

DR. JANE HUFFMAN WILDLIFE GENETICS INSTITUTE

EAST STROUDSBURG UNIVERSITY
562 INDEPENDENCE ROAD, SUITE 114,
EAST STROUDSBURG, PA 18301
570-422-7891

DNA EVALUATION REPORT

April 13, 2022

Submitted by:

Kevin Hynes
NYSDEC Wildlife Health Unit
108 Game Farm Road
Delmar, New York 12054-9767

Laboratory ID # NY-UNK-P-003

Case #: NY201998

Services Requested: Species Identification –Hybrid

Date Received at DNA Lab: January 4, 2022

Description of Evidence Submitted: Evidence was submitted to the Dr. Jane Huffman Wildlife Genetics Institute on January 4, 2022. Evidence included: (Item 1) blood swab.

Summary of Methods: Evidence from unknown species was submitted to the Dr. Jane Huffman Wildlife Genetics Institute on January 4, 2022. Following standards and procedures, DNA was extracted from evidence item 1. Using a canid specific microsatellite reaction, an individual genotypic profile was determined (Table 1). To determine maternal lineage, a portion of the mitochondrial cytochrome b and cytochrome oxidase subunit I gene were targeted. Successful sequence fragments were analyzed using the National Centers for Biotechnology Information (BLAST) database.

Summary of Results: DNA was successfully extracted from evidence item 1. Successful allele calls were made at 16/17 microsatellites for evidence item 1 (Table 1). Using a Bayesian clustering program STRUCTURE, the genomic profile of the specimen was compared to a set of domestic dogs, known wolves, and known coyotes. Evidence item 1 was identified as 65.2 percent match to wolf and 34.8 percent match to coyote (Table 2 and Figure 1). The maternal lineage of evidence item 1 was identified as 99.9 percent coyote, *Canis latrans*. The final species determination of evidence item 1 is coyote (*Canis latrans*).

Table 1: Microsatellite genotype profiles for multiplex I and II.

Sample	121	172	103	20	377	173	109	200	250	2001	2010	2062	225	403	2145	2054	2004
003-1	115	156	72	116	152	97	145	210	X	142	224	129	162	275	167	294	301
	115	156	86	122	154	97	145	216	X	142	224	129	166	277	167	294	301

Table 2: Q-values of association for evidence item 1 with three species clusters.

Item 1	Species Assignment	Q-Value	Cluster Percentage
	<i>Canis lupus familiaris</i> (dog)	0.000	0.00% ± 0.6%
	<i>Canis latrans</i> (coyote)	0.348	34.8% ± 0.6%
	<i>Canis lupus</i> (gray wolf)	0.652	65.2% ± 0.6%
Final Species Determination:		Coyote (<i>Canis latrans</i>)	

Detailed Explanation of Methods: The Microsatellite DNA profile was first constructed for evidence item 1 using two multiplexes for a total of 16 microsatellite loci amplifying out of 17. These profiles were then entered into the Bayesian clustering program STRUCTURE^{1, 2} (version 2.3.4) to determine the species. The methodology used; Bayesian clustering analysis is consistent with those found throughout the scientific literature. The methods found in Bohling and Waits (2011) and Bohling, Adams, and Waits (2012) were followed, while information from Verardi, Lucchini, and Randi (2006), and Randi (2008) was also used for the final analysis.

In order to use Bayesian clustering analysis to determine the species of an unknown sample, known control sets were used. These known controls allow the program, in this case STRUCTURE, to “learn” what the gene frequencies of each species looks like by utilizing the USEPOPINFO = 1 flag. For this analysis, a control set of 36 known *Canis lupus* (gray wolf) individuals, a set of 43 known *Canis lupus familiaris* (dog), and 17 coyote (*Canis latrans*) individuals were genotyped at 17 microsatellite loci to create genotypic profiles for these animals. These genotypic profiles were then used to train STRUCTURE in the previously mentioned manner. Once the training sets were analyzed, a number of “blind controls” and the genotype for evidence item 1 were run through STRUCTURE to determine the Q-values, or the probability that any sample would be associated with a particular species. The “blind controls” were known individuals of a certain species that were run through the simulation to ensure that STRUCTURE accurately gave them high Q-values for association with their actual species.

After multiple simulations were run of the same and different models, the final determination is evidence item 1 can be best classified as a coyote (*Canis latrans*). Bohling and Waits (2011), Verardi, Lucchini, and Randi (2006), and Randi (2008) found that when using STRUCTURE for hybridization analysis, at the very minimum, to classify a canid as a hybrid, a 10% or greater match was required for more than one species. However, the eastern coyote, which this sample was identified as, is a natural hybrid of wolves. This is shown in this analysis as wolf DNA assignment is estimated at 65.2% and coyote assignment at 34.8%. The material blood line of evidence item 1 was identified as originating from coyote (*Canis latrans*).

Below, the graphical printout of the Q-values found from the STRUCTURE analysis of the control set of samples, the blind controls, and our unknown specimen (003 – Sample). A detailed explanation of the figure is also included below.

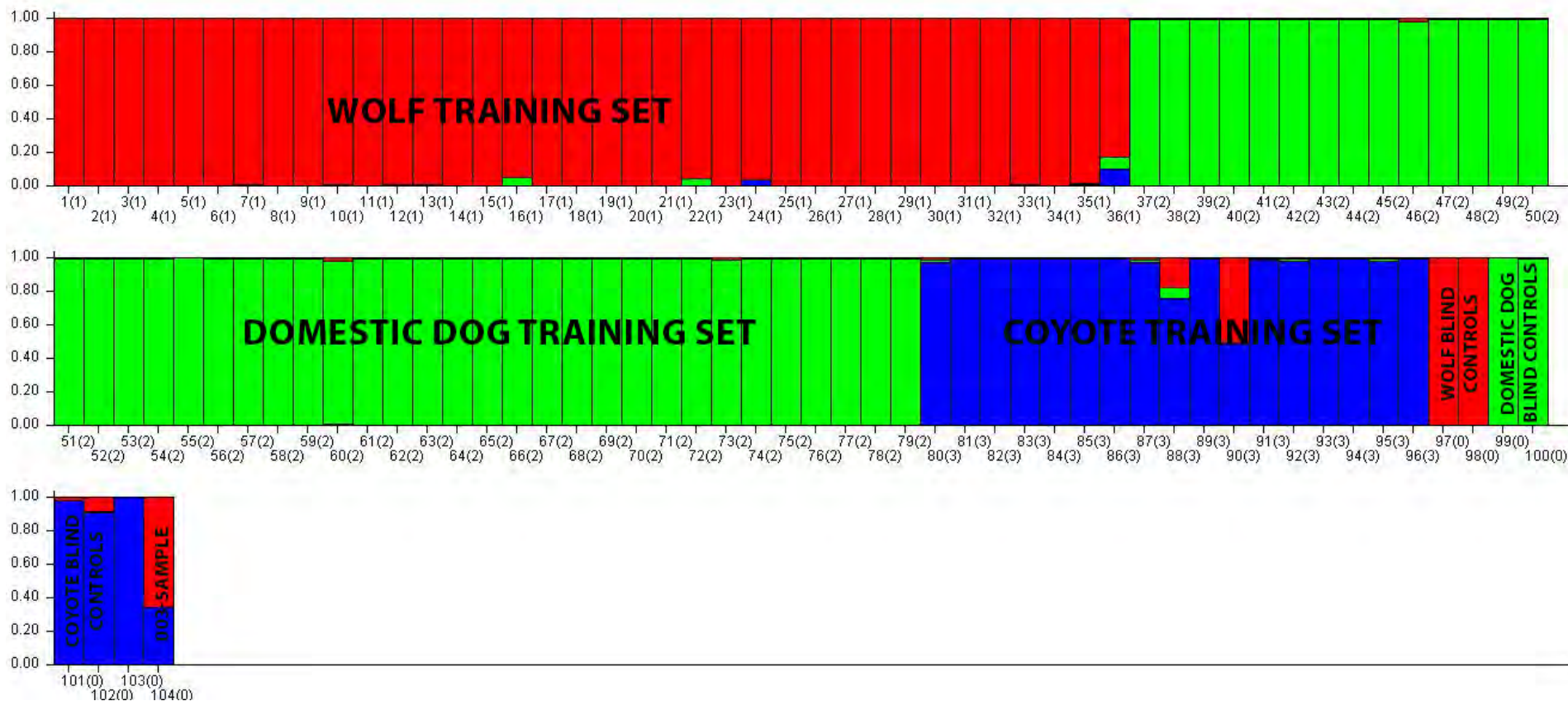


Figure 1: Graphical output of STRUCTURE’s Bayesian clustering method where each individual bar represents a separate sample. The height of the bar (y-axis) indicates the magnitude of the Q-value for that particular clustering assignment. Q-values range from 0.000, which indicates no probability of clustering, up to 1.000, which indicates a 100% probability with clustering to a particular group. Along the x-axis are the numbers of the samples that were entered into the simulation and the numbers in parentheses are the purported population, or species, assigned when entering data into the program. Population 1 indicates known wolves, population 2 indicates known domestic dogs, and population 3 indicates known coyotes which were used as a training set for STRUCTURE with the USEPOPINFO = 1 setting. The color of the bar on the graph indicates the cluster to which that Q-value is associated. Red bars indicate association with the *Canis lupus* (wolf) cluster, green bars indicate association with the *Canis lupus familiaris* (dog), and blue bars indicate association with *Canis latrans* (coyotes) cluster. STRUCTURE properly assigned the samples from each training set to their appropriate cluster as is indicated by the arrangement and color of the bars located under the three different training set headings. The samples located at the far right of the image with a value of zero in parentheses were entered into the program with no associated population or species information, which was determined by STRUCTURE by allocating Q-values. All blind controls that were analyzed were successfully determined to be the appropriate species with very high levels of certainty. Evidence item 1 is indicated by number 104 on Figure 1.

Literature Cited

- Bohling JH, Waits LP (2011) Assessing the prevalence of hybridization between sympatric *Canis* species surrounding the red wolf (*Canis rufus*) recovery area in North Carolina. *Molecular Ecology*, **20**, 2142–2156.
- ²Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotypedata: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.
- ¹Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Randi, E (2008) Detecting Hybridization between wild species and their domesticated relatives. *Molecular Ecology*, **17**, 285–293.
- Verardi A, Lucchini V, Randi E (2006) Detecting introgressive hybridization between free-ranging domestic dogs and wild wolves (*Canis lupus*) by admixture linkage disequilibrium analysis. *Molecular Ecology*, **15**: 2845–285.



Nicole Chinnici, MS, C.W.F.S
Certified Wildlife Forensic Scientist
Laboratory Director
Dr. Jane Huffman Wildlife Genetics Institute