

From: [Snyder, Caitlin](#)
To: [Grizzle, Betty](#)
Subject: Re: Question re previous SSA - AK wolf
Date: Tuesday, March 14, 2017 10:50:19 AM
Attachments: [GeneticsOverview_Olsen.pdf](#)

Hi Betty,

I went back and looked at my files from AA wolf. I didn't participate in any genetics panel, but I did participate in two meetings: one an internal FWS meeting where we discussed general AA wolf information, including genetics and a second meeting with partners where we discussed the wolf population model (this model helped inform our SSA). I'm attaching the presentation Jeff & John gave at the internal FWS meeting on AA wolf genetics. It might give you a better idea of the type of information they were analyzing -- as I mentioned, they reviewed the available literature and helped explain to us the differences between the techniques used by the researchers.

Thanks,
Caitlin

Caitlin Snyder
Unified Listing Team
U.S. Fish & Wildlife Service
MS: ES
5275 Leesburg Pike
Falls Church, VA 22041-3803
phone: 703 358 2673

On Tue, Mar 14, 2017 at 12:22 PM, Snyder, Caitlin <caitlin_snyder@fws.gov> wrote:

Hi Betty,

I'm working from home today, but my phone is forwarded (703 358 2673). I have a webinar meeting starting at 2:00, but am free beforehand.

Thanks,
Caitlin

Caitlin Snyder
Unified Listing Team
U.S. Fish & Wildlife Service
MS: ES
5275 Leesburg Pike
Falls Church, VA 22041-3803
phone: 703 358 2673

On Tue, Mar 14, 2017 at 11:07 AM, Grizzle, Betty <betty_grizzle@fws.gov> wrote:

Hi Caitlin - Are you in the office today? (I understand severe weather is hitting the NE) I would like to talk to you about the Alex. Arch Wolf effort that (I think) you were involved in.

I am teleworking from home so would be easier for me to call you if you are in the office.

Thanks.

--

Betty J. Grizzle, D.Env.
Fish and Wildlife Biologist
U.S. Fish and Wildlife Service
Carlsbad Fish and Wildlife Office
2177 Salk Ave, Suite 250
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Brief overview of genetic analyses and data relevant to the Alexander Archipelago wolf



Forces influencing genetic variation

Selection

a change in allele frequency because of heritable fitness differences among genotypes

✓ Mutation

a change in allele frequency because of change in DNA sequence

✓ Genetic Drift

a change in allele frequency because only a finite number of gamete pairs are created each generation

✓ Gene flow (migration/connectivity)

a change in allele frequency because of gene flow (introgression) from one population to another

Types of genetic studies

Phylogenetics

- the study of **genealogical lineages**
- define evolutionary relationships
- species, subspecies, population groups

Time scale/Forces

Longer/mutation, drift

Phylogeography

- extension of phylogenetics
- processes influencing distribution of lineages

Longer/mutation, drift

Population Genetics

- genetic diversity within and among populations

Shorter/drift, gene flow

Types of genetic markers

mtDNA

- mitochondrial DNA
- maternally inherited
- Phylogenetics / phylogeography / population genetics

Microsatellites

- Bi-parentally inherited (autosomal)
- Repetitive DNA, non-coding
- Phylogenetics / phylogeography / population genetics

SNP

- Single nucleotide polymorphism
- Coding and non-coding
- Phylogenetics / phylogeography / population genetics

Genetic Analyses

- Coastal wolf genetic studies launched in mid-90s, increasing annually
- Several studies comparing coastal and interior wolves, but few compare within-coastal populations (Alaska and BC)
- Researchers used different techniques and markers
- Various interpretations of genetic results, especially applied to subspecific designation



Genetic Analyses

Study	SEAK	Coastal BC	Type	Key findings
Shields 1995	Yes	No	mtDNA	<i>C.l. ligoni</i> distinct, no subpopulations
Weckworth et al. 2005	Yes	No	microsat	Coastal distinct from interior; 2 clusters within SEAK – POW and others
Breed 2007	Yes	Yes	microsat	3 units – coastal BC, POW, and other SEAK
Munoz-Fuentes et al. 2009	No	Yes	mtDNA	Coastal distinct from interior
Weckworth et al. 2010	Yes	No	mtDNA	Independent evolutionary histories – coastal and interior
Von Holdt et al. 2011	No	Yes	SNPs	Coastal distinct from interior
Weckworth et al. 2011	Yes	Yes	mtDNA	Coastal distinct from interior; SEAK and BC wolves related
Chambers et al. 2012	Yes*	Yes*	All*	Meta-analysis* of NA; coastal wolves not a subspecies
Cronin et al. 2014	Yes	No	SNPs	SEAK different from other wolves, but not at unprecedented levels of variation
Stronen et al. 2014	No	Yes	microsat	Coastal distinct from interior – genetics and ecology

Phylogenetic / Phylogeographic

Weckworth et al. 2011

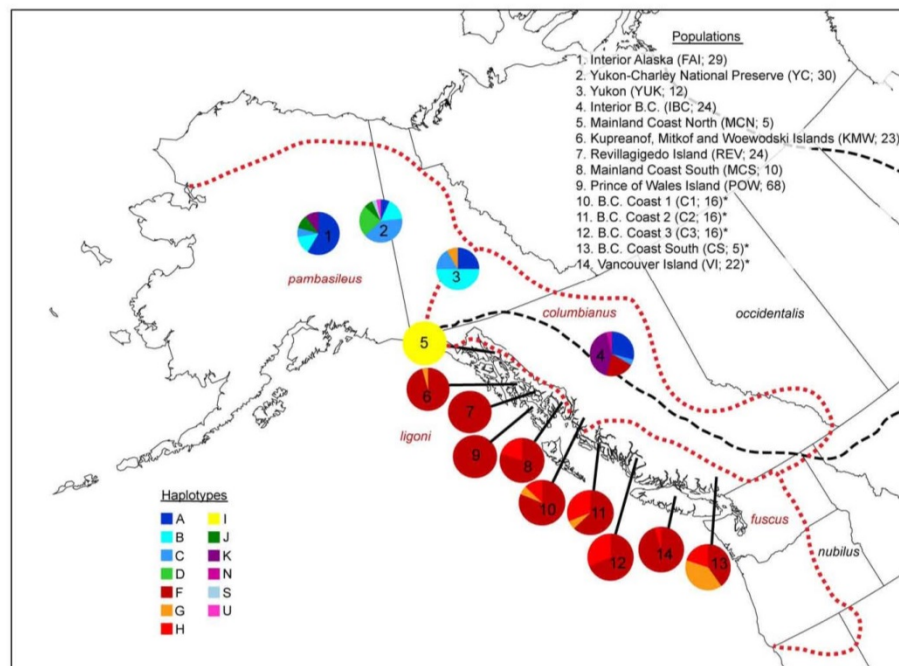


Figure 1. Map of sampling locations in Northwest North America. In the upper right, each population name is listed with its associated abbreviation and sample size. Asterisks indicate populations from Muñoz-Fuentes et al. [15]. Pie diagrams for each population indicate haplotype frequency, with color legend in the lower left. Black dashed lines indicate subspecies in the Pacific Northwest according to Nowak [33] and red dotted lines indicate the range of subspecies according to Hall and Kelson [32]. The 7 Southern United States (SUS) samples are not shown. doi:10.1371/journal.pone.0019582.g001

Munoz-Fuentes et al. 2009

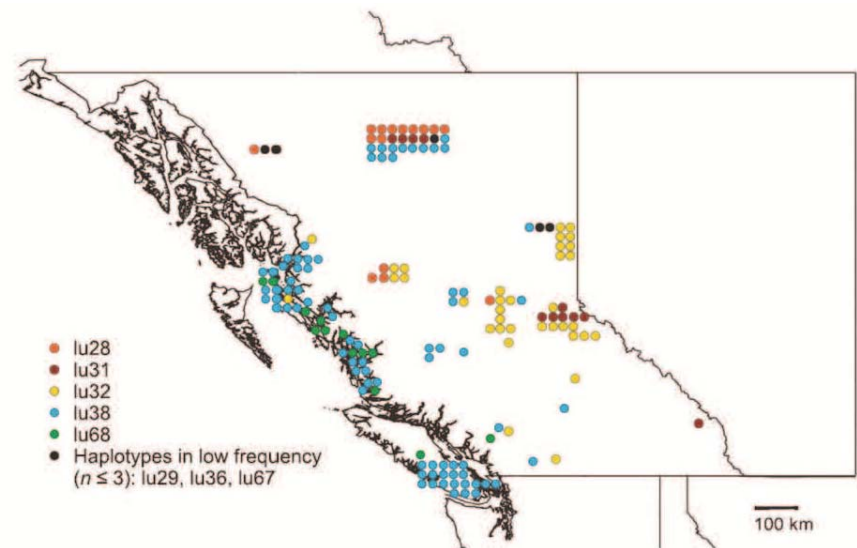


Figure 11. Distribution of control-region mtDNA haplotypes of *Canis lupus* in British Columbia, illustrating that phylogenetic divergence has been maintained between coastal and inland wolves that have been in geographically extensive and long-term contact (figure 3 of Muñoz-Fuentes et al. 2009). ©John Wiley and Sons. Used with permission.

Phylogenetics / Phylogeographic

Results support coastal and inland genetic lineages

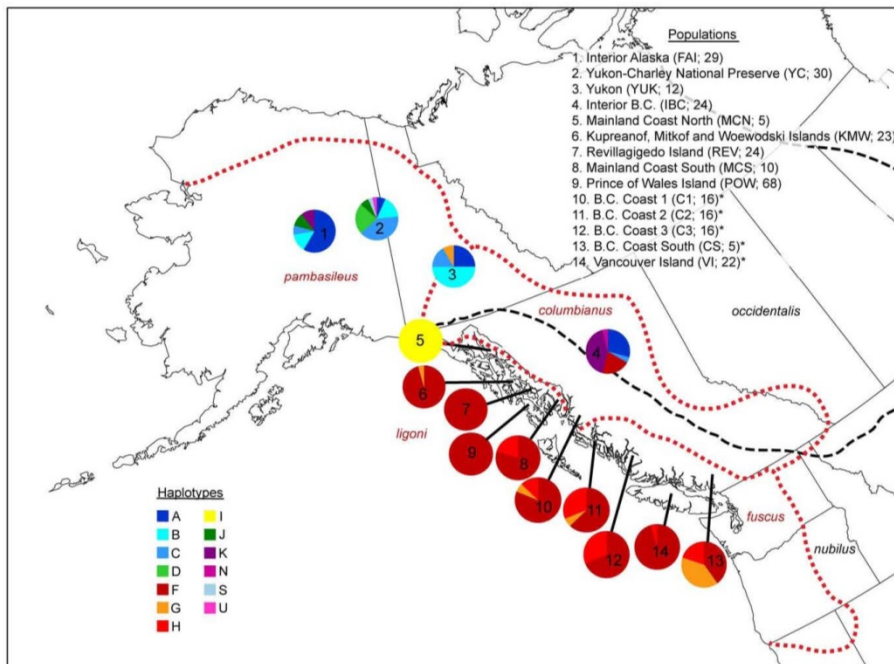


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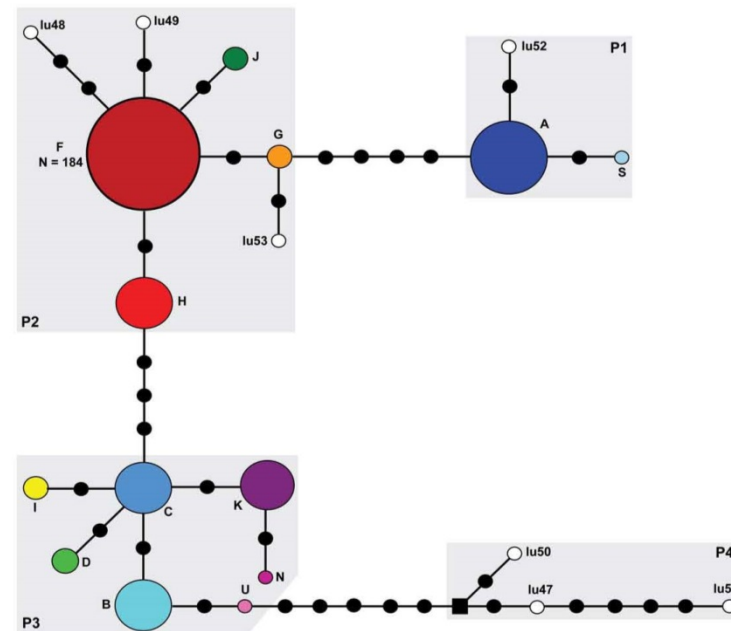


Figure 2. Haplotype network. Haplotype network for 20 haplotypes from 426 bp of mtDNA. Each black circle represents a single mutational change. The white circles indicate haplotypes from extirpated populations in the southern U.S. [25]. The coloured circles and haplotype labels are consistent with Figure 1. The size of each circle is proportional to the observed frequency of a given haplotype. The maximum circle size is for N=50, if N>50 the actual frequency is indicated. The black square represents a missing or hypothetical haplotype. Shaded regions define phylogroups 1, 2, 3 and 4 are labeled P1, P2, P3 and P4, respectively. doi:10.1371/journal.pone.0019582.g002

Genetic Analyses Summary

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Population Genetics

Sample Locations

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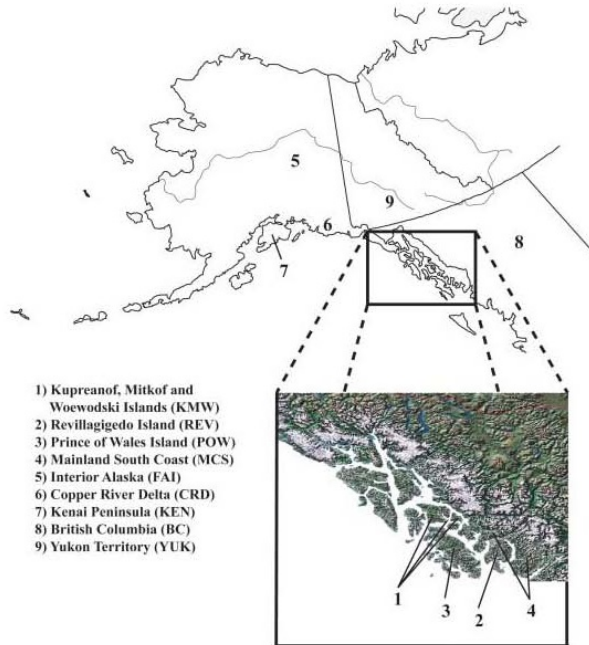
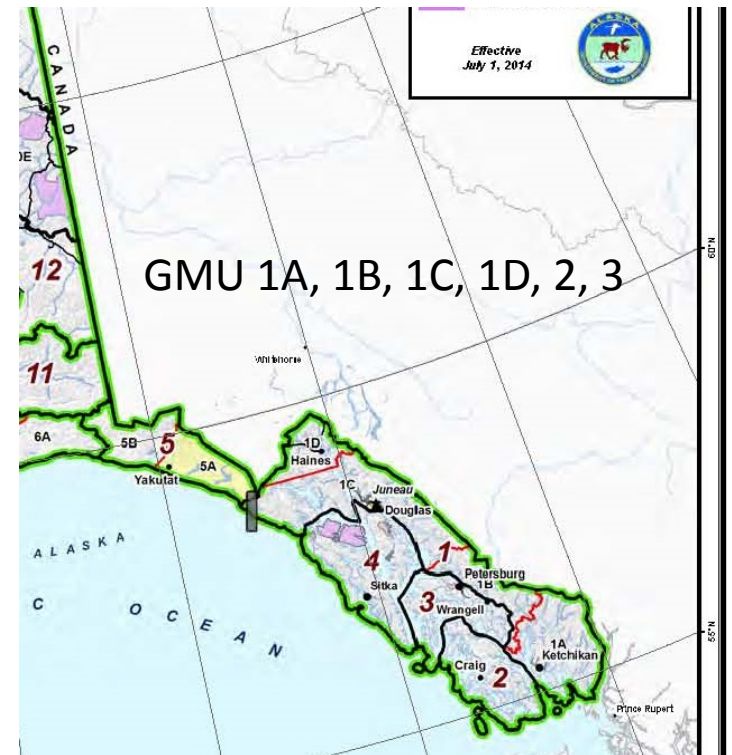


Fig. 1 Map of the Pacific Northwest with southeast Alaska expanded. Sampling locations and abbreviations are indicated.

1 = GMU3
 2 = GMU1A
 3 = GMU2
 4 = GMU1A

Weckworth et al. 2005



Cronin et al. 2014

Population Genetics

Sample Locations

Breed 2007

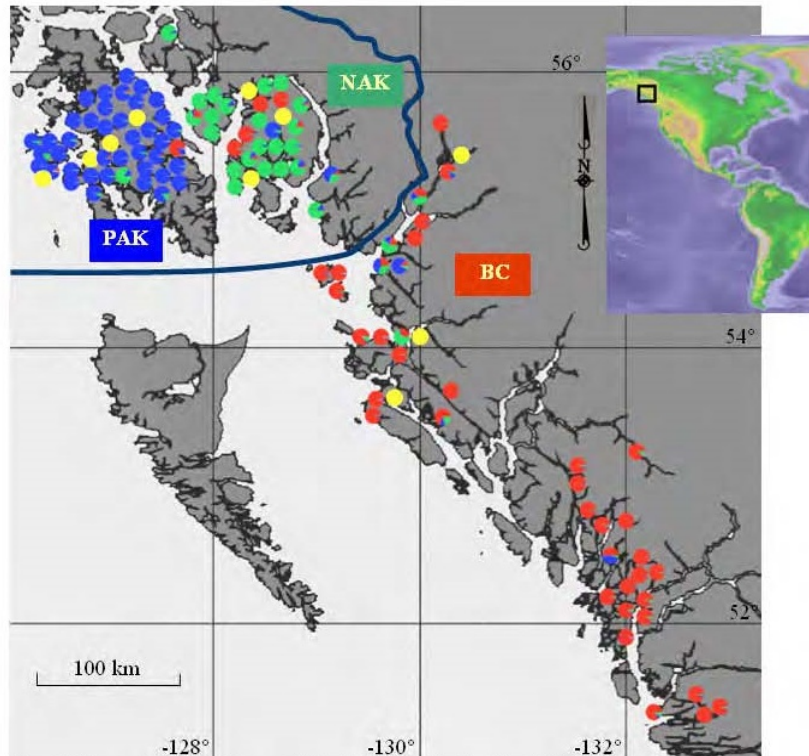
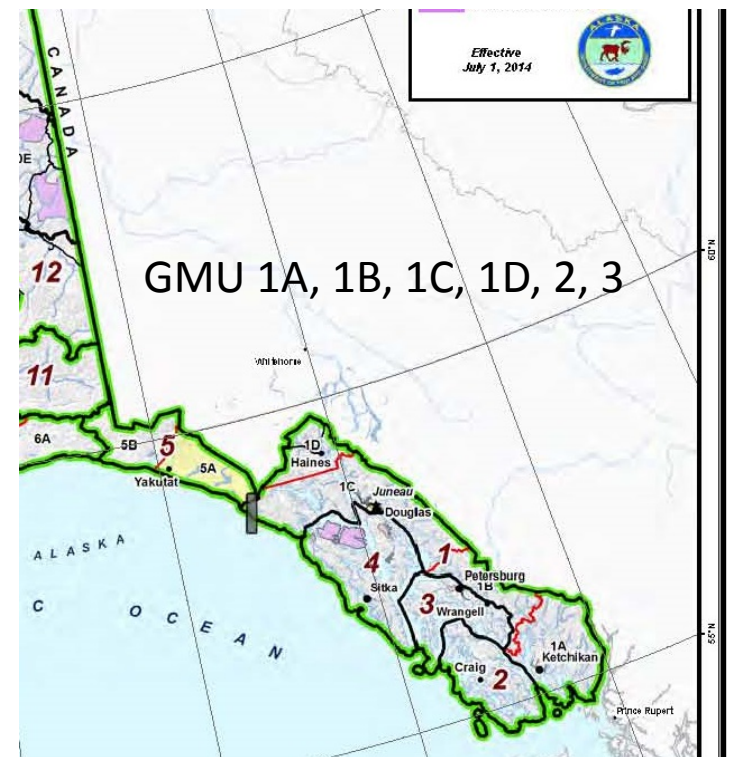


Figure 1. Representation of wolf geographic localities (1 circle = 1 wolf) and their cluster probabilities based on STRUCTURE 2.2 analyses with $K = 3$. The 10 wolves excluded by the GENECLASS 2.0.6 analysis are shown in yellow. The thick dark line represents the jurisdictional boundary of Alaska to the north and British Columbia to the south. Each MU is labelled with its specified acronym: BC = British Columbia; NAK = mainland coastal Alaska; PAK = Prince of Wales Island, Alaska. Each circle was moved manually a minimal distance to make way for proximate circles.

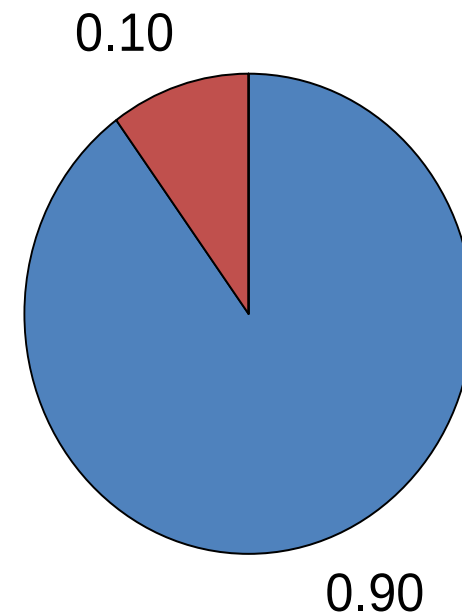
Cronin et al. 2014



Population subdivision is measurable

F_{ST} is the fraction of the total genetic variation that is due to variation between populations and is most commonly attributed to genetic drift and gene flow.

-  Fraction of total genetic variation in all populations
-  F_{ST} - Fraction of total genetic variation between populations



Population Genetics

Summary of Fst values

Weckworth et al. 2005

Table 4 Pairwise estimates of F_{ST} genetic distances for all population pairs. Population abbreviations as in Table 1

	KMW	REV	POW	MCS	FAI	CRD	KEN	BC
REV	0.04							
POW	0.20	0.10						
MCS	0.06	0.02	0.14					
FAI	0.22	0.16	0.22	0.12				
CRD	0.18	0.11	0.17	0.12	0.07			
KEN	0.19	0.13	0.23	0.13	0.10	0.12		
BC	0.19	0.11	0.19	0.09	0.08	0.09	0.13	
YUK	0.19	0.12	0.20	0.11	0.04	0.08	0.09	0.06

Cronin et al. 2014

Table 2 Summary of F_{ST} values derived from 123,801 SNP genotypes among wolf, coyote, and dog populations with $N > 1$

Populations compared	F_{ST}	F_{ST}	F_{ST}
	Mean (SE) ^a	Range	95% CL ^b
Intraspecies			
Among groups of dogs	0.0843 (0.0350)	0.0145–0.1251	0.1509
Among coyote locations	0.1105 (0.0211)	0.0188–0.2927	0.0453
Among all wolf locations	0.1525 (0.0101)	0.0124–0.3903	0.0202
Comparisons of southeast Alaska wolves			
Among all 6 Southeast AK locations	0.1268 (0.0184)	0.0344–0.2811	0.0394
Among 5 Southeast AK locations excluding GMU2	0.1147 (0.0225)	0.0344–0.2463	0.0508
GMU2 versus 5 other Southeast AK locations	0.1511 (0.0326)	0.1139–0.2811	0.0904
Southeast AK versus BC wolves	0.1195 (0.0215)	0.0607–0.1849	0.0552
Southeast AK versus MN wolves	0.2012 (0.0206)	0.1436–0.2638	0.0529
Southeast AK versus northern wolves (interior AK, ID, MT, WY)	0.1501 (0.0122)	0.0430–0.2441	0.0252
Southeast AK versus NM wolves	0.3448 (0.0158)	0.2810–0.3903	0.0407
Comparisons of North American populations			
Among northern wolves (interior AK, ID, MT, WY)	0.0451 (0.0109)	0.0124–0.0784	0.0279
MN wolves versus northern wolves (interior AK, ID, MT, WY)	0.1154 (0.0068)	0.0981–0.1271	0.0217
MN wolves versus NM wolves	0.2527 (N/A)	N/A	N/A
Northern wolves (interior AK, ID, MT, WY) versus NM wolves	0.2330 (0.0102)	0.2065–0.2526	0.0325
Comparisons with BC ^c			
BC wolves versus NM wolves	0.2219 (N/A)	N/A	N/A
BC wolves versus northern wolves (interior AK, ID, MT, WY)	0.0390 (0.0065)	0.0240–0.0535	0.0208
BC wolves versus Northern Rockies wolves (ID, MT, WY)	0.0342 (0.0062)	0.0240–0.0454	0.0267
BC wolves versus MN wolves	0.1031 (N/A)	N/A	N/A
Interspecies			
Dog versus coyote	0.3198 (0.0185)	0.2061–0.5222	0.0391
Dog versus wolf	0.3379 (0.0113)	0.2228–0.5153	0.0228
Wolf versus coyote	0.2790 (0.0111)	0.0817–0.4480	0.0221

^aMean (and standard error) values of pairwise F_{ST} (Supplementary Table 3 online) of the groups identified in Table 1 with $N > 1$.

^b95% Confidence level of the mean.

^cBC (British Columbia).

Among 3 SEAK excluding POW: 0.0400

POW versus 3 SEAK: 0.1467

Among 3 SEAK excluding GMU2: 0.0512

GMU2 versus 3 SEAK: 0.1173

Population Genetics

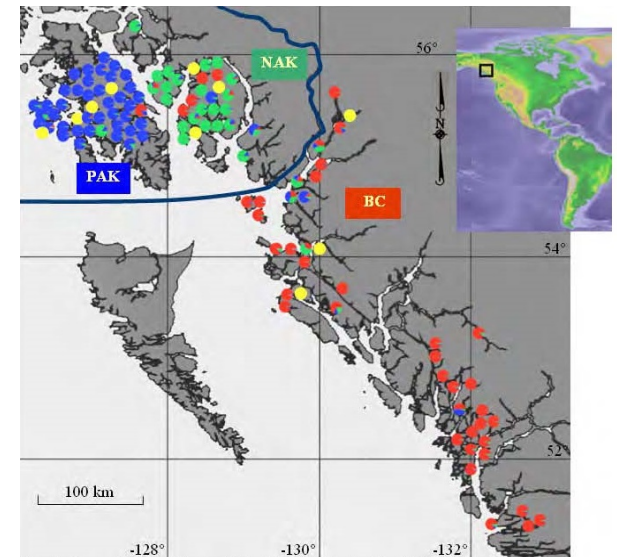
Summary of Fst values

Breed 2007

Table 8. Pairwise F_{ST} using θ (below diagonal) for each pair of MUs and their significance of differentiation (above diagonal). Significant results are denoted by *** ($p < 0.001$).

	BC	NAK	PAK
BC	-	***	***
NAK	0.087	-	***
PAK	0.149	0.122	-

- PAK formed most clearly defined cluster



Population Genetics

Cluster analysis

Breed 2007

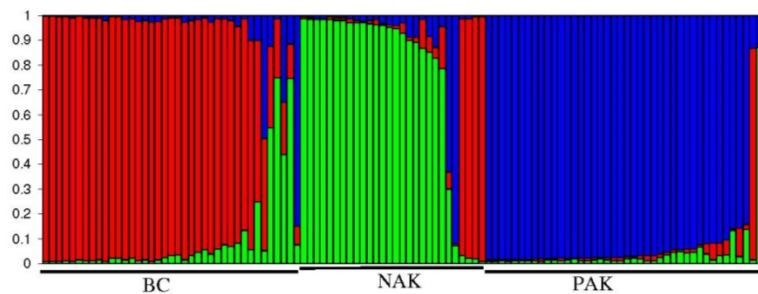


Figure 6.a. Bar plot output from *STRUCTURE* 2.2 of $K=3$. Likelihood of assignment to each cluster was represented by the colours (main BC cluster = red; main NAK cluster = green; main PAK cluster = blue). The x-axis denotations represent the sampling origin of each wolf.

Cronin et al. 2014

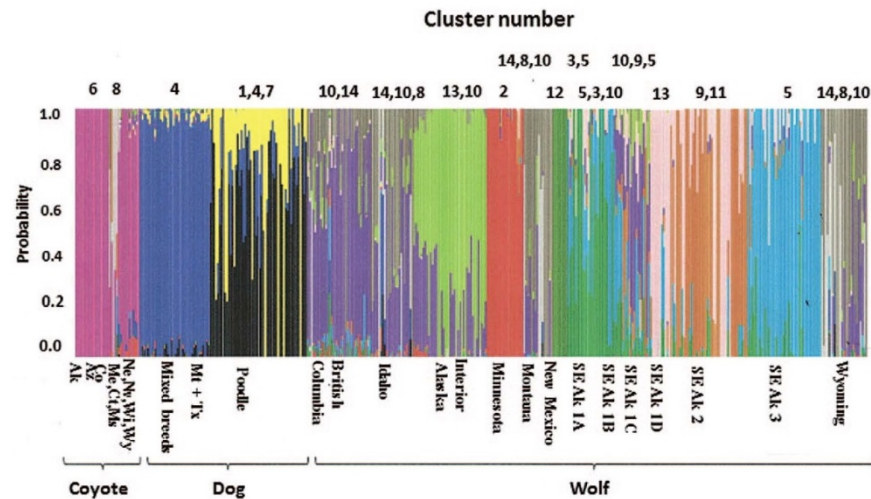


Figure 3. Histogram of ADMIXTURE analysis for $K = 14$ of 123 801 SNP genotypes for 431 wolves, coyotes, and dogs.

Weckworth et al. 2005

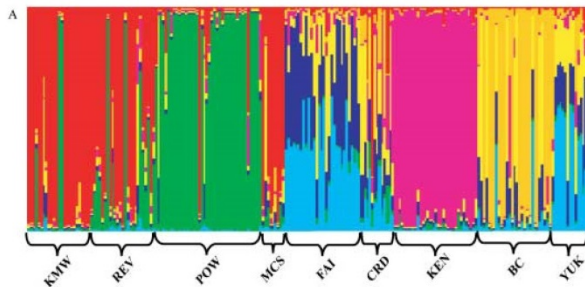


Fig. 4 Histograms of *STRUCTURE* assignment tests. Each vertical bar represents an individual and its assignment proportion into one of seven (A) or two (B) clusters. More than one colour per individual indicates admixture. Individuals are arranged in order by populations; abbreviations follow Table 1.

Population Genetics

Summary of Fst values

Weckworth et al. 2011

Table 1. AMOVA results.

Model	Hypothesized groupings	Φ_{SC}	Φ_{ST}	Φ_{CT}	% among groups	p Φ_{CT}
A	[KMW, MCN, MCS, POW, REV, VI, CBC, CS] [IBC, YC, FAI, YUK]	0.300	0.655	0.507	50.69	0.00196
B	[KMW, MCN, MCS, POW, REV] [VI, CBC, CS] [IBC, YC, FAI, YUK]	0.331	0.613	0.433	42.21	0.00782
C	[KMW, MCN, MCS, POW, REV] [VI, CBC, CS, IBC, YC, FAI, YUK]	0.448	0.605	0.287	28.56	0.05376
D	[KMW, MCN, MCS, POW, REV, VI, CBC, CS] [YC, FAI] [IBC, YUK]	0.322	0.628	0.451	45.09	0.00391
E	[KMW, MCN, MCS, POW, REV] [VI, CBC, CS] [YC, FAI] [IBC, YUK]	0.343	0.582	0.365	36.47	0.01760

Analysis of molecular variance for five *a posteriori* models of groupings according to different subspecies designations previously identified using morphological data.
doi:10.1371/journal.pone.0019582.t001

Among-group (Φ_{CT}) structure greatest when pooled into coastal and inland groups

Population Genetics

Gene Flow

Weckworth et al. 2005

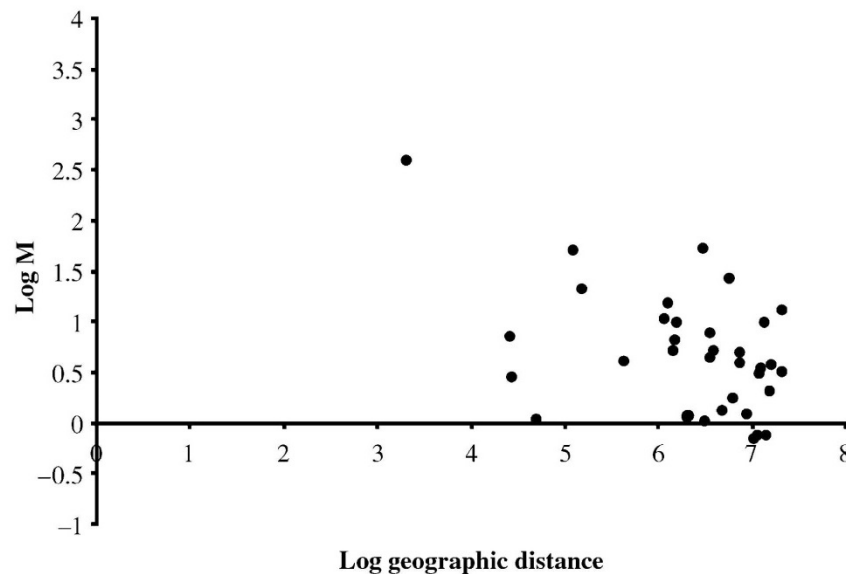


Fig. 5 Relationships between pairwise geographical distances and estimates of gene flow (M) based on F_{ST} for microsatellites.

No evidence of isolation by distance

- Dispersal distances are large and/or
- Barriers to dispersal are more important than distance

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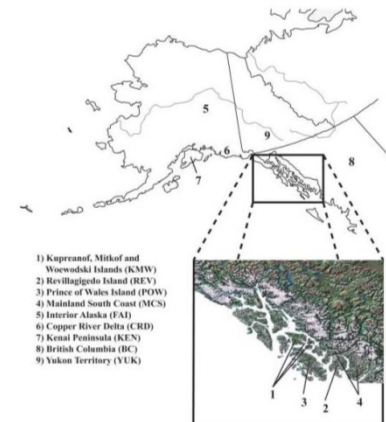


Fig. 1 Map of the Pacific Northwest with southeast Alaska expanded. Sampling locations and abbreviations are indicated.

Population Genetics

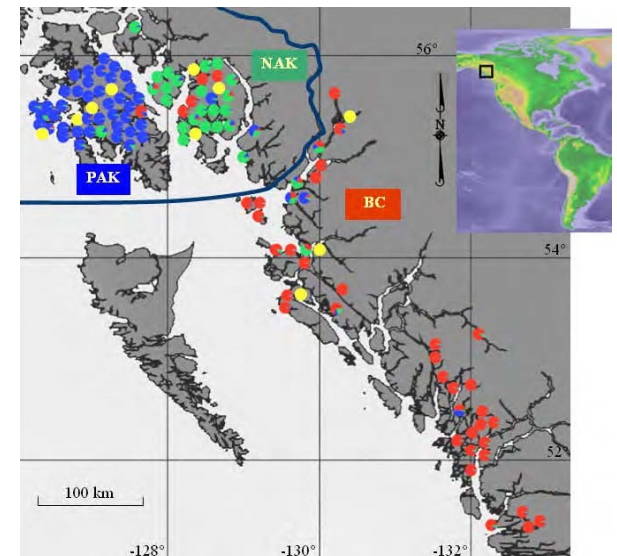
Gene Flow

Breed 2007

Table 9. Pairwise Pearson chi-square analysis p-values of differences in ratios of residents:immigrants. Significant differences are indicated by '*’.

	BC	NAK
NAK	0.041*	
PAK	0.701	0.056

- PAK formed most clearly defined cluster
- Low levels of immigration into PAK from NAK and BC
- BC source to PAK and NAK sink



Population Genetics

Population diversity

Weckworth et al. 2005

Table 1 Descriptive statistics for *Canis lupus* populations and clades

Populations	Abbr.	<i>n</i>	Alleles	Richness	H_E	H_O	F_{IS}	F_{ST}	M-ratio
Coastal group		101	5.00	3.21	0.52	0.48	0.05	0.12	0.795
Kupreanof, Mitkof, and Woewodski Islands, SE AK	KMW	26	3.73	3.00	0.46	0.43	0.08		0.747
Revillagigedo Island, SE AK	REV	24	4.09	3.46	0.57	0.59	-0.04		0.801
Prince of Wales Island, SE AK	POW	42	3.82	2.93	0.48	0.42	0.12		0.702
Mainland coast, SE AK	MCS	9	3.45	3.45	0.58	0.61	-0.04		0.737
Continental group		120	7.09	4.06	0.62	0.59	0.06	0.09	0.895
Fairbanks Quadrant, interior AK	FAI	29	5.55	4.62	0.64	0.59	0.08		0.846
Copper River Delta, coastal AK	CRD	14	3.64	3.43	0.58	0.53	0.10		0.737
Kenai Peninsula, coastal AK	KEN	33	3.55	3.17	0.55	0.55	0.01		0.750
British Columbia	BC	30	6.00	4.69	0.69	0.62	0.11		0.835
Yukon Territories	YUK	14	4.91	4.39	0.65	0.69	-0.06		0.825

Abbreviations (abbr.), sample size (*n*), mean number of alleles per locus (alleles), allelic richness (richness), expected heterozygosity (H_E), observed heterozygosity (H_O), F_{IS} , F_{ST} for the group comparison, and Garza & Williamson's (2001) M-ratios.

Cronin et al. 2014

Table 1 Numbers and locations of samples and observed heterozygosity of wolves, coyotes, and dogs that were genotyped with the Illumina170K CanineBeadChip

Species	Population name	Location	Subspecies/breed	Heterozygosity	Number	Total
Wolf	SEAK ^a wolf	GMU2 Southeast Alaska ^b	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.190	55	305
	SEAK wolf	GMU3 Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.181	38	
	SEAK wolf	GMU1A Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.195	22	
	SEAK wolf	GMU1B Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.193	8	
	SEAK wolf	GMU1C Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.230	13	
	SEAK wolf	GMU1D Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.259	2	
	BCWolf	British Columbia Canada	<i>Canis lupus nubilus</i> or <i>occidentalis</i>	0.244	35	
	INTAK ^c wolf	Interior Alaska	<i>Canis lupus occidentalis</i>	0.239	40	
	WY ^e wolf	Wyoming	Transplanted <i>C. l. occidentalis</i>	0.276	25	
	ID ^f wolf	Idaho	Transplanted <i>C. l. occidentalis</i>	0.270	23	
Coyote	MT ^g wolf	Montana	Transplanted <i>C. l. occidentalis</i>	0.251	16	35
	MN wolf	Minnesota	<i>Canis lupus harrisi</i> or <i>nubilus</i>	0.237	20	
	NM wolf	New Mexico	<i>Canis lupus baileyi</i>	0.172	8	
	AK Coyote	Alaska	<i>Canis latrans</i>	0.131	2	
	CT Coyote	Connecticut	<i>Canis latrans</i>	0.372	1	
	AZ coyote	Arizona	<i>Canis latrans</i>	0.152	15	
	ME coyote	Maine	<i>Canis latrans</i>	0.281	4	
	WY Coyote	Wyoming	<i>Canis latrans</i>	0.212	6	
	NE coyote	Nebraska	<i>Canis latrans</i>	0.165	1	
	NV coyote	Nevada	<i>Canis latrans</i>	0.157	1	
	WI coyote	Wisconsin	<i>Canis latrans</i>	0.177	2	
	CO coyote	Colorado	<i>Canis latrans</i>	0.162	1	
	MS coyote	Mississippi	<i>Canis latrans</i>	0.228	2	
	MTx	Montana and Texas	<i>C. familiaris</i> Australian shepard, Border collie	0.334	2	
Dog	Mixed		<i>Canis familiaris</i> Mixed breed	0.351	36	91
	Poodle		<i>Canis familiaris</i> Poodle	0.324	53	
Total						431

Lower diversity in SEAK wolves

- But not statistically significant

Population Genetics

Population diversity

Table 3. MU's H_E , H_O and MNA.

	MNA	H_E	H_O
BC	5.54	0.62	0.58
NAK	4.38	0.55	0.35
PAK	3.85	0.48	0.40

Table 4. Pairwise Wilcoxon's signed-ranks tests between expected (a) and observed (b) heterozygosities (df = 13). Significant differences after sequential Bonferroni correction indicated by '*'.

(a)	BC	NAK	(b)	BC	NAK
NAK	0.116		NAK	0.0019*	
PAK	0.013*	0.087	PAK	0.0071*	0.25

Table 6. Pairwise Wilcoxon's signed-ranks test between each MU's AR (df = 13). Significant differences after sequential Bonferroni correction indicated by '*'.

	BC	NAK
NAK	0.023*	
PAK	0.014*	0.044*

Table 5. Total number of alleles and private allele numbers per MU and the entire dataset as a whole. Frequency of all private alleles by MU shown in parentheses, with a homozygote for a private allele scored as 2.

	Alleles present	Private Alleles
BC	72	18 (76)
NAK	57	4 (7)
PAK	50	2 (2)
Total	79	24

Breed 2007

- BC = coastal British Columbia
- NAK = SEAK mainland and islands without POW
- PAK = POW

Final Thoughts

Ecological uniqueness

- Morphology
- Prey specialization
- Other specific behaviors

Genetic uniqueness

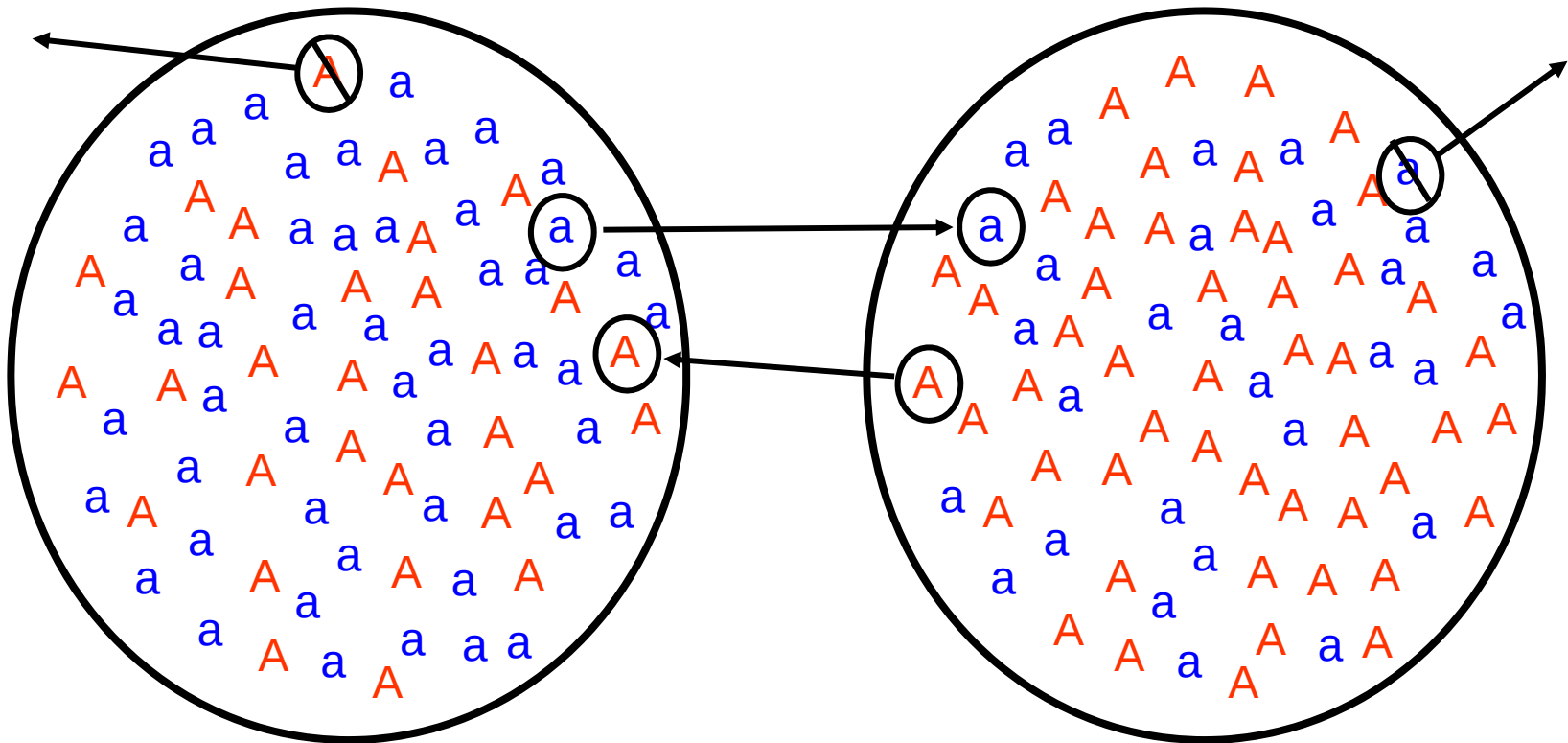
- Strong genetic differentiation between coastal and interior continental populations
- SEAK and BC wolves share common and unique haplotypes
- SEAK wolves monotypic; BC wolves had multiple haplotypes
- Barriers to gene flow vs isolation by distance?



**What is an AA wolf and
what are its
distributional limits?**

Genetic drift and migration

The most common interpretation of F_{ST} is as a measure of the genetic variation between populations due to the opposing forces of genetic drift and migration - the “drift-migration” model.



Evolutionary forces influencing genetic diversity in populations

impact

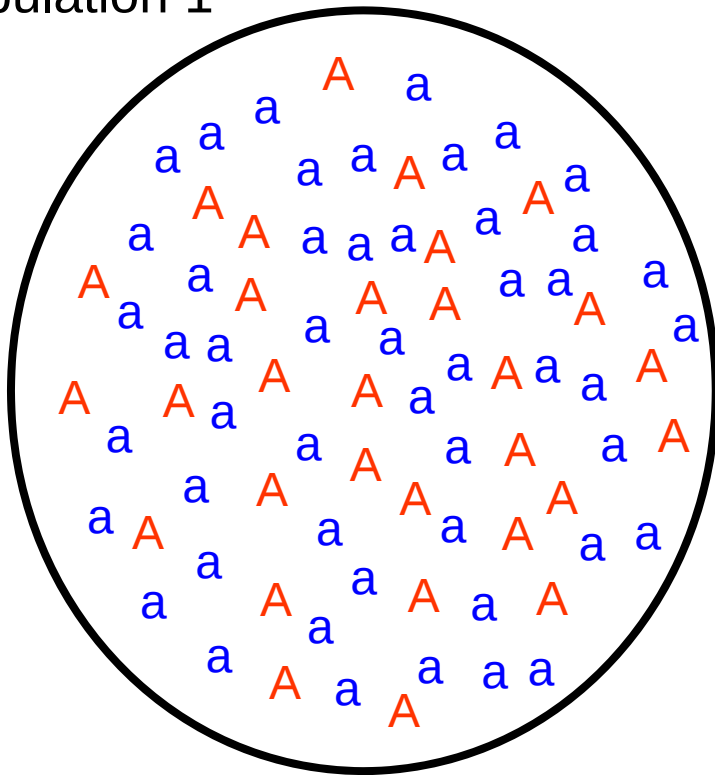
	locus	populations
selection	varies by locus	differentiate/homogenize
mutation	varies by locus	differentiate
genetic drift	same for all loci	differentiate
migration	same for all loci	homogenize

Most population genetic studies measure genetic drift and migration.

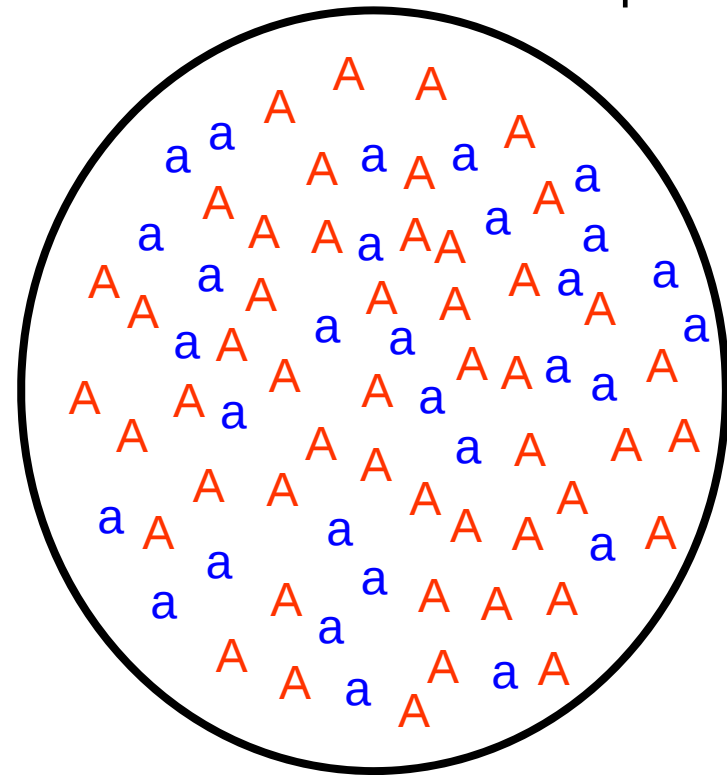
Population subdivision

Population subdivision exists when a **barrier to gene flow** prevents random mating between two or more populations. These populations generally display different allele and genotype frequencies.

Population 1



Population 2



Quick overview of genetic methods and data

Types of studies:

Phylogenetics	Phylogeography	Population Genetics
the study of genealogical lineages in order to define the evolutionary relationships among groups of organisms (species, subspecies, populations).	the study of the processes governing the geographic distribution of genealogical lineages .	the study of the evolutionary forces (selection, mutation, genetic drift, gene flow) influencing genetic diversity within and among populations?
Time scale – longer Forces – mutation, drift	Time scale – longer Forces – mutation, drift	Time scale – shorter Forces – drift, gene flow

Selected Wolf Genetic Papers

Study	SEAK	Coastal BC	Type	Key findings
Weckworth et al. 2005	Yes	No	microsat	Coastal distinct from interior; 2 clusters within SEAK – POW and others
Breed 2007	Yes	Yes	microsat	3 units – coastal BC, POW, and other SEAK
Munoz-Fuentes et al. 2009	No	Yes	mtDNA	Coastal distinct from interior
Weckworth et al. 2010	Yes	No	mtDNA	Independent evolutionary histories – coastal and interior
Weckworth et al. 2011	Yes	Yes	mtDNA	Coastal distinct from interior; SEAK and BC wolves related
Cronin et al. 2014	Yes	No	SNPs	SEAK different from other wolves, but not at unprecedented levels of variation
Stronen et al. 2014	No	Yes	microsat	Coastal distinct from interior – genetics and ecology

Weckworth et al. 2005

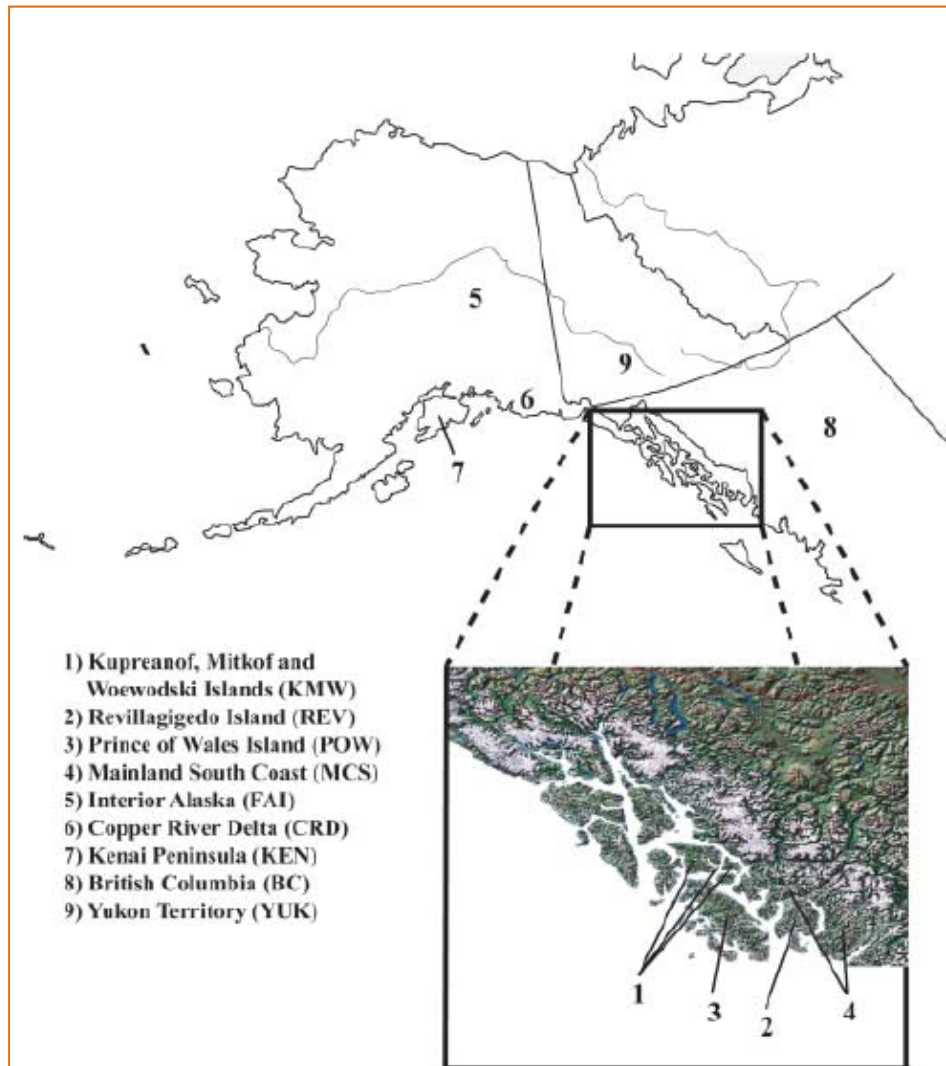


Fig. 1 Map of the Pacific Northwest with southeast Alaska expanded. Sampling locations and abbreviations are indicated.

Table 4 Pairwise estimates of F_{ST} genetic distances for all population pairs. Population abbreviations as in Table 1

	KMW	REV	POW	MCS	FAI	CRD	KEN	BC
REV	0.04							
POW	0.20	0.10						
MCS	0.06	0.02	0.14					
FAI	0.22	0.16	0.22	0.12				
CRD	0.18	0.11	0.17	0.12	0.07			
KEN	0.19	0.13	0.23	0.13	0.10	0.12		
BC	0.19	0.11	0.19	0.09	0.08	0.09	0.13	
YUK	0.19	0.12	0.20	0.11	0.04	0.08	0.09	0.06

Weckworth et al. 2005

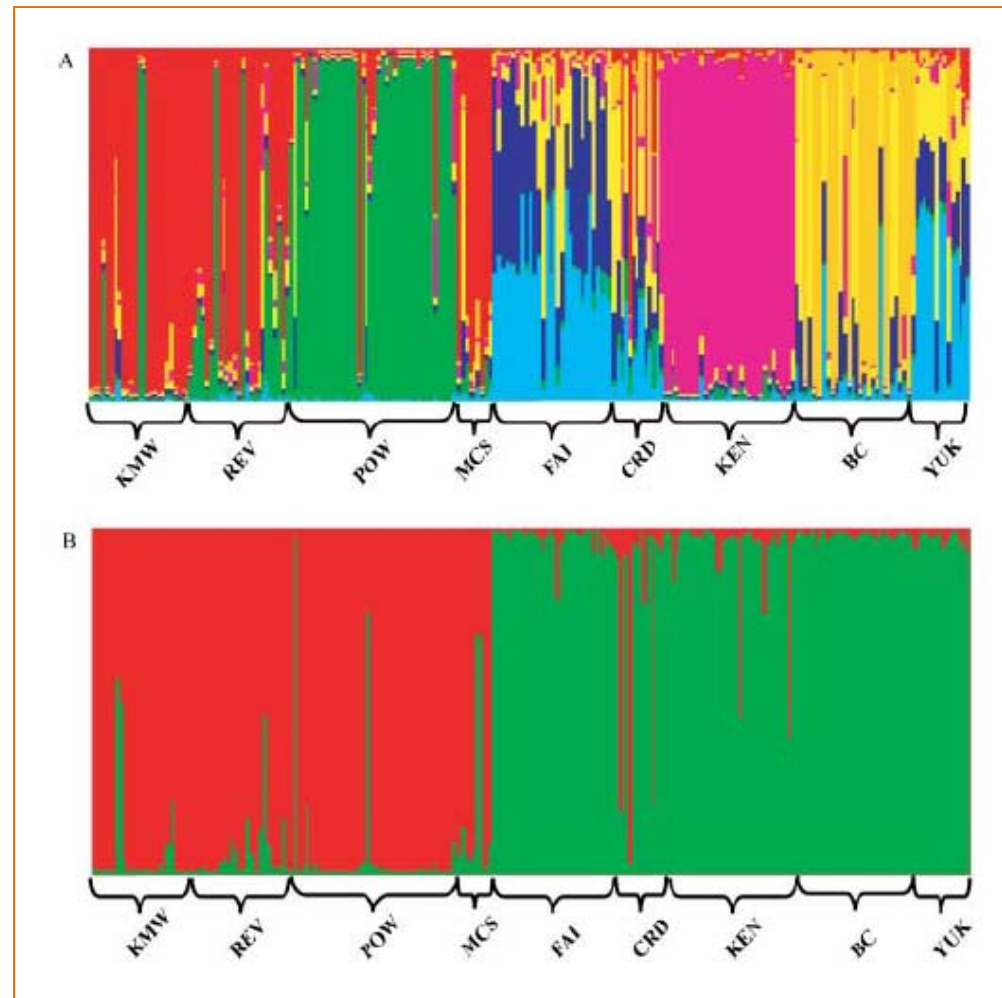
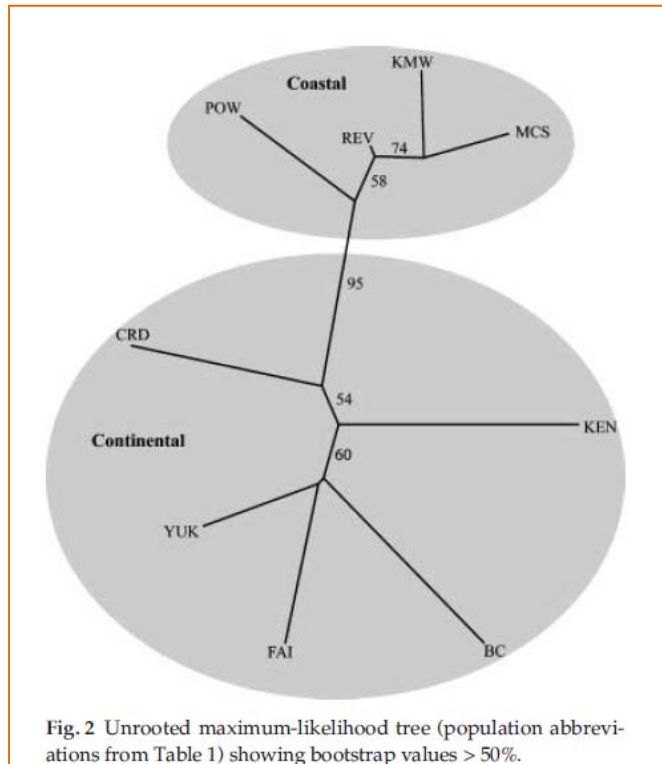


Fig. 4 Histograms of STRUCTURE assignment tests. Each vertical bar represents an individual and its assignment proportion into one of seven (A) or two (B) clusters. More than one colour per individual indicates admixture. Individuals are arranged in order by populations; abbreviations follow Table 1.

Munoz-Fuentes et al. 2009

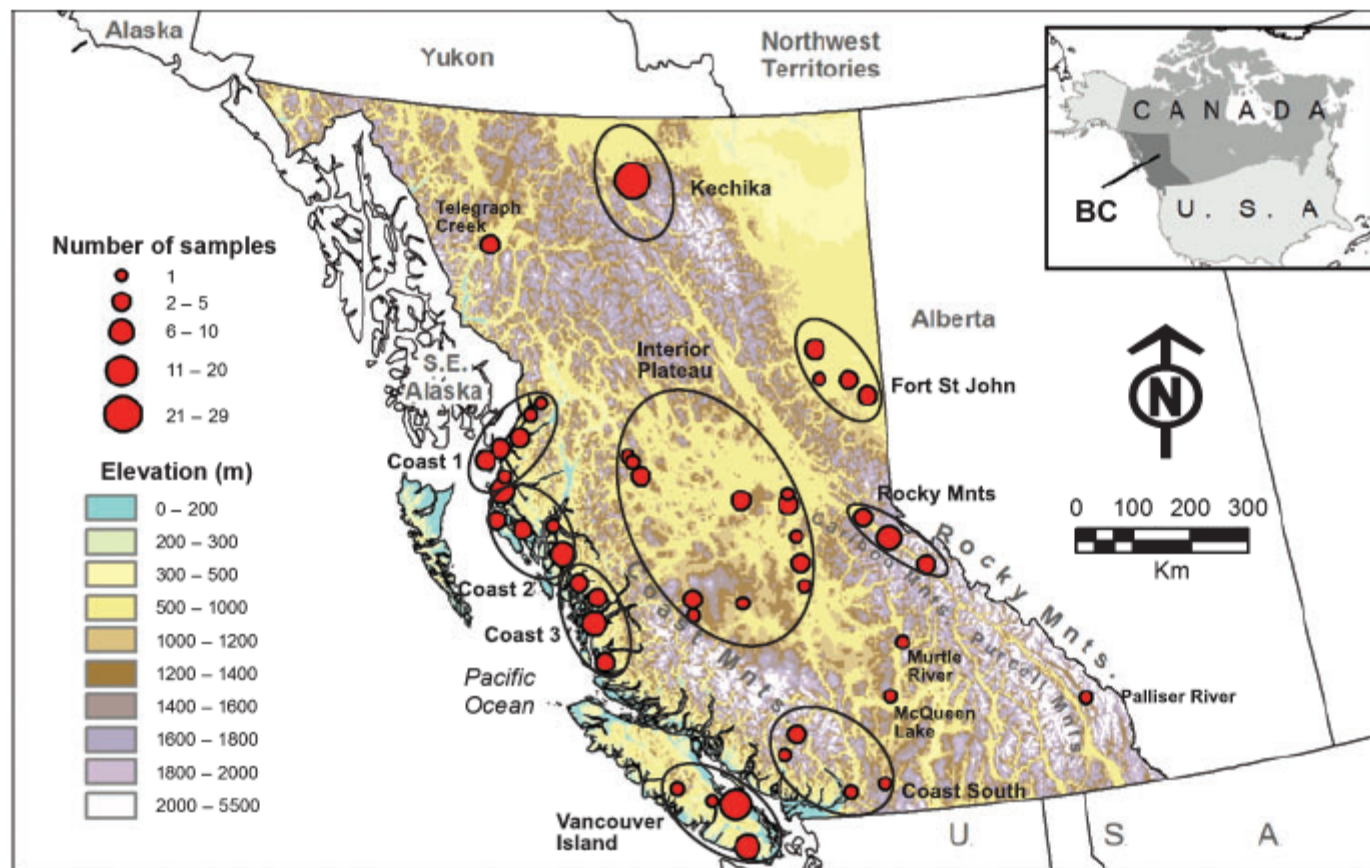
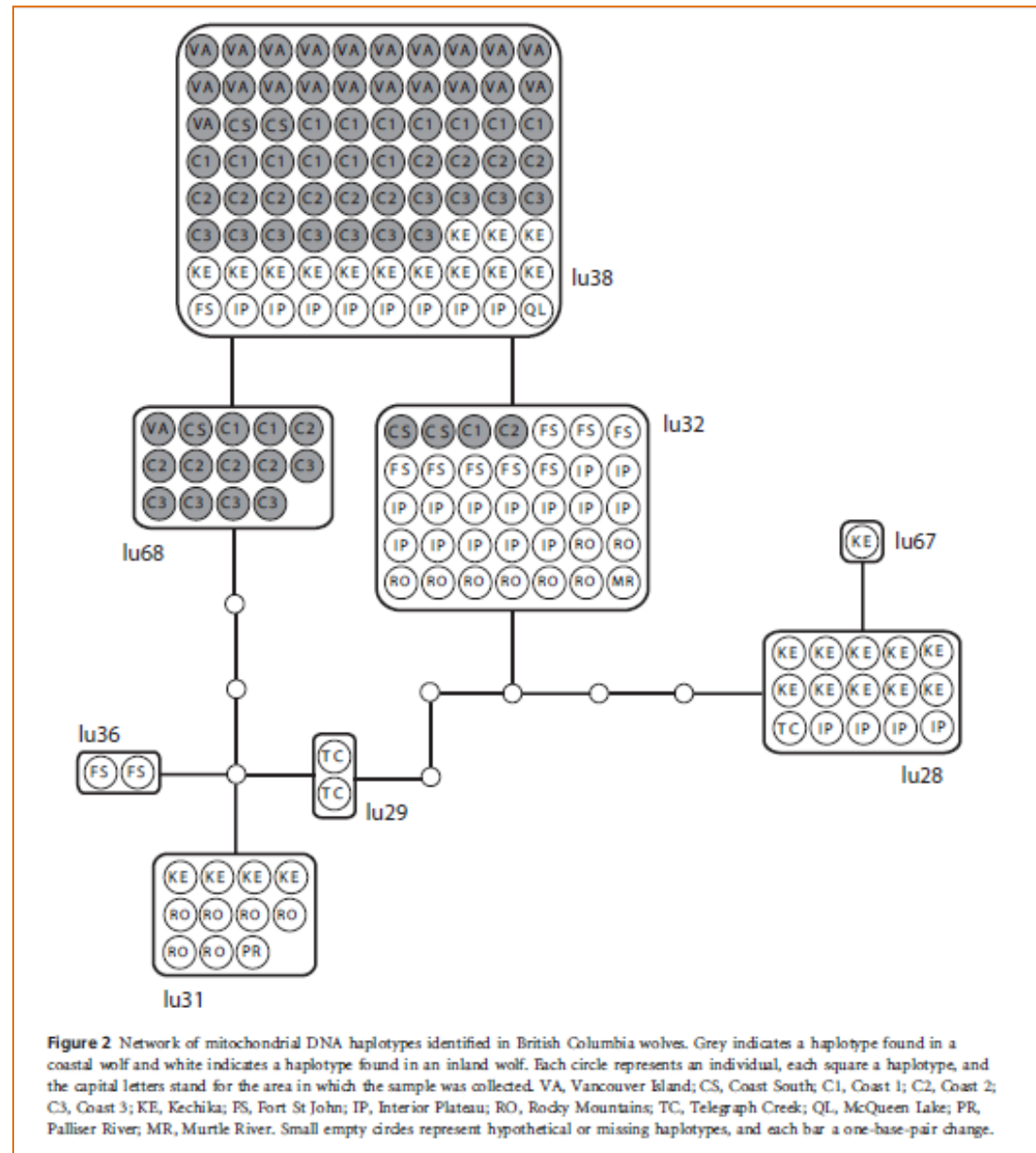
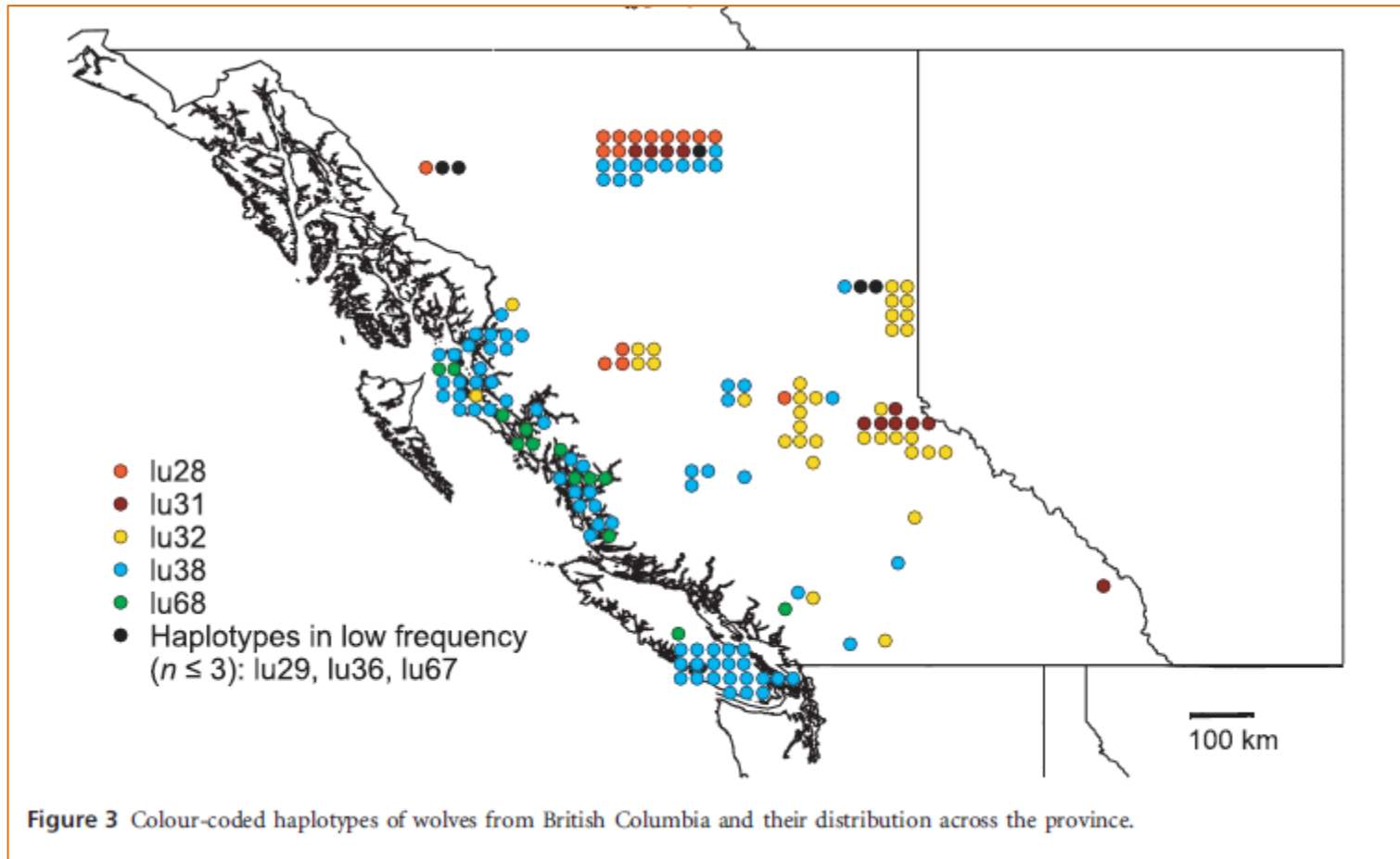


Figure 1 Map of British Columbia showing the elevation and distribution of wolf sampling localities, as indicated by red circles. Ellipses indicate grouping of the sampling localities based on geographical proximity into sampling areas for analyses. Sampling localities in Coast 1, Coast 2, Coast 3, Coast South and Vancouver Island are found in coastal British Columbia, east of the Coast Mountains, and the remaining sampling localities are in inland British Columbia.

Munoz-Fuentes et al. 2009



Munoz-Fuentes et al. 2009



Munoz-Fuentes et al. 2009

Table 4 Pairwise Φ_{ST} values between wolves from regions within British Columbia. Probability values were based on 1023 permutations. Overall $\Phi_{ST} = 0.305$, $P < 0.0001$. n.s., non-significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Negative values were converted to zero. Significant values remained significant at the 0.05 level after implementing the false discovery rate control of Benjamini & Hochberg (1995).

	Vancouver Island	Coast 1	Coast 2	Coast 3	Coast South	Kechika	Fort St John	Interior Plateau	Rocky Mountains
Vancouver Island	—								
Coast 1	0.000 ^{n.s.}	—							
Coast 2	0.167 ^{n.s.}	0.012 ^{n.s.}	—						
Coast 3	0.192 ^{n.s.}	0.032 ^{n.s.}	0.000 ^{n.s.}	—					
Coast South	0.396*	0.100 ^{n.s.}	0.054 ^{n.s.}	0.146 ^{n.s.}	—				
Kechika	0.303***	0.248**	0.248***	0.262**	0.118 ^{n.s.}	—			
Fort St John	0.463***	0.323***	0.298***	0.350***	0.000 ^{n.s.}	0.150*	—		
Interior Plateau	0.383***	0.293***	0.311***	0.353***	0.031 ^{n.s.}	0.132**	0.054 ^{n.s.}	—	
Rocky Mountains	0.472***	0.370***	0.333***	0.359**	0.147 ^{n.s.}	0.159*	0.090 ^{n.s.}	0.272***	—

Weckworth et al. 2010

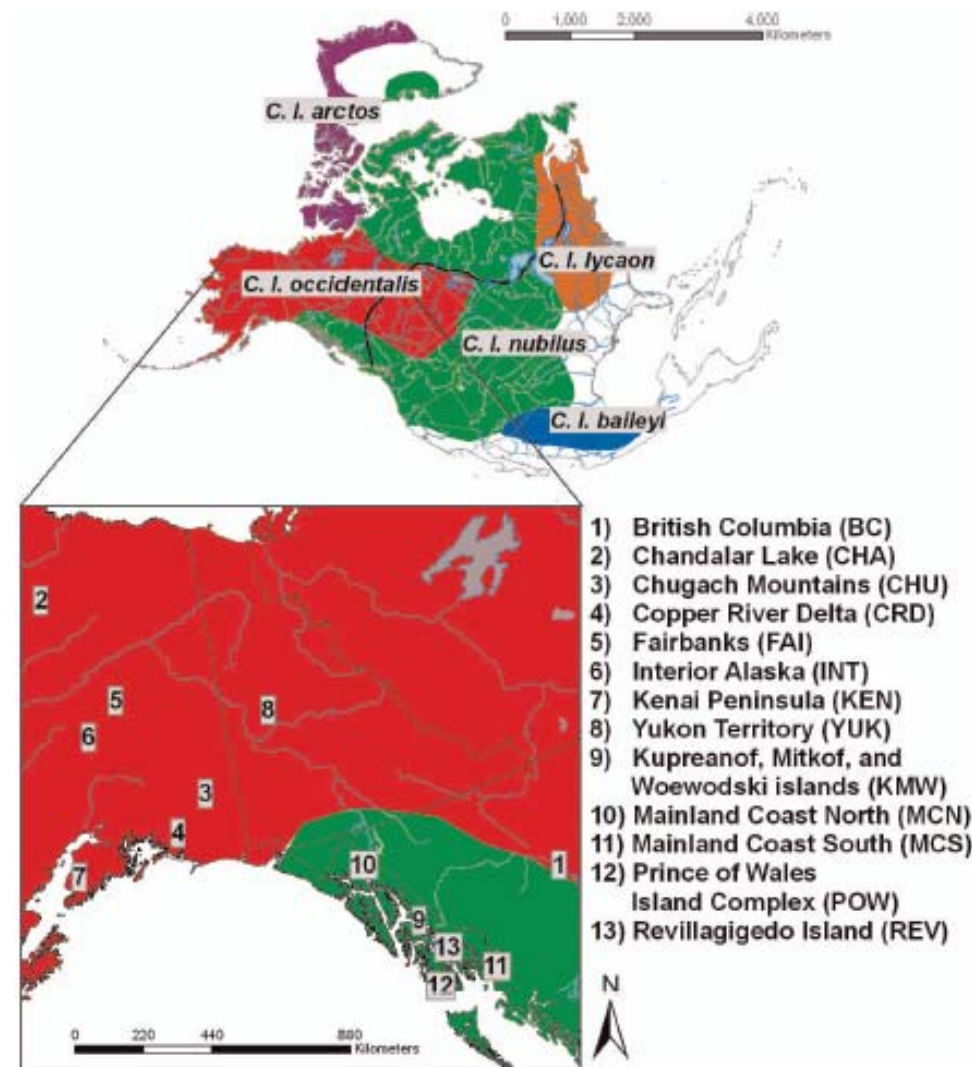


FIG. 1.—Map of North America (modified from Leonard et al. 2005) showing the pre-extirpation distribution of the gray wolf (*Canis lupus*) and the 5 subspecies based on morphological analysis (Nowak 1995). Solid black line marks the northern limit of eradication (Boitani 2003). Expanded inset of the Pacific Northwest indicates sampling locations and abbreviations.

Weckworth et al. 2010

TABLE 4.—Pairwise estimates of microsatellite F_{ST} (lower matrix—Weckworth et al. 2005) and mitochondrial DNA Φ_{ST} (upper matrix) for all population pairs consistent between 2 studies. Significant comparisons ($P < 0.01$) are in boldface type. Matrices are significantly correlated (Mantel test, $P = 0.0018$). Population abbreviations follow Table 1.

	KMW	REV	POW	MCS	FAI	CRD	KEN	BC	YUK
KMW	—	0.00	0.06	0.15	0.59	0.93	0.64	0.44	0.72
REV	0.04	—	0.00	0.25	0.60	0.94	0.65	0.46	0.75
POW	0.20	0.10	—	0.47	0.74	0.97	0.75	0.63	0.87
MCS	0.06	0.02	0.14	—	0.49	0.88	0.59	0.32	0.58
FAI	0.22	0.16	0.22	0.12	—	0.19	0.01	0.13	0.09
CRD	0.18	0.11	0.17	0.12	0.07	—	0.11	0.45	0.58
KEN	0.19	0.13	0.23	0.13	0.10	0.12	—	0.28	0.23
BC	0.19	0.11	0.19	0.09	0.08	0.09	0.13	—	0.08
YUK	0.19	0.12	0.20	0.11	0.04	0.08	0.09	0.06	—

Populations	Abbr.	<i>n</i>
Continental group	CNT	173
British Columbia	BC	24
Chandalar Lake	CHA	3
Chugach Mountains	CHU	3
Copper River Delta	CRD	16
Fairbanks Quad	FAI	29
Interior Alaska	INT	29
Kenai Peninsula	KEN	57
Yukon	YUK	12
Coastal group	CST	130
Kupreanof, Mitkof, and Woewodski islands, SE Alaska	KMW	23
Mainland Coast North, SE Alaska	MCN	5
Mainland Coast South, SE Alaska	MCS	10
Prince of Wales Island Complex, SE Alaska	POW	68
Revillagigedo Island, SE Alaska	REV	24
Russia	RUS	4
All		307

Weckworth et al. 2011

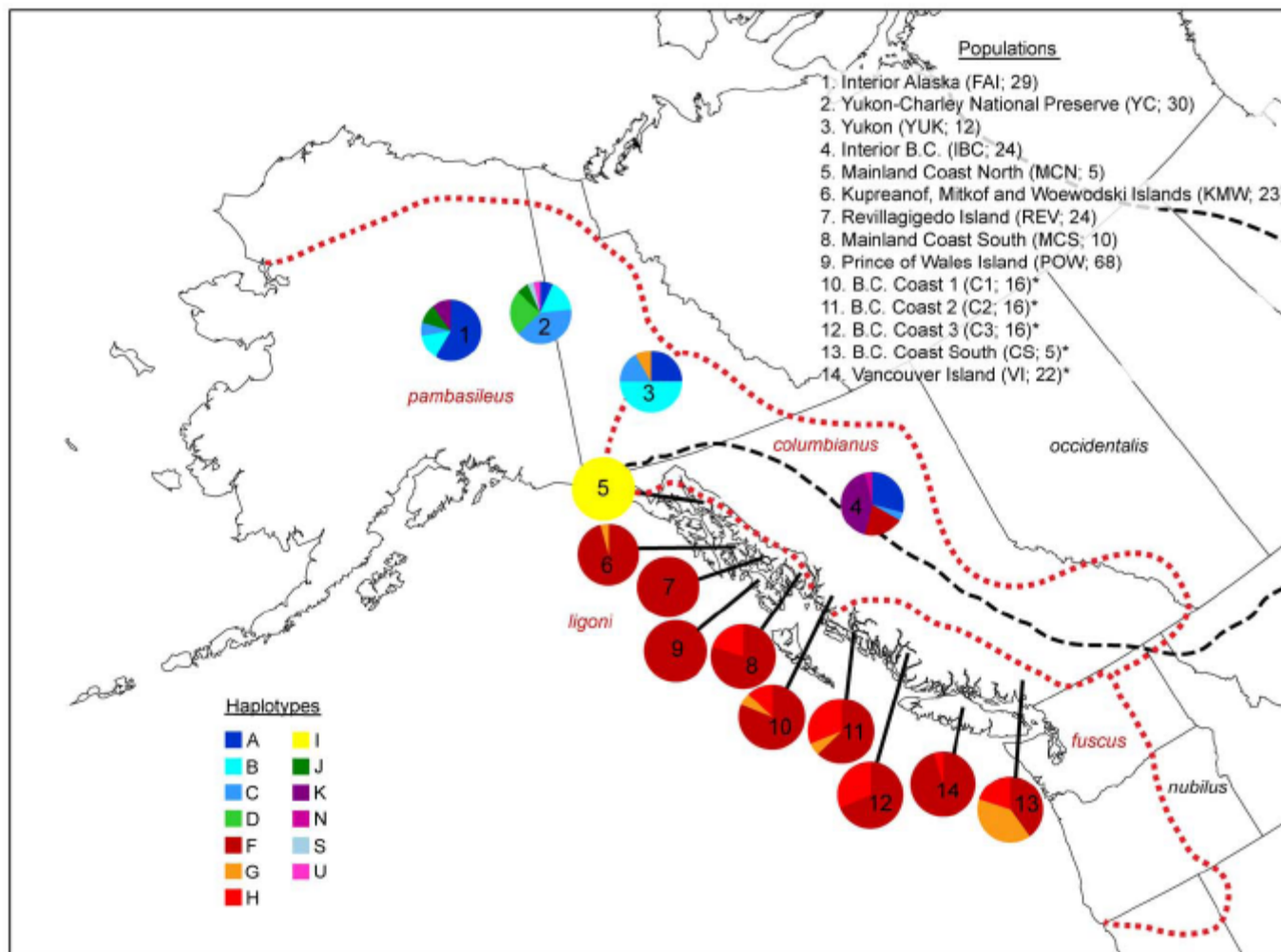


Figure 1. Map of sampling locations in Northwest North America. In the upper right, each population name is listed with its associated abbreviation and sample size. Asterisks indicate populations from Muñoz-Fuentes et al. [15]. Pie diagrams for each population indicate haplotype frequency, with color legend in the lower left. Black dashed lines indicate subspecies in the Pacific Northwest according to Nowak [33] and red dotted lines indicate the range of subspecies according to Hall and Kelson [32]. The 7 Southern United States (SUS) samples are not shown.
doi:10.1371/journal.pone.0019582.q001

Weckworth et al. 2011

Table 2. Φ_{ST} Population pairwise comparisons.

	VI	CBC	CS	IBC	YC	FAI	YUK	KMW	MCN	MCS	POW	REV
CBC	<i>0.084</i>											
CS	<i>0.396</i>	0.129										
IBC	<i>0.442</i>	<i>0.494</i>	<i>0.248</i>									
YC	<i>0.673</i>	<i>0.691</i>	<i>0.523</i>	<i>0.163</i>								
FAI	<i>0.534</i>	<i>0.590</i>	<i>0.336</i>	<i>0.121</i>	<i>0.351</i>							
YUK	<i>0.719</i>	<i>0.732</i>	<i>0.432</i>	0.086	<i>0.116</i>	<i>0.129</i>						
KMW	0.000	<i>0.130</i>	<i>0.380</i>	<i>0.451</i>	<i>0.681</i>	<i>0.536</i>	<i>0.724</i>					
MCN	<i>0.985</i>	<i>0.913</i>	<i>0.904</i>	<i>0.346</i>	<i>0.313</i>	<i>0.520</i>	<i>0.451</i>	<i>0.985</i>				
MCS	0.063	0.054	0.122	<i>0.330</i>	<i>0.581</i>	<i>0.444</i>	<i>0.584</i>	0.154	<i>0.949</i>			
POW	0.061	<i>0.241</i>	<i>0.775</i>	<i>0.637</i>	<i>0.808</i>	<i>0.701</i>	<i>0.872</i>	0.056	<i>1.000</i>	<i>0.472</i>		
REV	0.004	<i>0.149</i>	<i>0.551</i>	<i>0.465</i>	<i>0.692</i>	<i>0.552</i>	<i>0.746</i>	0.002	<i>1.000</i>	0.248	0.000	
SUS	<i>0.634</i>	<i>0.708</i>	<i>0.361</i>	<i>0.370</i>	<i>0.501</i>	<i>0.391</i>	<i>0.359</i>	<i>0.637</i>	<i>0.544</i>	<i>0.503</i>	<i>0.817</i>	<i>0.656</i>

Pairwise population comparisons calculating Φ_{ST} . Bold-italicized numbers indicate significant p-values ($\alpha=0.05$). Abbreviations as per text and Figure 1 (CBC = coastal British Columbia and is the combined data of C1, C2 and C3).

doi:10.1371/journal.pone.0019582.t002

Weckworth et al. 2011

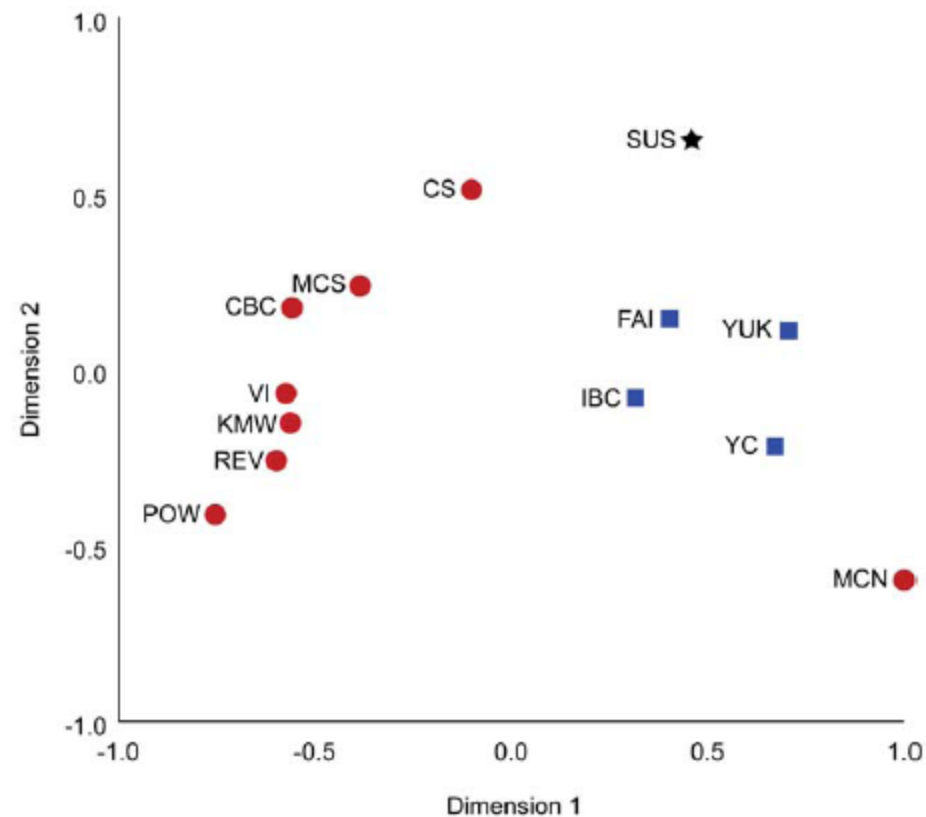


Figure 3. Multi-dimensional scaling plot. Non-metric multi-dimensional scaling plots of populations based on pairwise Φ_{ST} results. Red circles are coastal populations, blue squares are continental, and the black star represents the extirpated conterminous U.S. haplotypes. Population abbreviations are from Figure 1.
doi:10.1371/journal.pone.0019582.g003

Cronin et al. 2014

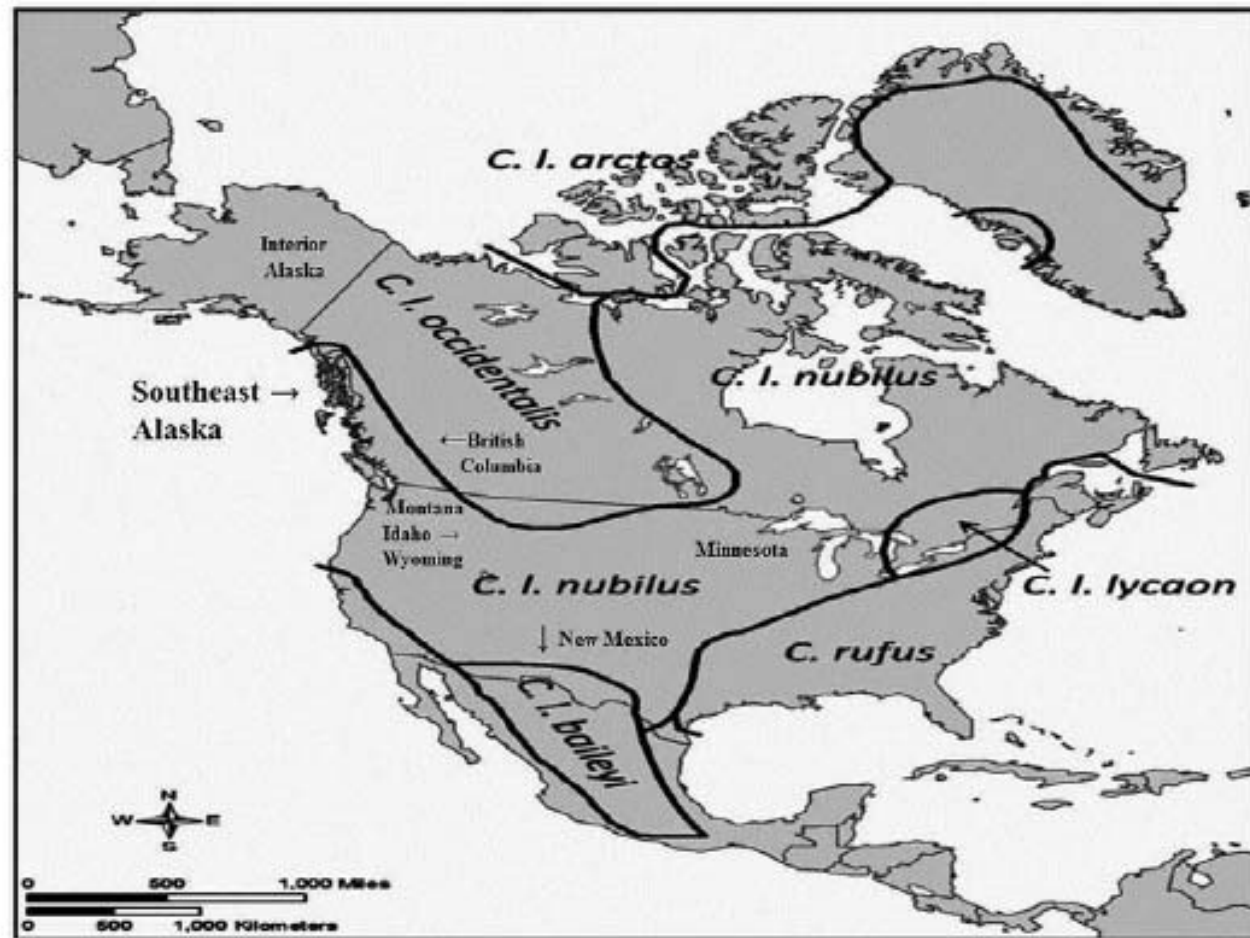


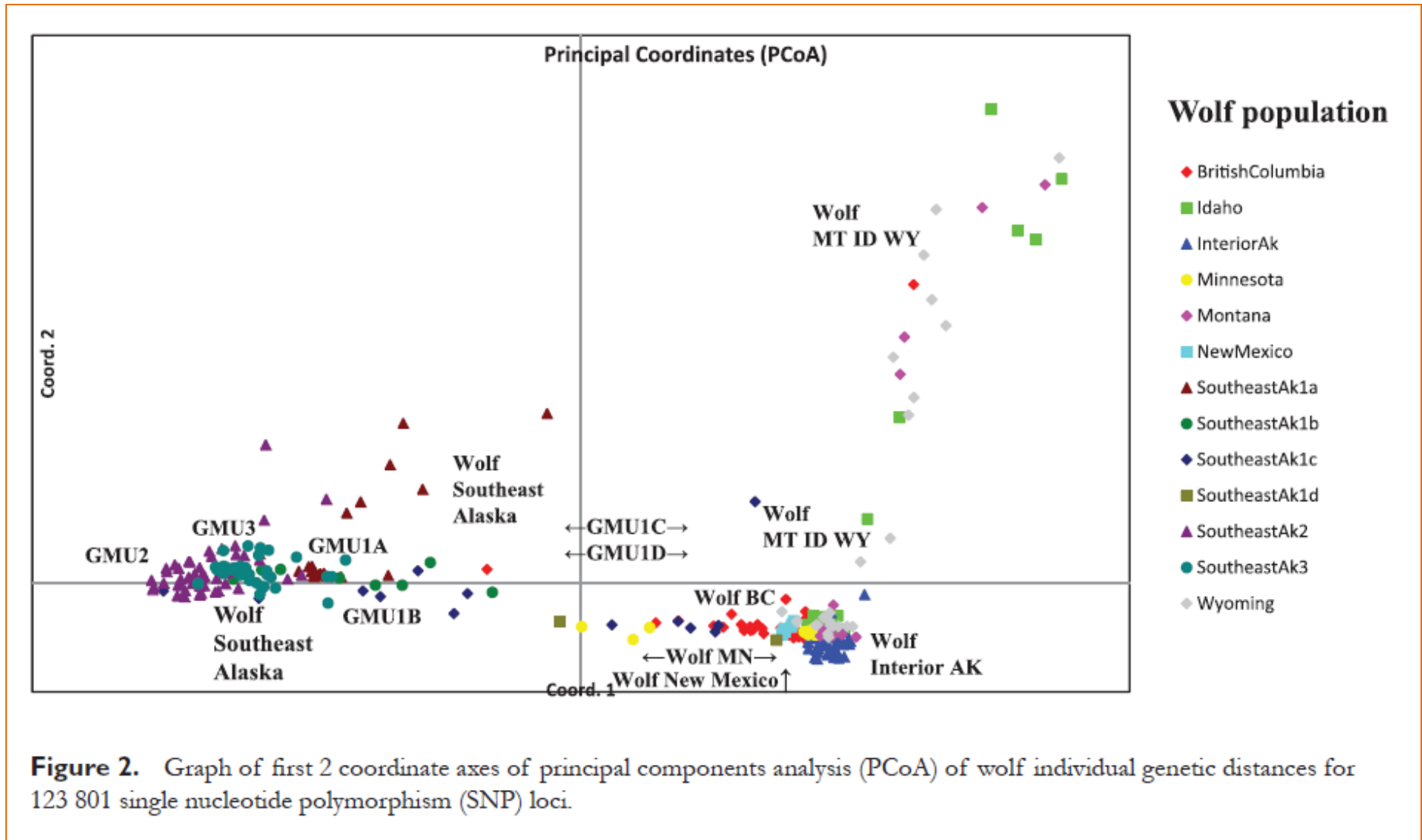
Figure 1. North American wolf (*Canis lupus*) sampling locations and subspecies distribution (Adapted from Nowak (1995, 2002) and Chambers et al 2012).

Cronin et al. 2014

Table 1 Numbers and locations of samples and observed heterozygosity of wolves, coyotes, and dogs that were genotyped with the Illumina170K CanineBeadChip

Species	Population name	Location	Subspecies/breed	Heterozygosity	Number	Total
Wolf	SEAK ^a wolf	GMU2 Southeast Alaska ^b	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.190	55	305
	SEAK wolf	GMU3 Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.181	38	
	SEAK wolf	GMU1A Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.195	22	
	SEAK wolf	GMU1B Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.193	8	
	SEAK wolf	GMU1C Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.230	13	
	SEAK wolf	GMU1D Southeast Alaska	<i>Canis lupus nubilus</i> or <i>ligoni</i>	0.259	2	
	BCWolf	British Columbia Canada	<i>Canis lupus nubilus</i> or <i>occidentalis</i>	0.244	35	
	INTAK ^c wolf	Interior Alaska	<i>Canis lupus occidentalis</i>	0.239	40	
	WY ^c wolf	Wyoming	Transplanted <i>C. l. occidentalis</i>	0.276	25	
	ID ^c wolf	Idaho	Transplanted <i>C. l. occidentalis</i>	0.270	23	
	MT ^c wolf	Montana	Transplanted <i>C. l. occidentalis</i>	0.251	16	
	MN wolf	Minnesota	<i>Canis lupus hyaen</i> or <i>nubilus</i>	0.237	20	
	NM wolf	New Mexico	<i>Canis lupus baileyi</i>	0.172	8	
Coyote	AK Coyote	Alaska	<i>Canis latrans</i>	0.131	2	35
	CT Coyote	Connecticut	<i>Canis latrans</i>	0.372	1	
	AZ coyote	Arizona	<i>Canis latrans</i>	0.152	15	
	ME coyote	Maine	<i>Canis latrans</i>	0.281	4	
	WY Coyote	Wyoming	<i>Canis latrans</i>	0.212	6	
	NE coyote	Nebraska	<i>Canis latrans</i>	0.165	1	
	NV coyote	Nevada	<i>Canis latrans</i>	0.157	1	
	WI coyote	Wisconsin	<i>Canis latrans</i>	0.177	2	
	CO coyote	Colorado	<i>Canis latrans</i>	0.162	1	
	MS coyote	Mississippi	<i>Canis latrans</i>	0.228	2	
Dog	MtTx	Montana and Texas	<i>C. familiaris</i> Australian shepard, Border collie	0.334	2	91
	Mixed		<i>Canis familiaris</i> Mixed breed	0.351	36	
	Poodle		<i>Canis familiaris</i> Poodle	0.324	53	
Total						431

Cronin et al. 2014



Cronin et al. 2014

Table 2 Summary of F_{ST} values derived from 123,801 SNP genotypes among wolf, coyote, and dog populations with $N > 1$

Populations compared	F_{ST}	F_{ST}	F_{ST}
	Mean (SE) ^a	Range	95% CL ^b
Intraspecies			
Among groups of dogs	0.0843 (0.0350)	0.0145–0.1251	0.1509
Among coyote locations	0.1105 (0.0211)	0.0188–0.2927	0.0453
Among all wolf locations	0.1525 (0.0101)	0.0124–0.3903	0.0202
Comparisons of southeast Alaska wolves			
Among all 6 Southeast AK locations	0.1268 (0.0184)	0.0344–0.2811	0.0394
Among 5 Southeast AK locations excluding GMU2	0.1147 (0.0225)	0.0344–0.2463	0.0508
GMU2 versus 5 other Southeast AK locations	0.1511 (0.0326)	0.1139–0.2811	0.0904
Southeast AK versus BC wolves	0.1195 (0.0215)	0.0607–0.1849	0.0552
Southeast AK versus MN wolves	0.2012 (0.0206)	0.1436–0.2638	0.0529
Southeast AK versus northern wolves (interior AK, ID, MT, WY)	0.1501 (0.0122)	0.0430–0.2441	0.0252
Southeast AK versus NM wolves	0.3448 (0.0158)	0.2810–0.3903	0.0407
Comparisons of North American populations			
Among northern wolves (interior AK, ID, MT, WY)	0.0451 (0.0109)	0.0124–0.0784	0.0279
MN wolves versus northern wolves (interior AK, ID, MT, WY)	0.1154 (0.0068)	0.0981–0.1271	0.0217
MN wolves versus NM wolves	0.2527 (N/A)	N/A	N/A
Northern wolves (interior AK, ID, MT, WY) versus NM wolves	0.2330 (0.0102)	0.2065–0.2526	0.0325
Comparisons with BC^c			
BC wolves versus NM wolves	0.2219 (N/A)	N/A	N/A
BC wolves versus northern wolves (interior AK, ID, MT, WY)	0.0390 (0.0065)	0.0240–0.0535	0.0208
BC wolves versus Northern Rockies wolves (ID, MT, WY)	0.0342 (0.0062)	0.0240–0.0454	0.0267
BC wolves versus MN wolves	0.1031 (N/A)	N/A	N/A
Interspecies			
Dog versus coyote	0.3198 (0.0185)	0.2061–0.5222	0.0391
Dog versus wolf	0.3379 (0.0113)	0.2228–0.5153	0.0228
Wolf versus coyote	0.2790 (0.0111)	0.0817–0.4480	0.0221

Cronin et al. 2014

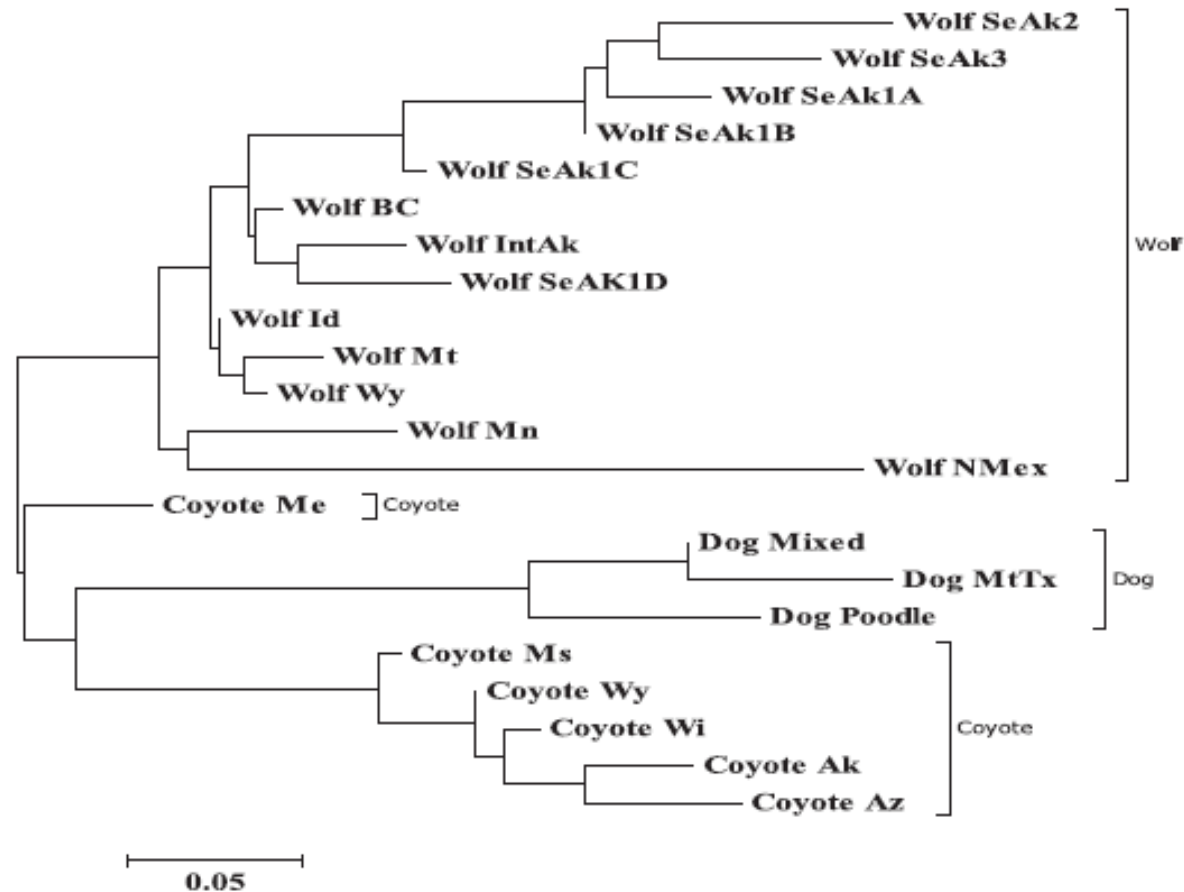
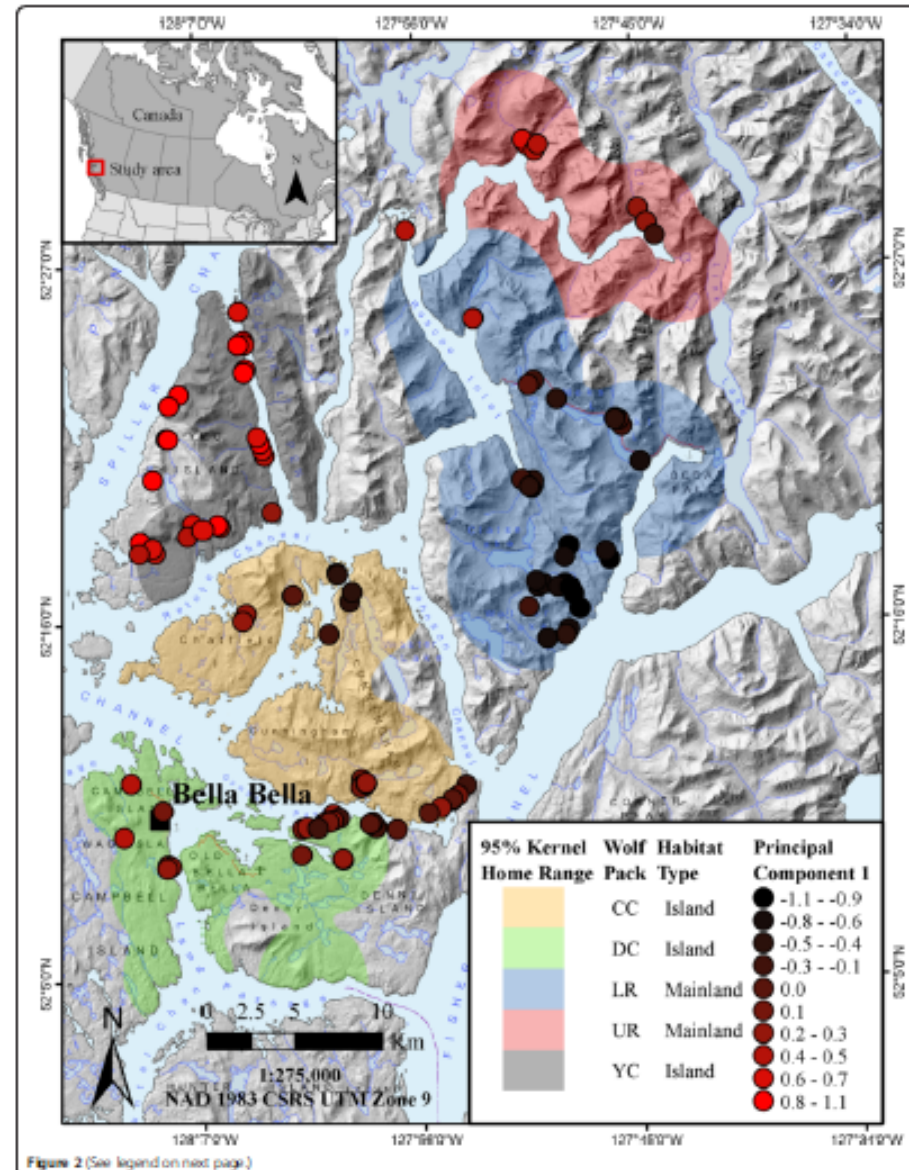


Figure 4. Neighbor-Joining tree of pairwise F_{st} values derived from 123 801 SNP genotypes among wolf, coyote, and dog populations identified in [Table 1](#) with $N > 1$.

Stronen et al. 2014



Stronen et al. 2014

Table 1 Genetic diversity measures for wolves (*Canis lupus*) from the central coast of British Columbia, Canada

Locus	#AllelesMA/IS	H _o MA	H _e MA	P-value + S.E. MA	F _{IS} MA	H _o IS	H _e IS	P-value + S.E. IS	F _{IS} IS
FH2054	6/8	0.465	0.595	0.016 + (0.0035)	0.220	0.456	0.720	0.000 + (0.0000)	0.368
FH2001	6/10	0.432	0.715	0.000 + (0.0000)	0.399	0.789	0.811	0.022 + (0.0069)	0.028
FH2096	4/3	0.318	0.334	0.171 + (0.0081)	0.047	0.592	0.502	0.276 + (0.0054)	-0.180
FH2010	3/6	0.211	0.319	0.023 + (0.0018)	0.342	0.355	0.538	0.000 + (0.0004)	0.342
FH2088	5/5	0.614	0.694	0.106 + (0.0050)	0.116	0.761	0.726	0.071 + (0.0054)	-0.049
FH2422	8/7	0.561	0.828	0.000 + (0.0000)	0.325	0.574	0.734	0.000 + (0.0001)	0.220
PEZ06	9/9	0.421	0.811	0.000 + (0.0000)	0.484	0.409	0.831	0.000 + (0.0000)	0.510
PEZ19	3/3	0.371	0.550	0.028 + (0.0021)	0.328	0.362	0.585	0.003 + (0.0005)	0.384
PEZ 15	8/12	0.605	0.796	0.000 + (0.0000)	0.243	0.452	0.755	0.000 + (0.0000)	0.403
FH3725	6/5	0.636	0.683	0.000 + (0.0001)	0.068	0.647	0.702	0.000 + (0.0002)	0.079
MEAN	5.8/6.8	0.463	0.632		0.264	0.539	0.690		0.211

Genetic variation across 10 microsatellite loci for individuals from mainland (MA, n = 44) and island (IS, n = 72) areas.

H_e values are calculated with correction for uneven samples sizes (Nei [32]).

*Bold font indicates significant *p*-values.

Stronen et al. 2014

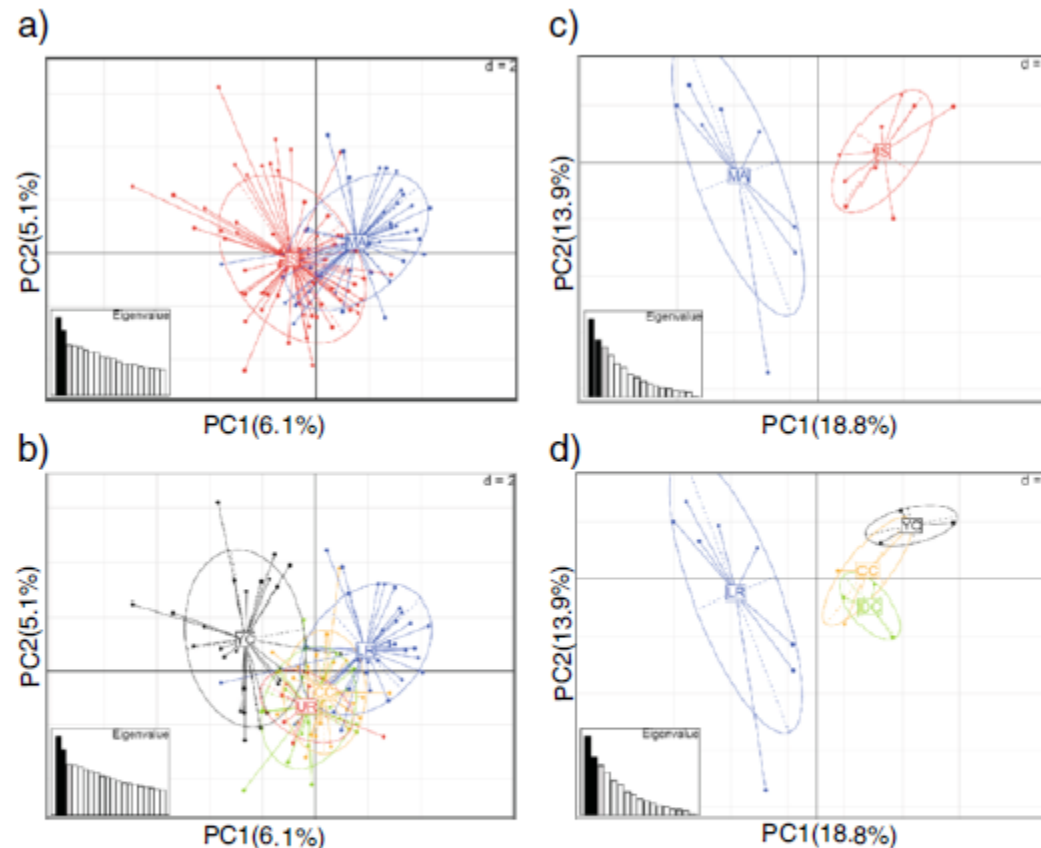


Figure 1 Principal component analyses of wolves (*Canis lupus*) from the central coast of British Columbia, Canada showing geographic distribution of individuals. **a)** Individual ($n = 116$) profiles based on ≥ 10 microsatellite loci labelled according to mainland (MA) and island (IS) sample locations. **b)** Individual profiles ($n = 116$) based on ≥ 10 microsatellite loci labeled according to membership in five wolf family groups: Upper Roscoe (UR) and Lower Roscoe (LR) on the mainland, and Yeo-Coldwell (YC), Cunningham-Chatfield (CC), and Denny-Campbell (DC) islands. Note that the label for DC (green colour) is overlapped by UR (red colour). **c)** A subsample of individual profiles ($n = 18$) with duplicated genotypes based on ≥ 5 loci labelled according to mainland and island sample locations. **d)** Individual profiles ($n = 18$) with duplicated genotypes based on ≥ 5 loci labelled according to membership in four wolf family groups LR, YC, CC, and DC (none from UR).