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Conservation Genetics

ISSN 1566-0621

Conserv Genet
DOI 10.1007/s10592-020-01280-8

Springer

Conservation Genetics
https://doi.org/10.1007/s10592-020-01280-8

RESEARCH ARTICLE

Population genomic diversity and structure at the discontinuous southern range of the Great Gray Owl in North America

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Received: 22 September 2019 / Accepted: 12 May 2020

Abstract

Species' distributions are often discontinuous near the edge of the range where the environment may be more variable than the core of the range. Range discontinuity can reduce or cut off gene flow to small peripheral populations and lead to genetic drift and subsequent loss of genetic diversity. The southern extent of the Great Gray Owl (*Strix nebulosa*) range in North America is discontinuous, unlike their northern core range across the boreal forests. We sampled owls from five different locations on the periphery of the range across the western US (Wyoming, Idaho, California, northern Oregon, and southern Oregon) to investigate genetic population structure and genetic diversity. Using a reduced-representation genomic sequencing approach to genotype 123 individuals at 4817 single nucleotide polymorphic loci, we identified four genetically differentiated populations: California, southern Oregon, northern Oregon, and Wyoming and Idaho grouped together as a single Rocky Mountain population. The four genetically differentiated populations of Great Gray Owls identified in this study display high differentiation and low genetic variation, which is suggestive of long-term isolation and lack of connectivity, potentially caused by range discontinuity. The populations that lack habitat connectivity to the rest of the breeding range (i.e. those in California and Oregon) had lower genetic diversity than the Rocky Mountain population that is connected to the core of the range. These factors and other risks (such as disease and human-caused mortality) heighten susceptibility of these range-edge populations to future habitat and climate changes, genetic diversity erosion, and potential extinction vortex. For these reasons, protecting and monitoring this species on the southern edge of their range is vital.

Keywords Conservation genomics · Nucleotide diversity · Population structure · Raptor · Single nucleotide polymorphism · *Strix nebulosa*

Introduction

Populations that exist on the periphery of the core of a species' range often exist in habitats with more variable biotic and abiotic factors (Kubisch et al. 2004), leading to distributions that are discontinuous near their edges (Saunders et al. 1991; Crooks et al. 2017). This separation from the core population can influence evolutionary processes such as gene flow, genetic drift, and natural selection, and can contribute to extinction vortices (Gilpin and Soulé 1986) for populations with discontinuous range-edge distributions. Gene flow between disconnected areas of a range can be obstructed by distance and lack of suitable connecting habitat between them. The resulting isolation can cause small population sizes and make genetic drift a dominant force in diminishing genetic diversity and creating genetically differentiated populations (Eckert et al. 2008; Athrey et al. 2012;

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10592-020-01280-8) contains supplementary material, which is available to authorized users.

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Published online: 31 May 2020

Tan et al. 2018). Low genetic diversity affects natural selection by reducing the potential for future adaptation (Robertson 1960; Johansson et al. 2007). Further, small isolated populations are more likely to experience inbreeding, which can result in inbreeding depression where the expression of harmful alleles decreases the fitness of individuals (Charlesworth and Willis 2009).

When faced with changes in their environment, species benefit from standing genetic diversity needed for adaptation, survival, and reproduction, particularly for populations at periphery of a range. In wild populations, the function of genetic variation in adaptive potential involves a complex combination of factors (Mills and Allendorf 1996). Some discontinuous populations can retain genetic diversity through mechanisms such as dispersal and de novo mutations, offsetting the negative genetic effects of being separated (Harrisson et al. 2012; Assis et al. 2013; Walsh et al. 2016; Bay et al. 2018). Local adaptations that occur near the limits of the species' habitat on the range-edge can be important to the overall adaptive potential of the species if the environment becomes more extreme throughout the range (Provan and Maggs 2012; Rehm et al. 2015; Bay et al. 2018). Especially when combined with other threats such as habitat destruction, climate change, or disease, decreased genetic diversity can contribute to extirpation of isolated populations (Frankham et al. 2017; Funk et al. 2018).

In the western United States, the Great Gray Owl (*Strix nebulosa*) has a discontinuous distribution on the southern extents of its range (Fig. 1) because they breed only in large stands of older-aged forests (Bull and Duncan 1993; Duncan and Hayward 1994; Wu et al. 2015). The core of their range lies in western Canada and extends south into Washington, Idaho, Montana, and Wyoming, becoming increasingly fragmented as the northern boreal forest transitions to montane forest and borders on sagebrush and desert in Oregon and California (Bull and Duncan 1993). Great Gray Owls are vagile raptors with the potential to disperse long distances, but the question of whether dispersal of individuals maintains gene flow between geographically separated areas remains largely unknown. Demographic data suggest that Great Gray Owl nest productivity has declined in some areas (Franklin 1988), however complementary genetic data describing population structure and evolutionary potential are lacking. A study employing microsatellite loci found genetic differentiation among owls in the Sierra Nevada, southern Oregon, northern Oregon, Idaho, and Alberta (Hull et al. 2010). Their findings led to the listing of *Strix nebulosa yosemitensis* as an endangered subspecies in California (Hull et al. 2014; California Department of Fish and Wildlife 2017). Low genetic diversity on the periphery of the range in California, northern Oregon, and southern Oregon compared to the core of the range in Alberta has also been documented (Hull et al. 2010).

Understanding population structure, diversity at genomic levels, and evolutionary potential are relevant to conservation status and management decisions about Great Gray Owls and their habitat. We generated genomic data for Great Gray Owls on the southern periphery of their breeding range in the western US. This study constitutes the first genomic work on Great Gray Owls and the first genetic analysis that includes Wyoming, which represents the southern extent of the species range in the Rocky Mountains and is impacted by habitat loss. Our goal was to extend genetic knowledge of the species by combining samples we collected from western Wyoming with samples collected by Hull et al. (2010) in central California, southern Oregon, northern Oregon, and eastern Idaho. We address two major questions in this study: (1) how are Great Gray Owls genetically structured across their discontinuous range edge? and (2) what are the levels of genome-wide genetic diversity across the southern range? We hypothesized that genetic diversity and population differentiation would be affected by range discontinuity. Given the distribution in the southern part of the Great Gray Owl breeding range (Fig. 1), we predicted that limited dispersal resulting from range discontinuity would cause higher differentiation and lower genetic diversity in the isolated parts of the range (i.e. California, northern Oregon, and southern Oregon) than parts of the range connected to the range core (i.e. Wyoming and Idaho).

Methods

Sampling and study area

We obtained 152 samples from five locations in western North America collected between 1992 and 2017 (Fig. 1, Appendix Table 4): southern Sierra Nevada Range in California (CA), southern Cascade Range in Oregon (ORS), Blue Mountain Range in northeastern Oregon (ORN), southeastern Idaho (west side of Teton Range) (ID), and northwest Wyoming (east side of Teton Range) (WY). Samples from western Wyoming were from owls either captured at nests sites as fledglings or associated with location data from telemetry from an ongoing study investigating their home range size and habitat selection (*pers. data)*. Blood was collected from the brachial vein and stored in Longmire's buffer solution (Longmire et al. 1997) or EDTA tubes and stored at −20 °C after transport. California, northern Oregon, southern Oregon, and Idaho samples are described in Hull et al. (2010).

Laboratory methods

DNA was extracted using Qiagen DNeasy Blood & Tissue kits (Qiagen, Valencia, CA, USA), with the following

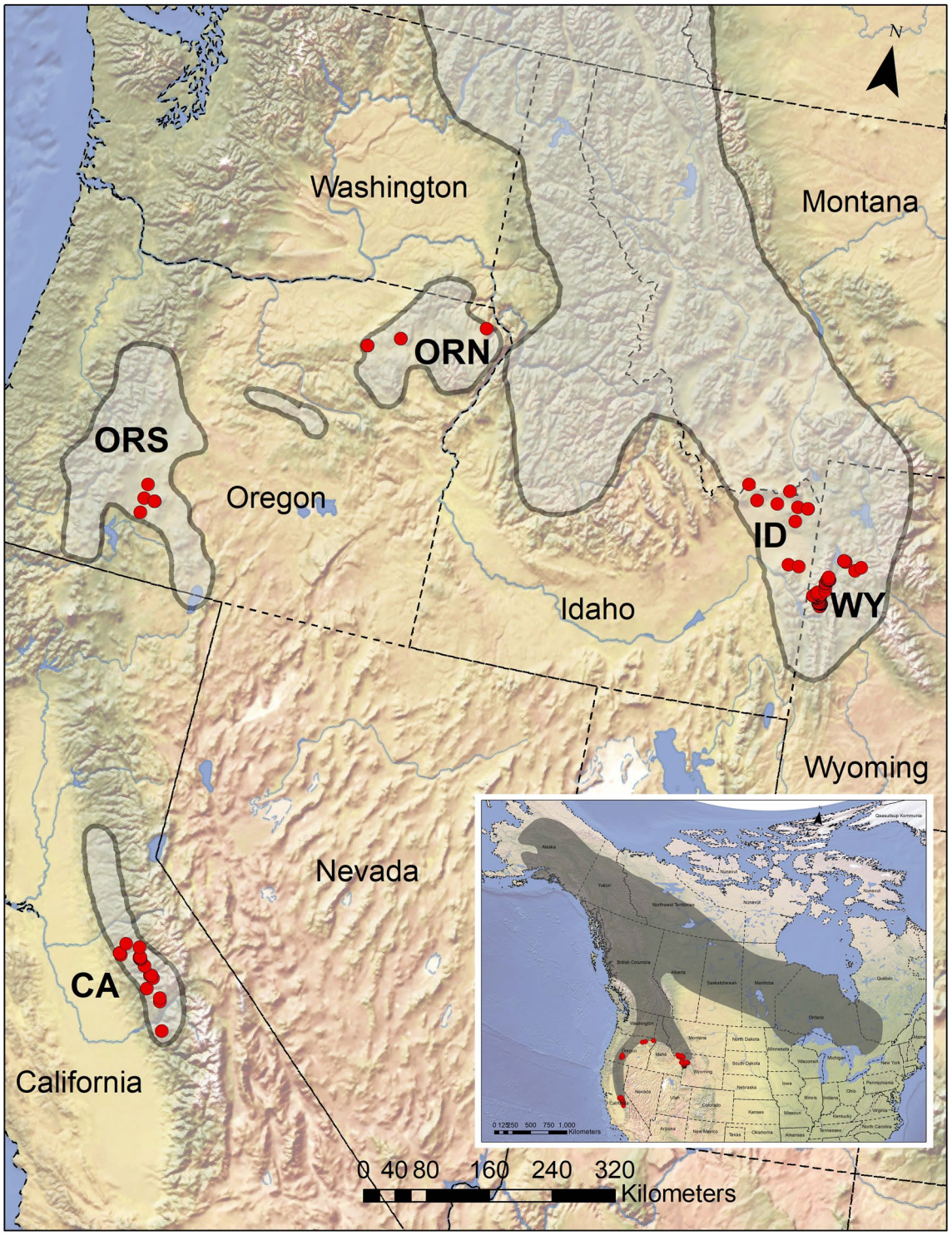

Fig. 1 Range map: The southern part of the Great Gray Owl breeding range in the western US. Dark gray outline delineates areas of documented breeding range in detail and does not include occurrences outside of the breeding season. The red points represent unique sampling locations, and the number of individuals sampled (by location) is California (CA) = 28, Idaho (ID) = 14, northern Oregon (ORN) = 8, southern Oregon (ORS) = 10, Wyoming (WY) = 92. The map was compiled from other studies and eBird observations (Franklin 1988; Bull and Duncan 1993; Sullivan et al. 2009; Thiemann and Fuller 2015; Wu et al. 2016). The inset map depicts the entirety of the Great Gray Owl North American range in dark gray (BirdLife International 2016), with our sampling locations in red

modifications for blood stored in Longmire's buffer: 12–36 h digestion times, 1–2 extra washes with AW1 and AW2 salt buffers, and doubled elution volumes. DNA was quantified using Qubit Fluorometer 3.0 (Invitrogen, Carlsbad, CA). To confirm adequate starting fragment sizes for high throughput (next generation) sequencing (NGS), we assessed a subset of DNA extracts across locations and years using gel electrophoresis. We found moderate degradation in some samples, likely from keeping samples at ambient temperatures for too long in the field. We normalized DNA to a target concentration range of 30–100 ng/µl by concentrating DNA with vacuum centrifugation in a SpeedVac Concentrator (Thermo Scientific, Waltham, MA) and ethanol precipitation (Sambrook and Russell 2006) or dilution in molecular grade water.

Reduced representation sequencing methods such as restriction-site associated DNA sequencing facilitate the identification of single nucleotide polymorphisms (SNPs) across the genome of many individuals for relatively low cost (Kraus and Wink 2015; Andrews et al. 2016; Toews et al. 2016b). We prepared two double digest restriction-site-associated DNA sequencing (ddRAD) sequencing libraries (pools of enriched DNA fragments ligated with sequencing adapters) according to Parchman et al. (2012). Briefly, we first digested the genomic DNA with two restriction enzymes—EcoRI and MseI (New England BioLabs, Ipswich, MA). Then we ligated Illumina sequencing adapters with overhangs matching the MseI and EcoRI cut sites (Sigma, St. Louis, MO) to the product. The EcoRI adapters included an 8–10 base pair barcode giving each sample a unique identifying sequence (minimum of 4 base difference between barcodes). These target fragments were amplified with Illumina PCR primers by polymerase chain reaction in duplicate reactions for each sample on thermal cyclers SimpliAmp (Applied Biosystems, Foster City, CA) with 30 cycles of 98 °C for 20 s, 60 °C for 30 s, 72 °C for 40 s, and a final extension of 72 °C for 10 min. We included an extra PCR step ensure double stranded DNA fragments (Parchman et al. 2012). Following PCR, all samples were pooled together. The pooled product was cleaned and concentrated with AMPure beads (Beckman Coulter, Brea, CA). We isolated DNA fragments between 350 and 450 bp with a Pippin Prep and 2% agarose gel (Sage Science PR00551, Beverly, MA) and verified size selection on Fragment Analyzer (Advanced Analytical, Santa Clara, CA). To increase sequencing coverage, we divided the samples between two sequencing libraries with replicates within each library and between libraries. The DNA libraries were sequenced in 2 lanes of 150 bp single-end reads on an Illumina HiSeq 4000 at The University of Texas at Austin Genomic Sequencing and Analysis Facility.

Quality filtering, variant calling and genotyping

We filtered the raw sequence data and discarded potential contaminants (*Escherichia coli*, *PhiX* control, adapter and primer dimers) using Bowtie2 (Langmead and Salzberg 2012). We used Trimmomatic (Bolger et al. 2014) to trim bases when the Phred quality score fell below 20 (99% accuracy) within a 4 bp sliding window and at the ends of reads (Illumina Inc 2000).

To demultiplex pooled libraries into individual samples by unique barcodes, we used a custom Perl script (DRYAD https://doi.org/10.5061/dryad.1rn8pk0qm). This script allows and corrects for one mismatch in the barcode and removes the adapter sequences from the reads, leaving only genomic DNA sequences. We then concatenated sequences from intra- and inter-library replicates for unique individuals (DRYAD https://doi.org/10.5061/dryad.1rn8pk0qm).

Since a reference whole genome for the Great Gray Owl was not available, we assembled sequences de novo (Willing et al. 2011). To do so, we created a synthetic reference from our data using the cd-hit-est package in CD-HIT to cluster sequences, as this has been shown to be the most accurate approach for de novo assembly of double digest genomic sequencing (Li and Godzik 2006; Fu et al. 2012; LaCava et al. 2019). We used the sequences from all individuals and a 0.95 sequence identity threshold, resulting in 1,099,773 unique contigs (DRYAD https://doi.org/10.5061/dryad.1rn8pk0qm). We verified our de novo reference by alignment to the Barn Owl (*Tyto alba*) genome using Bowtie2, which had an average 59% alignment rate. We removed 29 individuals with fewer than 400,000 reads, leaving 216,736,964 total reads across 123 individuals.

Reads from remaining individuals were mapped to the de novo reference using the BWA MEM algorithm (Li and Durbin 2009). The average assembly rate per individual was 97% and a total of 211,644,065 reads aligned. We filtered for sites with minimum base and mapping quality scores (Q-score) of 20, kept a max-depth of 100 reads per site per individual, and omitted insertions and deletions with SAMtools mpileup (Li 2011). We called biallelic SNPs with a Q-score of 20 or higher using BCFtools (Danecek et al. 2016) resulting in 222,753 sites. These sites were thinned using VCFtools (Danecek et al. 2011) to one SNP per 136 bp sequence read. In addition, we removed SNPs with a minor allele frequency less than 0.05, missing data in more than 25% of individuals, or a minimum read depth per site per individual less than 3. We converted the variant calls to genotype likelihoods and genotype point estimates (weighted average) using custom Perl scripts (DRYAD https://doi.org/10.5061/dryad.1rn8pk0qm). We checked for private SNPs in each library with PopGenReport package in R (Adamack and Gruber 2014; Gruber and Adamack 2015).

Population structure analysis

Unless otherwise specified, we did not remove known related individuals from analyses, as they are a natural component of populations (Waples and Anderson 2017; Hendricks et al. 2018). To visualize potential genetic clusters, we performed Principal Component Analysis (PCA) in custom R code (R Core Team 2017) using a genetic covariance matrix calculated from the point estimates of individual's genotypes (DRYAD https://doi.org/10.5061/dryad.1rn8pk0qm). We plotted the principal component vectors (PCs) that accounted for the majority of variance between individuals. For all PCAs, we used a subset of 78 putatively unrelated individuals after removing 45 Wyoming individuals identified as full- or half-siblings or parent–offspring from the field data, to prevent skewing the axes with first-degree relatives and to even out sample sizes among locations (McVean 2009). California, northern Oregon, southern Oregon, and Idaho samples did not contain any known relatives. To assign individuals to genetic clusters, we used STRUCTURE 2.3.4 (Pritchard et al. 2000). We parallelized the runs by implementing STRUCTURE using StrAuto (Chhatre and Emerson 2017) on the University of Wyoming Teton Computing Environment Intel x86_64 cluster (Advanced Research Computing Center 2018). Our model included the options for admixture and correlated allele frequencies. For each value of K = 1–8 (possible number of clusters), we ran 20 independent iterations with a burn-in of 100,000 steps, followed by a run of 1,000,000 Markov chain Monte Carlo (MCMC) steps to verify consistency and convergence of model parameters. We used STRUCTURE HARVESTER 0.6.94 (Earl and vonHoldt 2012) and Clumpak (Kopelman et al. 2015) to compile and visualize results. To infer the most likely number of genetic clusters, we used delta K (ΔK) (Evanno et al. 2005) and the probability of K (Pritchard et al. 2000). To plot results, we modified the function structurePlot from the StrataG package in R 2.0.2 (Archer et al. 2017).

We explored isolation-by-distance by calculating the geographic distance between all pairs of putatively unrelated samples (78) in FOSSIL in R (Vavrek 2011) and plotting this against the genetic covariance matrix created for the PCA and tested the Pearson's correlation using a Mantel test. We calculated population-specific Hudson's F_{ST} (Hudson et al. 1992) in R using a custom function for all pairs of sampling locations and for all loci (DRYAD https://doi.org/10.5061/dryad.1rn8pk0qm). This method for calculating F_{ST} measures the proportion of genetic divergence between subpopulations due to isolation and is highly correlated Weir and Cockerham's and Nei's F_{ST} and provides a reliable estimate of differentiation with uneven sample sizes (Bhatia et al. 2013).

Genetic diversity analyses

We used theta (θ) to estimate genome-wide nucleotide diversity. Theta estimates the amount of neutral variation at loci assuming an idealized Wright-Fisher population, and can be interpreted as the average number of mutations in the whole population per site per generation (Hahn 2018). Watterson's theta (θ_w) (Watterson 1975) and theta pi (θ_π) (Tajima 1989; Korneliussen et al. 2013) were calculated in ANGSD (Korneliussen et al. 2014). θ_π is defined as the average number of heterozygous sites for pairwise combinations of sequences, whereas θ_w uses the total number of heterozygous sites adjusted to the sample size (Hahn 2018). ANGSD uses a probabilistic approach to compute genotype likelihoods, the probability of the data given the genotype (Korneliussen et al. 2014), to account for uncertainty, and handles missing and low read data (Durvasula et al. 2016). We input nucleotide sequences from all 152 individuals from 5 locations that were sequenced, selecting for reads with mapping and base quality scores above 20.

Results

Quality filtering, variant calling, and genotyping

Our final dataset contained 4,817 biallelic SNPs (DRYAD https://doi.org/10.5061/dryad.1rn8pk0qm). After filtering, we discarded 29 samples from the SNP dataset, leaving 123 individuals from 5 locations (CA = 22, ORN = 8, ORS = 9, ID = 11, WY = 73). All individuals had genotype data at > 50% of SNPs, and all SNPs had data in > 75% of individuals. Average depth of coverage per SNP per individual was 12.3. After minor allele filtering, we found 69 total private alleles, 51 in California and 18 in Wyoming. Polymorphism was lowest in northern Oregon (71% of SNPs), followed by southern Oregon (76%), California (78%), Idaho (82%), and Wyoming had the highest polymorphism at 92%. Almost all loci were shared between libraries, with only 1 private SNP in library 1, and 7 in library 2.

Population structure

Principal components analysis of 78 owls among five different sampling locations suggested four genetic clusters (Fig. 2). Samples from Idaho and Wyoming formed one cluster, samples from California formed a second distinct cluster, while northern Oregon and southern Oregon formed the third and fourth clusters (northern and southern Oregon had no overlap on the first PC axis). The first axis explained the majority of the variation (70.4%) among genetic clusters. A separate PCA of individuals from Wyoming and Idaho did not provide evidence of substructure (Appendix Fig. 1 in Supplementary

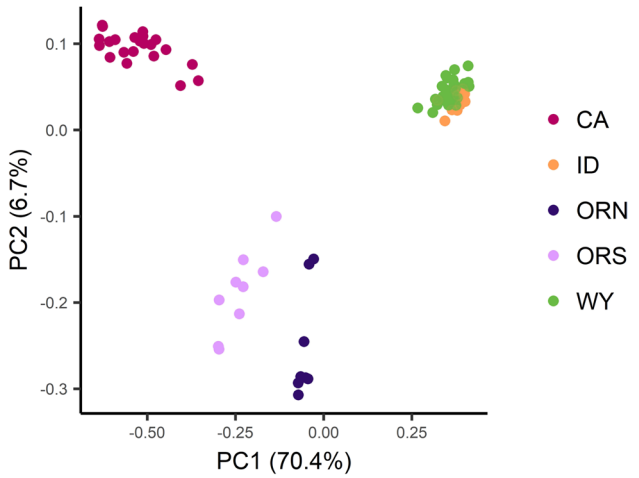

Fig. 2 Principal component analysis: PCA showing samples clustering into 4 separate groups based on genotype point estimates at 4817 SNPs. 78 individuals from 5 sampling locations: California (CA) n = 22, Idaho (ID) n = 11, northern Oregon (ORN) n = 8, southern Oregon (ORS) n = 9, Wyoming (WY) n = 28. The first 2 PC axes explain 77% of the variation between individuals. Subsequent PC axes accounted for little further difference (PC3 = 2.6%, PC4 = 0.7%, PC5 = 0.7%). The clusters correspond to geographical location of the samples

Materials). Despite the 30-year interval between sampling in Wyoming and Idaho, we did not detect differentiation between the locations that would have indicated genetic drift occurred over that time span. Great Gray Owls have a longevity of 13–30 years and generally do not breed until their third or fourth year of life, so we did not expect the sampling gap to be a problem (Bull and Duncan 1993).

STRUCTURE analysis best fit individuals into 3 genetic groups (K = 3): California, northern/southern Oregon, and Wyoming/Idaho (Fig. 3a, Appendix Fig. 2a in Supplementary Materials). We additionally implemented a hierarchical analysis, since the ΔK method can miss fine substructure (Evanno et al. 2005), and uneven small sample sizes combined with strong structure between California and Wyoming could have been masking substructure (Puechmaille 2016). Also, at K = 4, the model had minor modes, a further indication for hierarchical population structure. We ran STRUCTURE again on (1) a subset of only individuals from northern Oregon, southern Oregon, and California (Fig. 3b), and (2) a subset with only individuals from northern Oregon and southern Oregon (Fig. 3c). For northern Oregon, southern Oregon, and California together, Δ K showed K = 2 as optimal, while the probability of K was highest at K = 3, suggesting more substructure (Appendix Fig. 2b in Supplementary Materials). Further analysis of northern and

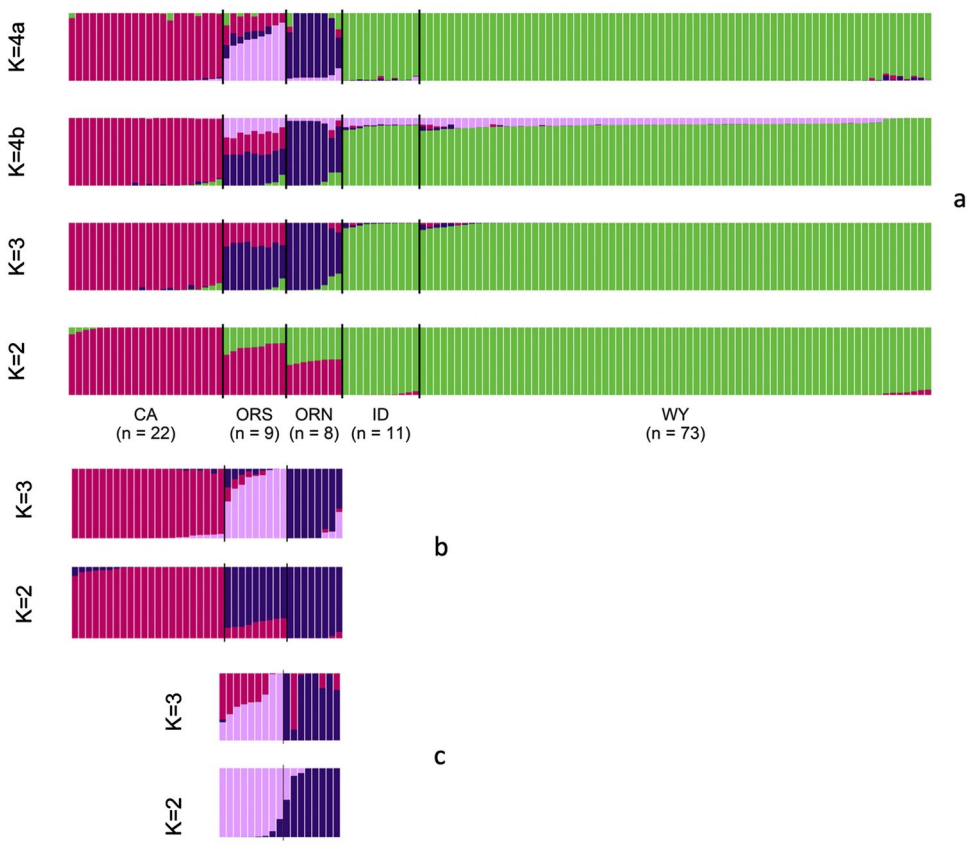

Fig. 3 Hierarchical STRUCTURE analysis. Each vertical bar represents an individual owl, organized by sampling location: California (CA), southern Oregon (ORS), northern Oregon (ORN), Idaho, (ID) and Wyoming (WY). Colors represent group membership. The proportion of each color in a bar represents the probability that the individual came from each of the groups. **a** STRUCTURE best fits all 123 individuals into 3 genetic clusters (K = 3). Results for K = 2 to 4 are shown, with K = 4a for the first minor mode (7/20 runs) and K = 4b the major mode (10/20 runs) (3/30 runs were a second minor mode, not shown). **b** Subset of individuals from California, northern Oregon, and southern Oregon cluster best into 2 or 3 genetic groups (K = 2 or K = 3). **c** Subset of individuals from northern Oregon and southern Oregon cluster best into 2 genetic groups (K = 2), strongly delineated by sampling location. K = 3 also had high probability

Table 1 Pairwise comparisons of genetic differentiation of Great Gray Owls (*Strix nebulosa*) between 5 sampling locations measured by Hudson's F_{ST}: California (CA) n = 22, northern Oregon (ORN) n = 8, southern Oregon (ORS) n = 9, Idaho (ID) n = 11, Wyoming (WY) n = 73. All pairs of localities except ID and WY are highly differentiated

	CA	ORN	ORS	ID	WY
CA	–	0.14	0.11	0.16	0.15
ORN	–	–	0.12	0.13	0.12
ORS	–	–	–	0.13	0.13
ID	–	–	–	–	0.032
WY	–	–	–	–	–

southern Oregon revealed distinct genetic structure between them (Fig. 3c), best assigning individuals to two clusters (Appendix Fig. 2c in Supplementary Materials). Visually, the models appear to differentiate between northern and southern Oregon individuals. STRUCTURE does not distinguish between discrete or continuous allele frequencies but shows that southern Oregon has intermediate allele frequencies to northern Oregon and California.

Population differentiation measured by pairwise F_{ST} was greatest between California and Idaho (0.16) (Table 1). F_{ST} between Idaho and Wyoming was an order of magnitude lower than between any other locations (0.032), indicating that these two locations were substantially less differentiated. Northern and southern Oregon had an F_{ST} of 0.12, indicating high differentiation between these two locations. An incremental change in F_{ST} was observed that loosely reflected the distance between the locations: lower values correspond to locations that are closer together geographically, and values increase as distance increases.

We found a significant negative correlation between geographic distance and genetic covariance using all pairwise combinations of 78 putatively unrelated individuals across 5 locations (Mantel test, r = − 0.91) (Fig. 4).

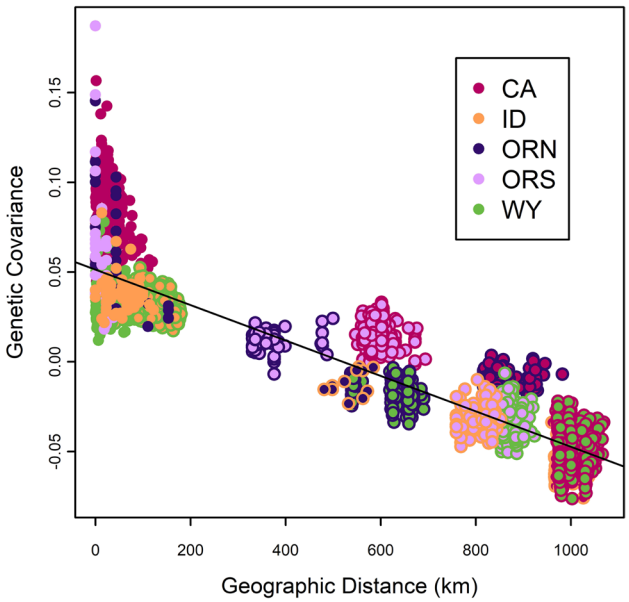

Fig. 4 Isolation-by-distance: Genetic similarity decreases as genetic distance increases. The black line represents negative correlation between geographic distance (km) and genetic covariance. Points represent pairwise comparisons between 78 individuals across 5 locations: California (CA), Idaho (ID), northern Oregon (ORN), southern Oregon (ORS), and Wyoming (WY). Colors correspond to origin of samples within each pair. Pairwise comparisons between samples from different origins are depicted by dual-colored points with one color from each location. Single color points (near distance of zero) are within-location pairs

Genetic diversity

Diversity statistics are reported in Table 2. Mean nucleotide diversity for each location was between 0.31×10^{-3} and 0.48×10^{-3} for Watterson's theta (θ_w) and between 0.40×10^{-3} and 0.43×10^{-3} for theta pi (θ_π) (Table 2). Wyoming had the highest nucleotide diversity for both metrics, while the four other locations had consistently lower values. Further, the locations separated from the rest of the range (California, northern Oregon, and southern Oregon) had a higher mean genetic covariance and higher variance around the mean than Idaho and Wyoming (Levene's test, F-value = 67.7, DF = 5, $p < 2.2 \times 10^{-16}$) (Fig. 5). 55% of loci were fixed in at least one sampling location. 76 SNPs were polymorphic in only one sampling location: 53 in California and 23 in Wyoming.

Table 2 Genetic diversity values for the Great Gray Owl (*Strix nebulosa*) sampled in 5 locations: California (CA), northern Oregon (ORN), southern Oregon (ORS), Idaho (ID), and Wyoming (WY). Wyoming has higher nucleotide diversity, measured by Watterson's theta (θ_w) and theta pi (θ_π), and lower mean covariance (i.e. genetic similarity) than other locations. Sample size (N) of θ includes all individual's nucleotide sequences, whereas *N* for covariance includes individuals after variant filtering. Mean covariance is reported with standard deviation

	N	θ_w ($\times 10^{-3}$)	θ_π ($\times 10^{-3}$)	*N*	Covariance
CA	28	0.34	0.41	22	0.077 ± .021
ORN	8	0.33	0.41	8	0.066 ± .033
ORS	10	0.31	0.40	9	0.060 ± .035
ID	14	0.31	0.40	11	0.040 ± .010
WY	92	0.48	0.43	28	0.036 ± .008

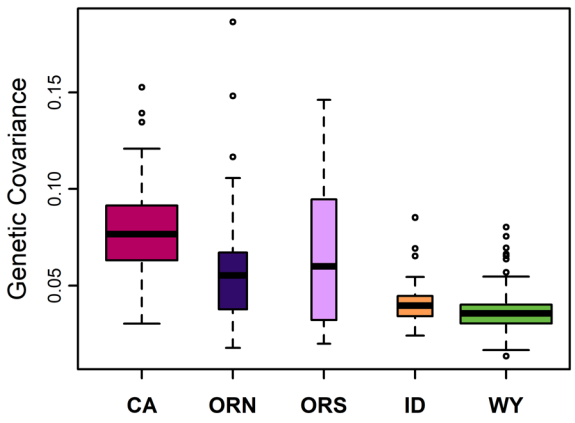

Fig. 5 Genetic covariance boxplot: Different locations differ significantly in genetic covariance (i.e., genetic similarity, measured by calculating the Pearson's covariance of the difference in each individual's genotype from mean genotype at each locus. Fragmented populations (California (CA), northern Oregon (ORN), southern Oregon (ORS)) have both higher mean covariance and variance than Wyoming (WY) and Idaho (ID). Black horizontal bars represent the median, boxes represent the interquartile range, the width of boxes scales to sample size, whiskers are the maximum and minimum, and circles are potential outliers

Discussion

Population structure

Genomic analysis along the southern edge of their range in western North America revealed that Great Gray Owls are not panmictic across their range, but rather occur in isolated populations. Our evidence shows three main genetic clusters of California, Wyoming/Idaho, and Oregon, with substructure in the Oregon cluster corresponding with north and south sampling locations. Thus, we propose four genetic populations: California, Wyoming/Idaho, northern Oregon, and southern Oregon. Wyoming and Idaho appear to be part of a single interbreeding population. Northern and southern Oregon are substantially delineated into separate genetic groups, with more differentiation than between California and southern Oregon, and almost as differentiated as California is to Wyoming/Idaho, our two most distinct populations (Table 1). Southern Oregon has allele frequencies intermediate between California and northern Oregon, as supported by its geographic location (Fig. 1) and the pattern of isolation-by-distance (Fig. 4). Breeding of Great Grey Owls in central Oregon has been documented in the Ochoco and Malheur National Forests (Thiemann and Fuller 2015) (Fig. 1). This region is geographically located between our northern and southern Oregon sites and may provide opportunities for gene flow, however samples from Central Oregon were not available for our study.

The California population has been proposed as a separate subspecies based on genetic, morphological and habitat data (Hull et al. 2014). We provide further genomic evidence supporting the distinctiveness of Sierra Nevada subspecies *Strix nebulosa yosemitensis* in California. The sub-structuring we identified between northern and southern Oregon suggests these Great Gray Owls may also qualify as distinct subspecies, although additional morphological and habitat analysis will be needed to support this suggestion. In comparison to other bird populations that are considered separate species and subspecies, F_{ST} values between Great Gray Owl populations are high (Table 3). Although we observed that genetic differentiation was higher with greater geographic distance between individuals, the absence of breeding pairs in the expanses between the four populations indicates there may be environmental

Table 3 F_{ST} for various bird populations calculated from SNP data

Population 1	Population 2	F_{ST}	Classification
Great Gray Owls in California	Great Gray Owls in Wyoming	0.15	Populations (proposed)
Great Gray Owls in northern Oregon	Great Gray Owls in southern Oregon	0.12	Populations (proposed)
Great Gray Owls in Idaho	Great Gray Owls in Wyoming	0.03	Same population (proposed)
Red-shafted flicker	Yellow-shafted flicker	0.02	Subspecies
Red-shafted flicker	Gilded flicker	0.12	Species
Greater Sage-Grouse	Gunnison Sage-Grouse	0.49	Species
Greater Sage-Grouse	Bi-state Sage Grouse	0.09	Possible subspecies
Yellow-rumped warbler	Non-hybridizing Yellow-rumped warbler taxon	0.08–0.26	Species
Yellow-rumped warbler	Hybridizing Yellow-rumped warbler taxon	0.02–0.06	Hybridizing species
Northern Spotted owl	California Spotted owl	0.06	Subspecies

Red-shafted (*Colaptes auratus cafer*), Yellow-shafted flickers (*C. a. auratus*) and Gilded flickers (*C. chrysoides*) from Aguillon et al. (2018), Gunnison Sage-Grouse (*Centrocercus minimus*), Greater Sage-Grouse (*Centrocercus urophasianus*) and Bi-state Sage-Grouse from Oyler-McCance et al. (2015), Yellow-rumped warbler (*Setophaga* spp.) from Toews et al. (2016a). Microsatellite analysis of the Northern Spotted (*Strix occidentalis caurina*) and California Spotted (*S. o. occidentalis*) owls from Miller et al. (2017) (recognizing that microsatellites are more variable and have higher mutation rates than SNPs, which could manifest as higher F_{ST})

obstacles causing the discontinuous shape of the range and preventing dispersal, rather than a continuous gradient of isolation-by-distance where distance is the only factor preventing panmixia. The degree of differentiation further indicates that it is likely caused by long-term isolation, possibly as far back as glacial refugia, and exacerbated by the effects of genetic drift.

Limited dispersal caused by the discontinuous distribution of high-quality breeding habitat in the American West may be contributing to isolation of the populations. The genomic evidence from our study shows that dispersal across range gaps is rare in Great Gray Owls, as gene flow is insufficient to remove signatures of genetic differentiation. Successful long-distance dispersal would likely have created connectivity between populations, as one successful dispersal per generation can prevent population differentiation and loss of genetic diversity (Spieth 1974; Wang 2004). Telemetry studies of Great Gray Owls have been limited in their ability to detect long-distance dispersal, although breeding dispersal distances of 450–700 km have been documented in Canada and Alaska across contiguous habitat (Duncan 1992; Nero and Copland 1997), while less than 30 km in Oregon have been documented (Bull et al. 1988; Bull and Henjum 1990). In contrast, barn owls, which have a continuous range across North America and have been reported to disperse up to 160 km (natal dispersal) (Marti 1999), have little genetic structure across their entire North American range (Huang et al. 2016). Similarly, Northern spotted owls in Oregon and Washington have been reported to disperse up to 111 km (Forsman et al. 2002) and show no significant genetic differentiation (Haig et al. 2004), whereas regional groups of fragmented Mexican spotted owl populations show significant genetic differentiation (Haig et al. 2004).

Genetic diversity

Great Gray Owl genetic diversity was lower in more isolated populations (California, northern Oregon, and southern Oregon) than in connected parts of the range (Wyoming and Idaho). Wyoming showed higher genetic diversity than any of the other locations and is on a peninsular part of the range that likely has more gene flow through connectivity to the more genetically diverse core of the range in Canada (Hull et al. 2010). Wyoming also has a larger estimated breeding population than California or Oregon, making it less susceptible to the effects of genetic drift that lead to diversity loss in small populations. The lower genetic diversity in California, northern Oregon, and southern Oregon is also reflected in higher individual genetic covariance, meaning individuals are genetically similar to one another, as would be expected in isolated populations with small population sizes (Fig. 5). Idaho showed low nucleotide diversity comparable to the isolated populations, but covariance similar to Wyoming, which could suggest that Idaho has a smaller breeding population than Wyoming or less connectivity to the core of the range. The high levels of F_{ST} between all populations also reflect low genetic diversity within populations, caused by isolation and genetic drift (Meirmans 2006).

Great Gray Owls in the western US have low levels of genetic diversity overall compared to some other avian species (Fig. 6). Avian genome diversity is commonly on the order of 10^{-3} to $\times 10^{-2}$, and many species reported θ_π from 1.0 to 5.0×10^{-3} (Ellegren 2013). In comparison, θ_π for Great Gray owls was an order of magnitude lower at 0.4×10^{-3}. Although genetic diversity alone does not necessarily represent adaptive variation, it does provide a metric for assessing adaptive potential in stressful environments (Funk et al. 2018).

Using genomic markers increases the resolution and accuracy of genetic diversity and population structure analyses (Narum et al. 2013; Benestan et al. 2016; Oyler-McCance et al. 2016). Measures of genetic diversity from genomic data provide more accurate estimates of genome-wide diversity than measures of heterozygosity that are commonly reported from microsatellite analyses (Fischer et al. 2017). We recommend that researchers using genomic sequence data report θ for genetic diversity statistics to make comparisons across species more meaningful. Although nucleotide diversity can

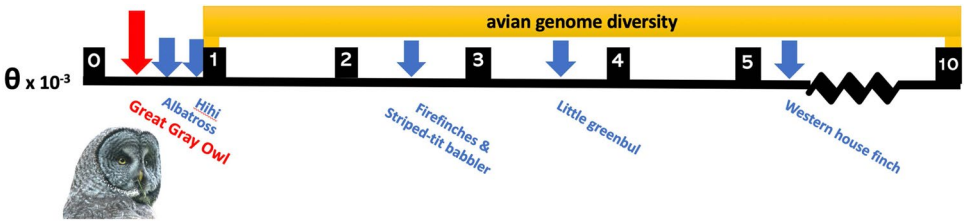

Fig. 6 Genetic diversity scale: Great Gray Owl nucleotide diversity (θ_π) is low compared to other avian species that reported nucleotide diversity and used similar methods: Black-footed Albatross (*Phoebastria nigripes*) $\pi = 0.0006$–0.00065 from ddRAD (Dierickx et al. 2015), hihi (*Notiomystis cincta*) $\pi = 0.00095$ from RADseq (de Villemereuil et al. 2019), Striped-Tit Babbler (*Mixornis gularis*) $\pi = 0.0025$ from ddRAD (Tan et al. 2018), Firefinches (*Lagonosticta*) $\pi = 0.0025$ from ddRAD (DaCosta and Sorenson 2016), little greenbul (*Andropadus virens*) $\pi = 0.0036$ from RADseq (Zhen et al. 2017), western House Finch (*Haemorhous mexicanus*) $\pi = 0.0050$–0.0054 from ddRAD (Shultz et al. 2016). Owl photo credit: B.M

vary across the genome (Dutoit et al. 2016., Ellegren 2013), making it possible that reduced-representation methods introduce an ascertainment bias by sub-sampling the genome (Arnold et al. 2013), our sequence data represents approximately 12% of the genome analyzed, which is a greater portion than can be analyzed using microsatellites.

Conservation

Breeding raptors are generally considered good indicators of biodiversity and environmental change (Burgas et al. 2014; Ibarra and Martin 2015), and populations of the Great Gray Owl identified through genetic data that warrant conservation could serve as a flagship species to help safeguard other less well-known species of the southern boreal forest. Threats to Great Gray Owl breeding range stemming from climate change (Siegel et al. 2014; National Audubon Society 2015; Schuetz et al. 2015) include increases in stand replacing fires (Westerling et al. 2003), insect outbreaks (Bentz et al. 2010), and changes in snowpack and prey availability (Mysterud 2016). As forecast warming temperatures cause potentially substantial range contractions and habitat loss for species such as the Great Gray Owl, northern, high elevation forest birds' distributions will likely move northward in latitude and upwards in elevation (Hitch and Leberg 2007; Langham et al. 2015; Siegel et al. 2014). These range shifts could signify the loss of important local adaptations and genetic diversity harbored in genetically distinct range-edge populations and decrease the evolutionary potential of species (Ralston and Kirchman 2013; Rehm et al. 2015).

Understanding population structure, genetic diversity, and evolutionary potential are relevant to conservation status and management decision making for Great Gray Owls and their habitat. Despite the expectation that Great Gray Owls could disperse across range gaps, our results indicate that the discontinuous Great Gray Owl range in the western US is also genetically disparate. The peripheral areas assessed here are effectively functioning as separate populations and should be managed and protected accordingly. The genetic differentiation of these populations highlights that even species capable of dispersal over long distances can become isolated and have low genetic diversity. Our finding of higher genetic diversity in the population with more suitable breeding habitat connecting it to the core of the range indicates that preserving those breeding grounds could help prevent low genetic diversity. Genetically isolated populations are at risk for further genetic diversity loss due to genetic drift or stochastic events, making them more susceptible to rapid environmental changes. Continued monitoring of these populations and preservation of existing connectivity and breeding habitat will contribute to the persistence and overall evolutionary potential of the Great Gray Owl across their circumboreal range.

Acknowledgements Financial support was provided by the Wyoming Game and Fish Department State Wildlife Grant, Meg and Bert Raynes Wildlife Fund, Wyoming Wildlife Foundation, Raptor Research Foundation Dean Amadon Grant, University of Wyoming Department of Veterinary Science, University of Wyoming College of Agriculture and Barbara and Richard Powell. The authors thank C.A. Buerkle for providing all custom R and Perl scripts, along with invaluable guidance on the molecular, analytical and theoretical aspects of this work. We thank C. Wagner, K. Gustafson, and V. Chhatre for theoretical and data analysis support. We thank Teton Raptor Center, K. Gura, B. Tanner, D. Holt, Wild Skies Raptor Center, Owl Research Institute, University of Wyoming Museum of Vertebrates, R.P. Gerhardt of Sage Science, USGS Bird Banding Laboratory, the USDA Forest Service, Yosemite National Park, US Bureau of Land Management Medford District, Wyoming Game and Fish Department, Grand Teton National Park, Bridger Teton National Forest, Craighead Beringia South, Calgary Wildlife Rehabilitation Society, California Department of Fish and Game, Lindsay Wildlife Hospital, UC Davis Raptor Center, UC Davis Veterinary Teaching Hospital, UC. Davis Museum of Wildlife and Fish Biology, J.J. Keane, W.K. Savage, S.A. Godwin, J.A Shafer, E.P. Jepsen, C. Stermer, N. Anderson, E. Bull, R. Byrnes, J. Duncan, A. Engilis, W. Farrier, C. Gallagher, D. Gerhardt, P. Krueger, J. Maurer, J. Medley, S. Patla, B. Stedman, S. Stock, L. Tierney, and S. Thompson for collecting and donating samples.

Author contributions B.M. and H.B.E. conceived the idea; B.M. and B.B. collected the data and performed experiments; H.B.E. supervised the research; B.M, B.B., S.M.L.S. and H.B.E. developed or designed methods; B.M., R.B.G, M.E.F.L and B.L.G. analyzed the data; B.M. wrote the paper; S.M.L.S., R.B.G. and H.B.E. substantially edited the paper; J.M.H., B.B., and H.B.E. contributed substantial materials and resources.

Data availability The datasets generated during and/or analyzed during the current study are available in Dryad: https://doi.org/10.5061/dryad.1rn8pk0qm.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures for Wyoming samples conformed to the protocols as approved by the National Park Service IACUC committee. Permit # IMR_GRTE_Bedrosian_GreatGreyOwl_2017.A3.

Appendix

See Table 4.

Table 4 Sample information for Great Gray Owls (*Strix nebulosa*) including the geographic region of the sampling location (**Location**), the number of samples (**N**), the collection period (**Year**), a more specific description of the location (**Location description**), a brief description of the forest (**Forest type**), and the range of elevation for the sampling location (**Elevation**)

Location	N	Year	Location description	Forest type	Elevation (m)
(WY) Western Wyoming	92	2013–2016	Bridger-Teton National Forest and Grand Teton National Park	Mixed conifer and aspen montane forest	1850–2350
(ID) Eastern Idaho	14	1992	Targhee National Forest	Lodgepole and aspen forest (Franklin 1988; Groves and Zehntner 1990)	1800–2200
(ORS) Southern Oregon	10	1994–1999	Winema National Forest	Mature mixed conifer, mainly Douglas fir (Goggans and Platt 1992)	600–1350
(CA) Central California	28	2005–2007	Stanislaus National Forest and Yosemite National Park	Mixed conifer with oak (Wu et al. 2015)	690–2420
(ORN) Northern Oregon	8	1992–1993	Umatilla National Forest and Wallowa-Whitman National Forest	Conifer forest (pine, fir, larch) with meadows and grasslands (Quintana et al. 2004)	930–1500

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