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Range-wide genetic differentiation among North American great gray owls (*Strix nebulosa*) reveals a distinct lineage restricted to the Sierra Nevada, California

Joshua M. Hull [a,b,*], John J. Keane [c], Wesley K. Savage [d], Steven A. Godwin [e], Jo Ann Shafer [f], Eric P. Jepsen [c], Rick Gerhardt [g], Chris Stermer [h], Holly B. Ernest [a,i]

[a] *Wildlife and Ecology Unit, Veterinary Genetics Laboratory, 258 CCAH, University of California, One Shields Avenue, Davis, CA 95616, USA*
[b] *Genomic Variation Laboratory, Department of Animal Science, University of California, Davis, CA 95616, USA*
[c] *United States Department of Agriculture, Forest Service, Pacific Southwest Research Station, Sierra Nevada Research Center, 1731 Research Park Drive, Davis, CA 95618, USA*
[d] *Department of Biological Sciences, Lehigh University, Bethlehem, PA 18015-4732, USA*
[e] *Ashland Resource Area, Medford BLM District, 3040 Biddle Road, Medford, OR 97504, USA*
[f] *P.O. Box 3415 Ashland, OR 97520, USA*
[g] *Sage Science, Inc. 319 SE Woodside Ct., Madras, OR 97741, USA*
[h] *California Department of Fish and Game, Wildlife Branch, 1812 Ninth Street, Sacramento, CA 95814, USA*
[i] *Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, One Shields Avenue/Old Davis Road, Davis, CA 95616, USA*

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ABSTRACT

Investigations of regional genetic differentiation are essential for describing phylogeographic patterns and informing management efforts for species of conservation concern. In this context, we investigated genetic diversity and evolutionary relationships among great gray owl (*Strix nebulosa*) populations in western North America, which includes an allopatric range in the southern Sierra Nevada in California. Based on a total dataset consisting of 30 nuclear microsatellite DNA loci and 1938-base pairs of mitochondrial DNA, we found that Pacific Northwest sampling groups were recovered by frequency and Bayesian analyses of microsatellite data and each population sampled, except for western Canada, showed evidence of recent population bottlenecks and low effective sizes. Bayesian and maximum likelihood phylogenetic analyses of sequence data indicated that the allopatric Sierra Nevada population is also a distinct lineage with respect to the larger species range in North America; we suggest a subspecies designation for this lineage should be considered (*Strix nebulosa yosemitensis*). Our study underscores the importance of phylogeographic studies for identifying lineages of conservation concern, as well as the important role of Pleistocene glaciation events in driving genetic differentiation of avian fauna.

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1. Introduction

Describing phylogeographic patterns is fundamental to understanding evolutionary processes and informing conservation efforts (e.g., O'Brien, 1994; Avise, 2004; Haig et al., 2004). While phylogeography is primarily a descriptive tool, important conservation insights can be revealed by estimating the relative phylogenetic depth of population divergence, and asking whether geographic populations are genetically distinct lineages, or subpopulations of larger regional genetic groups. Among wide-ranging avian taxa, broad-scale phylogeographic structure is often a result of past climatic and geological events (Avise and Walker, 1998), while fine-scale population differentiation is generally attributed to regional habitat specialization and local adaptation (e.g., Cicero and Johnson, 1998; Cicero, 2004; Hull et al., 2008a). In addition to describing phylogeographic relationships, population-level genetic analyses can aid in ascertaining the recent factors contributing to contemporary patterns of genetic diversity (e.g., estimating rates of recent gene flow) and provide the natural context for informed management approaches that seek to ameliorate further losses of diversity (Funk et al., in press).

The great gray owl (*Strix nebulosa*) is a large-bodied raptor species that ranges throughout Holarctic boreal forests and southward through several montane coniferous forests in Asia and North America (Bull and Duncan, 1993). Two subspecies are currently recognized based on plumage differences and distinct, non-overlapping distributions: *S. nebulosa nebulosa* in North America, and *S. n. lapponica* in Europe and Asia (Bull and Duncan, 1993). The breeding range of great gray owls in North America encompasses the boreal zones of Canada and Alaska, and extends southward into the Rocky Mountains and Cascade Range of the United States. The

* Corresponding author. Address: University of California, Wildlife and Ecology Unit, Veterinary Genetics Laboratory, 258 CCAH, One Shields Avenue, Davis, CA 95616, USA.
E-mail address: jmhull@ucdavis.edu (J.M. Hull).

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Klamath Basin, an important phylogeographic barrier identified in studies of plant and animal taxa (Soltis et al., 1997; Haig et al., 2004), separates the wider range of great gray owls to the north from a disjunct population at the terminus of the species range in the southern Sierra Nevada Mountains in California (Bull and Duncan, 1993). No differences in plumage or morphology have been observed across this barrier; however, the Sierra Nevada population differs from northern populations in migratory behavior, nest site selection, and prey preference (Bull and Duncan, 1993). The great gray owl is a State-endangered raptor in California due to a limited geographic distribution and an estimated statewide census population size of 100–200 individuals (Winter, 1980; Rich, 2000). The core breeding distribution of California great gray owls in the Sierra Nevada is centered in Yosemite National Park and the adjacent Stanislaus and Sierra National Forests. A few individuals have been sporadically documented further north in the Eldorado, Tahoe, and Plumas National Forests and to the south in Sequoia-Kings Canyon National Park and the Sequoia National Forest (Winter, 1986; Rich, 2000; Keane, 2001), but many hundreds of kilometers separate these sightings from the nearest population in southern Oregon.

The Sierra Nevada population of great gray owls may be at high risk of population decline or extinction due to a number of threats. Intense human development pressures at lower elevations threaten both breeding and wintering great gray owl habitat because the foothill regions of the Sierra Nevada are experiencing among the greatest human population growth rates of any region in California (Millar, 1996). Owl habitat at higher elevation is threatened by timber harvest, fire suppression, post-fire salvage harvest, grazing, and alteration of hydrological regimes that has reduced the number of large conifers and oak trees used for nesting, as well as the quality of meadows and forest stands used for foraging (Dull, 1999; Hutto and Gallo, 2006; Saab et al., 2009). West Nile virus infection is also a cause for concern in the Sierra Nevada owl population; while no indication of infection has yet been detected in the wild (Hull et al., 2010), Gancz et al. (2004) reported a 91.3% mortality rate in a captive colony of great gray owls in Ontario, Canada. Additional threats to Sierra Nevada great gray owls include mortality from car strikes, with 30 mortalities documented through 2008 (J. Mauer, Yosemite National Park, pers. comm.; J. Keane unpubl. data), increasing direct and indirect human disturbance resulting from recreational activities, stochastic extinction due to small population size, and increasing summer aridity and air temperatures resulting from climate change (Rauscher et al., 2008; Miller et al., 2009).

Given the small census population size, limited geographic distribution, and number of threats impacting great gray owls in the Sierra Nevada, documenting the phylogenetic relationships among North American populations is necessary to fully assess the conservation status of this isolated population. This information would also provide the context for identifying the level of conservation focus, research, and management action warranted for the Sierra Nevada population, and assist in development of appropriate management strategies. Our objectives for this study were to describe whether great gray owls, given their potential ability for long-range dispersal, are geographically structured across their western North American range. We approached this by generating a combined dataset of molecular markers that we used to address recent and historical population differentiation. With these data, we asked whether the Klamath Basin is a region of reduced gene flow in great gray owls, and explored temporal hypotheses to infer the evolutionary tempo of divergence among great gray owl lineages. We interpreted the results of these analyses in light of the conservation actions that may need to be considered for preserving the Sierra Nevada great gray owl population.

2. Materials and methods

2.1. Population sampling

We collected either whole blood and contour feathers or muscle samples from 93 individual great gray owls (*S. nebulosa*): 8 from western Canada, 25 from southern Oregon, 22 from eastern Idaho, 9 from northern Oregon, and 29 from the southern Sierra Nevada (Fig. 1). Sample collection was limited to adults and nestlings during the breeding season to ensure that samples were accurately associated with a specific geographic range. We only collected samples from individuals presumed to be unrelated. For nestlings, we took samples from a single individual and avoided sampling of adults. Alternatively, when sampling was from adults at nest sites, both parents were included and nestlings were excluded. We drew approximately 0.2 ml of blood from the medial metatarsal vein, and stored it in 1.2 ml of Longmire's solution. We also plucked two contour feathers from each breast. Muscle samples were collected from salvaged fresh great gray owl carcasses. For each of

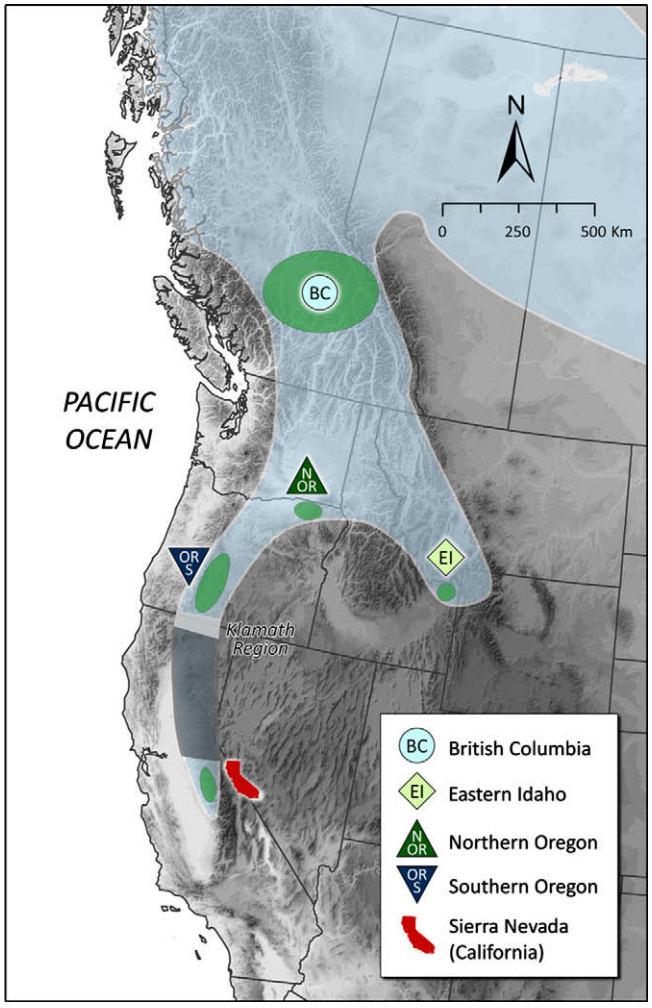

Fig. 1. Western North American range of great gray owls (*Strix nebulosa*; Ridgely et al., 2003). The circular areas illustrate the general sampled regions of population sampling for this study based on the five general regions: the Sierra Nevada, southern Oregon, northern Oregon, eastern Idaho, and western Canada in British Columbia. The Klamath Mountain region, a known phylogeographic barrier, is noted located by the white stippling below the southern Oregon sampling regions. The dark gray stippling identifies a gap in the distribution of great gray owls; this owl species has not been documented between southern Oregon and the southern Sierra Nevada Mountains in California.

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the 93 samples, we extracted genomic DNA using QIAGEN DNeasy kits (QIAGEN, Inc.) from 25 μl blood/buffer solution, a single feather calamus, or 5 mg of muscle.

2.2. Microsatellite data collection

We genotyped each of the 93 individual owls at 30 microsatellite loci (SneA001w, SneA011w, SneA012w, SneA105w, SneA107w, SneA113w, SneA116w, SneA117w, SneA127w, SneA201w, SneA 216w, SneA230w, SneA234w, SneA238w, SneA304w, SneA313w, SneA318w, SneA319w, SneA327w, SneA335w, SneA337w, SneA 338w, SneD116w, SneD123w, SneD202w, SneD203w, SneD218w, SneD223w, SneD224w, SneD236w) using the protocol described in Hull et al. (2008b). PCR fragments were size-separated on a 3730 DNA Analyzer (Applied Biosystems, Inc.), and alleles were scored with STRAND version 2.3.89 (Toonen and Hughes, 2001).

2.3. Mitochondrial data collection

We amplified a 915 bp fragment of the mitochondrial DNA *control region* using primers N1 and D12 (Barrowclough et al., 1999), and also amplified a 1005 bp segment of *nad*2 using primers L5219 and H6313 (Sorenson et al., 1999). We amplified both gene fragments for 80 individuals of the focal species (*S. nebulosa*) and six spotted owls (*S. occidentalis*) to use as the outgroup taxon in phylogenetic analyses. We selected the spotted owl as an outgroup because it is a co-distributed member of the genus *Strix* and a maximum likelihood phylogram of owls (Wink and Heidrich, 2000) suggests that branch lengths among *Strix* lineages are short. We prepared PCR products using 0.5 μl Exonuclease I and 1 μl Shrimp Alkaline Phosphatase per 25 μl reaction, and sequenced each on an ABI 3730 DNA Analyzer (Applied Biosystems, Inc.). We verified and aligned sequences using SEQUENCHER version 4.7 (Gene Codes Corporation).

2.4. Microsatellite data analysis

We calculated standard summary statistics using several software packages. We tested all loci for deviations from Hardy–Weinberg equilibrium and genotypic disequilibrium using GENEPOP version 3.4 (Raymond and Rousset, 1995). We tested for the presence of null alleles and scoring error using the program MICROCHECKER version 2.2.3 (van Oosterhout et al., 2004), and also through reanalyzing 10% of individuals at all loci. We calculated the number of private alleles using CONVERT version 1.31 (Glaubitz, 2004), while calculations of heterozygosity corrected for sample size and the mean number of alleles per locus were performed with the MICROSATELLITE TOOLKIT version 3.1.1 (Park, 2001). We calculated allelic richness (corrected for sample size) in FSTAT version 2.9.3.2 (Goudet, 1995) and pairwise population differentiation (F_{ST}) estimates between all sampling regions using ARLEQUIN version 3.11 (Excoffier et al., 2005). We also used the AMOVA framework in ARLEQUIN to test for degrees of differentiation between the *a priori* sampling groups of the southern Sierra Nevada region and the combined four northern sampling regions (western Canada, southern Oregon, eastern Idaho, and northern Oregon). We assessed significance for all calculations with sequential Bonferroni corrections for multiple tests (Rice, 1989).

We tested for pairwise genetic and geographic distance correlations among all sampling sites using Mantel tests (Mantel, 1967; Smouse et al., 1986) in ARLEQUIN. The dependent variable for this analysis was a matrix of transformed pairwise genetic distances expressed as $F_{ST}/(1 - F_{ST})$ (Rousset, 1997), while the independent matrix was composed of pairwise geographic distances between the centroids of each sampling region.

We used a multilocus Bayesian clustering algorithm (STRUCTURE version 2.2; Pritchard et al., 2000) to determine the number of population groups (K) and to probabilistically group individuals without using the known geographic location of sample collection. We used the population admixture model with a flat prior and assumed that allele frequencies were correlated among populations, and ran simulations for 750,000 iterations following a burnin period of 500,000. We used these initial settings to estimate the probability of one through 10 clusters (K), with each run replicated 10 times. We averaged the log $\Pr(X|K)$ statistics across the multiple runs for each of the 10 K estimates. We selected the K value of highest probability by identifying the set of values where the log $\Pr(X|K)$ value was maximized and subsequently selecting the minimum value for K that did not sacrifice explanatory ability (Pritchard and Wen, 2002; Waples and Gaggiotti, 2006). We defined membership to a cluster based upon the highest proportion of ancestry to each inferred cluster.

We tested for evidence of recent population size reductions in each sampling region with one-tailed Wilcoxon sign-rank tests for heterozygote excess in the program BOTTLENECK version 1.2.02 (Cornuet and Luikart, 1997); these tests were performed using both the infinite alleles (IAM) and two-phase (TPM, 70% step-wise mutation model and 30% IAM) models of microsatellite evolution. Because population bottlenecks can reveal a signal of reduced population sizes, we then estimated contemporary effective sizes (N_e) for each genetically distinct population based on gametic disequilibrium with sampling bias correction (Waples, 2006) using LDNE version 1.31 (Waples and Do, 2008). These analyses excluded alleles occurring at frequencies $\leqslant 0.05$, and for confidence in the effective size estimates we used the jackknife method to determine 95% confidence intervals (Waples, 2006).

2.5. Mitochondrial data analysis

We calculated the number of haplotypes, nucleotide diversity, and haplotype diversity in DNASP version 4.10.9 (Rozas et al., 2003) and estimated population differentiation through pairwise Φ_{ST} comparisons in ARLEQUIN. We tested for structure between southern Sierra Nevada and northern sites with an AMOVA in ARLEQUIN. To test for population expansion, we calculated the F_S statistic (Fu, 1997) in DNASP for each genetically distinct population.

We combined *nad*2 and *control region* sequences for each individual, and collapsed identical haplotypes into unique haplotypes for model inference and phylogeny reconstruction. Out of 86 sequences, we retained only the unique haplotypes to form a reduced dataset. In total, we analyzed 1938 bp alignment of mitochondrial sequence data for the two regions and 22 haplotypes (20 ingroup and 2 outgroup individuals).

The general forms of substitution models for the complete *control region* sequence and for each codon position in *nad*2 were specified by AIC calculated in MRMODELTEST version 2.3 (Nylander, 2004). We included each set of substitution models in a combined data partition scheme by codon (*nad2*) and gene region to conduct mixed model Bayesian phylogenetic inference in MRBAYES version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). We performed Bayesian analysis with two independent runs of 2×10^7 generations, each with 8 chains (7 heated), and with trees sampled along the chain every 1×10^3 generations. We evaluated convergence with both the PSRF (potential scale reduction factor; Gelman and Rubin, 1992) and log-likelihood scores, which both indicated early convergence among chains, prior to the first 100,000 generations. Despite early convergence, we used a conservative burnin of 10% (2000) of all sampled trees for each run (4000 burnin trees in total). Summarized trees and parameters across runs yielded 95% and 99% credible sets that contained 34,180 and 35,620 trees respectively.

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Table 1
Divergence times summary statistics estimated with relaxed phylogenetic analysis for the Sierra Nevada haplotypes of the great gray owl (*Strix nebulosa*), and the most inclusive clade containing all *S. nebulosa* haplotypes. Three different substitution rates were used to estimate clade ages, following the results reported in Nabholz et al. (2009): the average rate for 1571 avian taxa (0.027), a mean rate for two *Strix* species (0.034), and the average rate for all strigiform species (0.051). Substitution rates are third position codon changes measured in units of substitutions per site per million years (Nabholz et al., 2009). Posterior mean and median node ages estimated by relaxed Bayesian phylogenetic analysis (Drummond and Rambaut, 2007; Drummond et al., 2006) are listed in units of thousands of years before present, in addition to the highest posterior density intervals around the mean node ages. Also listed are standard errors (SE) for the mean ages, the posterior median ages, and the effective sample sizes (ESS) from the posterior distribution. See Fig. 3 for relative divergences times of great gray owl clades across the sampled regions.

Group	Substitution rate (subst/site/My)	Posterior mean age (95% HPD)	SE of mean	Posterior median age	ESS
Sierra Nevada	0.027	34.3 (2.5, 88.0)	0.350	25.8	8194
	0.034	26.7 (2.3, 69.6)	0.268	20.2	8080
	0.051	18.0 (1.5, 46.4)	0.173	13.6	8747
Strix nebulosa	0.027	174.3 (50.2, 381.8)	2.029	142.8	3133
	0.034	136.8 (40.0, 298.7)	1.643	112.2	3040
	0.051	92.0 (25.8, 203.1)	0.964	75.5	3612

We also conducted a maximum likelihood analysis of the combined mitochondrial sequences for topology reconstruction and a rapid bootstrap search using RAXML version 7.04 (Stamatakis, 2006; Stamatakis et al., 2008). The partitioned dataset (by codon for *nad2* and by gene region) was analyzed for 5×10^3 rapid bootstrap search replicates under the GTR+CAT substitution model, which accommodates rate heterogeneity among sites and partitions. We used the final Bayesian consensus phylogram to infer relationships, and Bayesian clade credibility ($\geqslant$0.95) and maximum likelihood bootstrap ($\geqslant$70%) values to evaluate support for focal ingroup clades of *S. nebulosa*.

Because the Sierra Nevada population of *S. nebulosa* is allopatric with respect to the larger species range, we tested the relative time since isolation (tmrca) from other great gray owl populations. We estimated divergence times using the relaxed clock method of Drummond et al. (2006), implemented in the program BEAST version 1.4.8 (Drummond and Rambaut, 2007). We partitioned the aligned sequence data by gene (*nad2* and *control region*) and by codon for *nad2*, with the same evolutionary model used in phylogenetic inference (above). We estimated divergence times under the uncorrelated relaxed lognormal clock model, with a coalescent tree prior and Jeffreys prior (Drummond and Rambaut, 2007) on the substitution model parameters. We used three different substitution rates taken from Nabholz et al. (2009) for three independent analyses (Table 1) with a lognormal prior on the substitution rate parameter; each analysis was performed five times for 20 million generations (100 million total generations for each substitution rate) to ensure that the chains were independently converging and not merely sampling local optima. Stationarity of the chains occurs when the effective sample size (ESS) is above 200 for all parameters (as per Drummond et al., 2006), which occurred prior to the first 2 million generations; these samples were discarded as burnin. We acknowledge that estimates of clade ages are based on a single locus (mtDNA), and that age estimation will be inherently biased to reflect the demographic history of the mitochondrial locus and likely not the true divergence time for a population/species. However, we are interested in estimating the relative tempo of divergences within the *S. nebulosa* group, and merely make inferences of divergences as such.

3. Results

3.1. Microsatellite data

Across all sampling sites, none of the 30 microsatellite loci deviated significantly from Hardy–Weinberg equilibrium expectations, nor was there evidence of linkage disequilibrium. Similarly, we found no evidence for the presence of null alleles or scoring errors across the complete multilocus dataset based on the 10% reruns. Among sampled regions, pooled samples from western Canada exhibited higher measures of genetic diversity, while those in the southern Sierra Nevada were the lowest (Table 2). Across the sampling scheme of the five western sample regions, we found a non-significant relationship between genetic and geographic distance (Mantel test; $P = 0.37$, $r = 0.18$). General summary statistics, including site-specific heterozygosities, number of alleles, allelic richness, and private alleles, are summarized in Table 2.

Pairwise F_{ST} values ranged between 0.06 and 0.17 across the dataset, and all estimates were significant after Bonferroni correction (Table 3). The highest pairwise F_{ST} comparisons were evident between the northern sampling regions and the southern Sierra Nevada. This result was supported by AMOVA comparisons of the southern Sierra Nevada site with the four northern sites, which indicated substantial differentiation ($F_{ST} = 0.13$, $P < 0.001$) and points to reduced gene flow between the Sierra Nevada populations and those to the north.

Bayesian clustering of the total dataset revealed very similar maximum log Pr($X|K$) for four and five clusters. At five K clusters, each sampling region was recovered as a distinct population cluster, whereas the northern and southern Oregon sampling sites

Table 2
Microsatellite and mitochondrial polymorphism data for great gray owls (*Strix nebulosa*) from five sampling regions. Listed for each sampling region are the number of sampled individuals for microsatellite data (N_{ms}), heterozygosities corrected for sample sizes (H_C), the average number of alleles at each locus (A/locus), allelic richness corrected for sample sizes (AR_C), the number of private alleles (A_P), the number of sampled individuals for mitochondrial data (N_{mt}), number of haplotypes per population (H_S), haplotype diversity (Hd), and nucleotide diversity (π). Note the generally low diversity measures for the Sierra Nevada relative to the other sampled regions. Sampling regions and map code correspond to the locations illustrated in Fig. 1.

Sampling region	Map code	Microsatellite DNA					Mitochondrial DNA			
		N_{ms}	H_C	A/locus	AR_C	A_P	N_{mt}	H_S	Hd	π
W. Canada	BC	8	0.63	4.2	4.2	14	8	6	0.89	0.00182
S. Oregon	ORS	25	0.58	4.4	3.6	16	21	4	0.74	0.00206
N. Oregon	NOR	9	0.53	3.2	3.2	4	7	2	0.29	0.00089
E. Idaho	EI	22	0.48	3.6	3.1	7	16	5	0.79	0.00181
Sierra Nevada	CA	29	0.47	3.3	2.8	3	28	3	0.67	0.00077

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Table 3
Pairwise comparisons of genetic differentiation between sampling regions of the great gray owl (*Strix nebulosa*) in western North America. Pairwise F_{ST} estimates (30 microsatellite loci) are above the diagonal; Φ_{ST} values (1938-base pairs of *nad2* and *control region* sequence data) are below the diagonal. All pairwise comparisons are significant following Bonferroni correction for the number of tests. Sampling regions and map code correspond to the locations illustrated in Fig. 1.

Sampling region	Map code	W. Canada	S. Oregon	N. Oregon	E. Idaho	Sierra Nevada
W. Canada	BC	–	0.06	0.10	0.13	0.14
S. Oregon	ORS	0.15	–	0.09	0.09	0.09
N. Oregon	NOR	0.22	0.29	–	0.16	0.17
E. Idaho	EI	0.16	0.21	0.36	–	0.17
Sierra Nevada	CA	0.71	0.63	0.79	0.62	–

merge for four *K* clusters (Fig. 2). Despite the similar maximum log Pr(*X*|*K*) for four and five clusters, the maximum was slightly higher for four clusters, and therefore we conservatively focus the remainder of the analysis and discussion on this estimate.

A significant excess of heterozygotes, indicating recent population bottlenecks, was detected at all sites (with the exception of the western Canada site) using both the IAM and TPM models of microsatellite evolution (Wilcoxon sign-rank test; Table 4). Similarly, all sampling sites except western Canada have very small effective size estimates (N_e ranged from 14 to 34; Table 4), indicating that there are either very few breeding individuals, or there has been a dramatic reduction in genetic diversity resulting from recent population bottlenecks.

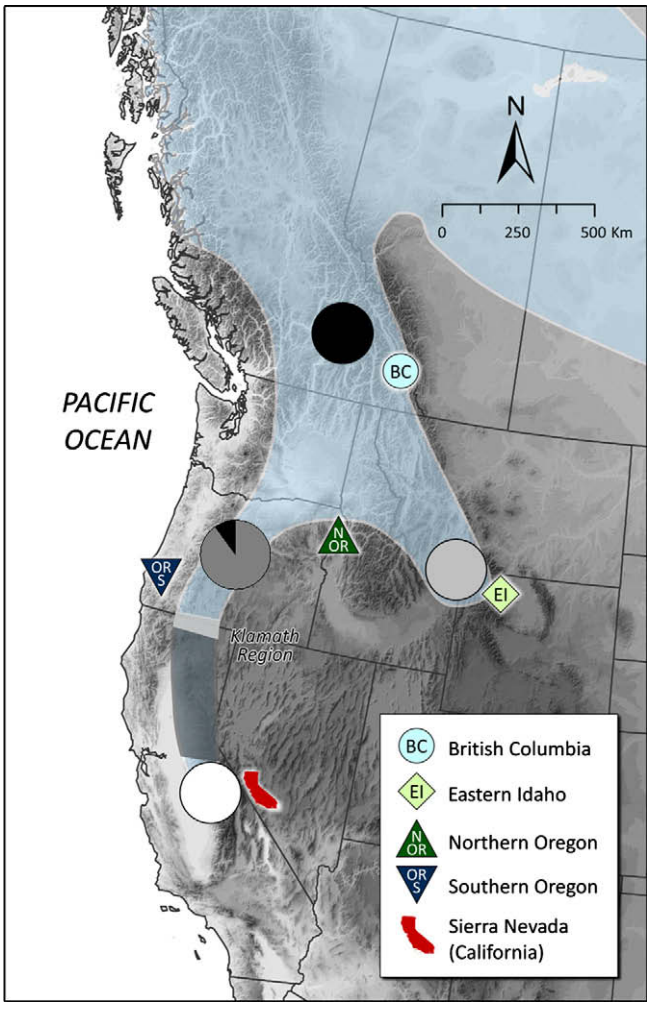

Fig. 2. Map of Bayesian population clustering of great gray owls based on a multilocus microsatellite genotype dataset. The four circle graphs next to the sampling regions illustrate the Bayesian population clustering estimate, *K*, from STRUCTURE, which grouped the two Oregon samples. Each pie chart represents a sampling location and the colors (white, light gray, gray, and black) represent the proportion of each Bayesian population cluster found at each sampling site. In general, regional sampling sites are composed of distinct clusters which suggest little evidence for recent gene flow among the southern Sierra Nevada (white), combined Oregon sampling regions (gray and black), eastern Idaho (light gray), and western Canada (British Columbia; black) regions. Some admixture is evident between British Columbia and the combined Oregon samples.

3.2. Mitochondrial data

Total haplotype diversity was 0.93 ± 0.01 and total nucleotide diversity was 0.003; site-specific diversity statistics are summarized in Table 2. We identified a total of 26 polymorphic sites (1.3%) in the sequenced region, and comparisons of all regions revealed significant pairwise differentiation (Table 3). An AMOVA between the southern Sierra Nevada and the four northern sites revealed significant differentiation ($\Phi_{ST} = 0.61$, $P < 0.001$). The F_S statistic was not significant in any of the populations (Table 4), suggesting that either the overall population of great gray owls in the Pacific Northwest and Sierra Nevada has not recently expanded, or that the signal of expansion has been degraded by recent bottlenecks (detected using microsatellite data; see above).

Out of 86 *nad2* and 86 *control region* sequences, there were 22 unique haplotypes, (Table 5; Genbank accession numbers GU784951–GU784978). Twenty ingroup haplotypes (combined gene region data) were unique and contained 26 polymorphic sites, only three of which were restricted to the three Sierra Nevada samples and 23 were variable among the remaining but unresolved *S. nebulosa* haplotypes. Two segregating sites were fixed between Sierra Nevada populations and all other great gray owls. Uncorrected *p*-distances were very low, indicative of little genetic differentiation across the range of *S. nebulosa*. For *nad2*, pairwise divergences ranged from 0.10% to 0.30% and from 0.11% to 0.87% for the *control region* sequences. Between *S. nebulosa* and *S. occidentalis*, sequences differed by 13.73–13.93% for *nad2* and 14.58–14.81% for the *control region*.

Bayesian and maximum likelihood phylogenetic inference methods resolved similar topologies for the combined mitochondrial sequences and supported the monophyly of *S. nebulosa*, and a lineage restricted to the Sierra Nevada Mountains (Fig. 3). North of the Sierra Nevada population, we found little resolution of geographical relationships among *S. nebulosa* haplotypes. Nodal support was sparing, with only two nodes supported by both inference methods.

We used three substitution rates to estimate divergence times, but focus on the rate average taken from two *Strix* species (0.034 subst/site/My; Nabholz et al., 2009). The Sierra Nevada population appears to be a recently derived monophyletic lineage that arose during Pleistocene events, approximately 26,700 years before present (95% HPD: 2300–69,600; Table 1; Fig. 4), and this pattern of recent diversification is similar across the other haplotypes and/or lineages sampled from the broader species range (Fig. 4). These results suggest owl populations were isolated in Pleistocene refugia (or where suitable habitat was available), and that subsequent dispersal was asymmetrical. The relative timing of lineage diver-

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Table 4
Population genetic statistics for great gray owl (*Strix nebulosa*) sampling regions. Listed by column are *P*-values (IAM and TPM) for population bottleneck testing and effective size (N_e) estimations (95% CI) based on data from 30 microsatellite loci, and *P*-values for Fu's F_S statistic based on 1938-base pairs of combined *nad2* and *control region* sequence data. The western Canada sampling site exhibited no evidence of a population bottleneck and had the highest effective size. In contrast, all of the more southern sampling sites showed evidence of recent population bottlenecks and very low effective size.

Sampling region	IAM *P*-values	TPM *P*-values	N_e (95% CI)	Fu's F_S *P*-values
W. Canada	0.04	0.450	481 (22.8–∞)	0.17
S. Oregon	<0.001	0.005	19 (13.4–30.5)	0.91
N. Oregon	<0.001	0.010	15 (6.1–53.9)	0.91
E. Idaho	<0.001	0.006	34 (17.9–128.7)	0.86
S. Sierra Nevada	<0.001	0.010	14 (10.2–21.5)	0.91

gences and lack of geographical congruence of haplotypes outside of the Sierra Nevada suggests that post-refugia dispersal was widespread in the northern range of the great gray owl, but excluded movement to the southern Sierra Nevada.

4. Discussion

4.1. Population divergence

Both nuclear and mitochondrial data revealed genetic differentiation between the Sierra Nevada population of great gray owls (*S. nebulosa*) and populations to the north, and moreover, indicate that the allopatric Sierra Nevada population is a distinct and independent evolutionary lineage. The microsatellite data showed significant differentiation among all Pacific Northwest sites which was not recovered with the mitochondrial data, suggesting relatively recent divergences among these populations. Collectively, the nuclear and mitochondrial data support the distinctiveness of an isolated lineage of great gray owls in the Sierra Nevada, and we base this conclusion on the high microsatellite F_{ST} differentiation, significant Φ_{ST} estimates, and genealogical exclusivity based on neutral mitochondrial DNA sequence data.

The magnitudes of observed F_{ST} and Φ_{ST} values between the Sierra Nevada and Pacific Northwest populations are equal to or greater than those observed among subspecies of many North American raptors. Notably, the degree of differentiation among Sierra Nevada and northern great gray owls is similar for subspecies of spotted owls (*S. occidentalis*; Haig et al., 2004), and eastern and western subspecies of red-shouldered hawks (*Buteo lineatus*) which are separated by thousands of miles (Hull et al., 2008c). Taken together, these results suggest that the southern Sierra Nevada population is demographically isolated from populations to the north, and has likely been isolated for an extensive period of time.

Table 5
Summary of great gray owl haplotype occurrences by sampling site and Genbank Accession Nos. BC = British Columbia, EI = eastern Idaho, NOR = northern Oregon, ORS = southern Oregon, and SN = Sierra Nevada. Haplotype labels correspond to labels in Figs. 3 and 4.

Haplotype label	Occurrences (site, # of occurrences)	Genbank Accession Nos.
BC1	BC, 1	GU784953 + GU784963
BC2	BC, 1	GU784958 + GU784965
BC3	BC, 1	GU784956 + GU784971
BC4	BC, 1	GU784958 + GU784972
BC5	BC, 1	GU784957 + GU784972
BC6	BC, 3	GU784958 + GU784973
EI1	EI, 2	GU784952 + GU784963
EI2	EI, 6	GU784952 + GU784962
EI3	EI, 1	GU784952 + GU784961
EI4	EI, 4	GU784958 + GU784969
EI5	EI, 3	GU784958 + GU784974
NOR1	NOR, 1	GU784954 + GU784964
NOR2	NOR, 6; ORS, 1	GU784958 + GU784976
ORS1	ORS, 7	GU784958 + GU784970
ORS2	ORS, 1	GU784955 + GU784966
ORS3	ORS, 4	GU784958 + GU784966
ORS4	ORS, 8	GU784958 + GU784975
SN1	SN, 11	GU784951 + GU784967
SN2	SN, 6	GU784951 + GU784968
SN3	SN, 11	GU784951 + GU784960
S. occidentalis 1	Outgroup	GU784959 + GU784977
S. occidentalis 2	Outgroup	GU784959 + GU784978

4.2. Genetic diversity and historical demography

The widely distributed western Canada population exhibited the highest indices of standing genetic diversity and largest effective size estimate (N_e), which may be a result of the fact that this region was an important refugium during the Pleistocene glacial cycles and currently supports a large breeding population. It may also indicate that subsequent to the last glaciation, suitable habitat has been greater and capable of sustaining larger populations of owls with the retention of ancestral polymorphism. The higher genetic diversity in western Canada contrasts with the southern portions of the species range, where contemporary populations are lower in diversity and may be smaller in size. The effective size of western Canada differs with the very small estimates throughout the remainder of the sampled range, where available habitat tends to be more fragmented and regionally confined, and is at the southern range extent of the species. This is no more obvious than for the Sierra Nevada population, which is depauperate in genetic diversity for every measure we calculated (Table 4).

Contemporary small effective population sizes are generally associated with recent population bottlenecks. In this study we found significant excesses of rare alleles relative to expected equilibrium heterozygosities, indicative of the signal expected from recent population bottlenecks and the outcome for effective size reduction. These results are also consistent with recent founding events, in which populations would be expected to retain one or a few closely related mitochondrial haplotypes. However, the mitochondrial data illustrate that each of the Pacific Northwest populations we sampled consist of haplotypes that do not form exclusive geographic lineages (Fig. 2). Thus, the results are inconsistent with recent founding events for the Pacific Northwest sites and suggest that the significant excesses of heterozygotes detected from the microsatellite data are due to recent population bottlenecks.

Similar to what we found herein with great gray owls, population bottlenecks have recently been documented in northern spotted owls (*Strix occidentalis caurina*) in the Pacific Northwest (Funk et al., in press). In fact, the bottlenecks in spotted owls are presumably very recent events, possibly occurring within the past few decades in response to habitat loss and the presence of barred owls (*Strix varia*). The causes of the recent population bottlenecks detected in the microsatellite data for western great gray owls are unknown, but by extension of logic, habitat degradation, and fragmentation of western forests and landscapes driven by the influence of increasing human development and management

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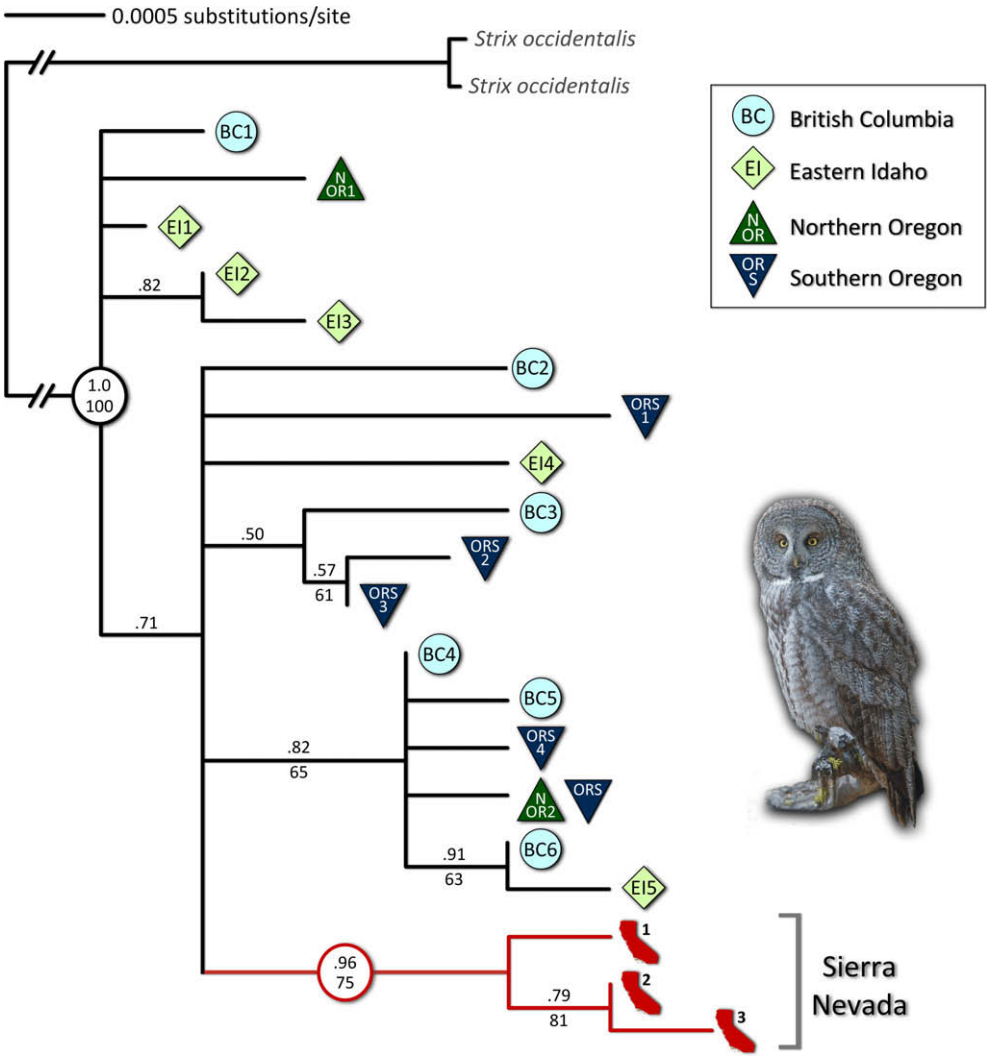

Fig. 3. Phylogenetic relationships of great gray owl based on 1938 bp alignment of *nad2* and *control region* sequence showing a monophyletic southern Sierra Nevada clade (indicated in red) and 17 other great gray owl haplotypes without geographic congruence. Numbers below branches are bootstrap values (maximum likelihood) and Bayesian posterior probabilities are above branches. Unique haplotype labels are indicated numerically by region (e.g., British Columbia, haplotype 1 = BC1); only one haplotype, NOR2, is shared between sampling sites (with Southern Oregon, SOR). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

activities may be associated with population declines in great gray owl populations across their western range in the Sierra Nevada and Pacific Northwest. Similar reductions in population size have been attributed to recent anthropogenic modifications of the natural landscape (e.g., Trzcinski et al., 1999; Butler et al., 2004). Regardless of cause, population bottlenecks and reductions in genetic diversity are important considerations to address for future management approaches for the great gray owl.

The relatively ancient phylogenetic pattern we observed in the mitochondrial data point to historical patterns of isolation and reduction in suitable habitat caused by past climatic and geologic events. Specifically, the Sierra Nevada lineage appears to have diverged in allopatry but has since remained isolated from the wider-ranging Pacific Northwest population (Fig. 3). While this estimate reflects the demographic history of the mitochondrial genome, the relative tempo of differentiation (Fig. 4) suggests that the Sierra Nevada lineage has experienced a long history of isolation from other great gray owl populations. The timing of divergence is similar for several other great gray owl haplotypes, although none of the other haplotypes are exclusive to specific geographic regions. Our interpretation of this pattern is that ancestral populations of great gray owls diverged recently, likely in allopatric refugia during the late Pleistocene, and that divergent haplotypes came into secondary contact following glacial retreat. In contrast, the Sierra Nevada population remained isolated through the Holocene and into the present. The signal of population bottlenecks is also apparent as indicated by the scarcity of shared haplotypes among populations. In the Pacific Northwest, haplotypes do not form exclusive geographic clusters, suggesting that prior to population bottlenecks, a widespread and interbreeding population existed in the Pacific Northwest. In contrast, Sierra Nevada haplotypes occur in a geographically clustered lineage which supports the interpretation of long-term isolation from the Pacific Northwest.

4.3. Taxonomy

The molecular phylogeny of western North American great gray owls suggests that a distinct evolutionary lineage is restricted to a small region of the Sierra Nevada Mountains in California. The mitochondrial data, consistent with the isolation detected in the more recent measures indicating reduced nuclear gene flow, suggest that the Sierra Nevada lineage has been isolated over the past 26,700 years (Table 1; Fig. 4). While the Sierra Nevada great gray

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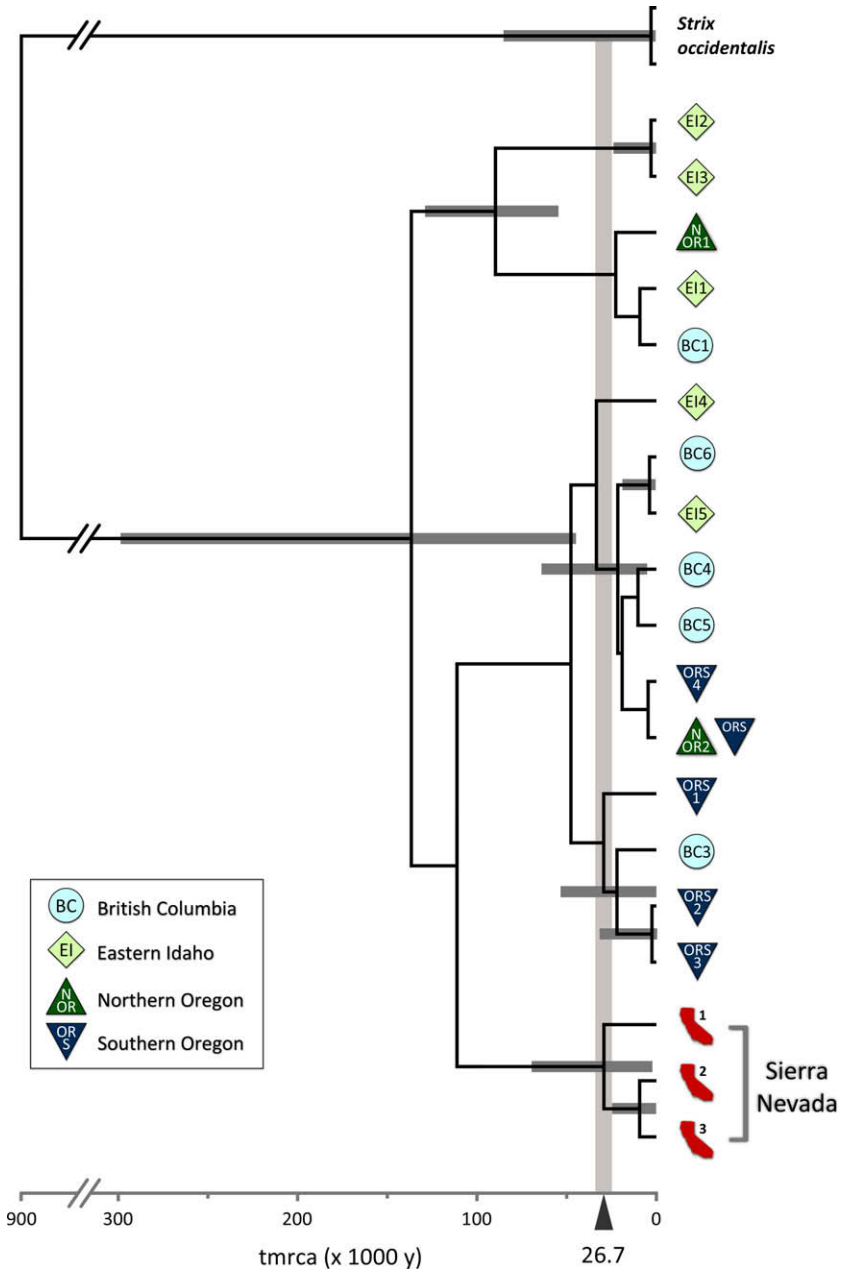

Fig. 4. Relaxed-clock Bayesian inference chronogram of great gray owls (*Strix nebulosa*) suggesting a Sierra Nevada lineage of approximately 26,700 years before present. Divergence time inference was conducted using the program BEAST version 1.4.8 (Drummond and Rambaut, 2007). Topology and node ages are based on the mean of 40 million post-burnin generations from five independent runs; horizontal bars indicate the 95% highest posterior densities for node ages. Dates were estimated using a substitution rate of 0.034 subst/site/My calculated for two *Strix* species (see Table 1; Nabholz et al., 2009).

owl is of very small effective size and an exclusive genealogical lineage, the lack of geographical concordance of mitochondrial haplotypes across the other portions of the species range can be explained by incomplete lineage sorting. Similar paraphyletic relationships have been documented among avian species (Funk and Omland, 2003), including recently diverged raptors that are recognized as full species (*B. swainsoni* and *B. galapagoensis*; Bollmer et al., 2006; Hull et al., 2008d).

Comparing our data with previous research on a similarly distributed congener, the spotted owl (*S. occidentalis*), provides some guidance in the interpretation of the taxonomic relationships within great gray owls. In the case of spotted owls, the Pacific Northwest subspecies (*S. o. caurina*) and the Sierra Nevada subspecies (*S. o. occidentalis*) are reciprocally monophyletic, while haplotypes are shared between *S. o. occidentalis* and *S. o. lucida* (Mexican spotted owl; Barrowclough et al., 1999; Haig et al., 2004). Moreover, the differentiation observed among *S. o. caurina*, *S. o. occidentalis*, and *S. o. lucida* (Φ_{CT} = 0.69, Haig et al., 2004) was similar to what we found between Sierra Nevada and Pacific Northwest populations of great gray owls (Φ_{ST} = 0.62–0.71; Table 3). Similarly, differentiation at nuclear loci among the three spotted owl subspecies (F_{ST} = 0.08–0.14; Funk et al., 2008) was equivalent to what we found between Sierra Nevada and Pacific Northwest great gray owl populations (F_{ST} = 0.09–0.17; Table 3). Unlike spotted owls where nuclear introgression was detected between *S. o. occidentalis* and *S. o. caurina*, and between *S. o. occidentalis* and *S. o. lucida* (Funk et al., 2008), we found no evidence of introgression between the Sierra Nevada and Pacific Northwest populations of great gray owls.

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The American Ornithologists Union (AOU) suggests that "subspecies should represent geographically discrete breeding populations that are diagnosable from other populations on the basis of plumage and/or measurements, but are not yet reproductively isolated" (AOU, 2010). Our data indicate that the Sierra Nevada population of great gray owls is clearly diagnosable with either mitochondrial or microsatellite loci, and therefore is effectively an independent, isolated lineage. Thus, our data support subspecies status for the Sierra Nevada great gray owl population under the AOU criterion. Similarly, the mitochondrial monophyly of the Sierra Nevada population clearly suggest that the population is a distinct evolutionary lineage under a genealogical view of species (Baum and Shaw, 1995). A similar pattern of differentiation has been observed among hook-billed kites (*Chondrohierax uncinatus*, Johnson et al., 2007). In this case, a monophyletic clade restricted to the island of Cuba has been suggested as a distinct species. However, given the similarity of divergence observed in western great gray owls with that documented among long-standing subspecies of a sympatric congener, the spotted owl, we recommend that the Sierra Nevada population of great gray owls should be designated as a separate subspecies from *S. nebulosa nebulosa*. This recommendation is not only supported by our molecular data, but also by life history differences in nesting behavior, prey selection, and migration behavior between great gray owls in the Sierra Nevada and the larger species range (Bull and Duncan, 1993). We suggest recognition of a new subspecies, *S. n. yosemitensis,* for the Sierra Nevada lineage of great gray owls, which should be considered by the AOU Checklist Committee (see Supplement). This designation would not only reflect the evolutionary history of the lineage, but would also provide important context for ongoing conservation and management efforts.

4.4. Conservation of the allopatric Sierra Nevada lineage

The unique evolutionary history of Sierra Nevada great gray owls (*S. nebulosa*) calls for a revised management plan that recognizes the importance of genetic diversity and genealogical exclusivity. Because of the range allopatry and extremely low census numbers, conservation efforts within the southern Sierra Nevada have primarily focused on protecting the extant population from threats to local demography. Current conservation actions include efforts aimed at reducing these threats, particularly from habitat loss, but also from potential West Nile virus infection and car strikes. In addition to managing against demographic threats, future conservation actions require considering how the limited genetic diversity may contribute to any instability of the Sierra Nevada population isolate. The maintenance of genetic diversity is a major concern because of the already low levels, as well as the low census numbers, the limited migration potential, and the potential for inbreeding depression. Development of a site-specific genetic management plan may assist in resolving some of these concerns, but without appropriate management that simultaneously addresses and ameliorates demographic and genetic threats, the Sierra Nevada lineage of great gray owls may decline to the point of extinction without the possibility of natural rescue.

Careful planning is required for actions aimed towards increasing census population size because of the potential for inbreeding depression while avoiding the loss of unique history by outcrossing with allopatric owl populations. Because of the low census and effective population sizes, agencies charged with protecting the Sierra Nevada population are challenged with preventing further population declines. Given the genetic distinctiveness documented here, as well as previously recognized differences in life history characteristics, translocations and crossing (through captive management and breeding) of owls from allopatric populations are inappropriate, and would diminish the distinct genetic characteristics of the southern Sierra Nevada population. Loss of locally-adapted forms would fail in meeting conservation objectives for maintaining existing distinct genetic lineages because homogenizing the gene pool could have potentially harmful results for persistence of the Sierra Nevada lineage.

4.5. Conclusion

Our findings strongly support recognition of a distinct evolutionary lineage of great gray owls restricted to the Sierra Nevada Mountains in California. Both nuclear and cytoplasmic genetic data suggest that the southern Sierra Nevada population is unique, and life history data also point to a demographically independent lineage that is minimally a separate subspecies due to its range allopatry. Given the small effective and census sizes, degrading habitat and environmental quality, and secondary threats concomitant with increasing human disturbance, a sound conservation program is essential to avoid local extinction of the Sierra Nevada great gray owl.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.02.027.

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