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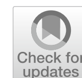
RESEARCH ARTICLE



A 37 K SNP array for the management and conservation of Golden Eagles (*Aquila chrysaetos*)

Megan E. Judkins[1,2] · Gary W. Roemer[3,15] · Brian A. Millsap[4] · Joseph G. Barnes[5] · Bryan E. Bedrosian[6] · Stephen L. Clarke[7] · Robert Domenech[8] · Garth Herring[9] · Myles Lamont[10] · Brian W. Smith[11] · Dale W. Stahlecker[12] · Matthew J. Stuber[13] · Wesley C. Warren[14] · Ronald A. Van Den Bussche[1]

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Abstract

We describe the development of a custom 37 K Affymetrix Axiom myDesign single nucleotide polymorphism (SNP) array for a culturally and ecologically important apex predator, the golden eagle (*Aquila chrysaetos*). Using this SNP array, we performed population genomic analysis on 154 individuals of known natal localities and detected three genetic clusters that we designated as Taiga/High Arctic, Great Basin, and Rocky Mountains/Great Plains. Each of these clusters appears to display clinal variation within these geographic regions. After determining genetic structure, we performed an assignment test of 32 individuals, five of which were siblings of individuals used in the assessment of genetic structure, three had associated telemetry data, and the remaining individuals were of unknown natal locations. Using this array, four siblings were correctly assigned to the same geographic region as their sibling and the genetic assignment of the radio telemetered birds agreed with the expected movement patterns displayed by these individuals. For the remaining individuals, we were able to assign all but five individuals to one of the three genetic clusters. Our genetic assignments illustrates the utility of this SNP array to accurately assign most individuals to predesignated geographical regions. While further compiling genetic and other data types, we can increase the power of this tool for identifying those breeding populations that may need assistance due to anthropogenic stressors that negatively impact their population viability. The use of this genetic resource will help substantiate decisions by multiple conservation groups that seek to preserve the natural population structure of the golden eagle.

Keywords Golden Eagle · *Aquila chrysaetos* · SNPs · Assignment tests · Conservation genetics

Introduction

Wide-ranging and highly mobile species, by definition, can traverse large areas and cross geographic boundaries that might impede other taxa. Owing to the suite of life history traits that make them so vagile, determining biologically relevant units for their management can be particularly difficult (Viengkone et al. 2016). An example of one such species is the golden eagle (*Aquila chrysaetos*). In western North America, golden eagles inhabit a diversity of habitats ranging from the Arctic Tundra of Canada and Alaska to the deserts of southern Mexico (D'Addario et al. 2019, Watson 2011). The distribution and density of golden eagles in this region is influenced by the availability of key prey resources (Simes et al. 2015) and by overlapping life history stages of breeding and natal dispersal, subadult floating, and migration and overwintering. For example, although golden eagles have been shown to exhibit strong natal philopatry (Millsap et al. 2014), subadult eagles can enter a pre-breeding dispersal stage (Murphy et al. 2017) where they have been shown to move through diverse geographic regions that may differ from where they eventually breed and nest. In addition to movements related to dispersal, some golden eagles, representing all life stages, exhibit migratory behavior creating situations where eagles from all parts of western North

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✉ Megan E. Judkins
mjudkins@iowanation.org

Extended author information available on the last page of the article

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America could be spending time in the same geographic area, such as when juvenile and adult eagles from the Arctic overwinter in the southwestern United States (US) and northern Mexico with individuals from other parts of the west (Bedrosian et al. 2018; McIntyre et al. 2008).

In the US, golden eagles are protected under the Migratory Bird Treaty Act (16 U.S.C. 703–712) and the Bald Eagle and Golden Eagle Protection Act (16 U.S.C. 668; hereafter referred to as Eagle Act). These Acts collectively protect eagles from disturbance and illegal take and delegate to the US Fish and Wildlife Service (USFWS) the authority and responsibility for management of eagles in the US. To aid in managing and conserving golden eagles, the USFWS adopted an adaptive management strategy partitioning the country into Eagle Management Units (EMUs). For golden eagles, these EMUs coincide with the Migratory Bird Flyways (Anderson and Padding 2015) with the exception that: (1) the Atlantic and Mississippi flyways have been combined into a single EMU, and (2) due to migratory patterns, populations of Alaskan golden eagles are considered to be part of both the Pacific and Central Flyways (USFWS 2016a). The decision to manage golden eagles using three EMUs was based on comparison of where eagles were initially banded and the location of subsequent band recoveries, with the objective of including natal areas and areas of eventual death in the same management units (USFWS 2016b). Defined this way, these EMUs link natal locations with areas of increased risk from anthropogenic factors, and thereby provide increased opportunities for management activities that can reduce or mitigate death or injury during seasonal migrations and dispersal events (USFWS 2016a,b).

Brown et al. (2017) utilized satellite movement data from 571 golden eagles to evaluate whether these data supported the USFWS management decision of three EMUs or whether alternative management scenarios that contained more units (Bird Conservation Regions, Landscape Conservation Cooperatives, and Migratory Bird Joint Ventures) might be more appropriate. Although their analyses did not support any of the four systems entirely, they did detect support for clusters of individuals associated with the Great Basin, Northern Rockies, California, Desert, and Southern Rockies Landscape Conservation Cooperatives (Brown et al. 2017) objectives were somewhat different than those of USFWS because their assessment was focused on overall movements, not linking natal areas with regions of greatest risk.

Natal dispersal is a population-level characteristic that is expected to influence gene flow and phylogeographic structure. Eagles move great distances, however, both band recovery and telemetry-based studies reveal strong natal philopatry. Estimates of golden eagle natal dispersal distances from band recovery data showed that the average dispersal distance was 46 km (Millsap et al. 2014). Most empirically recorded distances were less than 100 km, and only a few birds dispersed over 200 km from where they hatched. Similarly, natal dispersal distances were estimated using radio-telemetered golden eagles and revealed that male and female natal dispersal distances averaged 41 and 64 km, respectively (Murphy et al. 2019). These studies suggest that genetic structure could emanate from such strong natal philopatry, however, some long-distance movements were observed which could still homogenize populations. Though eagles may move to breeding sites close to their natal location over their lifetime, gene flow occurs across landscapes over generations.

Population genomic information can be used to describe phylogeographic patterns, delineate unique population segments, and estimate gene flow. All of which are important information for describing units worthy of concerted or unique management action (Benestan et al. 2016, Hendricks et al. 2019, Zimmerman et al. 2019). To assess a variety of population genetic variables, genome-wide SNP arrays have been developed for several wildlife species including the great tit (*Parus major*; Van Beers et al. 2012), Atlantic salmon (*Salmo salar*; Bourret et al. 2013), bottlenose dolphins (*Tursiops truncates*; Cammen et al. 2015), polar bears (*Ursus maritimus*; Malenfant et al. 2015; Viengkone et al. 2016), and bald eagles (*Haliaeetus leucocephalus*; Judkins et al. 2020). These and other studies have revealed the power of a large number of SNPs for elucidating fine-scale genetic structuring (e.g., Benestan et al. 2015, Benestan et al. 2016, Hendricks et al. 2019, Schweizer et al. 2016a, Schweizer 2016b). A SNP array has the potential to determine natal origins of migrating individuals (Ruegg et al. 2014) and could be applied to determine the origins of individuals that are injured or killed (Katzner et al. 2016). We believe that development of a medium density SNP array would be a beneficial approach for the generation of data relevant to the management and conservation of golden eagles and to address whether the restricted natal dispersal patterns empirically observed based on band recovery and telemetry data would generate a fine-scale pattern of genetic structure across the landscape.

Herein, we describe the development of an Axiom myDesign custom SNP array for golden eagles that contains over 37,000 SNPs distributed throughout the golden eagle genome and are representative of both inter-and intragenic regions. We used this SNP array to perform a population genomic assessment of golden eagles of known natal origins to determine levels of population subdivision. Relative to the two previous golden eagle genomic studies (Doyle et al. 2016, Van Den Bussche et al. 2017) we increased both the geographic scope of the study as well as the genetic coverage of the utilized markers to examine finer-scaled genetic structuring across western North America.

A second aim of this study was to perform an assignment test that included individuals with known natal localities

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as well as a sample of individuals from the National Eagle Repository (operated by USFWS) without known natal localities. Many golden eagle deaths occur during seasonal migrations and often occur hundreds to thousands of kilometers from their natal area (McIntyre 2012). Thus, the ability to probabilistically determine natal origins of migrating individuals would aid in golden eagle management by determining the impact that anthropogenic factors are having on specific breeding populations.

Materials and methods

SNP array development

SNP isolation and identification was performed using data from a sample of 32 golden eagles originally described by Van Den Bussche et al. (2017). These data provided 1.8 million SNPs for development of a golden eagle Axiom myDesign custom array. To screen for SNPs suitable for array development, we utilized several options in the PLINK software package (Purcell et al. 2007). Filtering options included a minor allele frequency (MAF) of 0.05 and a minimal genotype frequency of 0.3. We tested all loci for deviations from Hardy-Weinberg Equilibrium (HWE) and any locus that deviated significantly ($P < 0.001$) from these expectations was removed from consideration. All loci that passed this set of criteria were further thinned to retain only a single SNP in each 10KB sliding window using options in VCFTools (Danecek et al. 2011). SNPs remaining after these filtering steps, as well as 160 nuclear SNPs and a SNP for sex determination previously identified for golden eagles (Doyle et al. 2016) were sent to Affymetrix (Santa Clara, CA) for additional quality control measures such as binding specificity and strength to ensure each SNP was appropriate for array probe construction. SNPs remaining after quality control steps performed by Affymetrix were annotated against the golden eagle reference genome (https://www.ncib.nlm.nih.gov/nuccore/NW_011950869.1) using the program SnpEff (Cingolani et al. 2012). Finally, we prioritized SNPs located in genes that other studies found to be ecologically relevant (Malenfant 2015, Van Beers 2012), were upstream or downstream of genes, or were in intergenic regions and not necessarily closely associated with known genes.

To evaluate the accuracy of the sex determination SNP (Doyle et al. 2016), we performed standard PCR-based molecular sexing of 57 individuals that were also genotyped using the SNP array. For the PCR-based sex determination, we followed the PCR reaction conditions and thermal profile of Ito et al. (2003). PCR products were electrophoresed through a 3% agarose gel to determine if one (male) or two (female) bands were present. If the reaction did not amplify using primers MP and P2 of Ito et al. (2003), primers CHD1Wr and CHD1Zr (Banhos et al. 2008) were substituted using the reaction conditions and thermal profile of Ito et al. (2003). This second set of primers amplifies a 100 bp shorter region of the CHD gene and therefore is better able to amplify degraded DNA.

Sample selection for assessment of genetic structure

DNA was isolated from whole blood samples or tissues from 186 golden eagles (Online Resource 1). All samples were collected during previous regional or local studies designed to examine golden eagle exposure to contaminants, health, and movements or were obtained from the National Eagle Repository. These studies were conducted by permitted biologists and were collected and stored prior to the start of this study. An aliquot of these previously collected and stored samples were sent to our lab for analysis under permit USFWS MB82801A and MB58285B-0 which allowed us to subsample the stored samples. Blood aliquots were either stored in lysis buffer or dried on Whatman cards while tissue samples were received from the samples collected at the National Eagle Repository. All samples were sent to our laboratory at Oklahoma State University where whole genomic DNA was extracted following the method of Longmire et al. (1997), the DNEasy Blood and Tissue kit with manufacturer suggested protocol (Qiagen, Valencia, CA), or Chelex 100 with the suggested DNA extraction methods for Whatman cards (Sigma Aldrich, St. Louis, MO). A 1% agarose gel was used to assess DNA quality and DNA was quantified using a NanoDrop 3300 Spectrophotometer (Thermo Scientific, Waltham, MA). Our analyses for describing phylogeographic structure included 154 of the 186 eagles. Of the 154 individuals, 142 were hatchling/fledgling golden eagles with known natal locations (latitude and longitude of their nest was recorded at time of tissue sampling) from Arizona ($n = 3$), California ($n = 8$), Colorado ($n = 24$), Idaho ($n = 5$), Montana ($n = 4$), Nebraska ($n = 9$), Nevada ($n = 5$), New Mexico ($n = 18$), Oklahoma ($n = 1$), Oregon ($n = 30$), Texas ($n = 4$), Utah ($n = 3$), Washington ($n = 1$), Wyoming ($n = 13$), and Nunavut, Canada ($n = 14$). Additionally, 12 individuals were captured during their southern migration into the US and either isotope analysis performed on their feathers (Domenech et al. 2015) or GPS transmitter data suggested that these individuals were migrating from Alaska ($n = 9$) or British Columbia ($n = 3$). For 8 of these 12 individuals, we were able to determine summer/nesting locality and recorded latitude/longitude of this region. The latitude/longitude of the remaining 4 individuals represent locations of death, all of which occurred in their summer/nesting locality.

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Samples for population assignment

Following the laboratory methods described above, whole genomic DNA was isolated from the following 32 samples: Arizona (n = 1), California (n = 1), Colorado (n = 4), Idaho (n = 3), Nebraska (n = 1), Nevada (n = 1), New Mexico (n = 3), Nunavut (n = 1), Oregon (n = 2), Utah (n = 6), and Wyoming (n = 9). Five individuals (two from Colorado, and one each from Nevada, New Mexico, and Nunavut) were siblings of individuals used in the analyses of genetic structure, whereas the remaining 27 samples represented individuals from the National Eagle Repository for which natal locality was unknown.

SNP genotyping

DNA aliquots of the 186 golden eagles were sent to Eurofins (River Falls, WI) for genotyping using our golden eagle custom SNP array. All individuals were genotyped using options in the Axiom Analysis Suite v5.1.1 and scored SNPs were filtered to remove any loci that poorly clustered using the Bayesian scoring in the Axiom Analysis Suite, had a MAF < 0.01, or were monomorphic.

To determine genotyping error of our SNP array, 15 arbitrarily chosen individuals were genotyped a second time. Genotypes of the duplicate runs were scored independently and compared to identify discrepancies between the two runs. We evaluated the following two types of genotyping errors: the error rate of no call at a SNP in one run versus a base call for the same SNP in the alternative run and errors in which different bases were called for the same SNP in independent runs. Means and medians for these two types of errors were calculated independently and then combined for an overall error rate.

Assessment of population structure

To assess genetic structure, Structure v2.3.4 (Pritchard et al. 2000), FastStructure 1.0 (Raj et al. 2014), and Admixture v1.3 (Alexander et al. 2009) were used to analyze all SNPs that were scored and passed quality control standards. All three of these programs are model-based approaches and thus depend upon specific assumptions that, if violated, potentially impact clustering results (Evanno et al. 2005; Latch et al. 2006; Ruiz-Gonzalez et al. 2015). For example, it has been shown that Structure does not accurately identify the actual number of subpopulations (K) when F_{ST} is ≤ 0.3 (Latch et al. 2006). Because little is known about the genetic structure of golden eagles in North America, we were concerned with relying solely on results from these programs and therefore performed a discriminant analysis of principal components (DAPC; Jombart 2008).

Structure analysis

Structure was run using the admixture model with correlated allele frequencies for 10,000 burn-in iterations, 50,000 runs with K, the number of genetic clusters ranging from 1 to 8 using eight independent runs per K. The output from Structure was analyzed to determine if convergence had been achieved and was further analyzed using Structure Harvester v0.693 (Earl and Von Holdt 2012) and CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007). Results were summarized using Structure Harvester where delta K was visualized (Evanno et al. 2005) to infer the optimal K. We assigned individuals to a cluster if their Q-value ≥ 0.5 (Balkenhol et al. 2014). To assess the similarity between sampling location and genetic cluster assignment, we plotted all samples (based on latitude and longitude of nest location or summer/nesting territory) and used color-coded pie charts to represent the frequency of each genetic cluster within individuals using ArcMap version 10.6.1 (ESRI, Redlands, CA, USA).

Admixture analysis

Admixture was run with the number of groups, K, ranging from 1 to 8 in five separate iterations. The cross-validation error was compared between each run to ensure selection of the best K. Standard errors for each run were obtained using a bootstrap approach with 200 iterations. Following the same approach used for Structure, individuals were assigned to the cluster for which their Q-value was ≥ 0.5.

FastStructure analysis

FastStructure was run 10 times with K ranging from 1 to 8 using the simple prior method. To determine the most appropriate K, the command chooseK.py was utilized. Q values for each individual and each K were averaged across the 10 runs. Average Q values were visualized to determine the best K and individuals were assigned to a cluster if their Q-value was ≥ 0.5.

DAPC analysis

DAPC was run using Adegenet 2.1.0 in program R 3.6.3, an approach that has been shown to better handle hierarchical genetic structure as well as clinal variation due to isolation by distance (Jombart et al. 2010; Kalinowski 2011). We evaluated and compared the results of DAPC analyses performed with and without prior group assignments (Quemere et al. 2016). For prior group assignments we used state or Canadian province or territory of nest

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location. For the analysis without prior locality information, we ran successive K groupings with the number of clusters ranging from 1 to 8 to visualize the results. Ultimately, the optimal number of clusters was determined using the find.clusters() option. To avoid retaining too many principal components, which can result in an overly complex model and poor predictive power, the optimal number of principal components (n = 13) was retained according to the α-score. These principal components were used in the find.clusters() data set (Online Resource 2). Finally, Arlequin v3.5 (Excoffier and Lischer 2010) was used to perform an AMOVA and to calculate pairwise F_{ST}-values with significant differentiation being determined using a P-value of ≤ 0.05.

Analysis of genetic groups

Adegenet in program R was used to obtain observed (H_o), expected (H_e) heterozygosities and allelic richness (A_R) for each cluster. For the determination of A_R, unequal sample size was corrected using rarefaction to standardize groups to the smallest cluster size. Additionally, Adegenet was used in a test for isolation by distance among all individuals genotyped. The geographic distance matrix was calculated based on individual latitude and longitude coordinates of sampled nests (n = 144), summer/nesting territory (n = 8), or place of death on summer/nesting territory (n = 4) and the genetic distance matrix was based on the co-ancestry coefficients. Genepop 4.4.3 (Raymond and Rousset 1995) was used to test for HWE for each SNP within each cluster with significant departures from HWE determined via a sequential Bonferroni correction.

Assignment tests

After determining the most appropriate number of genetic clusters for individuals with known natal localities, we re-ran Structure but this time setting K to the number of genetic clusters determined in previous analyses and the USEPOPINFO option. For this analysis, individuals previously used in the analyses of genetic structure were coded with respect to the population they clustered in, whereas the remaining 32 individuals were coded as 0. In the new set of 32 individuals, there were 5 individuals that were siblings of birds in the original dataset consisting of 154 individuals. For these 5 sibling pairs, we removed the sibling from the larger, geographic data set of 154 individuals (reducing this data set to 149 individuals) so that we could assess the ability of our SNP chip to assign these individuals to one of the three genetic groups.

Results

SNP array development, Sample Scoring, and Error Rate

After quality control filtering was completed, the Axiom myDesign custom array consisted of 37,562 SNPs with the following composition – one sex determination locus, 4,719 genic, and 32,686 intergenic loci (EVA Accession: Project: PRJEB60512, Analyses: ERZ16299910). We successfully amplified, electrophoresed and determined the sex of all 57 individuals from the PCR products and the array. The sex determination methods were 100% concordant (27 females, 30 males). Of the 186 individuals genotyped, 98 were female and 88 were male based on the SNP sex determination locus.

Analysis of the 37,562 SNPs from the 154 individuals used in the determination of population structure revealed 139 monomorphic SNPs, 173 SNPs with a MAF < 0.01, and 836 SNPs that were poorly clustered using the Axiom Analysis software leaving 36,414 SNPs for downstream analysis. Genotyping error for the array was assessed using 15 arbitrarily chosen DNA samples that were genotyped twice. Errors where a SNP was called as a base in one run and the same SNP had a non-distinguishable base called in the second run occurred at an average of 0.60% and a median of 0.46%. Errors in which a different base was called for the same SNP in two independent runs occurred at an average of 0.23% with a median of 0.19%. Thus, the overall mean and median error was 0.82% and 0.64%, respectively.

Population structure

The model-based approaches (Structure, Admixture, FastStructure) indicated the existence of three genetically-distinct, regional clusters (Figs. 1 and 2, Online Resource 3, 4, 5). Analyses of population structure using DAPC failed to detect any genetic structure (K = 1) when utilizing the find.clusters() command. However, when visualizing the DAPC output it is evident that some genetic structure exists among these samples (Fig. 3). There appears to be a southern to northern cline along discriminant axis (DA) 1 and the most northerly samples, those from Nunavut, Alaska, and British Columbia, are separated from the contiguous US samples along DA 2. The AMOVA revealed significant partitioning of genetic variation among the three large clusters (F_{ST} = 0.023; $P < 0.001$). Hereafter, the three clusters will be referred to as: Taiga/High Arctic (Alaska and Canada), Great Basin (northern California, southern Oregon, southern Idaho, Nevada, Washington),

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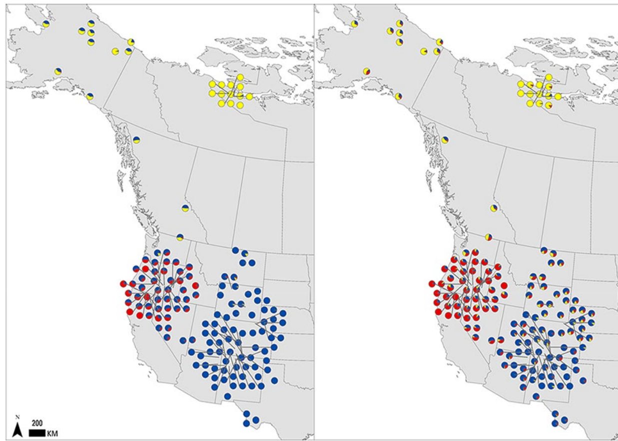

Fig. 1 Population structure for golden eagles based on 154 individuals genotyped at 36,414 SNPs and K = 3 as determined by both Structure (left) and FastStrucure (right) computer programs. This figure shows the geographic location of the sampled nest (except for individuals from Alaska and British Columbia – See Methods) and their genetic group assignment: yellow = Taiga/High Arctic, red = Great Basin, blue = Rocky Mountains/Great Plains

and Rocky Mountains/Great Plains (Arizona, Colorado, Montana, Nebraska, New Mexico, Oklahoma, Texas, Utah, Wyoming). When examining Q-values for the three groups, only six, six, and zero individuals were not assigned to one of the three groups with a Q-value ≥ 0.5 from Admixture, Structure, and FastStructure, respectively.

The Taiga/High Arctic cluster contained individuals from Nunavut, Alaska, and British Columbia and had a mean level of group membership of 81–85% (Table 1). The Great Basin cluster contained individuals west of the Rocky Mountains and had an overall group membership of 45–73% (Table 1). The Rocky Mountains/Great Plains cluster contained the greatest number of individuals and had an overall group membership of 79–100% (Table 1). All F_{st}_values were significant (p-value < 0.05): Taiga/High Arctic and Great Basin F_{st} = 0.04, Taiga/High Arctic/ and Rocky Mountain/Great Plains F_{st} = 0.04, and Rocky Mountain/Great Plains and Great Basin F_{st} = 0.01. Testing matrices of pairwise co-ancestry values versus geographic distance for all individuals revealed a significant (P = 0.001) effect of isolation by distance (Online Resource 5). Based on the above three groupings, none of the SNPs deviated significantly from HWE.

Results of assignment tests

To test the ability of our SNP array to assign individuals to likely areas of natal origin, we genotyped 32 individuals, 20 females and 12 males. Among the individuals used in the assignment test, five individuals were siblings of individuals used in the analysis of genetic structure. Four of the five individuals were assigned to the same genetic cluster as their sibling. Of the remaining 27 individuals, Structure was able to assign all but five individuals to one of the three genetic clusters with an assignment probability ≥ 0.5. Moreover, 11 of these 27 individuals were assigned to the cluster comprising the geographic area that they were collected. Eight individuals were assigned to the Taiga/High Arctic cluster (mean assignment probability = 71%), three individuals were assigned to the Great Basin cluster (mean assignment probability = 60%), and 12 individuals were assigned to the Rocky Mountains/

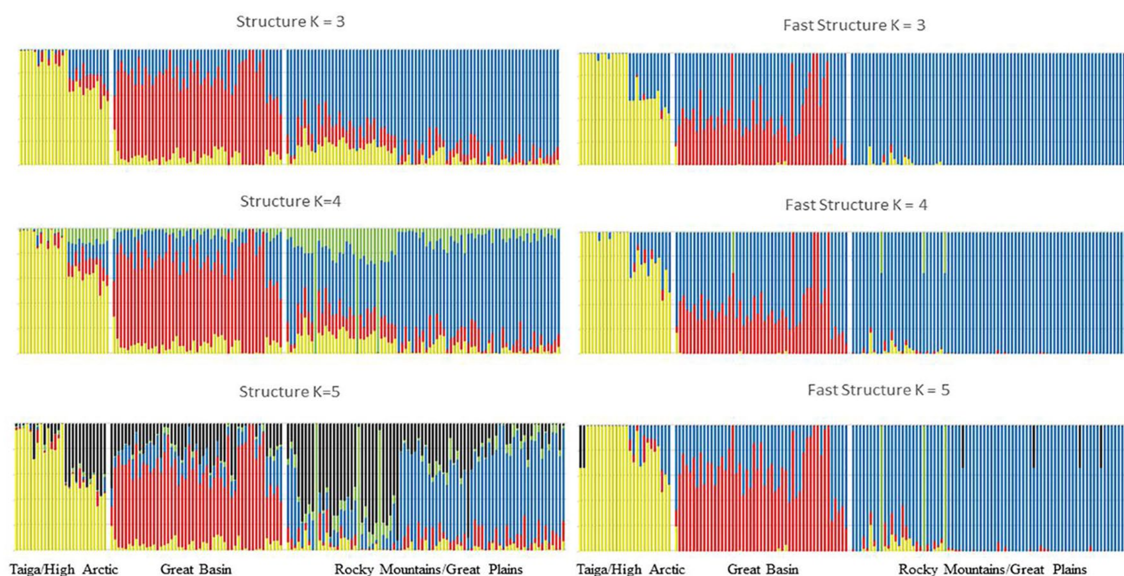

Fig. 2 Results of Structure and FastStructure Analysis (K = 3–5) based on 154 individuals genotyped at 36,414 SNPs

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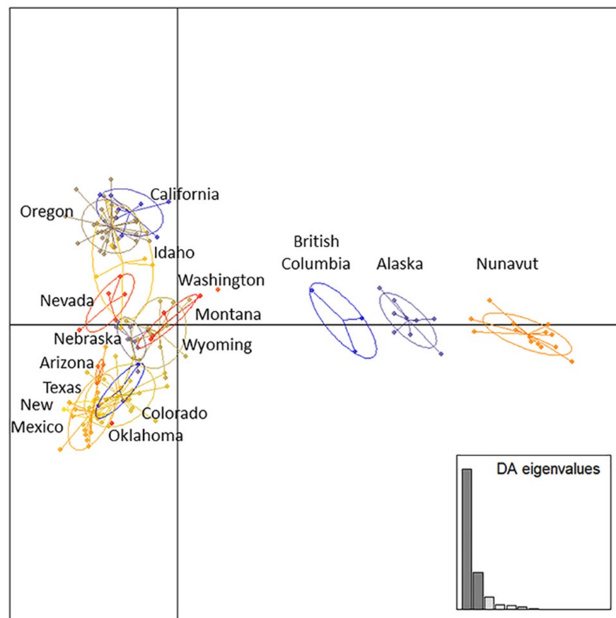

Fig. 3 Results of the discriminate analysis of principal components (DAPC) performed in Adegenet. Results were obtained using the optimal number of discriminant functions (n = 13) as determined by the α-score

Great Plains cluster (mean assignment probability = 60%). Mean assignment values are similar to the mean assignment values for these same three clusters based on the 154 individuals of known natal locality (Table 1). Five individuals, a female from Idaho, a female from Oregon and two individuals (1 male and 1 female) from Wyoming could not be assigned to any of the three clusters with a probability ≥ 50%. The assignment test did place the individual from Idaho within the Great Basin cluster with a probability of 46% and the two individuals from Wyoming were assigned to the Rocky Mountain/Great Plains cluster with probabilities of 38% and 45%, respectively. Finally, the individual from Oregon was assigned to the Taiga/High Arctic cluster with a probability of 45%.

Discussion

Continental genetic diversity

Golden eagles show strong natal philopatry (Millsap et al. 2014; Murphy et al. 2019) and this might predict that they would also show a pattern of genetic variation consistent with isolation by distance. Our analyses support the view that these large, vagile birds of prey, which can traverse large areas, show genetic structure with three clusters manifest at large regional scales. We observed relatively high levels of admixture, as evidenced by both mixed ancestry within and low F_{st} values among the regional clusters, but the use of a large number of SNP loci exposed what appears to be clinal variation within clusters in addition to the large-scale phylogeographic pattern across the continent, both consistent with the empirical pattern of natal dispersal distances evident from band recovery and telemetry data (Millsap et al. 2014; Murphy et al. 2019). The clinal variation within clusters may be explained by the frequent and typically short natal dispersal distances observed in golden eagles, whereas the mixed ancestry and weak genetic structure among the large regional clusters fits a pattern of isolation by distance caused by infrequent, long-distance natal dispersal.

In all three genetic structure analyses (Structure, FastStructure, Admixture), admixture occurred between each of the three regional clusters with FastStructure reporting the least amount of interregional admixture (Figs. 1 and 2; Table 2). Similar to other raptors, such as the Cooper's hawk (*Accipiter cooperi*; Sonsthagen et al., 2012), sharp-shinned hawk (*A. striatus*; Hull & Girman 2004), and red-tailed hawk (*Buteo jamaicensis;* Hull et al. 2008), an east to west division of genotypes was observed. This east to west division correlated with the Rocky Mountains, a pattern also observed in other avian species such as Wilson's warblers (*Cardellina pusilla*; Kimura et al. 2002) and wood ducks (*Aix sponsa*; Peters et al. 2005) as well as in previously published golden eagle studies (Doyle et al. 2016, Van Den Bussche 2017). In addition to this geographic filter, the evolutionary history of golden eagles may have had substantial impacts on the genetic structure observed. Judkins and Van Den Bussche (2018) found that the 19 mtDNA golden eagle haplotypes sequenced in 115 golden eagles sampled across North America supported a more recent population expansion and only intermediate divergence in Nearctic golden eagles compared to Palearctic and Mediterranean golden eagle populations. A recent population expansion, the presence of a geographic filter in the form of the Rocky Mountains, and the potential for gene flow arising from long-distance natal dispersal may homogenize the North American population enough to create large regional clusters but prevent isolated genetic groupings at smaller geographic scales.

Within clusters

Taiga/High Arctic –Our analyses supported the genetic distinctiveness of golden eagles from the most northern sampling areas including parts of Nunavut, British Columbia, and Alaska. The average group membership for this genetic cluster was 82% in Structure and 83% in FastStructure and represented the most homogeneous cluster out of the three that were found; the samples from Nunavut were the least admixed samples of all localities. This was similar to the results of Van Den Bussche (2017). All of the program's

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Table 1 Genetic characteristics of golden eagles representing the Taiga/High Arctic, Great Basin, and Rocky Mountain/Great Plains genetic groups

	N	Taiga/High Arctic	Great Basin	Rocky Mountain/ Great Plains	H_o	H_e	A_R
Taiga/High Arctic	26	0.807/0.802/0.850	0.077/0.006/0.105	0.116/0.192/0.105	0.29	0.29	1.95
Great Basin	49	0.071/0.005/0.078	0.713/0.448/0.730	0.216/0.547/0.193	0.30	0.30	1.99
Rocky Mountain/Great Plains	79	0.086/0.007/0.096	0.120/0.002/0.119	0.794/0.997/0.785	0.30	0.30	1.98

Frequencies represent the results from Structure, FastStructure and Admixture, respectively. N, Ho, He, and AR refer to number of golden eagles genotyped, observed heterozygosity, expected heterozygosity, and allelic richness, respectively.

Table 2 Results of individual assignment tests. ID = Individual identification; Location = where the bird was found/collected; values under Taiga/High Arctic, Great Basin, Rocky Mountains/Great Plains are the probability that the individual originated from that genetic cluster; Comments reflect cause of death or other information known about the individual

ID	State/Province/Territory	Date	Taiga/ High Arctic	Great Basin	Rocky Mountains/ Great Plains	Comments
0709–02973	Colorado	12/3/2014	0.03	0.60	0.38	Captured/fitted with telemetry collar/released
0799 – 00543	Wyoming	12/4/2014	0.68	0.07	0.26	Captured/fitted with telemetry collar/released
13-1-1645	Oregon	4/18/2013	0.45	0.32	0.24	Lead poisoning
13-1-795	Colorado	2/6/2013	0.82	0.05	0.13	Electrocution
14-1-1367	Arizona	2/20/2013	0.08	0.32	0.60	Roadkill
14-1-314	Oregon	11/4/2009	0.59	0.23	0.19	Roadkill
14-1-441	Utah	9/30/2013	0.10	0.26	0.64	Electrocution
14-1-548	Utah	2/19/2013	0.19	0.53	0.28	Electrocution
14-1-935	Wyoming	1/29/2013	0.30	0.20	0.50	Roadkill
14-1-967	Wyoming	1/5/2012	0.36	0.26	0.38	Emaciated
15-1-1690	Idaho	1/21/2014	0.26	0.39	0.35	Roadkill
15-1-637	Utah	3/11/2014	0.27	0.23	0.50	Roadkill
16-1-1591	Idaho	3/4/2015	0.21	0.46	0.33	Roadkill
16-1-58	California	8/8/2015	0.01	0.67	0.33	Electrocution
17-1-1892	Utah	10/11/2016	0.07	0.19	0.73	Roadkill
17-1-2075	Idaho	12/20/2016	0.28	0.06	0.66	Electrocution
18-1-0825	Wyoming	1/11/2018	0.26	0.23	0.51	Roadkill
18-1-1316	Nebraska	3/14/2018	0.73	0.05	0.22	Electrocution
18-1-1543	Wyoming	11/21/2017	0.34	0.22	0.45	Electrocution
18-1-2134	Wyoming	6/7/2018	0.67	0.04	0.29	Wind turbine strike
18-1-2589	Utah	3/16/2018	0.04	0.20	0.76	Roadkill
18-1-951	Wyoming	5/22/2017	0.25	0.20	0.56	Wind turbine strike
18-2-2043	Wyoming	5/30/2018	0.13	0.17	0.71	Wind turbine strike
18-2-2060	Wyoming	5/30/2018	0.73	0.01	0.26	Wind turbine strike
18-2-2588	Utah	1/26/2018	0.01	0.29	0.71	Electrocution
629-50755	New Mexico	6/15/2012	0.01	0.03	0.96	Sibling used in analysis of genetic structure
709–07061	Nevada	5/3/2018	0.10	0.39	0.52	Sibling used in analysis of genetic structure
709–3881	Colorado	6/6/2016	0.04	0.01	0.95	Sibling used in analysis of genetic structure
799 – 750	Colorado	6/5/2016	0.01	0.14	0.85	Sibling used in analysis of genetic structure
799–01208	Nunavut	7/24/2017	1.00	0.00	0.00	Sibling used in analysis of genetic structure
James Dean	New Mexico	4/25/2013	0.76	0.05	0.20	Rehab/ fitted with telemetry collar/released
623	New Mexico	2/22/2012	0.02	0.36	0.54	Captured/fitted with telemetry collar/released

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output indicated gene flow with the Rocky Mountains/Great Plains cluster albeit Nunavut had 8 of 14 individuals that were almost genetically pure with a Q-value greater than 98% in Structure and 12 of 14 with a Q-value of greater than 99% in FastStructure. The Structure and Admixture analyses showed a bidirectional movement, whereas the FastStructure analysis showed a unidirectional movement of alleles from the southern clusters into the Taiga/High Arctic cluster. These genetic patterns may correlate to the movement patterns observed by both Brown et al. (2017) and McIntyre et al. (2008, 2012) where individuals from Alaska migrated into the Rocky Mountain and Great Plains area and in Brown et al. (2017) where some individuals from the Nunavut area migrated into the Great Plains. While these individuals that represent Nunavut can be construed as having lower admixture, our sample size is small, and more samples should be collected to determine if this pattern is representative of this entire region. Future studies should focus on better sampling across Canada. In addition to the well-studied eagle population in eastern Canada (Brodeur et al. 1996; Katzner et al. 2012; Millsap and Vana 1984) there are nesting areas known from Manitoba, Saskatchewan and the Northwest Territories, with both cliff nesting and tree nesting populations (Asselin et al. 2013; Poole and Bromley 1988; Santy 1958; Scott and Bollinger 2015). Our samples from Alaska and British Columbia were sampled on their annual southern migration and had been fitted with radio transmitters to estimate natal origins. Future studies should focus on greater sampling of nests across this vast geographic region to help further elucidate genetic structuring and clarify relationships with the contiguous U.S. samples. Samples from nesting regions in North and South Dakota might also prove particularly useful in this regard (Allen 1987).

Great basin –This cluster contained individuals west of the Rocky Mountains and was also detected with a SNP analysis by Van Den Bussche et al. (2017) and supported by movement data from telemetered golden eagles (Brown et al. 2017). These samples cover Washington, Oregon, Northern California, Idaho, and Nevada. The average group membership within this cluster was 80% in Structure and 89% in FastStructure. Within this cluster, the Nevada and Washington samples were the most admixed in the Structure results, with an average group membership of 50% and 39% respectively, while the FastStructure results had average group memberships of 85% and 80%, respectively. As Washington was represented by a single sample, this individual could represent an anomaly for the area, or it could represent an area of change from the Great Basin cluster to the more northern Taiga/High Arctic cluster. When considering this regional cluster of individuals, the level of admixture may be influenced by other key topographic features including the Columbia and Snake River valleys and the Columbia Plateau. Furthermore, the impact of the topography of the Rocky Mountains on this region may be having a major impact on the gene flow between the Great Basin cluster with the Rocky Mountains/Great Plains cluster. Due to the Rocky Mountains ability to act as a true filter between these two groups breaking down at the northern and southern ends of the range, there could easily be ongoing gene flow between these groups. This gene flow could be contributing to the patterns of admixture observed in our dataset. Doyle et al. (2016) sampled 29 individuals from California, spanning nearly the entire north – south gradient and found that those individuals represented a distinct genetic entity. We were able to sample individuals from northern California and Nevada but without representatives from the remainder of California it is unclear how our results compare to those of Doyle et al. (2016). Again, more sampling is needed to better understand the genetic structure within the Great Basin cluster and to further refine its genetic relationship to other clusters.

Rocky mountains/great plains –This cluster contained the highest number of individuals. Individuals within this cluster had an average group membership of 75% in Structure and 72% in FastStructure and similar to groupings of golden eagles detected by Doyle et al. (2016) and Van Den Bussche et al. (2017). When considering the substructure within this cluster and gene flow with other clusters, the Structure, Admixture, and DAPC results significantly differed from the FastStructure results. The Structure, Admixture, and DAPC results all indicate a north to south cline that is supported by significant isolation by distance, while the FastStructure results show a more homogeneous population. Within the Structure analysis this region had bidirectional gene flow with both the Taiga/High Arctic and the Great Basin clusters whereas the FastStructure analysis revealed a unidirectional gene flow from this cluster to both of the other clusters. It should be noted that movements of telemetered golden eagles from Alaska, and both the Arctic and West coasts of Canada funnel south along both sides of the Rocky Mountains (Brown et al. 2017).

Eagle management units

When comparing our results with previously published results and the USFWS EMUs, the Great Basin and the Rocky Mountain/Great Plains clusters align with the Pacific and Central Flyways, as the continental divide is used to define and separate these flyways, and the Rocky Mountains could limit breeding interactions between eagles contributing to the clusters we observed. The Taiga/High Arctic cluster is not represented by a single flyway or EMU, but instead, encompasses an expansive breeding area which includes areas that occur outside the scope of the EMU management plan (i.e., Canada). Movement data shows that eagles from this region migrate along three different flyways:

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Pacific, Central, and Mississippi, with some smaller geographic locations using multiple flyways. For example, the samples from Nunavut were genetically homogeneous but limited movement data clearly shows that eagles from this area migrate south along two different flyways, the Central and Mississippi Flyways (Brown et al. 2017). Our genetic data further supports this pattern of movement based on the admixture patterns observed within the Taiga/High Arctic cluster.

Genetic assignment

Utilizing a combination of genetic and isotopic data for golden eagle assignments, Katzner et al. (2016) showed that the Altamont Pass Wind Resource Area (APWRA) caused the death of not only local populations of golden eagles, but importantly, this facility likely caused mortality of golden eagles from a much larger area of the western US. It is not only collisions with wind turbines that kill golden eagles, however. In part because of their extensive geographic range, which spans across western North America and long-distance movements during various life history stages (Bedrosian et al. 2018; Brown et al. 2017; McIntyre et al. 2008; Murphy et al. 2019), golden eagles are also exposed to and killed by electrocution, collisions with automobiles, lead poisoning, illegal shooting, and habitat loss (which affects their prey base) (Mojica et al. 2018, Millsap et al. *In Press*, Russell and Franson 2014). Given that our study has provided added insight into the genetic structuring of golden eagles across western North America, we were interested in determining if the SNP array that we developed would be a valuable management tool to probabilistically assign recovered eagle carcasses to their natal locality.

We assigned 27 of 32 individuals (84%) to one of the three regional clusters with a probability ≥ 50% (Table 2). For eight of the individuals used in the assignment test, we had additional data supporting their assignment. Five were siblings of individuals used in the analysis of genetic structure and three individuals were fitted with radio transmitters, which provided information regarding their seasonal movements. The remaining samples represented individuals killed due to electrocutions, wind turbine strikes, or vehicle collision (Table 2). The siblings from Nunavut, and those from Colorado and New Mexico were correctly assigned to the Taiga/High Arctic and Rocky Mountains/Great Plains clusters, respectively, with very high probabilities (Table 2). The sibling from Nevada was not correctly assigned to the Great Basin cluster, rather it was assigned to the Rocky Mountains/Great Plains cluster with a probability of 0.52. Nevada was a highly admixed area in the genetic structure analyses, so this incorrect cluster assignment could be a result of this ambiguity. Further support for our approach of being able to assign "unknown" individuals to likely natal areas comes from three individuals in our assignment test that had associated radio telemetry data. One individual (band number 0709–02973) was sampled in Colorado on 3 December 2014, fitted with a radio telemetry unit and movement data was collected from this individual for about one year. The genetic data assigned this individual (a male based on the sex determination SNP) to the Great Basin cluster, with a probability of 60%. Radio telemetry data indicated that this individual spent the majority of the breeding season north and east of Reno, NV (collected and tagged by DWS, USFWS unpubl. data). A second individual (band number 0799 – 00543, a male based on the sex determination SNP), had tissue samples obtained on 4 December 2014 from a locality in Wyoming. Based on the genetic data, this individual was assigned to the Taiga/High Arctic cluster with a probability of 68%. This individual was fitted with a radio telemetry unit along the coast of the Beaufort Sea on the north slope of Alaska (captured and tagged by Mike Lockhart, USFWS unpubl. data) and approximately three years of movement data indicated that this male made annual trips to this region during the breeding season. Thus, excluding the siblings and individuals that could not be assigned due to a high degree of admixture, we had 22 individuals that could be genetically assigned and compared to where they were recovered. Of these 22 individuals, 12 were recovered in a state that resides within the genetic cluster they were assigned and suggests that the area they were recovered represented their natal geographic area. The remaining 10 individuals were recovered in a state outside the genetic cluster they were assigned to. These results are consistent with golden eagle movement patterns and illustrates that this genetic assignment method can be used to determine if anthropogenic factors in the US are impacting birds both within and outside their natal region. The correct assignment of most of the siblings as well as those individuals with substantial telemetry data supports the use of our genetic data to probabilistically assign individuals to regions of the country that encompass their natal area.

Conservation implications

We describe the development of a genotyping array for golden eagles and the application of this array for understanding the genetic structuring of golden eagles across much of their western North American range. Elucidating the partitioning of genetic variation in golden eagles across western North America is meant to augment EMUs established by the USFWS (2016b) through improved alignment of genetic information with opportunities to manage and

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mitigate risk. The long-distance movements that golden eagles may make across western North America, especially individuals from populations living in northerly latitudes, expose them to anthropogenic stressors across the continent (Katzner et al. 2016; McIntyre 2012) used stable isotope and genetic data to show that greater than 25% of the golden eagles killed at the Altamont Pass Wind Resource Area (APWRA) were most likely recent immigrants, the average distance from the APWRA for the immigrant eagles was 146 km with one individual coming from an estimated distance greater than 800 km away. Being able to identify the natal origins of birds impacted by anthropogenic factors could aid the USFWS in developing, or refining guidelines for take within their golden eagle management plan. Our genetic assignment test illustrates that our SNP array was able to assign nearly 50% of the "unknown" eagles to a genetic cluster that does not include their recovery location and indicates that anthropogenic factors can impact locally breeding vs. distantly breeding birds and that the genetic assignment can be used to evaluate this impact. Additional sampling and genotyping of golden eagles across their range to further refine the population genetic structure of golden eagle and combining genetic data with other data, such as isotopes (Ruegg et al. 2014, 2017), can further increase the power of this tool. Finally, our SNP Array helps identify the natal origins of deceased golden eagles and determine if these deaths are disproportionately impacting the breeding population to a level that is unsustainable.

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Author contributions RAVDB, MEJ, WCW, and SLC designed the golden eagle SNP array. RAVDB, MEJ, GWR and BAM were responsible for study design. GWR, BAM, JGB, BEB, RD, GH, ML, BWS, DWS, and MJS collected golden eagle blood for other ongoing studies and provided these samples to this study. MEJ and RAVDB performed all aspects of data analysis. MEJ and RAVDB took the initial lead for the interpretation of results from the genetic analyses and wrote the initial draft of the manuscript. All authors provided insight into the biological and management implications of the data and all authors made significant contributions to the manuscript.

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Data availability All SNP data will be available from the EVA database once the manuscript has been accepted.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interest to disclose

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Authors and Affiliations

Megan E. Judkins[1,2] · Gary W. Roemer[3,15] · Brian A. Millsap[4] · Joseph G. Barnes[5] · Bryan E. Bedrosian[6] · Stephen L. Clarke[7] · Robert Domenech[8] · Garth Herring[9] · Myles Lamont[10] · Brian W. Smith[11] · Dale W. Stahlecker[12] · Matthew J. Stuber[13] · Wesley C. Warren[14] · Ronald A. Van Den Bussche[1]

[1] Department of Integrative Biology, Oklahoma State University, Stillwater, OK 74078, USA

[2] Grey Snow Eagle House, Iowa Tribe of Oklahoma, Perkins, OK 74059, USA

[3] Department of Fish, Wildlife and Conservation Ecology, New Mexico State University, Las Cruces, NM 88003, USA

[4] Division of Migratory Bird Management, U.S. Fish and Wildlife Service, Albuquerque, NM 87113, USA

[5] Nevada Department of Wildlife, 6980 Sierra Center Parkway, Suite 120, Reno, NV 89511, USA

[6] Teton Raptor Center, Wilson, WY 83014, USA

[7] Department of Nutritional Sciences, Oklahoma State University, Stillwater, OK 74078, USA

[8] Raptor View Research Institute, Missoula, MT 59806, USA

[9] United States Geological Survey, Forest and Rangeland Ecosystem Science Center, Corvallis, OR 97331, USA

[10] TerraFauna Wildlife Consulting, 19313 Zero Ave, Surrey, BC V3Z9R9, US

[11] U.S. Fish and Wildlife Service, Division of Migratory Bird Management, Mountain Prairie Region, Denver, CO 80225, USA

[12] Eagle Environmental Inc, Santa Fe, NM 87508, USA

[13] Division of Migratory Bird Management, U.S. Fish and Wildlife Service, Columbia Pacific Northwest Region, OR, Portland 97232, USA

[14] Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA

[15] Present Address: Las Cruces, USA

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