

Assessing relatedness between wolves in Washington
Final Report
2/5/18

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Objectives– 1) Determine the family relationships between packs and individuals for 43 tissue and 8 mouth swab samples, provided by Scott Becker, Washington Department of Fish and Wildlife

Methods

Laboratory Analysis

DNA was extracted from the tissue and swab samples using the DNeasy Blood and Tissue Kit (Qiagen, Inc.) in a laboratory dedicated to high quality DNA samples. One negative control was included in each extraction to monitor for contamination of reagents. A genotype was generated using eighteen microsatellite loci. Multiplex one contained 0.04 μM of C09.173, FH2088 and FH2137, 0.05 μM of FH2054 and FH2611, 0.09 μM of CXX.119, FH2001 and FH3725, 0.10 μM of FH2670 and 0.11 μM of FH2004, 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 and 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 CXX172, C05.377, and FH2010, 0.10 μM of AHT121 and C09.250, 0.16 μM of CXX200, 0.20 μM of CXX109, 0.70 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 tissue and swab samples were amplified twice for each multiplex. 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) 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 51 individuals from Washington were included in the analysis as well as individuals detected in the Strawberry (n=8), Nc'icn (n=10), Whitestone (n=3) and Huckleberry (n=11) packs provided by the Colville and Spokane Tribes. 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 400,000 MCMC repetitions. The total number of genetic

groups was determined using the highest log likelihood and greatest change in log likelihood methods as implemented in Structure Harvester (Earl & vonHoldt 2012).

Results

Microsatellite genotypes were obtained for the tissue and swab samples. Results are presented by pack assignments made during capture or scat sampling. 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. Light gray shading shows pairwise relatedness values between the breeders while dark gray shading shows pairwise relatedness values between the breeders and their offspring.

Strawberry Pack:

There were 8 individuals associated with the Strawberry pack. These individuals were originally detected through scat sampling. Individuals 13-09 and 14-40 are the breeders of the pack; as determined through the method of exclusion and their relatedness value is zero. The offspring of the breeders all have zero genotype mismatches except 14-42 who has one genotype mismatch to the breeders. The relatedness values between the breeders and other members of the pack ranged from 0.50 to 0.62, which is indicative of a parent/offspring relationship.

	Status	13-09	14-40	14-16	14-42	14-43	14-47	14-50	CCT16-1
13-09	B*	-							
14-40	B	0	-						
14-16	S [§]	0.5	0.5	-					
14-42	S	0.52	0.5	0.5	-				
14-43	S	0.62	0.51	0.51	0.47	-			
14-47	S	0.5	0.5	0.26	0.55	0.43	-		
14-50	S	0.5	0.5	0.66	0.44	0.4	0.12	-	
CCT16-1	S	0.5	0.51	0.33	0.34	0.58	0.35	0.26	-

* B= breeder

§ S= subordinate

Nc'icn Pack:

There were 8 individuals associated with the Nc'icn pack. These individuals were originally detected through scat sampling. Individuals 12-06m and 12-21f are the breeders of the pack; as determined through the method of exclusion and their relatedness value is zero. All offspring had zero genotype mismatches to the breeders with the exception of individual WA10M who had one genotype mismatch. The relatedness values between the breeders and other members of the pack ranged from 0.30 to 0.60, which is indicative of a parent/offspring relationship. The lower relatedness values for individuals WA10m and 13-18f are likely due to missing genotype data.

	Status	12-6	12-21	14-03	WA10M	13-16	13-18	13-22	CCT16-26
12-06	B	-							
12-21	B	0	-						
14-03	S	0.5	0.5	-					
WA10M	S	0.39	0.5	0.37	-				
13-16	S	0.51	0.5	0.44	0.53	-			

13-18	S	0.3	0.6	0.49	0.52	0.45	-		
13-22	S	0.56	0.5	0.6	0.66	0.53	0.34	-	
CCT16-26	S	0.52	0.42	0.69	0.54	0.57	0.34	0.47	-

Whitestone Pack:

There was only one individual associated with this pack. This individual was originally detected through scat sampling. Individual 14-09f is closely related to 004m, 006f and 008f (r-values of 0.46, 0.50 and 0.43 respectively), which might indicate that she originated in the Diamond pack.

Huckleberry Pack:

There were 14 individuals associated with this pack. Eleven of the individuals were originally detected through scat sampling. Individuals 035m and S1012f are the breeders of the pack; as determined through the method of exclusion and their relatedness value is zero. All offspring of the breeders had zero genotype mismatches. Relatedness values between the breeders and other members of the pack ranged from 0.41 to 0.60, which is indicative of a parent/offspring relationship. Individuals S095m and 054m appear to be less related to the breeders in the pack, with six and nine genotype mismatches respectively and lower relatedness values from 0.09 to 0.38. These individuals, however, are closely related to some offspring of the breeders (highlighted in blue in the table below). Individual S095m and 054m are related to each other at the half sibling level. One possible explanation for the observed relatedness values in this pack is a turnover of one of the breeders. Another possible explanation is an offspring of the breeders dispersed and founded a new pack and S095m and 054m are offspring from the new pack. This explanation does not account for the presence of S095m and 054m within the Huckleberry pack's territory. S095m and 054m are not highly related to any other individuals in the population so there is no evidence they originated in any of the other sampled wolf packs in Washington.

	Status	035m	S1012	S12	S19	S31	S36	S1016	S1082	S1083	S095	042m	051f	052f	054m
035m	B	-													
S1012	B	0	-												
S12	S	0.5	0.5	-											
S19	S	0.5	0.5	0.51	-										
S31	S	0.5	0.5	0.5	0.57	-									
S36	S	0.5	0.5	0.21	0.4	0.08	-								
S1016	S	0.5	0.5	0.46	0.71	0.73	0.41	-							
S1082	S	0.41	0.5	0.46	0.46	0.5	0.28	0.29	-						
S1083	S	0.5	0.58	0.5	0.38	0.45	0.44	0.4	0.45	-					
S095	U*	0.11	0.16	0.12	0.32	0.32	0.3	0.54	0.08	0.13	-				
042m	S	0.51	0.5	0.27	0.41	0.34	0.33	0.49	0.19	0.33	0.24	-			
051f	S	0.5	0.5	0.57	0.45	0.24	0.29	0.37	0.57	0.39	0.09	0.38	-		
052f	S	0.6	0.5	0.6	0.55	0.5	0.5	0.16	0.51	0.6	0	0.39	0.42	-	
054m	U	0.09	0.38	0.05	0.32	0.62	0.01	0.5	0	0.11	0.31	0.44	0.11	0	-

*U= Uncertain pack association

Diamond Pack:

There were six individuals associated with this pack, all of them sampled via captures. Individual 004m is a breeder in the pack as determined in the field and as evidenced by his high relatedness values (r-values 0.43 to 0.57) to all other members of the pack. Individual 013f is listed as the breeder from 2011 to 2014 according to field data, however, she can be excluded as the mother of 039m (genotype mismatches at 4 loci) who was born in 2013. In fact, 013f appears to be an offspring of 004m as their relatedness value is 0.44. Furthermore, there are no genotype mismatches between 004m and 006f, 007m,

010f, 013f, 039m, and 008f. If 013f and 004m did breed it would be an inbred father/daughter relationship. With the current samples from the Diamond pack there is no evidence to suggest that 013f and 004m ever bred. Thus, the genetic data do not support the field data that 013f is the breeder in this pack. It appears the true breeding female has not been sampled. Individual 008f was born into the Diamond pack and later dispersed to become the breeder in the Goodman Meadows pack.

	Status	004m	006f	007m	010f	013f	039m	008f
004m	B	-						
006f	S	0.53	-					
007m	S	0.43	0.47	-				
010f	S	0.57	0.51	0.47	-			
013f	B	0.44	0.52	0.48	0.58	-		
039m	S	0.45	0.45	0.41	0.37	0.32	-	
008f	S	0.48	0.65	0.63	0.5	0.42	0.56	-

Dirty Shirt Pack:

There were four individuals associated with this pack, all of them sampled via captures. Individual 055f is a breeder in the pack as determined in the field and as evidenced by her high relatedness values (r-values 0.60 to 0.65) to all other members of the pack. All offspring have zero genotype mismatches to 055f with the exception of individual 048f who has two genotype mismatches to 055f. This may mean that a different, unsampled female bred in 2014. The Dirty Shirt pack also has elevated relatedness values (r-values 0.13 to 0.42) with the Smackout pack. The median value of 0.33 falls within the range of grandparents/grandchildren, half siblings or aunt/uncle relationships. This value means it is possible that the other breeder in the Dirty Shirt pack, who has not yet been sampled, originated in the Smackout pack.

	Status	048f	055f	068m	070f
048f	S	-			
055f	B	0.6	-		
068m	S	0.4	0.65	-	
070f	S	0.47	0.64	0.59	-

Goodman Meadows:

There were six individuals associated with this pack, four of them were sampled via captures. The other two WA-013607 and WA-013608 were part of a poaching case. Individuals 008f and 041m are the breeders in the pack; as determined in the field, through the method of exclusion and their relatedness value is 0.16. The breeders have zero mismatches to 062m, 069m, WA013607 and WA013608. The breeders are closely related to the other members of the pack (r-values 0.48 to 0.59). As stated above 008f was born into the Diamond pack and then became the breeder of the Goodman Meadows pack. This means that the Goodman Meadows pack has elevated relatedness values (0 to 0.39) to the Diamond pack, which would be expected given 008f is originally from the Diamond pack.

	Status	008f	041m	062m	069m	WA-013607	WA-013608
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	-

Lookout Pack:

There were four individuals associated with this pack, all of them sampled via captures. Individuals 001f and 002m were the breeders in the pack until 2010 when their radio collar signals disappeared. Their breeding status was confirmed via field data and their relatedness value is zero. Before 001f and 002m disappeared they produced 049f with relatedness values of 0.38 and 0.66 respectively and zero genotype mismatches. 049f became the breeder in the pack as determined by field data and she produced 040f with a relatedness value of 0.54 and zero genotype mismatches. 040f has relatedness values to 001f and 002m of 0.26 and 0.28. This is consistent with 001f and 002m being her grandparents.

	Status	001f	002m	040f	049f
001f	B	-			
002m	B	0	-		
040f	S	0.26	0.28	-	
049f	B	0.38	0.66	0.54	-

Loup Loup Pack:

There were four individuals associated with this pack, all sampled via captures. Individuals 060f and 061m are the breeders in the pack as confirmed by field data. 060f and 061m have a relatedness value of 0.39. This is within the range seen for both full siblings and half siblings so these individuals could be an inbred pair. Individuals 059f and 071f are both offspring of 060f and 061m as evidenced by their relatedness values (r-values 0.42 to 0.67) and through the method of exclusion. 059f has no genotype mismatches to the breeding pair while 071f has one genotype mismatch to the breeding pair.

	Status	059f	060f	061m	071f
059f	S	-			
060f	B	0.67	-		
061m	B	0.56	0.39	-	
071f	S	0.52	0.42	0.62	-

Profanity Peak Pack:

There were three individuals associated with this pack, all sampled via captures. Individual 047f is the breeder in the pack as confirmed by field data. Individual 063m appears to possibly be the other breeder in the pack due to his low relatedness value (0.14) with 047f. 064f is likely an offspring of 047f and 063m as shown by her relatedness values to the pair, 0.44 and 0.53 respectively. This relationship was confirmed through the method of exclusion as 064f has one genotype mismatch to 047f and 063m. The breeding female 047f originated in the Nc'icn pack with r-values ranging from 0.38 to 0.55. According to field data 047f left Profanity Peak pack to become the breeder in the Sherman pack.

	Status	047f	063m	064f
047f	B	-		
063m	B	0.14	-	

064f	S	0.44	0.53	-
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Ruby Creek Pack:

There were two individuals associated with this pack. These individuals, 036f and 037f, are highly related to each other (r-value= 0.72). It is possible that these individuals are sisters or a mother and daughter. These individuals are also highly related to 028m, the breeder from the Smackout pack with relatedness values of 0.50. Through the method of exclusion 028m cannot be excluded as the father of these two individuals as there are no genotype mismatches between the three. Did 028m maintain two different breeding females, one in the Smackout pack and one in the Ruby Creek pack?

Salmo Pack:

There was only one individual associated with this pack, and he was sampled by capture. Individual 011m is not highly related to any other individual in Washington and therefore possibly came from out of state.

Sherman Pack:

There were three individuals associated with this pack with two sampled by capture and one sampled by harvest. As noted by field data 047f left the Profanity Peak pack and formed the Sherman pack with 053m. Individual 1137Zf is likely the daughter of the breeding pair as evidenced by her relatedness values of 0.51 and 0.59 to the pair and through the method of exclusion. 1137Zf has no genotype mismatches with 047f and 053m.

	Status	047f	053m	1137Zf
047f	B	-		
053m	B	0.09	-	
1137Zf	S	0.51	0.59	-

Smackout Pack:

There were eight individuals associated with this pack, all sampled via capture. Individual 028m was determined to be the breeding male in the pack via field data until his death in 2014. 028m cannot be excluded as the father of individuals 014f, 045f and 046f and there are no genotype mismatches between these individuals. It appears there may have been two different breeding females that produced 014f, 045f and 046f. This was determined by the presence of three alleles at two loci. Once the male allele is accounted for there should only be two different alleles that the female can contribute to the offspring. The presence of a third allele would indicate that a second female was involved. 028m can be excluded as the father of 056f, 057f, 065m and 067f at two to five loci. The two sets of offspring appear to be half siblings with a median relatedness value of 0.26. This would make sense as 028m was replaced with a new male breeder.

	Status	014f	028m	045f	046f	056f	057f	065m	067f
014f	S	-							
028m	B	0.5	-						
045f	S	0.23	0.5	-					
046f	S	0.46	0.52	0.39	-				
056f	S	0.13	0.13	0.26	0.17	-			
057f	S	0.22	0.1	0.37	0.41	0.61	-		

065m	S	0.17	0.02	0.28	0.32	0.8	0.76	-	
067f	S	0.4	0.41	0.24	0.6	0.28	0.47	0.32	-

Teanaway Pack:

There were 11 individuals associated with this pack with 10 sampled by capture and 1 sampled as a mortality. Teanaway is highly related to the Lookout pack with relatedness values ranging from 0.20 to 0.64. It is possible that the original Lookout pair who disappeared in 2010 moved and created the Teanaway pack. 001f and 002m from Lookout have high relatedness values to 012f and 032m (r-values 0.44 to 0.60), which are consistent with a parent/offspring relationship. In addition there is only one genotype mismatch between each individual and the Lookout pair. Therefore it is highly likely that the Lookout pair bred in Teanaway and produced 012f and 032m. The next breeding pair in Teanaway was 012f and 032m who produced 015f, 016f, 031f, 033m, 038f, 072f and M016-13WA. This is based on the high relatedness values (r-values 0.41 to 0.62) between these individuals. In addition there are zero genotype mismatches between 012f, 032m and 015f, 016f, 031f, 033m, 038f, 072f and M016-13WA, which means that 012f and 032m cannot be excluded as the parents. The pairing of 012f and 032m is an inbreeding event as the pair are full siblings. The next breeding pair in Teanaway was 038f and 032m who produced 043f and 044m. This is based on the high relatedness values (r-values 0.59 to 0.69) between 038f, 032m, 043f and 044m. In addition, there are no genotype mismatches between 038f, 032m, 043f and 044m which means that 038f and 032m cannot be excluded as the parents. The pairing of 038f and 032m is also an inbreeding event as the pair are father and daughter. The Teanaway pack is the most geographically isolated pack in the state of Washington. It could be that this isolation has caused a lack of gene flow from unrelated dispersing individuals. Sample M016-13WA an individual of unknown origin is from Teanaway with relatedness values of 0.42 to 0.57.

	Status	001f	002m	012f	015f	016f	031f	032m	033m	038f	043f	044m	072f	M016-13WA
001f	B	-												
002m	B	0	-											
012f	B	0.44	0.6	-										
015f	S	0.28	0.6	0.62	-									
016f	S	0.34	0.49	0.62	0.52	-								
031f	S	0.61	0.26	0.67	0.46	0.67	-							
032m	B	0.5	0.54	0.35	0.61	0.56	0.47	-						
033m	S	0.37	0.59	0.75	0.67	0.77	0.68	0.6	-					
038f	S	0.4	0.43	0.43	0.5	0.48	0.39	0.56	0.4	-				
043f	S	0.2	0.48	0.41	0.56	0.48	0.29	0.67	0.45	0.69	-			
044m	S	0.26	0.49	0.51	0.72	0.51	0.5	0.59	0.59	0.63	0.61	-		
072f	B	0.36	0.5	0.57	0.65	0.36	0.4	0.72	0.47	0.52	0.63	0.58	-	
M016-13WA	S	0.39	0.37	0.5	0.53	0.42	0.43	0.53	0.5	0.57	0.46	0.55	0.56	-

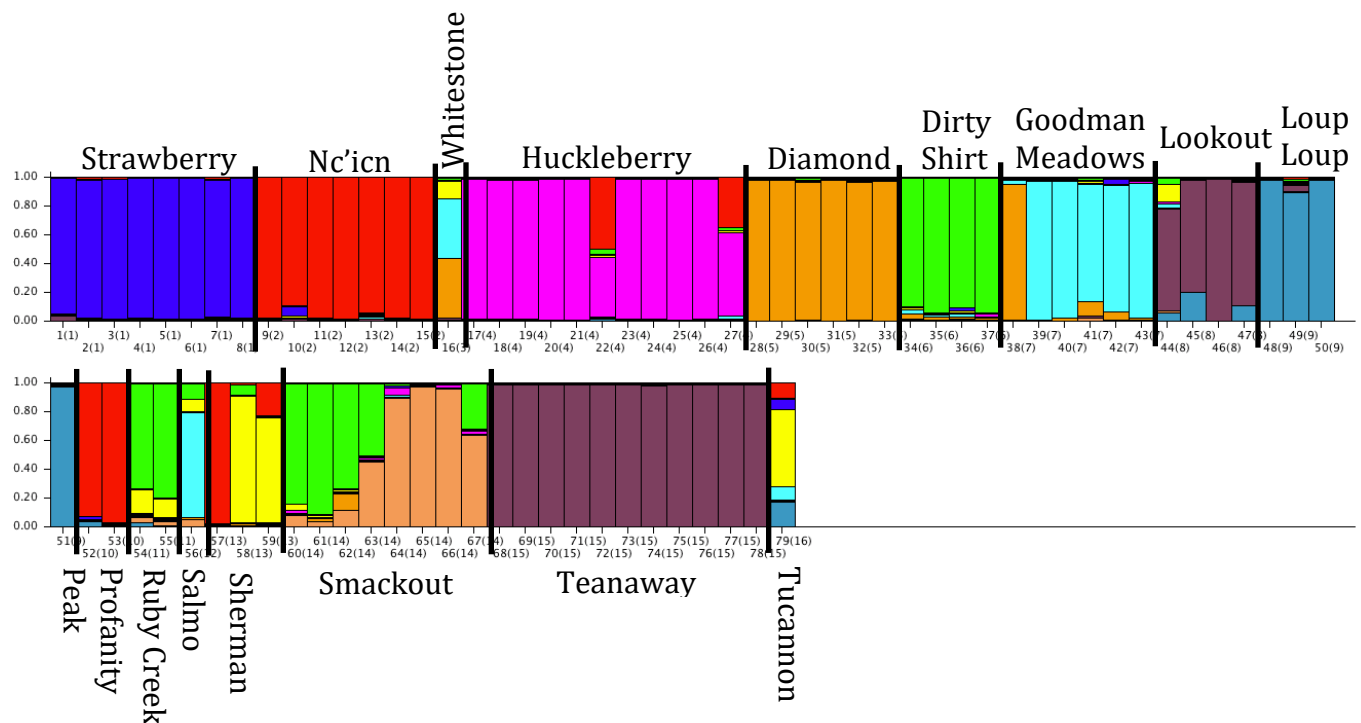
Tucannon Pack:

There was one individual associated with this pack and was sampled as a capture. 058f is not highly related to any individuals in Washington and so a new relatedness analysis with wolves from Oregon was performed. During the analysis it was discovered that 058f was a match to Oregon wolf W041F. This individual originated in the Wenaha pack. This individual was identified via scat sampling in 2013.

Results of the Structure analysis:

The total number of genetic groups as determined by the highest log likelihood method was ten. The greatest change in log likelihood method found that there were three genetic groups represented, however, the second greatest change in log likelihood was at ten genetic groups. Results are shown for ten genetic groups. There are a total of 16 packs represented so some lumping of packs occurred.

- 1) The Whitestone female appears to be a mix between Diamond and Goodman Meadows. This is in part backed up by the relatedness values, which indicated she might have originated in the Diamond pack.
- 2) The two individuals in the Huckleberry pack that appear to be mixed between Nc'icn and Huckleberry are the two individuals who appear unrelated to the pack.
- 3) In the Goodman Meadows pack the first individual is the breeding female who originated in the Diamond pack thus the reason that she is lumped in the Structure analysis.
- 4) The Lookout pack was lumped with the Teanaway pack, which is corroborated by the relatedness results.
- 5) The Ruby Creek individuals group with both the Dirty Shirt and the Smackout packs. This is supported by the relatedness analysis, which indicated the breeding male of Smackout was also the father of the Ruby Creek individuals. The relationship between the Dirty Shirt pack and the Smackout pack could be because one of the breeders of Dirty Shirt appeared to originate from the Smackout pack.
- 6) The Salmo individual is lumped with the Goodman Meadows pack, however the relatedness analysis does not support this relationship.
- 7) The Sherman pack consists of a breeding female from the Nc'icn pack and a breeding male who is not significantly related to any other wolves in Washington. The offspring appears as a mix between between the breeders.
- 8) The Smackout pack is a mix of two genetic groups, which makes sense because a new breeding pair replaced the original breeding pair.
- 9) The Tucannon individual is a mix of many genetic groups. This is likely due to the individual being from Oregon whose population is not represented in this Structure analysis.



Citations

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