Genomic and morphological analysis of an American crow hybrid zone

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Abstract

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The Northwestern Crow (*Corvus caurinus*) and American Crow (*Corvus brachyrhynchos*) are continuously distributed sister taxa that lack reliable traditional characters for identification near their range boundary. We used genomic SNPs (nuDNA) and mtDNA to investigate the degree of genetic differentiation and hybridization between these crows. Our results indicate that American and Northwestern crows have distinct evolutionary histories, supported by two nuDNA ancestry clusters and two 1.1%-divergent mtDNA clades dating to the late Pleistocene, when glacial advances may have isolated crow populations in separate refugia. We document extensive current hybridization, with geographic overlap of mtDNA clades and admixture of nuDNA across >900 km of western Washington and western British Columbia. This broad hybrid zone consists of late-generation hybrids and backcrosses, but not recent (e.g., F1) hybrids. Nuclear DNA and mtDNA clines had concordant widths and were both centered in southwestern British

Columbia, farther north than previously postulated. Overall, our results suggest a history of reticulate evolution in American and Northwestern crows.

The two major hypotheses that have been put forward to explain the origin of this broad hybrid zone differ in both mechanism and timing. The first hypothesis is that post-glacial expansion allowed crows to expand from formerly isolated Pleistocene refugia into newly available habitats, leading to lineage fusion. The second is that the hybrid zone is a more recent artifact of anthropogenic habitat changes, resulting from the relatively recent forest fragmentation and land use changes associated with European colonization. Our objective was to differentiate between these two hypotheses by sequencing mtDNA from >160 years of museum specimens and assessing the timing of secondary contact within the hybrid zone. Sequencing a 90-bp fragment of mtDNA ND2 diagnostic for American and Northwestern haplogroups, we detected breeding season co-occurrence between American and Northwestern haplogroups at three different localities in 1889-1892, prior to the bulk of European-associated land use changes. We also detected geographic overlap of haplogroups in the southern Puget Sound of Washington in the 1850s, but not all of these individuals were sampled during the breeding season. We did not detect significant changes in haplogroup frequency within localities over time. Overall, our results are most consistent with the hybrid zone resulting from non-anthropogenic habitat changes since the last glacial maximum, or from anthropogenic influences of Native American/First Nations peoples prior to the late 19th century. Although our power to detect mtDNA haplogroup overlap and changes in haplogroup frequency over time was limited by sample size, using historical museum specimens is a direct and promising way of assessing changes in secondary contact over time.

The broad, sigmoidal molecular cline across the hybrid zone is consistent with a general pattern of decreasing body size along the Pacific coast reported by an earlier morphological study. However, the data in the morphological study were not presented in a way that facilitates a quantitative comparison with the shape of the molecular cline. In this study, we reassessed morphological variation in American/Northwestern crows along the Pacific coast and across North America, and compared morphological and molecular variation along the Pacific coast using modern cline methods. The cline for body size was centered near Seattle, 355 km and 158 km south of the clines for mtDNA and nuDNA, respectively. The ± 2 LL intervals for cline width overlapped between body size and both the mtDNA and nuclear DNA clines. The smallest crows in North America occur in Alaska, coastal British Columbia, coastal Washington, and coastal Oregon, respectively, mirroring the American/Northwestern crow hybrid cline based on molecular data. The largest crows are resident in Florida. Overall, body size variation in the American/Northwestern crow complex provides a counterexample to Bergmann's rule, which states that animals have larger body size at higher latitudes and/or in regions with cooler climates. One alternative explanation for geographic variation in body size of American crows is ecological character displacement as a consequence of competition with other co-occurring *Corvus* species. The smaller and gregarious Fish Crow reaches its peak abundance in Florida, where American Crows are raven-like, achieving their largest body size and tending to be more solitary. Conversely, the larger Common Raven is abundant in Alaska, where it co-occurs coastally with the smallest American (Northwestern) crows.

Table of Contents

Chapter One

Cryptic and extensive hybridization between ancient lineages of American crows

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ABSTRACT

Most species and therefore most hybrid zones have historically been defined using phenotypic characters. However, both speciation and hybridization can occur with negligible morphological differentiation. Recently developed genomic tools provide the means to better understand cryptic speciation and hybridization. The Northwestern Crow (*Corvus caurinus*) and American Crow (*Corvus brachyrhynchos*) are continuously distributed sister taxa that lack reliable traditional characters for identification. In this first population genomic study of Northwestern and American crows, we use genomic SNPs (nuDNA) and mtDNA to investigate the degree of genetic differentiation between these crows and the extent to which they may hybridize. Our results indicate that American and Northwestern crows have distinct evolutionary histories, supported by two nuDNA ancestry clusters and two 1.1%-divergent mtDNA clades dating to the late Pleistocene, when glacial advances may have isolated crow populations in separate refugia. We document extensive hybridization, with geographic overlap of mtDNA clades and admixture of nuDNA across >900 km of western Washington and western British Columbia. This broad hybrid zone consists of late-generation hybrids and backcrosses, but not recent (e.g., F1) hybrids. Nuclear DNA and mtDNA clines had concordant widths and were both centered in southwestern British Columbia, farther north than previously postulated. Overall, our results suggest a history of reticulate evolution in American and Northwestern crows, perhaps due to recurring neutral expansion(s) from Pleistocene glacial refugia followed by lineage fusion(s). However, we do not rule out a contributing role for more recent potential drivers of hybridization, such as expansion into human-modified habitats.

INTRODUCTION

Phenotypic characters have historically been a primary basis for distinguishing between species (Bickford et al. 2007), but the increasing availability of DNA evidence has made clear that speciation is not always accompanied by morphological change (Fišer et al. 2018). Genomic analyses of morphologically conserved groups have uncovered cryptic species across the tree of life (e.g., Pfenninger and Schwenk 2007, Satler et al. 2013, Hotaling et al. 2016, Larsen et al. 2017), yet not all morphologically diagnosed species show genomic differences (e.g., Mason and Taylor 2015).

Genomic tools provide ways to understand the population genetic structure and evolutionary history of cryptic species. Examples include identifying geographic clusters of genetically homogenous individuals, identifying independent evolutionary lineages, establishing phylogenetic relatedness of lineages, and estimating divergence dates (Struck et al. 2018). In geographic areas where genetically differentiated individuals co-occur, the presence or absence of genomic admixture indicates either hybridization or reproductive isolation (e.g., Scordato et al. 2017, Pulido-Santacruz et al. 2018, Linck et al. 2019).

Where species interbreed extensively, hybrid zones offer natural laboratories in which to investigate the speciation continuum (Harrison 1993, de Queiroz 1998). This is just as true for cryptic hybrid zones, because genomic inquiry is productive regardless of morphological differences between parental species. Geographic-genomic patterns at cryptic hybrid zones can illuminate speciation processes like the influence of differing levels of gene flow and selection, and the fusion of lineages that can occur when previously allopatric populations experience secondary contact. A narrow cline may suggest a pronounced selection gradient across a hybrid

zone, perhaps on morphologically cryptic but biologically important characters, whereas a broad cline is more consistent with neutral processes (Mallet et al. 1990). Comparing widths of mtDNA and nuDNA clines can elucidate additional speciation mechanisms, such as the presence of sex-biased asymmetries in dispersal or the effects of Haldane's rule (Toews and Brelsford 2012). The relative age of a hybrid zone can also be inferred, because the heterozygosity of admixed individuals decreases with each generation of backcrossing (Milne and Abbott 2008, Bouchemousse et al. 2016). In addition, the presence of pronounced peaks of differentiation across the genome may indicate elevated selection within certain genomic regions, possibly related to the evolution of morphologically cryptic reproductive isolating mechanisms. Fewer peaks, on the other hand, may suggest a predominant role for neutral processes like genetic drift (Irwin et al. 2018).

However, because most species have historically been diagnosed morphologically, most hybrid zone studies to date have likewise focused on morphologically distinct parental species (Barton and Hewitt 1989). By comparison, much less is known about what is happening in cryptic hybrid zones, and research on this topic has begun to appear only recently (e.g., Pfenninger and Nowak 2008, Herrera-Aguilar et al. 2009, Patel et al. 2015, Quilodrán et al. 2018, Pulido-Santacruz et al. 2018). These few studies have already documented a variety of evolutionary patterns and processes operating in the absence of morphological differences, including cryptic reticulate evolution (Kearns et al. 2018). However, additional genomic studies of cryptic hybrid zones are needed to better synthesize how evolutionary processes may differ at hybrid zones with and without substantial morphological differentiation. For example, because the hybrid zone literature has mostly focused on morphologically distinct parentals, it may be

underestimating the importance of non-morphological characters in the evolution of reproductive isolation.

The Northwestern Crow (*Corvus caurinus*) and American Crow (*Corvus brachyrhynchos*) are candidates for cryptically hybridizing at their contact zone. These crows are sister taxa (Haring et al. 2012) that have long been considered separate species (Baird 1858). However, they are nearly identical morphologically, and collectively they have a continuous distribution along the Pacific coast of North America (Figure 1; Johnston 1961, Clements et al. 2017). The traditional, phenotypic characters for identifying these all-black corvids are based on putative differences in morphology, voice, and ecology, and have always been controversial (Johnston 1961, also see Discussion). Uncertainty in their identification has moreover led to uncertainty about the location of their range boundary and confusion regarding the nature and extent of a secondary contact zone. It has remained unclear whether, for example, there is assortative mating of discrete forms in sympatry (Brooks 1917, 1942) or clinal variation without diagnosable differences (Johnston 1961), and little new information has surfaced during the past half century. The only prior molecular study sequenced mtDNA from just a handful of American and putative Northwestern crows (Haring et al. 2012), and all three "Northwestern" Crow samples were from near the range boundary, leaving doubts as to whether these were Northwestern Crows, American Crows, or hybrids. After more than a century of uncertainty, genomic data now provides an opportunity to assess the geographic distribution of Northwestern and American crows and to determine the extent to which they mate assortatively, hybridize, or exhibit clinal variation.

In this study, our objective was to use genomic data and geographically robust sampling of American and Northwestern crows to better understand the evolutionary history of these

presumptive species that lack well-defined phenotypic characters. Specifically, we set out to 1) assess whether Northwestern and American crows represent independently evolving evolutionary lineages, and, if so, 2) determine the extent to which they might hybridize or exhibit reproductive isolation, 3) test the role of specific geographic barriers in potentially structuring gene flow, and 4) better understand their evolutionary and biogeographic history.

MATERIALS AND METHODS

Sample collection and DNA extraction

To conduct a population genetic survey of American Crows and Northwestern Crows near their range boundary and across North America, we sampled frozen tissue (n=218), blood (Alaska; n=35), or feather material (Idaho; n=6) from crows identified *a priori* as either species (Table S1). We also included two Carrion Crow (*Corvus corone*) tissue samples as outgroups (Haring et al. 2012). Tissue samples were obtained from natural history museums and were generally associated with vouchered specimens (Table S1). Blood samples were obtained under permits and approvals from the US Fish and Wildlife Service, the Alaska Department of Fish and Game, and the Institutional Animal Care and Use Committees at the University of Alaska Fairbanks and the US Geological Survey Alaska Science Center. Feather samples were collected under permits from the US Fish and Wildlife Service and the Idaho Department of Fish and Game, following recommended protocols in the Guidelines to the Use of Wild Birds in Research (Gaunt et al. 1997). We extracted total genomic DNA with a DNeasy tissue extraction kit (Qiagen, Valencia, CA) following manufacturer protocol.

Mitochondrial DNA (mtDNA) sequencing

To survey a large sample of crows across the putative contact zone and throughout North America and to conduct divergence dating, we amplified 1,041 base pairs (bp) of mtDNA NADH dehydrogenase subunit 2 (ND2) from 259 individuals (Table S1). We used primers L5215 (Hackett 1996) and TrC (Miller et al. 2007) and 12.5 μL PCR reactions on a T100 thermal cycler (Bio-Rad, Hercules, CA) as follows: 94°C for 2.5 min, 35 cycles of (94°C for 30 s, 54°C annealing for 30 s, 72°C for 1 min), 10 min at 72°C, 10°C hold. We sent PCR products to the High-Throughput Genomics Unit at the University of Washington for cleanup and sequencing. We unambiguously aligned complementary strands with Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI) and downloaded a Carrion Crow ND2 sequence from GenBank as an outgroup (Table S1).

mtDNA phylogeny, divergence dating, and haplotype network

We compared models of ND2 sequence evolution using jModeltest 2.1.4 (Posada 2008) and conducted ND2 divergence dating in BEAST 1.8.4 (Suchard et al. 2018) using the HKY+G model, an uncorrelated relaxed clock with lognormal distribution, and a constant size coalescent tree prior. We fixed the uncorrelated relaxed clock mean parameter *ucld.mean* to 0.0145, half the ND2 rate of 2.9×10^{-2} substitutions site^{-1} My^{-1} derived from Hawaiian honeycreepers based on sequential uplift dates of the Hawaiian island chain (Lerner et al. 2011). We ran a chain length of 50 million, saving 10,000 posterior trees. We used Tracer 1.6.0 to verify convergence (ESS scores > 200) and generated a maximum clade credibility tree with TreeAnnotator 1.8.4 after discarding the first 2,500 trees as burn-in.

To verify the ND2 tree topology across phylogenetic methods, we also inferred a maximum likelihood tree in RAxML 8.0.2 (Stamatakis 2014) using the GTRGAMMA model with 500 rapid bootstrap replicates and an SVDquartets tree (Chifman and Kubatko 2014) in PAUP* 4.0a165 (Swofford 2002), sampling 100,000 quartets with a shared tree model and 100 bootstrap replicates. We constructed a median-joining ND2 haplotype network (Bandelt et al. 1999) in PopArt 1.7 (Leigh and Bryant 2015) and calculated mean pairwise distance between haplotype groups in R (R Core Team 2018) using *ape* 3.5 (Paradis et al. 2004). We omitted one sample (*gws4003_Pierce_Co*) from the haplotype network and pairwise divergence calculations because only a partial ND2 sequence was obtained.

Nuclear DNA (nuDNA) SNP library preparation

To assess whether Northwestern and American crows show distinct evolutionary histories in the nuclear genome and to determine the extent to which these crows hybridize, we generated reduced representation double digest restriction-associated DNA (ddRAD) SNP libraries. We generated these genomic SNP libraries from a subset of 62 American/Northwestern Crow individuals for which we had also sequenced mtDNA ND2, and two Carrion Crows as an outgroup (Table S1). To maximize our power to detect potentially distinct, sympatric lineages, we used mtDNA results as a guide for selecting individuals for nuDNA analysis. At localities containing both of the major mtDNA ND2 haplogroups in our broad population genetic survey, we selected individuals for ddRAD sampling to reflect the approximate overall ratio of mtDNA haplogroups at each locality. We followed the ddRAD sequencing protocol of Peterson et al. (2012) after verifying high molecular weight DNA on a gel. We digested 350-500 ng of DNA with SbfI-HF and MspI restriction endonucleases (New England BioLabs, Ipswich, MA), pooled

sets of 8 samples, and size-selected 415-515 bp DNA fragments on a BluePippin machine (Sage Science, Beverly, MA). We multiplexed 96 avian ddRAD libraries from this study and another study, obtaining two runs of single-end 50-bp reads from Illumina HiSeq 2500 at the Computational Genomics Research Laboratory at the University of California (Berkeley, CA).

nuDNA sequence assembly

We used *process_radtags* in STACKS 1.42 (Catchen et al. 2013) with default settings to demultiplex reads, discard low-quality reads, discard reads with an uncalled base, rescue barcodes, and rescue SbfI RAD tags. To align reads to an American Crow reference genome (Zhang et al. 2014), we built a genome database using default settings for *gmap_build* in GMAP (Wu and Watanabe 2005). We aligned reads to the reference genome with GSNAP version 2016-09-23 (Wu and Nacu 2010), specifying ≥ 90% coverage, ≤ 3 mismatches, and default settings for other parameters. We converted alignments to BAM format with SAMtools (Li et al. 2009) and created loci and called SNPs from the aligned reads with *ref_map.pl* in STACKS 1.42 (Catchen et al. 2013). We constructed a 64-crow alignment including the two Carrion Crow outgroup samples and a 62-crow alignment containing only American and Northwestern crows. For each dataset, we retained stacks with a depth of ≥ 3 identical reads, loci with a coverage of ≥ 4 samples, and bi-allelic SNPs with a minor allele count ≥ 2 and heterozygosity ≤ 0.5. We output Structure files for downstream analysis. We used a custom R script to generate 64-crow and 62-crow alignments of unlinked SNPs retaining one random SNP per locus, a 62-crow alignment without missing data, and 48-crow alignments of Pacific coastal birds with and without missing data.

Mapping nuDNA loci to chromosomes

To infer chromosomal positions for our reference-aligned ddRAD loci from STACKS, we aligned the American Crow reference genome with the chromosome-annotated, repeat-masked genome of the Zebra Finch (*Taeniopygia guttata*; *http://hgdownload.cse.ucsc.edu/goldenPath/taeGut2/bigZips/taeGut2.fa.masked.gz*). This approach takes advantage of the high degree of synteny in birds at the chromosomal level (Ellegren 2010). We aligned the American Crow and Zebra Finch genomes with nucmer in MUMmer 3.23 (Kurtz et al. 2004), customizing a shell script by C.J. Battey (https://github.com/cjbattey/truseq_assembly). After excluding aligned regions < 1000 bp in length, we assigned an American Crow scaffold to a chromosome when >75% of all >1000 bp alignment regions in that scaffold mapped to the same Zebra Finch chromosome.

nuDNA cluster analyses

We used two independent methods to determine K, the number of nuDNA ancestry clusters. First, we used the K-means clustering algorithm implemented in the *find.clusters* function in *adegenet* 2.0.1 (Jombart et al. 2010) to calculate the Bayesian information criterion (BIC) for K=1 through K=10. We used the 62-sample dataset with no missing data after transforming by principal components analysis (PCA), retaining all principal components.

Second, we also used the Bayesian admixture model with correlated allele frequencies implemented in Structure 2.3.4 (Pritchard et al. 2000) to conduct 5 replicate runs each for K=1 to K=4 with 200,000 generations and a burn-in of 20,000. We estimated the optimal number of clusters in the unlinked 62-sample dataset with missing data by analyzing the rate of change in the likelihood distribution between successive K values in Structure Harvester 0.6.94 (Evanno et

al. 2005, Earl and vonHoldt 2012, Leaché et al. 2017). We combined results from replicate runs while accounting for permutations and label switching using CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007).

To test for a potential effect of Z chromosome copy number on nuDNA ancestry inference, we conducted a Welch's two-sample t-test comparing K=2 Structure ancestry proportions for males and females. To test for potential effects of different numbers of loci and different amounts of missing data in the alignments used for *adegenet* PCA and Structure, we plotted K=2 ancestry proportions from Structure/CLUMPP against transformed genomic PC1 values from *adegenet* for the 48 Pacific coastal samples, and conducted a simple linear regression. We applied a custom linear transformation to genomic PC1 values for this analysis such that our southernmost and northernmost localities had population means of 0 and 1, respectively.

Isolation by distance

We tested for isolation by distance in the 62-sample continent-wide dataset and in the 48-sample Pacific coastal dataset (both without missing data) by conducting Mantel tests (Mantel 1967) in *ade4* (Dray and Dufour 2007) with 1,000 Monte-Carlo permutations. We used Euclidean geographic distance and calculated Edwards' genetic distance (Edwards 1971) in *adegenet* (Jombart 2008).

Recent-generation vs. late-generation hybrids

We compared genomic hybrid indices to inter-taxon heterozygosities to determine whether individual crows were recent-generation hybrids or descendants of long-admixed

populations (Milne and Abbott 2008, Bouchemousse et al. 2016). We designated crows as parental American or parental Northwestern if these respective ancestry proportions exceeded 0.98 in our combined K=2 Bayesian clustering runs (Scordato et al. 2017). We used a custom R script to subset the 62-sample SNP alignment containing missing data to include only parental individuals and only variable, unlinked SNPs present for ≥75% of individuals. With this alignment we calculated genome-wide and SNP-specific F_{ST} between parental populations with R package Hierfstat (Goudet 2005). We considered SNPs with $F_{ST} > 0.6$ to be ancestry-informative (Scordato et al. 2017) and further limited our alignment to these SNPs using a custom R script. For each individual crow, we calculated a maximum likelihood estimate of the genomic hybrid index and the average inter-taxon heterozygosity across ancestry-informative loci using R package Introgress (Gompert and Buerkle 2010). F1 hybrids have an expected hybrid index of 0.5 and expected heterozygosity of 1.0 for loci fixed in parental individuals. Heterozygosity is reduced in later-generation hybrids and backcrosses. We considered crows with hybrid index >0.25 and <0.75 and heterozygosity >0.5 to be recent-generation hybrids, individuals with hybrid index >0.25 and <0.75 but with heterozygosity <0.5 to be later-generation hybrids, and birds with hybrid index <0.25 or >0.75 to be backcrosses (Milne and Abbott 2008, Larson et al. 2014, Scordato et al. 2017, Toews et al. 2018).

Fitting and comparing clines for mtDNA, overall nuDNA, and individual SNPs

We fit cline models for mtDNA and overall nuDNA along the Pacific Coast using R package *hzar* (Derryberry et al. 2014). We assigned transect distances to each Pacific coastal population using a smoothed curve drawn parallel to the Pacific coastline in QGIS 2.8.3 (QGIS Development Team 2015). For mtDNA, we fit a cline model for population frequencies of

American mtDNA (range 0 to 1) using *hzar.doMolecularData1DPops* and *hzar.makeCline1DFreq*. For overall nuDNA, we fit a cline model to means and variances of population ancestry proportions (*q* values) from combined K=2 Bayesian clustering runs using *hzar.doNormalData1DPops* and *hzar.makeCline1DNormal* (Leaché et al. 2017, Scordato et al. 2017, Linck et al. 2019). For both mtDNA and overall nuDNA, we fit models without exponential tails, restricted parameter search space to a liberal yet reasonable range of values (cline center >500 km and <3500 km, cline width <3500 km; Derryberry et al. 2014) and fixed means, variances, and allele frequencies at cline ends to the observed values of the terminal populations (Linck et al. 2019, Lipshutz et al. 2019). We ran the Markov chain Monte Carlo (MCMC) optimizer for 3 iterative cycles using *hzar.chain.doSeq*, retaining the third run for subsequent analysis (Derryberry et al. 2014). We used *hzar.get.ML.cline* and *hzar.getLLCutParam* to obtain maximum likelihood estimates for widths, centers, and ± 2 log likelihood (LL) intervals. We used these intervals to test for coincidence of centers and concordance of widths (Derryberry et al. 2014, Lipshutz et al. 2019).

We also used *hzar* to fit frequency-based clines to 905 individual genomic SNPs with no missing data across the 48 Pacific coastal samples. We fit clines with free endpoint scaling and no exponential tails while reasonably restricting parameter search space (cline center > 0 km and < 4000 km; cline width < 4000 km; Derryberry et al. 2014). We conservatively identified the SNPs with the best-supported clines ($\Delta AIC_c \geq 6$ between the cline model and the null model) and used a chi-squared test to assess whether the chromosomal distribution of these SNPs differed from the chromosomal distribution of all 905 SNPs examined.

Assessing heterogeneity of admixture near potential barriers to and corridors for gene flow

To provide an *ad hoc* assessment of potential differences in gene flow and admixture between Vancouver Island crows and those across the water barrier on the adjacent mainland, we compared nuDNA ancestry proportions of individuals from Vancouver Island (*vic* + *nvi* localities, n=8) to those of nearby mainland populations (*yvr* + *cbc* localities, n=8). We compared Northwestern nuDNA ancestry from the combined K=2 Structure runs using a Student's t-test with a two-tailed hypothesis and pooled variances. We also compared ND2 haplogroup proportions of crows between Vancouver Island the adjacent mainland using a Pearson's chi-squared test with 2,000 Monte Carlo replicates (n=35 for *nvi* + *vic*; n=34 for *cbc* + *yvr*).

To assess the Skeena River valley of British Columbia as a potential corridor for gene flow across the Coast Mountains, we conducted an *ad hoc* comparison of the within-population variance in nuDNA K=2 ancestry proportions between two localities nearest to the Skeena River and all other localities. We fitted two nested mixed effects models in *nlme* (Pinheiro et al. 2017) and compared them with a likelihood ratio test. Both models included the Northwestern nuDNA ancestry proportion as the response variable, membership in *nbc* or *cbc* (the localities nearest to the Skeena River) as a fixed effect, and locality as a random effect. The more complex model incorporated a variance function parameter allowing the Skeena River localities to take on a common within-population variance estimate that differed from a within-population variance estimate common to all other populations.

RESULTS

Sequence alignments

nuDNA. We obtained 181,580,191 raw sequencing reads across 64 samples in 8 pools, retaining 150,638,152 reads (83.0%) after discarding reads with ambiguous barcodes, ambiguous RAD tags, or low quality scores. The Bayesian admixture model readily distinguished the two Carrion Crow samples from the 62 American and Northwestern crows, and we excluded these Carrion Crows from subsequent analyses. The 62-sample alignment contained 7,292 loci with 9,563 SNPs before random subsampling to 7,292 unlinked SNPs. The 62-sample alignment with no missing data contained 738 unlinked SNPs, and the 48-sample alignment of Pacific coastal samples with no missing data contained 905 unlinked SNPs.

We conservatively mapped 89% (6,494/7,292) of ddRAD loci in the 62-sample crow alignment to Zebra Finch chromosomes, revealing that our SNPs were widely dispersed across the genome and relatively concentrated on small, GC-rich chromosomes (Figure S2, Figure S3). Of the 6,494 mapped loci, 206 (3.2%) mapped to the AT-rich Z chromosome, which comprises 5.9% of the Zebra Finch genome (https://www.ncbi.nlm.nih.gov/genome/367).

mtDNA. We obtained 259 mtDNA ND2 sequences, including 258 full-length ND2 sequences (1041 bp) and one partial sequence identifiable to haplogroup.

American and Northwestern crows have distinct evolutionary histories

Genomic SNPs and mtDNA sequences both revealed distinct evolutionary histories for Northwestern and American crows. Two different clustering methods both supported two nuDNA ancestry clusters corresponding to Northwestern and American crows (Figure 1). First,

the K-means algorithm in *adegenet* minimized the BIC at K=2 (256.85 for K=1, 255.12 for K=2, and ≥255.81 for 3≤K≤10; Figure S4). Second, the Evanno method also selected K=2 as the best-fit model for the number of nuDNA ancestry clusters (Table S2). Combined results from replicate K=2 Bayesian clustering runs in Structure are presented in Figure 1. There was no effect of sex on K=2 Structure ancestry proportions (t=0.46, p=0.64). Structure analysis of the 7,292-SNP alignment with missing data and PCA analysis of the 905-SNP alignment without missing data yielded the same genomic signal despite different numbers of loci, different amounts of missing data, and different underlying analyses ($R^2 = 0.95$ and $p < 10^{-15}$ for regression of Structure K=2 ancestry vs. genomic PC1, Figure S5).

We recovered two major lineages of mtDNA ND2 with an estimated divergence time of ~443,000 years ago (95% HPD interval 268-649 kya; Figure S6, Figure S7). We hereafter refer to these ND2 lineages as Northwestern (n=95) and American (n=164) haplogroups. The Northwestern and American haplogroups were separated by 3 fixed differences and a mean uncorrected pairwise distance of 1.10% (range 0.58% to 1.63%). Bayesian posterior probability, maximum likelihood bootstrap, and quartets bootstrap values were 100%, 89%, and 74% for the American haplogroup clade and 77%, 66%, and 82% for the Northwestern haplogroup clade (Figure S8, Figure S9, Figure S10).

American and Northwestern Crow mtDNA haplogroups overlap geographically

Overall, population frequencies of the Northwestern mtDNA haplogroup increased with latitude west of the Cascades and Coast Ranges. All Alaskan crows had the Northwestern mtDNA haplogroup, and all crows from California, east of the Cascades of Oregon and Washington, and east of the Coast Mountains of British Columbia had the American mtDNA

haplogroup (Figure 1). Individual crows with American and Northwestern mtDNA haplogroups co-occurred within a >900 km overlap zone on the Pacific slope of Washington and British Columbia. We found no additional geographic-genetic structuring within the Northwestern or American mtDNA haplogroups.

American and Northwestern crows hybridize extensively

Of the crows for which we sampled nuDNA, all individuals from Alaska had pure (>98%) Northwestern ancestry, and all from California and east of the Cascades/Coast Mountains had pure American ancestry (Figure 1). Hybrid crows with nuDNA ancestry >2% and <98% occupied a Pacific coastline distance of >900 km and included all of the crows that we sampled from coastal Washington to coastal British Columbia at the approximate latitude of northern Haida Gwaii (locality *nbc*). Among four crows at the northern limit of the hybrid zone, two had pure Northwestern ancestry, one hybrid had 82% Northwestern ancestry, and another hybrid had 81% American ancestry.

The first two genomic PCA axes explained 6.8% and 3.3% of nuDNA variation, respectively (Figure S11). The first axis closely approximated the Pacific coastal hybrid cline, separating crows from California and east of the Cascades from crows northwest of the Cascades in Washington, British Columbia, and Alaska. The second axis separated American Crows of eastern and western North America and also provided additional resolution of crows along the Pacific coastal hybrid cline. Isolation by distance was evident for continent-wide sampling (Figure S12; $n=62$, $p < 0.001$) and within the Pacific coastal samples alone (Figure S13; $n=48$, $p < 0.0005$).

The hybrid zone consists of late-generation hybrids and backcrosses

Among 62 Northwestern and American crows with nuDNA SNP data, 10 were parental Northwestern, 18 were parental American, and 34 were hybrids. Our alignment of the 28 parental crows contained 3,582 unlinked SNPs present in ≥75% of individuals, with a genome-wide F_{ST} of 0.13. Thirty-five SNPs were ancestry-informative ($F_{ST} > 0.6$) between parental American and parental Northwestern crows, including 2 SNPs with fixed differences ($F_{ST} = 1$). Two of these 35 ancestry-informative SNPs (5.7%) mapped to the Z chromosome, which did not significantly differ from the Z-chromosome proportion of all mapped loci (3.2%, $X^2 = 0.67$, $p = 0.63$).

All 34 hybrids we sampled had low inter-taxon heterozygosities at ancestry-informative SNPs given their respective hybrid indices, indicating that they were late-generation hybrids and backcrosses and not F1s or early-generation hybrids (Figure 2).

Nature and extent of mitonuclear concordance and discordance

Nuclear DNA ancestry was generally concordant with mtDNA haplogroup. Crows with pure (>98%) nuDNA ancestry never had a "mismatched" mtDNA haplogroup, but some mitonuclear discordance was evident (Figure 1). Individuals with the American mtDNA haplogroup (n=40) had up to 62% Northwestern nuDNA ancestry (K=2), and crows with the Northwestern mtDNA haplogroup (n=22) had up to 60% American nuDNA ancestry. At six localities, we sampled nuDNA from crows with both American and Northwestern mtDNA haplogroups. Five of these six localities contained only hybrids, and the sixth locality contained two hybrids and two pure Northwesterns.

Cline characteristics for mtDNA, overall nuDNA, and individual nuDNA SNPs

We generated Pacific coastal cline models using 218 mtDNA samples from 13 populations (range = 9-31 per population) and nuDNA ancestry from 48 individuals in 12 populations (4 per population). The mtDNA and overall nuDNA clines were both centered in southwestern British Columbia and had overlapping ± 2 LL intervals for cline width (Figure 3). The mtDNA cline was centered near the latitude of central Vancouver Island (2445 km ± 2 LL range 2340-2530 km) with a width of 825 km (± 2 LL range 609-1158 km). The nuDNA cline was centered near the latitude of Vancouver (2642 km ± 2 LL range 2585-2700 km) with a width of 918 km (± 2 LL range 560-1266 km).

Of the 905 individual SNPs for which we calculated Pacific coastal clines, 94 SNPs exhibited a "strong" clinal pattern ($\Delta AIC_c \geq 6$ between the cline model and the null model; Figure S14). Clines for these 94 individual SNPs had a median center of 2401 km (median ± 2 LL range 1913-2853 km) and a median width of 379 km (median ± 2 LL range 0.4 km to 2905 km). The 94 SNPs with "strong" clinal patterns did not differ in chromosomal distribution from the full set of 905 SNPs (Figure S15; $X^2 = 22.0$, $p = 0.88$).

Heterogeneous admixture near potential barriers to and corridors for gene flow

Overall, both mtDNA and nuDNA results indicated a trend of increasing Northwestern ancestry with latitude west of the Cascades and Coast Mountains (Figure 1). However, Vancouver Island crows had a higher frequency of the Northwestern ND2 haplogroup (74%) than crows on the nearby mainland (26%; $X^2=15.8$, $p<0.0005$). Crows on Vancouver Island also averaged 44% more Northwestern nuDNA ancestry than adjacent mainland crows (74% vs. 51%, $t=3.13$, $df=14$, $p<0.01$).

Crows within most localities were fairly homogeneous in their ancestry proportions, but localities closest to the Skeena River valley were a notable exception (Figure 1), where within-population ancestry variation was about 9 times higher at these localities than elsewhere (variance parameter = 9.02, likelihood ratio = 85.02, df=1, $p<10^{-19}$).

DISCUSSION

To better understand a potential case of cryptic speciation and hybridization, we conducted a population genomic study of American and Northwestern crows. We found both mtDNA and nuDNA evidence that these crows represent two historically divergent evolutionary lineages, but we also identified a >900 km-wide cryptic hybrid zone consisting of a late-generation hybrid swarm. Overall, our results suggest that this system represents a compelling example of reticulate evolution (Huson and Bryant 2006, Sessa et al. 2012).

Pleistocene divergence, historical biogeography, and gene flow

Climatic oscillations can have profound effects on the speciation process (Hewitt 2004). During the late Pleistocene, when American and Northwestern mtDNA are estimated to have diverged ~443,000 years ago, North America was undergoing extensive glacial advances and retreats at regular ~100,000-year Croll-Milankovich intervals (Muller and MacDonald 1997, Clark et al. 2009). Much of the Pacific Northwest was covered in ice sheets during the glacial periods, isolating terrestrial organisms south of the ice sheets or in ice-free northern refugia such as Beringia, Haida Gwaii, or the Alexander Archipelago (Galbreath and Cook 2004, Burg et al. 2006, Anderson et al. 2006, Godbout et al. 2008, Shafer et al. 2010, Geraldes et al. 2019). During

the interglacial periods, terrestrial organisms expanded from refugial populations into newly ice-free habitats, leading to secondary contact and potentially renewed gene flow between closely related, previously allopatric forms (Shafer et al. 2010). These repeated cycles of isolation and secondary contact created complex and/or reticulate population genetic histories in many of the region's terrestrial organisms (Hewitt 2004; e.g., Omland et al. 2000, Latch et al. 2009, Kearns et al. 2018).

Northwestern and American crows may have diverged following a similar pattern, with Northwestern Crow populations evolving in isolation in one or more of the ice-free northern refugia while American Crow populations remained south of the ice sheets. Today, Northwestern and American Crow mtDNA haplogroups overlap across most of coastal Washington and all of coastal British Columbia, consistent with post-glacial expansion of previously isolated populations into newly available habitat during one or more interglacial periods. Further sampling of crows on Haida Gwaii, the Alexander Archipelago, and other putative northern refugia might uncover additional genetic diversity within the Northwestern mtDNA haplogroup, which would corroborate the Pleistocene refugia hypothesis (e.g., Krosby and Rohwer 2009, Geraldes et al. 2019).

Mountain ranges can also represent significant barriers to gene flow. Our results show that most gene flow between American and Northwestern Crows has occurred on a north-south axis to the west of the Coast Mountains and Cascades. These 2-5 My-old ranges would have restricted east-west gene flow in crows during Pleistocene interglacial periods as they do today (Shafer et al. 2010). Even though geography and genetic data both suggest predominantly north/south gene flow on the Pacific slope of the Coast Mountains and Cascades, we note the potential for limited east-west gene flow across these ranges, especially at major drainages with

low passes such as the Fraser and Skeena River valleys. Indeed, the relatively high variation we found in nuDNA ancestry proportions near the Skeena River is consistent with more recent gene flow and backcrossing there compared to other localities. However, despite the higher variance in nuDNA ancestry proportions near the Skeena, the hybrid crows we sampled there all appeared to be late-generation hybrids and backcrosses, as found at all other localities sampled. The elevated Northwestern Crow ancestry in Vancouver Island hybrids compared to mainland birds also suggests that gene flow along the Pacific coastline may vary based on the geography of islands and water barriers.

Selection vs. neutral processes across the hybrid zone

The width of a hybrid zone depends in part on whether the hybrid zone is primarily structured by selection or by neutral processes (Mallet et al. 1990). The >900 km-wide hybrid zone in American/Northwestern crows is >7 times wider than typical avian hybrid zones in North America (130 ± 44 km, mean ± SD, n=8; Hoffman et al. 1978, Rohwer and Wood 1998, Ruegg 2008; Irwin et al. 2009, Mettler and Spellman 2009, Brelsford and Irwin 2009, Toews et al. 2011, Seneviratne et al. 2012), suggesting a prominent role for neutral processes at the scale of the whole genome.

Peaks of differentiation within the genome can also indicate a role for selection across a hybrid zone. At the chromosome level, we found no evidence that some chromosomes had more SNPs with pronounced clines than did other chromosomes, again consistent with neutral processes. However, we note that although we sampled SNPs from diverse regions of the genome, the total fraction of the genome we sampled was small. Our marker density was thus insufficient to rule out smaller "islands" of genomic divergence within chromosomes (e.g.,

Toews et al. 2018), and it remains possible that selection is important at isolated loci (e.g., Poelstra et al. 2014). Across a morphologically cryptic hybrid zone like American/Northwestern crows, loci under strong selection could be associated with biologically important but less visually salient traits, e.g., those related to physiology or cognition.

A very broad hybrid zone consisting of late-generation hybrids and backcrosses is consistent overall with a prolonged period of neutral expansion. However, we also cannot rule out more recent processes. These crows are human commensals that thrive in disturbed landscapes (Verbeek and Butler 1999, Verbeek and Caffrey 2002), and indigenous peoples have inhabited the land situated within this hybrid zone for millennia. More recently, European settlers and their descendants have heavily modified the landscape through deforestation, agriculture, and urbanization. One hypothesis posits that recent land use changes and associated increased habitat heterogeneity may have removed habitat barriers to dispersal that existed before the time of European settlement, increasing opportunities for hybridization between a more maritime Northwestern Crow and a more agrarian American Crow (Marzluff and Angell 2005, Haring et al. 2012). Under this scenario, more than a century of crow generations may have been sufficient genetic recombination to dilute highly heterozygous F1s and recent-generation hybrids out of the population.

Mitonuclear concordance and hybrid zone processes

Strong cline discordance between mtDNA and nuDNA can indicate that Haldane's rule (Haldane 1922, Coyne and Orr 1989, Devis et al. 1997, Dasmahapatra et al. 2002, Carling and Brumfield 2008, McCormack et al. 2011), sex-biased asymmetries (Toews and Brelsford 2012), and/or a selective gradient acting on mtDNA (Cheviron and Brumfield 2009) are important

processes in a hybrid zone. However, in the Pacific coastal hybrid zone between American/Northwestern crows, mtDNA and nuDNA clines had concordant widths and were both centered in southwestern British Columbia. This overall mitonuclear concordance, a pattern observed in ~82% of published studies (Toews and Brelsford 2012), suggests that the above three processes likely do not play major roles in structuring this particular hybrid zone.

Reproductive isolation and phenotypic characters

Extensive genomic admixture constitutes strong evidence that reproductive isolation is lacking. Indeed, our population genomic study clarifies that American/Northwestern crows are not reproductively isolated, a question that had remained unresolved in the ornithological literature for >160 years. In light of our results, past claims of two distinct crow species breeding assortatively in sympatry (Brooks 1917, 1942) appear to have been overly ambitious, seemingly arising from the misapplication of subjective identification criteria. Traditional phenotypic characters for distinguishing American and Northwestern crows have included size, ecology, and voice, but these were always controversial when subjected to scrutiny. In the hindsight of our genomic study showing extensive admixture, it is now easier to see why these characters were unreliable. Historically, Northwestern Crows were considered to be diagnostically smaller than American Crows (Baird 1858). In actuality, however, size variation in coastal crow populations is clinal, with northern birds averaging smaller, but with great overlap in measurements among individuals, especially near the range boundary (Rhoads 1893, Johnston 1961). Likewise, intertidal habitat use, once thought to be a distinguishing feature of Northwestern Crow (Baird 1858), might simply reflect adaptive responses to local food availability (Cooper 1870). Purported vocal differences (Baird 1858, Suckley and Cooper 1860, Brooks 1917, 1942;

Hellmayr 1934) do not seem to correlate with size (Rhoads 1893) or habitat (Johnston 1961) near the range boundary, and individual birds have been observed giving typical vocalizations of both taxa (Johnston 1961). Moreover, crows are oscine passerines that can learn vocalizations (Beecher and Brenowitz 2005), and individual crows can even change vocalizations when joining a new social group (Brown 1985).

The broad genomic hybrid zone we uncovered corroborates the work of some previous researchers who documented a continuous morphological cline in American/Northwestern crows along the Pacific Northwest coast (Rhoads 1893, Johnston 1961). Various authorities have been inconsistent regarding the southern range limit of Northwestern Crow, placing it anywhere from California (e.g., American Ornithologists' Union 1895) to Oregon (e.g., American Ornithologists' Union 1983) to Washington State (e.g., Ridgway 1904, Verbeek and Butler 1999, Verbeek and Caffrey 2002, Clements et al. 2017). These difficulties in identifying a discrete range boundary now make sense given the existence of a broad genomic cline. Notably in our study, however, both mtDNA and nuDNA analyses placed the center of the hybrid zone in southwestern British Columbia, farther north than previous hypotheses based on traditional phenotypic characters (e.g., American Ornithologists' Union 1998).

The lack of geographic-genetic structuring within American Crow mtDNA (Figure S1, Figures S6-S10) was somewhat surprising given that American Crows are widespread and morphologically variable across their North American distribution (Ridgway 1904, Johnston 1961). However, widespread migration in American Crows (Verbeek and Caffrey 2002, Townsend et al. 2018), combined with occasional long-distance female dispersal (McGowan 2001, Withey and Marzluff 2005), provide a ready ecological mechanism for homogenizing gene flow.

Prevalence and discovery of cryptic hybrid zones

This first population genomic analysis of the contact zone between American and Northwestern crows revealed a broad, cryptic hybrid zone. This avian hybrid zone in North America involves a well-known taxonomic group in an intensively studied geographic region, so the fact that it remained enigmatic for so long suggests that the global frequency of cryptic hybrid zones is greatly underestimated. In the case of these crows, >160 years of muddled and conflicting ornithological literature based on subjective and variable phenotypic characters hinted at the potential existence of a cryptic hybrid zone. We expect that comprehensive population genomic surveys of other morphologically austere taxa will reveal many additional cryptic hybrid zones. Furthermore, we encourage researchers to carefully characterize these cryptic hybrid zones to further facilitate comparisons and syntheses of speciation and hybridization processes in both morphologically conserved and morphologically distinctive organisms.

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DATA ACCESSIBILITY

Input files and scripts for running analyses and producing figures are available on GitHub at https://github.com/slager/crow_hybrid_zone. Raw reads, barcodes, aligned reads, detailed sample information, and a snapshot of the above GitHub repository are available on Dryad at https://doi.org/10.5061/dryad.rr4xgxd5f. Mitochondrial DNA ND2 sequences are available under GenBank accession numbers MN830547-MN830805. Demultiplexed and quality-filtered nuDNA reads are available at the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA595997 and BioSample accession numbers SAMN13608537-SAMN13608600.

AUTHOR CONTRIBUTIONS

DLS, RRH, SR, and JK conceived the study; DLS and JK designed the study; SR, CW, and CVH collected field samples; DLS and KLE conducted the lab work. DLS analyzed the data, interpreted the data, wrote the manuscript, and revised the manuscript with input from all co-authors.

FIGURES AND TABLES

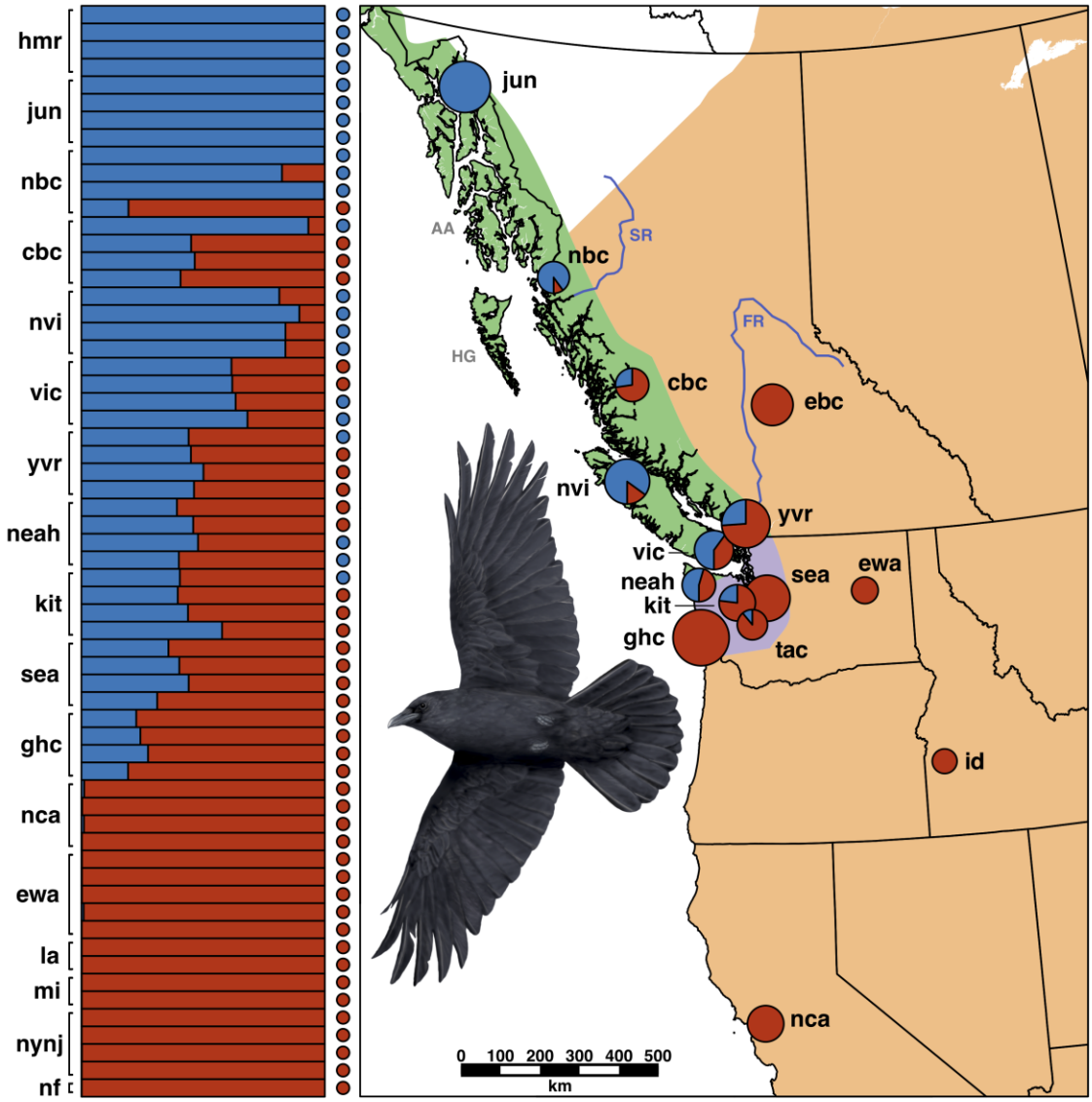

Figure 1. Extent of hybridization between Northwestern Crow (blue) and American Crow (red). At left, bars show nuDNA K=2 ancestry proportions and adjacent circles indicate mtDNA haplogroup for the same 62 individuals. At right, locality pies depict mtDNA haplogroup proportions from the full mtDNA dataset (n=6-31 per locality), and background colors indicate range maps for Northwestern Crow (green), American Crow (orange), and the overlap zone (purple; BirdLife 2013). The map shows the Pacific Northwest of North America from southeastern Alaska to northern California. Sample localities outside this mapped region contain 100% Northwestern (*hmr*) or 100% American (*sca*, *la*, *mi*, *nynj*, *nf*) mtDNA haplogroups. For mtDNA haplogroup proportions across the full geographic range of American/Northwestern crows, see Figure S1. Sample IDs for bars at left run numerically from top to bottom (e.g., mi01 above mi02; see Table S1). Labels indicate locations of the Alexander Archipelago (AA), Haida Gwaii (HG), the Skeena River (SR), and the Fraser River (FR). Original crow illustration by Kevin L. Epperly.

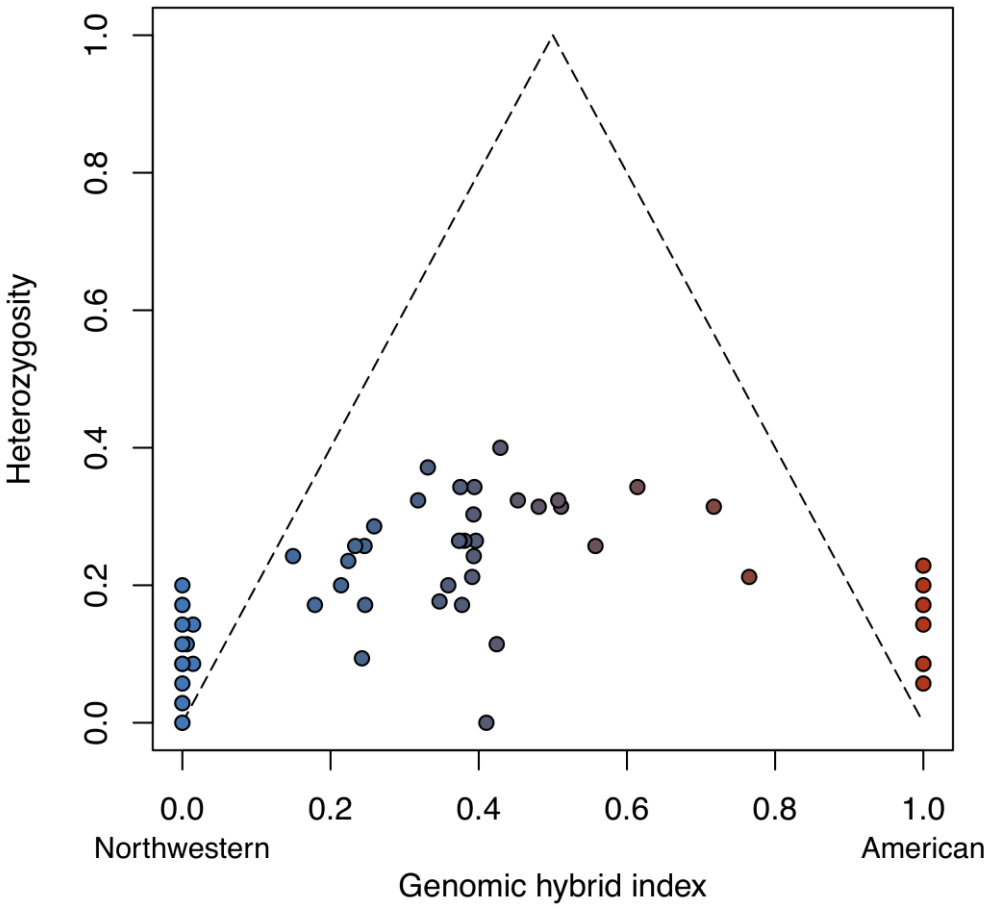

Figure 2. Genomic hybrid index vs. inter-taxon heterozygosity across the Northwestern Crow (blue) and American Crow (red) hybrid zone, based on 34 ancestry-informative nuDNA SNPs (see text). F1 hybrids are expected to have a hybrid index of 0.5 and a heterozygosity of 1.0 for loci fixed in parental individuals. Dotted lines indicate the expected reduction in heterozygosity due to backcrossing (see Methods).

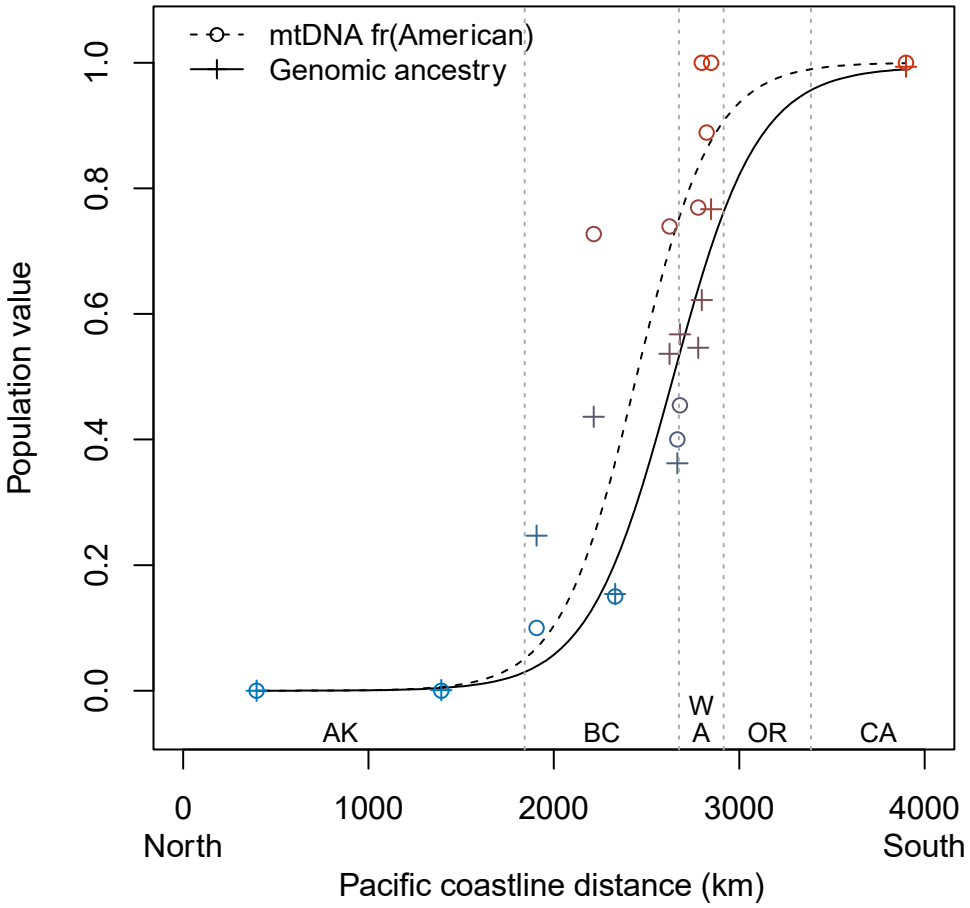

Figure 3. Pacific coastal clines spanning the Northwestern Crow (blue) and American Crow (red) hybrid zone. Circles indicate population frequencies of the American mtDNA haplogroup and crosses indicate population means for nuDNA K=2 ancestry (see Methods). Dashed and solid lines represent best-fit cline models (see Methods), and dotted vertical lines indicate state and provincial boundaries.

Table S1. Detailed sampling information. Crows from the *rus* locality are *Corvus corone*; all other samples are *Corvus brachyrhynchos* or *Corvus caurinus*. The *Corvus corone* with specimen number IPMB 156 corresponds to GenBank number JQ864447. Museum abbreviations: UWBM = University of Washington Burke Museum, AMNH = American Museum of Natural History, LSUMZ = Louisiana State University Museum of Zoology, MVZ = Museum of Vertebrate Zoology at Berkeley, UMMZ = University of Michigan Museum of Zoology.

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117914	sar8229	A	-
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117915	sar8230	A	-
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117916	sar8231	N	-
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.8	10-May-2014	UWBM 117917	sar8232	N	-
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.8	10-May-2014	UWBM 117918	sar8233	A	-
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.8	10-May-2014	UWBM 117919	sar8234	A	-
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117920	sar8235	N	cbc01
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117921	sar8236	A	cbc02
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117922	sar8237	A	cbc03
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117923	sar8238	A	cbc04
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117924	sar8239	A	-
ebc	BRITISH COLUMBIA: Williams Lake	52.0	-122.3	09-May-2014	UWBM 117910	sar8225	A	-
ebc	BRITISH COLUMBIA: Riske Creek	52.0	-122.6	09-May-2014	UWBM 117911	sar8226	A	-
ebc	BRITISH COLUMBIA: Puntzi Lake	52.1	-124.1	09-May-2014	UWBM 117912	sar8227	A	-
ebc	BRITISH COLUMBIA: Puntzi Lake	52.1	-124.1	09-May-2014	UWBM 117913	sar8228	A	-
ebc	BRITISH COLUMBIA: Alexis Creek	52.1	-123.4	11-May-2014	UWBM 117925	sar8240	A	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
ebc	BRITISH COLUMBIA: Alexis Creek	52.1	-123.4	11-May-2014	UWBM 117926	sar8241	A	-
ebc	BRITISH COLUMBIA: Vanderhoof	54.0	-123.9	11-May-2014	UWBM 117927	sar8242	A	-
ebc	BRITISH COLUMBIA: Tintagel	54.2	-125.6	11-May-2014	UWBM 117928	sar8243	A	-
ebc	BRITISH COLUMBIA: Tintagel	54.2	-125.6	11-May-2014	UWBM 117929	sar8244	A	-
ebc	BRITISH COLUMBIA: Tintagel	54.2	-125.6	11-May-2014	UWBM 117930	sar8245	A	-
ebc	BRITISH COLUMBIA: Smithers	54.9	-127.3	13-May-2014	UWBM 117941	sar8256	A	-
ebc	BRITISH COLUMBIA: Houston	54.4	-126.7	13-May-2014	UWBM 117942	sar8257	A	-
ebc	BRITISH COLUMBIA: Quesnel	53.0	-122.5	14-May-2014	UWBM 117943	sar8258	A	-
ebc	BRITISH COLUMBIA: Quesnel	53.0	-122.5	14-May-2014	UWBM 117944	sar8259	A	-
ebc	BRITISH COLUMBIA: Pavilion	50.9	-121.8	14-May-2014	UWBM 117945	sar8260	A	-
ebc	BRITISH COLUMBIA: Pavilion	50.9	-121.8	15-May-2014	UWBM 117946	sar8261	A	-
ebc	BRITISH COLUMBIA: Boston Bar	49.8	-121.4	15-May-2014	UWBM 117947	sar8262	A	-
ewa	WASHINGTON: Okanogan Co.; Oroville	48.9	-119.5	17-Jul-1997	UWBM 58553	plg200	A	-
ewa	WASHINGTON: Asotin Co.; Asotin	46.0	-117.3	21-Jun-1995	UWBM 59039	csw5193a	A	-
ewa	WASHINGTON: Asotin Co.; Asotin	46.1	-117.3	20-Jun-1995	UWBM 59059	sar7000	A	ewa01
ewa	WASHINGTON: Asotin Co.; Asotin	46.0	-117.4	21-Jun-1995	UWBM 59089	svd982	A	ewa05
ewa	WASHINGTON: Okanogan Co.; Loomis	48.7	-119.7	01-Jun-1999	UWBM 62080	smb77	A	ewa02
ewa	WASHINGTON: Okanogan Co.; Loomis	48.7	-119.7	01-Jun-1999	UWBM 62081	smb78	A	ewa03
ewa	WASHINGTON: Okanogan Co.; Loomis	48.7	-119.7	01-Jun-1999	UWBM 62082	smb79	A	ewa04

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
ghc	WASHINGTON: Pacific Co.; Bay Center	46.7	-123.9	28-Apr-2013	UWBM 117091	csw8543	A	-
ghc	WASHINGTON: Pacific Co.; Bay Center	46.7	-123.9	28-Apr-2013	UWBM 117092	csw8544	A	-
ghc	WASHINGTON: Pacific Co.; Bay Center	46.7	-123.9	28-Apr-2013	UWBM 117093	csw8545	A	-
ghc	WASHINGTON: Pacific Co.; Bay Center	46.6	-124.0	28-Apr-2013	UWBM 117094	csw8546	A	-
ghc	WASHINGTON: Pacific Co.; Long Beach	46.4	-124.1	28-Apr-2013	UWBM 117095	csw8547	A	-
ghc	WASHINGTON: Pacific Co.; Long Beach	46.4	-124.1	28-Apr-2013	UWBM 117096	csw8548	A	-
ghc	WASHINGTON: Pacific Co.; Long Beach	46.4	-124.1	28-Apr-2013	UWBM 117097	csw8549	A	-
ghc	WASHINGTON: Pacific Co.; Long Beach	46.5	-124.1	28-Apr-2013	UWBM 117098	csw8550	A	-
ghc	WASHINGTON: Pacific Co.; Long Beach	46.5	-124.1	28-Apr-2013	UWBM 117099	csw8551	A	-
ghc	WASHINGTON: Pacific Co.; Long Beach	46.5	-124.1	28-Apr-2013	UWBM 117100	csw8552	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117101	csw8602	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117102	csw8603	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117103	csw8604	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117104	csw8605	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117105	csw8606	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117106	csw8607	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117107	csw8608	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117108	csw8609	A	-
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117109	csw8610	A	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117898	csw8821	A	-
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117899	csw8814	A	ghc01
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117900	csw8815	A	ghc02
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117901	csw8816	A	ghc03
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117902	csw8817	A	ghc04
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117903	csw8818	A	-
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117904	csw8819	A	-
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117905	csw8820	A	-
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117906	csw8822	A	-
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117907	csw8823	A	-
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117908	csw8824	A	-
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117909	csw8825	A	-
hom	ALASKA: Homer	59.6	-151.5	24-Mar-2007	-	USGS 70575018	N	hmr01
hom	ALASKA: Homer	59.6	-151.5	24-Mar-2007	-	USGS 70575019	N	hmr02
hom	ALASKA: Homer	59.6	-151.5	24-Mar-2007	-	USGS 70575024	N	hmr03
hom	ALASKA: Homer	59.6	-151.5	24-Mar-2007	-	USGS 70575025	N	-
hom	ALASKA: Homer	59.6	-151.5	24-Mar-2007	-	USGS 70575027	N	hmr04
hom	ALASKA: Homer	59.6	-151.5	22-Feb-2008	-	USGS 70575055	N	-
hom	ALASKA: Homer	59.6	-151.5	23-Feb-2008	-	USGS 70575059	N	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
hom	ALASKA: Homer	59.6	-151.5	23-Feb-2008	-	USGS 70575060	N	-
hom	ALASKA: Homer	59.6	-151.5	23-Feb-2008	-	USGS 70575063	N	-
hom	ALASKA: Homer	59.6	-151.5	23-Feb-2008	-	USGS 70575064	N	-
hom	ALASKA: Homer	59.6	-151.5	23-Feb-2008	-	USGS 70575065	N	-
hom	ALASKA: Homer	59.6	-151.5	24-Feb-2008	-	USGS 70575068	N	-
hom	ALASKA: Homer	59.6	-151.5	24-Feb-2008	-	USGS 70575070	N	-
hom	ALASKA: Homer	59.6	-151.5	24-Feb-2008	-	USGS 70575075	N	-
hom	ALASKA: Homer	59.6	-151.5	24-Feb-2008	-	USGS 70575076	N	-
id	IDAHO: Ada Co.; Eagle	43.7	-116.4	summer of 1996 or 1997	-	BC002	A	-
id	IDAHO: Ada Co.; Eagle	43.7	-116.4	summer of 1996 or 1997	-	BCM3	A	-
id	IDAHO: Ada Co.; Eagle	43.7	-116.4	summer of 1996 or 1997	-	BCN4	A	-
id	IDAHO: Ada Co.; Eagle	43.7	-116.4	summer of 1996 or 1997	-	BCS1	A	-
id	IDAHO: Ada Co.; Eagle	43.7	-116.4	summer of 1996 or 1997	-	BCW1	A	-
id	IDAHO: Ada Co.; Eagle	43.7	-116.4	summer of 1996 or 1997	-	BCY2	A	-
jun	ALASKA: Haines	59.2	-135.4	01-Sep-2007	-	USGS 70575028	N	-
jun	ALASKA: Haines	59.2	-135.4	01-Sep-2007	-	USGS 70575029	N	-
jun	ALASKA: Haines	59.2	-135.4	11-Apr-2008	-	USGS 105506111	N	-
jun	ALASKA: Juneau	58.4	-134.6	13-Apr-2008	-	USGS 105506122	N	-
jun	ALASKA: Juneau	58.4	-134.6	13-Apr-2008	-	USGS 105506123	N	-
jun	ALASKA: Juneau	58.4	-134.6	13-Apr-2008	-	USGS 105506124	N	-
jun	ALASKA: Juneau	58.4	-134.6	13-Apr-2008	-	USGS 105506129	N	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
jun	ALASKA: Juneau	58.4	-134.6	13-Apr-2008	-	USGS 105506130	N	-
jun	ALASKA: Juneau	58.4	-134.6	14-Apr-2008	-	USGS 105506133	N	jun04
jun	ALASKA: Juneau	58.4	-134.6	14-Apr-2008	-	USGS 105506134	N	-
jun	ALASKA: Juneau	58.4	-134.6	14-Apr-2008	-	USGS 105506137	N	-
jun	ALASKA: Juneau	58.4	-134.6	14-Apr-2008	-	USGS 105506138	N	jun01
jun	ALASKA: Juneau	58.4	-134.6	14-Apr-2008	-	USGS 105506141	N	jun02
jun	ALASKA: Juneau	58.4	-134.6	14-Apr-2008	-	USGS 105506142	N	jun03
jun	ALASKA: Juneau	58.4	-134.6	14-Apr-2008	-	USGS 105506146	N	-
jun	ALASKA: Juneau	58.4	-134.6	15-Apr-2008	-	USGS 105506147	N	-
jun	ALASKA: Juneau	58.4	-134.6	15-Apr-2008	-	USGS 105506150	N	-
jun	ALASKA: Juneau	58.4	-134.6	15-Apr-2008	-	USGS 105506151	N	-
jun	ALASKA: Haines	59.2	-135.4	17-Apr-2008	-	USGS 105506167	N	-
jun	ALASKA: Haines	59.2	-135.4	17-Apr-2008	-	USGS 105506168	N	-
jun	ALASKA: Juneau; Juneau	58.3	-134.4	01-Jul-2012	UWBM 116771	csw8450	N	-
jun	ALASKA: Haines; Haines; above Lutak Inlet	59.3	-135.4	08-Jun-2006	UWBM 84789	csw7048	N	-
jun	ALASKA: Haines; Haines; above Lutak Inlet	59.3	-135.4	08-Jun-2006	UWBM 84867	sez083	N	-
jun	ALASKA: Juneau; Juneau	58.3	-134.4	06-Jul-2007	UWBM 87091	aei110	N	-
jun	ALASKA: Juneau; Juneau	58.3	-134.5	05-Jul-2008	UWBM 87796	evl1154	N	-
jun	ALASKA: Juneau; Juneau	58.3	-134.4	05-Oct-2008	UWBM 88685	evl1183	N	-
kit	WASHINGTON: Kitsap Co.; Poulsbo	47.7	-122.6	11-Jun-2013	UWBM 117848	kle720	A	-
kit	WASHINGTON: Kitsap Co.; Kingston	47.9	-122.5	09-Jun-2013	UWBM 117849	kle719	N	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
kit	WASHINGTON: Kitsap Co.; Port Orchard	47.5	-122.6	15-Jun-2013	UWBM 117853	kle723	N	kit01
kit	WASHINGTON: Kitsap Co.; Silverdale	47.6	-122.7	18-Jun-2013	UWBM 117854	kle722	N	-
kit	WASHINGTON: Kitsap Co.; Bremerton	47.6	-122.6	15-Jun-2013	UWBM 117855	kle721	A	-
kit	WASHINGTON: Kitsap Co.; Poulsbo	47.7	-122.6	08-Jun-2013	UWBM 117856	kle718	A	-
kit	WASHINGTON: Kitsap Co.; Kingston	47.8	-122.6	29-Jun-2013	UWBM 117859	kle729	A	-
kit	WASHINGTON: Kitsap Co.; Bremerton	47.6	-122.6	08-Jul-2005	UWBM 117861	kle731	A	-
kit	WASHINGTON: Kitsap Co.; Bremerton	47.6	-122.6	02-Jul-2013	UWBM 117862	kle732	A	kit02
kit	WASHINGTON: Kitsap Co.; Port Orchard	47.5	-122.6	26-Jun-2013	UWBM 117863	kle733	A	kit03
kit	WASHINGTON: Kitsap Co.; Silverdale	47.7	-122.7	01-Jul-2013	UWBM 117864	kle734	A	kit04
kit	WASHINGTON: Kitsap Co.; Silverdale	47.7	-122.7	10-Jun-2005	UWBM 117865	kle735	A	-
kit	WASHINGTON: Kitsap Co.; Seabeck	47.6	-122.8	06-Jul-2005	UWBM 117866	kle736	A	-
la	LOUISIANA: East Baton Rouge Parish; Baton Rouge	30.5	-91.1	22-Jun-1999	UWBM 80702	mng174	A	la02
la	LOUISIANA: East Baton Rouge Parish; Baton Rouge	30.5	-91.1	21-Jun-1999	UWBM 84785	sez034	A	la01
mi	MICHIGAN: Tuscola Co.; Caro	43.5	-83.4	11-Jun-1987	UMMZ 227423	227423	A	mi01
mi	MICHIGAN: Washtenaw Co.; Ann Arbor	42.3	-83.7	02-Jun-1994	UMMZ 233637	233637	A	mi02
nbc	BRITISH COLUMBIA: Skeena River; Telegraph Point	54.2	-129.7	12-May-2014	UWBM 117931	sar8246	N	-
nbc	BRITISH COLUMBIA: Port Edwards	54.2	-130.2	12-May-2014	UWBM 117932	sar8247	N	-
nbc	BRITISH COLUMBIA: Port Edwards	54.2	-130.3	13-May-2014	UWBM 117933	sar8248	N	-
nbc	BRITISH COLUMBIA: Port Edwards	54.2	-130.3	13-May-2014	UWBM 117934	sar8249	N	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
nbc	BRITISH COLUMBIA: Port Edwards	54.2	-130.3	13-May-2014	UWBM 117935	sar8250	N	-
nbc	BRITISH COLUMBIA: Port Edwards	54.2	-130.3	13-May-2014	UWBM 117936	sar8251	N	-
nbc	BRITISH COLUMBIA: Port Edwards	54.2	-130.3	13-May-2014	UWBM 117937	sar8252	N	nbc01
nbc	BRITISH COLUMBIA: Skeena River; Telegraph Point	54.2	-129.7	13-May-2014	UWBM 117938	sar8253	N	nbc02
nbc	BRITISH COLUMBIA: Skeena River west of Kwinitsa River mouth	54.2	-129.6	13-May-2014	UWBM 117939	sar8254	N	nbc03
nbc	BRITISH COLUMBIA: Skeena River west of Kwinitsa River mouth	54.2	-129.6	13-May-2014	UWBM 117940	sar8255	A	nbc04
nca	CALIFORNIA: Shasta Co.; Redding	40.6	-122.4	10-Jul-1995	AMNH DOT 9976	prs1180	A	-
nca	CALIFORNIA: Marin Co.; Kentfield	38.0	-122.5	15-Oct-1998	MVZ 179732	2156	A	-
nca	CALIFORNIA: Contra Costa Co.; Bethel Island	38.0	-121.6	13-Feb-1999	MVZ 179991	2063	A	nca03
nca	CALIFORNIA: Contra Costa Co.; Martinez	38.0	-122.1	23-Oct-1999	MVZ 179992	2170	A	nca04
nca	CALIFORNIA: Contra Costa Co.; Walnut Creek	37.9	-122.0	02-Mar-1999	MVZ 179993	2176	A	-
nca	CALIFORNIA: Contra Costa Co.; Lafayette	37.9	-122.1	22-Oct-1999	MVZ 179994	2171	A	-
nca	CALIFORNIA: Contra Costa Co.; Sam Ramon	37.8	-122.0	21-May-1999	MVZ 179995	1847	A	nca01
nca	CALIFORNIA: Alameda Co.; Livermore	37.7	-121.8	30-May-1999	MVZ 179996	1858	A	nca02
nca	CALIFORNIA: Solano Co.; Benicia	38.1	-122.2	01-Nov-2000	MVZ 180198	2180	A	-
nca	CALIFORNIA: San Joaquin Co.; Tracy	37.7	-121.4	28-Feb-2000	MVZ 180199	2177	A	-
nca	CALIFORNIA: Contra Costa Co.; Concord	38.0	-122.0	16-Mar-2000	MVZ 180200	2175	A	-
nca	CALIFORNIA: Contra Costa Co.; Crockett	38.1	-122.2	23-Jul-2000	MVZ 180201	2181	A	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
nca	CALIFORNIA: Alameda Co.; Livermore	37.7	-121.8	10-Mar-2000	MVZ 180202	2179	A	-
neah	WASHINGTON: Clallam Co.; Lake Crescent	48.1	-123.8	02-Apr-2014	UWBM 117871	csw8704	A	-
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.4	02-Apr-2014	UWBM 117872	csw8705	N	-
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	02-Apr-2014	UWBM 117873	csw8706	A	neah01
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	02-Apr-2014	UWBM 117874	csw8707	A	neah02
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	02-Apr-2014	UWBM 117875	csw8708	N	-
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.4	03-Apr-2014	UWBM 117876	csw8709	A	-
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	03-Apr-2014	UWBM 117877	csw8710	N	neah03
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	03-Apr-2014	UWBM 117878	csw8711	N	neah04
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	03-Apr-2014	UWBM 117879	csw8712	N	-
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	03-Apr-2014	UWBM 117880	csw8713	N	-
neah	WASHINGTON: Clallam Co.; Sappho	48.1	-124.3	02-Apr-2014	UWBM 117881	csw8714	A	-
nf	NEWFOUNDLAND: Avalon Bay	47.4	-53.4	25-Apr-2000	UWBM 66786	gkd129	A	nf01
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117951	sar8266	N	nvi01
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117952	sar8267	N	nvi02
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117953	sar8268	N	nvi03

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117954	sar8269	N	nvi04
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117955	sar8270	N	-
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117956	sar8271	N	-
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117957	sar8272	N	-
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117958	sar8273	N	-
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117959	sar8274	N	-
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117960	sar8275	N	-
nvi	BRITISH COLUMBIA: Gold River, Nesook Bay	49.8	-126.4	17-May-2014	UWBM 117961	sar8276	N	-
nvi	BRITISH COLUMBIA: Gold River, Nesook Bay	49.8	-126.4	17-May-2014	UWBM 117962	sar8277	N	-
nvi	BRITISH COLUMBIA: Gold River, Nesook Bay	49.8	-126.4	17-May-2014	UWBM 117963	sar8278	N	-
nvi	BRITISH COLUMBIA: Tahsis	49.9	-126.7	17-May-2014	UWBM 117964	sar8279	N	-
nvi	BRITISH COLUMBIA: Vancouver Island; Royston	49.7	-125.0	05-Jul-2008	UWBM 87433	csw7279	N	-
nvi	BRITISH COLUMBIA: Vancouver Island; Comox	49.7	-124.9	07-Jul-2008	UWBM 87444	csw7290	N	-
nvi	BRITISH COLUMBIA: Vancouver Island; Campbell River	50.0	-125.3	28-May-2008	UWBM 87449	csw7295	A	-
nvi	BRITISH COLUMBIA: Vancouver Island; Campbell River	50.0	-125.3	27-Apr-2008	UWBM 87450	csw7296	A	-
nvi	BRITISH COLUMBIA: Vancouver Island; Courtenay	49.7	-125.0	28-Jun-2008	UWBM 87451	csw7297	N	-
nvi	BRITISH COLUMBIA: Vancouver Island; Campbell River	50.0	-125.3	02-Jul-2008	UWBM 87452	csw7298	A	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
nynj	NEW YORK: Suffolk Co.; Lloyd Harbor	40.9	-73.5	03-Jul-2004	AMNH DOT 13647	pac1225	A	-
nynj	NEW JERSEY: Union Co.; Westfield	40.7	-74.3	21-Jul-2004	AMNH DOT 13715	pac1300	A	nynj04
nynj	NEW YORK: Suffolk Co.; Northport	40.9	-73.3	17-Jul-2005	AMNH DOT 13858	pac1346	A	nynj02
nynj	NEW YORK: Nassau Co.; Wantagh	40.7	-73.5	11-Jun-2005	AMNH DOT 13859	pac1347	A	nynj03
nynj	NEW YORK: Queens Co.; JFK Airport	40.6	-73.8	10-Jun-2000	AMNH DOT 7202	pac348	A	nynj01
rus	-	-	-	-	IPMB 156*	-	-	-
rus	RUSSIA: Magadanskaya Oblast; Magadan, 45 km S, 70 km E	59.1	151.9	29-Jun-1992	UWBM 44190	jmb1014	-	rus01
rus	RUSSIA: Magadanskaya Oblast'; Magadan, 45 km S, 70 km E	59.1	151.9	29-Jun-1992	UWBM 44191	jmb1015	-	rus02
sca	CALIFORNIA: San Bernardino Co.; Harper Lake	35.0	-117.3	21-Nov-1991	LSUMZ B22990	b22990	A	-
sea	WASHINGTON: King Co.; Kent	47.4	-122.2	16-Jul-2013	UWBM 117850	kle726	A	-
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	11-Jun-2005	UWBM 117870	kle740	A	-
sea	WASHINGTON: Snohomish Co.; Edmonds	47.9	-122.3	06-Aug-1997	UWBM 58600	csw5724	A	-
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	09-Apr-1993	UWBM 65073	csw4779	A	-
sea	WASHINGTON: King Co.; Seattle	47.6	-122.3	16-Jun-1993	UWBM 65094	jmb1416	A	sea02
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	13-May-1992	UWBM 65101	jmb960	A	sea03
sea	WASHINGTON: Snohomish Co.; Lynnwood	47.9	-122.3	17-Apr-1998	UWBM 68201	gkd23	A	-
sea	WASHINGTON: King Co.; Redmond	47.7	-122.1	03-Apr-1996	UWBM 71418	rya82	A	-
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	07-Jul-2000	UWBM 79625	jml017	A	-
sea	WASHINGTON: King Co.; Seattle	47.6	-122.3	14-Mar-2005	UWBM 81644	ejm002	A	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	09-Jul-2005	UWBM 81681	gjy002	A	-
sea	WASHINGTON: King Co.; Des Moines	47.4	-122.3	27-Jun-2006	UWBM 84163	car003	A	-
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	25-May-2006	UWBM 84174	kle003	A	-
sea	WASHINGTON: King Co.; Seattle	47.5	-122.4	15-Mar-2005	UWBM 84204	meh29	A	-
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	01-Apr-2003	UWBM 84228	rjr001	A	-
sea	WASHINGTON: King Co.; SeaTac	47.4	-122.3	18-May-2003	UWBM 84277	cxj001	A	sea01
sea	WASHINGTON: King Co.; SeaTac	47.4	-122.3	31-May-2002	UWBM 85982	wss002	A	-
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	24-May-2007	UWBM 86268	kle065	A	sea04
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	10-Aug-2009	UWBM 90348	csw7570	A	-
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	24-Jun-2009	UWBM 90350	csw7572	A	-
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	11-Jun-2009	UWBM 90351	csw7573	A	-
tac	WASHINGTON: Pierce Co.; Gig Harbor	47.3	-122.6	10-Jul-2013	UWBM 117851	kle725	A	-
tac	WASHINGTON: Pierce Co.; Vaughn	47.3	-122.8	11-Jul-2013	UWBM 117852	kle724	N	-
tac	WASHINGTON: Pierce Co.; Lake Tapps	47.2	-122.2	22-Jul-2013	UWBM 117857	kle727	A	-
tac	WASHINGTON: Pierce Co.; Tacoma	47.2	-122.4	04-Jul-2013	UWBM 117867	kle737	A	-
tac	WASHINGTON: Pierce Co.; Gig Harbor	47.3	-122.6	01-Jun-2011	UWBM 117868	kle738	A	-
tac	WASHINGTON: Pierce Co.; University Place	47.2	-122.5	29-Jun-2013	UWBM 117869	kle739	A	-
tac	WASHINGTON: Pierce Co.; Tacoma	47.3	-122.5	29-Apr-2013	UWBM 117882	csw8715	A	-
tac	WASHINGTON: Pierce Co.; Tacoma	47.3	-122.5	29-Jul-2010	UWBM 117885	cec667	A	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
tac	WASHINGTON: Pierce Co.; Tacoma	47.3	-122.5	05-Jun-2013	UWBM 117886	gws4003	A	-
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	27-May-2008	UWBM 87422	csw7268	N	-
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	01-Jun-2008	UWBM 87423	csw7269	A	-
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	22-Jun-2008	UWBM 87427	csw7273	N	-
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	02-Jul-2008	UWBM 87445	csw7291	N	-
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	02-Jul-2008	UWBM 87446	csw7292	N	-
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	18-Jun-2008	UWBM 87473	csw7323	N	-
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	28-Jun-2008	UWBM 87475	csw7325	N	-
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	01-Jun-2008	UWBM 87477	csw7327	A	vic01
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	04-Jun-2008	UWBM 87478	csw7328	A	vic02
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	21-Jun-2008	UWBM 87484	csw7334	N	vic03
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	01-Jun-2008	UWBM 87485	csw7335	A	-
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	25-Jun-2008	UWBM 87486	csw7336	A	-
vic	BRITISH COLUMBIA: Vancouver Island; Esquimalt	48.4	-123.4	29-Jul-2008	UWBM 87496	csw7348	N	-
vic	BRITISH COLUMBIA: Vancouver Island; Esquimalt	48.4	-123.4	29-Jul-2008	UWBM 87497	csw7350	N	vic04

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
vic	BRITISH COLUMBIA: Vancouver Island; Esquimalt	48.4	-123.4	29-Jul-2008	UWBM 87498	csw7351	A	-
yvr	BRITISH COLUMBIA: Hope	49.4	-121.5	15-May-2014	UWBM 117948	sar8263	N	-
yvr	BRITISH COLUMBIA: Hatzic	49.2	-122.2	15-May-2014	UWBM 117949	sar8264	A	-
yvr	BRITISH COLUMBIA: Hatzic	49.2	-122.2	15-May-2014	UWBM 117950	sar8265	A	-
yvr	BRITISH COLUMBIA: Richmond	49.2	-123.1	19-Jun-2008	UWBM 87425	csw7271	A	-
yvr	BRITISH COLUMBIA: Coquitlam	49.3	-122.8	15-Jun-2008	UWBM 87434	csw7280	A	-
yvr	BRITISH COLUMBIA: Delta	49.1	-122.9	03-Jul-2008	UWBM 87438	csw7284	N	-
yvr	BRITISH COLUMBIA: Surrey	49.1	-122.8	01-Jul-2008	UWBM 87439	csw7285	N	yvr01
yvr	BRITISH COLUMBIA: Delta	49.1	-122.9	09-Jul-2008	UWBM 87441	csw7287	A	yvr02
yvr	BRITISH COLUMBIA: Surrey	49.1	-122.8	09-Jul-2008	UWBM 87443	csw7289	N	-
yvr	BRITISH COLUMBIA: Burnaby	49.2	-123.0	16-Jul-2008	UWBM 87453	csw7299	A	yvr03
yvr	BRITISH COLUMBIA: Coquitlam	49.3	-122.8	17-Jul-2008	UWBM 87454	csw7300	N	-
yvr	BRITISH COLUMBIA: Coquitlam	49.3	-122.8	14-Jul-2008	UWBM 87455	csw7301	A	yvr04
yvr	BRITISH COLUMBIA: Agassiz	49.2	-121.8	16-Jul-2008	UWBM 87457	csw7303	A	-
yvr	BRITISH COLUMBIA: Agassiz	49.2	-121.8	16-Jul-2008	UWBM 87458	csw7305	A	-
yvr	BRITISH COLUMBIA: Chilliwack	49.1	-121.9	20-Jul-2008	UWBM 87459	csw7306	A	-
yvr	BRITISH COLUMBIA: Delta	49.1	-122.9	11-Jul-2008	UWBM 87462	csw7311	A	-
yvr	BRITISH COLUMBIA: Langley	49.1	-122.7	17-Jul-2008	UWBM 87465	csw7314	A	-
yvr	BRITISH COLUMBIA: Richmond	49.2	-123.1	16-Jul-2008	UWBM 87467	csw7317	A	-
yvr	BRITISH COLUMBIA: Richmond	49.2	-123.1	16-Jul-2008	UWBM 87468	csw7318	A	-

Locality	Specific locality	Lat.	Long.	Date	Museum specimen no.	Field no.	mtDNA haplo.	ddrad no.
yvr	BRITISH COLUMBIA: Port Moody	49.3	-122.8	21-Jul-2008	UWBM 87469	csw7319	A	-
yvr	BRITISH COLUMBIA: Langley	49.1	-122.7	23-Jul-2008	UWBM 87489	csw7339	N	-
yvr	BRITISH COLUMBIA: Chilliwack	49.1	-121.9	24-Jul-2008	UWBM 87495	csw7347	A	-
yvr	BRITISH COLUMBIA: Chilliwack	49.1	-121.9	05-Aug-2008	UWBM 87509	csw7362	A	-

K	Reps	Mean LnP(*K*)	Stdev LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Delta *K*
1	5	-174866.22	11.0312	NA	NA	NA
2	**5**	**-163293.36**	**8.8534**	**11572.86**	**9353.32**	**1056.464364**
3	5	-161073.82	64.3754	2219.54	1681.9	26.126456
4	5	-160536.18	277.2055	537.64	NA	NA

Table S2. Selection of the optimal value of *K* for the American/Northwestern crow hybrid zone using Structure Harvester. The optimal result is shown in bold.

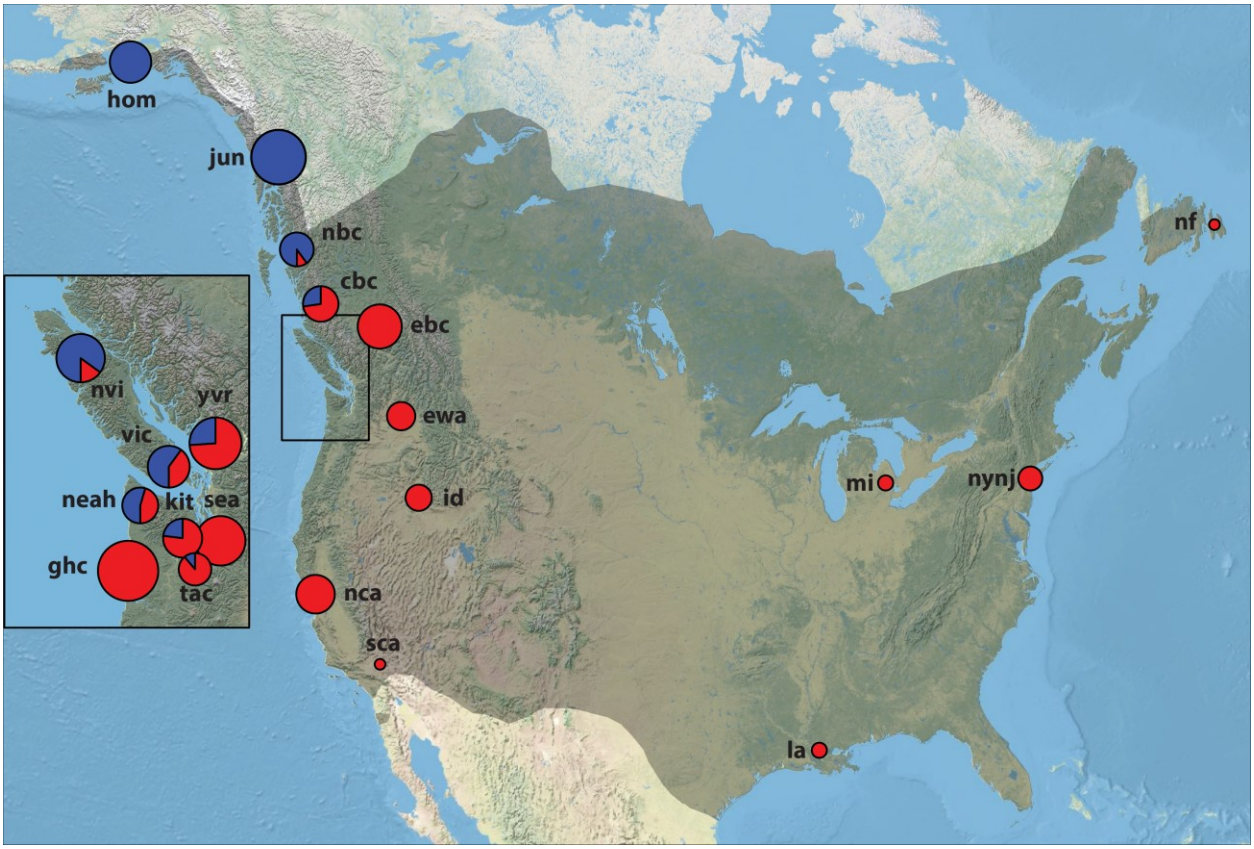

Figure S1. Full map of sampling localities in North America (see Table S1). The shaded area shows the combined range of American and Northwestern crows. Pie charts show proportion of American (red) and Northwestern (blue) mtDNA ND2 haplogroups at each locality, and total pie area is proportional to mtDNA ND2 sample size.

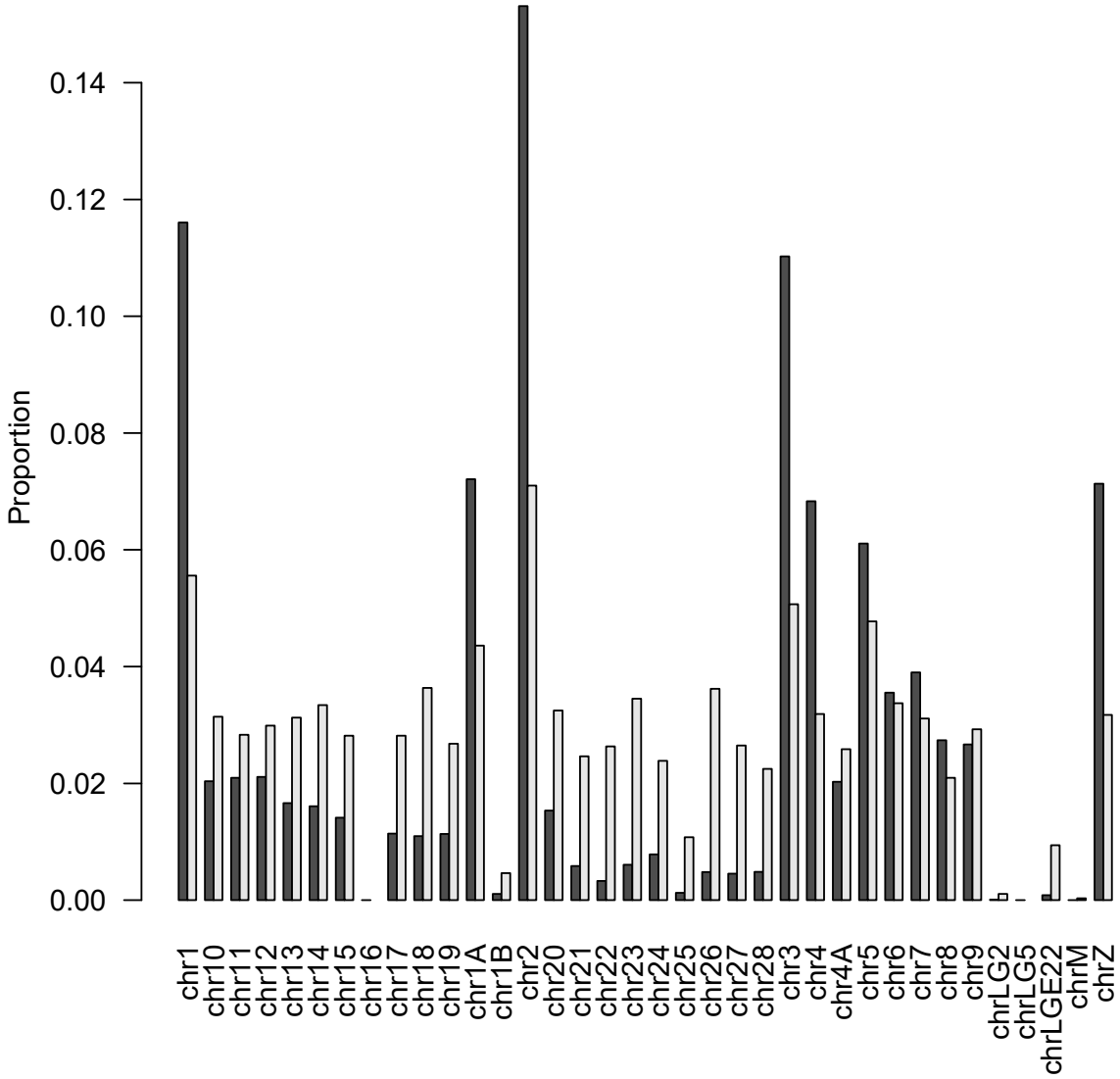

Figure S2. Chromosomal proportions of the Zebra Finch (*Taeniopygia guttata*) genome (black) and American/Northwestern crow ddRAD loci (gray).

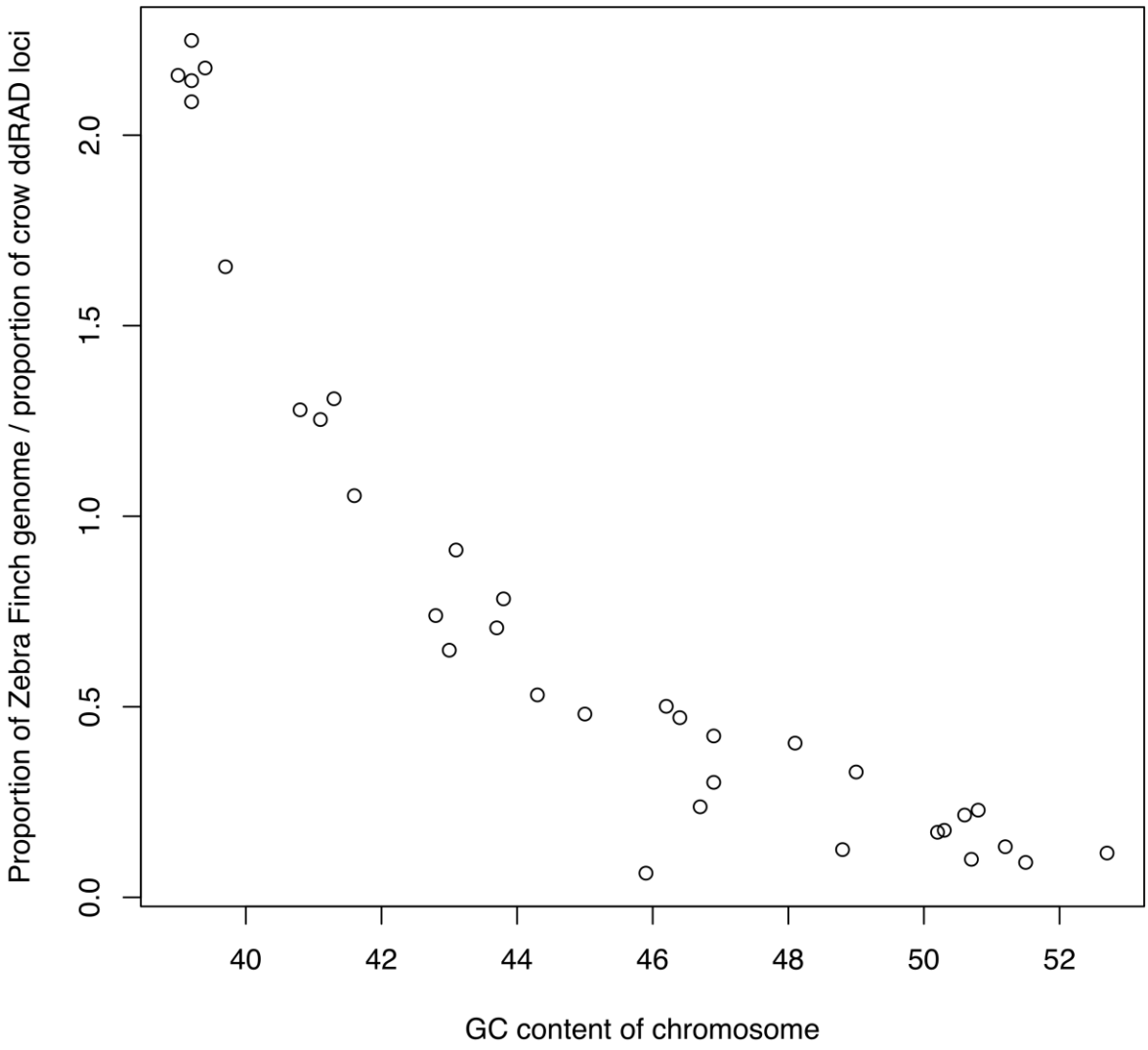

Figure S3. Relative chromosomal proportions in the Zebra Finch (*Taeniopygia guttata*) genome and American/Northwestern crow ddRAD loci in relation to percent chromosomal GC content.

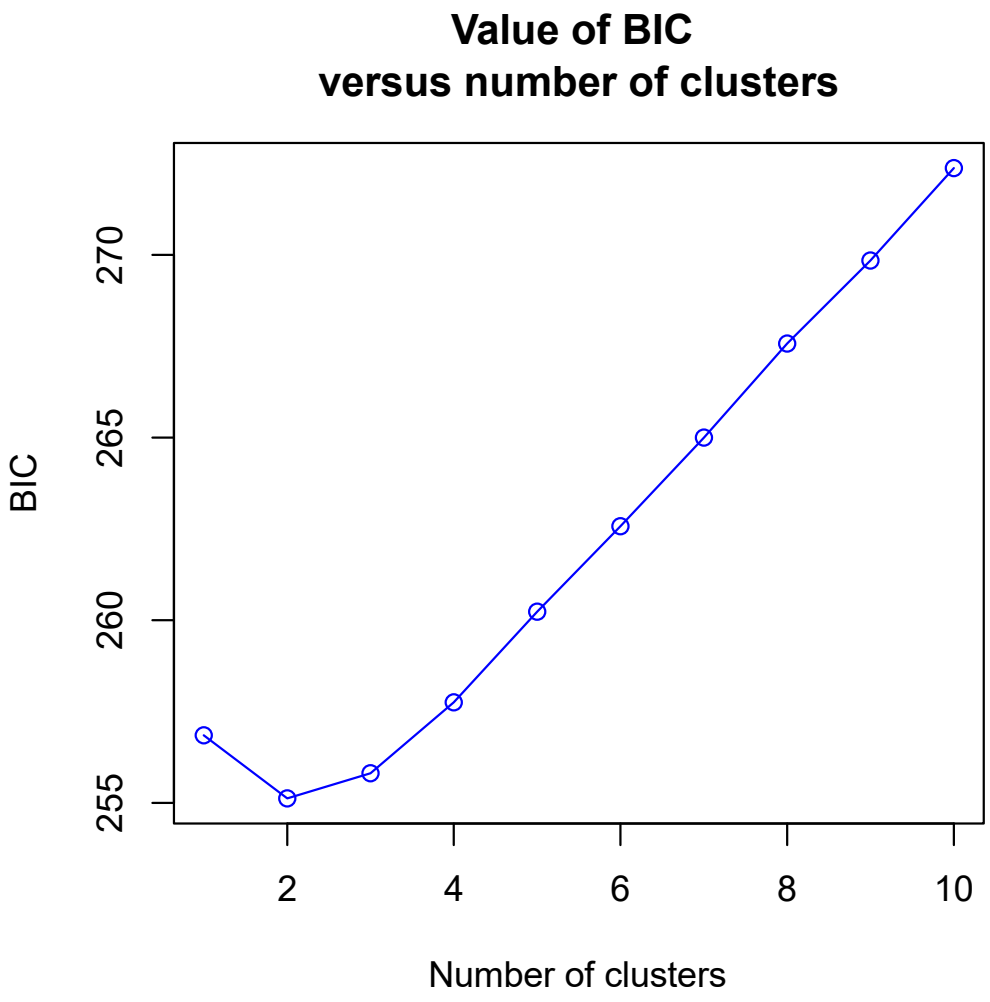

Figure S4. Bayesian Information Criterion (BIC) values for K=1 to K=10 in the K-means clustering algorithm implemented in the *find.clusters* function in *adegenet* 2.0.1 (Jombart et al. 2010). This analysis used the continent-wide, 62-sample alignment with no missing data after transforming by principal components analysis (PCA), retaining all principal components.

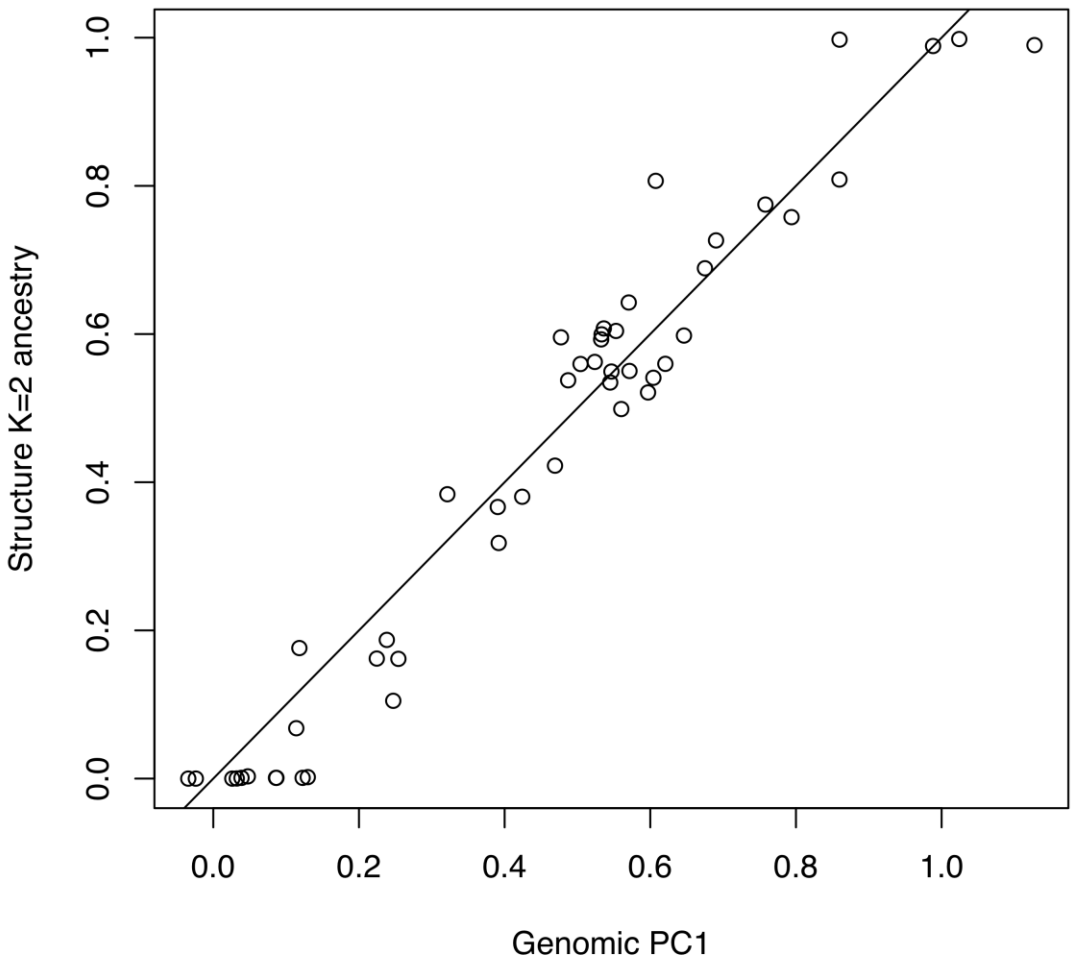

Figure S5. Correlation between Structure K=2 ancestry proportions and transformed genomic PC1 for the 48 Pacific coastal samples ($R^2 = 0.95$, $p < 10^{-15}$), with y=x line shown for reference. The Structure analysis used an alignment of 7,292 SNPs including missing data, with coverage for ≥ 4 of 62 samples continent-wide. The PCA analysis used an alignment of 905 SNPs with no missing data for the 48 Pacific coastal samples.

Figure S6. Median-joining haplotype network of mtDNA ND2 in Northwestern and American crows (n=258) and *Corvus corone* outgroup (n=1; see Table S1). Bayesian posterior probability, maximum likelihood bootstrap, and quartets bootstrap values were 100%, 89%, and 74% for the American haplogroup clade and 77%, 66%, and 82% for the Northwestern haplogroup clade, respectively (see Methods).

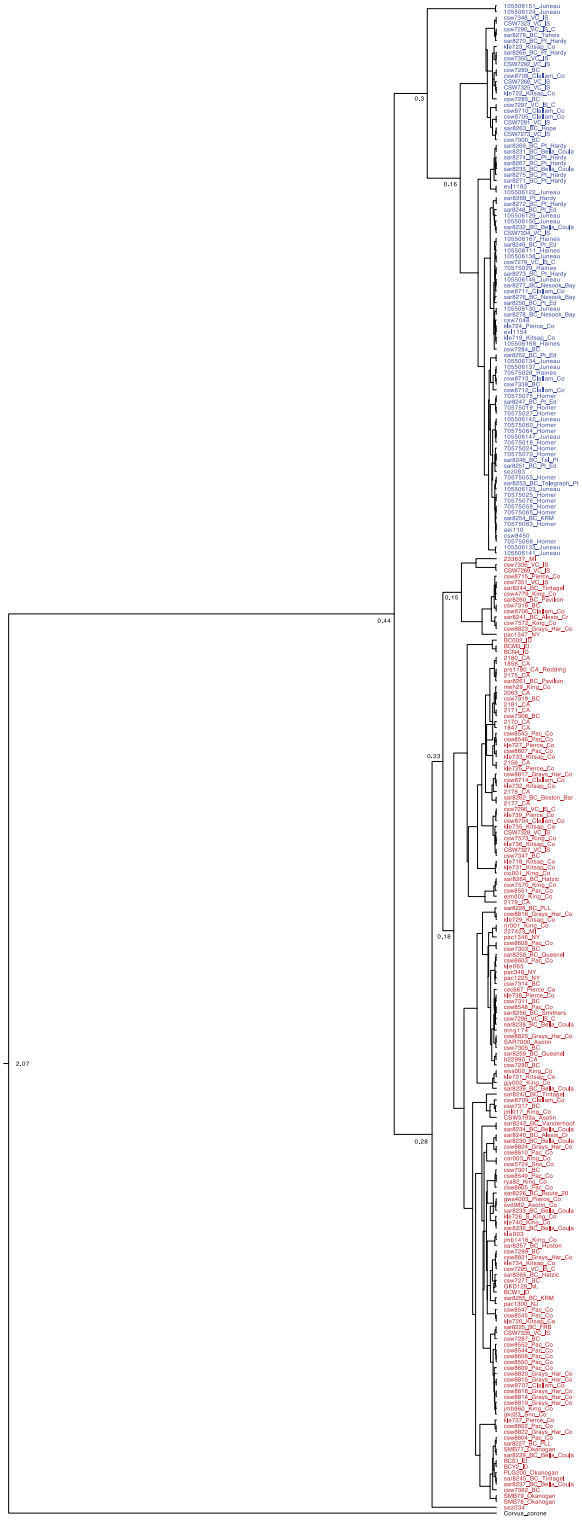

Figure S7. Time-calibrated mtDNA ND2 BEAST phylogeny of American/Northwestern crows with divergence dates (Mya).

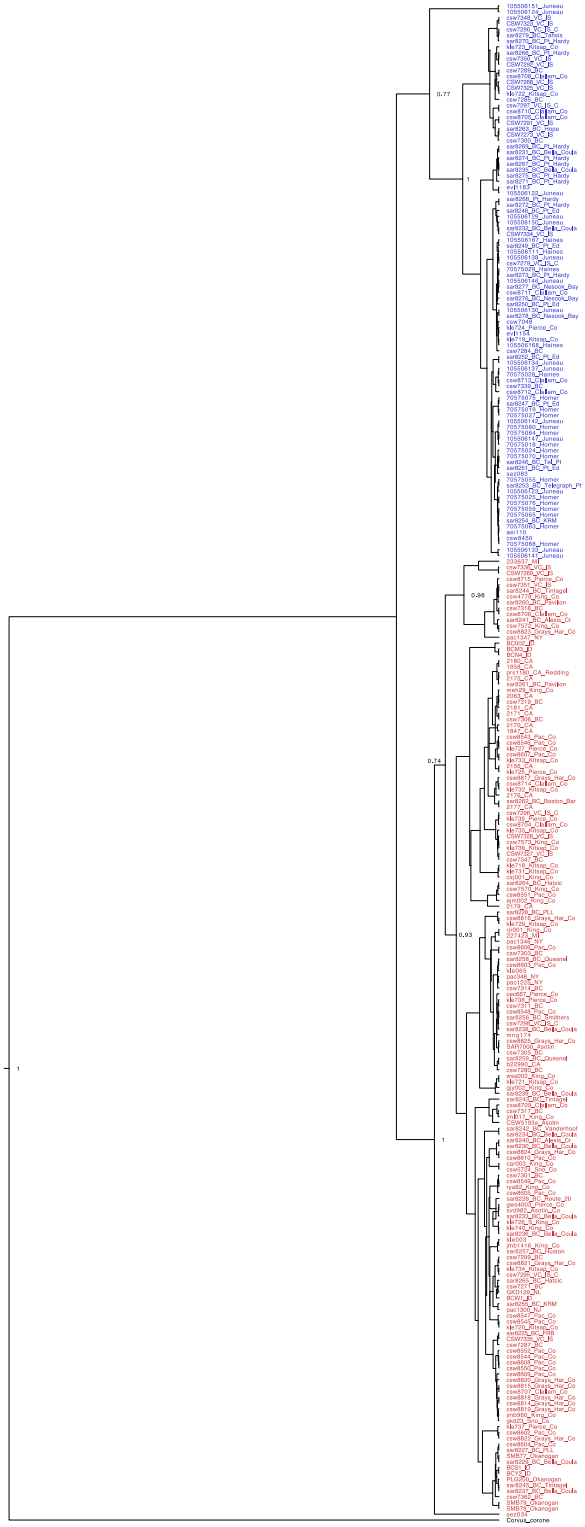

Figure S8. Time-calibrated mtDNA ND2 BEAST phylogeny of American/Northwestern crows with Bayesian posterior probabilities.

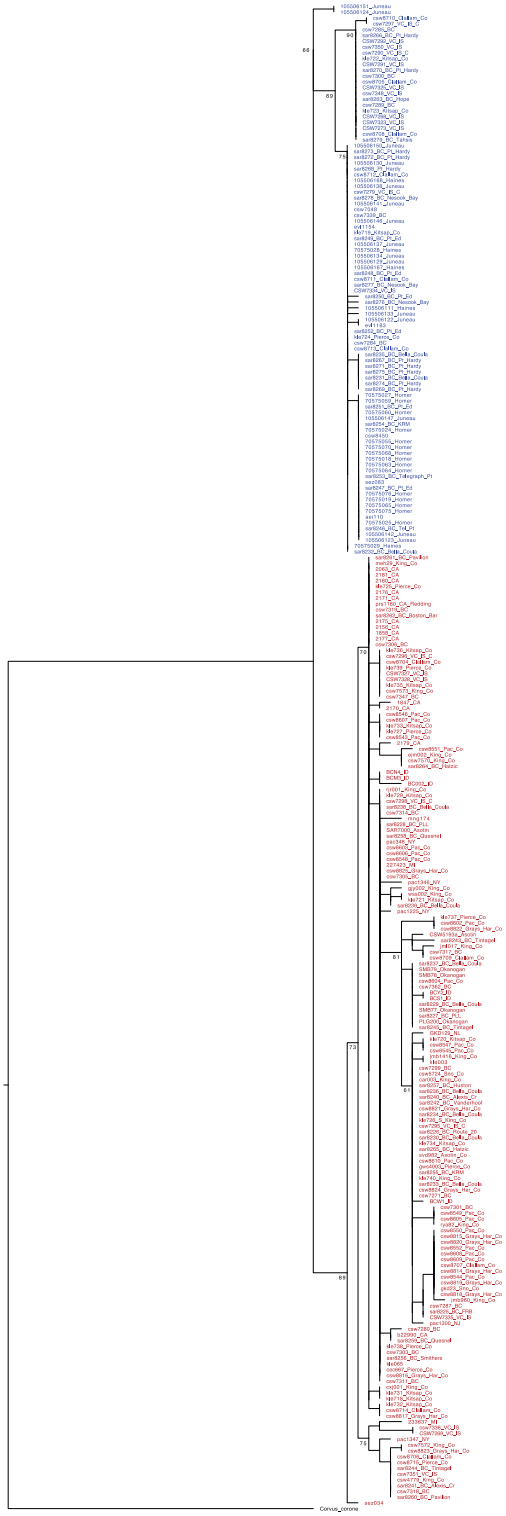

Figure S9. Maximum likelihood mtDNA RAxML tree of American/Northwestern crows with bootstrap values.

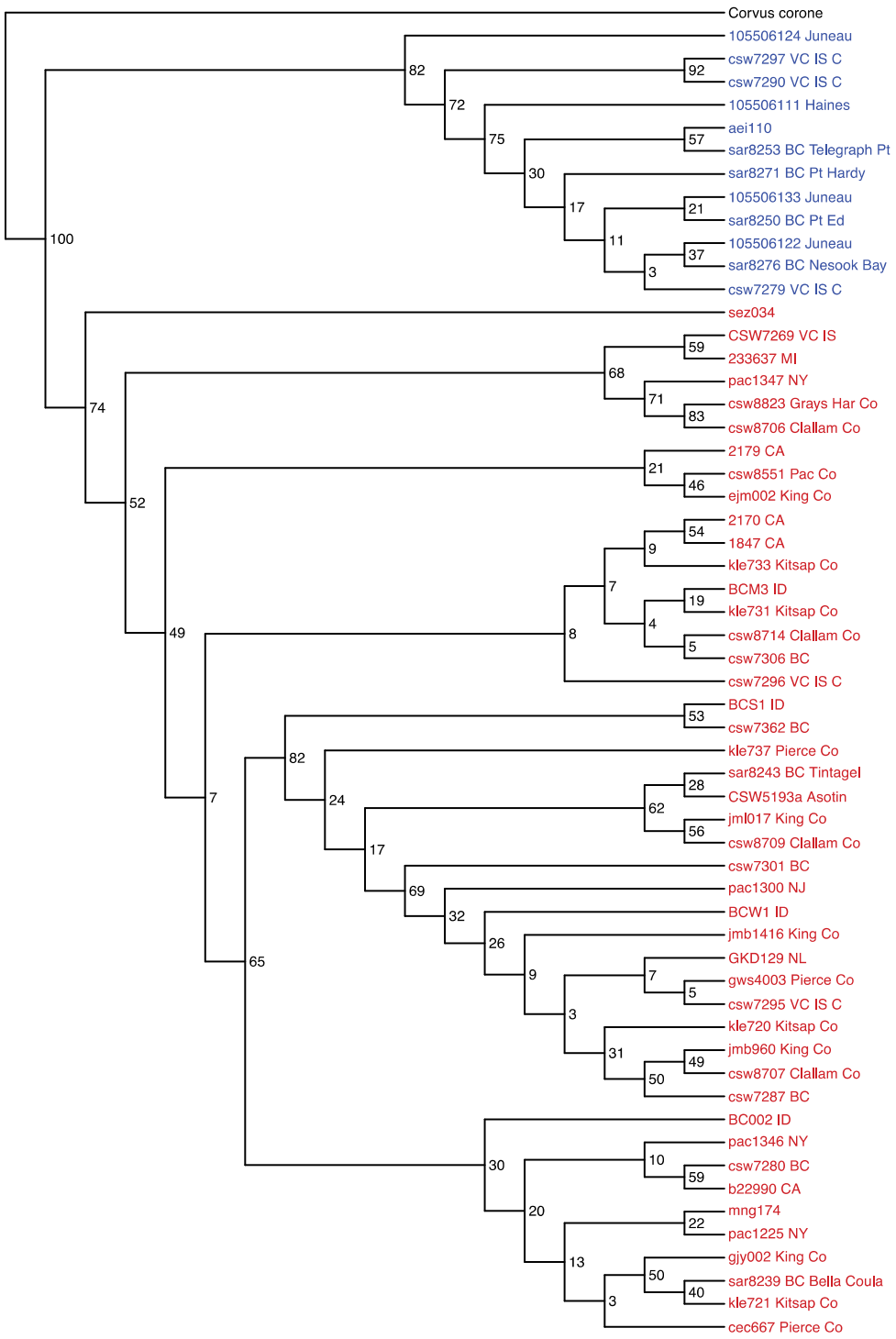

Figure S10. SVDquartets mtDNA ND2 tree of American/Northwestern crows with bootstrap values.

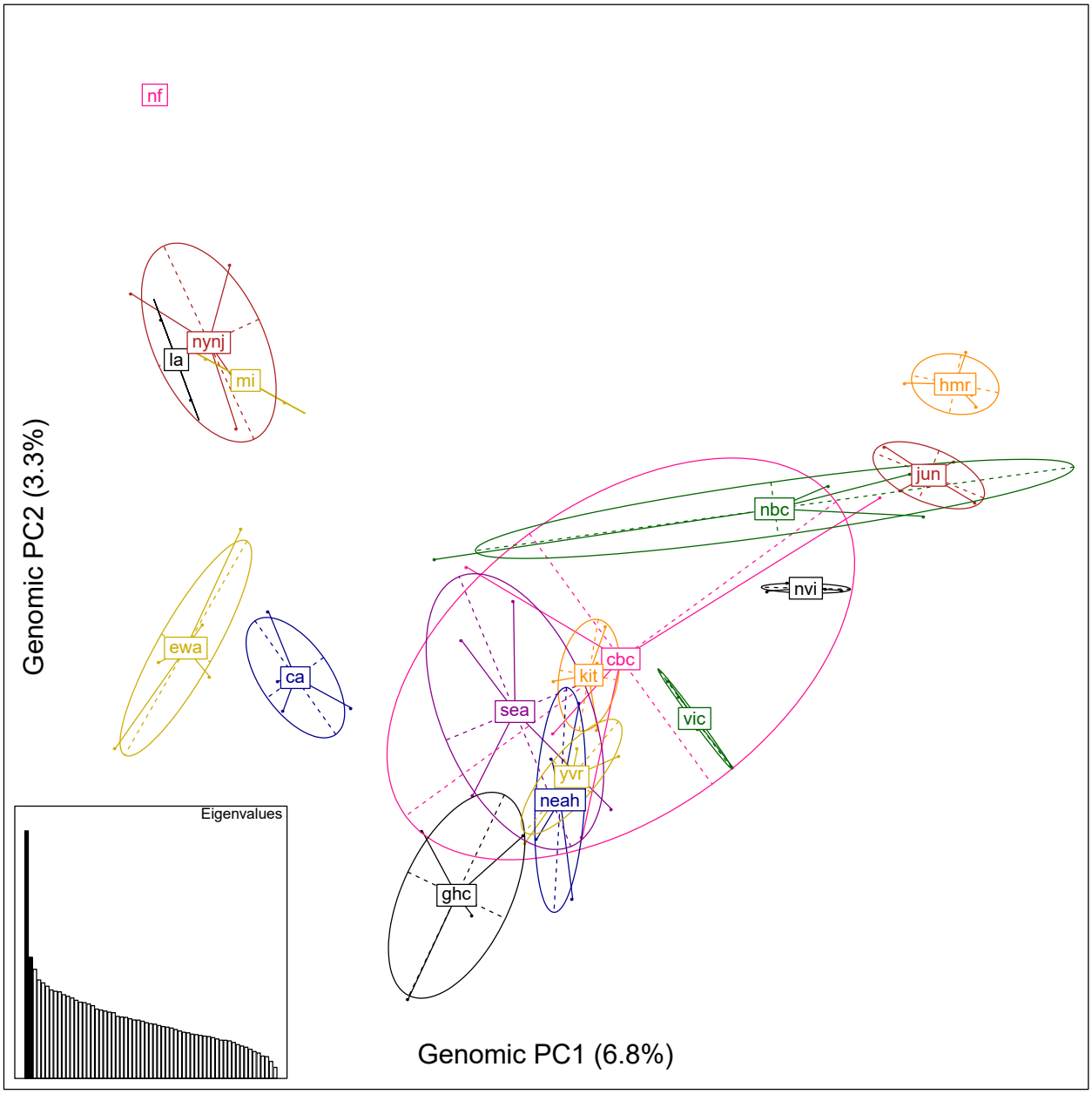

Figure S11. Genomic variation in American/Northwestern crows along the first and second principal components analysis axes (PC1 and PC2). Values in parentheses indicate the amount of genomic variation explained by each principal components axis. For names and locations of the colored localities, please see Table S1 and Figure S1.

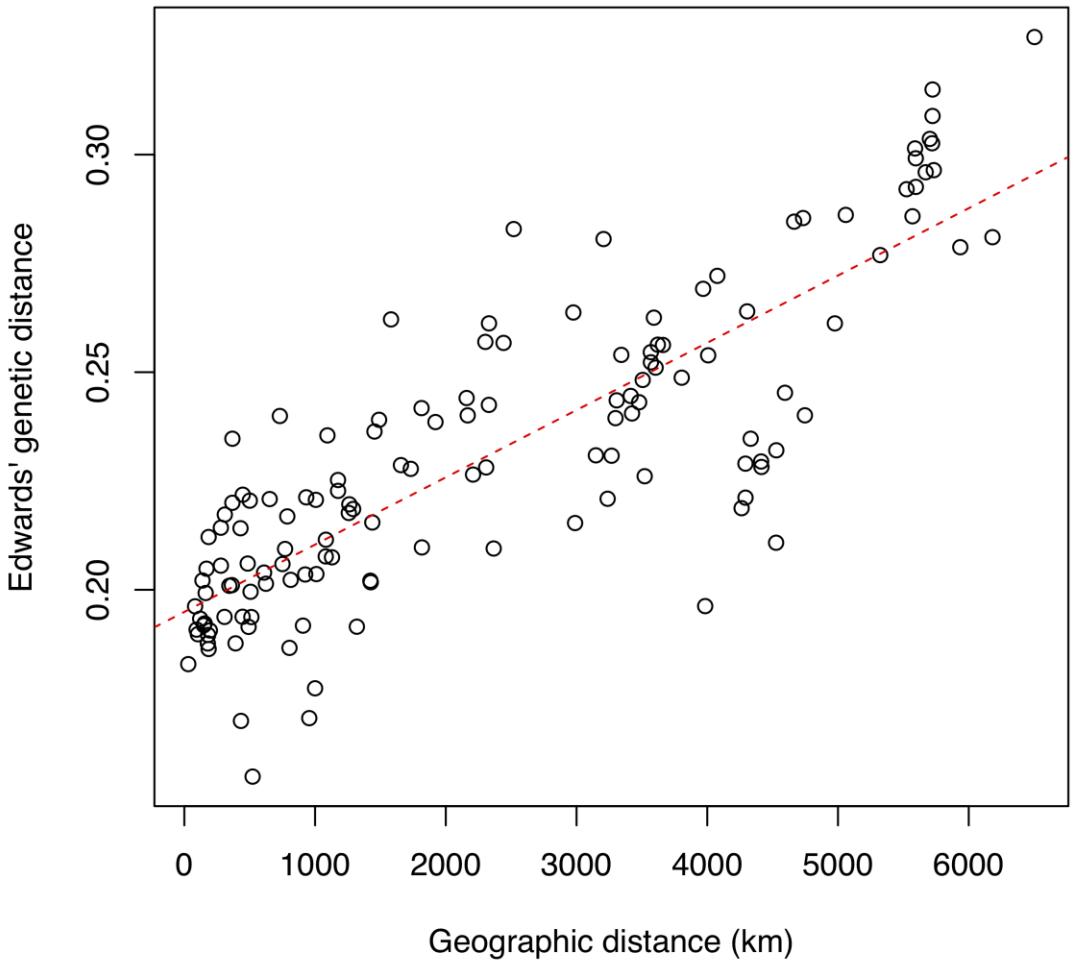

Figure S12. Continent-wide isolation by distance in American/Northwestern crows (Mantel test, n=62, $p < 0.001$).

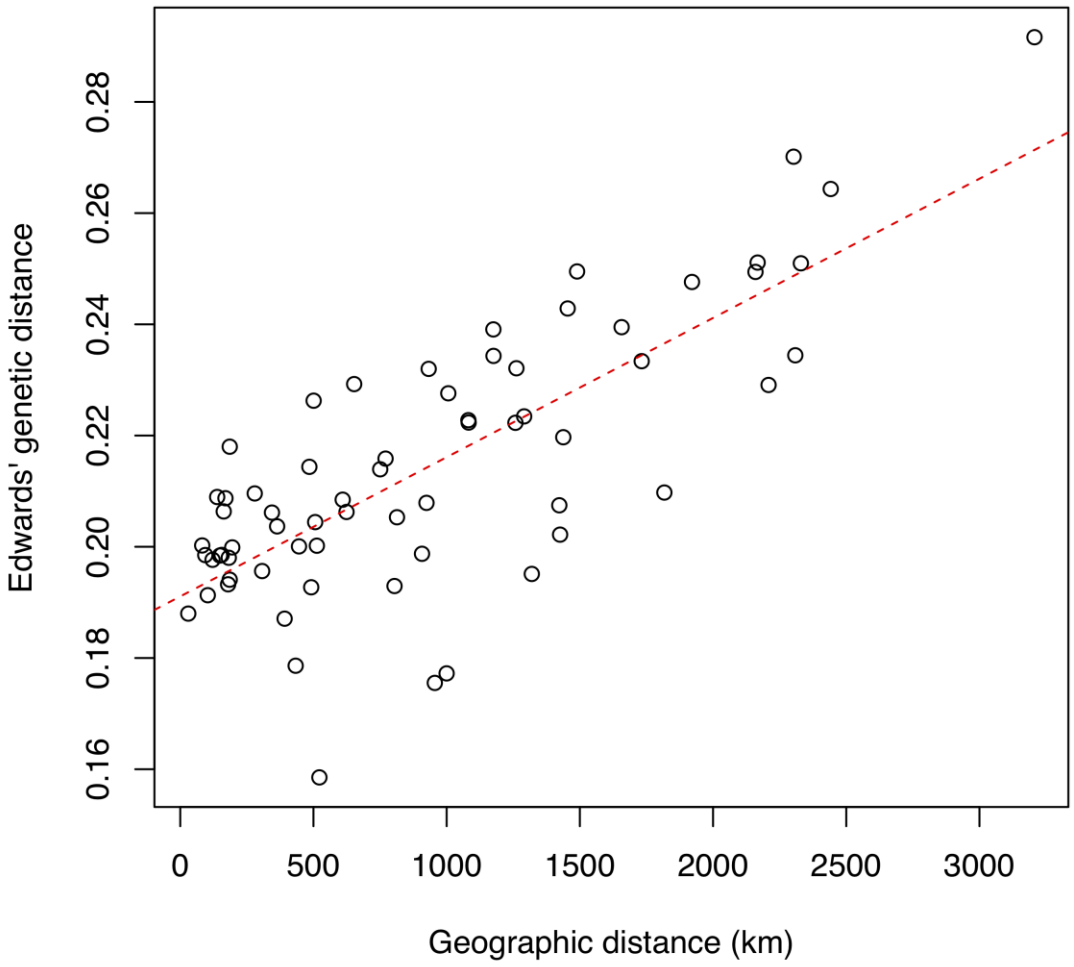

Figure S13. Pacific coastal isolation by distance in American/Northwestern crows (Mantel test, n=48, $p < 0.0005$).

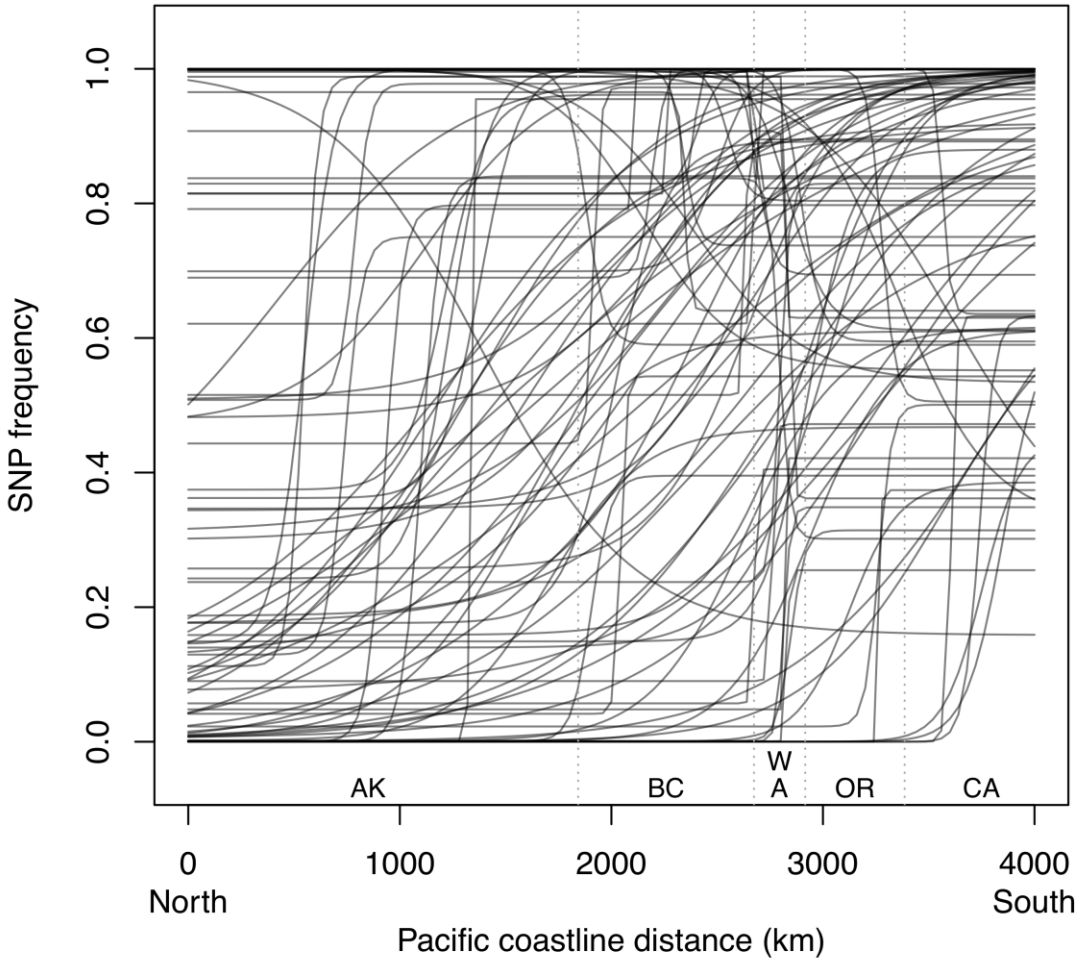

Figure S14. Pacific coastal clines for 94 genomic SNPs with a "strong" clinal pattern ($\Delta AIC_c \geq 6$ between the cline model and the null model).

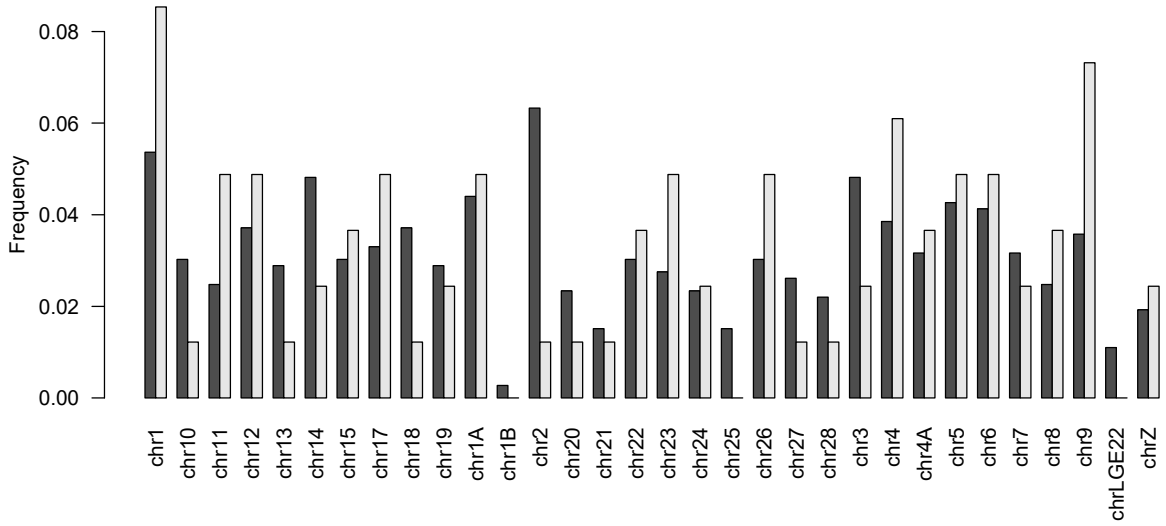

Figure S15. Chromosomal distribution of the subset of 94 genomic SNPs with a "strong" clinal pattern ($\Delta AIC_c \geq 6$ between the cline model and the null model; gray) vs. all 905 SNPs with no missing data along the Pacific coastal transect (black). These distributions did not significantly differ ($X^2 = 22.0$, $p = 0.88$).

Chapter Two

Investigating historical secondary contact between American/Northwestern crows using DNA from museum specimens

ABSTRACT

Northwestern Crows (*Corvus caurinus*) and American Crows (*Corvus brachyrhynhcos*) diverged in the late Pleistocene but today hybridize along >900 km of the Pacific Northwest coast of North America. The two major hypotheses that have been put forward to explain the origin of this broad hybrid zone differ in both mechanism and timing. The first hypothesis is that post-glacial expansion allowed crows to expand from formerly isolated Pleistocene refugia into newly available habitats. The second is that the hybrid zone is a more recent artifact of anthropogenic habitat changes, resulting from the relatively recent forest fragmentation and land use changes associated with European colonization. Our objective was to differentiate between these two hypotheses by sequencing mtDNA from >160 years of museum specimens and placing constraints on the timing of secondary contact within the hybrid zone. Sequencing a 90-bp fragment of mtDNA ND2 diagnostic for American and Northwestern haplogroups, we detected breeding season co-occurrence between American and Northwestern haplogroups at three different localities in 1889-1892, prior to the bulk of European-associated land use changes. We also detected geographic overlap of haplogroups in the southern Puget Sound of Washington in the 1850s, but not all of these individuals were sampled during the breeding season. We did not detect significant changes in haplogroup frequency within localities over time. Overall, our results are most consistent with the hybrid zone resulting from non-anthropogenic habitat changes since the last glacial maximum, or from anthropogenic influences of Native American/First Nations peoples prior to the late 19th century. Although our power to detect mtDNA haplogroup overlap and changes in haplogroup frequency over time were limited by

sample size, using historical museum specimens is a direct and promising way of assessing changes in secondary contact over time.

INTRODUCTION

Northwestern Crows (*Corvus caurinus*) and American Crows (*Corvus brachyrhynhcos*) are closely related and morphologically similar taxa that hybridize extensively along the northwest coast of North America (Slager et al. 2020). Mitochondrial DNA divergence dating suggests that the two mtDNA lineages associated with Northwestern and American crows diverged in the Pleistocene, ~443,000 years ago (Slager et al. 2020). Today, however, today these mtDNA haplotype groups show broad geographic overlap in the Pacific Northwest. Furthermore, SNPs from the nuclear genome indicate a swarm of late-generation hybrids occupying >900 km of Pacific coastline in Washington and British Columbia (Slager et al. 2020).

Two different hypotheses have been put forward to explain the origin of this broad hybrid zone, and these hypotheses differ in both mechanism and timing. The first is that the current hybrid zone is a result of ongoing expansion from formerly isolated Pleistocene refugia (Slager et al. 2020). Consistent with this hypothesis, most of the current hybrid zone occupies terrain that was covered in thick ice sheets during Pleistocene glaciations. Subsequent melting and re-vegetation of the Pacific Northwest coast would have allowed crows to re-colonize this intervening area and experience secondary contact, possibly multiple times.

The second hypothesis is that the Northwestern/American Crow hybrid zone is a more recent artifact of anthropogenic habitat changes (Marzluff and Angell 2005, Haring et al. 2012).

Consistent with this hypothesis, crows are human commensal species and are intelligent urban adapters that thrive in disturbed areas with cleared forests, agriculture, and population centers (Verbeek and Butler 1999, Verbeek and Caffrey 2002). Furthermore, crow populations have been rapidly increasing in urban and agricultural areas (Marzluff et al. 1994, 2001). According to this hypothesis, extensive areas of contiguous forest after de-glaciation but before anthropogenic deforestation would have prevented secondary contact between American and Northwestern crows. Increased habitat heterogeneity associated with deforestation and agriculture following European colonization would have removed barriers to gene flow that existed previously, leading to secondary contact and the opportunity for hybridization (Marzluff and Angell 2005).

One way to directly differentiate between these two alternate hypotheses is to investigate the timing of secondary contact between American/Northwestern crows. Natural history museum collections contain a time series of American/Northwestern crow specimens at a geographic and temporal scale suitable for directly testing whether secondary contact between Northwestern and American crows occurred prior to, during, or after the majority of European-associated land use changes in the Pacific Northwest. However, because individuals of the parental species are not morphologically distinguishable near the hybrid zone (Johnston 1961), it has not been possible to use historical museum specimens to establish the timing of secondary contact using traditional morphological methods. Genetic tools provide a promising way to assess the historic extent of secondary contact between American and Northwestern crows (Slager et al. 2020).

In this study, we investigate the historic extent of secondary contact between American and Northwestern crows in the Pacific Northwest. We sequenced mitochondrial DNA from museum specimens spanning a >160 year time series, including the bulk of European-associated

land use changes in the Pacific Northwest, to differentiate between the above two alternative hypotheses for the age of secondary contact in American/Northwestern crows.

METHODS

Historical samples

To investigate the history of secondary contact between American and Northwestern crows, we obtained toe pad and/or skin clip samples from historical museum specimens (n=37; Table 1). These included five from the 1850s, thirty-one from 1889-1918, and one from an unknown date in the 19th century (ANSP 2875; see Discussion).

Inferring secondary contact

American and Northwestern crows collectively possess two mtDNA ND2 haplogroups that are separated by a mean 1.1% pairwise sequence divergence and 3 fixed differences (Slager et al. 2020). Hybrid American x Northwestern crows can represent either haplogroup, but parental American or Northwestern crows (>98% nuclear genomic ancestry) always represent the respective "American" or "Northwestern" haplogroup (Slager et al. 2020). Thus, we consider co-occurrence of these haplogroups during the breeding season to indicate secondary contact between American and Northwestern crows.

Characterizing seasonal migration and long-distance dispersal

Inferring secondary contact between crow mtDNA haplogroups requires accounting for the length of the breeding season and potential seasonal movements of individuals. Although

Northwestern Crows are not thought to migrate (Verbeek and Butler 1999), at least some American Crows do (Verbeek and Caffrey 2002, Townsend et al. 2018). Geographic patterns of migratory connectivity are poorly characterized for crows in the Pacific Northwest, and it is possible that crows from different haplogroups overlap outside of the breeding season. With this in mind, and in order to conservatively infer secondary contact, all modern crow samples included in this study (Slager et al. 2020) were collected during the breeding season (April-August; Verbeek and Butler 1999, Verbeek and Caffrey 2002). However, some historical samples were collected at other times of year, potentially representing migrants and/or wintering individuals from other populations. American Crows typically initiate fall migration in September, with the last individuals arriving on the breeding grounds by mid-April (Verbeek and Caffrey 2002). To conservatively infer secondary contact among breeding crows, we only included April-August individuals in our analyses.

To assess potential dispersal or seasonal migration of crows in the Pacific Northwest, we queried the North American database of mark-recapture data for American and Northwestern crows (USGS Bird Banding Laboratory 2018). We excluded records with latitude or longitude of 0 or NA and assigned banding and encounter locations to the nearest Bird Conservation Region (BCR; Bird Studies Canada and NABCI 2014) using a custom R script. We retained records for which the banding and/or encounter location was assigned to the Northern Pacific Rainforest BCR. This BCR encompasses the Pacific slope from northern California to the Seward Peninsula of Alaska. In Washington and British Columbia, the eastern boundary of this BCR coincides with the crest of the Cascades and Coast Mountains, ranges that likely represent a geographic barrier to east-west gene flow in American/Northwestern crows (Slager et al. 2020). To search for instances of dispersal or seasonal migration at scales relevant to our sampling scheme, we

filtered for records that had a band-encounter distance of >30 miles or that crossed the boundary of the Northern Pacific Rainforest BCR. We manually examined records in the resulting dataset to verify geo-referencing. We discarded one record that only ostensibly crossed the Northern Pacific Rainforest BCR boundary because its encounter coordinates were imprecisely plotted as the provincial centroid of British Columbia**.** For records of special interest, we obtained and verified original scanned copies of band-encounter reports (Danny Bystrak, pers. comm.).

Extracting historical DNA

We extracted historical DNA in a clean pre-PCR lab used primarily for anthropological extractions. To remove potential surface contaminants prior to mincing samples, we conducted two wash cycles. For each wash cycle, we vortexed the sample in 100% EtOH for 2 pulses and 10 seconds, and then pipette-washed the sample with 500 mL of 100% EtOH. We then extracted DNA following the MacManes salt extraction protocol (MacManes 2013).

Identifying mtDNA haplogroups from historical samples

To assign historical samples to American and Northwestern mtDNA haplogroups, we amplified a haptogroup-diagnostic 90-bp mtDNA ND2 fragment using custom-designed primers (crow_int_ND2_F4: AAA GCA CCT TCA CTT AGC AC; crow_int_ND2_R1: GAG TCA TTT TGG GAG GAA GCC). This fragment included one of the three fixed differences between the full-length American and Northwestern ND2 haplogroups (position 769) and a second highly informative SNP (position 729) that diagnosed American and Northwestern haplogroups in 258 of 259 full-length, 1041-bp ND2 samples (but not sample sez034 from Slager et al. 2020).

Amplifying and sequencing historical mtDNA

To amplify the diagnostic mtDNA ND2 fragment, we used 25 μL PCR reactions as follows: 95°C for 5 min, 35 cycles of (94°C for 1 min, 56°C for 1 min, 72°C for 1 min), 10 min at 72°C, 4°C hold. We sent PCR products to the Seattle facility of Genewiz (South Plainfield, NJ) for cleanup and sequencing, and we unambiguously aligned complementary strands with Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI).

Modern samples

To characterize the mtDNA signal of modern secondary contact, we obtained specimen information, mtDNA ND2 sequences, and mtDNA haplogroups of modern samples (1992-2014; n=176; Table 2) from a prior study of the modern American/Northwestern crow hybrid zone (Slager et al. 2020). Some of these modern mtDNA samples are from specimens with known hybrid/parental ancestry based on nuclear genomic SNPs (Slager et al. 2020).

Historical vs. modern comparisons

To compare mtDNA haplogroup proportions and the extent of secondary contact between historical and modern periods, we conducted X^2 tests in R, simulating p-values with 2,000 Monte-Carlo replicates (Hope 1968). We conducted these comparisons between timepoints at localities where sufficient sample sizes were available for comparing between timepoints. In the southern Puget Sound, we made all three possible pairwise comparisons among 1850s samples from Fort Steilacoom, 1890s samples from Nisqually, and modern samples from the Tacoma area (*tac* locality). In the Vancouver area, we compared modern samples (*yvr* locality) with 1889-1892 samples, using several chi-squared tests to conduct a sensitivity analysis of including

locations successively farther east along the lower Fraser River. In the Victoria area, we compared 1892 samples with modern samples (*vic* locality). In eastern British Columbia, we compared 1892 samples with modern samples (*ebc* locality).

Census data

For an index of European-associated urbanization we used official government census data for Washington (1850-2000; United States Census Bureau 2002, 2010) and British Columbia (1851-2001; Statistics Canada 2006).

RESULTS

mtDNA sequences

We were able to sequence a 90-bp fragment of mtDNA ND2 from 36 of 37 historical specimens, and this fragment was diagnostic for identifying American vs. Northwestern mtDNA (Table 1). Collectively, these 36 sequences were represented by two 90-bp haplotypes that were identical at 88 bp, with two concordant SNPs that identified 19 crows representing the Northwestern mtDNA haplogroup and 17 crows representing the American mtDNA haplogroup. These same two 90-bp haplotypes also predominate in modern American/Northwestern crow samples, with the historical American (n=157) and Northwestern (n=95) haplotypes together comprising 252 of 259 modern, continent-wide sequences (97.3%) at this 90-bp fragment (Slager et al. 2020).

Band-encounter data

Of 2,247 American/Northwestern Crow band encounters, 173 had a banding and/or encounter location assigned to the Northern Pacific Rainforest BCR. Of these, thirteen had band-encounter locations separated by >30 miles, seven by > 50 miles, five by > 100 miles, two by > 300 miles, and one by > 500 miles. One of these crows (#48519268) crossed the Northern Pacific Rainforest BCR boundary. This individual was banded during the breeding season east of the Coast Mountains at Stum Lake, British Columbia on 17 June 1950 and encountered during the non-breeding season in November 1950 west of the Cascades at Whidbey Island, Washington (see also Campbell et al. 1997). The other 12 band-encounter records included 11 within coastal Washington and/or coastal British Columbia and one 567-mile movement within coastal Alaska, from Homer on 24 March 2007 to Haines on 10 July 2008. No records meeting the filtering criteria had banding or encounter localities in Oregon or California, despite the Northern Pacific Rainforest region extending south into these states.

Co-occurrence of mtDNA haplotypes across time and space

Modern crows (1992-2014) In 176 modern crows, American and Northwestern mtDNA haplogroups co-occurred during the breeding season at multiple localities west of the Coast Mountains and Cascades from Bella Coola, British Columbia to the southern Puget Sound of Washington (Figure 1).

Centennial crows (1889-1918) Among 31 centennial crows, 19 were breeders and 12 were potential non-breeders (Figure 1). During this time period, members of the American and Northwestern haplogroups co-occurred in the breeding season at Nisqually in the southern Puget

Sound, along the Fraser River Delta near Vancouver, and at Lac La Hache in eastern British Columbia (Figure 1).

Sesquicentennial crows (1854-1856) We characterized the mtDNA haplogroup assignment for 4 crows from the 1850s. Two of these were breeders from the southern Puget Sound (Fort Steilacoom) and both were assigned to the Northwestern haplogroup (Figure 1). The two birds collected outside the breeding season represented the American haplogroup. One was from the southwestern outer coast of Washington, and one was from Fort Steilacoom (Figure 1). An additional specimen from Fort Steilacoom, possibly also collected in the 1850s, represented the Northwestern haplogroup (ANSP 2875; see Discussion).

Comparing haplogroup proportions within localities across timepoints

Within localities, there were no differences in mtDNA haplogroup proportions between breeding crows across modern, centennial, and sesquicentennial time points. This was true in the southern Puget Sound for sesquicentennial vs. centennial (Fort Steilacoom vs. Nisqually $X^2 = 2.06$, $p = 0.43$), centennial vs. modern (Nisqually vs. *tac* $X^2 = 2.12$, $p = 0.27$), and sesquicentennial vs. modern (Fort Steilacoom vs. *tac* $X^2 = 6.52$, $p = 0.06$). Differences between centennial and modern haplogroup proportions in Victoria or eastern British Columbia were also not significant (Victoria $X^2 = 1.8$, $p = 0.52$; eastern British Columbia $X^2 = 2.44$; $p = 0.32$). Haplogroup proportions also did not significantly differ between breeding samples of centennial vs. modern crows in the Vancouver area and along the lower Fraser River, regardless of how far east from Vancouver samples were included in the comparison (Vancouver-area only $X^2 = 4.5$, $p = 0.07$; including Mt. Lehman $X^2 = 2.25$, $p = 0.25$; including Hatzic $X^2 = 2.85$, $p = 0.26$;

including Chilliwack $X^2 = 3.75$, $p = 0.08$; including Agassiz $X^2 = 4.34$, $p = 0.07$; including Hope $X^2 = 3.67$, $p = 0.10$).

DISCUSSION

Over a century of secondary contact between American and Northwestern crows

Our finding of overlap between American and Northwestern mtDNA haplotype groups at three localities in 1889-1892 during the breeding season shows that secondary contact between American and Northwestern crows occurred prior to most European-associated human population growth in the Pacific Northwest. If European anthropogenic changes did drive an increase in secondary contact during the breeding season, then this could only have occurred very rapidly, prior to ~1890. Overall, our data are more consistent with the idea that the present biogeography of the American/Northwestern crow hybrid zone results from non-anthropogenic habitat changes since the last glacial maximum, or from anthropogenic influences of Native American/First Nations peoples prior to the late 19th century. It is also possible that recent anthropogenic factors did not increase the geographic extent of secondary contact, but broke down behavioral reproductive isolating mechanisms that were more effective prior to European-associated land use changes.

The spatial and temporal extent of secondary contact we report is conservative

We found evidence of century-long secondary contact between American and Northwestern crows despite the limitation that our approach may underestimate the magnitude of secondary contact. First, a limited number of historical museum specimens were available, and

few historical localities contained multiple individuals, limiting our power to detect co-occurrence of American and Northwestern mtDNA haplogroups across space and time. Second, hybrid American x Northwestern crows with intermediate hybrid indices can represent either the American or Northwestern mtDNA haplogroup (Slager et al. 2020). Thus, at historical localities containing only one sampled haplogroup (e.g., Victoria), some or all crows could have been hybrids, and we would not have detected this stage of secondary contact using our binary, haploid, non-recombining mtDNA marker. Finally, crows in the coastal Pacific Northwest are thought to generally be non-migratory (Verbeek and Butler 1999). Thus, some or all of the "non-breeders" that we excluded from our formal comparisons may have been resident birds. If so, had these been sampled during the breeding season, our power to detect secondary contact would have increased.

Secondary contact preceded a century of anthropogenically driven numerical increases in crow populations

Our finding of secondary contact between crows with American and Northwestern haplogroups as early as ~1890 also counters the idea that genetic "swamping" of Northwestern Crows by American Crows results primarily from more recent numerical increases in crow populations (Marzluff and Angell 2005). This hypothesis seems to stem from the observation that crow population densities in the Pacific Northwest tend to track human population densities, with smaller territory sizes and denser populations in urban areas like Seattle compared to those in more wild areas like the Olympic peninsula (Marzluff et al. 2001). As such, crow population trends have also tracked that of humans, increasing in rapidly growing urban areas, while remaining more constant in exurban areas and wildlands (Marzluff et al. 2001). Crows in the

Pacific Northwest rapidly increased in urban areas in the second half of the 20th century, including a 57% increase in Seattle from 1960-1996 (Marzluff et al. 2001) and a >10-fold increase in Vancouver from 1957-1993 (Campbell et al. 1997). An increase in numbers was also reported in the early 20th century (Brooks 1925). However, the secondary contact we documented at three localities in the late 19th century predates these increases, and indeed most European-associated population growth in the Pacific Northwest. The 1890-1891 census population of Washington and British Columbia was just 4.6% that of the 2000-2001 census population (Figure 2).

Likelihood of hybridization in 1889-1892 given breeding season secondary contact

Co-occurrence of American and Northwestern crows and/or their backcrosses during the breeding season is necessary for hybridization, but does not constitute direct evidence of hybridization (Crispo et al. 2011). While our finding of co-occurring mtDNA haplogroups in the late 19th century indicates that crows representing different haplogroups had the opportunity to hybridize, our results cannot definitively rule out assortative mating (e.g., Brooks 1942). However, we do consider it unlikely that these co-occurring crows mated assortatively. In modern sampling, at eight localities where both American and Northwestern haplogroups co-occurred during the breeding season, all eight localities had at least some hybrid crows with admixed nuclear DNA, with seven of these eight localities having only hybrids (Slager et al. 2020). Moreover, we are not aware of any reproductive isolating mechanisms that would have likely been in place during the 19th century but not today.

Potential secondary contact and hybridization in the 1850s, given non-breeding season secondary contact

Although we did not uncover direct mtDNA evidence for breeding season secondary contact at Fort Steilacoom in the 1850s, we did find crows with Northwestern haplotypes there during the breeding season and a crow with the American haplogroup there during the non-breeding season. Even if the crow with the American haplotype was only a winter transient, its presence still illustrates that crows with American haplotypes did occur along the Puget Sound in the 1850s at some point in the year. Moreover, because crows in the coastal Pacific Northwest are mostly non-migratory and because pair bonds typically form during the non-breeding season (Verbeek and Butler 1999), the opportunity for mixed pair formation was clearly already present in the 1850s. This is also true of crows sampled on Vashon Island in 1918, where both haplogroups also co-occurred in the non-breeding season.

American and Northwestern crows prior to European colonization

Secondary contact between American and Northwestern crows may have occurred well prior to European colonization of the Pacific Northwest. In Washington in the 1850s, crows were already abundant human commensals that fed on discarded food at indigenous settlements (Suckley and Cooper 1860), suggesting a long history of synanthropism with Native American/First Nations peoples prior to European colonization (Verbeek and Butler 1999). Today in the forested wildlands of the Olympic Peninsula where crows are generally rare, they frequent small wooded campgrounds and will fly tens of kilometers to feed on anthropogenic food (Marzluff et al. 2001). These wide-ranging habits, combined with occasional long-distance dispersal evident from band-encounter distances of >100 km within Washington and British

Columbia, provide a ready mechanism for contact between maritime and inland crows prior to European colonization and related land use changes. Given their ability to disperse across forest and the presence of suitable and contiguous crow habitat along rivers and coastlines, the extensive deforestation and agriculture that followed European colonization were likely never prerequisites for gene flow between American and Northwestern crows.

Crow movements across the Cascades and Coast Mountains

Our survey of banding-encounter data revealed a crow moving from eastern British Columbia in June to the Puget Sound in November. This record illustrates that crows sometimes move seasonally across the Coast Mountains/Cascades, despite its apparent significance as a barrier to east-west gene flow during the breeding season (Slager et al. 2020). This and multiple instances of longer-distance dispersal within the hybrid/overlap zone illustrate that non-breeding crow populations in western Washington and western British Columbia may include migrants or wintering birds from elsewhere, affirming our methodological approach of using only breeding season samples to conservatively assess secondary contact.

American and Northwestern crow mtDNA haplogroups co-occurred during the breeding season a century ago in eastern British Columbia, where no evidence for haplogroup overlap was noted in a more extensive modern survey (Slager et al. 2020). Coincidentally, Lac La Hache in eastern British Columbia, where we detected the centennial breeding crow with a Northwestern haplotype, is only ~120 km "as the crow flies" from Stum Lake, the June 1950 banding location of a crow re-encountered in November at the Puget Sound. Major river valleys near low passes such as Skeena and Fraser may represent crow migration/dispersal routes across the Coast Mountains and Cascades (Campbell et al. 1997, Slager et al. 2020). Indeed, crows of unknown

identity reportedly follow fish spawning runs up to 120 km upriver on the Fraser, Skeena, and Nass (Campbell et al. 1997). By contrast, in the large number of modern samples, the easternmost breeding crow with a Northwestern haplotype was at Hope, British Columbia, in the Fraser River valley along the southern flank of the Coast Mountains (Slager et al. 2020).

Strengths and limitations of approach and markers

Natural history museum specimens are a unique and powerful resource for longitudinal studies of secondary contact and hybridization between cryptic taxa, as long as the time series of available specimens matches the time scale of the research questions, as in this study. Although the ability to sequence genetic information from mid-19th century museum specimens constitutes an amazing resource for longitudinal studies, sample sizes are limited to what is extant in collections. In our study, this limited both our power to detect mitochondrial haplotype overlap at a given date/locality and our power to detect small changes in the frequencies of haplotypes over time.

Mitochondrial DNA markers have the advantage of being high in copy number, and the short, yet diagnostic fragment we amplified had the advantage of being short enough to sequence from >160 year old degraded DNA. Our ongoing research using large numbers of short nuclear DNA reads from whole-genome shotgun sequencing will allow for calculating a hybrid index for individual historical specimens, enabling a finer-scale look at hybridization itself, rather than secondary contact, as in this present study.

Taxonomic considerations

The 1850s samples we obtained represent all 5 of the original series of 8 cotypes for *Corvus caurinus* (Baird 1858) that are known to be extant (Deignan 1961), and we successfully sequenced mtDNA from four (Table 1). One of the remaining three cotypes (#10309) was likely destroyed in 1923 by the Great Kantō Earthquake and associated fires (Isao Nishiumi, pers. comm., Deignan 1961). Another (#10312) was presumably destroyed by the 1978 fire at the Museum at Lisbon (Judite Alves, pers. comm., Deignan 1961). The whereabouts of the third missing cotype (#10308) are unknown, but it is possibly the same specimen as the old, undated skin ANSP 2875 with a locality of Fort Steilacoom (Nate Rice, pers. comm., Deignan 1961).

All eight of the original cotypes for *Corvus caurinus* were collected within the modern American/Northwestern crow hybrid zone, in areas where pure Northwestern Crows do not currently breed (Baird 1858, Slager et al. 2020). Furthermore, of the 4 *Corvus caurinus* cotypes from which we successfully sequenced mtDNA, 2 had mtDNA from the Northwestern haplogroup but 2 had mtDNA from the American haplogroup. Therefore, these two birds from the American haplogroup were either hybrids or American crows, whereas the other two individuals were either hybrids or Northwestern Crows. If all the type specimens for *Corvus caurinus* had American haplotype mtDNA, then under ICZN rules, *Corvus caurinus* would be regarded as an unavailable taxonomic name (if hybrid) or as a junior synonym of *brachyrhynchos* (if pure American). However, the uncertainty regarding the hybrid index of these individual specimens and the mix of mtDNA haplogroups from different specimens makes the taxonomic situation complicated. Furthermore, both cotypes we sequenced with the American haplogroup were collected outside the breeding season, and it is thus possible that they had dispersed or migrated from a distant breeding area. Overall, these results point to the need

for additional taxonomic work involving sequencing of nuclear genomic DNA from these type specimens (Slager et al., in progress), and underscore the importance of selecting type material from 1) a single locality and 2) during the breeding season in mobile organisms like birds.

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FIGURES AND TABLES

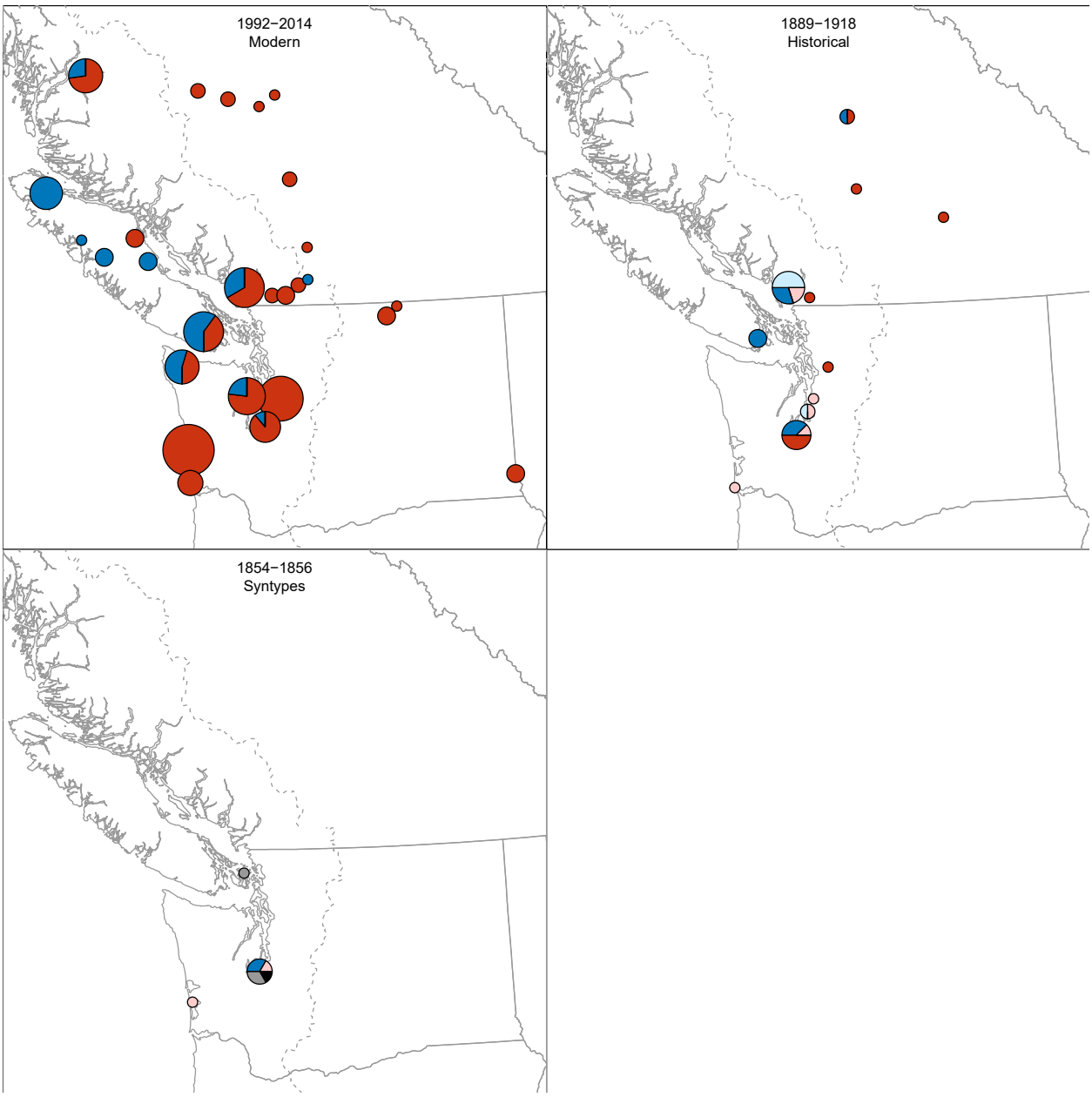

Figure 1. Map of historical and modern samples of American and Northwestern crows. Red and blue indicate American and Northwestern breeding season samples, respectively, and pink and light blue indicate samples outside the breeding season. Black and gray colors indicate unsequenced breeding season and non-breeding season cotypes, respectively. Pie size is proportional to sample size at each locality. Mystery sample ANSP 2875 is mentioned in the text but not shown here.

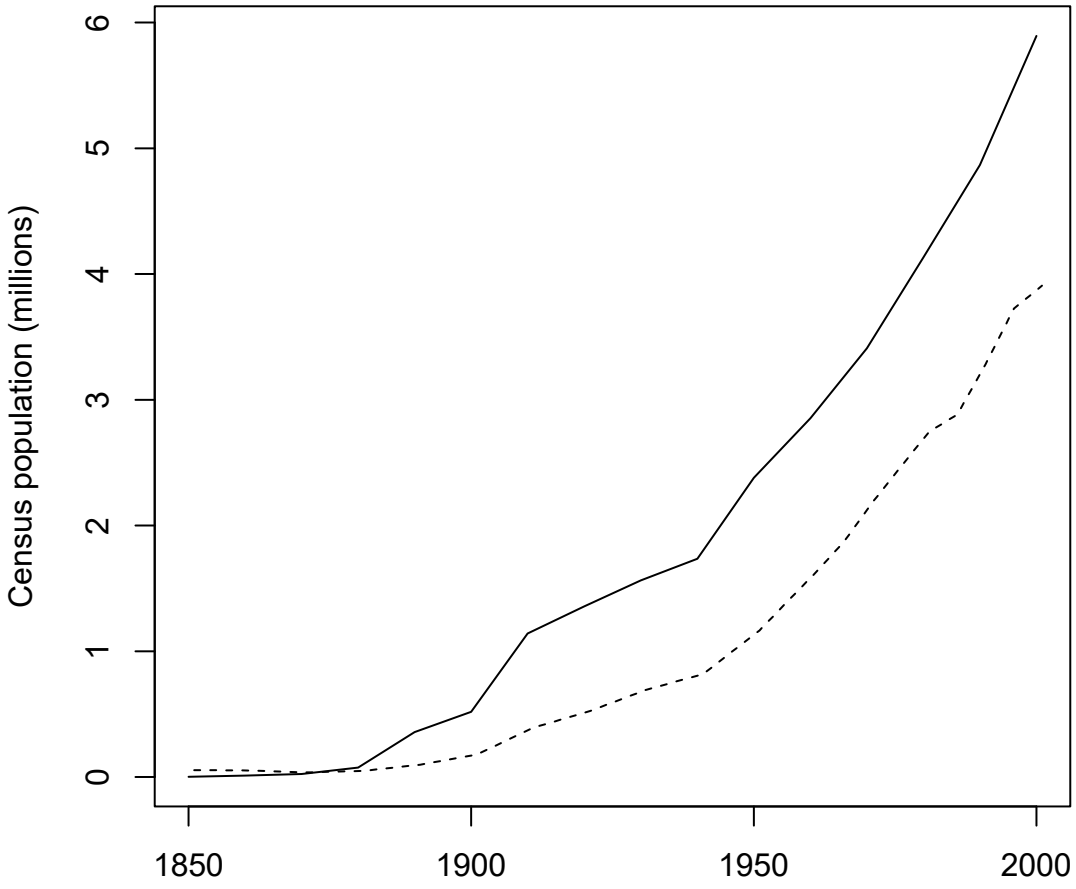

Figure 2. Government (human) census data from Washington (solid line) and British Columbia (dashed line).

Table 1. List of modern samples (from Slager et al., 2020; n=176).

local-ity	specific locality	lat	long	date	mus-eum no.	field no.	mt DNA
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117914	sar8229	A
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117915	sar8230	A
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117916	sar8231	N
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.8	10-May-2014	UWBM 117917	sar8232	N
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.8	10-May-2014	UWBM 117918	sar8233	A
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.8	10-May-2014	UWBM 117919	sar8234	A
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117920	sar8235	N
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117921	sar8236	A
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117922	sar8237	A
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117923	sar8238	A
cbc	BRITISH COLUMBIA: Bella Coola	52.4	-126.7	10-May-2014	UWBM 117924	sar8239	A
ebc	BRITISH COLUMBIA: Williams Lake	52.0	-122.3	09-May-2014	UWBM 117910	sar8225	A
ebc	BRITISH COLUMBIA: Riske Creek	52.0	-122.6	09-May-2014	UWBM 117911	sar8226	A
ebc	BRITISH COLUMBIA: Puntzi Lake	52.1	-124.1	09-May-2014	UWBM 117912	sar8227	A
ebc	BRITISH COLUMBIA: Puntzi Lake	52.1	-124.1	09-May-2014	UWBM 117913	sar8228	A
ebc	BRITISH COLUMBIA: Alexis Creek	52.1	-123.4	11-May-2014	UWBM 117925	sar8240	A
ebc	BRITISH COLUMBIA: Alexis Creek	52.1	-123.4	11-May-2014	UWBM 117926	sar8241	A
ebc	BRITISH COLUMBIA: Vanderhoof	54.0	-123.9	11-May-2014	UWBM 117927	sar8242	A

ebc	BRITISH COLUMBIA: Tintagel	54.2	-125.6	11-May-2014	UWBM 117928	sar8243	A
ebc	BRITISH COLUMBIA: Tintagel	54.2	-125.6	11-May-2014	UWBM 117929	sar8244	A
ebc	BRITISH COLUMBIA: Tintagel	54.2	-125.6	11-May-2014	UWBM 117930	sar8245	A
ebc	BRITISH COLUMBIA: Smithers	54.9	-127.3	13-May-2014	UWBM 117941	sar8256	A
ebc	BRITISH COLUMBIA: Houston	54.4	-126.7	13-May-2014	UWBM 117942	sar8257	A
ebc	BRITISH COLUMBIA: Quesnel	53.0	-122.5	14-May-2014	UWBM 117943	sar8258	A
ebc	BRITISH COLUMBIA: Quesnel	53.0	-122.5	14-May-2014	UWBM 117944	sar8259	A
ebc	BRITISH COLUMBIA: Pavilion	50.9	-121.8	14-May-2014	UWBM 117945	sar8260	A
ebc	BRITISH COLUMBIA: Pavilion	50.9	-121.8	15-May-2014	UWBM 117946	sar8261	A
ebc	BRITISH COLUMBIA: Boston Bar	49.8	-121.4	15-May-2014	UWBM 117947	sar8262	A
ewa	WASHINGTON: Okanogan Co.; Oroville	48.9	-119.5	17-Jul-1997	UWBM 58553	plg200	A
ewa	WASHINGTON: Asotin Co.; Asotin	46.0	-117.3	21-Jun-1995	UWBM 59039	csw5193a	A
ewa	WASHINGTON: Asotin Co.; Asotin	46.1	-117.3	20-Jun-1995	UWBM 59059	sar7000	A
ewa	WASHINGTON: Asotin Co.; Asotin	46.0	-117.4	21-Jun-1995	UWBM 59089	svd982	A
ewa	WASHINGTON: Okanogan Co.; Loomis	48.7	-119.7	01-Jun-1999	UWBM 62080	smb77	A
ewa	WASHINGTON: Okanogan Co.; Loomis	48.7	-119.7	01-Jun-1999	UWBM 62081	smb78	A
ewa	WASHINGTON: Okanogan Co.; Loomis	48.7	-119.7	01-Jun-1999	UWBM 62082	smb79	A
ghc	WASHINGTON: Pacific Co.; Bay Center	46.7	-123.9	28-Apr-2013	UWBM 117091	csw8543	A
ghc	WASHINGTON: Pacific Co.; Bay Center	46.7	-123.9	28-Apr-2013	UWBM 117092	csw8544	A
ghc	WASHINGTON: Pacific Co.; Bay Center	46.7	-123.9	28-Apr-2013	UWBM 117093	csw8545	A

ghc	WASHINGTON: Pacific Co.; Bay Center	46.6	-124.0	28-Apr-2013	UWBM 117094	csw8546	A
ghc	WASHINGTON: Pacific Co.; Long Beach	46.4	-124.1	28-Apr-2013	UWBM 117095	csw8547	A
ghc	WASHINGTON: Pacific Co.; Long Beach	46.4	-124.1	28-Apr-2013	UWBM 117096	csw8548	A
ghc	WASHINGTON: Pacific Co.; Long Beach	46.4	-124.1	28-Apr-2013	UWBM 117097	csw8549	A
ghc	WASHINGTON: Pacific Co.; Long Beach	46.5	-124.1	28-Apr-2013	UWBM 117098	csw8550	A
ghc	WASHINGTON: Pacific Co.; Long Beach	46.5	-124.1	28-Apr-2013	UWBM 117099	csw8551	A
ghc	WASHINGTON: Pacific Co.; Long Beach	46.5	-124.1	28-Apr-2013	UWBM 117100	csw8552	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117101	csw8602	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117102	csw8603	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117103	csw8604	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117104	csw8605	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117105	csw8606	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117106	csw8607	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117107	csw8608	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117108	csw8609	A
ghc	WASHINGTON: Pacific Co.; Leadbetter Point	46.6	-124.1	May-2013	UWBM 117109	csw8610	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117898	csw8821	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117899	csw8814	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117900	csw8815	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117901	csw8816	A

ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117902	csw8817	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117903	csw8818	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117904	csw8819	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117905	csw8820	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117906	csw8822	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117907	csw8823	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117908	csw8824	A
ghc	WASHINGTON: Grays Harbor Co.; Grayland	46.8	-124.1	10-Jun-2014	UWBM 117909	csw8825	A
kit	WASHINGTON: Kitsap Co.; Poulsbo	47.7	-122.6	11-Jun-2013	UWBM 117848	kle720	A
kit	WASHINGTON: Kitsap Co.; Kingston	47.9	-122.5	09-Jun-2013	UWBM 117849	kle719	N
kit	WASHINGTON: Kitsap Co.; Port Orchard	47.5	-122.6	15-Jun-2013	UWBM 117853	kle723	N
kit	WASHINGTON: Kitsap Co.; Silverdale	47.6	-122.7	18-Jun-2013	UWBM 117854	kle722	N
kit	WASHINGTON: Kitsap Co.; Bremerton	47.6	-122.6	15-Jun-2013	UWBM 117855	kle721	A
kit	WASHINGTON: Kitsap Co.; Poulsbo	47.7	-122.6	08-Jun-2013	UWBM 117856	kle718	A
kit	WASHINGTON: Kitsap Co.; Kingston	47.8	-122.6	29-Jun-2013	UWBM 117859	kle729	A
kit	WASHINGTON: Kitsap Co.; Bremerton	47.6	-122.6	08-Jul-2005	UWBM 117861	kle731	A
kit	WASHINGTON: Kitsap Co.; Bremerton	47.6	-122.6	02-Jul-2013	UWBM 117862	kle732	A
kit	WASHINGTON: Kitsap Co.; Port Orchard	47.5	-122.6	26-Jun-2013	UWBM 117863	kle733	A
kit	WASHINGTON: Kitsap Co.; Silverdale	47.7	-122.7	01-Jul-2013	UWBM 117864	kle734	A
kit	WASHINGTON: Kitsap Co.; Silverdale	47.7	-122.7	10-Jun-2005	UWBM 117865	kle735	A

kit	WASHINGTON: Kitsap Co.; Seabeck	47.6	-122.8	06-Jul-2005	UWBM 117866	kle736	A
neah	WASHINGTON: Clallam Co.; Lake Crescent	48.1	-123.8	02-Apr-2014	UWBM 117871	csw8704	A
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.4	02-Apr-2014	UWBM 117872	csw8705	N
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	02-Apr-2014	UWBM 117873	csw8706	A
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	02-Apr-2014	UWBM 117874	csw8707	A
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	02-Apr-2014	UWBM 117875	csw8708	N
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.4	03-Apr-2014	UWBM 117876	csw8709	A
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	03-Apr-2014	UWBM 117877	csw8710	N
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	03-Apr-2014	UWBM 117878	csw8711	N
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	03-Apr-2014	UWBM 117879	csw8712	N
neah	WASHINGTON: Clallam Co.; between Sekiu and Neah Bay	48.3	-124.5	03-Apr-2014	UWBM 117880	csw8713	N
neah	WASHINGTON: Clallam Co.; Sappho	48.1	-124.3	02-Apr-2014	UWBM 117881	csw8714	A
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117951	sar8266	N
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117952	sar8267	N
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117953	sar8268	N
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117954	sar8269	N

nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117955	sar8270	N
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117956	sar8271	N
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117957	sar8272	N
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117958	sar8273	N
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117959	sar8274	N
nvi	BRITISH COLUMBIA: Port Hardy	50.6	-127.2	16-May-2014	UWBM 117960	sar8275	N
nvi	BRITISH COLUMBIA: Gold River, Nesook Bay	49.8	-126.4	17-May-2014	UWBM 117961	sar8276	N
nvi	BRITISH COLUMBIA: Gold River, Nesook Bay	49.8	-126.4	17-May-2014	UWBM 117962	sar8277	N
nvi	BRITISH COLUMBIA: Gold River, Nesook Bay	49.8	-126.4	17-May-2014	UWBM 117963	sar8278	N
nvi	BRITISH COLUMBIA: Tahsis	49.9	-126.7	17-May-2014	UWBM 117964	sar8279	N
nvi	BRITISH COLUMBIA: Vancouver Island; Royston	49.7	-125.0	05-Jul-2008	UWBM 87433	csw7279	N
nvi	BRITISH COLUMBIA: Vancouver Island; Comox	49.7	-124.9	07-Jul-2008	UWBM 87444	csw7290	N
nvi	BRITISH COLUMBIA: Vancouver Island; Campbell River	50.0	-125.3	28-May-2008	UWBM 87449	csw7295	A
nvi	BRITISH COLUMBIA: Vancouver Island; Campbell River	50.0	-125.3	27-Apr-2008	UWBM 87450	csw7296	A
nvi	BRITISH COLUMBIA: Vancouver Island; Courtenay	49.7	-125.0	28-Jun-2008	UWBM 87451	csw7297	N
nvi	BRITISH COLUMBIA: Vancouver Island; Campbell River	50.0	-125.3	02-Jul-2008	UWBM 87452	csw7298	A
sea	WASHINGTON: King Co.; Kent	47.4	-122.2	16-Jul-2013	UWBM 117850	kle726	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	11-Jun-2005	UWBM 117870	kle740	A

sea	WASHINGTON: Snohomish Co.; Edmonds	47.9	-122.3	06-Aug-1997	UWBM 58600	csw5724	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	09-Apr-1993	UWBM 65073	csw4779	A
sea	WASHINGTON: King Co.; Seattle	47.6	-122.3	16-Jun-1993	UWBM 65094	jmb1416	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	13-May-1992	UWBM 65101	jmb960	A
sea	WASHINGTON: Snohomish Co.; Lynnwood	47.9	-122.3	17-Apr-1998	UWBM 68201	gkd23	A
sea	WASHINGTON: King Co.; Redmond	47.7	-122.1	03-Apr-1996	UWBM 71418	rya82	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	07-Jul-2000	UWBM 79625	jml017	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	09-Jul-2005	UWBM 81681	gjy002	A
sea	WASHINGTON: King Co.; Des Moines	47.4	-122.3	27-Jun-2006	UWBM 84163	car003	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	25-May-2006	UWBM 84174	kle003	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	01-Apr-2003	UWBM 84228	rjr001	A
sea	WASHINGTON: King Co.; SeaTac	47.4	-122.3	18-May-2003	UWBM 84277	cxj001	A
sea	WASHINGTON: King Co.; SeaTac	47.4	-122.3	31-May-2002	UWBM 85982	wss002	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.3	24-May-2007	UWBM 86268	kle065	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	10-Aug-2009	UWBM 90348	csw7570	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	24-Jun-2009	UWBM 90350	csw7572	A
sea	WASHINGTON: King Co.; Seattle	47.7	-122.4	11-Jun-2009	UWBM 90351	csw7573	A
tac	WASHINGTON: Pierce Co.; Gig Harbor	47.3	-122.6	10-Jul-2013	UWBM 117851	kle725	A
tac	WASHINGTON: Pierce Co.; Vaughn	47.3	-122.8	11-Jul-2013	UWBM 117852	kle724	N
tac	WASHINGTON: Pierce Co.; Lake Tapps	47.2	-122.2	22-Jul-2013	UWBM 117857	kle727	A

tac	WASHINGTON: Pierce Co.; Tacoma	47.2	-122.4	04-Jul-2013	UWBM 117867	kle737	A
tac	WASHINGTON: Pierce Co.; Gig Harbor	47.3	-122.6	01-Jun-2011	UWBM 117868	kle738	A
tac	WASHINGTON: Pierce Co.; University Place	47.2	-122.5	29-Jun-2013	UWBM 117869	kle739	A
tac	WASHINGTON: Pierce Co.; Tacoma	47.3	-122.5	29-Apr-2013	UWBM 117882	csw8715	A
tac	WASHINGTON: Pierce Co.; Tacoma	47.3	-122.5	29-Jul-2010	UWBM 117885	cec667	A
tac	WASHINGTON: Pierce Co.; Tacoma	47.3	-122.5	05-Jun-2013	UWBM 117886	gws4003	A
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	27-May-2008	UWBM 87422	csw7268	N
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	01-Jun-2008	UWBM 87423	csw7269	A
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	22-Jun-2008	UWBM 87427	csw7273	N
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	02-Jul-2008	UWBM 87445	csw7291	N
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	02-Jul-2008	UWBM 87446	csw7292	N
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	18-Jun-2008	UWBM 87473	csw7323	N
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	28-Jun-2008	UWBM 87475	csw7325	N
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	01-Jun-2008	UWBM 87477	csw7327	A
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	04-Jun-2008	UWBM 87478	csw7328	A
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	21-Jun-2008	UWBM 87484	csw7334	N
vic	BRITISH COLUMBIA: Vancouver Island; Victoria	48.4	-123.4	01-Jun-2008	UWBM 87485	csw7335	A
vic	BRITISH COLUMBIA: Vancouver Island; Saanich	48.5	-123.4	25-Jun-2008	UWBM 87486	csw7336	A

vic	BRITISH COLUMBIA: Vancouver Island; Esquimalt	48.4	-123.4	29-Jul-2008	UWBM 87496	csw7348	N
vic	BRITISH COLUMBIA: Vancouver Island; Esquimalt	48.4	-123.4	29-Jul-2008	UWBM 87497	csw7350	N
vic	BRITISH COLUMBIA: Vancouver Island; Esquimalt	48.4	-123.4	29-Jul-2008	UWBM 87498	csw7351	A
yvr	BRITISH COLUMBIA: Hope	49.4	-121.5	15-May-2014	UWBM 117948	sar8263	N
yvr	BRITISH COLUMBIA: Hatzic	49.2	-122.2	15-May-2014	UWBM 117949	sar8264	A
yvr	BRITISH COLUMBIA: Hatzic	49.2	-122.2	15-May-2014	UWBM 117950	sar8265	A
yvr	BRITISH COLUMBIA: Richmond	49.2	-123.1	19-Jun-2008	UWBM 87425	csw7271	A
yvr	BRITISH COLUMBIA: Coquitlam	49.3	-122.8	15-Jun-2008	UWBM 87434	csw7280	A
yvr	BRITISH COLUMBIA: Delta	49.1	-122.9	03-Jul-2008	UWBM 87438	csw7284	N
yvr	BRITISH COLUMBIA: Surrey	49.1	-122.8	01-Jul-2008	UWBM 87439	csw7285	N
yvr	BRITISH COLUMBIA: Delta	49.1	-122.9	09-Jul-2008	UWBM 87441	csw7287	A
yvr	BRITISH COLUMBIA: Surrey	49.1	-122.8	09-Jul-2008	UWBM 87443	csw7289	N
yvr	BRITISH COLUMBIA: Burnaby	49.2	-123.0	16-Jul-2008	UWBM 87453	csw7299	A
yvr	BRITISH COLUMBIA: Coquitlam	49.3	-122.8	17-Jul-2008	UWBM 87454	csw7300	N
yvr	BRITISH COLUMBIA: Coquitlam	49.3	-122.8	14-Jul-2008	UWBM 87455	csw7301	A
yvr	BRITISH COLUMBIA: Agassiz	49.2	-121.8	16-Jul-2008	UWBM 87457	csw7303	A
yvr	BRITISH COLUMBIA: Agassiz	49.2	-121.8	16-Jul-2008	UWBM 87458	csw7305	A
yvr	BRITISH COLUMBIA: Chilliwack	49.1	-121.9	20-Jul-2008	UWBM 87459	csw7306	A
yvr	BRITISH COLUMBIA: Delta	49.1	-122.9	11-Jul-2008	UWBM 87462	csw7311	A
yvr	BRITISH COLUMBIA: Langley	49.1	-122.7	17-Jul-2008	UWBM 87465	csw7314	A

yvr	BRITISH COLUMBIA: Richmond	49.2	-123.1	16-Jul-2008	UWBM 87467	csw7317	A
yvr	BRITISH COLUMBIA: Richmond	49.2	-123.1	16-Jul-2008	UWBM 87468	csw7318	A
yvr	BRITISH COLUMBIA: Port Moody	49.3	-122.8	21-Jul-2008	UWBM 87469	csw7319	A
yvr	BRITISH COLUMBIA: Langley	49.1	-122.7	23-Jul-2008	UWBM 87489	csw7339	N
yvr	BRITISH COLUMBIA: Chilliwack	49.1	-121.9	24-Jul-2008	UWBM 87495	csw7347	A
yvr	BRITISH COLUMBIA: Chilliwack	49.1	-121.9	05-Aug-2008	UWBM 87509	csw7362	A

Table 2. List of historical samples (n=37).

pop	locality	year	mo.	day	museum no.	cotype	mtDNA
ebc	BC: Ashcroft	1892	6	10	ANSP 30945	-	A
ebc	BC: Lac La Hache	1892	6	24	ANSP 30946	-	N
ebc	BC: Lac La Hache	1892	7	1	ANSP 30947	-	A
ebc	BC: Vernon	1892	7	23	ANSP 30948	-	A
ghc	WA: Pacific Co.; Shoalwater Bay	1854	9	14	USNM A10306	yes	A
ghc	WA: Cape Disappointment	1889	11	4	AMNH 47532	-	A
kit	WA: Vashon Island	1918	12	18	USNM 269171	-	A
kit	WA: Vashon Island	1918	12	18	USNM 269172	-	N
sea	WA: Snohomish Co, Granville	1897	7	6	USNM 157969	-	A
sea	WA: Seattle	1897	9	2	USNM 157967	-	A
tac	WA: Ft. Steilacoom	1856	2	NA	USNM A10310	yes	A
tac	WA: Ft. Steilacoom	1856	4	25	AMNH 42372	yes	N
tac	WA: Ft. Steilacoom	1856	4	25	USNM A10307	yes	N
tac	WA: Ft. Steilacoom	NA	NA	NA	ANSP 2875	?	N
tac	WA: Nisqually Flats	1892	3	25	ANSP 30934	-	A
tac	WA: Nisqually Flats	1892	4	3	ANSP 30935	-	A
tac	WA: Nisqually Flats	1892	4	5	ANSP 30936	-	A
tac	WA: Nisqually Flats	1892	4	5	ANSP 75706	-	N
tac	WA: Nisqually Flats	1892	4	9	ANSP 75705	-	A
tac	WA: Nisqually Flats	1892	4	14	ANSP 30937	-	N
tac	WA: Nisqually Flats	1892	4	14	ANSP 30938	-	N
tac	WA: Nisqually Flats	1892	4	19	ANSP 30939	-	A
vic	BC: Victoria	1892	5	19	ANSP 30942	-	N
vic	BC: Victoria	1892	5	23	ANSP 30943	-	N
vic	BC: Victoria	1892	5	23	ANSP 30944	-	N
yvr	BC: Westminster	1889	5	NA	AMNH 47523	-	N
yvr	BC: Mt. Lehman	1889	6	7	AMNH 47518	-	A
yvr	BC: Vancouver	1889	10	4	AMNH 47529	-	N
yvr	BC: Vancouver	1889	10	7	AMNH 47526	-	N
yvr	BC: Vancouver	1889	10	7	AMNH 47527	-	A
yvr	BC: Vancouver	1889	10	7	AMNH 47530	-	N
yvr	BC: Vancouver	1889	10	9	AMNH 47525	-	N

yvr	BC: Vancouver	1889	10	9	AMNH 47528	-	N
yvr	BC: Westminster	1889	NA	NA	AMNH 47524	-	A
yvr	BC: Lulu Island	1892	5	30	ANSP 30940	-	N
yvr	BC: Lulu Island	1892	5	30	ANSP 30941	-	N
-	WA: Orcas Island	1857	12	20	USNM A9511	yes	-

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Chapter Three

Morphological-molecular cline concordance in an American crow hybrid zone

ABSTRACT

American and Northwestern crows hybridize across nearly 1,000 km of their coastal distribution in Washington and British Columbia. The broad, sigmoidal molecular cline across this hybrid zone is consistent with a general pattern of decreasing body size along the Pacific coast reported by an earlier morphological study. However, the data in the morphological study were not presented in a way that facilitates a quantitative comparison with the shape of the molecular cline. In this study, we reassessed morphological variation in American/Northwestern crows along the Pacific coast and across North America, and compared morphological and molecular variation along the Pacific coast using modern cline methods. The cline for body size was centered 355 km and 158 km south of the clines for mtDNA and nuDNA, respectively. The ± 2 LL intervals for cline width overlapped between body size and both the mtDNA and nuclear DNA clines. The smallest crows in North America occur in Alaska, coastal British Columbia, coastal Washington, and coastal Oregon, respectively, mirroring the American/Northwestern crow hybrid cline based on molecular data. The largest crows are resident in Florida. Overall, body size variation in the American/Northwestern crow complex provides a counterexample to Bergmann's rule, which states that animals have larger body size at higher latitudes and/or in regions with cooler climates. One alternative explanation for geographic variation in body size of American crows is ecological character displacement as a consequence of competition with other co-occurring *Corvus* species. The smaller and gregarious Fish Crow reaches its peak abundance in Florida, where American Crows are raven-like, achieving their largest body size and tending to be more solitary. Conversely, the larger Common Raven is abundant in Alaska, where it co-occurs coastally with the smallest American (Northwestern) crows.

INTRODUCTION

Cline models offer a way to quantitatively compare the introgression of different traits across a hybrid zone (Barton and Hewitt 1985). For example, a trait with strong introgression relative to others may be under strong selection. In a hybrid zone involving Neotropical manakins, two male plumage traits introgressed much farther than 8 morphological and molecular traits that were concordant in center and width, potentially suggesting strong sexual selection on male plumage (Brumfield et al. 2001). By contrast, when clines are coincident, it may suggest that those traits are experiencing a similar selection-dispersal equilibrium (Barton and Hewitt 1985) and/or strong epistasis (e.g., Dasmahapatra et al. 2002).

The American Crow (*Corvus brachyrhynchos*) and Northwestern Crow (*C. caurinus*) are morphologically similar taxa that come into secondary contact along the Pacific Northwest coast of North America. A recent population genetic study revealed a relatively linear, >900 km hybrid zone in coastal portions of Washington and British Columbia (Slager et al. 2020). Across this hybrid zone, mitochondrial DNA (mtDNA) and nuclear DNA clines were sigmoidal, concordant in width, and both were centered in southwestern British Columbia. Within most hybrid zone localities, hybrid indices were similar among individuals and closely correlated with latitude.

The broad north-south genetic cline described by Slager et al. (2020) is consistent with the pattern of geographic variation in body size reported by Johnston (1961). Johnston found gradually decreasing body size northward along the Pacific coast from California to Alaska, contrary to the pattern predicted by Bergmann's rule of generally increasing body size with higher latitudes and/or lower temperatures (Ashton 2002, Meiri and Dayan 2003). Despite carefully accounting for variation due to age and sex and limiting his analysis to breeding-season

birds to exclude confounding variation from migrants, he was unable to diagnose American and Northwestern crows morphologically within the hybrid zone. This raises the question of the degree to which morphological variation in crows along the Pacific coast mirrors the genetic cline documented by Slager et al. (2020).

Directly comparing published morphological data (Johnston 1961) with published genetic clines (Slager et al. 2020) is difficult because the morphological measurements are presented as summary statistics for broad geographic regions (e.g., California, Oregon, and Alaska; Johnston 1961). Although Johnston reported means, measures of variation, and sample sizes for these localities, his work predated quantitative cline models, and a data table of his individual specimen measurements is not available for reconstructing a morphological cline.

Our objectives in this study were to 1) measure morphological variation in American/Northwestern crows specimens collected across North America, focusing especially on the Pacific Northwest hybrid zone, 2) compare morphological and molecular variation quantitatively across the hybrid zone using cline models, and 3) place morphological variation across the Pacific Northwest hybrid zone in context of range-wide morphological variation in American/Northwestern crows.

METHODS

Aging and measuring museum specimens

To ensure accurate specimen metadata, the collection date, locality, sex, and museum number were simultaneously verified from the original specimen label and an electronic museum database.

Using linear measurements of an animal to study a hybrid zone requires accounting for ontogeny. DLS aged each study skin as hatch-year (HY), after hatch-year (AHY), second-year (SY), or after-second-year (ASY), following the calendar-year aging system (Pyle 1997). For fully-grown birds, age was generally readily assessed by examining the shape, coloration, molt status, and extent of wear of the rectrices under a bright light source (Emlen 1936, Pyle 1997). The coloration and extent of wear of the primaries and presence or absence of molt limits among the flight feathers and coverts was helpful corroborating information (Emlen 1936, Pyle 1997). The presence of bare skin or active molt at the gape and the presence of grayish to brownish non-iridescent feathers on the underparts, when present, were also used as corroborating evidence for aging birds as juveniles (Pyle 1997).

To minimize variation in measurements, a single researcher (DLS) took all linear measurements using the same Mitutoyo Absolute Digimatic caliper. The tarsus was measured as the distance from the joint between the tibiotarsus and tarsometatarsus to the distal edge of the most distal undivided scute at the base of the three forward toes (Baldwin et al. 1931, Johnston 1961). The bill was measured as the distance from the anterior edge of the nares to the tip of the maxilla (Baldwin et al. 1931, Johnston 1961). However, only tarsus measurements were used in analyses, because bill measurements, although correlated with tarsus, were highly variable due to the shape and length of the curved maxilla tip.

Specimen inclusion rules

For all analyses, we only included specimens that were collected during the April to September breeding period (Verbeek and Butler 1999, Verbeek and Caffrey 2002). This avoids confounding variation due to seasonal migration or dispersal away from nesting areas (Johnston

1961, Townsend et al. 2018). We also excluded hatch-year (HY) specimens to avoid confounding variation due to ontogeny (Johnston 1961).

Sex-adjusted tarsus length

American/Northwestern crows are sexually dimorphic in body size (Johnston 1961). To combine data across sexes for use in cline analyses, we calculated a sex-adjusted tarsus measurement separately for individuals comprising the Pacific coastal cline transect and for those across all of North America.

For the Pacific coastal cline transect, we first tested for an interaction between the effects of sex and latitude on tarsus length using the regression formula tarsus ~ sex * latitude. In light of the result that there was no significant interaction between sex and latitude on tarsus length, we calculated the offset between male and female tarsus length using the mixed model formula tarsus ~ sex, random = ~1|population, where the last term represents a random effect of coastal population assignment on tarsus length. We used the fixed effect of sex from this model as the male-female offset in tarsus length. To obtain the sex-adjusted tarsus measurement, we added half the offset value to female tarsus lengths and subtracted half the offset value from male tarsus lengths.

For analyzing crows across North America, we first tested for two-way and three-way interactions between the effects of sex, latitude, and longitude on tarsus length. Based on the result that none of these interaction terms were significant, we calculated the offset between male and female tarsus length using the mixed model formula tarsus ~ sex, random = ~1|state_province, where the last term represents a random effect of state/province on tarsus length. We included separate dummy states/provinces for the coastal and inland portions of

British Columbia, Washington, Oregon, and California, following the boundaries described in the "cline-fitting" section of the Methods. We used the fixed effect of sex from this model as the male-female offset in tarsus length. To obtain the sex-adjusted tarsus measurement, we added half the offset value to female tarsus lengths and subtracted half the offset value from male tarsus lengths.

Cline-fitting

Crows were included on the Pacific coastal cline transect on the basis of coastal biogeographic regions. Using a spatial overlay of Bird Conservation Regions (BCRs; Bird Studies Canada and NABCI 2014), we included all birds from Alaska, where crows are strictly coastal, birds from British Columbia, Washington, and Oregon on the Pacific slope of the Coast Mountains and Cascades, and birds from California and Baja California from west of the Sierra Nevada Mountains and west of the Mojave and Sonoran deserts.

Cline-fitting models for linear measurements require population means and variances at point locations. Therefore, we divided coastal samples into 30 geographically compact populations. Each population contained multiple adult individuals collected during the April-September breeding season.

Alaska samples were divided into 7 populations using boroughs or pooled adjacent boroughs (Kodiak Island, Kenai Peninsula, Valdez-Cordova, Yakutat, Hoonah-Angoon/Haines/Juneau, Sitka, Wrangell/Prince of Wales-Hyder/Ketchikan Gateway). British Columbia samples were divided into 3 populations based on island biogeography (Haida Gwaii, Vancouver Island, mainland). Washington samples were divided into 12 populations corresponding to counties or pooled adjacent counties (Clallam/Jefferson, Grays Harbor, Pacific,

San Juan, Whatcom, Skagit/Island, Snohomish, King, Pierce, Kitsap, Mason/Thurston/Lewis, Wahkiakum/Cowlitz/Clark). Oregon, California, and Baja California samples were demarcated into 8 populations using 1° bands of latitude or pooled adjacent bands depending on available sample size (32-33°+, 34-35°+, 36°+, 37°+, 38-39°+, 40-42°+, 43-44°+, 45°+).

For each population, we used the mean latitude and mean longitude of samples in that population as the basis for assigning a transect distance along the Pacific coastline. We assigned transect distances to each population using a smoothed curve drawn parallel to the Pacific coastline in QGIS 2.8.3 (QGIS Development Team 2015), following the same transect as Slager et al. (2020).

We fit cline models for tarsus along the Pacific Coast using R package *hzar* (Derryberry et al. 2014), following the methodology of Slager et al. (2020). We fit a cline model to means and variances of sex-adjusted tarsus lengths using *hzar.makeCline1DNormal*. We fit a model without exponential tails, restricted parameter search space to a liberal yet reasonable range of values (cline center >500 km and <3500 km, cline width <3500 km; Derryberry et al. 2014) and fixed means and variances at cline ends to the observed values of the terminal populations (Linck et al. 2019, Lipshutz et al. 2019). We ran the Markov chain Monte Carlo (MCMC) optimizer for 3 iterative cycles using *hzar.chain.doSeq*, retaining the third run for subsequent analysis (Derryberry et al. 2014). We used *hzar.get.ML.cline* and *hzar.getLLCutParam* to obtain maximum likelihood estimates for widths, centers, and ± 2 log likelihood (LL) intervals. We used these intervals to test for coincidence of centers and concordance of widths (Derryberry et al. 2014, Lipshutz et al. 2019) between sex-adjusted tarsus length and the corresponding molecular clines from Slager et al. (2020).

RESULTS

Pacific Coastal transect

We measured 206 adult crow specimens collected along the Pacific coast of North America from April-September, including 86 females and 120 males. There was no interaction effect between sex and latitude on tarsus length ($t = -0.919$, $p = 0.36$). The male-female offset in tarsus length from the mixed-effects model was 2.05 mm. Female tarsus length was 49.78 ± 0.41 mm (mean ± SE, n=86, range 42.53-56.91). Male tarsus length was 52.11 ± 0.34 mm (n=120, range 42.80-59.26). Sex-adjusted tarsus length was 50.97 ± 0.26 (n=206, range 41.77-58.23). The smallest and largest crow specimens along the Pacific coast in terms of sex-adjusted tarsus length were males collected at Kelp Bay, Sitka, Alaska on 15 May 1915 and at Alameda, California on 14 April 1904, respectively. The average number of samples per assigned coastal population was 6.9 (range 2-18 samples/population).

We recovered a sigmoidal cline in sex-adjusted tarsus length along the Pacific coast of North America (Figure 1). The cline was centered near Seattle, 355 km and 158 km south of the clines for mtDNA and nuDNA, respectively, and the ± 2 LL ranges for cline center did not overlap between morphological and molecular clines (Table 1). The ± 2 LL intervals for cline width overlapped between the sex-adjusted tarsus cline and both the mtDNA and nuclear DNA clines (Table 1).

Across North America

Across all of North America, we measured 598 adult crow specimens collected during April-September, including 258 females and 340 males. There were no interaction effects

between sex:latitude (t=-0.978, p = 0.33), sex:longitude (t=0.652, p=0.51), or sex:latitude:longitude (t=-0.621, p=0.54) on tarsus length. The male-female offset in tarsus length from the mixed-effects model was 2.21 mm. The raw tarsus length for females was 53.90 ± 0.26 mm (mean ± SE, n=258, range 42.53-62.21). For males, the raw tarsus length was 56.18 ± 0.24 mm (mean ± SE, n = 340, range 42.80-65.98). Sex-adjusted tarsus length was 55.04 ± 0.18 (n=598, range 41.69-64.87).

Among the sampled geographic regions across North America, the four with the shortest adjusted tarsus lengths were Alaska (random intercept = -9.64 mm), coastal British Columbia (-8.31 mm), coastal Washington (-5.80 mm), and coastal Oregon (-2.76 mm, Figure 2). The four regions with the longest adjusted tarsus lengths were Florida (+4.72 mm), Texas (+3.51 mm), Nova Scotia (+3.12 mm), and Maine (+2.83 mm). The smallest and largest crow specimens across North America in terms of sex-adjusted tarsus length were males collected at Kelp Bay, Sitka, Alaska on 15 May 1915 and at New River, Bradford County, Florida on 04 April 1896, respectively.

DISCUSSION

The crows with the smallest tarsus measurements were from Alaska, a result consistent with the trend in decreasing body size from Washington State to Alaska reported by Johnston (1961). Moreover, our cline analysis showed that the cline in tarsus length was concordant in width and roughly coincident in center with the molecular data clines calculated from either mtDNA or nuclear DNA (Slager et al. 2020). Overall, these morphological data provide

independent evidence for the broad cline formed by the American/Northwestern crow hybrid zone in the Pacific Northwest (Slager et al. 2020).

The crows with the largest tarsus measurements were from Florida. This is also consistent with the measurements and interpretation of Johnston (1961), who found the breeding crows of peninsular Florida to be the largest and most morphologically distinctive of all the American Crow populations he examined.

The overall geographic pattern exhibited by American crows, with smallest body size in Alaska and largest body size in Florida, provides a counter-example to Bergmann's rule, which predicts larger body size in animals inhabiting higher latitudes and/or cooler climates. Indeed, a sizable minority of bird species appears to counter Bergmann's rule. For example, one meta-analysis found that Bergmann's rule is a valid ecological generalization for birds, with 72% of species following Bergmann's rule (Meiri and Dayan 2003). Another meta-analysis found that 76% of bird species followed Bergmann's rule, and that the pattern was generally valid in terms of both latitude and temperature (Ashton 2002).

One alternative hypothesis to explain geographic variation in American crows is character displacement due to interspecific competition with congeners. Character displacement derives from the competitive exclusion principle (Hardin 1960) and is evident when closely related species are more divergent in traits in areas of overlap than in areas where only one of the species occurs (Brown and Wilson 1956). The Common Raven is a much larger *Corvus* species that overlaps with American/Northwestern crows along the Pacific coast of North America, and it is particularly common in Alaska, where it co-occurs with crows along the coast (Fink et al. 2020). Competition for similar food resources between these two omnivores, leading to Alaskan

crows evolving smaller body size through character displacement, may be one possible explanation for the contra-Bergmann's pattern observed in crows of the Pacific Northwest.

Conversely, the abundance of a smaller *Corvus* species, the Fish Crow, peaks in peninsular Florida (Fink et al. 2020), where American Crows reach their largest size, raising the possibility that character displacement in Florida is happening in the opposite direction. Fish Crows are a highly social species (McGowan 2001), and, interestingly, the American Crows of peninsular Florida, in addition to being very large, also differ behaviorally from other American Crows. In fact, American Crows in southern Florida seldom flock and mainly inhabit backcountry areas (Verbeek and Caffrey 2002), much like the Common Raven.

The overall concordance between morphological and molecular clines across the American/Northwestern crow hybrid zone suggests that body size and the overall genome are experiencing a similar strength of selection across the hybrid zone. Indeed, body size is a quantitative trait based on contributions from a large number of genes of small effect (Falconer 1953, Cheverud et al. 1996), and ddRAD loci are also widely distributed across the genome (Peterson et al. 2012, Slager et al. 2020). Therefore, it is perhaps expected that these different traits exhibited similar cline shapes, as has been found in some previous studies (e.g., Ruegg 2008, Seneviratne et al. 2012).

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FIGURES AND TABLES

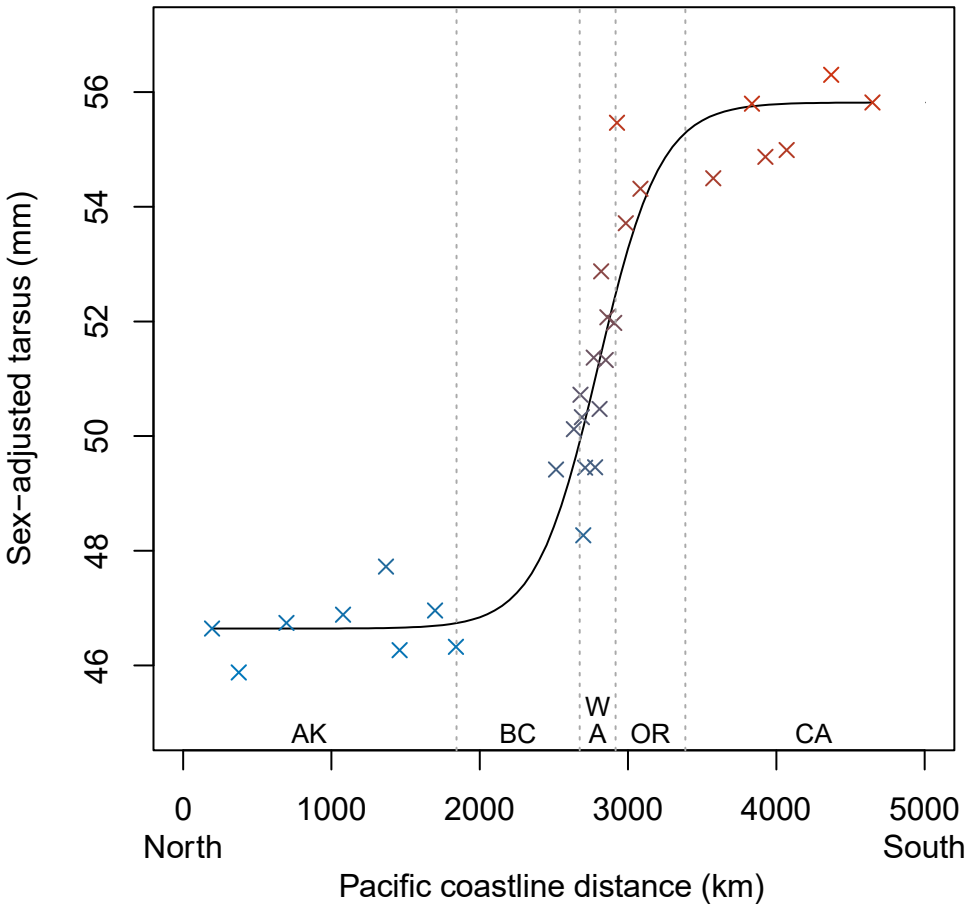

Figure 1. Pacific coastal cline spanning the Northwestern Crow (blue) and American Crow (red) hybrid zone. Markers indicate population mean sex-adjusted tarsus length (see Methods). The line represents the best-fit cline model (see Methods), and the dotted vertical lines indicate state and provincial boundaries.

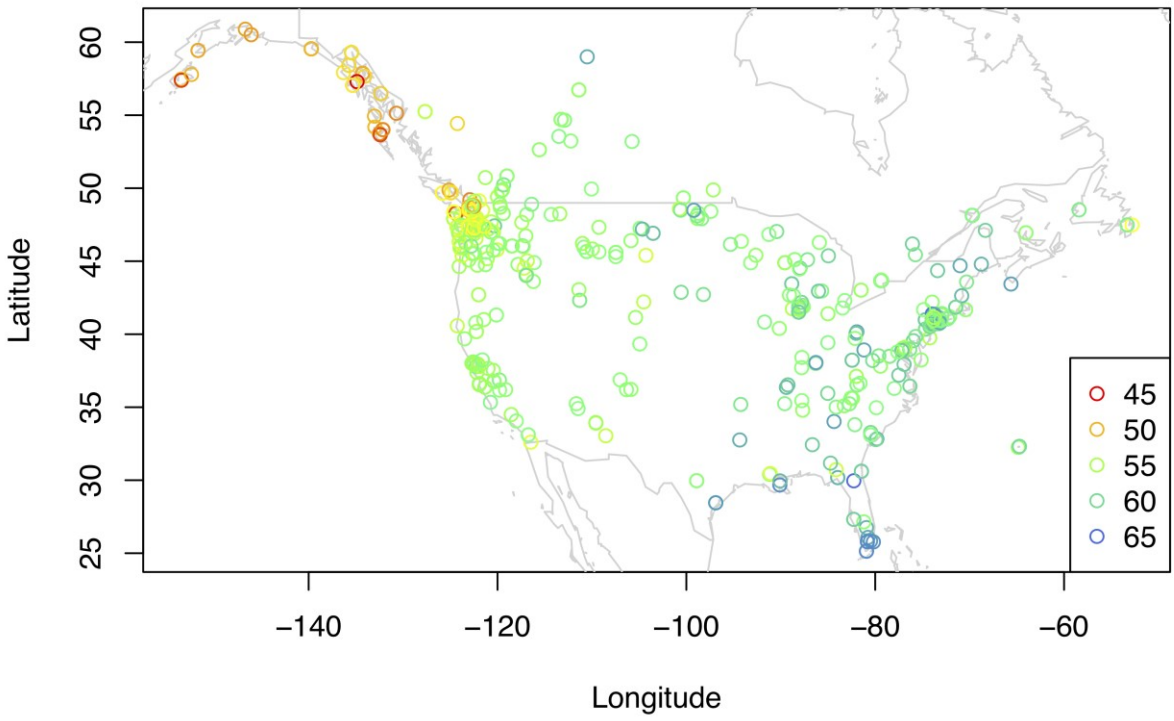

Figure 2. Map of sex-adjusted tarsus length (mm) for American/Northwestern crows across North America using a continuous color scale.

	center (± 2 LL range)	width (± 2 LL range)
mtDNA (Slager et al. 2020)	2,445 km (2,340-2,530)	825 km (609-1,158)
nuDNA (Slager et al. 2020)	2,642 km (2,585-2,700)	918 km (560-1,266)
tarsus	2,800 km (2,746-2,853)	839 km (588-1,426)

Table 1. Cline center and cline width with ± 2 log likelihood (LL) intervals for sex-adjusted tarsus, mtDNA, and nuDNA across the American/Northwestern crow hybrid zone.

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