(separate page) Title:

Deciphering cryptic population structure in Western Sandhill Crane subspecies (*Antigone canadensis*) of the Pacific Flyway

Corresponding author: Ruth Joy (rjoy@sfu.ca)

**Authors:**

**Ruth Joy1, Krista Roessingh2, Kathleen Meszaros1, Allyson Miscampbell3, Carol Ritland3, Matt Hayes4, Gary Ivey5, Mike Petrula6, Jeffrey B. Joy7,8,9**

1School of Environmental Science, Simon Fraser University, Burnaby, BC, Canada

2Citizen Scientist, Denny Island, BC, Canada

3Genetic Data Centre, University of British Columbia, Faculty of Forestry, Vancouver, BC, Canada

4Illinois Audubon Society, Springfield, Illinois, USA

5International Crane Foundation, 1350 SE Minam Ave., Bend, OR 97702, USA

6Alaska Department of Fish and Game, Division of Wildlife Conservation, Waterfowl Program, Anchorage, Alaska, USA

7Molecular Epidemiology and Evolutionary Genetics, BC Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada

8Bioinformatics Programme, University of British Columbia, Vancouver, BC, Canada

9Division of Infectious Diseases, Department of Medicine, University of British Columbia, Vancouver, BC, Canada

**(223/250 words) Abstract**

Population segregation catalyzes genetic differentiation and can lead to speciation. Population genetic structure is also critically important for population management, especially in species characterized by small, isolated populations. Sandhill Crane (*Antigone canadensis*) populations of the Pacific Flyway are made up of breeding populations nesting west of the Rocky Mountains, and isolated by intermediate mountain ranges. Current management policy in British Columbia treats all Sandhill Cranes as a single population, whereas in the western United States subpopulations are subject to population specific management. Here, we analyze microsatellite markers, mitochondrial DNA sequences, and mitochondrial haplogroups, derived from 203 individual Sandhill Cranes to elucidate population genetic structure of cranes migrating along the Pacific Flyway to summer breeding habitat on the North and Central Coast of British Columbia and southeast Alaska. STRUCTURE, AMOVA, FST, DAPC, and phylogenetic analyses reveal that geographically separated crane populations along the west coast of North America show substantial genetic differentiation in the Pacific Flyway. These findings are consistent with behavioural and ecological evidence - divergent diets, flyways and breeding habitats. We conclude that the unique characteristics of coastal Sandhill Crane populations deserve special management consideration relative to interior and southwestern Alaskan crane populations to safeguard the genetic diversity and adaptations within and between populations to mitigate deleterious impacts of current and future climate change scenarios. Special management of the Pacific Flyway birds can be accomplished by protecting the breeding, staging and wintering habitats of each population.

Keywords: population management, population genetics, gene flow, Pacific Flyway, Sandhill Cranes, non-invasive sampling

# 1 Introduction

A clear understanding of population genetic diversity and its effects on population genetic structure can be critically important for species management plans. Maintenance of intra-species genetic diversity facilitates species abilities to adapt to changing habitats and selective environments and thus should be a key goal of conservation and management planning (Sgrò et al. 2011). Population genetic structure depends on a variety of factors, including differential reproductive success and dispersal and settling patterns on breeding areas. For migratory species, these factors can be compounded by long-distance movements and intermixing of different genetic groups on staging and wintering areas (Faaborg et al. 2010). Significant population genetic structure results from reproductive isolation due to physical barriers such as geographic separation or behavioral barriers directed by natural or sexual selection. Given enough time, reproductive isolation can also result from genetic drift (Turbek et al. 2018). Genetic differentiation leading to reproductive isolation between allopatric breeding populations may result from selection, genetic drift, or their combination (Turbek et al. 2018). Determining the context of divergence and extent of gene flow among populations of migratory species is important for understanding the evolutionary trajectory of populations and, critically, for assigning conservation priority (Jetz et al. 2014, Stein et al. 2018).

Sandhill Cranes (*Antigone canadensis*) comprise a complex of geographically separated breeding populations with varying levels of conservation importance tied to national, state and provincial conservation priorities. This charismatic species is known for its elaborate courtship displays that include ritualised dancing and synchronised calls, useful for both establishing and maintaining pair bonds. Sandhill Cranes form long-term pair bonds, often staying together year-round. They display high fidelity to breeding territories and established migratory flyways (Walkinshaw 1973). Furthermore, young display strong philopatry (Walkinshaw 1949, Littlefield and Ivey 1995). However, some birds show punctuated long-distance dispersal especially at young ages (Hayes 2015). These life history properties coupled with specific habitat requirements (nesting in a variety of freshwater wetland types and feeding in various upland habitats) determine broad-scale genetic patterns of differentiation.

Six morphological and geographic subspecies of Sandhill Cranes have been described across their breeding distribution (Mirande and Harris 2019), from Siberia in Eastern Asia through North America to Cuba in the Caribbean. The three migratory subspecies have distributions that roughly follow a north-south ecotone with *A.c. canadensis* (‘Lesser’) breeding in the arctic, *A.c. rowani* (‘Canadian’) in the subarctic and the Pacific coast, and *A.c. tabida* (‘Greater’) in the south (Littlefield and Ivey 2002), and all are distinguished by morphology (bill size and body length) and geography (Johnsguard 1983). The validity of *A.c. rowani* as a genetically separate subspecies has been debated by several studies (Rhymer et al. 2001, Glenn et al. 2002, Petersen et al. 2003, and Jones et al. 2005) suggesting that it s morphological differences may be only clinal, however these studies omitted samples from cranes breeding west of the Rocky Mountain range.

Sandhill Cranes that migrate within the Pacific Flyway through British Columbia (BC) and Alaska for the most part share wintering grounds in California’s Central Valley, but during migration and breeding are split between the ‘interior’ migratory flyway and the ‘coastal’ flyway. The ‘interior’ migration corridor includes cranes that migrate east of the Cascade and Coast Mountains, members of the Central Valley Population (>8,600 cranes, mostly *A.c. tabida*) that breed from Northern California to central BC (Pacific Flyway Council 1997; Littlefield and Ivey 2002), as well as members of the Pacific Coast Population (>36,100 cranes, mostly *A.c. canadensis* and a smaller number of *A.c. rowani*) that breed in southwest Alaska (Caven 2023; Petrula and Rothe 2005). The Central Valley population prefers freshwater marsh or groundwater fens, grasslands, farm fields or grazing pastures, and are adapted to nesting and foraging in more terrestrial environments (Cooper 1996, Jones et al. 2005). Cranes that use the ‘coastal’ flyway are also members of the Pacific Coast Population, but they migrate up the west side of the Coast Mountains and breed from the north end of Vancouver Island to southeast Alaska, and are thought to be *A.c. rowani* (Ivey et al. 2005, Stinson 2017). Cranes from this coastal population utilize marine intertidal habitats, foraging on marine molluscs in estuaries and coastal beaches and nesting in bogs close to the ocean (Hearne and Hamel 2003, Roessingh 2012).

Prior to 2009, the BC government assigned all Sandhill Cranes elevated conservation status in the province, due to the paucity of information regarding their individual population status and potential threats to their breeding habitat (Cooper 1996). In 2009, the status changed to "apparently secure", without differentiating between different sized populations nor conservation concerns related to breeding habitats and land use management. Delisting was due to widespread breeding records and a rise in overall population and range since 1970 (BC Conservation Data Centre 2018). Meanwhile, all Sandhill Cranes remain on the endangered list in Washington State (Washington Department of Fish and Wildlife 2021), and *A.c. tabida* is listed as ‘sensitive’ in Oregon State (Oregon Department of Fish and Wildlife 2016) and ‘threatened’ in the State of California (California Natural Diversity Database 2021), where the wintering habitat of BC's cranes is facing loss and encroachment, as well as impacts of land-use and climate change.

Population genetic structure of cranes along the Pacific Flyway was quantified in Hayes (2015) using Amplified Fragment Length Polymorphism (AFLP) markers, mitochondrial DNA (mtDNA) haplotypes, and morphological data. Hayes (2015) found evidence of population genetic structure but gene flow between coastal-breeding birds and some interior Central Valley cranes in Oregon and California (Hayes 2015). This analysis was restricted to eight samples from the coastal breeding population, and zero from interior BC. We set out to build on Hayes (2015) analysis to address the information deficit in the population genetic structure of the Pacific Flyway Sandhill Crane populations west of the Rocky Mountains of British Columbia and southern Alaska. Specifically, we test the hypothesis that gene flow restriction between BC coastal and interior breeding populations, two regions separated by the Pacific Mountain System (also known as the ‘Coast Mountains’), which includes the Cascade Range in the United States, has resulted in genetic structure among these populations. We predict genetic markers derived from samples from coastal breeding cranes will show significant divergence from genetic markers derived from cranes breeding in the Interior. To test this hypothesis, we analyzed microsatellite markers and mtDNA control region sequences to compare Sandhill Cranes nesting across the region covering southwest to southeast Alaska, coastal and interior BC, mtDNA control region sequences were also grouped into haplogroups following Hayes et al. 2013. We also sought to test the efficacy of microsatellite primers designed for different crane species in amplifying markers from Sandhill Cranes.

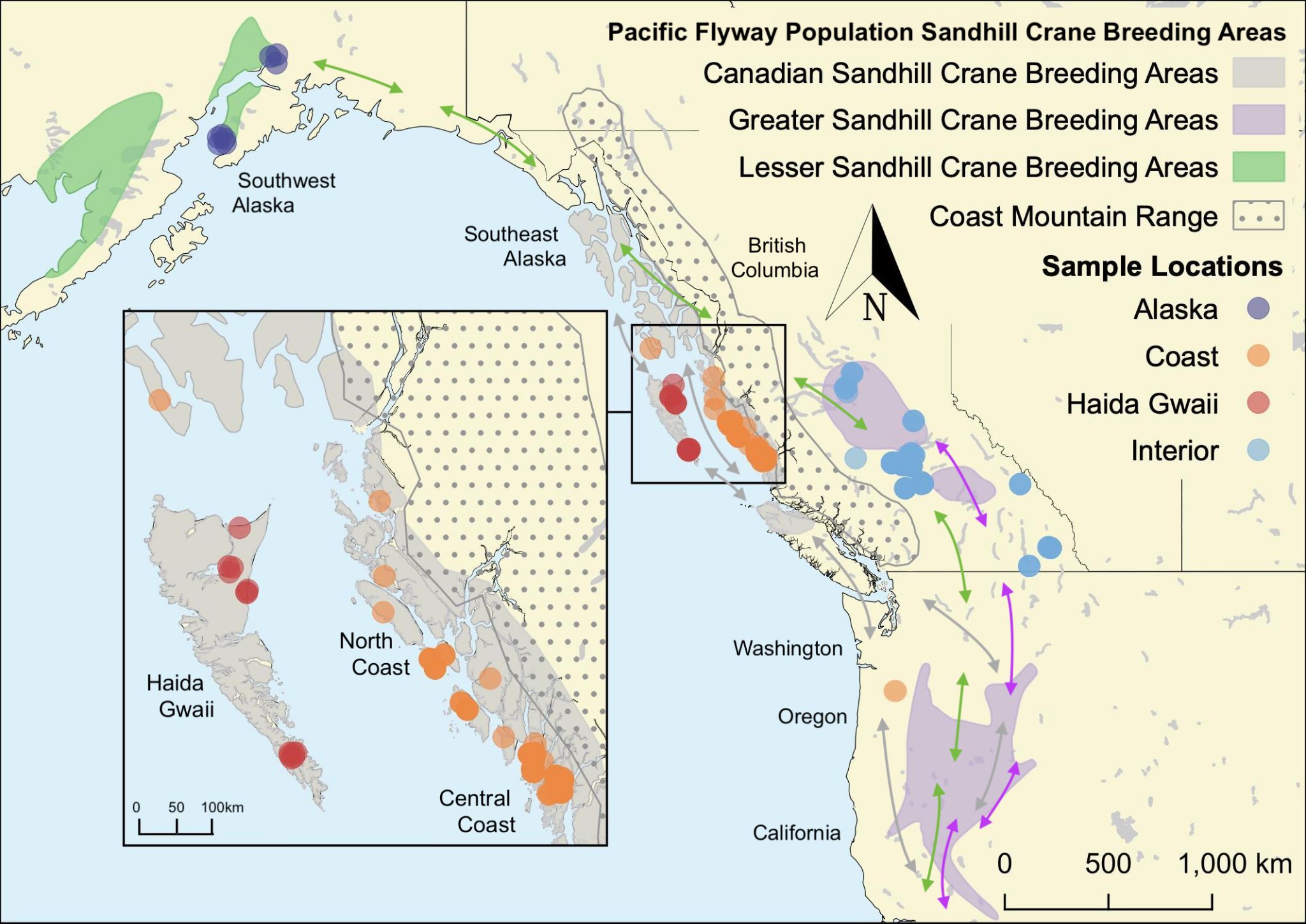
# 2 Methods

**2.1 Specimen Collections**

To elucidate differences between Sandhill Crane populations, we collected naturally-shed feathers from cranes within their breeding territories. The four sample collection areas were 1) the ‘Coast’ population including the islands of the remote Central and North Coast of BC and Southeast Alaska, 2) the offshore archipelago of ‘Haida Gwaii’, 3) the intermountain ‘Interior BC’ grassland regions between the Coast Mountains to the west and the Rocky Mountains to the east, and 4) southwest ‘Alaska’. Our field collection focused on naturally shed feathers, minimizing impact and disturbance to the cranes, and allowing us to maximize volunteer participation, a strategy enabling sample collection over a broad geographic aea (Segelbacher 2002). We used public outreach tools including a website, listservs, email, and public radio to reach residents and field workers. Public outreach enabled access to samples from private lands through the assistance and contributions of ranchers and other landowners who responded. We created a spatial-temporal RShiny tool (an open-source web framework for creating web applications in R) at adaptable resolution for the province of BC with archived spatial data. We mapped all available Sandhill Crane breeding location information on this site, including possible, probable, and confirmed breeding location records from 1960 to 2018 from eBird (eBird 2018), the BC Breeding Bird Atlas (Davidson et al. 2015), iNaturalist (iNaturalist 2018), as well as citizen science data conveyed to us directly. The map tool guided sample collection effort by highlighting known breeding areas, and encouraged volunteers to contribute their own knowledge of previously undocumented breeding sites.

Collection of moulted feathers is a non-invasive sampling method particularly well-suited to cranes as their feathers are large, easily spotted, and often identifiable by staining with iron-rich mud during the breeding season (Walkinshaw 1950). Participants were asked to collect the freshest and largest feathers, since larger wing, bustle, or tail feathers generally yield more DNA than coverts or body contour feathers (Johansson et al., 2012; Kelly 2019; Peters et al. 2020). Feathers were collected in July and August, after the nesting period, when chicks had left the nest, coinciding with the peak moulting season of 2017 and 2018 (Walkinshaw 1950). We communicated individually with volunteers to ensure feathers were collected from known breeding areas rather than staging areas of migratory cranes. Moulted feathers were retrieved within 100m of nest sites wherever possible to prevent the collection of feathers from migrating birds. Once feathers were collected, they were dried at room temperature to limit exposure to humidity, which has been related to lower PCR performance (Vili et al. 2013). Feathers were then wrapped in plastic and shipped by mail to the University of British Columbia's Genetic Data Centre, Department of Forest and Conservation Sciences. In total just over 500 feathers were donated from 3 geographic regions of BC.

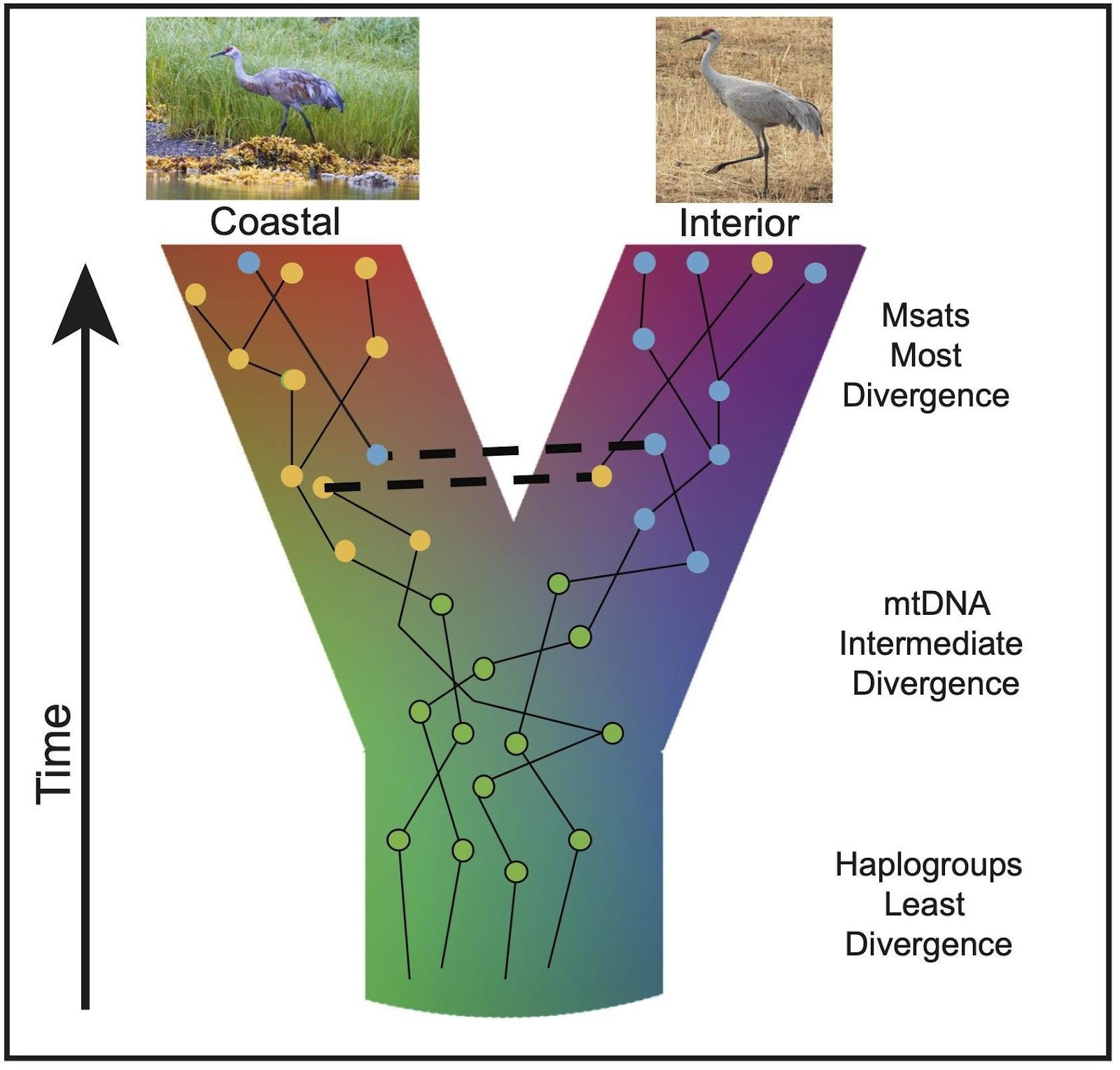
Overall, the authors and our collaboration of citizen scientists collected moulted Sandhill crane feathers and other genetic material from 123 individual cranes on the Central and North Coast of BC, and from 77 cranes at various sites east of the Coast Mountains, including samples collected by 14 volunteers at 12 different Interior BC sites (Figure 1, Table 1). Additionally, we received 13 contributions of moulted feathers from Haida Gwaii collected by nine volunteers from eight separate sites. We received nine blood samples from southwest Alaska’s breeding Sandhill Cranes collected during two previous studies (Petrula and Rothe 2005; G. Ivey, unpublished data). We also received blood samples from six cranes captured and tagged while staging in the Lower Columbia River region of Washington and Oregon, and may have been part of the small (3,000-5,000), discrete population that winters in that region (Ivey et al. 2005, Ivey et al. 2015, Stinson 2017). These birds travelled up the coastal Pacific Flyway and summered along the BC and Southeast Alaska coast (Ivey et al. 2005).

**Figure 1**. Pacific Flyway Sandhill crane breeding areas in Northwestern North America highlighting regions where the putative *A.c. canadensis (Lesser)*, *A. c. rowani* (Canadian) and *A. c. tabida* (Greater) subspecies range. Coloured arrows indicate migration routes for each subspecies (Ivey 2013, Petrula and Rothe 2005). DNA sample collection sites are represented by semi-transparent points, with colours corresponding to each of the four study regions.

**2.2 Molecular Methods**

We chose up to three feathers for DNA extraction if multiple feathers were collected from a single location. DNA was isolated from a total of 300 feather samples using the QIAamp DNA Investigator kit (Qiagen, Valencia, CA) following their protocol for isolation of total DNA from tissues. DNA was isolated from 15 blood samples following standard proteinase K-phenol-chloroform procedures (Sambrook et al. 1989). DNA concentration and quality was verified with a Nanodrop 2000c (Fisher Scientific, Toronto, ON, Canada). Comparison of microsatellites from each sample revealed no duplicate microsatellite patterns were observed, suggesting that samples came from unique individuals.

We investigated two genetic markers to look at different resolutions of divergence times: microsatellites (Msats) and mitochondrial DNA (mtDNA) sequences, mtDNA sequences were also grouped into haplotype groups based on combinations of mutations previously shown to be informative in defining genetic variation (Hayes 2015; Figure 2). We selected these markers to capture the breadth of genetic variation in the nuclear and mitochondrial genomes, and to investigate population structure and divergence dynamics along the Pacific Flyway over different time scales. We compared differences in these markers between the four geographic regions of ‘Coast’, ‘Haida Gwaii’, ‘Interior BC’ and ‘Southwest Alaska’.



**Figure 2**. Graphical depiction of context of divergence between Sandhill Crane (*Antigone canadensis*) populations. Expected resolution of divergence of rapidly evolving microsatellite (Msats), intermediately evolving mitochondrial DNA sequences (mtDNA) and slower evolving mitochondrial haplotype groups is demarcated on the right. Between population migration (introgression) is illustrated by horizontal dashed lines.

**2.2.1 Microsatellite Methods**

We screened 28 microsatellites characterized in Whooping C rane (*Grus americana*, Jones et al. 2010) and B lue C rane (*Grus paradisea*, Meares et al. 2008) for cross-species amplification and polymorphism. Of these, 8 microsatellite loci (Gram6, Gram11, Gram20, Gram22, Gram30, Gram 42 from Jones et al. (2010) and GjM15 and GjM48b from Meares et al. (2008)) amplified consistently and were polymorphic. Polymerase chain reaction (PCR) was performed in 10ul reaction volumes consisting of 2 pmol dNTP (New England Biolabs, Ipswich, MA, USA), 1X PCR buffer (Stratagene, La Jolla, CA, USA), 1.5 units of Paq5000 (Stratagene), 1 pmol each forward and reverse primer (Eurofins MWG Operon, Louisville, KY, USA), 0.3 pmol M13 IRDye® labelled primer (Integrated DNA Technologies, Skokie, IL, USA), with 5-50ng DNA template. PCR conditions followed Meares et al. (2008) and Jones et al. (2010).

Amplified products from 222 samples were denatured and run on a 5% polyacrylamide gel electrophoresed for 2.5 hours on a LI-COR 4300 automated sequencer (LICOR Inc., Lincoln, NE, USA) with a minimum of four size standards (50-350 bp or 50-700 bp LICOR) per 64 well gel and two to four reference samples. Gels were scored using SAGA 3.3 Microsatellite Analysis software (LICOR). Due to challenges associated with extraction and amplification of DNA from feather samples we were unable to amplify DNA from several feathers; these samples were eliminated from further analysis. Our final count included 203 samples (Table 1) from the four geographic regions west of the Rocky Mountains.

**2.2.3 Mitochondrial Sequence Methods (mtDNA and Haplotypes)**

We amplified a 437 bp portion of the mtDNA control region, using primers L-17 and H393 (Glenn et al. 2002) for 52 crane samples 41 feathers and 11 blood samples (Table 1). Polymerase chain reaction (PCR) was performed in 50 𝜇l reaction volumes consisting of 0.2 mM dNTP (New England Biolabs, Ipswich, MA, USA), 1X PCR buffer (Stratagene, La Jolla, CA, USA), 2.0 units of Paq5000 (Stratagene), 50 pmol each forward and reverse primers (Integrated DNA Technologies, Skokie, IL, USA), 1.25 mg BSA, with 40 ng DNA template. PCR amplification followed Glenn et al (2002); purification and sequencing of PCR products followed Hersh et al. (2022).

Sequence chromatograms were imported into Geneious 8.1.9 (Biomatters, Inc. San Diego, CA, USA). We edited and assembled the forward and reverse sequences for each sample into individual contigs, checking that results were the same for the complementary strands. We inspected alignments by eye for ambiguous base calls, paying particular attention to confirm nucleotide polymorphisms. Consensus sequences were aligned and exported from Geneious (Geneious 8.1.9) in FASTA format for haplotype analysis and assessed with DNAdiffer (Ritland 2012) to assign haplotypes.

Fifty-two individual mtDNA sequences, 437bp in length, were analysed (Geneious 8.1.9, DNAdiffer 2012). These sequences represented 22 unique haplotypes, which were aligned with reference sequences from Genbank (Accession AF367871, Group A; AF367890, Group B; AF367905, Group C as described by Glenn et. al 2002 ). Glenn et al 2002 used restriction enzyme digests (HaeIII) to define these groups and we also searched our sequences for HaeIII restriction enzyme sites using Geneious version 8.1.9. We found 3 haplogroups which directly correlated to groups of Glenn et al. 2002 (Supplementary Material).

**2.3 Population Genetic Structure**

For each of the four sampling regions (SW Alaska, Coast, Haida Gwaii, and Interior BC), we used GenoDive 3.05 (Meirmans 2020) to calculate microsatellite allelic richness, allelic richness standardized by sample size (AR), expected heterozygosity (He), and observed heterozygosity (Ho). We assessed each of the four populations for degree of inbreeding by calculating the proportion of the variance in the population contained in an individual (FIS). Deviations from Hardy Weinberg e quilibrium were evaluated for each population at each locus, and across all populations at all loci.

**2.3.1 Analysis of Molecular Variance**

We investigate population structure and genetic differentiation in microsatellites between regional population groupings using an analysis of molecular variance (AMOVA; Excoffier et al. 1992). We tested for significant differences among populations in measures of within and between genetic diversity using an AMOVA as implemented in GenoDive 3.05, following methods of Excoffier et al. (1992) and Meirmans (2020) using an infinite allele model and 1000 permutations. Species comparisons were further evaluated for significance with post hoc pairwise tests. Post-hoc (FST) comparisons of populations allowed us to evaluate pairwise population differences (Coast vs Interior; Coast vs SW Alaska; Interior vs SW Alaska; Coast vs Haida Gwaii, Interior vs Haida Gwaii, SW Alaska vs Haida Gwaii). Statistical significance was tested using 1000 permutations, and tested at significance level .

**2.3.2 Isolation by Distance**

To evaluate isolation by distance between Sandhill Crane populations, we first estimated pairwise genetic differentiation between all four sampling regions in this study using Wright’s F-statistic (FST, Wright 1943), calculated using GenoDive 3.05 (Meirmans 2020). We then calculated the geographic centroid for each study population, and evaluated the relationship between pairwise geographic distances and pairwise genetic distances in R (R Core Team 2023). Mantel tests were performed as implemented in adegenet (Jombart 2008).

**2.3.3 Discriminant Analysis of Principal Components**

Discriminant Analysis of Principal Components (DAPC; Jombart and Collins 2015) was used to investigate genetic structure of biological populations. DAPC integrates the sampling region prior into a variance optimization function through the rotation of axes that maximises the between-region variance while minimising the within-region variance (Jombart 2008). We ran successive K-means clustering analyses on linear discriminant functions of one, two, and four sampling clusters, and used the Bayesian Information Criterion (BIC; Schwarz 1978) to assess the best supported model. We used the package ADEGENET that implements DAPC in R (Jombart 2008).

**2.3.4 Bayesian Cluster Analysis**

To compare against a priori grouping of individuals in the above analyses, further examination of microsatellite genetic structure between and within crane populations was performed using a Bayesian model-based cluster analysis implemented in Structure V2.3.4 (Pritchard et al. 2000). The methods in the Structure clustering approach are individual based and are aimed to reduce the amount of bias that sampling and grouping has on determination of population structure (Falush et al. 2003), particularly in comparison to the DAPC method above. Structure implements Bayesian methods by calculating the likelihood that the data can be divided into a number of clusters, with each cluster trying to maximise Hardy-Weinberg equilibrium and linkage equilibrium (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009). By optimising the likelihood for different numbers of genetic clusters, we test for the optimal number in our samples.

We performed 1000 Bayesian model-based cluster simulations using the admixture and correlated allele frequency models for different numbers of clusters (K) for K=1 through K=8 . We ranked models to determine the most likely cluster number based on the Bayesian information statistic (BIC) where smaller BIC indicated better parsimony (Falush et al. 2003; Hubisz et al. 2009). For each model, we set the burnin period to 100,000 and used 200,000 Markov chain Monte Carlo repetitions.

**2.3.5 Phylogenetic Inference**

Phylogenetic trees were inferred for both microsatellite markers and mitochondrial DNA sequences. For microsatellite markers, genetic distance matrices of Sandhill Crane populations were inferred using Nei’s genetic distance (GST, Nei 1973) and Wright’s F-statistic (FST) distance (Wright 1951). Nei’s genetic distance and FST distances were calculated as implemented in PoptreeW (Takezaki et al. 2014). Phylogenetic trees were inferred from the genetic distance matrices via neighbour joining, and nodal support values were calculated based on 1000 bootstrap replicates, (Takezaki et al. 2014). The resulting phylogenetic trees were visualised using FigTree (Rambaut 2012).

All obtained mitochondrial sequences (n=52) were aligned using MAFFT v7.490 (Katoh and Standley 2009) and visually inspected using AliView v1.26 (Larsson 2014). A maximum likelihood phylogeny was inferred using IQTREE2 (Minh et al. 2020) following selection of the most appropriate substitution model using model finder (Kalyaanamoorthy et al. 2017) and nodal support values were based on 1000 bootstraps.

# 3 Results

**3.1 Specimen Collection**

The amplification rate of blood/tissue samples (0.810, N=18) was marginally better than that of feather samples (0.75, N=185), but this difference in amplification was not significant (p=0.13). However, blood samples were always amplified at some loci, whereas there were 19 feathers from which no genetic markers could be extracted (these feathers were removed from the dataset).

**3.2 Microsatellite Genetic Diversity and Structure**

Mean amplification rate varied both across sampling regions (Table 1) and between loci (Table 2). Mean amplification ranged from 0.682 to 0.885 among the four geographic regions. Of the eight loci examined, the rate of amplification varied from 0.581 to 0.818 with Gram11 and GiM15 having the highest amplification rates (Table 2).

**Table 1**. Sample types per population and number of successfully amplified samples. Descriptive statistics for each population derived from multilocus microsatellite DNA genotypes (n=203).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample Types and Descriptive Statistics | Population | | | | Overall |
|  | Coast | Haida Gwaii | Interior BC | Alaska |  |
| Number of feathers | 117 | 13 | 77 | 0 | 207 |
| Number of blood samples | 6 | 0 | 0 | 9 | 15 |
| Samples genotyped | 123 | 13 | 77 | 9 | 222 |
| Samples used in genotype analysis | 108 | 13 | 73 | 9 | 203 |
| Samples used in mtDNA sequence and haplotype analysis | 20 | 5 | 20 | 7 | 52 |
| Samples successfully genotyped | 108 | 13 | 73 | 9 | 203 |
| Average alleles per locus | 9.375 | 5.500 | 9.125 | 5.375 | 7.34 |
| Standardized allelic richness | 3.920 | 3.630 | 4.200 | 4.340 | 4.02 |
| Mean amplification rate | 0.601 | 0.885 | 0.779 | 0.736 | 0.747 |
| Expected heterozygosity | 0.725 | 0.688 | 0.748 | 0.754 | 0.730 |
| Observed heterozygosity | 0.584 | 0.678 | 0.547 | 0.642 | - |
| Inbreeding within subpopulation (FIS) | 0.194 | 0.015 | 0.269 | 0.149 | - |
| p-value associated with FIS | \*\*0.001 | 0.395 | \*\*0.001 | \*0.022 | - |

\*\*significant at=0.05 using a Benjamini-Yekutieli correction

Average alleles per locus and standardised allelic richness per locus were 7.34 and 4.02 respectively, with an expected heterozygosity of 0.73 across loci (Table 1). Within the samples of the four geographic regions, we found various levels of genetic diversity. Higher standardised allelic richness (AR) was observed in the Alaska (AR = 4.34) and Interior (AR = 4.20) populations, with lower richness observed on the Coast (AR = 3.92) and Haida Gwaii populations (AR = 3.63).

3.2 Population Structure

With the exception of Haida Gwaii, all regions showed a significant deficit in heterozygosity (inbreeding coefficient significantly different from zero , FIS, P < 0.05, Table 1). The inbreeding coefficient (FIS) was highest in samples from Interior BC (FIS = 0.27, p=0.001) and Coast (FIS = 0.188, p=0.001), populations corresponding to regions with larger sample sizes (and more statistical power). The cranes from Haida Gwaii had the lowest degree of inbreeding with an FIS value close to 0 (FIS=0.015) and the lowest homozygosity rate (32.2%). The inbreeding coefficient, FIS, can be sensitive to allelic dropout (Soulsbury et al. 2007), but since population FIS values did not align with dropout rates, our results do not indicate that allelic dropout was a significant factor.

Degree of differentiation between populations as measured by FST indicates that 5% (p=0.001) of the total genetic variability can be attributed to differences among populations. The overall AMOVA results suggest significant differences between sampling regions (p=0.001). Post hoc, pairwise analyses for differences between sampling regions suggested that Haida Gwaii and Coastal BC samples were significantly different from samples from Alaska and Interior BC (p<0.05, Figure 3). By contrast, birds from Interior BC/Alaska sampling regions and Haida Gwaii/Coastal BC sampling regions were not statistically different (p=0.307; Figure 3).

Private alleles occurred in 7 of 8 loci (Table 2). Within the Coast population, 12.6% of alleles (12 of 96 alleles) were private, the Interior BC population had 10.4% (10 of 96 alleles) private, Alaska had 2.1% (2 of 96 alleles) and Haida Gwaii had 1.1% (1 of 96 alleles). When the most similar populations were grouped (Alaska with Interior, Coastal with Haida Gwaii) six of the loci contained private alleles in each of the Alaska/Interior and the Coast/Haida Gwaii populations (Table 2). The majority of alleles were shared between both grouped populations (62 of 95). However, 18.9% of alleles were private (unique) to Coastal cranes (Coast and Haida Gwaii cranes) and 15.9% were unique to the SW Alaska and Interior BC cranes (Table 2).

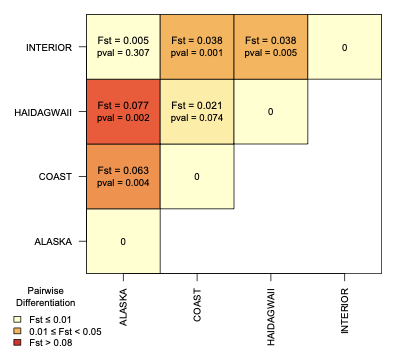
Table 2. Number of shared and private alleles across four populations: Alaska, Interior BC, Coastal BC and Haida Gwaii as well as those shared between populations grouped based on similarity (Alaska/Interior and Coast/Haida Gwaii). The rate of amplification at each of the 8 loci is reported with a mean amplification across all alleles of 74.7 %. The significance of the test for Hardy Weinberg Equilibrium (HWE) for all 8 loci is shown; the overall p-value of all samples was <0.001. .

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Locus Name | Gram-  6 | Gram-11 | Gram-20 | Gram-22 | Gram-30 | Gram-42 | GjM-15 | GjM-48b |
| Number of Alleles | 12 | 17 | 22 | 6 | 21 | 6 | 4 | 8 |
| Shared Alleles Across Four Populations (%) | 8.3 | 23.5 | 18.1 | 50 | 23.8 | 33.3 | 50 | 25 |
| Private Alleles in Alaska (%) | 16.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Private Alleles in Interior (%) | 0 | 5.9 | 31.8 | 0 | 4.7 | 0 | 0 | 12.5 |
| Allele in grouped Alaska/Interior Population (%) | 0 | 5.9 | 0 | 16.7 | 0 | 0 | 0 | 0 |
| Private Alleles in Coast (%) | 8.3 | 5.9 | 13.6 | 0 | 23.8 | 33.3 | 0 | 0 |
| Private Alleles in Haida Gwaii (%) | 0 | - | 4.5 | 0 | 0 | 0 | 0 | 0 |
| Private Allele in grouped Coast/Haida Gwaii Population (%) | 8.3 | - | 4.5 | 0 | 9.5 | 0 | 0 | 0 |
| Amplification Rate (%) | 78.3 | 81.8 | 72.4 | 76.8 | 78.8 | 73.9 | 81.8 | 58.1 |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Test for HWE p-values\*\* | <0.001 | <0.001 | <0.001 | 0.019 | <0.001 | <0.001 | <0.001 | <0.001 |

\*\* p-values are corrected using the Benjamini and Yekutieli (2001) correction.

There were no significant differences (p > 0.05) between FST  values of cranes sampled in Alaska vs the Interior populations nor between the Coastal BC and Haida Gwaii populations (Figure 3). Moderate (p ≤ 0.05) significant differences were found between the Interior and Haida Gwaii populations, the Alaska and Haida Gwaii populations, and between the Coastal and Alaska populations. Highly significant differences (p ≤ 0.001) were found between the Interior and Coast populations. Moderate genetic differentiation was found between the Alaska and Haida Gwaii populations (FST = 0.077) and between the Alaska and Coastal BC populations (FST = 0.064) (Figure 3).



**Figure 3**. Values of FST for pairwise differentiation of populations where darker colours represent populations more divergent with p-values below the significance threshold indicating evidence of deviations from HWE. FST values between 0.05-0.25 indicate moderate genetic differentiation (Wright 1978).

3.2.1 Discriminant Analysis of Principal Components

Overall, the discriminant analysis of principal components (DAPC) model was able to assign individuals to correct sampling regions 92.2% of the time if a four-population model was assumed (Table 3).

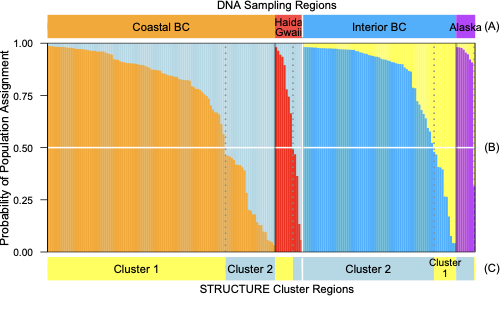
**Table 3**. Comparison of proportion of DAPC assigned to correct group (i.e., probability of a sample being correctly identified to its geographic sampling region), compared to the Structure cluster analysis’ assignment to Cluster 1 and Cluster 2.

|  |  |  |  |
| --- | --- | --- | --- |
|  | DAPC Results | | |
| Assigned Population | Modelled proportion of samples in Coast/Haida Gwaii cluster | Modelled proportion of samples in Interior/  Alaska cluster | Percent assigned to correct geographic sampling region |
| Coast | 0.968 | 0.032 | 97.1% |
| Haida Gwaii | 1.00 | 0.00 |  |
| Interior BC | 0.156 | 0.844 | 84.9% |
| Alaska | 0.111 | 0.889 |  |

We evaluated evidence for different numbers of populations (‘1’: All sampling regions, ‘2’: Coast/HG vs Alaska/Interior; and ‘4’ populations corresponding to four sampling regions; and ‘8’ subpopulations) and found the most support for the model with two populations (smallest BIC statistic: 1028.8, compared to 1 population (BIC: 1029.0) or 4 populations (BIC: 1029.3). The best rate of correct assignment was observed in the Coast and Haida Gwaii populations (0.968 and 1.0, Table 3).

3.2.2 Bayesian Cluster Analysis

Using the Bayesian analysis software ‘Structure’, we tested evidence for the number of clusters in the genetic samples for each of K=1 to K=8. The best model with the lowest BIC was the model that returned two population clusters (K=2; BIC: 1370.6 relative to the next closest model of K=3, BIC: 1374.4). The majority of assignments aligned with geographic DNA sampling regions (Figure 4) however, some admixture among populations is occurring. Cluster 1 contains the majority of Coast and Haida Gwaii samples (76%), while Cluster 2 contains the majority of Interior BC and Alaska samples (85.4%). Broken down by sampling regions, we found 78% of Coastal BC and 62% of Haida Gwaii birds are grouped in Cluster 1, while Cluster 2 contained 85% and 89% of the Interior BC and Alaska samples, respectively (Figure 4).



**Figure 4.** Bayesian cluster analysis of the microsatellite DNA genetic structure extracted from Sandhill Crane samples collected in four different DNA sampling regions (A). Individual admixture proportion of ancestry assigned to individuals in each of the K=2 subgroups is plotted for each individual (B). Individuals are ordered by DNA sampling region, and then by model-based likelihood (admixture proportion) for Cluster 1 for the Coastal BC (orange) and Haida Gwaii (red) sampling regions. For Interior BC (blue) and Alaska (purple) sampling regions, individuals are ordered by the likelihood for Cluster 2. (C) If an individual has a higher admixture proportion of belonging to Cluster 1 relative to Cluster 2 it is coloured yellow, if an individual has a higher admixture proportion for Cluster 2, it is coloured light blue.

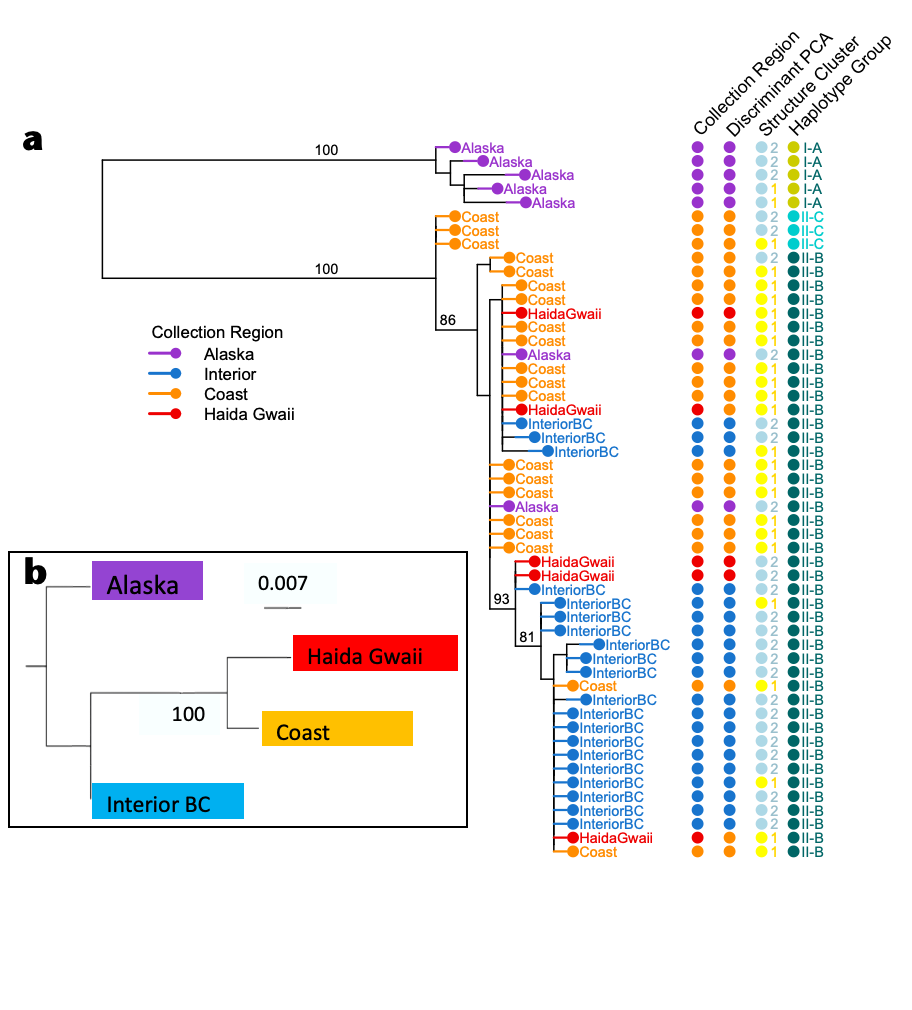
**3.3 Mitochondrial Genetic Diversity and Structure**

Sequences obtained for 52 individuals fell into 22 different haplotypes (Genbank accession numbers xxxxx-xxxxx; Figure 5a). The three most frequent haplotypes were a, c, and k. Haplotype “a” was shared by nine individuals (17% frequency): six from the Coast, two from Haida Gwaii and one from the Interior population. Seven Coastal cranes (13%) shared haplotype “c” while haplotype k was shared by seven other birds (13%); five Interior, one Haida Gwaii, and one Coast. Eleven haplotypes were unique (21%). Following Glenn et al. (2002) and Rhymer et al. (2001) reference groups, we identified three haplotype groups in our Sandhill Crane mitochondrial control region sequences from the four regions. These groups broadly correspond to clades in the phylogenetic tree. One clade consists of the highly diverged Alaska population (Group I-A), the second, coastal clade, at the base of the remaining samples in the phylogenetic tree (Group II-B), and the third (Group II-C) includes all other samples from the Coast, Haida Gwaii, Interior and Alaska groups (Figure 5).

**3.4 Overall Population Structure**

**3.4.1 Phylogenetic Inference**

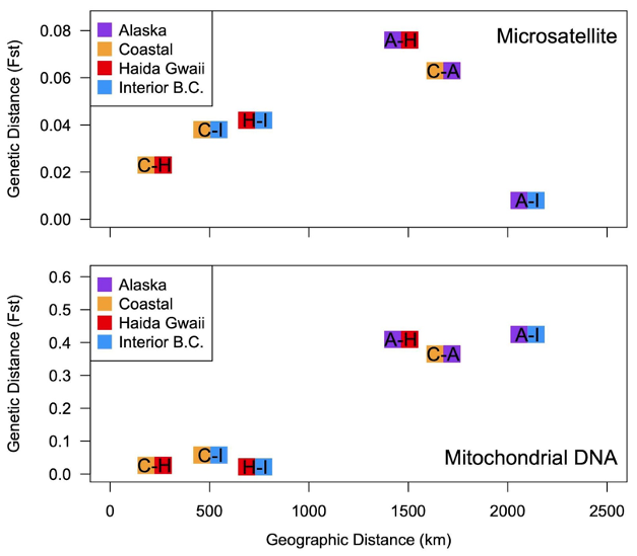
Inferred phylogenetic relationships among sampled Sandhill crane populations based on the mtDNA control region sequences (n=52) suggests there is population structure in the Pacific Flyway Sandhill Cranes (Figure 5). The analysis primarily splits individuals into clades based on breeding region of origin with limited admixture. Bootstrap support values reveal 100% nodal support for separation between the Alaska group and all populations of BC cranes, i.e., samples collected on Haida Gwaii, the Coast, and in Interior BC. There is good nodal support (93%) for the clade that is composed primarily of samples derived from the BC Interior breeding population (blue samples in the lower clade of Figure 5b). Coast and Haida Gwaii populations are largely separate from Alaska and Interior clades but without strong nodal support and with some introgression or incomplete lineage sorting.

An additional phylogenetic tree based on microsatellite markers inferred using FST distances (Figure 5b) is generally in broad agreement with the mtDNA phylogeny. However, the microsatellite phylogenies reveal strong support (nodal support of 100) for the node separating the Coastal BC and Haida Gwaii birds from the Interior BC and Alaska birds, indicating support for the split between the Coastal and Haida Gwaii populations relative to the Interior BC and Alaska populations. There was no difference in topology for trees inferred under FST distances and those inferred using Nei’s genetic distance (GST; Nei 1973).

**Figure 5**. Phylogenetic relationships among Pacific flyway Sandhill Cranes. Maximum likelihood phylogenetic tree of mtDNA inferred using IQTREE2, bootstrap support values are denoted at the nodes (if support > 80) (a). Each point represents an individual crane. Neighbour joining phylogenetic tree inferred from FST distances derived from microsatellite markers in PoptreeW (b). Nodal support value is based on 1000 bootstrap replicates. Each tip represents a population. Scale bar represents FST genetic distance.

**3.4.2 Isolation by Distance**

We estimated pairwise genetic differentiation for microsatellite and mitochondrial DNA, between the four sampling regions in this study. Differentiation of microsatellite FST followed the expected differentiation with geographic distance. The Interior BC and Alaskan populations were the farthest geographic distance but suggested the closest genetic distance (Figure 6, top panel). This pattern was not repeated in pairwise differentiation of mtDNA where the four populations displayed a pattern consistent with isolation by distance (Figure 6, bottom panel) in which the strong effect of large genetic distances and large geographic distances between all populations and Alaska was observed.



**Figure 6**. Correlation between genetic distance (as judged by FST) and geographic distance. The top panel shows this relationship for microsatellite data while the bottom panel shows the relationship for mtDNA. In both cases there is evidence of large genetic distances and large geographic distances between all populations and Alaska.

# 4 Discussion

Our integrated analyses across a range of genetic markers reveal differences in degree of genetic differentiation between geographically separated breeding populations of Sandhill Cranes in the Pacific Flyway. These results support results of previous studies analyzing morphological, habitat, and genetic data. Previous genetic and morphological studies have identified migratory cranes in regions east of the Rocky Mountains as belonging to two distinct subspecies, *A.c. tabida*, and *A.c. canadensis*, with samples from *A.c. rowani* either grouping together with those from *A.c. tabida* (Rhymer et al. 2001, Glenn et al. 2002) or being intermediate (Petersen et al. 2003, Jones et al. 2005). Ivey et al. (2005) found that cranes wintering in Sauvie Island and Ridgefield National Wildlife Refuge (Lower Columbia River, Washington State) migrated west of the coast mountains to summer breeding grounds ranging from the central coast of BC to southeast Alaska (Dall Island and Prince of Wales Island). The morphological data and general features (large flat head profiles on intermediate body size; shorter unfeathered portion of legs) suggest that these birds are from the *A.c. rowani* subspecies. A genetic analysis using amplified fragment length polymorphism (AFLP) of eight coastal breeding cranes found they grouped together and were separate from Alaskan cranes, but with incomplete separation from interior (Oregon and California) nesting cranes (Hayes 2015) although no Interior BC cranes were included in that study.

Analysis of the assignment of mitochondrial control region sequences into haplogroups reveals variation between the southwest Alaska population of *A.c. canadensis* and all regions sampled in British Columbia and southeast Alaska. Phylogenetic trees of mtDNA and microsatellites illustrate population structure emerging among geographically isolated coastal and interior breeding populations with limited introgression or incomplete lineage sorting. Overall, more rapidly evolving genetic markers (microsatellites) show higher levels of genetic differentiation among geographically isolated breeding populations compared to intermediate mtDNA sequence and more conserved mtDNA haplogroups that show the least differentiation.

Multiple lines of evidence suggest coastal populations of Sandhill Cranes are genetically differentiated from the *A.c. tabida* of interior BC, and the *A.c. canadensis* found in southwest Alaska. The results of AMOVA, DAPC and Structure k-means clustering algorithms of microsatellite loci all suggest significant differentiation between Coastal BC regions and the Interior BC and Alaska regions. Phylogenetic trees based on both microsatellite and mtDNA sequence data displayed broad agreement in separation of major clades by geographic region, with mtDNA showing limited introgression and weak nodal support values. Microsatellite phylogenies display strong support for the node separating coastal cranes from Interior BC and Alaska birds, indicating support for the split between the Coast and Haida Gwaii population relative to the Interior BC and Alaska populations. Finally, genetic distance (FST, GST of microsatellites) was correlated with genetic distance between Coastal and Interior populations but did not correlate with geographic distance between Sandhill Crane populations of the coastal region of Alaska and Interior BC. Overall, these analyses of available genetic markers highlight population structure within the Pacific Flyway population that broadly corresponds with ecological divisions, and the importance of considering the distinctive ecological context of Coastal and Interior Sandhill Crane populations in conservation and management planning.

Genetic divergence between Coastal, Interior and southwestern Alaskan cranes could be due to historical or contemporary factors or a combination. Jones et al. (2005) postulated that a glacial barrier during the Pleistocene era caused a geographic constraint that may have been responsible for the initial separation of the northern *A.c. canadensis* and southern *A.c. tabida* subspecies, however, the separation time was not long enough for full reproductive isolation and speciation. This breeding isolation may have been long enough to give rise to the haplotype variation between Sandhill Cranes subspecies west of the Rocky Mountains. If the vicariant glacial barrier of this era was responsible for the formation of these two subspecies, then its subsequent melting, beginning 18,000 years ago at the western margin (Darvill et al. 2018), might have resulted in these two subspecies coalescing, depending on the degree of gene flow between populations.  Haplotype group I-A typically associated with *A.c. canadensis* (Glenn et al 2002, Rhymer et al 2001) was only detected in the Alaskan Bristol Bay and Cook Inlet breeding birds, whereas in our study there were two cranes with haplotype group II haplotype typically associated with *A.c. rowani* and *A.c. tabida* found in southwest Alaska. Both cranes were male radio-tagged birds, suggesting there may be male-mediated dispersal from the southern populations up into Alaska.

It is possible that Coastal and Haida Gwaii cranes persisted through the Last Glacial Maximum of the Pleistocene in glacial refugia on the outer coasts of southeast Alaska and BC’s northern coast or recolonized these areas early afterwards. Recent studies of pollen in glacial sediments suggest that the Hecate Plain, now submerged under Hecate Strait, between Haida Gwaii and the North and Central Coast, provided a glacial refugium that may have supported a diverse flora and fauna during the Fraser glaciation (Mathewes and Clague 2017). Part of the coastal crane habitat, the island archipelago of Haida Gwaii, may have been ice-free during the last ice age. The existence of Haida Gwaii as a glacial refugium has been suggested by the presence of multiple endemic taxa (Foster 1963, Heusser 1990) and possibly unique genetic lineages (O'Reilly et al. 1993, Soltis et al. 1997, Byun et al. 1997, Clarke et al. 2001, Smith et al. 2001, Fleming and Cook 2002, Topp and Winker 2008). For example, divergence estimates for endemic subspecies of the sedentary Northern Saw-whet Owl, Hairy Woodpecker, and Steller's Jay provide evidence that these likely survived throughout the last glacial maximum in the area of Haida Gwaii (Pruett et al. 2013). Evidence of a forested glacial refuge along the western edge of the Alexander Archipelago in Southeast Alaska was also suggested by genomically distinct clades within Pacific martens (*Martes caurina*) on the North Pacific Coast (Colella et al. 2020). Pollen records show the development of pine (*Pinus contorta* subsp. *contorta*) parkland vegetation in the Western Alexander Archipelago of southeast Alaska soon after the LGM (∼15,240–14,040 ybp), suggesting recolonization from coastal refugia (Ager 2019).

Another possibility that could explain historical genetic differentiation of coastal cranes is the early recolonization of coastal areas that maintained unusually stable sea levels over the past 15,000 years. The Dundas Island Archipelago, northeast of Haida Gwaii (McLaren 2008), as well as the Hakai Passage area of the Central Coast (McLaren et al. 2014) were identified through core sampling as places with stable shorelines over this period, occupying a sea level hinge, with land depressed underwater by glaciers to the east and uplifted above sea level to the west by tectonic forces (Clague et al. 1983). Both of these areas remain important breeding habitats for coastal cranes (Roessingh 2012).

Continued contemporary divergence could be driven by different habitat selection, foraging strategies, and behaviours for predator avoidance of Coastal and Haida Gwaii cranes compared to Interior cranes. Although Sandhill cranes are omnivorous and occupy a wide range of habitats across North America, cranes breeding in coastal BC and Haida Gwaii nest and roost in bog complexes and feed on marine molluscs within estuaries and on coastal beaches (Hearne and Hamel 2003, Roessingh 2012). Microsatellite data suggests that coastal breeding Sandhill Cranes are continuing to diverge from interior birds despite some introgression. Ecological selection can cause rapid divergence in candidate genes depending on how it affects survival and reproductive rates, but neutral markers generally show slower rates of genetic divergence relative to markers under selection. Some level of reproductive isolation may result in genetic distinction despite low levels of gene flow when combined with sufficiently strong or divergent ecological selection. Adaptation to different breeding environments can lead to increasing reproductive isolation despite sympatry during non-breeding periods, even without extended isolation of allopatric breeding populations (Winker 2010). Ecological traits, such as habitat associations, may correlate with patterns of genetic diversity and divergence, as shown in comparison of closely related upland and floodplain species of Amazonian birds (Harvey et al. 2017). In cranes, such divergent ecologies may drive local adaptation and/or reproductive isolation leading to genetic differences in alleles between coastal and interior cranes over time.

While samples collected and included in this study covered a wide geographic region in British Columbia, to gain an understanding of the whole Pacific population samples of feather or blood from the neighbouring Pacific US states of Alaska, Washington, Oregon and California would strengthen and build a complete subspecies level inference putting into context the population structure observed in this British Columbia-focused study.

However, overall, the significant between-population genetic differentiation observed in this study coupled with differences in migration route, diet, and breeding habitat distinguish Coastal cranes from Interior and Alaskan populations, suggesting that Coastal cranes are on an independent evolutionary trajectory, and may meet the Committee on the Status of Endangered Wildlife in Canada’s criteria for a Designatable Unit (COSEWIC 2020). Overwintering habitat for western cranes in Washington , Oregon , and California is protected, but critical crane breeding habitat in BC currently has no protection. Cranes are a migratory species with summer and winter habitat separated by the Canada-US international border, complicating conservation efforts. In BC, proposed protection of Sandhill Cranes continues to be based on a single-species approach, rather than at the ecosystem level that integrates genetic structure into how conservation of cranes and their habitat is managed. With the climate shifting across British Columbia to warmer, dryer and more fire-prone summers, we anticipate the native range of Sandhill Cranes and other species dependent on marshlands for breeding, to become a transient and dynamic property. In the coming decades, ensuring the fullest range of adaptations within local populations will ensure the resiliency of the species and will be an important prerequisite to effective stewardship (Suding and Hobbs 2009).

# Acknowledgements

We would like to acknowledge the many Indigenous nations from whose territory we gathered samples, in particular the Heiltsuk, Kitasoo/Xai'xais, and Gitga'at Nations of the Central and North coast of BC, and the Tsilhqot’in, the St’át’imc, Nlaka’pamux of the Cariboo-Chilcotin region of interior British Columbia. Citizen scientists were critical to obtaining sufficient numbers of samples to test the hypothesis of genetic isolation of coastal birds from other crane populations, providing 52 of the 203 samples used for analysis. These volunteers were also key to sampling as broad a geographic area as possible, locating previously undocumented breeding habitat, accessing samples from private lands through the assistance and contributions of ranchers and other landowners, and educating members of the public about the life history and conservation needs of Sandhill Cranes. We are grateful to the National Geographic Society/Waitt Grants Foundation and to the Public Conservation Assistance Fund through the BC Habitat Conservation Trust Fund for the funding they provided to this project.

# Literature Cited

Ager, T. A. (2019). Late Quaternary vegetation development following deglaciation of northwestern Alexander Archipelago, Alaska Frontiers.  Earth Science, 7(104), 1–25.

Benjamini, Y., Yekutieli, D., (2001) The control of false discovery rate under dependency. Ann Stat 29:1165–1188.

BC Conservation Data Centre. (2018). Conservation Status Report: *Antigone canadensis*. BC Minist. Of Environment. Available:<https://a100.gov.bc.ca/pub/eswp/>. Accessed Dec. 15, 2021.

Byun, S. A., Koop, B. F., and Reimchen, T. E. (1997). North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. Evolution, 51(5), 1647-1653.

California Natural Diversity Database (CNDDB). (2021). State and Federally listed endangered and threatened animals of California; October 2021. State of California, Natural Resources Agency, Department of Fish and Wildlife, Biogeographic Data Branch.

Caven, A.J. (2023). An Updated Minimum Estimate of the Global Sandhill Crane Population. Platte River Natural Resource Reports eJournal 2:1-14.

Clague, J. J., Luternauer, J. L., and Hebda, R. J. (1983). Sedimentary environments and postglacial history of the Fraser Delta and lower Fraser Valley, British Columbia. Canadian Journal of Earth Sciences, 20(8), 1314-1326.

Clarke, T. E., Levin, D. B., Kavanaugh, D. H., and Reimchen, T. E. (2001). Rapid evolution in the Nebria gregaria group (Coleoptera: Carabidae) and the paleogeography of the Queen Charlotte Islands. Evolution, 55(7), 1408-1418.

Colella, J. P., Wilson, R. E., Talbot, S. L., and Cook, J. A. (2019). Implications of introgression for wildlife translocations: the case of North American martens. Conservation Genetics, 20(2), 153-166.

Cooper, J. M. (1996). Status of the sandhill crane in British Columbia. Wildlife Bulletin B-83, British Columbia Ministry of Environment, Lands and Parks, Wildlife Branch, Victoria, British Columbia, Canada.

COSEWIC (2020). COSEWIC guidelines for recognizing designatable units. Committee on the Status of Endangered Wildlife in Canada. Available:https://www.cosewic.ca/index.php/en/reports/preparing-status-reports/guidelines-recognizing-designatable-units.html. Accessed Jun. 21, 2024.

Darvill, C. M., Menounos, B., Goehring, B. M., Lian, O. B., and Caffee, M. W. (2018). Retreat of the western Cordilleran Ice Sheet margin during the last deglaciation. Geophysical Research Letters, 45, 9710– 9720.

Davidson, P.J.A., R.J. Cannings, A.R. Couturier, D. Lepage, and C.M. Di Corrado (eds.). (2015). The Atlas of the Breeding Birds of British Columbia, 2008-2012. Bird Studies Canada, Delta, BC < http://www.birdatlas.bc.ca/ e > Accessed Jun. 21, 2024.

eBird. (2018). eBird: An online database of bird distribution and abundance [web application]. eBird, Cornell Lab of Ornithology, Ithaca, New York. Available: http://www.ebird.org. Accessed Sep. 30, 2018.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Faaborg, J., Holmes, R.T., Anders, A.D., Bildstein, K.L., Dugger, K.M., Gauthreaux Jr, S.A., Heglund, P., Hobson, K.A., Jahn, A.E., Johnson, D.H. and Latta, S.C. (2010). Conserving migratory land birds in the New World: Do we know enough? Ecological Applications, 20(2), 398-418.

Fleming, M. A., and Cook, J. A. (2002). Phylogeography of endemic ermine (Mustela erminea) in southeast Alaska. Molecular Ecology, 11(4), 795-807.

Falush, D., M. Stephens and J. K. Pritchard. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164, 1567-1587.

Foster, J. (1963). The evolution of the native land mammals of the Queen Charlotte Islands and the problem of insularity (Doctoral dissertation, University of British Columbia).

Geneious 8.1.9 [https://www.geneious.com](http://www.geneious.com/)

Glenn, T.C., Thompson, J.E., Ballard, B.M., Roberson J.A. and French, J.O. (2002). Mitochondrial DNA variation among wintering midcontinent gulf coast Sandhill Cranes. The Journal of Wildlife Management, 66(2), 330-348. doi: https://www.jstor.org/stable/3803166

Harvey, M. G., Aleixo, A., Ribas, C. C., and Brumfield, R. T. (2017). Habitat association predicts genetic diversity and population divergence in Amazonian birds. The American Naturalist, 190(5), 631-648.

Hayes, M. (2015). Dispersal and population genetic structure in two flyways of Sandhill Cranes (*Grus canadensis*). Ph.D. dissertation, University of Wisconsin-Madison, Madison, WI

Hearne, M., and P. Hamel. (2003). The sandhill cranes of Haida Gwaii. Report prepared for the West Coast Crane Working Group. <http://www.wccwg.nacwg.org/researchprojects/queencharlottes.htm> Accessed Feb. 24 2008.

Hersh E.W., Wheeler E.J., Constanzo B. Ramakrishnan A.P; Miscampbell A.E., Ritland C., Whitton J., Gorrell J.C. and Harrower W. (2022). Diversity among rare and common congeneric plant species from the Garry oak and Okanagan shrub-steppe ecosystems in British Columbia: implications for conservation. Botany 101(1), 1-14.

Heusser, C. J. (1990). Late quaternary vegetation of the Aleutian Islands, southwestern Alaska. Canadian Journal of Botany, 68(6), 1320-1326.

Hubisz, M. J., D. Falush, M. Stephens and J. K. Pritchard. (2009). Inferring weak populations structure with the assistance of sample group information. Molecular Ecology Resources 9, 1322-1332.

iNaturalist. (2018). Coastal Crane Atlas project observations. Available from https://www.inaturalist.org/projects/coastal-crane-atlas. Accessed Sep. 30, 2018.

Ivey, G.L., Herziger, C.P. and Hoffmann, T.J. (2005). Annual movements of Pacific coast sandhill cranes. North American Crane Workshop Proceedings. 13.

Ivey, G.L. (2013). Pacific Flyway Sandhill Crane Migration Map. International Crane Foundation.

Ivey, G.L., Dugger, B.D., Herziger, C.P., Casazza, M.L. and Fleskes, J.P. (2015). Wintering ecology of sympatric subspecies of Sandhill Crane: Correlations between body size, site fidelity, and movement patterns. The Condor: Ornithological Applications, 117(4), 518-529.

Jetz, W., Thomas, G.H., Joy, J.B., Redding, D.W., Hartmann, K., Mooers, A.O. (2014). Global distribution and conservation of evolutionary distinctness in birds. Current Biology 24(9), 919-930.

Johansson, M.P., Mcmahon, B.J., Höglund, J. and Segelbacher, G. (2012). Amplification success of multilocus genotypes from feathers found in the field compared with feathers obtained from shot birds. Ibis, 154(1), 15-20.

Johnsgard, P. A. (1983). Cranes of the world: Eurasian crane (*Grus grus*). Cranes of the World, by Paul Johnsgard, 17.

Jombart, T. (2008). adegenet: an R package for the multivariate analysis of genetic markers. Bioinformatics 24: 1403-1405.

Jombart, T., and Collins, C. (2015). Analysing genome-wide SNP data using adegenet 2.0.0. <<https://adegenet.r-forge.r-project.org/files/tutorial-genomics.pdf>> Accessed Jul. 23, 2024.

Jones, K.L., Krapu, G.L., Brandt, D.A. and Ashley, M.V. (2005). Population genetic structure in migratory sandhill cranes and the role of Pleistocene glaciations. Molecular Ecology, 14(9), 2645-2657.

Jones K. L. Henkel J. R. Howard J. J. Lance S. L. Hagen C. Glenn T. C. (2010). Isolation and characterization of 14 polymorphic microsatellite DNA loci for the endangered Whooping Crane (Grus americana) and their applicability to other crane species. Conservation Genetics Resources 2, 251-254.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods.,14(6), 587-9.Epub 2017/05/10. doi: 10.1038/nmeth.4285. PubMed PMID: 28481363; PubMed Central PMCID: PMCPMC5453245.

Katoh K, and Standley DM. (2009). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013, 30(4), 772-80. Epub 2013/01/19. doi: 10.1093/molbev/mst010. PubMed PMID: 23329690; PubMed Central PMCID: PMCPMC3603318.

Kelly, C. D. (2019). Do birds of a feather flock together: An investigation of sandhill crane (*Antigone canadensis*) populations using non-invasive feathers as a source of DNA (MSc thesis). Trent University, Peterborough, Ontario, Canada.

Larsson A. (2014). AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics. 2014, 30(22), 3276-8. Epub 2014/08/07. doi: 10.1093/bioinformatics/btu531. PubMed PMID: 25095880; PubMed Central PMCID: PMCPMC4221126.

Littlefield, C. D., and Ivey, G. L. (2002). Sandhill Crane recovery plan. Washington Department of Fish and Wildlife, Olympia, Washington.

Littlefield, C. D., and G. L. Ivey. (1995). An unusual record of Sandhill Crane philopatry. Wilson Bulletin 107, 766.

Mathewes, R. W., and Clague, J. J. (2017). Paleoecology and ice limits of the early Fraser glaciation (marine isotope stage 2) on Haida Gwaii, British Columbia, Canada. Quaternary Research, 88(2), 277-292.

McLaren, D. (2008). Sea Level Change and Archaeological Site Locations on the Dundas Island Archipelago of North Coastal British Columbia. (PhD Disssertation, University of Victoria).

McLaren, D., D. Fedje, M. B. Hay, Q. Mackie, I. J. Walker, D. H. Shugar, J. B.R. Eamer, O. B. Lian, C. Neudorf. (2014). A post-glacial sea level hinge on the central Pacific coast of Canada. Quaternary Science Reviews, 97, 148-169.

Meares K, Dawson D, Horsburgh G, Glenn T, Jones K, Braun M, Perrin M, Taylor T. (2008). Microsatellite loci characterized in three African crane species (Gruidae, AVES). Molecular Ecology Resources 9, 308–311.

Meirmans, P.G. (2012). AMOVA-based clustering of population genetic data. Journal of Heredity 103, 744–750.

Meirmans, P.G. (2020). GenoDive version 3.0: Easy-to-use software for the analysis of genetic data of diploids and polyploids. Molecular Ecology Resources. Jul 20(4), 1126-31.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Von Haeseler, A. and Lanfear, R. (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, *37*(5), 1530-1534.

Mirande, C., and J. Harris. (2019). Crane Conservation Strategy. International Crane Foundation, Baraboo, Wisconsin, USA.

Nei M. (1973). Analysis of gene diversity in subdivided populations. Proceeding National Academy of Science USA 70, 3321-3323.

Oregon Department of Fish and Wildlife. (2016). Oregon Conservation Strategy. Salem, Oregon. <<https://oregonconservationstrategy.com/strategy-species/greater-sandhill-crane/?highlight=sandhill%20crane>> Accessed Dec. 13, 2021.

O'Reilly, P., Reimchen, T. E., Beech, R., and Strobeck, C. (1993). Mitochondrial DNA in Gasterosteus and pleistocene glacial refugium on the Queen Charlotte Islands, British Columbia. Evolution, 47(2), 678-684.

Pacific Flyway Council. (1997). Pacific Flyway management plan for the Central Valley Population of Greater Sandhill Cranes. Pacific Flyway Study Committee (c/o Pacific Flyway Representative USFWS), Portland, Oregon, USA.

Peters, C., Nelson, H., Rusk, B. and Muir, A. (2020). A novel method to optimise the utility of underused moulted plumulaceous feather samples for genetic analysis in bird conservation. Conservation Genetics Resources, 12(3), 457-467.

Petersen, J. L., Bischof, R., Krapu, G. L., and Szalanski, A. L. (2003). Genetic variation in the midcontinental population of sandhill cranes, *Grus canadensis*. Biochemical Genetics, 41, 1-12.

Petrula, M. J. and T.C. Rothe. (2005). Migration chronology, routes, and winter and summer range of Pacific Flyway Population of lesser Sandhill Cranes. Proceedings North American Crane Workshop 9, 53-68.

Pritchard, J. K., Stephens and P. Donnelly. (2000). Inference of population structure using multilocus genotype data. Genetics 155, 945-959.

Pruett, C. L., C. M. Topp, J. M. Maley, K. G. McCracken, S. Rohwer, S. Birks, Spencer G. Sealy, K. Winker. (2013). Evidence from the Genetics of Landbirds for a Forested Pleistocene Glacial Refugium in the Haida Gwaii Area, The Condor,115(4), 725–737,<https://doi.org/10.1525/cond.2013.120123>

Rambaut, A. (2012). FigTree: Tree Figure Drawing Tool Version 1.4.4 [Software]. Available from<http://tree.bio.ed.ac.uk/software/figtree/>

Rhymer J.M., Fain, M.G., Austin, J.E., Johnson, D.H. and Krajewski, C. (2001). Mitochondrial phylogeography, subspecific taxonomy, and conservation genetics of sandhill cranes (*Grus canadensis*; Aves: Gruidae). Conservation Genetics 2, 203-218. doi: <http://dx.doi.org/10.1023/A:1012203532300>

Ritland, K. (2012). DNADiffer. http://gdc.forestry.ubc.ca/downloads.

R Core Team. (2023). R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <[https://www.R-project.org/](https://www.r-project.org/)>

Roessingh, K. (2012). Nesting habitat and diet studies of sandhill cranes (*Grus canadensis*) from the central and north coast of British Columbia (MSc Thesis, University of Victoria, Canada).

Sambrook J. Fritsch E. F. Maniatis T. (1989). Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schwarz, G. (1978). Estimating the dimension of a model. Annals of Statistics. 6, 461-4.

Segelbacher, G. (2002). Noninvasive genetic analysis in birds: testing reliability of feather samples. Molecular Ecology Notes, 2(3), 367-369.

Sgrò CM, Lowe AJ, Hoffmann A.A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. Evolutionary applications,4(2), 326-37.

Smith, C. T., Nelson, R. J., Wood, C. C., and Koop, B. F. (2001). Glacial biogeography of North American coho salmon (Oncorhynchus kisutch). Molecular Ecology, 10(12), 2775-2785.

Soltis, D. E., Gitzendanner, M. A., Strenge, D. D., and Soltis, P. S. (1997). Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. Plant Systematics and Evolution, 206, 353-373.

Soulsbury, C. D., Iossa, G., Edwards, K. J., Baker, P. J., and Harris, S. (2007). Allelic dropout from a high-quality DNA source. Conservation Genetics, 8, 733-738.

Stein, R.W., Mull, C.G., Kuhn, T.S., Aschliman, N.C., Davidson, L.N., Joy, J.B., Smith, G.J., Dulvy, N.K. and Mooers, A.O. (2018). Global priorities for conserving the evolutionary history of sharks, rays and chimaeras. *Nature ecology and evolution*, *2*(2), 288-298.

Stinson, D. W. (2017). Periodic status review for the Sandhill Crane. Washington Department of Fish and Wildlife, Olympia, Washington.

Suding, K. N., and Hobbs, R. J. (2009). Models of ecosystem dynamics as frameworks for restoration ecology. New models for ecosystem dynamics and restoration, 3-21.

Takezaki N, Nei M, Tamura K. (2014). POPTREEW: Web Version of POPTREE for Constructing Population Trees from Allele Frequency Data and Computing Some Other Quantities.

Topp, C. M., and Winker, K. (2008). Genetic patterns of differentiation among five landbird species from the Queen Charlotte Islands, British Columbia. The Auk, 125(2), 461-472.

Turbek, S. P., Scordato, E. S., and Safran, R. J. (2018). The role of seasonal migration in population divergence and reproductive isolation. Trends in Ecology and Evolution, 33(3), 164-175.

Vili, N., Nemesházi, E., Kovács, S., Horváth, M., Kalmár, L., and Szabó, K. (2013). Factors affecting DNA quality in feathers used for non-invasive sampling. Journal of Ornithology, 154(2), 587–595. <<https://doi.org/10.1007/s10336-013-0932-9>>

Walkinshaw, L. H. (1950). The Sandhill Crane in the Bernard W. Baker Sanctuary, Michigan. The Auk, 67(1), 38-51.

Walkinshaw, L.N. (1973). Cranes of the world. Winchester Press, New York, USA.

Washington Department of Fish and Wildlife. (2021). State Listed Species. Revised October 2021. <<https://wdfw.wa.gov/sites/default/files/2021-10/statelistedcandidatespecies_10132021.pdf>> Accessed Dec. 13, 2021.

Winker, K. (2010). Chapter 1: Subspecies represent geographically partitioned variation, a gold mine of evolutionary biology, and a challenge for conservation. Ornithological Monographs, 67(1), 6-23.

Wright, S. (1951). “The Genetical Structure of Populations”. Annals of Eugenics 15(1) 323– 354.

Wright, S. (1978). The relation of livestock breeding to theories of evolution. Journal of Animal Science, 46(5), 1192-1200.

Data accessibility

All mitochondrial DNA sequences are deposited in GenBank under the accession numbers XXX-XXX. All metadata associated with collection of feather and blood samples collected and used in the microsatellite, mitochondrial sequence and haplotype analyses are uploaded as online supplemental material (Appendix A). The microsatellite dataset used in our analyses are deposited in the Dryad database at: http://www.xxx.xxx.

Benefit Sharing Statement

Benefits generated from this research arise from the sharing of our data and results on public databases, as described above. The results of this study will help motivate conservation and management policy along the Pacific Flyway for Sandhill Cranes.

Author Contributions

Ruth Joy and Krista Roessingh conceived and funded the project. Ruth Joy, Krista Roessingh, Matt Hayes, and Gary Ivey collected samples of British Columbia cranes, and Mike Petula contributed samples from Alaska. Allyson Miscampbell and Carol Ritland contributed to the lab procedures. Ruth Joy, Kathleen Meszaros and Jeffrey Joy performed the data analysis and interpreted the results. Ruth Joy, Krista Roessingh and Kathleen Meszaros wrote the first draft and all coauthors revised successive versions. All authors improved and approved the final manuscript.

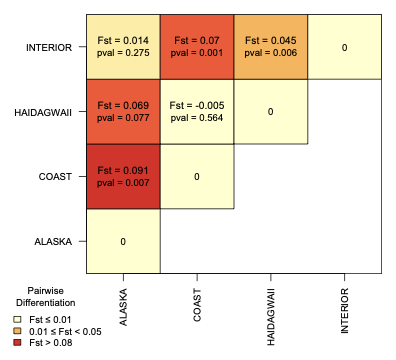
SUPPLEMENTARY MATERIAL

Supplementary Table 1: Reanalysis of Table 1 that includes 69 samples that had no null-alleles. Sample types per population and number of successfully amplified samples. Descriptive statistics for each population derived from multilocus microsatellite DNA genotypes.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample Types and Descriptive Statistics** | **Population** | | | | **Overall** |
| **Coast** | **Haida Gwaii** | **Interior BC** | **Alaska** |
| Number of feathers | 24 | 7 | 31 | 0 | 62 |
| Number of blood samples | 5 | 0 | 0 | 2 | 7 |
| Samples genotyped | 29 | 7 | 31 | 2 | 69 |
| Samples used in genotype analysis | 29 | 7 | 31 | 2 | 69 |
| Samples used in mtDNA sequence and haplotype analysis | 20 | 5 | 20 | 7 | 52 |
| Samples successfully genotyped | 29 | 7 | 31 | 2 | 69 |
| Average alleles per locus | 7.125 | 4.750 | 8.500 | 2.625 | 5.750 |
| Standardized allelic richness | 3.858 | 3.756 | 4.200 | 4.298 | 4.028 |
| Mean amplification rate | 1.000 | 1.000 | 1.000 | 1.000 | 1.00 |
| Expected heterozygosity | 0.618 | 0.680 | 0.581 | 0.688 | 0.642 |
| Observed heterozygosity | 0.724 | 0.721 | 0.753 | 0.781 | 0.745 |
| Inbreeding within subpopulation (FIS) | 0.190 | 0.070 | 0.241 | -0.041 | - |
| p-value associated with FIS | \*\*0.001 | 0.229 | \*\*0.001 | 0.160 | - |

Supplemental Table 2. Amplification rates by loci for the blood/tissue samples, feathers, and for all samples (combined amplification rate).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Gram6 | Gram11 | Gram20 | Gram22 | Gram30 | Gram42 | GjM15 | GjM48b | Overall |
| Blood/Tissue (n=18) | 0.78 | 0.83 | 0.72 | 0.89 | 0.89 | 0.67 | 0.83 | 0.83 | 0.81 |
| Feathers (n=185) | 0.78 | 0.82 | 0.72 | 0.76 | 0.78 | 0.75 | 0.82 | 0.57 | 0.75 |
| All (Blood & Feathers, n=203) | 0.78 | 0.82 | 0.72 | 0.77 | 0.79 | 0.74 | 0.82 | 0.58 | 0.75 |



Supplemental Figure 1. Values of FST for pairwise differentiation of populations for a dataset restricted to only individuals with complete coverage of all alleles (no null-alleles) whereby darker colours represent higher FST values indicating evidence of deviations from HWE. p-values for each comparison are denoted below the FST value for each population pair.