Landscape Features Shape Genetic Structure in Threatened Northern Spotted Owls

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Abstract

Several recent studies have shown that landscape features can strongly affect spatial patterns of gene flow and genetic variation. Understanding landscape effects on genetic variation is important in conservation for defining management units and understanding movement patterns. The landscape may have little effect on gene flow, however, in highly mobile species such as birds. We tested for genetic breaks associated with landscape features in the northern spotted owl (*Strix occidentalis caurina*), a threatened subspecies associated with old forests in the U.S. Pacific Northwest and extreme southwestern Canada. We found little evidence for distinct genetic breaks in northern spotted owls using a large microsatellite dataset (352 individuals from across the subspecies' range genotyped at 10 loci). Nonetheless, dry low-elevation valleys and the Cascade and Olympic Mountains restrict gene flow, while the Oregon Coast Range facilitates it. The wide Columbia River is not a barrier to gene flow. In addition, inter-individual genetic distance and latitude were negatively related, likely reflecting northward colonization following Pleistocene glacial recession. Our study shows that landscape features may play an important role in shaping patterns of genetic variation in highly vagile taxa such as birds.

Introduction

A fundamental question in evolutionary biology is how landscape features such as mountains, rivers, and environmental heterogeneity affect spatial patterns of genetic variation (Manel and others, 2003; Storfer and others, 2007). Understanding how landscapes mold genetic variation is also important in conservation genetics for defining demographically independent management units (Moritz, 1994; Palsbøll and others, 2007) and understanding movement patterns. The simultaneous development of highly variable molecular markers, increasing computer power for computationally intensive genetic analyses, and Geographic Information System (GIS) technology has improved the potential to address these questions. As a result, the field of landscape genetics has emerged, providing a research framework for investigating the influence of landscape and environmental features on genetic structure, genetic discontinuities, and gene flow (Manel and others, 2003; Holderegger and Wagner, 2006; Storfer and others, 2007). Landscape genetics is currently in an exponential growth phase (Holderegger and Wagner, 2006). Only four years after the term 'landscape genetics' was first coined (Manel and others, 2003), several studies have demonstrated the importance of landscape and environmental features in structuring genetic variation in many taxa, including mammals (Geffen and others, 2004; Coulon and others, 2006; Cushman and others, 2006; Miller and others, 2006), amphibians (Funk and others, 2005; Spear and others, 2005; Giordano and others, 2007; Marsh and others, 2007), reptiles (Manier and Arnold, 2006), fish (Neraas and Spruell, 2001; Crispo and others, 2006), and insects (Finn and others, 2006).

Although landscape features may have a strong influence on gene flow in terrestrial or aquatic animals, it is less clear if gene flow in highly mobile animals such as birds is similarly influenced. Some studies of birds that are strong fliers indicate high levels of gene flow over large areas (for example, Koopman and others, 2007; Funk and others, 2007b; Draheim and Haig, unpub. data, reviewed in Avise, 2000), whereas other studies of less-mobile birds suggest that landscape features such as water bodies (Broderick and others, 2003), habitat breaks (Piertney and others, 1998), mountains (Caizergues and others, 2003), and anthropogenic habitat fragmentation (Bouzat, 2001; Veit and others, 2005) can restrict gene flow. These results suggest that the influence of landscape features on gene flow in birds varies, depending on a number of factors, including flying ability, whether or not the species is migratory, degree of habitat specialization, home-range size, mating system, and latitude (Martin and McKay, 2004). In addition, repeated population isolation and expansion associated with Pleistocene climatic fluctuations has played an important role in structuring intraspecific genetic variation in northern temperate birds (Avise and Walker, 1998; Klicka and Zink, 1999; Milá and others, 2000).

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The northern spotted owl (*Strix occidentalis caurina*) is a threatened subspecies associated with old forests in the U.S. Pacific Northwest and southwestern British Columbia, Canada. The subspecies was listed as threatened under the U.S. Endangered Species Act (ESA) in 1990, primarily because of declining habitat and populations (U.S. Fish and Wildlife Service [USFWS], 1990), and is considered endangered in Canada (Committee on the Status of Endangered Wildlife in Canada [COSEWIC], 2000). Despite ESA-listing and dramatic reductions in forest harvest under the Northwest Forest Plan (Noon and Blakesley, 2006), the species is still experiencing declines in demographic (Anthony and others, 2006) and genetic effective population sizes (Funk and others, 2008a).

There are several landscape features in the range of northern spotted owls that could affect their movements and gene flow. These include the large, non-forested valleys of western Oregon (Willamette, Rogue, and Umpqua Valleys);

the Yakima Valley in central Washington; high mountain ridges formed by the Cascade and Olympic Mountains; and wide water bodies such as the Columbia River and Puget Sound (fig. 1). Although northern spotted owls can disperse long distances, most birds disperse only a few territories from natal sites (Forsman and others, 2002) with lowland valleys such as the Willamette Valley, Oregon acting as barriers to dispersal. In addition, Pleistocene glaciations are thought to have isolated some forest species into multiple forest refugia in the Pacific Northwest, resulting in independent lineages (Soltis and others, 1997; Brunsfeld and others, 2001; Carstens and others, 2005; Steele and Storfer, 2006). Thus, current landscape features and historic isolation may have resulted in distinct genetic groups, or management units, of northern spotted owls. Identification of any such management units is important for conservation of this threatened subspecies. Previous genetic analyses of northern spotted owls using random amplified polymorphic DNA (Haig and others, 2001)
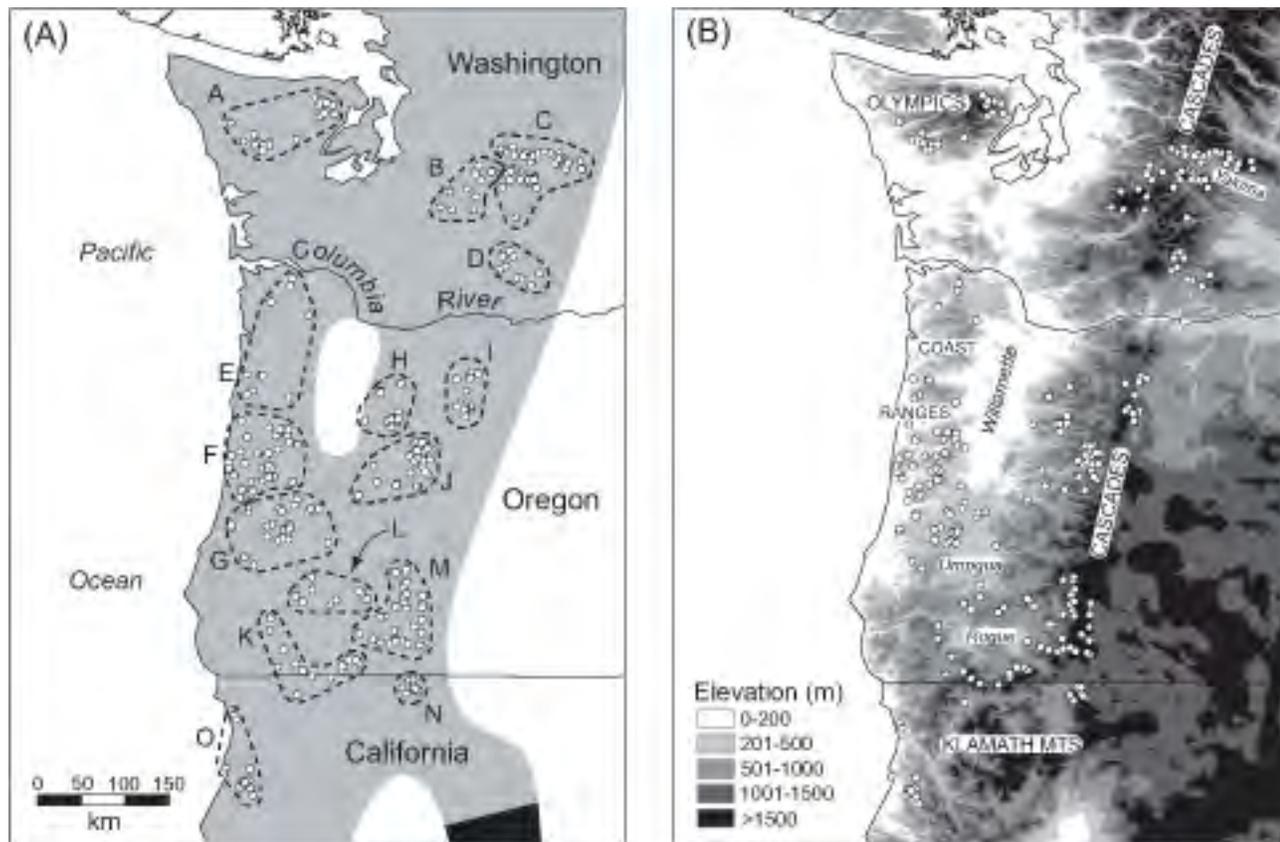
Figure 1. Map illustrating the distribution of northern spotted owl samples included in landscape genetic analyses. (A) Ranges of northern (gray) and California (black) spotted owls and boundaries of 15 study areas (dashed lines). Subspecies ranges are based on Gutiérrez and others (1995). Study area locations (and sample sizes) are: A. Olympic Peninsula, WA (n = 22); B. Western Washington Cascades (13); C. Cle Elum (eastern Cascades), WA (51); D. Yakima (eastern Cascades), WA (18); E. Northern Oregon Coast Range (12); F. Middle Oregon Coast Range (47); G. Southern Oregon Coast Range (31); H. Northwestern Oregon Cascades (15); I. Warm Springs (eastern Cascades), OR; J. Western Oregon Cascades (28); K. Siskiyou Mountains, OR and CA (17); L. South Umpqua River area, OR (10); M. Southern Oregon Cascades (32); N. Klamath National Forest, CA (14); O. Humboldt, CA (28). (B) Prominent mountain ranges (capital letters) and valleys (italicized) in the range of northern spotted owls.

and mitochondrial DNA (mtDNA, Barrowclough and others, 1999; Haig and others, 2004; Barrowclough and Gutiérrez, 2005) did not uncover such discontinuities, but these markers are likely not as powerful as hypervariable microsatellite markers coupled with larger sample sizes for identifying genetic discontinuities.

The goal of the present study was to investigate the landscape genetics of northern spotted owls using microsatellite loci. Our two specific questions were: (1) Are there any genetic discontinuities in northern spotted owls; and (2) Do mountains, non-forested valleys, or water bodies restrict gene flow?

Materials and Methods

Sampling

We collected blood samples from 352 northern spotted owls from 15 study areas from across the subspecies' range in Washington, Oregon, and California from 1990 to 2006 (fig. 1). Study areas were bounded by landscape features such as mountain ridges, rivers, and non-forested habitat. Our choice of study area boundaries, however, does not affect our conclusions because most analyses were individual-based and, thus, do not require *a priori* definition of sampling units. No known close relatives (parent-offspring or siblings) were included. Samples were collected following the protocol of the American Ornithologists' Union (Gaunt and Oring, 1997). Blood was stored in cryogenic tubes containing a buffer solution (100 mM Tris—HCl, pH 8.0; 100 mM EDTA, pH 8.0; 10 mM NaCl; 0.5% SDS) and frozen at -80^0 C until analyses.

Microsatellite Genotyping

DNA extraction, PCR, and fragment analysis were performed as described previously (Funk and others, 2007a). All owls were genotyped at 10 variable microsatellite loci developed for Mexican spotted owls (loci: 6H8, 15A6, 13D8, and 4E10.2; Thode and others, 2002), Lanyu scops owls (*Otus elegans botelensis*, loci: Oe37, Oe53, Oe128, Oe129, and Oe149, Hsu and others, 2003, 2006), and ferruginous pygmy-owls (*Glaucidium brasilianum*; locus: FEPO5, Proudfoot and others, 2005). One of these loci (Oe128) and an additional microsatellite marker (Bb126; Isaksson and Tegelström, 2002) are diagnostic of spotted versus barred owls (Funk and others, 2007a) and were genotyped to assure that no spotted owl-barred owl hybrids were included in the analysis. PCR conditions and annealing temperatures were the same as those described in the original primer notes.

Standard Population Genetic Analyses

We calculated exact probabilities for Hardy-Weinberg proportions, genotypic disequilibrium, genic differentiation, and Weir and Cockerham's (1984) F_{ST} values among study areas and subspecies using GENEPOP 3.4 (Raymond and Rousset, 1995). Expected heterozygosities were calculated with MICROSATELLITE ANALYZER 2.39 (Dieringer and Schlötterer, 2003). Critical α values for pairwise tests of allelic differentiation were determined using the sequential Bonferroni adjustment (Rice, 1989). We also tested for isolation-by-distance among individuals using a Mantel test implemented in ALLELES IN SPACE (AIS, Miller, 2005). Landscape genetic analyses

In a previous analysis including all three spotted owl subspecies (Funk and others, 2008a), we found evidence for introgression between California and northern spotted owls (primarily in southern Oregon and northern California) and between Mexican and northern spotted owls (centered in Washington) using a clustering algorithm implemented in STRUCTURE (Pritchard and others, 2000). Because the goal of the current study was to investigate the genetic structure of northern spotted owls alone, we excluded individuals estimated by STRUCTURE to have greater than 50 percent membership in California or Mexican spotted owl genetic clusters (clusters 3 and 4, respectively, in Funk and others, 2008a) from all subsequent analyses. This left 304 northern spotted owls for analysis.

The first method we used to test for genetic discontinuities in northern spotted owls is a Bayesian clustering method implemented in GENELAND 2.0.0 (Guillot and others, 2005a). This method is similar to that of STRUCTURE, except that GENELAND explicitly incorporates geographic coordinates of animals in the analysis and generates maps of population ranges. Populations are assumed to conform to Hardy-Weinberg proportions and to be spatially organized through the so-called colored Poisson-Voronoi tessellation (Lantuéjoul, 2002). GENELAND is designed to identify genetic breaks in populations that are spatially contiguous, and therefore is well suited for analysis of northern spotted owls, which have a fairly contiguous distribution (fig. 1A). The method has previously been tested using simulated datasets and has shown to perform well at estimating the number of populations, assigning individuals to populations, and drawing borders between populations (Guillot and others, 2005b). For inferring the number of populations (K), we used 100,000 Markov chain Monte Carlo MCMC) iterations, thinning interval of 100, minimum K at 1, and maximum K at 15 (equivalent to the total number of study areas, fig. 1A). We ran the MCMC five times to verify the consistency of results.

The next method we used to test for genetic barriers in northern spotted owls was Monmonier's maximum difference algorithm (Monmonier, 1973) using AIS. This procedure locates barriers to gene flow by iteratively identifying sets of contiguous, large genetic distances along a connectivity network. The steps of this analysis consist of: (1) connecting adjacent geographical positions of individuals using Delaunay triangulation (Brassel and Reif, 1979; Watson and others, 1992), resulting in a connectivity network; (2) calculating genetic distances between neighboring samples and associating these distances to each edge of the network; and (3) using Monmonier's maximum difference algorithm to identify boundaries, as described in detail elsewhere (Monmonier, 1973; Manel and others, 2003). Because there was substantial variation in geographic distances between sampled individuals, we followed the recommendation of Manni and others (2004) and performed this analysis using residual genetic distances derived from the linear regression of genetic versus geographic distances.

We then used AIS to generate a three-dimensional surface plot of genetic distance patterns across the range of northern spotted owls, termed a 'genetic landscape shape' (Miller, 2005). Unlike the GENELAND clustering algorithm or Monmonier's algorithm, this method provides a visual representation of genetic divergence across the entire landscape as opposed to only identifying distinct genetic breaks or barriers. This procedure involves three main steps: (1) construction of a connectivity network of sampled individuals and assignment of calculated interindividual genetic distances to landscape coordinates at midpoints of the connectivity edges; (2) use of an interpolation procedure (inverse distance-weighted interpolation, Watson, 1992; Watson and Philips, 1985) to infer genetic distances at locations on a uniformly spaced grid overlaid on the sampled landscape; and (3) generation of a three-dimensional surface plot where *X* and *Y* coordinates correspond to geographic locations on a rectangular grid and surface plot heights (*Z*) reflect genetic distance. Details of the procedure are provided in Miller (2005). We used several different distance weighting values ($a = 0.25–2$), grid sizes, and raw and residual genetic distances to make sure that interpretations were not sensitive to these parameters.

Our previous STRUCTURE analysis (Funk and others, 2008a) indicated that two genetic clusters (clusters 1 and 2) were unevenly represented on either side of the Columbia River, suggesting that this river might restrict gene flow. Therefore, we also performed a *post hoc* analysis of molecular variance (AMOVA, Excoffier and others, 1992) in ARLEQUIN 3.01 (Excoffier and others, 2005) to test whether separating northern spotted owl study areas into groups north versus south of the Columbia River explained a significant portion of the variance. Ten thousand permutations were used to determine significance of variance components.

Results

Standard Population Genetic Analyses

Genotypic frequencies within study areas generally corresponded to expected Hardy-Weinberg proportions. Only 7 out of 150 tests for deviations from Hardy-Weinberg proportions were significant at the $\alpha = 0.05$ level, which is less than the 7.5 ($= 0.05 \times 150$) expected to deviate by chance. No loci had a consistent excess of homozygotes, suggesting no null alleles were present. Similarly, 31 out of 665 possible tests for departure from linkage equilibrium were significant, less than the 33.25 tests expected to be significant by chance. No pairs of loci consistently departed from linkage equilibrium across study areas, thus loci were independent. Expected heterozygosity (H_E) was similar across study areas, ranging from 0.685–0.764 (mean = 0.736).

Pairwise F_{ST} values among northern spotted owl study areas ranged from 0.001–0.061. Allelic differentiation was significant at the $\alpha = 0.05$ level for 61 out of 105 (58.1%) of these pairwise comparisons after correcting for multiple tests. The correlation coefficient of the Mantel test of isolation-by-distance was only $r = 0.089$, but still statistically significant ($P = 0.001$).

Landscape Genetics

Within northern spotted owls, only one population was inferred using the clustering method implemented in GENELAND. In all five MCMC runs, support was higher for a single cluster (mean posterior probability = 65.0%) than two or more clusters (mean = 35.0%), suggesting an absence of distinct genetic discontinuities in the range of northern spotted owls.

Monmonier's maximum difference algorithm identified a circular area in the center of the southern Oregon Cascades (fig. 1*A*) as the strongest genetic barrier in the range of northern spotted owls. This area corresponds to the eastern portion of the Rogue River Valley (fig. 1*B*). The genetic landscape shape generated in AIS also revealed that a number of landscape features were associated with increased or decreased inter-individual genetic distance (fig. 2), suggesting that these features restrict or facilitate gene flow, respectively. First, genetic distance peaked in the centers of three dry, low-elevation valleys, namely the Rogue River and Umpqua River Valleys in southern Oregon (south section of fig. 2) and the Yakima River Valley near Cle Elum, Washington (northeast section of fig. 2). Second, genetic distances were high in the Cascade and Olympic Mountains (eastern edge and northwest corner of fig. 2, respectively), but low in the Oregon Coast Range (in northwest Oregon). Finally, there was an overall negative relationship between genetic distance and latitude (that is, higher genetic distances towards the south). The same general patterns were observed regardless of whether raw or

residual genetic distances were used and regardless of the choice of grid size and distance weighting parameter.

Grouping northern spotted owl study areas based on which side of the Columbia River they were on explained less than 1% of the variation in the AMOVA, although this grouping was still significant at the $\alpha = 0.05$ level (table 1). The small proportion of variation explained by this grouping suggests that the Columbia River is not an effective barrier to gene flow.

Table 1.   Results from analysis of molecular variance (AMOVA) for northern spotted owls breeding north versus south of the Columbia River.

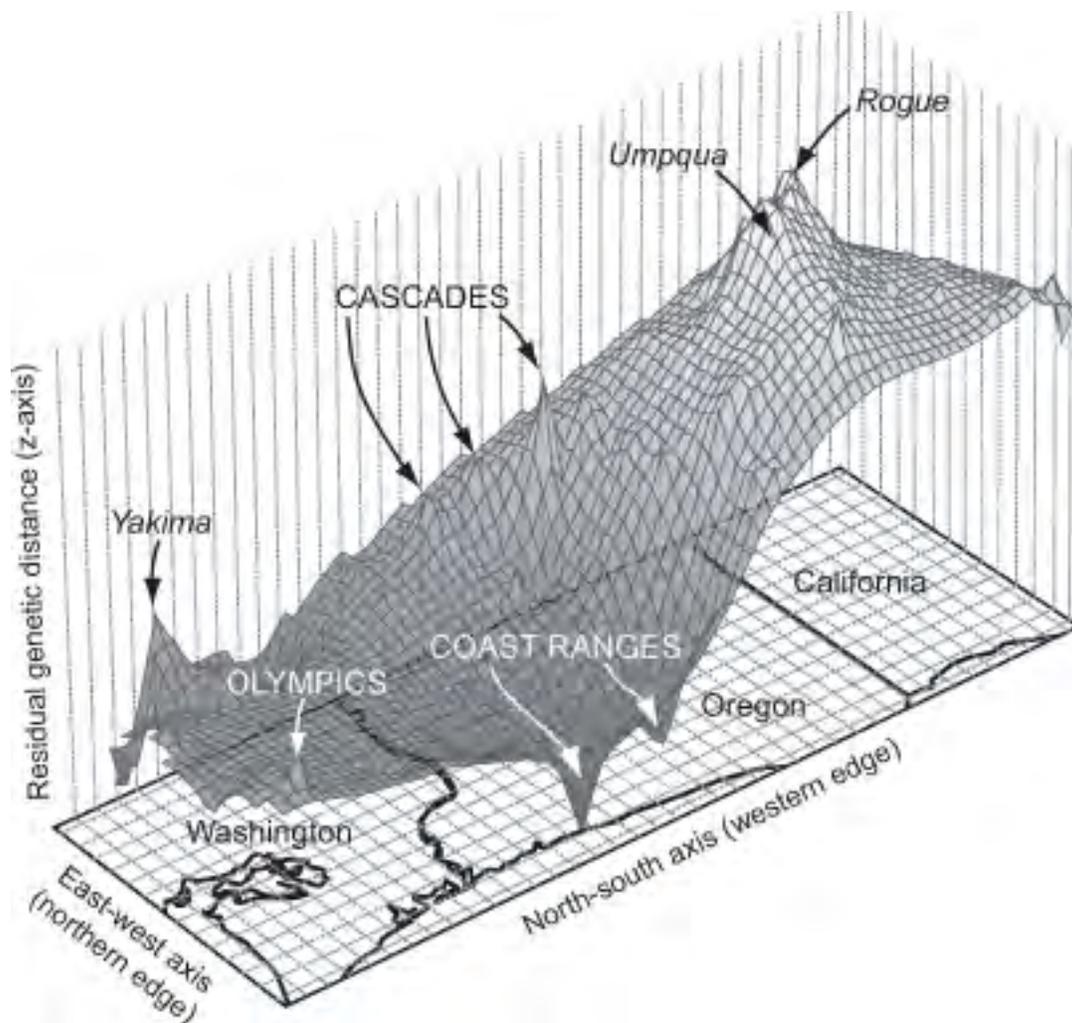| Grouping	Number of groups	Variance components	Percent of variation	*P*-value
North versus south of Columbia River	2	Among groups	0.62	0.018
		Among study areas	1.83	<0.001
		Within study areas	97.55	<0.001

Figure 2.   Results of genetic landscape shape interpolation analysis for northern spotted owls using a distance weighting parameter (*a*) of 0.25 and residual genetic distances. A 29 × 80 grid was used so that each square in the genetic landscape shape is equivalent to approximately 10 × 10 km (and each square in the base is equivalent to 20 × 20 km). *X* and *Y* axes correspond to geographic locations and surface plot heights reflect genetic distances. Higher genetic distances have lighter shading, and lower genetic distances darker shading. Qualitatively similar results were obtained using raw genetic distances, different grid sizes, and a range of distance weighting parameters (*a* = 0.25–2). Mountains (capital letters) and valleys (italicized) associated with specific genetic peaks and troughs are shown.

Discussion

Landscape Genetics of Northern Spotted Owls

We found no evidence for distinct genetic breaks in northern spotted owls, but several landscape features influenced patterns of gene flow and genetic variation. This demonstrates that even in vagile bird species such as the northern spotted owl, topography and environmental heterogeneity can shape genetic variation. The field of landscape genetics is thus relevant to a wide variety of taxa, not only dispersal-limited species such as small mammals (Miller and others, 2006); amphibians (Funk and others, 2005; Spear and others, 2005; Giordano and others, 2007; Marsh and others, 2007); reptiles (Manier and Arnold, 2006); and fish (Neraas and Spruell, 2001; Crispo and others, 2006).

Low-elevation valleys were particularly important in structuring genetic variation, especially the Rogue River and Umpqua River Valleys in southern Oregon and the Yakima River Valley on the east side of the Washington Cascades (fig. 2). Interestingly, the largest valley in the range of northern spotted owls, the Willamette Valley sandwiched between the Oregon Coast Range and Cascade Mountains in northwestern Oregon, did not have any obvious effects on genetic variation. One difference between the Willamette Valley and the other three valleys is that it receives more precipitation. The Willamette Valley receives approximately 75–200 cm of precipitation (Daly and Taylor, 1998), primarily in the form of rain, whereas the Rogue, Umpqua, and Yakima Valleys only receive 38–100 cm, 75–100 cm, and 50–75 cm, respectively. These drier valleys may act as greater physiological or behavioral barriers to northern spotted owls, which are adapted to cool, mesic forest conditions, than the relatively wet Willamette Valley. In contrast, the Willamette Valley has been shown to reduce gene flow in red tree voles (Miller and others, 2006), a prey species of northern spotted owls.

A large-scale dispersal study of northern spotted owls conducted from 1985 to 1996 found that all of these valleys, the Willamette, Rogue, Umpqua, and Yakima Valleys, acted as important barriers to dispersal (Forsman and others, 2002). This effect was most pronounced for the Willamette Valley. It is not clear why the Willamette Valley was a barrier to dispersal in the Forsman and others (2002) study, but was not a barrier to gene flow in our genetic analysis. One possible explanation is that since genetic structure is influenced by current and historical gene flow, restricted dispersal across the Willamette Valley is a new phenomenon caused by the dramatic anthropogenic alteration of the valley. Approximately 70 percent of Oregon's human population resides in the Willamette Valley (Willamette Valley Livability Forum, 1999) and the valley has been extensively modified for agriculture and urbanization since European settlement in the past century

and a half (Gibson, 1985; Robbins, 1997; Taft and Haig, 2003). Thus, lack of a genetic barrier effect of the Willamette Valley may reflect high levels of gene flow prior to European settlement. Alternatively, lack of a barrier effect may be due to owls flying around the southern end of the Willamette Valley, as observed by Forsman and others (2002), resulting in high gene flow between the Cascade Mountains and Oregon Coast Range. This seems like an unlikely explanation for lack of a barrier effect of the Willamette Valley, however, because owls are also capable of flying around the other three valleys that act as barriers (Forsman and others, 2002).

Mountains also influenced the genetic structure of northern spotted owls, but interestingly, different mountains affected genetic structure in opposite directions (fig. 2). The Cascade and Olympic Mountains were associated with increased genetic distance, thus apparently restricting gene flow. The Oregon Coast Range, on the other hand, was associated with genetic-distance troughs, indicating high levels of gene flow. We suspect that the reason that these mountains have different effects on gene flow is due to the fact that the Oregon Coast Range consists of low-elevation mountains (maximum elevation = 1,249 m) covered by forests, whereas the Cascade and Olympic Mountains are dissected by high alpine ridges and peaks (maximum elevations of 4,392 m and 2,428 m, respectively) that probably represent formidable barriers to movement. Mountains have also been shown to act as barriers to gene flow in black grouse (Caizergues and others, 2003), as well as several other vertebrate taxa (Funk and others, 2005; Cushman and others, 2006; Giordano and others, 2007).

The Columbia River did not act as a barrier to gene flow for northern spotted owls. Although the two northern spotted owl clusters previously identified by STRUCTURE were unevenly distributed across the Columbia (Funk and others, 2008a), neither the GENELAND nor AMOVA analyses here supported a barrier effect of this river. We are unaware of any study showing restricted gene flow across rivers in a temperate bird species, although there are several such examples for tropical birds (Aleixo, 2004; Hayes and Sewlal, 2004; Höglund and Shorey, 2004; Cheviron and others, 2005).

A final pattern observed in the genetic landscape shape was increasing genetic distance towards the south (fig. 2). This pattern is consistent with northward colonization following Pleistocene glacial recession, a phenomenon that is supported by previous phylogenetic studies in the Pacific Northwest (Matocq 2002, Carstens and others, 2004; Spinks and Shaffer, 2005; Steele and Storfer, 2006). This interpretation is also consistent with genetic tests for changes in effective population size in northern spotted owls, which show a strong signal of Pleistocene population expansion in Washington and a weaker signal of expansion in Oregon (Funk and others, 2008b). The negative relationship between inter-individual

genetic distance and latitude seen here in northern spotted owls mirrors a similar negative relationship between inter-population genetic distance and latitude observed among many taxa on a global scale (Martin and McKay, 2004).

A limitation of the genetic landscape shape analysis conducted in AIS is that it is largely descriptive and only identifies geographic areas of low and high inter-individual genetic divergence. It does not explicitly test the effects of specific landscape features on genetic structure. Thus, the current study should be treated as a coarse-scale foundation for future, finer-scale research including additional samples. Sampling focused around specific landscape features (e.g., valleys and mountains) would allow dissection of the effects of specific landscape layers (e.g., forest cover, rivers, and topography) on genetic structure using an explicit hypothesis-testing framework (Cushman and others, 2006).

Conservation Implications

Lack of evidence for genetic discontinuities in the range of northern spotted owls suggests that the subspecies represents one genetic population or deme. Nonetheless, geographically separated parts of the subspecies' range may function as demographically independent populations, because only a few dispersers per generation are sufficient to homogenize gene pools (Wright, 1931; Slatkin, 1985; Mills and Allendorf,1996). In addition, minimal genetic structure in neutral markers such as microsatellites does not indicate lack of adaptive genetic differences across the subspecies' range. Adaptive differences can be maintained with ongoing gene flow (Haldane, 1930, Lewontin and Krakauer, 1975). Substantial variation in life histories and ecology has been documented across the range of northern spotted owls (Gutiérrez and others, 1995), and it is likely that at least some of this variation is genetically based and adaptive. Maintaining this variation is important for persistence in the face of environmental change.

The finding that high-elevation mountains and dry valleys restrict gene flow is also relevant for conservation. In particular, this result suggests that dispersal is similarly restricted by these landscape features. Thus, northern spotted owls separated by mountain ridges and valleys may be less demographically connected, have fewer opportunities for gene flow from other populations, and consequently be at higher risk of local extinction. For northern spotted owls, areas that appear to be isolated from the rest of the subspecies' range by mountains or valleys include the eastern Cascades, the Olympic Peninsula, and the eastern edge of the Rogue River Valley. Finer-scale genetic analyses with additional samples will help determine the degree to which these and other areas are isolated by topography and habitat breaks.

Conclusions

Our analysis of the genetic structure of northern spotted owls across most of the range of the subspecies allowed us to test for genetic discontinuities and identify landscape features that influence the subspecies' genetic structure. Although no distinct genetic breaks were found in northern spotted owls, several landscape features were important in structuring genetic variation. Dry, low elevation valleys and the high elevation Cascade and Olympic Mountains restricted gene flow, while the lower Oregon Coast Range facilitated gene flow, acting as a "genetic corridor." The Columbia River did not act as a barrier, suggesting owls readily fly over this large river. Thus, even in taxa such as northern spotted owls with potential for long-distance dispersal, landscape features can have an important impact on gene flow and genetic structure.

Acknowledgments

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