diversity

MDPI

Article

Movement and Genomic Methods Reveal Mechanisms Promoting Connectivity in a Declining Shorebird: The Lesser Yellowlegs

Katherine Christie [1,*,†], Robert E. Wilson [2,3,*,†], James A. Johnson [4], Christian Friis [5], Christopher M. Harwood [6], Laura A. McDuffie [7], Erica Nol [8] and Sarah A. Sonsthagen [9]

1 Alaska Department of Fish and Game, Threatened, Endangered and Diversity Program, Anchorage, AK 99518, USA
2 School of Natural Resources, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
3 Nebraska State Museum, University of Nebraska-Lincoln, Lincoln, NE 68588, USA
4 U.S. Fish and Wildlife Service, Division of Migratory Birds, Anchorage, AK 99503, USA
5 Environment and Climate Change Canada, Canadian Wildlife Service, Toronto, ON M3H 5T4, Canada
6 U.S. Fish and Wildlife Service, Kanuti National Wildlife Refuge, Fairbanks, AK 99701, USA
7 U.S. Geological Survey, Alaska Science Center, Anchorage, AK 99508, USA
8 Biology Department, Trent University, Peterborough, ON K9L 0G2, Canada
9 U.S. Geological Survey, Nebraska Cooperative Fish and Wildlife Research Unit, School of Natural Resources, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
* Correspondence: katie.christie@alaska.gov (K.C.); rwilson43@unl.edu (R.E.W.)
† These authors contributed equally to this work.

Abstract: Integrating tracking technology and molecular approaches provides a comprehensive picture of contemporary and evolutionary mechanisms promoting connectivity. We used mitochondrial DNA and double digest restriction-site associated DNA (ddRAD) sequencing combined with satellite telemetry to investigate the connectivity of geographically disparate breeding populations of a declining boreal shorebird, the lesser yellowlegs (*Tringa flavipes*). We were able to track 33 individuals on their round-trip migrations to Central and South America and back to the boreal wetlands of North America. Nearly all (93%) adults captured on the breeding grounds returned to within 5 km of the original capture site, with a median dispersal distance of 629 m. While our telemetry data revealed limited breeding dispersal in adults, genetic data uncovered significant interconnectedness across the species' range. Very little genetic structure was estimated at ddRAD autosomal (Φ_{ST} = 0.001), *Z*-linked (Φ_{ST} = 0.001), and mtDNA loci (Φ_{ST} = 0.020), and maximum likelihood-based clustering methods placed all individuals in a single cluster regardless of capture location, indicating the species is panmictic. Our data indicate that large-scale juvenile dispersal is the main mechanism maintaining connectivity in this species, resulting in the absence of genomic structure.

Keywords: *Tringa flavipes*; genetic diversity; boreal; connectivity; harvest; shorebird; lesser yellowlegs; double digest restriction-site associated DNA; ddRAD

Citation: Christie, K.; Wilson, R.E.; Johnson, J.A.; Friis, C.; Harwood, C.M.; McDuffie, L.A.; Nol, E.; Sonsthagen, S.A. Movement and Genomic Methods Reveal Mechanisms Promoting Connectivity in a Declining Shorebird: The Lesser Yellowlegs. *Diversity* **2023**, *15*, 595. https://doi.org/10.3390/d15050595

Academic Editor: Gary Voelker

Received: 14 February 2023
Revised: 12 April 2023
Accepted: 20 April 2023
Published: 26 April 2023

1. Introduction

For declining species, the existence of genetically distinct sub-populations with limited dispersal can be cause for concern because they may have lower genetic diversity and increased opportunities for inbreeding and, therefore, may be less able to adapt to environmental perturbations [1]. Furthermore, when small, genetically distinct sub-populations are exposed to a threat such as harvest or human disturbance, populations may be at greater risk of becoming extirpated [2–4]. In contrast, large, interbreeding populations with very little genetic structure are thought to be more resilient because localized stochastic events and declines may be buffered by immigration of individuals from other regions [5].

Molecular approaches such as DNA sequencing and the identification of thousands of single-nucleotide polymorphisms (SNPs) can help inform conservation and management of declining bird species. These molecular approaches complement traditional movement and banding data by providing information on natal dispersal and genomic connectivity (dispersal followed by successful reproduction) across a broader time frame while allowing for inferences on a population's genetic diversity. For example, genomic data have been used to identify genetically distinct populations that display unique migratory strategies (e.g., Wilson's warbler (*Cardellina pusilla*) [6], greater white-fronted goose (*Anser albifrons*) [7]). Genomic data can provide additional evidence if certain populations or lineages are evolutionarily and/or demographically independent, resulting in the delineation of conservation units [8–11].

The lesser yellowlegs (*Tringa flavipes*) is a declining shorebird species that breeds in the boreal biome of North America from Alaska, U.S.A., to Quebec, Canada, and spends the boreal winter in the southern United States and throughout much of Mexico, Central America, and South America [12]. The species has experienced a steep decline (~63%) throughout its range [13,14], and as a result, has been listed as threatened in Canada [15] and a species of high conservation concern in the United States [16]. Lesser yellowlegs migrate long distances, which exposes them to multiple and likely concomitant threats across the annual cycle [16]. Threats include unsustainable harvest in South America [17], habitat loss [18,19], pesticides [20], and wetland drying [21].

In the Atlantic Americas Flyway, between 110,000 and 243,000 shorebirds are harvested for sport and subsistence each year [22]. The lesser yellowlegs comprised the majority (53–67%) of the harvest in Barbados [23] and were frequently sold in markets in Guyana [24]. Collectively, harvest mortality in these and other Caribbean and South American countries is thought to be unsustainable [25]. Recent evidence based on satellite tracking data showed that individuals breeding in central and eastern Canada had substantially greater probabilities of occurring in the harvest zone during hunting season than individuals originating in western Canada and Alaska [26]. The migration tracking data also indicated that individuals from geographically disparate breeding locations used different flyways during migration but overlapped in the non-breeding area, resulting in weak migratory connectivity overall [27]. These conclusions indicate that breeding birds using the Atlantic Americas Flyway are disproportionately affected by harvest, but it is unknown whether these birds represent a genetically distinct breeding population segment warranting special attention.

Dispersal is one of the primary mechanisms by which genetic diversity is maintained in wild populations, and in most bird species, natal dispersal exceeds adult dispersal [28]. Nevertheless, there is a great deal of variation in adult breeding site fidelity among shorebird species due to the diversity of mating systems within this group. Socially monogamous species typically have higher fidelity than polygamous or polyandrous species [29,30]. High site fidelity in monogamous species is thought to facilitate mate reunion, territory formation, and nest initiation early in the breeding season [31]. Lesser yellowlegs are monogamous for the duration of the breeding season but have low pair retention rates in subsequent years [12].

In this study, we evaluated the genetic distinctiveness and connectivity of populations across the breeding distribution of lesser yellowlegs using two approaches. First, we tagged birds on the breeding grounds using satellite transmitters and later tracked birds to their subsequent breeding destinations to quantify adult breeding site fidelity. Although moderate or high site fidelity may be observed among adult birds, high site fidelity by both sexes (>60%) may not equate to population structure (e.g., Barrow's goldeneye (*Bucephala islandica*) [32]). Second, using samples collected across the breeding range, we used complementary molecular approaches, including double digest restriction-site associated DNA (ddRAD), which recovered autosomal and Z-linked loci, and mitochondrial DNA (mtDNA) control region sequence data, to assess the spatial variation in genetic diversity

within lesser yellowlegs and to determine if significant population genetic structure exists and corresponds to movement data.

2. Materials and Methods

2.1. Breeding Site Fidelity via GPS

From May to August of 2018–2020, we captured and attached GPS tracking devices to 110 adult lesser yellowlegs at sites across the breeding range (U.S.A.—Alaska: Anchorage [including Joint Base Elmendorf-Richardson (JBER)], Kanuti National Wildlife Refuge; Canada—Northwest Territories: Yellowknife, Manitoba: Churchill, Ontario: James Bay, Quebec: Mingan; Figure 1). Birds were captured using audio lures and mist-nets, bow-nets, or whoosh nets. All birds were captured during the breeding season except at James Bay and Mingan, where trapping occurred post-breeding and potentially during migration. Tracking devices (Lotek Pinpoint Argos GPS-75, 4 g) were attached to birds via leg-loop harness [33]. Transmitters were programmed to transmit for approximately 1 year and record locations at 1- to 4-day intervals during southward migration and 4-day intervals during northward migration [27]. For birds that returned to breeding areas with active transmitters during the subsequent breeding season, the mean arrival date was calculated for each site and sex [34]. We did not use the locations of birds that stopped transmitting before this date because they were potentially in migratory status. Therefore, dispersal distances were calculated strictly for birds considered to be in breeding status. Transmitter schedules prevented us from determining exact nesting locations. For each bird that was transmitting during the breeding season, we calculated the geodesic distance between the initial capture location and the closest return location in ArcGIS (ArcMap version 10.5.1, [35]). For birds tagged during southbound migration in James Bay and Mingan, we measured the distance between the initial capture location and the closest return location during the subsequent breeding period (late May–early July; [12]). This location corresponded to where the bird was nesting or foraging during the breeding period.

2.2. Samples and DNA Extraction

We obtained blood samples from adult lesser yellowlegs captured during the breeding season from May 22 to July 7 or from juvenile or adult birds during southbound migration from July 18 to August 30 (2017–2019) (Figure 1, see Table 1 for sample size). Samples were stored in vials containing Longmire's solution and frozen at the Alaska Department of Fish and Game, Anchorage office. To provide additional geographical representation, breeding season samples from Alberta were obtained through museum tissue loaned from the Royal Ontario Museum. Genomic DNA was extracted using a DNeasy Blood and Tissue kit following the manufacturer's protocols (Qiagen, Valenica, CA, USA). Extractions were quantified using a High Sensitivity Quant-iT dsDNA Assay Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.3. ddRAD Library Preparation and Read Assembly

Sample preparation for ddRAD sequencing and bioinformatic pipelines follows DaCosta and Sorenson ([36]; Python scripts available at http://github.com/BU-RAD-seq/ddRAD-seq-Pipeline, accessed on 15 August 2021). The genomic DNA (~1 µg) was digested with SbfI and EcoRI restriction enzymes. Sequencing adaptors containing unique dual indices were ligated to sticky ends, and fragments were size selected (size range 300–450 base pairs; bp) using double-sided SPRI beads (Beckman Coulter, Inc., Indianapolis, IN, USA). Size-selected fragments were amplified with 20 cycles using Phusion high-fidelity DNA polymerase (Thermo Scientific, Pittsburgh, PA, USA), and excess primers and dNTPs were removed with AMPure XP beads (Beckman Coulter, Inc.). Libraries were pooled in equimolar amounts based on fluorometry (High Sensitivity Quant-iT dsDNA Assay Kit). Single-end (150 bp) sequencing was completed on an Illumina HiSeq 4000 at the University of Oregon Core Genomics Facility. Raw reads are accessed on the

National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject PRJNA940525; biosample accessions: SAMN33686961-SAMN33687143).

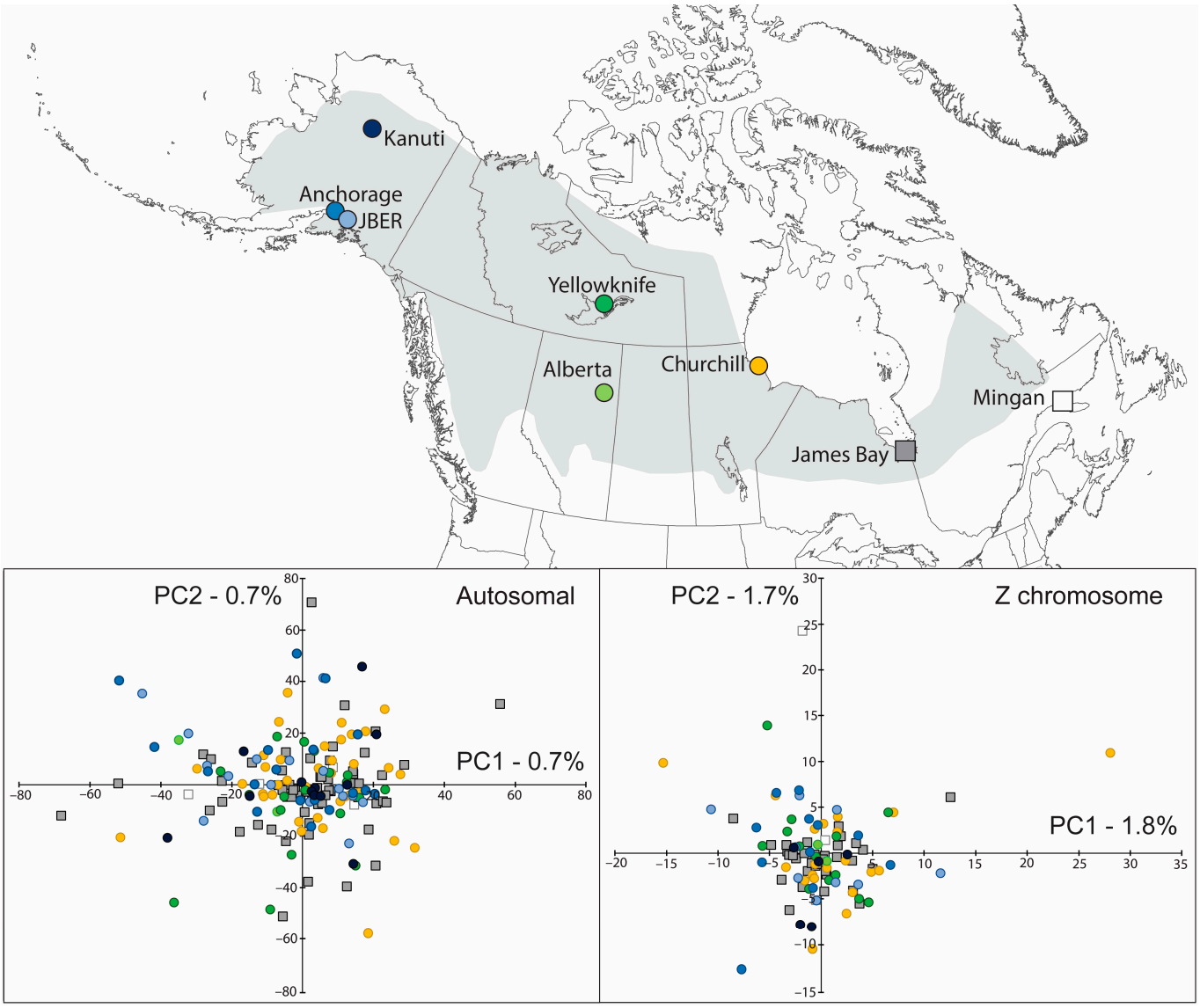

Figure 1. Map and scatter plot of lesser yellowlegs sample site locations. The scatter plots are of the first two principal components plotted for haplotypic data, with the proportion of variance explained, from 5816 autosomal and 281 Z-linked loci (males only). Sites on the map denoted with circles are areas where birds were sampled during the breeding season, and sites denoted with squares are for birds that were sampled during non-breeding. The area shaded in gray represents an approximate distribution during the breeding season.

Libraries were demultiplexed at the core facility using bcl2fastq 2.20 software (Illumina, San Diego, CA, USA). After clustering of similar sequences into putative loci, genomic positions relative to the *Calidris pugnax* reference genome (GenBank assembly 1.0, GCA_001431845.1) were determined using blastn v.2, allowing clusters representing the same locus to be combined, thereby improving the detection of polymorphic insertions and deletions (indels). Generally, individual genotypes at each locus were scored as heterozygous when two distinct haplotypes (i.e., alleles) accounted for more than 29% of sequence reads for a given sample and as homozygous when >93% of reads were consistent with a single haplotype. Putative heterozygotes with only 20–29% of reads representing a second allele were also allowed if that allele was present in other individuals. If not, the genotype

was flagged as ambiguous. When only 7–20% of reads represented a second allele or there was evidence of more than two alleles in a given sample, genotypes were also flagged as ambiguous. Loci with a median per-sample sequencing depth ≥10, <10% missing genotypes, and <10% flagged genotypes were retained for downstream analyses. Autosomal and Z chromosome-linked loci were identified as outlined in [37], with assignments based on differences in sequencing depth and homozygosity between males and females. Females have only one Z chromosome, and therefore, Z-linked markers are expected to appear homozygous and have half the sequencing depth as males.

Table 1. Indices of genetic diversity: nucleotide (π) and haplotype (h) diversity, number of haplotypes (H), haplotypic richness (HR), tests of selective neutrality (Fs and D), and sample size (*n*) based on autosomal (A) and Z-linked double digest restriction-associated DNA (ddRAD) and mitochondrial DNA (mtDNA) control region loci for lesser yellowlegs sampled across breeding (B) and non-breeding (NB) sample sites in Alaska and Canada. The single standard deviation is in parentheses. Significant metrics are in bold text. HR is based on a minimum sample size of 4 individuals. JBER is Joint Base Elmendorf-Richardson.

		ddRAD					mtDNA				
Location	**Type**	**π–A**	**π–Z**	***n***	**H**	**HR**	**π**	**h**	**Fs**	**D**	***n***
Kanuti	B	0.0060	0.0038	12	5	3.1	0.0012 (0.0011)	0.538 (0.161)	**−3.1**	**−1.8**	13
Anchorage	B	0.0061	0.0037	20	4	2.1	0.0006 (0.0007)	0.298 (0.133)	**−2.7**	**−1.7**	19
JBER	B	0.0061	0.0037	20	4	2.7	0.0015 (0.0013)	0.500 (0.122)	−1.0	−0.7	20
Yellowknife	B	0.0061	0.0037	18	6	2.9	0.0010 (0.0010)	0.468 (0.140)	**−4.5**	**−2.0**	19
Alberta	B	0.0053	0.0029	3	3	3.0	0.0020 (0.0019)	0.833 (0.222)	−0.9	−0.7	4
Churchill	B	0.0062	0.0038	37	4	1.9	0.0007 (0.0008)	0.252 (0.092)	−1.7	**−1.5**	37
James Bay	NB	0.0062	0.0038	66	10	2.4	0.0008 (0.0008)	0.331 (0.075)	**−11.1**	**−2.0**	66
Mingan	NB	0.0057	0.0036	5	3	2.6	0.0016 (0.0016)	0.700 (0.218)	−0.8	−1.0	5
Overall	–	0.0063	0.0038	181	19	–	–	–	–	–	183

2.4. mtDNA Sequencing

Lesser yellowlegs individuals were sequenced at the mtDNA control region. A 512-bp fragment was amplified using primer pairs LEYE_CR323L (5′-CACATATAACGTMCTAAACCC-3′) and LEYE_CR842H (5′-CAGTGTTGATATGATTCCCC-3′). PCR amplifications and post-sequencing processing followed Sonsthagen et al. [38], with one exception: excess dNTPs and primers were removed using ExoSAP-IT (ThermoFisher Scientific, Waltham, MA, USA). PCR products were cycle-sequenced at Functional Biosciences, Inc. (Madison, WI, USA). For quality control purposes, we amplified and sequenced 10% of the samples in duplicate. No inconsistencies in mtDNA sequences were observed between replicates. Sequence data are accessioned on GenBank (accession numbers: OQ680686-OQ680708).

2.5. Genomic Diversity and Divergence

Nucleotide diversity (π) and composite pairwise estimates of relative divergence (Φ_{ST}) among sample location pairs and overall were calculated for each ddRAD locus using a custom Python script (out2phistA.py; available at http://github.com/BU-RAD-seq/Out-Conversions, accessed on 15 August 2021).

Diversity indices (nucleotide π and haplotype *h*) and divergence (F_{ST} and Φ_{ST}) among location pairs and overall were calculated for mtDNA in Arlequin 2.0 [39]. We also tested the hypothesis of selective neutrality and the evidence of population expansion using Fu's

Fs [40] and Tajima's D [41] as implemented in Arlequin. An unrooted network for mtDNA was constructed in Network 10 [42] using the reduced median method [43] to illustrate reticulations in the gene tree because of homoplasy or recombination.

2.6. Population Genetic Structure

We applied three complementary methods to assess genetic relationships among the sampled sites. We visualized major trends in the distribution of genetic variation using a nonparametric method, principal components analysis (PCA), using the R package 'adegenet' [44] on haplotypic data sets for both autosomal and Z-linked loci. Only male individuals (n = 99) were included in the analyses of Z-linked loci. We plotted individuals relative to the first two principal components to determine the degree to which genetically similar individuals cluster into distinct geographic groups. We tested for the presence of close familial relatives within each sampled site, as genetic similarities among family groups can generate a stronger signal than population-level signatures using COLONY v2.0.6.8 [45]. Each unique haplotype at a given locus was scored as an allele. Priors for allelic dropout and genotyping errors did not influence familial relationship estimates because of the large number of loci analyzed; that is, results were identical across a range of priors. For the PCA analysis, we retained one individual from each set of close familial relatives, resulting in the exclusion of five individuals (see Supplemental Table S1 for a summary of COLONY results). For other analyses, the exclusion of close relatives did not have an appreciable effect on results; all samples were included in the remaining analyses.

Maximum likelihood estimates of population assignments for each individual were obtained using the parametric method implemented in ADMIXTURE [46,47] based on the autosomal ddRAD data set. Rare SNPs observed in only one individual were excluded from the analysis without a priori assignment of individuals to populations. The SNP data set was formatted in PLINK [48] following Alexander et al. [49]. We ran 100 iterations per analysis for each K (K = 1–10) and tested for optimal values of K using a 10-fold cross-validation (CV) procedure and quasi-Newton algorithm. The optimum K was based on the average of CV-errors across the 100 analyses per K. Additional Ks were explored to assess further population structure resolution that is consistent with the biology of this species [50]. CLUMPP v.1.1 [51] was used to determine the robustness of assignments of individuals to clusters at each K using the Large Greedy algorithm and 1000 random permutations to estimate final admixture populations for each K with per-sample assignment probabilities based on the 100 replicates.

We used fineRADstructure [52] to infer population structure based on shared co-ancestry (first coalescence) based on the autosomal data set using a haplotype-based approach (i.e., all SNPs were retained). FineRADstructure focuses on the most recent coalescence events, which provide information on recent sample relatedness, which in turn can be informative for evaluations of contemporary gene flow. Samples were assigned to populations using 1,000,000 iterations sampled every 1000 steps with a burn-in of 100,000. We used 10,000 iterations of the tree-building algorithm to assess relationships among clusters.

2.7. Spatial Genetic Structure

We used the program EEMS [53] to estimate rates of gene flow (m) and genetic diversity (q) relative to geographic distance across the landscape based on the autosomal ddRAD data set. EEMS uses a stepping-stone model to assess regions where genetic dissimilarity decays more quickly or slowly than expected under a model of isolation by distance based on individual gene flow rates. A migration (gene flow) surface is interpolated from these gene flow rates across the landscape to identify barriers or corridors to movement. Based on preliminary runs, we adjusted parameters so that the accepted proposal variances were between 10% and 40%. We ran three independent analyses using 1,000,000 burn-in steps followed by 5,000,000 Markov chain Monte Carlo (MCMC) iterations that sampled every 1000 steps for each deme (100, 250, and 500). We checked convergence and visualized gene flow and diversity surfaces using the 'rEEMSplots' package in R [53]. Only sites sampled

during the breeding period (Anchorage, Kanuti, Alberta, Yellowknife, and Churchill) were included in the analysis, with the addition of Mingan, which comprised individuals that originated from unsampled breeding locations (e.g., northern Quebec, Canada).

3. Results

3.1. Breeding Site Fidelity via GPS

Of the 110 lesser yellowlegs fitted with tracking devices, 33 birds were still transmitting the following breeding season. Of 27 birds captured on the breeding grounds and still transmitting the following breeding season, the median dispersal distance between capture and return locations was 629 m (range: 88–423,143 m; Figure 2, Table 2). All but two birds (93%) returned to within 5 km of their capture location. One female from Anchorage returned to a location 17 km from the original capture location. The remaining bird (a male) from Churchill returned to a location 423 km from where it was captured the year before. This bird was captured while foraging on the shoreline of Hudson Bay one day after the mean arrival date; therefore, it is possible that it was still migrating to the breeding area. Predictably, birds (n = 7) captured during southbound migration in James Bay and Mingan returned to breeding locations north or northwest of the capture location, with a median distance of 414 km (range: 236–1850 km; Figure 2, Table 2).

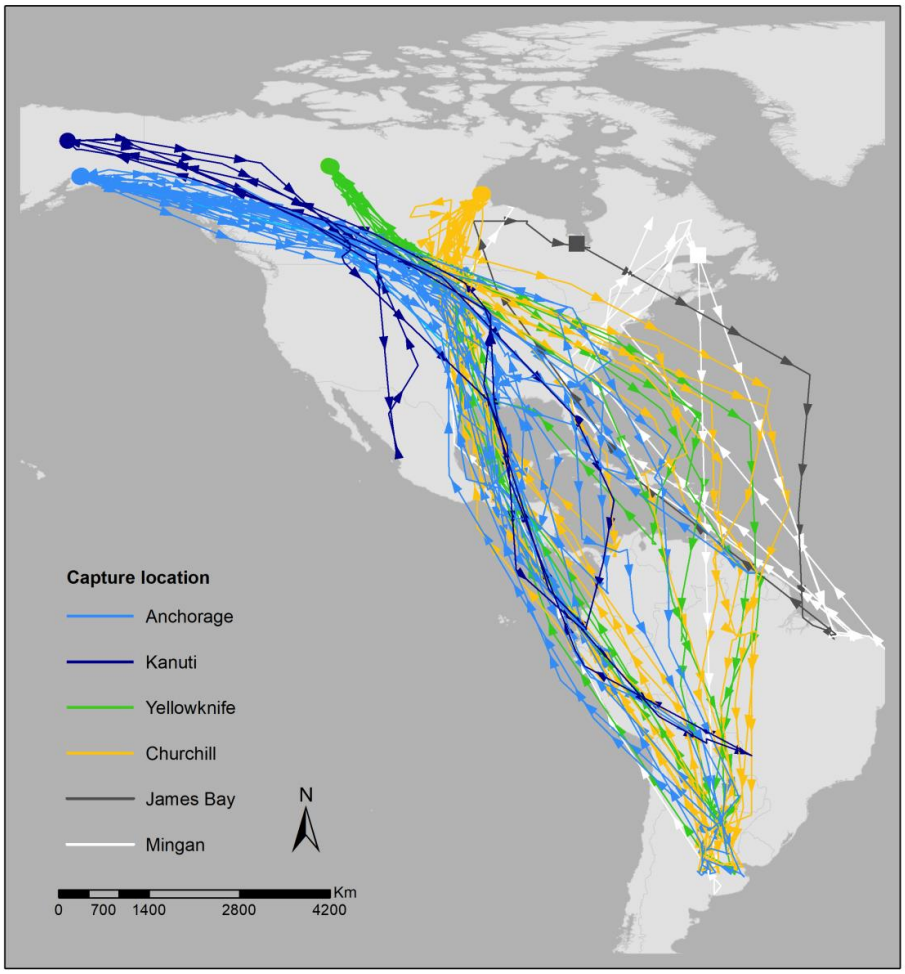

Figure 2. Southbound and northbound migrations of adult lesser yellowlegs that were tagged across their breeding range in 2018–2020. Only birds (n = 33) with functioning transmitters during the breeding season following capture are shown. Capture locations are denoted by circles (breeding areas) or squares (post-breeding migratory stopovers). Arrows indicate the direction of travel (southbound or northbound migration).

Table 2. Distance between capture location on the breeding grounds and the closest known return location (based on GPS transmitters) during the subsequent breeding season for adult lesser yellowlegs in Anchorage (including Joint Base Elmendorf-Richardson, JBER), Kanuti, Yellowknife, and Churchill. All birds were captured during the breeding period except for those from James Bay and Mingan.

Deployment Location	Location Type	Minimum Distance (m)	Maximum Distance (m)	Median Distance (m)	*n*
Anchorage	Breeding area	200.2	17,821.8	776.9	11
Kanuti	Breeding area	341.1	1101.6	753.5	3
Yellowknife	Breeding area	88.6	405.5	246.1	5
Churchill	Breeding area	212.5	423,143.6	547.1	8
All breeding	Breeding area	88.6	423,143.6	629.2	27
James Bay	Migratory stopover	235,722.2	235,722.2	235,722.2	1
Mingan	Migratory stopover	297,447.0	1,849,575.8	489,060.1	5
All non-breeding	Migratory stopover	235,722.21	1,849,575.80	413,628.23	6

3.2. Read Assembly and Loci Identification

We obtained 222,985,791 raw sequencing reads (median = 1,185,836 reads per individual, range 487,543–2,506,696) with a maximum read length of 150 bp. After initial exploration of genotyping results, two sample pairs were deemed to be of close familial relationship (e.g., siblings) based on preliminary PCAs and fine-RAD structure results and field notes (location, date, and age of individuals sampled). In both instances, the sample pairs were hatch-year-aged birds captured at the James Bay site. A single individual from each pair with a close familial relationship was removed from subsequent analyses. A total of 6097 putative single-copy loci met the depth/genotype threshold and passed automated checks for alignment quality or passed thresholds after manual edits that yielded 35,254 SNPs or insertions/deletions. Of those, 5816 loci were assigned to the autosomal gene and 281 were assigned to the Z chromosome. The final data sets comprised loci with a median sequencing depth of 67 reads per locus per individual, and on average 99.1% (a minimum of 80.0%) of alleles per individual per locus were scored.

We detected six pairs or trios of close familial relatives, including full siblings and parent-offspring relationships, in the Alaska-sampled populations of JBER, Anchorage, and Kanuti, as well as the non-breeding sampling locations of James Bay and Mingan (Supplemental Table S1). The Anchorage and JBER sampling locations included two pairs of full siblings as well as one parent-offspring pair. The parent in the parent-offspring pair (JBER location) was also a full sibling to the Anchorage individual in one of the full sibling pairs. In James Bay, we observed two pairs of full siblings. Lastly, a male from Mingan was inferred to be the parent of an individual breeding 5000 km away in Kanuti.

3.3. Genomic Diversity and Divergence

Genomic diversity was relatively uniform across autosomal and Z-linked ddRAD loci and populations, though Alberta exhibited lower levels of π (15.9 and 23.7% less) than other sites (Table 1). Alberta also had the highest proportion of loci with no variation at both autosomal (28.9%) and Z-linked (45.7%) loci, followed by Mingan (19.7 and 37.8%, respectively). Among the remaining sites, the proportion of non-variable loci ranged from 4.9–13.4% at autosomal and 10.8–24.8% at Z-linked loci, with James Bay having the lowest and Kanuti having the highest proportion of loci at both marker types. We note that Alberta (n = 4) and Mingan (n = 5) have low sample sizes.

Divergence among sampled sites was subtle (overall Φ_{ST}Autosomal = 0.001; Φ_{ST}Z-linked = 0.001), with pairwise estimates from −0.006 to 0.008 and −0.016 to 0.007 for autosomal and Z-linked loci, respectively (Supplemental Table S2). Some of the highest degrees of divergence were estimated between Alberta and Mingan and other sampled locations, and this is reflected in the number of loci with elevated Φ_{ST}Z-linked (>0.1; n = 285–714 autosomal; n = 23–41 Z-linked). Comparisons among locations with larger

sample sizes only had a small proportion of loci with elevated divergence (<2%, n = 4–109 autosomal; <4%, n = 1–11 Z-linked).

We recovered 19 unique mtDNA haplotypes characterized by 16 variable sites. The diversity metrics (number of haplotypes, haplotype richness, π, and h) were generally similar across sampled locations (Table 1), though Anchorage, Churchill, and James Bay exhibited lower levels than other sampled sites. Fs and D were negative for all locations and significantly negative for all except JBER, Alberta, and Mingan (Table 1), indicative of historical population expansion. A single haplotype group was observed, with one high-frequency haplotype that comprised individuals from all sampled locations and contained >78% of sampled individuals (Figure 3). The remaining haplotypes were of low frequency and represented by <3.3% of sampled individuals (n = 1–2) with private haplotypes (only observed at a single location). Population divergence was uncovered, albeit at low levels. The overall estimate was significant when a nucleotide substitution model was applied to the dataset (F_{ST} = 0.013, p = 0.159; Φ_{ST} = 0.020, p = 0.046). Estimates for the pairwise comparisons ranged from −0.175–0.221 for F_{ST} and −0.119–0.127 for Φ_{ST}; two comparisons between JBER and Churchill and JBER and James Bay were significant at Φ_{ST} (0.052 and 0.065, respectively; Supplemental Table S2).

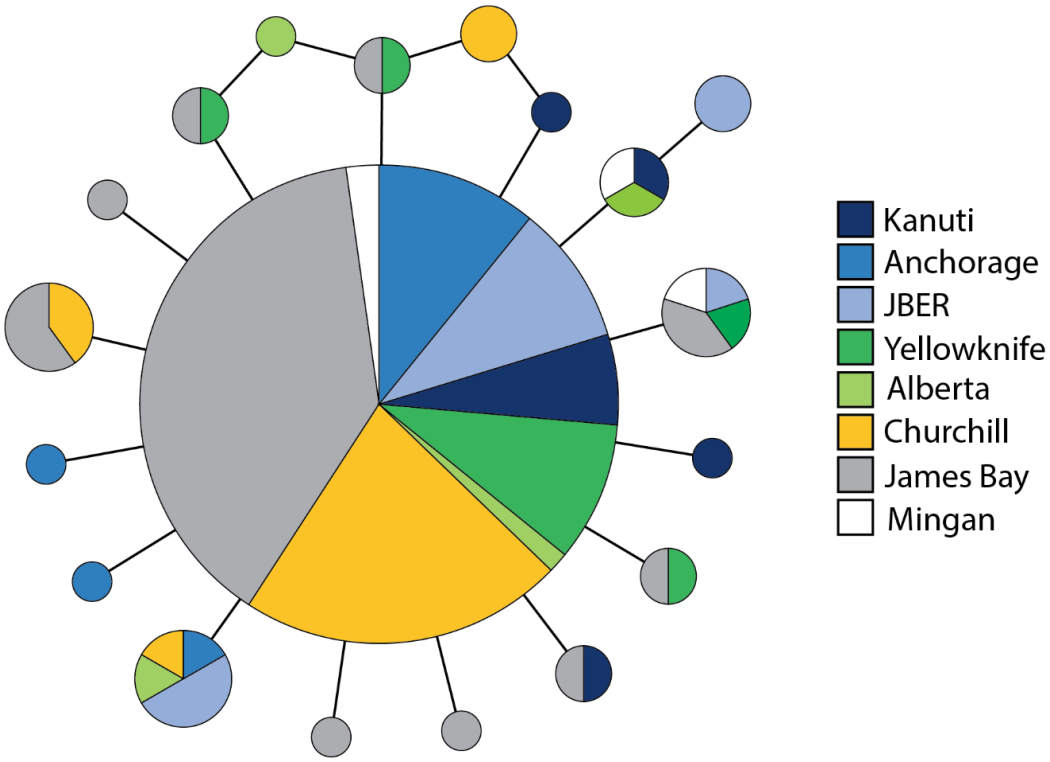

Figure 3. Unrooted network illustrating relationships among mitochondrial DNA (mtDNA) control region haplotypes assayed from lesser yellowlegs residing in Alaska and Canada. The size of the circle is proportional to the number of individuals representing that haplotype.

3.4. Population Genetic Structure

The limited structure was recovered across sampled sites and analyses. No discernible clusters were uncovered when samples were plotted relative to the first two principal component axes based on autosomal or Z-linked loci (Figure 1). Structure was not uncovered based on the maximum likelihood approach as implemented in ADMIXTURE (Supplemental Figure S1). Further, nearly all (n = 164/181; 91%) individuals clustered together in a single group based on recent genetic ancestry in the fineRADstructure analysis (Supplemental Figure S2). The remaining recovered clusters comprised two to three individuals with relatively greater co-ancestry, reflecting kin associations confirmed with

COLONY analysis. Most of these clusters comprised individuals sampled at geographically close sites (Anchorage and JBER or within James Bay) with one exception: one member of a pair of lesser yellowlegs with high co-ancestry values was captured at the farthest west site, Kanuti, and the other member was captured at the farthest east site, Mingan.

3.5. Spatial Genetic Structure

The EEMS analysis did not detect any regions where genetic dissimilarity decayed more quickly or slowly than expected under a model of isolation by distance across all demes analyzed (Supplemental Figure S3). Genetic diversity, however, was high at breeding sites, with lower than expected diversity from birds sampled at Mingan based on an isolation-by-distance model of genetic dissimilarity (Supplemental Figure S3).

4. Discussion

4.1. Key Findings

Inferences of population distinctiveness in lesser yellowlegs differed between approaches. Lesser yellowlegs exhibited high breeding site fidelity (93%) based on GPS tracking data. Adults carrying GPS transmitters returned to the same breeding areas in subsequent years, with transmitting locations a median distance of only 246–777 m from their original capture location, although few birds (7%, $n = 2/27$) dispersed over greater distances (17–423 km). The high breeding site fidelity observed based on telemetry data is indicative of distinct breeding populations. Conversely, genomic diversity does not appear to be structured across the breeding range of lesser yellowlegs. At ddRAD autosomal, Z-linked, and mtDNA loci, little genetic structure was uncovered. Populations were only weakly diverged at ddRAD loci ($\Phi_{ST} < 0.007$; Supplemental Table S3), and only two comparisons were significant based on mtDNA sequence divergence ($\Phi_{ST} < 0.065$, $n = 56$ comparisons; Supplemental Table S2). Further, non-parametric and maximum likelihood-based clustering methods placed all individuals into a single cluster regardless of sample location, and no barriers to dispersal were uncovered. Furthermore, the parent/offspring relationship between one bird sampled in Mingan, Quebec, and another sampled approximately 5000 km away in Kanuti, Alaska, supports the hypothesis of long-distance dispersal of juveniles. The coalescent-based approach and COLONY analysis, however, also identified high co-ancestry (siblings and parent-offspring relationships) among birds sampled from the same geographic region. Combined, these findings highlight that dispersal decisions vary at the individual level and the degree of philopatry can be quite variable within species as it is strongly influenced by ecological factors ([54]; also see "mechanisms" section below). The lack of partitions in genomic diversity indicates that lesser yellowlegs breeding areas are connected through a network of gene flow. Given the high return rate observed at breeding areas for adult lesser yellowlegs, we hypothesize that the absence of genomic structure among sampled sites is attributable to juvenile dispersal as the main mechanism promoting genomic connectivity in this species.

Although the two approaches applied here appear to provide conflicting results regarding the distinctiveness of populations, telemetry- and genomics-based methods are measuring different aspects of connectivity (genomic vs. migratory). Specifically, in our study, movement data provided a direct measure of the dispersal of breeding adults. Genomic data provide an indirect measure of natal and breeding dispersal and subsequent successful reproduction. Movement- and genomic-based methods are therefore complementary and allow for greater inference regarding mechanisms promoting or restricting dispersal among areas. Through the evaluation of results from both approaches, we were able to identify the cohort likely promoting gene flow in lesser yellowlegs as juvenile birds. If only a single approach had been applied, we could have overestimated the amount of structure present in lesser yellowlegs. This study adds to the growing body of literature highlighting the importance of employing multiple approaches that assess natal and breeding dispersal for evaluations of population distinctiveness (e.g., [7,32,55]).

4.2. Mechanisms

The observed lack of genetic structure in lesser yellowlegs is akin to that found in reed warblers (*Acrocephalus scirpaceus*) and mallards (*Anas platyrhynchos*), which have widespread distributions and gene flow across populations [5,56]. Natal dispersal is likely contributing to significant gene flow in lesser yellowlegs. Migratory birds often have greater breeding site fidelity than natal philopatry [28,54]. Dispersal in juveniles typically exceeds adult dispersal except for species occurring in highly isolated environments (e.g., islands; [57]). For monogamous species, returning to familiar breeding areas facilitates reunion with mates and defense of territories [58]. Juveniles, conversely, do not have established territories or mates. Dispersal among breeding areas may provide a mechanism to avoid mating with close relatives. As a result, the benefits of returning to natal nesting areas are somewhat lower for this age group, especially if breeding habitat is widespread [31]. Instead, juveniles are thought to "follow the leader" from wintering areas to breeding grounds that support high densities of conspecifics [58], although see [59]. Juvenile lesser yellowlegs have been reported to return to natal sites at a rate of 19% (4/21 birds; [12]), and this taken together with typically low juvenile shorebird survival rates [60,61] indicates that relatively infrequent long-distance natal dispersal events are maintaining gene flow. Alternatively, the absence of genomic structure at the three marker types assayed herein may be attributable to incomplete lineage sorting post-divergence [62]. Given (a) the concordant signal in patterns of genomic variation across three marker types with differing modes of inheritance and effective population sizes, (b) the detection of close-familial relationships between individuals sampled at distant sites, and (c) the high adult return rates, juvenile dispersal is likely a more parsimonious hypothesis regarding differences in patterns of structure between genomic and direct observations of movement than incomplete lineage sorting in lesser yellowlegs.

4.3. Comparisons with Boreal Avifauna

Presently, species nesting in the boreal biome have varied patterns of spatial genomic diversity ([63,64]; see [65] for a review). During the Pleistocene, much of the Boreal was covered in glacial ice sheets, and North American bird species breeding in boreal forests and wetlands occupied refugia north and (or) south of the ice sheets where populations diverged in allopatry [62]. Persistence in multiple glacial refugia leaves predictable patterns of genetic diversity that were not observed in lesser yellowlegs. Lesser yellowlegs are weakly structured across their range, and the mtDNA haplotype network has a star-like pattern, consistent with expansion from a single glacial refugium post-Pleistocene [62]. When we compare the pattern of structure among other boreal species at sites that occur within the lesser yellowlegs distribution, we see a similar absence of genetically distinct haplotype groups (e.g., boreal chickadee (*Poecile hudsonicus*), golden-crowned kinglet (*Regulus satrapa),* Swainson's thrush (*Catharus ustulatus*), yellow-rumped warbler (*Setophaga coronata),* and blackpoll warbler (*Setophaga striata*), see Table 1 and Figure 1 in [65]). Species with more complex patterns of spatial genetic structure (e.g., rusty blackbird (*Euphagus carolinus*) [64], Canada jay (*Perisoreus canadensis*), yellow-bellied flycatcher (*Empidonax flaviventris*) [65]) have breeding distributions that expand to areas that often harbor unique genomic diversity (e.g., Newfoundland, southwestern United States). Comparisons of genomic diversity among species in locations shared with lesser yellowlegs yields a similar pattern—weak spatial genomic structuring across the central and western boreal.

4.4. Conservation Implications

Genetic diversity is fundamental to the ability of a species to adapt to changing environments, climate change, and other natural or anthropogenic stressors (e.g., disease, pollution). Panmictic species with moderate to high immigration likely have the advantage of being partially buffered from localized population declines and resulting declines in genetic diversity (see [66]). However, such species may also have the disadvantage of the cascading effects of threats on the entire population (e.g., disease and harvest [5]).

The boreal biome in North America is composed of a mixture of wetland complexes and forests that is projected to contract at its southern edge [67] along with substantial decreases in population size and ranges of bird species [68–70]. Under different climate-scenario models, the lesser yellowlegs' vulnerability to climate change has been categorized as high [69].

Lesser yellowlegs have a broad distribution, covering much of the Western Hemisphere throughout their annual cycle, breeding across most of the continental boreal biome, and migrating south to nonbreeding grounds in South America. As a potential consequence of weak migratory connectivity, most of our sampling sites had higher than expected genetic diversity (EEMS analysis, Supplementary Figure S3). This may provide lesser yellowlegs with a degree of adaptive capacity to climate change and other anthropogenic activities that continue to alter the landscape. Moreover, current research has shown that populations in central and eastern Canada are disproportionately affected by harvest [26]. Nevertheless, our results demonstrate that these birds do not represent a genetically distinct population segment. The observed weak migratory connectivity suggests that eastern Canadian populations may benefit from dispersing first-year breeders from western populations. Conversely, western populations are not completely isolated from the threat of harvest because individuals hatched in this region may use the Atlantic Americas Flyway during southbound migration as adults, where exposure to harvest is highest [26]. Thus the vulnerability to stressors of a population can be positively or negatively affected by connectivity [71].

The synchronized migration schedules and/or cultural transmission of migratory behaviors among juveniles from similar geographic areas are thought to promote localized adaptation and genetic structure in some species of migratory birds [6,7]. This does not appear to be the case for lesser yellowlegs, even though migration and breeding schedules differ between eastern and western populations [34]. Genomic connectivity through natal dispersal is likely facilitated by the amount of geographical and temporal overlap during migration and the boreal winter [27]. However, genomic connectivity could be disrupted as breeding and boreal wintering areas continue to become fragmented [72–75]. Future efforts to identify and conserve important areas where individuals from multiple breeding populations converge during the non-breeding period would likely have a high conservation benefit for the species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15050595/s1, Table S1. Summary of COLONY analysis results showing familial relationships among samples of lesser yellowlegs.; Table S2: Pairwise estimates of population genetic divergence for lesser yellowlegs at mtDNA control region: mtDNA F_{ST} is below the diagonal and Φ_{ST} is above. Significant comparisons are in bold text. Table S3. Pairwise estimates of population divergence (Φ_{ST}) for lesser yellowlegs at ddRAD loci: estimates based on Z-linked loci are below the diagonal, and autosomal loci are above. Figure S1. Average membership coefficients of lesser yellowlegs individuals from sampled sites divided into two clusters inferred from 5816 autosomal double digest restriction-site associated DNA (ddRAD) loci. A plot of cross-validation (CV) error estimates for each K is shown. Colors correspond to sample locations indicated in Figure 1. Figure S2. A fine-RAD structure co-ancestry matrix indicating pairwise genetic similarity between individual lesser yellowlegs. Inferred populations are indicated by clustering in the dendrogram. Colors correspond to sample locations indicated in Figure 1. Figure S3. The estimated effective gene flow (A) and genetic diversity (B) surface between sites (black circles) sampled for lesser yellowlegs. White areas indicate gene flow rates consistent with isolation by distance expectations, whereas shaded areas have dispersal rates that are higher (blue, corridors) or lower (orange, barriers) than expected under isolation by distance.

Author Contributions: Conceptualization, S.A.S., J.A.J., K.C. and R.E.W.; Resources, K.C., S.A.S., C.M.H., C.F., E.N. and L.A.M.; Methodology, R.E.W., K.C., J.A.J. and S.A.S.; Software, R.E.W., K.C. and S.A.S.; Formal Analysis, R.E.W., K.C., J.A.J. and S.A.S.; Investigation, R.E.W., K.C., J.A.J. and S.A.S.; Writing—original draft, K.C., R.E.W. and S.A.S.; Writing—review and editing, R.E.W., K.C., J.A.J., S.A.S., L.A.M., E.N., C.F. and C.M.H.; Funding acquisition, J.A.J. and K.C.; Project administration,

S.A.S., J.A.J. and K.C.; Data curation, R.E.W., S.A.S., J.A.J., K.C. and L.A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the 673 CES/CEIEC, U.S. Department of the Air Force (project numbers FXSB46058118, FXSB4658119, and FXSBA53216120), the Alaska Department of Fish and Game, Bird Studies Canada, Environment and Climate Change Canada, Parks Canada (Mingan), the Canadian Wildlife Service-Quebec Region, Canadian Wildlife Service, Ontario Region, the U.S. Fish and Wildlife Service (WSFR—SWG Grants T-32-1, T-33-2020), the USFWS Candidate Conservation Species grant, and the 2018 and 2019 Churchill Northern Studies Centre Northern Research Fund.

Institutional Review Board Statement: This research was conducted in compliance with Institutional Animal Care and Use Committee standards (USFWS 2016-07 and 2019-05, ADF&G 0058-2018-2, and UAA 1388604) and the Canadian Animal Care Committee (Trent U 25284, Environment Canada 18CF01, 19CF01, and 19YA01).

Data Availability Statement: Sample information and the data used in the present study are accessible in the NCBI Sequence Read Archive (BioProject PRJNA940525; biosample accessions: SAMN33686961-SAMN33687143). Scripts for population genomics bioinformatics and infile conversion are available on GitHub (http://github.com/BU-RAD-seq/ddRAD-seq-Pipeline, accessed on 4 April 2023). Mitochondrial control region sequence data are accessioned on NCBI GenBank (accession numbers: OQ680686-OQ680708).

Acknowledgments: This research used resources provided by the Core Science Analytics, Synthesis, and Libraries (CSASL) Advanced Research Computing (ARC) group at the U.S. Geological Survey. We would like to thank the many field technicians who helped collect the data for this study. We also thank the Royal Ontario Museum for sharing specimens from Alberta. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the USFWS. This article has been peer reviewed and approved for publication in accordance with USGS Fundamental Science Practices.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Saccheri, I.; Kuussaari, M.; Kankare, M.; Vikman, P.; Hanski, I. Inbreeding and Extinction in a Butterfly Metapopulation. *Nature* **1998**, *392*, 491–494. [CrossRef]
2. Kinley, T.A.; Apps, C.D. Mortality Patterns in a Subpopulation of Endangered Mountain Caribou. *Wildl. Soc. Bull.* **2001**, *29*, 158–164. [CrossRef]
3. Currey, R.J.C.; Dawson, S.M.; Slooten, E. An Approach for Regional Threat Assessment under IUCN Red List Criteria That Is Robust to Uncertainty: The Fiordland Bottlenose Dolphins Are Critically Endangered. *Biol. Conserv.* **2009**, *142*, 1570–1579. [CrossRef]
4. Norman, S.A.; Hobbs, R.C.; Goertz, C.E.C.; Burek-Huntington, K.A.; Shelden, K.E.W.; Smith, W.A.; Beckett, L.A. Potential Natural and Anthropogenic Impediments to the Conservation and Recovery of Cook Inlet Beluga Whales, *Delphinapterus leucas*. *Mar. Fish. Rev.* **2016**, *77*, 89–105. [CrossRef]
5. Kraus, R.H.S.; Van Hooft, P.; Megens, H.J.; Tsvey, A.; Fokin, S.Y.; Ydenberg, R.C.; Prins, H.H.T. Global Lack of Flyway Structure in a Cosmopolitan Bird Revealed by a Genome Wide Survey of Single Nucleotide Polymorphisms. *Mol. Ecol.* **2013**, *22*, 41–55. [CrossRef] [PubMed]
6. Ruegg, K.C.; Anderson, E.C.; Paxton, K.L.; Apkenas, V.; Lao, S.; Siegel, R.B.; Desante, D.F.; Moore, F.; Smith, T.B. Mapping Migration in a Songbird Using High-Resolution Genetic Markers. *Mol. Ecol.* **2014**, *23*, 5726–5739. [CrossRef] [PubMed]
7. Wilson, R.E.; Sonsthagen, S.A.; Dacosta, J.M.; Sorenson, M.D.; Fox, A.D.; Weaver, M.; Skalos, D.; Kondratyev, A.V.; Scribner, K.T.; Walsh, A.; et al. As the Goose Flies: Migration Routes and Timing Influence Patterns of Genetic Diversity in a Circumpolar Migratory Herbivore. *Diversity* **2022**, *14*, 1067. [CrossRef]
8. Funk, C.W.; McKay, J.K.; Hohenlohe, P.A.; Allendorf, F. Harnessing Genomics for Delineating Conservation Units. *Trends Ecol. Evol.* **2012**, *27*, 489–496. [CrossRef]
9. Klicka, L.B.; Kus, B.E.; Title, P.O.; Burns, K.J. Conservation Genomics Reveals Multiple Evolutionary Units within Bell's Vireo (*Vireo bellii*). *Conserv. Genet.* **2016**, *17*, 455–471. [CrossRef]
10. Waples, R.S.; Lindley, S.T. Genomics and Conservation Units: The Genetic Basis of Adult Migration Timing in Pacific Salmonids. *Evol. Appl.* **2018**, *11*, 1518–1526. [CrossRef]
11. Forester, B.R.; Murphy, M.; Mellison, C.; Petersen, J.; Pilliod, D.S.; Van Horne, R.; Harvey, J.; Funk, W.C. Genomics-informed Delineation of Conservation Units in a Desert Amphibian. *Mol. Ecol.* **2022**, *31*, 5249–5269. [CrossRef] [PubMed]

12. Tibbitts, T.L.; Moskoff, W. Lesser Yellowlegs, *Tringa flavipes*. In *Birds of the World*; Poole, A., Ed.; Cornell Lab of Ornithology: Ithaca, NY, USA, 2020.
13. Rosenberg, K.V.; Dokter, A.M.; Blancher, P.J.; Sauer, J.R.; Smith, A.C.; Smith, P.A.; Stanton, J.C.; Panjabi, A.; Helft, L.; Parr, M.; et al. Decline of the North American Avifauna. *Science* **2019**, *366*, 120–124. [CrossRef]
14. Handel, C.M.; Sauer, J.R. Combined Analysis of Roadside and Off-Road Breeding Bird Survey Data to Assess Population Change in Alaska. *Condor* **2017**, *119*, 557–575. [CrossRef]
15. COSEWIC. *COSEWIC Assessment and Status Report on the Lesser Yellowlegs Tringa flavipes in Canada*; Committee on the Status of Endangered Wildlife in Canada, Environment and Climate Change Canada: Ottawa, ON, Canada, 2020.
16. Clay, R.; Lesterhuis, A.; Centron, S. *Conservation Plan for the Lesser Yellowlegs*; Manomet Center for Conservation Sciences: Manomet, MA, USA, 2012.
17. Atlantic Flyway Shorebird Initiative Harvest Working Group. *A Plan to Address the Sustainability of Shorebird Harvest in the Western Atlantic Flyway*; Atlantic Flyway Shorebird Harvest Group: Hadley, MA, USA, 2016. Available online: https://www.shorebirdplan.org/wp-content/uploads/2018/08/Shorebird-Harvest-Atlantic-Flyway-Plan-May-2016.pdf (accessed on 20 April 2023).
18. Studds, C.E.; Kendall, B.E.; Murray, N.J.; Wilson, H.B.; Rogers, D.I.; Clemens, R.S.; Gosbell, K.; Hassell, C.J.; Jessop, R.; Melville, D.S.; et al. Rapid Population Decline in Migratory Shorebirds Relying on Yellow Sea Tidal Mudflats as Stopover Sites. *Nat. Commun.* **2017**, *8*, 14895. [CrossRef] [PubMed]
19. Kleijn, D.; Schekkerman, H.; Dimmers, W.J.; Van Kats, R.J.M.; Melman, D.; Teunissen, W.A. Adverse Effects of Agricultural Intensification and Climate Change on Breeding Habitat Quality of Black-Tailed Godwits *Limosa* l. *limosa* in the Netherlands. *Ibis* **2010**, *152*, 475–486. [CrossRef]
20. Strum, K.M.; Hooper, M.J.; Johnson, K.A.; Lanctot, R.B.; Zaccagnini, M.E.; Sandercock, B.K. Exposure of Nonbreeding Migratory Shorebirds to Cholinesterase-inhibiting Contaminants in the Western Hemisphere. *Condor* **2010**, *112*, 15–28. [CrossRef]
21. Galbraith, H.; DesRochers, D.W.; Brown, S.; Reed, J.M. Predicting Vulnerabilities of North American Shorebirds to Climate Change. *PLoS ONE* **2014**, *9*, e108899. [CrossRef]
22. Atlantic Flyway Shorebird Initiative Harvest Working Group. Actions for the Atlantic Flyway Shorebird Initiative's Shorebird Harvest Working Group 2020–2025. 2020, pp. 1–16. Available online: https://www.shorebirdplan.org/wp-content/uploads/2020/09/AFSI-Shorebird-Harvest-Action-Plan-2020_25-April-2020.pdf (accessed on 20 April 2023).
23. Wege, D.C.; Burke, W.; Reed, E.T. *Migratory Shorebirds in Barbados: Hunting, Management and Conservation*; BirdLife International: Cambridge, UK, 2014.
24. Andres, B.A.; Moore, L.; Cox, A.R.; Frei, B.; Roy, C. A Preliminary Assessment of Shorebird Harvest in Coastal Guyana. *Wader Study* **2022**, *129*, 1–9. [CrossRef]
25. Watts, B.D.; Turrin, C. Assessing Hunting Policies for Migratory Shorebirds throughout the Western Hemisphere. *Wader Study* **2016**, *123*, 6–15. [CrossRef]
26. McDuffie, L.A.; Christie, K.S.; Harrison, A.-L.; Taylor, A.R.; Andres, B.A.; Laliberté, B.; Johnson, J.A. Eastern-Breeding Lesser Yellowlegs Are More Likely than Western-Breeding Birds to Visit Areas with High Shorebird Hunting during Southward Migration. *Ornithol. Appl.* **2022**, *124*, 1–16. [CrossRef]
27. McDuffie, L.A.; Christie, K.S.; Taylor, A.R.; Nol, E.; Friis, C.; Harwood, C.M.; Rausch, J.; Laliberte, B.; Gesmundo, C.; Wright, J.R.; et al. Flyway-scale GPS Tracking Reveals Migratory Routes and Key Stopover and Non-breeding Locations of Lesser Yellowlegs. *Ecol. Evol.* **2022**, *12*, e9495. [CrossRef] [PubMed]
28. Greenwood, P.J.; Harvey, P.H. The Natal and Breeding Dispersal of Birds. *Ann. Rev. Ecol. Syst.* **1982**, *13*, 1–21. [CrossRef]
29. Kwon, E.; Valcu, M.; Cragnolini, M.; Bulla, M.; Lyon, B.; Kempenaers, B. Breeding Site Fidelity Is Lower in Polygamous Shorebirds and Male-Biased in Monogamous Species. *Behav. Ecol.* **2022**, *33*, 592–605. [CrossRef]
30. Kempenaers, B.; Valcu, M. Breeding Site Sampling across the Arctic by Individual Males of a Polygynous Shorebird. *Nature* **2017**, *541*, 528–531. [CrossRef]
31. Adkins-Regan, E.; Tomaszycki, M. Monogamy on the Fast Track. *Biol. Lett.* **2007**, *3*, 617–619. [CrossRef]
32. Brown, J.I.; Lavretsky, P.; Wilson, R.E.; Haughey, C.L.; Boyd, W.S.; Esler, D.; Talbot, S.L.; Sonsthagen, S.A. High Site Fidelity Does Not Equate to Population Genetic Structure for Common Goldeneye and Barrow's Goldeneye in North America. *J. Avian Biol.* **2020**, *51*, e02600. [CrossRef]
33. Rappole, J.H.; Tipton, A.R. New Harness Design for Attachment of Radio Transmitters to Small Passerines. *J. F. Ornithol.* **1991**, *62*, 335–337.
34. McDuffie, L.A. Migration Ecology and Harvest Exposure Risk of Lesser Yellowlegs. Ph.D. Thesis, University of Alaska Anchorage, Anchorage, AK, USA, 2021.
35. ESRI Environmental Systems Research Institute. ArcMap 10.5.1, Redlands, CA, USA. 2011. Available online: https://www.esri.com/en-us/arcgis/products/arcgis-desktop/resources (accessed on 20 April 2023).
36. DaCosta, J.M.; Sorenson, M.D. Amplification Biases and Consistent Recovery of Loci in a Double-Digest RAD-Seq Protocol. *PLoS ONE* **2014**, *9*, e106713. [CrossRef] [PubMed]
37. Lavretsky, P.; Dacosta, J.M.; Hernández-Baños, B.E.; Engilis, A.; Sorenson, M.D.; Peters, J.L. Speciation Genomics and a Role for the Z Chromosome in the Early Stages of Divergence between Mexican Ducks and Mallards. *Mol. Ecol.* **2015**, *24*, 5364–5378. [CrossRef] [PubMed]

38. Sonsthagen, S.A.; Talbot, S.L.; White, C.M. Gene Flow and Genetic Characterization of Northern Goshawks Breeding in Utah. *Condor* **2004**, *106*, 826–836. [CrossRef]
39. Schneider, S.; Roessli, D.; Excofier, L. Arlequin: A Software for Population Genetic Data Analysis. 2000. Available online: http://cmpg.unibe.ch/software/arlequin3/ (accessed on 20 April 2023).
40. Fu, Y.X. Statistical Tests of Neutrality of Mutations against Population Growth, Hitchhiking and Background Selection. *Genetics* **1997**, *147*, 915–925. [CrossRef]
41. Tajima, F. The Effect of Change in Population Size on DNA Polymorphism. *Genetics* **1989**, *123*, 597–601. [CrossRef]
42. Fluxus Technology Ltd. NETWORK 10.1. 2022. Available online: www.fluxus-engineering.com (accessed on 20 April 2023).
43. Bandelt, H.J.; Forster, P.; Sykes, B.C.; Richards, M.B. Mitochondrial Portraits of Human Populations Using Median Networks. *Genetics* **1995**, *141*, 743–753. [CrossRef]
44. Jombart, T. Adegenet: A R Package for the Multivariate Analysis of Genetic Markers. *Bioinformatics* **2008**, *24*, 1403–1405. [CrossRef] [PubMed]
45. Jones, O.; Wang, J. COLONY: A Program for Parentage and Sibship Inference from Multilocus Genotype Data. *Mol. Ecol. Resour.* **2010**, *10*, 551–555. [CrossRef] [PubMed]
46. Alexander, D.H.; Lange, K. Enhancements to the ADMIXTURE Algorithm for Individual Ancestry Estimation. *BMC Bioinform.* **2011**, *12*, 246. [CrossRef] [PubMed]
47. Alexander, D.H.; Novembre, J.; Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **2009**, *19*, 1655–1664. [CrossRef]
48. Purcell, S.; Neale, B.; Todd-Brown, K.; Thomas, L.; Ferreira, M.A.R.; Bender, D.; Maller, J.; Sklar, P.; De Bakker, P.I.W.; Daly, M.J.; et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **2007**, *81*, 559–575. [CrossRef]
49. Alexander, D.; November, J.; Lange, K. Admixture 1.22 Software Manual 2012. Available online: https://dalexander.github.io/admixture/download.html (accessed on 20 April 2023).
50. Janes, J.K.; Miller, J.M.; Dupuis, J.R.; Malenfant, R.M.; Gorrell, J.C.; Cullingham, C.I.; Andrew, R.L. The K = 2 Conundrum. *Mol. Ecol.* **2017**, *26*, 3594–3602. [CrossRef]
51. Jakobsson, M.; Rosenberg, N.A. CLUMPP: A Cluster Matching and Permutation Program for Dealing with Label Switching and Multimodality in Analysis of Population Structure. *Bioinformatics* **2007**, *23*, 1801–1806. [CrossRef]
52. Malinsky, M.; Trucchi, E.; Lawson, D.J.; Falush, D. RADpainter and FineRADstructure: Population Inference from RADseq Data. *Mol. Biol. Evol.* **2018**, *35*, 1284–1290. [CrossRef]
53. Petkova, D.; Novembre, J.; Stephens, M. Visualizing Spatial Population Structure with Estimated Effective Migration Surfaces. *Nat. Genet.* **2016**, *48*, 94–100. [CrossRef] [PubMed]
54. Weatherhead, P.J.; Forbes, M.R.L. Natal Philopatry in Passerine Birds: Genetic or Ecological Influences? *Behav. Ecol.* **1994**, *5*, 426–433. [CrossRef]
55. Webster, K.L.; Mclaughlin, J.W. ScienceDirect Application of a Bayesian Belief Network for Assessing the Vulnerability of Permafrost to Thaw and Implications for Greenhouse Gas Production and Climate Feedback. *Environ. Sci. Policy* **2013**, *38*, 28–44. [CrossRef]
56. Procházka, P.; Stokke, B.G.; Jensen, H.; Fainová, D.; Bellinvia, E.; Fossøy, F.; Vikan, J.R.; Bryja, J.; Soler, M. Low genetic differentiation among Reed Warbler *Acrocephalus scirpaceus* populations across Europe. *J. Avian Biol.* **2011**, *42*, 103–113. [CrossRef]
57. Rönkä, N.; Pakanen, V.M.; Pauliny, A.; Thomson, R.L.; Nuotio, K.; Pehlak, H.; Thorup, O.; Lehikoinen, P.; Rönkä, A.; Blomqvist, D.; et al. Genetic Differentiation in an Endangered and Strongly Philopatric, Migrant Shorebird. *BMC Ecol. Evol.* **2021**, *21*, 125. [CrossRef] [PubMed]
58. Oring, L.W.; Lank, D.B. Breeding Area Fidelity, Natal Philopatry, and the Social Systems of Sandpipers. In *Shorebirds: Breeding Behavior and Populations*; Burger, J., Olla, B.L., Eds.; Plenum Publishing Corporation: Ann Arbor, MI, USA, 1984.
59. Lecomte, N.; Gauthier, G.; Giroux, J.F.; Milot, E.; Bernatchez, L. Tug of War between Continental Gene Flow and Rearing Site Philopatry in a Migratory Bird: The Sex-Biased Dispersal Paradigm Reconsidered. *Mol. Ecol.* **2009**, *18*, 593–602. [CrossRef]
60. Nol, E.; Williams, S.; Sandercock, B.K. Natal Philopatry and Apparent Survival of Juvenile Semipalmated Plovers. *Wilson J. Ornithol.* **2010**, *122*, 23–28. [CrossRef]
61. Pakanen, V.M.; Hagstedt, R.; Pauliny, A.; Blomqvist, D. Survival during the Pre-fledging Period Rather than during Post-fledging Drives Variation in Local Recruitment of an Endangered Migratory Shorebird, the Southern Dunlin *Calidris alpina schinzii*. *J. Ornithol.* **2021**, *162*, 119–124. [CrossRef]
62. Avise, J. *Phylogeography: The History and Formation of Species*; Harvard University Press: Cambridge, MA, USA, 2000.
63. Koopman, M.E.; Hayward, G.D.; McDonald, D.B. High Connectivity and Minimal Genetic Structure among North American Boreal Owl (*Aegolius funereus*) Populations, Regardless of Habitat Matrix. *Auk* **2007**, *124*, 690–704. [CrossRef]
64. Wilson, R.E.; Matsuoka, S.M.; Powell, L.L.; Johnson, J.A.; Demarest, D.W.; Stralberg, D.; Sonsthagen, S.A. Implications of Historical and Contemporary Processes on Genetic Differentiation of a Declining Boreal Songbird: The Rusty Blackbird. *Diversity* **2021**, *13*, 103. [CrossRef]
65. Ralston, J.; FitzGerald, A.M.; Burg, T.M.; Starkloff, N.C.; Warkentin, I.G.; Kirchman, J.J. Comparative Phylogeographic Analysis Suggests a Shared History among Eastern North American Boreal Forest Birds. *Ornithology* **2021**, *138*, 1–16. [CrossRef]

66. Ramírez, O.; Gómez-Díaz, E.; Olalde, I.; Illera, J.C.; Rando, J.C.; González-Solís, J.; Lalueza-Fox, C. Population Connectivity Buffers Genetic Diversity Loss in a Seabird. *Front. Zool.* **2013**, *10*, 1–5. [CrossRef]
67. Rehfeldt, G.E.; Crookston, N.L.; Sáenz-Romero, C.; Campbell, E.M. North American Vegetation Model for Land-use Planning in a Changing Climate: A Solution to Large Classification Problems. *Ecol. Appl.* **2012**, *22*, 119–141. [CrossRef]
68. Bateman, B.L.; Taylor, L.; Wilsey, C.; Wu, J.; LeBaron, G.S.; Langham, G. Risk to North American Birds from Climate Change-related Threats. *Conserv. Sci. Pract.* **2020**, *2*, 1–15. [CrossRef]
69. Bateman, B.L.; Wilsey, C.; Taylor, L.; Wu, J.; LeBaron, G.S.; Langham, G. North American Birds Require Mitigation and Adaptation to Reduce Vulnerability to Climate Change. *Conserv. Sci. Pract.* **2020**, *2*, 1–18. [CrossRef]
70. Stralberg, D.; Berteaux, D.; Drever, C.R.; Drever, M.; Lewis, I.N.; Schmiegelow, F.K.A.; Tremblay, J.A. Conservation Planning for Boreal Birds in a Changing Climate: A Framework for Action. *Avian Conserv. Ecol.* **2019**, *14*, 13. [CrossRef]
71. Seaborn, T.; Griffith, D.; Kliskey, A.; Caudill, C.C. Building a Bridge between Adaptive Capacity and Adaptive Potential to Understand Responses to Environmental Change. *Glob. Chang. Biol.* **2021**, *27*, 2656–2668. [CrossRef] [PubMed]
72. Iwamura, T.; Possingham, H.P.; Chadès, I.; Minton, C.; Murray, N.J.; Rogers, D.I.; Treml, E.A.; Fuller, R.A. Migratory Connectivity Magnifies the Consequences of Habitat Loss from Sea-level Rise for Shorebird Populations. *Tohoku J. Exp. Med.* **2013**, *230*, 1–8. [CrossRef]
73. Xu, Y.; Si, Y.; Wang, Y.; Zhang, Y.; Prins, H.H.T.; Cao, L.; de Boer, W.F. Loss of Functional Connectivity in Migration Networks Induces Population Decline in Migratory Birds. *Ecol. Appl.* **2019**, *29*, 1–10. [CrossRef]
74. Wells, J.V.; Dawson, N.; Culver, N.; Reid, F.A.; Morgan Siegers, S. The State of Conservation in North America's Boreal Forest: Issues and Opportunities. *Front. For. Glob. Chang.* **2020**, *3*, 1–18. [CrossRef]
75. Medan, D.; Torretta, J.P.; Hodara, K.; de la Fuente, E.B.; Montaldo, N.H. Effects of Agriculture Expansion and Intensification on the Vertebrate and Invertebrate Diversity in the Pampas of Argentina. *Biodivers. Conserv.* **2011**, *20*, 3077–3100. [CrossRef]