

Species identification of individuals who are suspected to have red wolf ancestry
Final Report
2/6/19

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Objectives– 1) Determine the species composition of four hair samples collected from the Albemarle Peninsula provided by Andrea Shipley, Mammalogist, North Carolina Wildlife Resources Commission.

Methods

Laboratory Analysis

DNA was extracted from the hair samples using the DNeasy Blood and Tissue Kit (Qiagen, Inc.) in a laboratory dedicated to low quality DNA samples. One negative control was included in the extraction to monitor for contamination of reagents. Microsatellite genotypes were generated for each sample using 17 loci divided into two PCR multiplexes. Multiplex one contained the following loci, AHT103, AHT121, CXX20 CXX109, CXX172, CXX173, CXX200, CXX250 and CXX377 (Ostrander *et al.* 1993; Holmes *et al.* 1995; Mellersh *et al.* 1997). Multiplex two contained the following loci, CXX225, CXX403, FH2001, FH2004, FH2054, FH2062, FH2010, and FH2145 (Ostrander *et al.* 1993, 1995; Mellersh *et al.* 1997). Two to four replicate microsatellite PCRs were performed each hair sample. PCR products were visualized using a 3130xl DNA Sequencer (Applied Biosystems) and allele sizes were scored using Genemapper 5.0 (Applied Biosystems).

Statistical Analysis

The genetic ancestry of the samples was estimated using the Bayesian assignment test approach implemented in the software STRUCTURE 2.2 (Pritchard *et al.* 2000; Falush *et al.* 2003). The samples were combined with genotypes from 36 dogs, 62 coyotes from North Carolina and Virginia and 121 red wolves from the wild population in North Carolina. The number of populations (K) was set to 3 (ie. A red wolf population, coyote population and a dog population), and the percentage of ancestry (q) was estimated from each population. Program STRUCTURE 2.2 was run for 5 replicates with the admixed model of ancestry and the correlated allele frequency model with a burn-in length of 100,000 repetitions and 400,000 MCMC repetitions.

Results

DNA was successfully isolated for all 4 of the samples (Table 1 and Appendix 1, attached excel spreadsheet). Results of the STRUCTURE analysis indicate that two individuals shared $\geq 90\%$ of their ancestry with coyotes (Table 1). Individual MV-18-015F shared 72% of its ancestry with the red wolf group, 16% of its ancestry with the dog group and 12% of its ancestry with the coyote group. Individual MV-18-017F shared 87% of its ancestry with the red wolf group and 12% of its ancestry with the

coyote group. Both of these individuals could be classified as red wolf backcrossed individuals. Individual MV-18-017F is close to a 7/8th red wolf (where a 75% red wolf backcrosses to the red wolf population).

Regarding the presence of dog ancestry in one RWBC, we believe the dog ancestry is coming from admixed coyotes as evidenced by the presence of a coyote/dog F1 in the report from 101118.

Citations

- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.
- Holmes NG, Dickens HF, Parker HL *et al.* (1995) Eighteen canine microsatellites. *Animal Genetics*, **26**, 131–132.
- Mellersh CS, Langston AA, Acland GM *et al.* (1997) A linkage map of the canine genome. *Genomics*, **46**, 326–336.
- Ostrander EA, Mapa FA, Yee M, Rine J (1995) One hundred and one new simple sequence repeat-based markers for the canine genome. *Mammalian Genome*, **6**, 192–195.
- Ostrander EA, Sprague GF, Rine J (1993) Identification and characterization of dinucleotide repeat (CA)_n markers for genetic mapping in dog. *Genomics*, **16**, 207–213.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Table 1. Species identification results from microsatellite genotype data (Microsat Species).

Sample	Type	Microsat Species	Ancestry Red Wolf	Ancestry Dog	Ancestry Coyote
MV-18-015F	hair	RWBC*	72.1%	15.9%	11.9%
MV-18-016M	hair	coyote	0.30%	4.7%	95.1%
MV-18-017F	hair	RWBC ^o	86.8%	1.0%	12.2%
CC18003M	hair	coyote	0.20%	0.70%	99.1%

* 75% red wolf, 16% dog and 12% coyote

^o 75% red wolf and 25% coyote