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Spotted owl (*Strix occidentalis*) microsatellite variation in California

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SPOTTED OWL (*STRIX OCCIDENTALIS*)
MICROSATELLITE VARIATION IN CALIFORNIA

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Andrea L. Henke

May 2005

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ABSTRACT

SPOTTED OWL (*STRIX OCCIDENTALIS*) MICROSATELLITE VARIATION IN CALIFORNIA

By Andrea Henke

Genetic diversity and population structure were characterized for four spotted owl (*Strix occidentalis*) populations present in California using six microsatellite loci derived from the Mexican spotted owl (*S. o. lucida*) and optimized for Northern (*S. o. caurina*) and California (*S. o. occidentalis*) spotted owls. Modified protocols to extract feather DNA and sex owls are described. Populations isolated along coastal ranges were less diverse and more divergent than populations sampled within interior ranges. The coastal population of *S. o. occidentalis* showed extremely low genetic diversity, implying a past bottleneck or founding event. Equilibrium deviations within *S. o. occidentalis* in the Sierra Nevada suggested inbreeding and/or population subdivision. A large genetic divergence was detected between *S. o. caurina* and *S. o. occidentalis*, although there was evidence for subspecies mixing in the interior populations. Microsatellite results supported current subspecies taxonomy, with evidence of limited gene flow between subspecies and among all populations sampled.

APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

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Andrea Henke

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	vii
LIST OF FIGURES.....	ix
FULL ABSTRACT.....	1
INTRODUCTION.....	3
METHODS.....	5
RESULTS.....	12
DISCUSSION.....	23
REFERENCES.....	35
APPENDICES	
A. MICROSATELLITE ALLELE LENGTHS.....	40
B. FEATHER COLLECTION PROTOCOL.....	41

LIST OF TABLES

	<i>Page(s)</i>
TABLE 1. Spotted owl (<i>Strix occidentalis</i>) sampling.....	5
TABLE 2. Genetic diversity indices of observed heterozygosity (H_O), gene diversity (H), inbreeding values (F_{IS}), allele size ranges in each subspecies (SR), and number of observed alleles (A) for six microsatellite loci.....	13
TABLE 3. Population differentiation based on F_{ST} and R_{ST}	17
TABLE 4. Bayesian modeling with population inference from the genotypes of sampled individuals.....	20
TABLE 5. Results of cluster analysis with prior population information showing the proportional membership of each predefined population in inferred genetic clusters I - IV.....	21
TABLE 6. Inferred ancestry probabilities for 13 spotted owls.....	21
TABLE 7. Subspecies assignment tests.....	22

LIST OF FIGURES

	<i>Page(s)</i>
FIGURE 1. Spotted owl (<i>Strix occidentalis</i>) sampling areas in California. (01) Monterey County, (02) El Dorado National Forest, (03) Marin County, (04) Mendocino National Forest.....	6
FIGURE 2. Comparison of allelic frequency variation among (A) <i>S. o. caurina</i> populations and (B) <i>S. o. occidentalis</i> populations.....	16
FIGURE 3. Neighbor-joining tree of two Northern spotted owl (NSO) populations and two California spotted owl (CSO) populations based on Nei's D_A	18
FIGURE 4. Unrooted neighbor-joining tree of individual owls based on Nei's D_A .	19
FIGURE 5. Spotted owl subspecies unique alleles.....	20

FULL ABSTRACT

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Key words: microsatellite, endangered species, feather DNA, conservation genetics, population bottleneck, hybridization, spotted owl, *Strix occidentalis*

Genetic diversity and population structure were characterized in four populations of spotted owls (*Strix occidentalis*) present in California using six microsatellite loci derived from the Mexican spotted owl (*S. o. lucida*) and optimized for Northern (*S. o. caurina*) and California (*S. o. occidentalis*) spotted owls. Genetic samples were obtained non-invasively by collecting naturally molted feathers at owl nest and roost sites. Modified protocols to extract feather DNA and sex owls are described.

Populations isolated along coastal ranges were less diverse and more divergent than spotted owl populations sampled within interior mountain ranges. Effective population sizes (N_E) estimated for coastal populations were low, and genetic variation was especially low for *S. o. occidentalis*, although both subspecies showed reduced genetic diversity compared to *S. o. lucida*. There were large F_{ST} values between coastal and interior *S. o. occidentalis* populations (27%), and also evidence for restricted gene flow between *S. o. caurina* populations (9%). The coastal population of *S. o. occidentalis* showed extremely low genetic diversity (with two of six loci fixed) implying a past bottleneck or founding event. Equilibrium deviations in the Sierra Nevada population of *S. o. occidentalis* suggested inbreeding and/or population subdivision. A large genetic

divergence (35%) was detected between *S. o. caurina* and *S. o. occidentalis*, although there was some evidence for subspecies mixing in the interior populations.

Microsatellite results supported current subspecies taxonomy, with evidence of limited gene flow between subspecies and among all populations sampled.

INTRODUCTION

In this study, microsatellite markers designed for the Mexican spotted owl (*Strix occidentalis lucida*) are used to examine gene flow and genetic variation in both subspecies of spotted owl present in California (Thode *et al.* 2002). Although microsatellite DNA primer sequences have been published for several species of owl, including Mexican spotted owls, boreal owls, burrowing owls, eagle owls, and Lanyu scops owls (Thode *et al.* 2002; Isaksson & Tegelström 2002; Korfanta *et al.* 2002; Hsu *et al.* 2003; Koopman *et al.* 2004), this is the first use of microsatellite markers to assess genetic parameters in California (*S. o. occidentalis*) and Northern spotted owls (*S. o. caurina*). Population genetic analysis with microsatellite markers can infer (1) the distribution of genetic variation within and among sampled populations, (2) the extent of population subdivision, (3) taxonomic divisions, (4) historical processes such as bottlenecks or founder's effects, (5) deviations from equilibrium conditions caused by inbreeding, drift, and/or fragmentation, and (6) the presence of introgressive hybridization (Schlötterer & Pemberton 1998). This study expands upon previous genetic research by using non-invasive sampling and microsatellite genotyping to genetically characterize populations as well as subspecies. This is one of the first applications of microsatellite markers to the study of conservation genetics in any *Strigiform* species worldwide.

Molecular markers have revealed extremely low levels of genetic diversity within spotted owls compared to most other avian taxa (Avice 2004). Assays of 23 allozyme loci in 107 owls representing all three subspecies (Barrowclough & Gutiérrez 1990) only revealed a single variable locus in the Mexican spotted owl. Similarly, screening of 400 RAPD primers in the Northern spotted owl only detected 13 polymorphic primer sets (Haig *et al.* 2001). Barrowclough *et al.* (1999) and Haig *et al.* (2004a) found that mitochondrial DNA haplotype diversity varied among subspecies, with California spotted owls displaying fewer haplotypes than either the Mexican or the Northern subspecies.

The three subspecies of spotted owl that occur in North America all show evidence of population declines (LaHaye *et al.* 1994; Franklin *et al.* 1999; Seamans *et al.* 1999; Blakesley *et al.* 2001; Seamans *et al.* 2001; Anthony *et al.* 2004). The Northern spotted owl and the Mexican spotted owl are federally listed as threatened species. The California spotted owl was denied listing status under the U.S. Endangered Species Act in 2003, although its status is currently under review by the U.S. Fish & Wildlife Service. Recent genetic research has suggested subspecies hybridization, with California spotted owl mitochondrial haplotypes identified in Northern spotted owls sampled from northwestern California (Barrowclough *et al.* 1999, Haig *et al.* 2004a). The California spotted owl is found inland throughout the Sierra Nevada range and is distributed along isolated coastal mountain ranges stretching south from Monterey Bay. The southern limit of the Northern spotted owl's range is north of San Francisco Bay in

Marin County. Inland, the defined ranges of the two subspecies nearly overlap in Shasta County, where neighboring populations of each subspecies are separated by less than 50 km, forming a subspecies contact zone (Gutiérrez & Barrowclough 2005). Research on Northern spotted owls suggests that owls will disperse across mosaics of fragmented habitats and that 10% of juvenile owls will disperse farther than 50 km (Forsman *et al.* 2002). Notably, recent morphological studies on museum specimens were unable to differentiate the California from the Northern subspecies based on a combination of eight diagnosable characters (Courtney *et al.* 2004). Clinal variation may be a factor, with evidence for subspecies clines in plumage coloration and pattern (Barrowclough & Flesness 1996).

METHODS

Sampling Areas

Northern spotted owls ($N = 74$) were sampled at the southern limits of their range within the Mendocino National Forest and in Marin

Table 1. Spotted Owl (*Strix occidentalis*) sampling.

Subspecies/Population	Area (km ²)	# of Territories	Years	<i>N</i>
<i>S. o. caurina</i>				
Mendocino	400	21	02-03	36
Marin	120	25	99-00	38
<i>S. o. occidentalis</i>				
Monterey	275	31	98-01	38
El Dorado	250	25	00-01	41

County (Figure 1, Table 1). The Marin coastal population is dense and relatively isolated from other owl populations. In contrast, the Mendocino interior population has an uneven distribution and is located at the southern end of a continuous forested region stretching northward towards the Klamath basin (Figure 1).

California spotted owls ($N = 79$) were sampled in the El Dorado National Forest and along the Pacific coast in Monterey County (Figure 1, Table 1). Within the California coastal ranges, Monterey is near the northern end of a series of ~20 small, isolated populations with estimated carrying capacities varying from six (San Luis Obispo area) to 266 (Los Angeles area). Monterey population carrying capacities were estimated as 100 owls in the northern region and 60 owls further south (LaHaye *et al.* 1994)

Figure 1. Spotted Owl (*Strix occidentalis*) sampling areas in California.
(01) Monterey County, (02) El Dorado National Forest, (03) Marin County, (04) Mendocino National Forest.

Feather Sampling

In areas with historical detections, spotted owl territories were night surveyed to locate owls. Owls were re-located during the day at day roosts and/or nest sites and aged as juvenile, subadult, or adult (Pyle 1997). Non-invasive genetic sampling methods were followed, with the exception of Marin which also included feathers plucked from owls during banding. Over 1500 naturally molted feather samples were collected from spotted owl nesting and roosting areas and stored individually in labeled envelopes at room temperature. Care was taken to

collect feathers in core owl areas directly under known day roosts or nests. Some feathers were collected directly from roosting owls as they molted.

Molted feathers collected from owl territories containing breeding pairs were divided into adult and juvenile patterned samples (if collected before ~ June/July). If several related individuals from the same territory were fingerprinted, no siblings or parent-offspring combinations were used in the analysis. Fifty-six juvenile and parental pedigrees from 32 parent-offspring pairs were compared to confirm alleles and Mendelian inheritance patterns. Alleles were also re-amplified from separate feather samples collected at the same owl site to corroborate individual DNA fingerprints. Nearly all owls were sexed by DNA analysis. DNA sexing was required to consistently sample both members of a territorial pair from collections of molted feathers. For comparison, a single barred owl (*Strix varia varia*) from Marin County was also genotyped.

Microsatellite Analysis from Feather Samples

A standard PureGene Tissue DNA Isolation Kit protocol (Gentra Systems) was modified to maximize DNA yield from feathers as follows: Cold cell lysis solution (0.3 ml) containing 20 mg/ml polyvinylpyrrolidone 40 (Sigma) was added to ~1 cm of dried calamus (cut in half). The tissue was crushed with a plastic pestle for 2 min. After adding 1.5 µl of 20 mg/ml Proteinase K, samples were incubated at 58°C for 3.5 h, at 65°C for 15 min, and then iced. After adding cold protein precipitation solution (0.1

ml), the samples were vortexed and incubated on ice for 30 min. The supernatants were centrifuged (13,000xg) at 4°C for 10 min and transferred to siliconized 1.5 ml tubes. Equal volumes of cold isopropanol and 1 µl of 20 mg/ml glycogen was added and the samples were stored overnight at -20°C. Pellets were washed with 70% ethanol, air-dried for 10 min, and dissolved in 20 µl of DNA hydration solution.

Amplification conditions for six microsatellite loci (15A6, 8G11, 13D8, 1C6, 4E102, and 6H8) characterized from the Mexican spotted owl by Thode *et al.* (2002) were optimized for Northern and California spotted owls. PCR reactions (20 µl) contained 1X PCR Buffer II (Applied Biosystems), 1.25 U of AmpliTaq Gold (Applied Biosystems) and 0.2 mM each dNTP (except for locus 4E102 which used 0.3 mM). Primer and magnesium concentrations varied by locus: 15A6 (1.5 mM MgCl₂, 0.5 µM primer), 8G11 (1.75 mM MgCl₂, 0.5 µM primer), 13D8 (2.0 mM MgCl₂, 0.5 µM primer), 1C6 (2.25 mM MgCl₂, 0.5 µM primer), 4E102 (2.25 mM MgCl₂, 0.75 µM primer), and 6H8 (2.5 mM MgCl₂, 1.0 µM primer).

Loci were amplified using an Eppendorf Mastercycler Gradient thermal cycler. An initial polymerase activation step of 93°C (9 min) was followed by 35 cycles of 94°C (30 s), optimized annealing temperatures (45 s), and 72°C (45 s) for all loci except 4E102 which had annealing and extension times of 60 s. A final extension was performed at 72°C for 6 min. Optimized annealing temperatures were 52°C (6H8), 53°C (1C6, 8G11), 55°C (4E102), and 56°C (13D8, 15A6). PCR products (alleles)

were resolved on 6.5% polyacrylamide gels with Spreadex™ polymer (Elchrom Scientific) and their sizes estimated by comparison with M3 markers (Elchrom Scientific).

Sex Determination

DNA samples were tested to determine the sex of each owl by amplifying a portion of the sex-linked CHD gene present in all avian species using a modification of the method of Griffiths *et al.* (1998). The published P2 and P8 primers did not amplify the CHD-W and CHD-Z genes in spotted owls consistently, so both primers were modified based on the boreal owl (*Aegolius funereus*) CHD sequences (Fridolfsson & Ellegren 2000, GenBank AF181824, AF181825). PCR reactions (20 µl) contained 1X PCR Buffer II, 1.25 U AmpliTaq Gold, 0.3 mM each dNTP, 2.5 mM MgCl₂, and 0.75 µM each modified primer (P2Owl: 5'-ATCTCTGCATCRCTAAATCC-3'; P8Owl: 5'-TCCCAAGGATGAGAAACTG-3'). PCR conditions mimicked microsatellite 4E102, except with an annealing temperature of 48°C. As Griffiths *et al.* (1998) discovered for the tawny owl (*Strix aluco*), it was necessary to restrict the 380 bp PCR products with *Hae*III (Promega) in order to discriminate similarly sized introns in female owls. Females (ZW) produced a two-band restriction pattern (310 and 380 bp) while males (ZZ) produced a one-band pattern (310 bp) when electrophoresed on 2.5% agarose gels.

Statistical Analysis

Allelic frequencies, heterozygosity estimates, and the expected number of alleles under two microsatellite mutation models were calculated from MICROSatellite ANALYZER (MSA, Dieringer & Schlötterer 2002). Individual microsatellite fingerprints were compared within populations using CERVUS (Marshall *et al.* 1998). Exact Hardy-Weinberg (HW) tests were run on all six loci for each population with GENEPOP 3.1 (Raymond & Rousset 1995). Inbreeding coefficients (F_{IS}) were calculated in GENEPOP (Weir & Cockerham 1984). Differences in allele distribution among populations were tested in GENEPOP using Fisher's exact test.

Pairwise F_{ST} (Weir & Cockerham 1984) and R_{ST} (Michalakis & Excoffier 1996) were calculated in GENEPOP. Although both statistics measure population subdivision based on variance, F_{ST} uses the variance in allele frequencies, while R_{ST} measures are based on the variance in repeat number. Both F_{ST} and R_{ST} can infer average levels of gene flow, but R_{ST} is subject to bias when sample sizes are low (< 50) and few loci are scored (Gaggiotti *et al.* 1999; Balloux & Goudet 2002). A procedure to estimate the contribution of stepwise mutations to population differentiation was followed as described in Hardy *et al.* (2003). Subspecies divergence was measured by R_{ST} statistics. All genetic distance measures reported are from MSA. N_m estimation was based on the private alleles method in GENEPOP. Neighbor-joining phylograms were constructed from the microsatellite data (POPULATIONS 1.2.28; O. Langella) using Nei's D_A chord distance matrix option.

Bayesian modeling to infer population structuring was completed in STRUCTURE 2.1 (Pritchard *et al.* 2000; Falush *et al.* 2003). Cluster modeling approaches are different from standard genetic distances because they function by inferring parameters that can best describe a given data set. Modeling parameters in a population allows for hypothesis testing. Bayesian clustering procedures were followed to infer the extent of genetic structuring among subspecies and populations and to test assignment of a single *S. varia* sample. All results were based on runs of 10^6 iterations with a burn-in period of 15,000. Two reciprocal assignment tests were analyzed at the subspecies level: (1) Northern spotted owls were assigned to a single taxon (USEPOPINFO=1), and California spotted owls were clustered without a designated population of origin, and (2) California spotted owls were assigned to a single taxon (USEPOPINFO=1), while Northern spotted owls clustered.

The program BOTTLENECK tested all four spotted owl populations for a recent decline in their effective population sizes, N_E (Cornuet & Luikart 1996). The Two-Phase Model (TPM) of microsatellite evolution is based on the Stepwise Mutation Model (SMM) but allows for some multi-step mutations (DiRienzo *et al.* 1994). A 70% TPM mutation model was selected based on precedent (Hoelzel *et al.* 2002) and the following empirical observation. When the number of alleles observed for each locus was compared with the expected number of alleles under both the SMM and Infinite Alleles Model (IAM), the observed allele number within each population corresponded better to

the expected allele count under the SMM in 68% of the comparisons, with the remainder falling under the IAM.

A linkage disequilibrium (D) approach was used as a single-generational estimate of effective population size (N_E) (Hill 1981). This method measures the correlation (r) among alleles at different loci in a population of sample size (s) according to the formula $N_E(D) = 1/[3 \times (r^2 - 1/s)]$. Random-mating, infinite, idealized populations show no correlation, but real populations have positive values of D due to drift, migration, selection, and linkage. The small and isolated coastal populations of Marin and Monterey approximate island models, and the assumptions necessary to estimate N_E from D were more closely adhered to (*i.e.* neutral alleles, no migration, no subpopulation structure, and random sampling of the entire population). The program N_E ESTIMATOR (Molecular Fisheries Lab, Queensland) calculated D and associated confidence intervals from population allelic frequency data.

RESULTS

Genetic Diversity

Allele size ranges varied between subspecies at four of six loci (Table 2). All six microsatellite loci were polymorphic in three of four populations sampled. In Monterey, two of the loci (1C6 and 8G11) were fixed for a single allele (Table 2, Figure 2). Microsatellite 1C6 showed low polymorphism in both subspecies. Across all loci, a

Table 2. Genetic diversity indices of observed heterozygosity (H_o), gene diversity (H), inbreeding values (F_{IS}), allele size ranges in each subspecies (SR), and number of observed alleles (A) for six microsatellite loci.

California Spotted Owl																		
Northern Spotted Owl																		
Locus	Interior: Mendocino				Coastal: Marin				Interior: El Dorado				Coastal: Monterey					
	H_o	H	F_{IS}	A	SR	H_o	H	F_{IS}	A	H_o	H	F_{IS}	A					
6H8	0.83	0.82	-0.01	7	94-118	0.84	0.75	-0.13	6	0.44	0.51	+0.14	5	94-118	0.53	0.47	-0.13	2
15A6	0.58	0.68	+0.14	4	146-162	0.42	0.39	-0.08	4	0.63	0.77	+0.17	5	146-162	0.53	0.54	+0.03	4
1C6	0.31	0.27	-0.12	3	112-124	0.13	0.13	-0.05	3	0.53	0.45	-0.16	4	112-127	0.00	0.00	0.00	1
8G11	0.81	0.78	-0.04	7	138-166	0.53	0.47	-0.12	5	0.61	0.68	+0.10	5	146-162	0.00	0.00	0.00	1
4E102	0.72	0.83	+0.13	8	214-249	0.70	0.75	+0.07	5	0.59	0.66	+0.11	6	214-239	0.40	0.55	+0.27	4
13D8	0.72	0.83	+0.13	9	171-207	0.76	0.74	-0.03	5	0.66	0.73	+0.09	7	175-199	0.30	0.26	-0.15	3
Mean	66%	70%	+0.04	6.3	94-249	56%	54%	-0.06	4.7	58%	63%	+0.08	5.3	94-239	29%	30%	+0.01	2.5

total of 41 alleles were detected, with a minimum of 15 in Monterey and a maximum of 38 in Mendocino. The mean number of alleles found in each population was higher for interior populations compared to coastal populations (Table 2). Of the four areas sampled, alleles unique to a population were present only in El Dorado (2) and Mendocino (7). Overall, 93% of all alleles observed were present in Mendocino, 78% in El Dorado, and 68% in Marin. In contrast, spotted owls in Monterey were much less diverse, only displaying 37% of the total alleles sampled (Figure 2). All owls analyzed displayed unique individual fingerprints within populations in identity tests except for several spotted owls in Monterey (Marshall *et al.* 1998). Probability of identity (*PID*) values were 2.3% (Monterey) and 0.0042% (El Dorado) for the California subspecies, and 0.00018% (Mendocino) and 0.015% (Marin) for the Northern subspecies.

Observed heterozygosity values (H_o) at six loci ranged from 13 - 84 % in the Northern spotted owl, and from 0 - 66% in the California spotted owl (Table 2). Within subspecies, gene diversity (H) was 30% higher in Mendocino (0.70) compared to Marin (0.54), and 108% higher in El Dorado (0.63) compared to Monterey (0.30). Genetic diversity (measured by allelic diversity and heterozygosity) was lowest in Monterey and highest for Mendocino with the larger populations of Mendocino and El Dorado more genetically diverse than the smaller, more restricted coastal populations of Marin and Monterey.

Hardy-Weinberg exact tests were performed to determine if the observed genotypic frequencies matched those expected under HW equilibrium conditions. HW probability tests relying on a Markov chain showed all six loci to be in equilibrium in all populations. Results for all loci were insignificant ($P > 0.05$), before adjustments for the number of pairwise comparisons were applied. Global exact tests, run separately for each population, showed a multilocus deviation from HW equilibrium in El Dorado with a significant excess of homozygous individuals ($P \leq 0.001$). Inbreeding coefficients (F_{IS}) showed positive trends (indicating an excess of homozygotes) at five loci in El Dorado, and primarily negative trends (indicating an excess of heterozygotes) at five loci in Marin (Table 2). Global tests failed to show a heterozygote excess in any population.

Population Genetic Structure

Allele distributions (Figure 2) were highly significantly different ($P \leq 0.0001$; Fisher's exact test) at three loci (6H8, 15A6, and 8G11) among all populations. For Northern spotted owl populations, locus 13D8 was only moderately differentiated ($P < 0.05$), while for 1C6 and 4E102 the allelic distributions were not statistically different. Monterey and Marin showed a lack of allelic differentiation at 1C6, although this is likely an artifact of reduced polymorphism at this locus in both populations.

Results of allele size permutation testing were insignificant for population comparisons at multilocus or single locus levels, indicating that allele size is uninformative for

(A) NORTHERN SPOTTED OWL POPULATIONS

(B) CALIFORNIA SPOTTED OWL POPULATIONS

Figure 2. Comparison of allelic frequency variation among (A) *S. o. caurina* populations and (B) *S. o. occidentalis* populations. Alleles are designated by letters and corresponding loci are indicated below. Arrows represent alleles that were not found in coastal populations. Asterisks denote alleles present in only one subspecies. Allele lengths are given in Appendix A.

discerning population differentiation. Therefore pairwise F_{ST} values were favored over R_{ST} for analysis at the population level (Hardy *et al.* 2003). For subspecies comparisons, multilocus R_{ST} estimates and the R_{ST} value for locus 6H8 showed a significant contribution of stepwise mutations to subspecies differentiation. This suggests that microsatellite genetic differentiation at the subspecies level is dependent on stepwise mutation processes and that the roles of drift and/or migration are less significant than mutation.

Evidence of reduced gene flow was present for all four spotted owl populations sampled (Table 3). Northern spotted owl

Table 3. Population differentiation based on F_{ST} (lower left matrix) and R_{ST} (upper right matrix).

	<i>California Spotted Owl</i>		<i>Northern Spotted Owl</i>	
	MONTEREY	EL DORADO	MENDOCINO	MARIN
MONTEREY	---	0.152	0.288	0.535
EL DORADO	0.266	---	0.172	0.407
MENDOCINO	0.278	0.106	---	0.055
MARIN	0.453	0.199	0.090	---

populations showed the smallest genetic divergence ($F_{ST} = 9\%$), while California spotted owl populations showed considerable allelic frequency changes ($F_{ST} = 27\%$). Comparisons across subspecies generated larger divergence values, with the two coastal populations highly differentiated ($R_{ST} = 54\%$).

Subspecies Differentiation

On average, a 35% genetic divergence (averaged across R_{ST} values) was detected between subspecies (Table 3). Distance measures estimated a subspecies divergence of 23% based on chord distance (D_A , Nei *et al.* 1983) and 48% based on the proportion of

shared alleles (D_{PS} , Bowcock *et al.* 1994). Neighbor-joining trees based on Nei's D_A showed strong support (99%) for the separation of Northern and California spotted owls (Figure 3). The coastal populations showed more divergence than the interior populations (indicated by longer branch lengths). When individual owls were displayed in an unrooted radial tree (Figure 4), the four populations were clearly defined, with a strong branching pattern in El Dorado and minimal differentiation in Monterey. Some owls in Mendocino were associated with the Marin population (represented by asterisks), although only 12 owls clustered in different populations from where they were sampled.

Figure 3. Neighbor-joining tree of two Northern Spotted Owl (NSO) populations and two California Spotted Owl (CSO) populations.

This phylogeny is based on Nei's D_A and shows 99% bootstrapping values after 1000 replications.

Figure 4. Unrooted neighbor-joining tree of individual owls based on Nei's D_A .

Both subspecies are labeled according to sampling area with (E) = El Dorado; (V) = Monterey; (M) = Mendocino; and (G) = Marin. A single Barred Owl (BO) is also shown. Owls that do not cluster with their respective populations are indicated by asterisks.

Several alleles were unique to each subspecies, with two alleles detected only in *S. o. occidentalis*, and nine alleles found strictly in *S. o. caurina* (Figure 5). The number of migrants per generation (N_m) between subspecies was estimated as 0.83 by the private alleles method (Slatkin 1985). This approximation should be interpreted cautiously because it is subject to sampling error and to various simplifying assumptions usually violated in natural populations (Whitlock & McCauley 1999).

Figure 5. *Spotted Owl subspecies unique alleles.* Asterisks indicate nearly private alleles.

Table 4. *Bayesian modeling.*

Population inference from the genotypes of sampled individuals. Each value of K populations is inferred from the genetic data.

K	Log $P(X/K)$	$P(K/X)$
1	-2553.6	~0
2	-2209.2	~0
3	-2403.7	~0
4	-2010.8	~1
5	-2058.6	~0

$P(K/X)$ represents the probability that the model fits the data.

Genetic Structuring from Bayesian Modeling

The number of total populations (K) represented by the raw genetic data was inferred following

procedures described in Pritchard *et al.* (2000).

The results of each clustering run without prior

population information (USEPOPINFO=0) at

different assumed number of populations ($K = 1-5$)

inferred four genetically distinct populations (from pooled genotypes) corresponding to the four geographic sampling areas (Table 4). If spotted owls were panmictic, a single cluster belonging to a single population ($K = 1$) would have resulted from the allelic frequency data. Instead, spotted owls showed evidence of genetic subdivision among all populations sampled. Of 151 spotted owls tested, only 19 owls were assigned a high probability (>25%) of membership (or ancestry) in a different population from where

they were located at the time of DNA sampling. All other owls formed genetic groupings corresponding to their populations of origin.

Next, individual owls were assigned to one of four geographic areas depending on both genetic *and* sampling locality information. Results of the clustering analysis using prior population information (USEPOPINFO=1) assigned most owls to their population of origin (Table 5). Owls

Table 5. Results of cluster analysis. Bayesian modeling with prior population information (USEPOPINFO=1) showing the proportional membership of each predefined population in inferred genetic clusters I - IV.

POPULATION	<i>S. o. occidentalis</i>		<i>S. o. caurina</i>	
	I	II	III	IV
Monterey	0.998	0.001	0.001	0.000
<i>S. o. occidentalis</i>				
El Dorado	0.005	0.982	0.009	0.004
<i>S. o. occidentalis</i>				
Mendocino	0.004	0.002	0.981	0.014
<i>S. o. caurina</i>				
Marin	0.000	0.002	0.005	0.994
<i>S. o. caurina</i>				

assigned to multiple areas have a probability of admixture, or ancestry in more than one

Table 6. Inferred ancestry probabilities for 13 Spotted Owls.

Each owl shows a 95% or lower probability of full ancestry exclusively from the population in which the owl was sampled. Admixture probabilities are split between the present and parental generations.

Owl	Sex	Age	Origin Population	Ancestry in Origin Population (%)	Sampled Generation: Inferred Immigrant Ancestry (%)	Parental Generation: Inferred Immigrant Ancestry (%)
<i>California Spotted Owls</i>						
SW-1	♂	A	Monterey (V)	93	---	3.5 (M) 3.5 (E)
19-3*	♀ ^C	A	El Dorado (E)	95	---	5 (G)
45-8	♀ ^C	A	El Dorado (E)	32	10 (M)	36 (M) 22 (G)
83-13	♂	A	El Dorado (E)	86	8 (V)	6 (V)
115-10*	♂	A	El Dorado (E)	95	---	2.5 (M) 2.5 (G)
<i>Northern Spotted Owls</i>						
644*	♀ ^N	A	Mendocino (M)	93	1 (G)	2 (E) 4 (G)
459*	♀ ^C	A	Mendocino (M)	92	---	8 (G)
109	?	A	Mendocino (M)	90	5 (G)	5 (G)
62	♂	SA	Mendocino (M)	76	---	24 (V)
262*	♂	A	Mendocino (M)	76	18 (G)	6 (G)
541*	♂	A	Mendocino (M)	94	---	6 (G)
BP-6	♀	A	Marin (G)	86	1 (M)	11 (M) 2 (E)
HT-3	♀	A	Marin (G)	94	---	2 (M) 4 (E)

(A) = Adult; (SA) = Subadult; (*) Indicates that the owl did not cluster with the population it was sampled from in Figure 4; (C) Indicates that the owl carried a California Spotted Owl mitochondrial haplotype; (N) Indicates that the owl carried a Northern Spotted Owl mitochondrial haplotype (T. Chi, unpubl).

population, represented by q (defined as the proportion of membership in an inferred cluster). Thirteen owls showed small probabilities of recent ancestry in other areas (GENESBACK=1; MIGRPRIOR(v)=0.05; $K=4$; Table 6).

Table 7. *Subspecies assignment tests.*

	<i>S. o. occidentalis</i>	<i>S. o. caurina</i>	To test genetic differences among subspecies, Northern spotted owls were
NORTHERN SPOTTED OWL ASSIGNMENT TEST	I	II	
<i>S. o. caurina</i>	0.161	0.839	
<i>S. o. occidentalis</i>	0.980	0.020	
CALIFORNIA SPOTTED OWL ASSIGNMENT TEST	I	II	
<i>S. o. occidentalis</i>	0.604	0.396	
<i>S. o. caurina</i>	0.006	0.994	

designated as a single taxon while California spotted owls were assigned to one of two possible genetic clusters ($K = 2$). The test was repeated for California spotted owls (designated as a single group) while the Northern subspecies was assigned. Assignment results are reported in Table 7 and show higher q values associated with Northern subspecies assignments (0.839) compared to California subspecies assignments (0.604).

Bottlenecks and Effective Population Size (N_E)

Bottlenecks are severe, temporary reductions in population size. As individuals are removed from a population, allelic diversity is reduced faster than heterozygosity. This leads to an increase in H_O over H_E , under an assumption of mutation-drift equilibrium (Cornuet & Luikart 1996). Bottlenecks were not detected in either coastal population, although the power to detect a bottleneck in Marin was low (~11%). Statistical power in Monterey was greater (~64%) but the ability to detect an effect was limited by low polymorphism. The probability of deviation from mutation-drift equilibrium in

Monterey was low but insignificant under the Wilcoxon test. Statistical power in Mendocino (~100%) was greatest and the Mendocino population did show a significant excess of heterozygotes ($P < 0.05$; $n = 36$), suggesting a recent bottleneck event. However, only the Wilcoxon sign-rank test (Luikart *et al.* 1998) was significant, with sign and mode-shift tests not showing an effect (Cornuet & Luikart 1996). This result may be artificial and possibly influenced by recent immigration of hybrids and/or owls from genetically divergent populations (Cornuet & Luikart 1996). Additionally, the range of mitochondrial haplotypes found in Mendocino appears to be inconsistent with a bottleneck hypothesis (T. Chi, unpubl.).

Estimates of a single-generation N_E for the two coastal spotted owl populations were both relatively low based on linkage disequilibrium calculations. Both populations are small and likely contain fewer than 200 owls. The Monterey effective population size was estimated between 13 and 393 owls, with a most likely value of 32 ($D = 3.72$). For the Northern subspecies in Marin, N_E was 37 ($D = 3.53$) with confidence intervals of 22 to 81 owls.

DISCUSSION

Northern Spotted Owls

The two populations of Northern spotted owls were genetically differentiated, implying restricted gene flow (Figure 2A). Mitochondrial control region sequences also show

distinct haplotype distributions in the same populations, indicating relatively divergent gene pools (T. Chi, unpubl.). The levels of genetic independence found for each population are unexpected given that there is less than a 120 km distributional gap (Figure 1). There is no reason to assume that spotted owls in Marin and Mendocino were historically reproductively isolated. In the past, semi-forested corridors and suitable habitat along coastal mountains should have provided dispersal routes for owls moving in either direction. In an extensive dispersal study of Northern spotted owls in Washington and Oregon, Forsman *et al.* (2002) reported that owls would cross fragmented areas consisting of clear-cuts, roads, and variable forest cover types, and that only significant landscape features such as large non-forested valleys or bays acted as barriers to dispersal.

Alleles sampled from Marin form a smaller subset of the allelic diversity present in Mendocino, with the exception of a single allele. These results suggest possible population fragmentation followed by the introduction of additional genetic variation to Mendocino and/or a loss of genetic variation in Marin, with shifts in allelic frequencies over time. Several genotypes sampled in Mendocino appeared to share ancestral histories with Marin (Figure 4, Table 6), suggesting that some owls in Mendocino are closely related to spotted owls sampled from Marin. Marin is densely populated and could act as a source population in comparison to Mendocino, or alternatively traces of historical gene flow between the two populations may have been retained in Mendocino and lost from Marin by such mechanisms as drift. In Marin, genetic isolation, lower

variability ($H = 54\%$), and a small N_E estimate ($N_E = 37$) elevate the conservation priority of this population.

California Spotted Owls

The inland and coastal California spotted owl populations show considerable genetic differences, primarily because allelic diversity in Monterey is extremely low. Similar to the Northern subspecies, alleles present in Monterey form a small subset of the allelic diversity present in El Dorado (Figure 2B). The degree of differentiation within the California spotted owl is very high and comparable to inter-island or island vs. mainland avian populations (Mundy *et al.* 1997; Tarr *et al.* 1998; Hille *et al.* 2003). There are genetic consequences for small population sizes. As population size decreases, genetic variation is also reduced (Frankham 1996). Spotted owls in Monterey showed levels of genetic variability similar to critically endangered avian species restricted to small islands, such as the San Clemente loggerhead shrike (Mundy *et al.* 1997). Low diversity in the California spotted owl coastal population may have been caused by founding events and/or population bottlenecks as proposed by Barrowclough *et al.* (1999), and genetic diversity may have remained low or was further reduced by inbreeding, isolation, and small N_E . The estimated N_E for both coastal spotted owl populations is well under thresholds required to prevent genetic decline from inbreeding depression and drift (Avis 2004). The lack of a detectable bottleneck suggests a more ancient population crash or alternatively population expansion from a small group of founders. Low genetic variation is likely present

throughout the entire network of south coastal California spotted owl populations, as suggested by a single mitochondrial haplotype detected in 10 owls from Southern California (Barrowclough *et al.* 1999) and in 15 owls from Monterey (T. Chi, unpubl.). It appears that gene flow with genetically divergent source populations is non-existent, or at a level where drift overwhelms infrequent migration events. This is consistent with dispersal estimates for isolated Southern California spotted owl populations based on census data (LaHaye *et al.* 1994).

The south coastal network of California spotted owls has been described as a metapopulation (Noon & McKelvey 1992; LaHaye *et al.* 1994; Gutiérrez & Harrison 1996), yet linkages have not been examined genetically. Monterey would qualify as the most northern documented patch, although prior to intense development pressure and pre-1900 logging it seems reasonable to assume that Santa Cruz and San Mateo Counties (to the north of Monterey) would have been occupied by spotted owls and currently represent an increasingly isolated habitat island. It is important within metapopulations to preserve suitable *unoccupied* habitat patches as well as *occupied* ones, which in many theoretical models contribute to the long-term persistence of the entire metapopulation (Hanski 1996). Spotted owls within the Monterey area have been described as separate northern and southern subpopulations (LaHaye *et al.* 1994). Microsatellite analysis divided the Monterey population into two major lineages (Figure 4), but owls were intermingled among the lineage groupings and clustering analysis did

not identify any population substructure, indicating that owls within Monterey easily traverse the Santa Lucia range.

In the Sierra Nevada, the El Dorado population was more genetically diverse but contained a significant excess of homozygotes. Common explanations for Hardy-Weinberg equilibrium departure include the presence of selection or null alleles, inbreeding effects, non-random mating patterns, or population subdivision (Wahlund's effect). Null (or non-amplifying) alleles are a concern for DNA isolated from feathers, but undetected alleles are unlikely to result in equilibrium deviations in only a single population and null alleles were not detected on pedigree analysis. There was some evidence for population subdivision in El Dorado based on Bayesian clustering analysis. Limited dispersal (*i.e.* a nearest-neighbor breeding scenario) could lead to inbreeding in small populations with low N_E . In addition, if recruitment is low and site-specific, only a small percentage of the breeding pairs will contribute to the population gene pool. Over time this could skew the adult allelic frequencies and reinforce inbred lineages. Extreme variation in reproductive success has been documented in spotted owls (Blakesley *et al.* 2001) and only a limited number of juvenile feather samples were collected in El Dorado compared to other sampled areas. An inbreeding result would not exclude the possibility of subpopulation structuring or non-random mating effects. Interestingly, there was some limited evidence for non-random mating patterns based on a narrow distribution of rare alleles associated with the Northern subspecies among paired male and female California spotted owls.

Genetic Diversity

Genetic diversity measures were similar for spotted owl populations located in interior California and lower for the smaller coastal populations. Because natural barriers and developed areas can isolate spotted owls in coastal regions, lower gene flow exchange rates with other surrounding owl populations are anticipated compared with owl populations located in large contiguous forested areas. In small populations, genetic variation is lost as a result of genetic drift. If coastal populations receive fewer immigrants, eventually lower polymorphism and heterozygosity will result due to the tendency of alleles to drift toward fixation. Low heterozygosity is not only an indication of a potential loss of adaptive genetic variation, but can also suggest reduced reproductive fitness, which increases the risk of extinction (Reed & Frankham 2003). All average heterozygosity (H) values for the Northern and California subspecies (30%-70%) were lower than the same six-locus estimate of 78% reported for the threatened Mexican spotted owl (Thode *et al.* 2002), and observed heterozygosity (H_O) was only 43% in the California subspecies and 61% in the Northern subspecies. Allele counts and size ranges were also greater in the Mexican spotted owl (*S. o. lucida* = 54 alleles, 84-265 bp range; *S. o. caurina* = 39 alleles, 94-249 bp range; *S. o. occidentalis* = 32 alleles, 94-239 bp range).

Subspecies Divergence and Introgression

Subspecies genetic differentiation estimates were large for a raptor with the capacity for long-distance dispersal (Forsman *et al.* 2002). The Northern and the California

subspecies showed a significant genetic divergence represented by large allelic frequency shifts ($R_{ST} = 35\%$). Private alleles detected for both subspecies include several common alleles found exclusively in either Northern or California spotted owls (Figure 5). Microsatellite genetic variation clearly supports the current subspecies taxonomic divisions defined by the American Ornithologists' Union and summarized in Gutiérrez *et al.* (1995) and Gutiérrez & Barrowclough (2005). Mitochondrial DNA also supports significant genetic differences among subspecies (Barrowclough *et al.* 1999; Haig *et al.* 2004a, T. Chi, unpubl.). The Northern and the California spotted owl traditional ranges nearly overlap in Shasta County within the Klamath bioregion, forming a subspecies contact zone where mixing and introgression of the two subspecies is more likely. Subspecies genetic divergence estimates between the central Sierra (El Dorado) and the north interior (Mendocino) were 58% lower than similar estimates between *S. o. occidentalis* in the central Sierra (El Dorado) and *S. o. caurina* along the coast (Marin), implying higher gene flow nearer to the subspecies contact zone. Multiple genetic markers have indicated that the two subspecies may not be strictly reproductively isolated in the Klamath region and directional subspecies introgression is suggested by several California spotted owl mitochondrial haplotypes sampled within the traditional range of the Northern spotted owl (Barrowclough *et al.* 1999; Haig *et al.* 2004a; T. Chi, unpubl.).

First generation subspecies hybrids have not yet been genetically identified, although several owls are suspected hybrids based on mitochondrial DNA haplotypes

(Barrowclough *et al.* 1999; Haig *et al.* 2004a). Documentation of subspecies hybrids in the field based on morphology is unlikely because of the similarities between Northern and California spotted owls. In this study, several owls showed small probabilities of mixed ancestry across subspecies boundaries (Table 6) and a subspecies hybrid was identified by combining microsatellite and mitochondrial haplotype data (T. Chi, unpubl.). A California spotted owl breeding female in the Sierra Nevada showed significant *S. o. caurina* ancestry in the parental generation (58%, Table 6), and possessed a common *S. o. occidentalis* mitochondrial haplotype suggesting that this owl may be a first generation subspecies hybrid between a male *S. o. caurina* and a female *S. o. occidentalis*.

In Washington and Oregon, 47 barred and Northern spotted owl hybrids have been identified and some have produced offspring by backcrossing with either species (Kelly & Forsman 2004; Haig *et al.* 2004b). Backcrossed hybrids are difficult to classify in the field (Hamer *et al.* 1994), and there may be detection bias because second generation hybrids can closely resemble a non-hybrid parent. Although the primary threat from recent barred owl sympatry is related to resource competition, the genetic effects of species introgression are important to address for spotted owl conservation. Barred owls and mixed-species hybrids may be capable of greater long distance dispersal, and in combination with backcrossing, could transfer alleles across populations or subspecies. The historical presence of mixed-species pairs in Mendocino suggests that introgression could account for a small portion of the higher allelic

diversity found in this population. Several spotted owls in Mendocino carried relatively rare alleles, and a barred owl genotype clustered with the Mendocino population of Northern spotted owls in both the individual phylogenetic tree (Figure 4) and in Bayesian modeling (data not shown).

Barred owl alleles were sampled at the same six microsatellite loci from *S. varia* feather samples from a single owl collected in Marin County. Seven of nine barred owl alleles sampled consisted of smaller size microsatellite repeats not detected in any spotted owl sampled. The barred owl also carried a heterozygous allele that was the same length as an allele common to both spotted owl subspecies. This may represent allelic overlap or may be evidence of spotted owl allelic introgression into the recently expanded barred owl gene pool. Additionally, the barred owl carried a homozygous allele detected in a heterozygous state in a breeding Northern spotted owl female from Mendocino, suggesting limited entry of barred owl alleles into the Northern spotted owl gene pool through introgression. This shared allele could alternatively represent allele frequency distribution overlap at this locus, or (less likely) may be evidence of spotted owl introgression into the founding barred owl populations. Barred owls have expanded their range south over successive generations and may accumulate spotted owl genetic variation through hybridization and introgression over time. The barred owl present in Muir Woods National Monument, Marin County may have slight mixed-species ancestry, although morphologically it appeared identical to *S. varia*.

Relation to other Phylogeographic Patterns

Spotted owl phylogeographic patterns in California are strikingly similar to population genetic breaks identified for several native *ground-dwelling* taxa (Calsbeek *et al.* 2003). Deep phylogenetic splits in several native plants, mammals, reptiles, and amphibians occur north of San Francisco Bay, south of Monterey Bay, or along east/west gradients between the coastal and Sierra Nevada ranges. Spotted owl genetic structure contrasts with the molecular diversification of several native bird species, and is instead more similar to phylogeographic patterns present in many woodland and/or riparian-dependent mammals such as ornate shrews or dusky footed woodrats (Calsbeek *et al.* 2003). These similarities may reflect generalized population dynamics, genetic drift effects, and gene flow patterns across diverse groups of species. Spotted owl population genetic differentiation, and the concordance with multiple ground-restricted taxa, suggest high isolation and significant genetic independence uncommon in avian species. This diversification model in spotted owls should be evaluated in comparison to additional molecular information (as it develops) for other specialized forest-dependent avian species.

Conclusions on Conservation and Status

It is important to consider genetic factors in the present management of spotted owls. In some populations the limiting factors, reproductive rates, survival rates, and recruitment rates are unknown. Microsatellites suggest low levels of gene flow among different

regions. Genetic variability is low across several genetic markers, especially for the California spotted owl, increasing the risk of inbreeding depression. Reduced genetic variability could lead to lower disease resistance. Spotted owls sampled in coastal areas appear to be on tiny habitat islands with small breeding populations and limited gene pools. Small effective population sizes (N_E) can magnify genetic effects such as drift, hybridization, or inbreeding. Reliance on small numbers of successfully breeding pairs can also change population dynamics via demographic stochasticity. In a large population mating effects, natural variation in sex-ratios, and changes in reproductive and mortality rates all tend to average out. In small populations, fluctuations and interactions of these factors may accelerate declines. At any time, demographic and/or environmental stochastic factors can interact and combine with genetic factors to further impact population status (Lande 1999).

Microsatellite variation strongly supports subspecies designations. Within subspecies, there appear to be substantial barriers to gene flow with reduced genetic variability in coastal areas. The extremely low genetic variability detected in the coastal population of California spotted owls suggests that the south coast populations are in need of increased protection and monitoring. Conservation efforts should focus on demographic assessment (to prevent collapse) and monitoring (to assess potential threats including West Nile virus and sudden oak death). Because of the significant genetic divergence found among inland and coastal populations of the California spotted owl, these areas likely qualify as distinct population segments (U.S. Fish &

Wildlife Service, 1996). Distinct population segments must be substantially reproductively isolated and must represent a significant component in the evolutionary history of a species. Bayesian clustering analysis did not show strong support for grouping coastal and interior California spotted owl populations in subspecies assignment tests (Table 7). Isolation within California's south coastal ranges appears great enough to support local adaptation and the maintenance of an independent evolutionary lineage. In the Sierra Nevada, genetic diversity and population connectivity should be assessed on a larger scale. All owl populations sampled were genetically divergent indicating degrees of isolation and fragmentation not often observed in avian species. Isolation in combination with other factors (such as subspecies and/or species hybridization and introgression) could produce a cascade of genetic effects. Non-invasive feather sampling provides a simple method of reassessing populations to monitor genetic trends. These issues should be followed closely through further genetic research and conservation planning.

REFERENCES

- Anthony RG, Forsman ED, Franklin AB, Anderson DR, Burnham KP, White GC, Schwarz CJ, Nichols J, Hines J, Olson GS, Ackers SH, Andrews S, Biswell BL, Carlson PC, Diller LV, Dugger KM, Fehring KE, Fleming TL, Gerhardt RP, Gremel SA, Gutiérrez RJ, Happe P, Herter DR, Higley JM, Horn RB, Irwin LL, Loschl PJ, Reid JA, Sovern SG (2004) Status and trends in demography of Northern Spotted Owls, 1985-2003. Draft Report, Interagency Regional Monitoring Program, Portland, Oregon.
- Avice JC (2004) *Molecular Markers, Natural History, and Evolution*, 2nd edn. Sinauer Associates, Massachusetts.
- Balloux F, Goudet J (2002) Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. *Molecular Ecology*, **11**, 771-783.
- Barrowclough GF, Gutiérrez RJ (1990) Genetic variation and differentiation in the Spotted Owl (*Strix occidentalis*). *Auk*, **107**, 737-744.
- Barrowclough GF, Flesness NR (1996) Species, subspecies, and races: the problem of units of management in conservation. In: *Wild Mammals in Captivity: Principals and Techniques*. (eds. Kleiman DG, Allen ME, Thompson KV, Lumpkin S, and Harris H), pp. 247-254. University of Chicago Press, Chicago.
- Barrowclough GF, Gutiérrez RJ, Groth JG (1999) Phylogeography of Spotted Owl (*Strix occidentalis*) populations based on mitochondrial DNA sequences: gene flow, genetic structure, and a novel biogeographic pattern. *Evolution*, **53**, 919-931.
- Blakesley JA, Noon BR, Shaw DH (2001) Demography of the California Spotted Owl in Northeastern California. *Condor*, **103**, 667-677.
- Bowcock AM, Ruíz-Linares A, Tomfohrde J, Minch E, Kidd J, Cavalli-Sforza LL (1994) High resolution human evolutionary trees with polymorphic microsatellites. *Nature*, **368**, 455-457.
- Calsbeek R, Thompson JN, Richardson JE (2003). Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. *Molecular Ecology*, **12**, 1021-1029.
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001-2014.

- Courtney SP, Blakesley JA, Bigley RE, Cody ML, Dumbacher JP, Fleischer RC, Franklin AB, Franklin FJ, Gutiérrez RJ, Marzluff JM, Sztukowski L (2004) Scientific evaluation of the status of the Northern Spotted Owl. Final Report, Sustainable Ecosystems Institute, Portland, Oregon.
- Dieringer D, Schlötterer C (2002) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, **3**, 167-169.
- Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences of the USA*, **91**, 3166-3170.
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated frequencies. *Genetics*, **164**, 1567-1587.
- Forsman ED, Anthony RG, Reid JA, Loschl PJ, Sovern SG, Taylor M, Biswell BL, Ellingson A, Meslow EC, Miller GS, Swindle KA, Thrailkill JA, Wagner FF, Seaman DE (2002) Natal and breeding dispersal of Northern Spotted Owls. *Wildlife Monographs*, **149**, 1-35.
- Frankham R (1996) Relationship of genetic variation to population size in wildlife. *Conservation Biology*, **10**, 1500-1508.
- Franklin AB, Burnham KP, White GC, Anthony RG, Forsman ED, Schwartz C, Nichols JD, Hines J (1999) Range-wide status and trends in Northern Spotted Owl populations. Colorado Cooperative Fish & Wildlife Research Unit, Colorado State University, Fort Collins, Colorado and Oregon Cooperative Fish & Wildlife Research Unit, Oregon State University, Corvallis, Oregon.
- Fridolfsson AK, Ellegren H (2000) Molecular evolution of the avian CHD1 genes on the Z and W sex chromosomes. *Genetics*, **155**, 1903-1912.
- Gaggiotti OE, Lange O, Rassmann K, Gliddons C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology*, **8**, 1513-1520.
- Griffiths R, Double MC, Orr K, Dawson RG (1998) A DNA test to sex most birds. *Molecular Ecology*, **7**, 1071-1075.
- Gutiérrez RJ, Franklin AB, LaHaye WS (1995) Spotted Owl. *The Birds of North America*, **179**, 1-28.

- Gutiérrez RJ, Harrison S (1996) Applying metapopulation theory to Spotted Owl management: A history and critique. In: *Metapopulations and Wildlife Conservation* (ed. McCullough DR), pp. 167-185. Island Press, Washington D.C.
- Gutiérrez RJ, Barrowclough G (2005) Redefining the distributional boundaries of the Northern and California spotted owls: implications for conservation. *The Condor*, **107**, 182-187.
- Haig SM, Wagner RS, Forsman ED, Mullins TD (2001) Geographic variation and genetic structure in Spotted Owls. *Conservation Genetics*, **2**, 25-40.
- Haig SM, Mullins TD, Forsman ED (2004a) Subspecific relationships and genetic structure in the Spotted Owl. *Conservation Genetics*, **5**, 683-705.
- Haig SM, Mullins TD, Forsman ED (2004b) Genetic identification of Spotted Owls, Barred Owls, and their hybrids: Legal implications of hybrid identity. *Conservation Biology*, **18**, 1347-1357.
- Hanski I (1996). Metapopulation Ecology. In: *Population Dynamics in Ecological Space and Time* (eds. Rhodes OE, Chesser RK, Smith MH), pp. 13-43. University of Chicago Press, London.
- Hardy OJ, Charbonnel N, Freville H, Heuertz M (2003). Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. *Genetics*, **163**, 1467-1482.
- Hill NG (1981) Estimation of effective population size from data on linkage disequilibrium. *Genetic Research*, **38**, 209-216.
- Hille SM, Nesje M, Segelbacher G (2003). Genetic structure of kestrel populations and colonization of the Cape Verde archipelago. *Molecular Ecology*, **12**, 2145-2151.
- Hoelzel AR, Fleischer RC, Campagna C, LeBoeuf BJ, Alvord G (2002) Impact of a population bottleneck on symmetry and genetic diversity in the northern elephant seal. *Journal of Evolutionary Biology*, **15**, 567-575.
- Hsu YC, Severinghaus LL, Lin YS, Li SH (2003) Isolation and characterization of microsatellite DNA markers from the Lanyu scops owl (*Otus elegans botelensis*). *Molecular Ecology Notes*, **3**, 595-597.
- Isaksson M, Tegelström H (2002) Characterization of polymorphic microsatellite markers in a captive population of the eagle owl (*Bubo bubo*) used for supportive breeding. *Molecular Ecology Notes*, **2**, 91-93.

- Kelly EG, Forsman ED (2004) Recent records of hybridization between barred owls (*Strix varia*) and Northern spotted owls (*S. occidentalis caurina*). *The Auk*, **121**, 806-810.
- Koopman, ME, Schable, NA, Glenn, TC (2004) Development and optimization of microsatellite DNA primers for boreal owls (*Aegolius funereus*). *Molecular Ecology Notes*, **4**, 376-378.
- Korfanta NM, Schable, NA, Glenn TC (2002) Isolation and characterization of microsatellite DNA primers in burrowing owl (*Athene cunicularia*). *Molecular Ecology Notes*, **2**, 584-585.
- LaHaye WS, Gutiérrez RJ, Akcakaya HR (1994) Spotted Owl metapopulation dynamics in Southern California. *Journal of Animal Ecology*, **63**, 775-785.
- Lande R (1999) Extinction risks from anthropogenic, ecological, and genetic factors. In: *Genetics and the Extinction of Species* (eds. Landweber L, Dobson A), pp. 1-22. Princeton University Press, Princeton, New Jersey.
- Luikart G, Allendorf FW, Cornuet J, Sherwin WB (1998) Distortion of allele frequency distribution provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238-247.
- Marshall TC, Slate J, Kruuk L, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639-655.
- Michalakis Y, Excoffier L (1996) A genetic estimation of population subdivision using distances between alleles with special references for microsatellite loci. *Genetics*, **142**, 1061-1064.
- Mundy NI, Winchell CS, Burr T, Woodruff DS (1997) Microsatellite variation and microevolution in the critically endangered San Clemente Island loggerhead shrike (*Lanius ludovicianus mearnsi*). *Proceedings of the Royal Society London B*, **264**, 869-875.
- Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution*, **19**, 153-170.
- Noon BR, McKelvey KS (1992) Stability properties of the Spotted Owl metapopulation in southern California. In: *The California Spotted Owl: A Technical Assessment of its Current Status* (ed. Verner J), pp. 187-206, General Technical Report PSW-GTR-133. USDA Forest Service, Albany, CA.

- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.
- Pyle P (1997) *Identification Guide to North American Birds. Part I Columbidae to Ploceidae*. Slate Creek Press, Bolinas, CA.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248-249.
- Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology*, **17**, 230-237.
- Schlötterer C, Pemberton J (1998). The use of microsatellites for genetic analysis of natural populations – a critical review. In: *Molecular Approaches to Ecology and Evolution* (eds. DeSalle R, Schierwater B), pp. 71-85, Birkhauser Verlag, Switzerland.
- Seamans ME, Gutiérrez RJ, May CA, Peery MZ (1999) Demography of two Mexican Spotted Owl populations. *Conservation Biology*, **13**, 744-754.
- Seamans ME, Gutiérrez RJ, Moen CA, Perry MZ (2001) Spotted Owl demography in the central Sierra Nevada. *Journal of Wildlife Management*, **65**, 425-431.
- Slatkin M (1985) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53-65.
- Tarr CL, Conant S, Fleischer RC (1998) Founder events and variation at microsatellite loci in an insular passerine bird, the Laysan finch (*Telespiza cantans*). *Molecular Ecology* **7**, 719-731.
- Thode AB, Maltbie M, Hansen LA, Green LD, Longmire JL (2002) Microsatellite markers for the Mexican Spotted Owl (*Strix occidentalis lucida*). *Molecular Ecology Notes*, **2**, 446-448.
- U.S. Fish & Wildlife Service (1996) *The Federal Register*, **61**, 4722.
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.
- Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 4Nm + 1$. *Heredity*, **82**, 117-125.

Appendix A. Microsatellite Allele Lengths

Table of allele lengths for six microsatellite loci isolated from the Mexican spotted owl by Thode <i>et al.</i> (2002). Allele lengths are measured by their electrophoretic mobility on polyacrylamide Spreadex™ gels in comparison to M3 markers. Each allele corresponds to a letter substitute used in the text. Allelic distributions are shown for each subspecies and sampled population of Northern spotted owls (NSO) and California spotted owls (CSO).							
Allele	Allele Length	NSO	CSO	CSO Coast	CSO Interior	NSO Coast	NSO Interior
6a8							
A	94	X	X	X	X	X	X
B	98	X	X	X	X	X	X
C	102	X	X		X	X	X
D	106	X	X		X	X	X
E	110	X				X	X
F	114	X					X
G	118	X	X		X	X	X
15A6							
H	146	X	X	X	X	X	
I	150	X	X	X	X	X	X
J	154	X	X	X	X	X	X
K	158	X	X	X	X	X	X
L	162	X	X		X		X
1c6							
M	112	X	X	X	X	X	X
N	121	X	X		X	X	X
O	124	X	X		X	X	X
P	127		X		X		
8c11							
Q	138	X					X
R	146	X	X		X	X	X
S	150	X	X	X	X	X	X
T	154	X	X		X	X	X
U	158	X	X		X	X	X
V	162	X	X		X	X	X
W	166	X					X
4e102							
X	214	X	X		X	X	X
Y	219	X	X		X	X	X
Z	224	X	X	X	X	X	X
AA	229	X	X	X	X	X	X
BB	234	X	X	X	X	X	X
CC	239	X	X	X	X		X
DD	244	X					X
EE	249	X					X
13d8							
FF	171	X				X	X
GG	175	X	X		X	X	X
HH	179	X	X	X	X	X	X
II	183	X	X	X	X	X	X
JJ	187	X	X	X	X	X	X
KK	191	X	X		X		X
LL	195		X		X		
MM	199	X	X		X		X
NN	203	X					X
OO	207	X					X

Appendix B. Feather Collection Protocol

I. Collecting Feathers from Captured Spotted Owls

If possible, collect feathers directly from owls during banding. We request a minimum of 2-3 feathers per owl. Feathers shed naturally during the banding process (instead of plucked) are suitable. Feathers collected from captured owls can be stored together in the same envelope. All feathers collected from banded owls must be kept separate from feathers collected from other owls or from the ground at owl sites. If possible, feathers should not be handled. Information requested for feather genetic samples collected during banding is as follows:

Location _____	Site Name/# _____
Collector _____	Date _____
Band # _____	Age Juvi ☐ SubAdult ☐ Adult ☐
Sex M ☐ F ☐ U ☐ Pair Y ☐ N ☐ U ☐	Known Hybrid? Y ☐ N ☐
Nesting Y ☐ N ☐ U ☐	# of nestlings/fledglings (if known) _____
Abnormal morphology/plumage? _____	
Notes: _____	

M=Male; F=Female; U=Unknown; Hybrids= If the genetic sample is from a known hybrid please indicate the origin (BO Female + SO Male or BO Male + SO Female); Notes= Include habitat associations or any other relevant information.

II. Collecting Feathers in Spotted Owl Nest or Roost Areas

Please collect naturally molted feathers from the ground at spotted owl sites. Often an entire family of owls can be genotyped from feathers collected during different months of the nesting season. Feathers also provide a historical genetic record for each owl territory. Each molted feather collected from the ground should be stored in a separate envelope and labeled with precise location information. If possible, feathers should not be handled. Information requested for feathers picked up from the ground at owl sites is as follows:

Location _____	Site Name/# _____
Collector _____	Date _____
Pair Y ☐ N ☐ U ☐	Known Hybrid at site? Y ☐ N ☐
Nesting Y ☐ N ☐ U ☐	# of nestlings/fledglings (if known) _____
Feather Collection at: Roost M ☐ F ☐ Nest ☐ Other Area ☐	
Notes: _____	

Notes = It is really helpful to state the exact location that the feather was collected from. For example, *male roost* or *20 feet west from nest* or *upslope of female roost*, etc.