

Assessing relatedness between wolves in Washington
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Objectives– 1) Determine the family relationships between packs and individuals for 16 tissue samples, provided by Ben Maletzke, Washington Department of Fish and Wildlife.

Methods

Laboratory Analysis

DNA was extracted from the tissue samples using the DNeasy Blood and Tissue Kit (Qiagen, Inc.). One negative was included in the extraction to monitor for contamination of reagents. A multi-locus genotype was generated for the sample using eighteen microsatellite loci and two sex markers (Seddon 2005). Multiplex one contained 0.04 μM FH2137 and DBY, 0.06 μM of FH2088, FH2054 and C09.173, 0.09 μM of FH2001 and FH2670 and 0.10 μM of FH2611, 0.11 μM of FH3725 and DBX, 0.16 μM of FH2004 and 0.23 μM of CXX.119, 1X Qiagen Multiplex PCR Kit Master Mix, 0.5X Q solution and 2 μl of DNA extract in a 7 μl reaction as in (Stenglein *et al.* 2010). The other eight loci (AHT103, AHT121 (Holmes *et al.* 1995), C09.250, C05.377 and FH2010 (Mellersh *et al.* 1997), CXX109, CXX172, and CXX200 (Ostrander *et al.* 1993) were multiplexed into one polymerase chain reaction. This reaction contained 0.06 μM of C05.377, and FH2010, 0.07 μM of CXX172, 0.10 μM of AHT121, 0.11 μM of C09.250, 0.13 μM of CXX109, 0.16 μM of CXX200, 0.36 μM of AHT103, 1X Qiagen Multiplex PCR Kit Master Mix, 0.5X Q solution and 1 μl of DNA extract in a 7 μl reaction volume. The sample was amplified twice at each multiplex to account for possible genotyping errors. PCR products were visualized using a 3130xl DNA Sequencer (Applied Biosystems) and allele sizes were scored using Genemapper 5.0 (Applied Biosystems). Assessment of sample quality and genotype screening methods followed those of Stenglein *et al.* (2010).

Statistical Analyses

In order to assess the familial relationships between packs and individuals relatedness values were calculated from a database containing gray wolves from Idaho (n=30) and British Columbia (n=20) and Washington (n=52) using the program ML-Relate (Kalinowski *et al.* 2006). Parentage was also determined using the method of exclusion (Chakraborty *et al.* 1974). The method of exclusion compares the genotypes between the breeders and offspring to count the number of genotype mismatches. In theory even one genotype mismatch would exclude an individual from being a parent but in practice one genotype mismatch is allowed to account for possible genotyping errors like allelic dropout or mutations that can occur. The genetic structure between packs was estimated using the Bayesian assignment test approach implemented in the software STRUCTURE 2.2 (Pritchard *et al.* 2000; Falush *et al.* 2003). All 68 individuals from Washington were included in the analysis. The purpose of this analysis is to determine how many genetic groups (for instance packs) are represented in the Washington wolf dataset. The value of K (genetic groups) varied from 1 to 15. Five replicates for each value of K were run with the admixed model of ancestry and the correlated allele frequency model with a burn-in length of 100,000 repetitions and 500,000 MCMC repetitions. The total number of genetic groups was determined using the highest log likelihood and greatest change in log likelihood method (Evanno *et al.* 2005) as implemented in Structure Harvester (Earl & vonHoldt 2012).

Results

Microsatellite genotypes were obtained at 16-18 loci for all the tissue samples. Results are presented by pack assignments made during capture. Pairwise relatedness values range from 0 to 1, 0 indicates unrelated, 0.25 indicates half siblings, grandparents/grandchild, and aunt uncle/niece nephew and 0.5 indicates full siblings or parent/offspring relationships. Below light gray shading of cells in the tables shows pairwise relatedness values between the breeders while dark gray shading shows pairwise relatedness values between the breeders and their offspring.

Carpenter Creek Pack:

There were three new individuals sampled in the Carpenter Creek pack, all of them via captures. It appears that 081m may be the breeder in the pack due to his age and the fact he has zero genotype mismatches with 082f and 1 genotype mismatch with 087m. Individual 081m has high relatedness values with the Smackout pack (r-values 0.47 to 0.64) which might indicate he originated in this pack. 081m and 082f are related to two members of the Dirty Shirt pack, 055f and 070f (r-values 0.40 to 0.55). In our previous report it was hypothesized that the male breeder of the Dirty Shirt pack came from the Smackout pack. This could explain the high relatedness values to this pack.

	Status	081m	082f	087m
081m	B?	-		
082f	S	0.50	-	
087m	S	0.41	0.38	-

Goodman Meadows Pack:

There were two new individuals sampled from the Goodman Meadows pack, 080m, 086f, both via captures. 080m and 086f have only zero and one genotype mismatches to the breeding pair of this pack. As mentioned in the previous report 008f originated in the Diamond pack and so 080m and 086f have elevated relatedness values to the Diamond pack (r-value 0.17 to 0.50).

	Status	008f	041m	062m	069m	WA-013607	WA-013608	080m	086f
008f	B	-							
041m	B	0.16	-						
062m	S	0.57	0.59	-					
069m	S	0.5	0.5	0.23	-				
WA-013607	S	0.52	0.5	0.7	0.1	-			
WA-013608	S	0.5	0.48	0.64	0.31	0.55	-		
080m	S	0.52	0.68	0.52	0.22	0.50	0.46	-	
086f	S	0.43	0.62	0.57	0.36	0.40	0.45	0.52	-

OPT Pack:

There were three new individuals sampled in the OPT pack. Field data suggested that M075-18WA was the breeder of this pack. His relatedness values would suggest that he is not the father of 083m and M074-18WA. Instead, 083m appears to be the offspring of the Sherman pack with zero genotype mismatches and high relatedness values (0.51 to 0.56). M074-18WA appears to be the son of 083m with zero genotype mismatches and a high relatedness value. M075-18W has elevated relatedness values with the Profanity Peak pack and with the Togo pack (r-values 0.42 to 0.45), however, M075-18W

is not related to the breeding female of the Profanity Peak pack making it unlikely he originates from that pack.

	Status	083m	M074-18WA	M075-18WA
083m	S	-		
M074-18WA	S	0.58	-	
M075-18WA	B	0.04	0.27	-

Stranger pack:

Only one member of the Stranger pack has been sampled. 085m is most closely related to 081m from the Carpenter Ridge pack (r-value= 0.50). 085m is not related to the other two members of the Carpenter Ridge pack and so it is not likely that 085m originated in this pack.

Togo pack:

Only one member of the Togo pack has been sampled. 084m is highly related to the Profanity Peak pack with r-values from 0.44 to 0.66. This suggests that 084m originated in the Profanity Peak pack.

Touchet pack:

Only one member of the Touchet pack has been sampled. 079m is not closely related to any other wolf sampled in Washington. Due to the Touchet packs location on the border with Oregon, it is possible 079m came from Oregon or parents that came from Oregon.

Tucannon pack:

One new member of the Tucannon pack was sampled. 076f is highly related to the other individual from the Tucannon pack 058f (r-value 0.80).

Individuals:

M081-18WA is most closely related to members of the Teanaway pack (r-values 0.30 to 0.50). M081-18WA is also closely related to members of the Lookout pack (r-values 0.36 to 0.69), which makes sense as the Teanaway pack was founded by members of the Lookout pack.

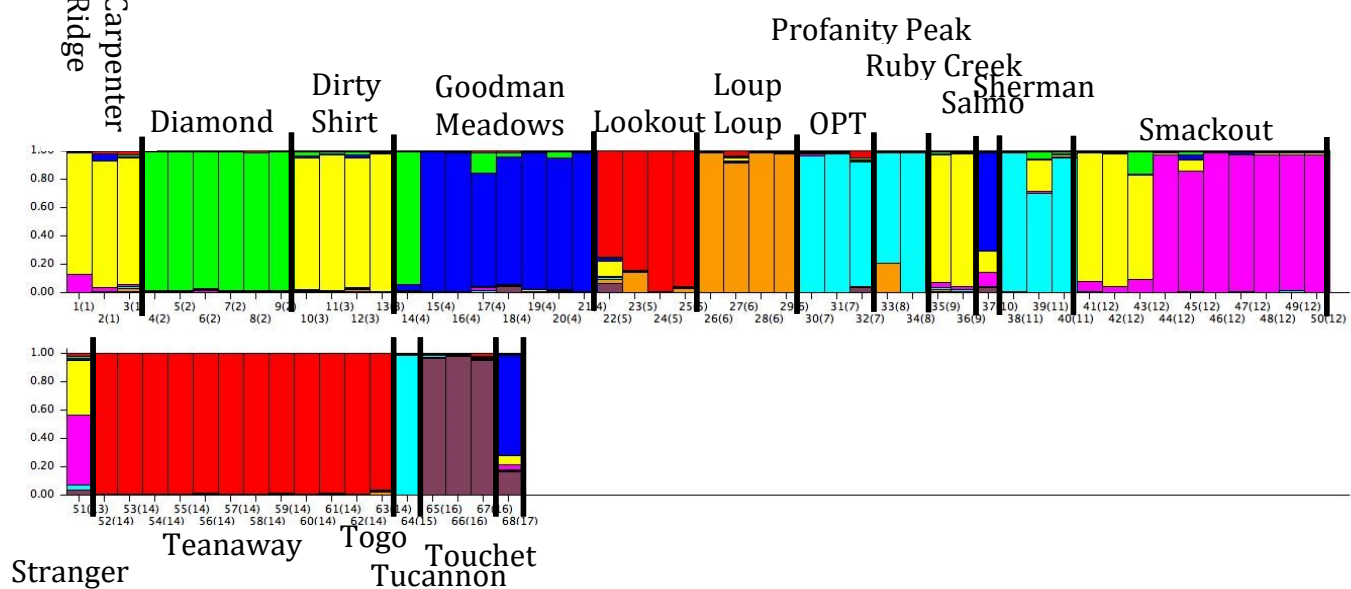
M082-19WA is most closely related to members of the Tucannon pack (r-values 0.43 and 0.57). This suggests that M082-19WA originated in this pack.

Results of Structure Analysis

The total number of genetic groups as determined by the highest log likelihood method was eight. The greatest change in log likelihood method found there were three genetic groups, however, the second greatest change in log likelihood was at eight genetic groups. Results are shown for eight genetic groups. There is a total of 17 packs represented so some lumping of packs occurred. Each column represents the ancestry of one individual. Packs are divided by black lines.

- 1) As shown in the relatedness results members of the Carpenter Ridge pack share ancestry with the Dirty Shirt, Ruby Creek and Smackout packs.
- 2) The Goodman Meadows breeding female is from the Diamond pack originally thus she shares ancestry with this group.
- 3) The OPT, Profanity, Sherman and Togo packs all share ancestry due to their relatedness to one another

- 4) The individual from the Stranger pack shares ancestry with the Carpenter Ridge, Dirty Shirt, Ruby Creek and Smackout packs. This individual was closely related to one member of the Carpenter Ridge pack but did not have high relatedness values to the Smackout pack.
- 5) The Touchet pack individual appears to share ancestry with the Goodman Meadows pack, however, it did not have high relatedness values with the Goodman Meadows pack.



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