध्ययन की संस्तुति की जाती है।