

PRELIMINARY MICROSATELLITE ANALYSIS OF TWO POPULATIONS OF NORTHERN SPOTTED OWLS (*Strix occidentalis caurina*)

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INTRODUCTION

Northern Spotted Owls (*Strix occidentalis caurina*) are one of the most publicized threatened animals in North America, yet very few studies have addressed questions of genetic variation and gene flow in this species. Barrowclough and Gutierrez (1990) were the first to report on genetic variation among the three subspecies of Spotted Owls using allozymes. They found no variation in 22 of 23 allozyme loci examined. In 1999, Barrowclough et al. sequenced 1105 bases of the mitochondrial control region (domain I and II) in 73 Spotted Owls from 13 populations, including all 3 of the designated subspecies. They identified 37 haplotypes or about one for every two owls sequenced. Homoplasy at several of the variable base pair sites obscured phylogenetic analysis among the subspecies. They found genetic variation within populations to be lower in the California Spotted Owl (*Strix occidentalis occidentalis*) compared with either the Northern or the Mexican Spotted Owl (*Strix occidentalis lucida*). However, limited sampling spread over a large area, and a high frequency of novel haplotypes, reduced the accuracy of the within-population variability estimates. Among subspecies, they identified one common California Spotted Owl haplotype present in a Northern Spotted Owl. Haig et al. (2001) performed RAPD analyses using 11 variable primer sets on 276 Spotted Owls representing all three subspecies. Within-population variation was found to be extremely low and geographically structured. Among subspecies, Haig et al. (2001) examined the same mitochondrial control regions as Barrowclough et al. (1999) and found support for a Mexican Spotted Owl subspecies, but detected California Spotted Owl haplotypes in designated Northern Spotted Owls at a rate of 13%.

Clearly, both the taxonomic status and population structure of the Spotted Owl requires further extensive and intensive genetic research. In this report we address questions of gene flow and genetic variation in a fine-scale genetic analysis of two populations of Northern Spotted Owls in California. Microsatellite markers were used to estimate genetic variability within and between populations. This preliminary research is submitted to the U.S. Fish & Wildlife Service in response to their request for information regarding inbreeding, genetic variability, and hybridization in Northern Spotted Owls. Results are presented for Northern Spotted Owls from Mendocino National Forest (MNF; $n=26$), and from Marin County (MC; $n=30$). Sampling areas are shown in Figure 1.

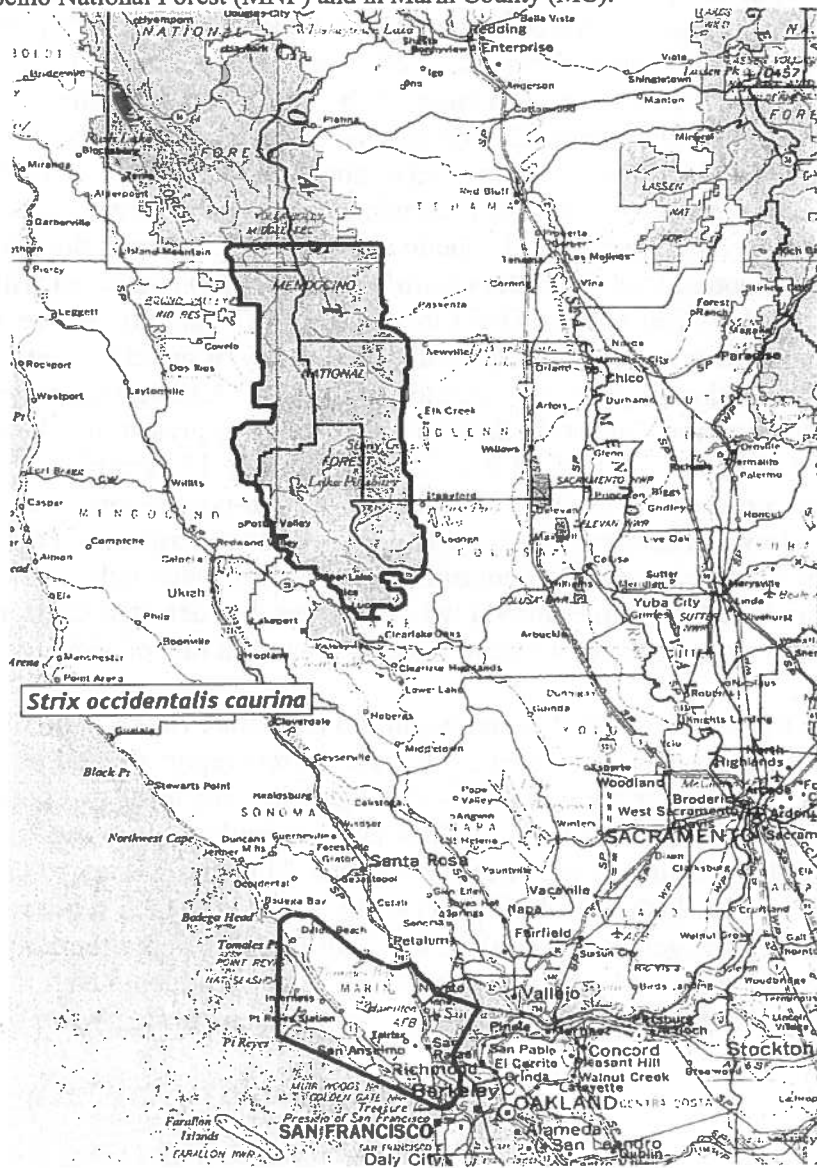
METHODS

Field

Historical Spotted Owl territories were night surveyed to locate either single or pairs of owls. Owls were re-located during the day at day roosts and/or nest sites. Naturally

molted feather samples were collected from the ground at Spotted Owl nesting and roosting areas for use in DNA fingerprinting. Care was taken to collect feathers in owl core areas directly under known day roosts or nests. Owl DNA samples from Marin County included a mixture of shed feather samples collected from the ground within active owl territories, as well as feathers directly plucked from owls during capture for leg banding (by National Park Service biologists). Feather samples obtained for MC represent 1999 and 2000 field seasons, while feathers collected from MNF are from 2002. Genetic samples were collected from a total of 21 Spotted Owl territories within MNF and 22 sites in MC located on a mixture of private and public landholdings.

Fig 1. Northern Spotted Owl sampling areas in California. The two populations are located in the Mendocino National Forest (MNF) and in Marin County (MC).



Laboratory

DNA extraction procedures for feathers were modified from protocols available from Puregene™ kits (Gentra Systems). PCR conditions for six sets of microsatellite primers

(6H8, 15A6, 1C6, 8G11, 4E10, and 4E10.2) derived from the Mexican Spotted Owl (*Strix occidentalis lucida*) by Thode et al. (2002) were optimized for Northern and California Spotted Owls. DNA samples were tested to determine the sex of the owl represented by amplifying a portion of the sex-linked CHD (chromobox-helicase-DNA-binding) gene present in all avian species using a modification of Griffiths' method (Griffiths et al. 1998). PCR amplification of each of six microsatellite loci was performed on DNA samples from each site that represented either 1) a juvenile or 2) one or both adult members of a territorial pair. PCR products (alleles) were resolved on 6.5% polyacrylamide gels with Spreadex™ polymer (Elchrom Scientific). Allele scoring ambiguity was minimized because of the use of large tri, tetra, and pentanucleotide microsatellite repeats.

Molted feathers collected from owl territories containing breeding pairs were divided into adult and juvenile patterned samples. Juvenile and parental pedigrees were compared with six or more markers to confirm alleles and Mendelian inheritance patterns. Some alleles were also re-amplified from separate (i.e. independent) feather samples collected at the same owl site to corroborate individual DNA fingerprints. Most owls included in this preliminary analysis were sexed as female or male. If several related individuals from the same territory were fingerprinted, no siblings or parent-offspring combinations were used in the analysis. Juveniles were only included if genetic samples for territorial pair members were unavailable.

Genetic Analysis

Allelic frequencies were calculated from Microsatellite Analyzer (MSA) (Dieringer & Schlötterer 2002) and GENEPOP 3.1 (Raymond & Rousset 1995). Expected heterozygosity (H) estimates and the expected number of alleles under two microsatellite mutation models were obtained from MSA. Exact Hardy-Weinberg tests were run on all six loci for each population using GENEPOP 3.1. Follow-up U score tests were run from GENEPOP 3.1 to assess deviations from Hardy-Weinberg equilibrium. Linkage disequilibrium testing over all loci and for each population was performed in GENEPOP 3.1. Inbreeding coefficients (F_{IS}) were calculated in GENEPOP 3.1 following Weir & Cockerham (1984).

Differences in allele distribution among populations were tested using Fisher's exact test in GENEPOP 3.1. Wright's fixation index (F_{ST}) was reported as Global F_{ST} . Associated confidence intervals and p -values were constructed by permutation tests in MSA. All genetic distance measures reported are from MSA. The number of migrants entering a population per generation (Nm) was estimated by the private alleles method in GENEPOP 3.1. Bayesian modeling to infer population structuring was completed in Structure 2.1 (Pritchard et al. 2000; Falush et al. 2003).

RESULTS

Genetic Diversity

Allele sizes for Northern Spotted Owls ranged from 94-260 base pairs (bp) over all loci (Table 1). Rare alleles (≤ 2 appearances in either population) were present in all six markers tested. The numbers of alleles per locus (over both populations) varied among microsatellite markers as follows: 1C6 (3), 15A6 (5), 6H8 (7), 8G11 (7), 4E102 (7), and 4E10 (13). Overall, 90% of all alleles observed were present in the MNF owl population. In contrast, Spotted Owls in MC were less diverse, only displaying 76% of the total alleles sampled.

Table 1. Genetic diversity indices at 6 microsatellite loci for two populations of Northern Spotted Owls in Mendocino National Forest (MNF) and Marin County (MC).

Locus	Allele Size Ranges (bp)	Mendocino National Forest			Marin County			H-W single locus deviations MNF/MC
		Heterozygosity H_o	Gene Diversity H	Inbreeding Coefficient F_{IS}	Heterozygosity H_o	Gene Diversity H	Inbreeding Coefficient F_{IS}	
6H8	94-118	0.85	0.84	-0.002	0.87	0.77	-0.124	0/0
15A6	146-162	0.65	0.68	+0.037	0.40	0.32	-0.166	0/0
1C6	112-124	0.13	0.13	-0.031	0.14	0.13	-0.057	0/0
8G11	142-166	0.80	0.79	-0.015	0.53	0.49	-0.092	0/0
4E10	195-260	0.74	0.86	+0.142	0.86	0.88	+0.016	*1/0
4E102	214-224	0.69	0.83	+0.164	0.69	0.75	+0.087	0/0
		$H_{o_{ave}} = 0.643$	$H_{ave} = 0.687$		$H_{o_{ave}} = 0.582$	$H_{ave} = 0.556$		

* $P < 0.05$

Observed heterozygosity values (H_o) ranged from 13 – 85% in the MNF population and from 14 – 87% in the MC population (Table 1). Average heterozygosity or gene diversity (H) was 24% higher in MNF (0.687) compared to MC (0.556).

Hardy-Weinberg exact tests (for each population and each locus) were performed to determine if the observed genotypic frequencies matched those expected under Hardy-Weinberg equilibrium conditions. Testing procedures relied on a Markov chain method. Five out of six loci tested by Hardy-Weinberg exact tests were in equilibrium in both populations sampled. For the MNF owl population, one locus (4E10) showed a significant excess of homozygote individuals ($p < 0.039$; $SE = 0.0038$, Table 1). Additional multisample score tests to assess the increased homozygosity observed resulted in a significant result for the MNF owl population at locus 4E10 ($p < 0.018$, $SE = 0.0024$). For both MNF and MC, global exact tests over all loci revealed no significant deviations from Hardy-Weinberg equilibrium. Inbreeding coefficients (F_{IS}) showed positive trends (indicating an excess of homozygotes) at two loci in both populations, and primarily negative trends (indicating an excess of heterozygotes) for most other loci (Table 1).

Confirmed linkage of microsatellite 4E10 with 4E10.2 was established by linkage disequilibrium testing in GENEPOP 3.1 ($\chi^2 = \infty$). Locus 4E10 was excluded from further population differentiation testing to satisfy independent assortment assumptions. Microsatellite 4E10.2 was retained because it amplified consistently and strongly.

Population Differentiation

Several measures of genetic structuring or allelic differentiation between the MNF and MC owl populations were assessed. Allele frequency distributions for both populations were tested for significant genetic differentiation using Fisher's exact test (GENEPOP3.1). This test estimates the probability (p-value) that the allele distributions across populations are identical. Allele histograms were highly significantly different at three of five loci ($p < 0.0001$). A fourth locus (4E10.2) showed only moderate differentiation among the two populations ($p < 0.05$), while for the fifth locus (1C6) the allele distribution was found to be statistically identical across populations.

Wright's F-statistics (F_{IS} , F_{ST} , F_{IT}) were calculated for five independent loci using two different estimation methods (Table 2). F_{ST} is an index of genetic differentiation ranging from 0 (representing no difference among populations) to 1 (fixation of alternative alleles in subpopulations). F_{ST} is therefore the percentage of genetic variation found among subpopulations. Global F-statistics were estimated in MSA by a variance-based statistical value theta (θ) proposed by Weir & Cockerham (1984). This method partitions the variance of allelic frequencies among subpopulations, individuals, and gametes. Population structuring is determined by comparing the correlation of alleles found within a subpopulation with allele patterns found over the entire population. As the allele frequencies in the subpopulations diverge, θ_{ST} approaches 1. Additional estimates of F_{ST} were obtained through a separate clustering analysis in Structure 2.1.

Global F_{ST} values are reported per locus and over all loci (Table 2). The probability that F_{ST} is significantly different from zero was estimated by permutation testing. Estimates of F_{ST} for each microsatellite varied. Smaller, insignificant values of F_{ST} ranging from 0-1% were found for microsatellites 4E10.2 and 1C6. Higher estimates of F_{ST} were noted at 15A6 and 8G11 (21% and 22%, respectively). MNF and MC showed significant genetic differentiation at 3 of 5 loci ($p \leq 0.0001$). This result is in agreement with Fisher's exact test described above. Global F_{ST} over all loci was 0.117, representing a 12% genetic divergence between owls in MNF and MC. In the overall estimation of F_{ST} , the large variability in the values of F_{ST} per locus (0-22%) resulted in broad 95% confidence intervals (0.014 - 0.212). Confidence values were calculated in MSA by resampling with 1000 replications.

Values of a variant of F_{ST} , defined as R_{ST} are also given in Table 2. R_{ST} statistics were estimated in GENEPOP 3.1 using Rho (ρ). Although both statistics measure population subdivision based on variance, F_{ST} uses the variance in allele frequencies, while R_{ST} measures (such as Rho) are based on the variance in repeat number. Rho values are provided here for comparison only (see discussion).

Table 2. Population differentiation estimates based on F_{ST} (θ) and R_{ST} (ρ), and Nei's genetic distance (D_a) for MNF and MC Northern Spotted Owls.

Locus	Global F_{ST}	Rho	D_a
6H8	0.069*	0.068	
15A6	0.210*	0.356	
1C6	-0.003	-0.012	
8G11	0.218*	0.010	
4E102	0.014	-0.020	
All Loci	0.117	0.047	0.165

* $P < 0.001$

P = the probability that the estimates are different from zero

Nei's chord distance measure (D_a), is also included in Table 2. This genetic distance among MNF and MC owls was similar to several F_{ST} values inferred from Bayesian cluster modeling (Pritchard et al. 2000). 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 also allows for hypothesis testing. Clustering procedures were followed to infer the extent of genetic structuring among Northern Spotted Owls in MNF and MC. Inferring the number of populations represented by the raw genetic data tested the hypothesis that the two populations were genetically distinct. The results of each clustering run at different assumed number of populations (K) are in Table 3. The quantity $P(K/X)$ represents the probability that the model fits the data.

Table 3. Cluster population modeling. Each value of K populations is inferred from the genetic data.

K	Log $P(X/K)$	$P(K/X)$
1	-674.9	0.0005
2	-667.3	0.9995
3	-818.1	0.0000
4	-713.7	0.0000

The number of migrants per generation (Nm) between the two populations sampled was estimated to be 1.5 by the private alleles method in GENEPOP 3.1. This estimate should be interpreted cautiously because it is subject to sampling error and to various simplifying assumptions that are usually violated in natural populations (Whitlock & McCauley 1999).

DISCUSSION

Genetic Variation Within Populations

Allelic Diversity

The total number of alleles per locus across both populations varied from 2 to 11 alleles. Microsatellite 1C6 showed low polymorphism in our samples of both Northern and California Spotted Owls, as a result of a high frequency of a single dominant allele (and a

few infrequent rare alleles) in all owls examined (unpublished). In Mexican Spotted Owls 1C6 was more polymorphic with observed heterozygosities of 67.8% (Thode et al. 2002). The total number of alleles reported for Mexican Spotted Owls (MSO) differed substantially from Northern Spotted Owl (NSO) allele counts at the same 6 loci (MSO = 63; NSO = 39). Similarly, Northern Spotted Owl allele size ranges (averaged over all loci) were 55% lower than those reported for the Mexican Spotted Owl. Because the sampling methods for Mexican Spotted Owl DNA samples are not reported and the results for Northern Spotted Owls are preliminary and limited, direct comparison of allelic diversity between the two subspecies is not possible.

Allelic diversity also varied between the two Northern Spotted Owl populations tested, with MC showing a 14% reduction in the total number of reported alleles when compared to MNF. This is an intuitively expected result because the owls located in Marin County north of San Francisco are more geographically isolated from other Spotted Owl populations compared with owls located in the Mendocino National Forest. The MNF Spotted Owl population is contiguous with the Yolla Bolla Wilderness area, the Trinity National Forest and the Six Rivers National Forest (Fig. 1). This population should show higher allelic diversity due to mixing with other adjacent Northern Spotted Owl populations. However, alternative explanations for the increase in allelic diversity should be explored. When rare allele frequency data are considered with respect to individual owls and territorial locations, it is apparent that rare alleles at more than one locus are present repeatedly in a few individual owls, and that these owls are clustered geographically. Rare alleles are defined here as ≤ 2 occurrences of an allele in a subpopulation. In MNF, 10 alleles (over 5 loci) met this criterion for rarity. Eight of these 10 rare alleles were distributed over only 7 individual owls *and* these owls were all found within the same general area of the Mendocino National Forest. This amounts to 7 owls displaying 76% of the rare allele frequencies detected over the sampled population. To visualize this effect, the locations of these owl territories are mapped along with the historical locations of Barred Owls (*Strix varia*) (Fig 2).

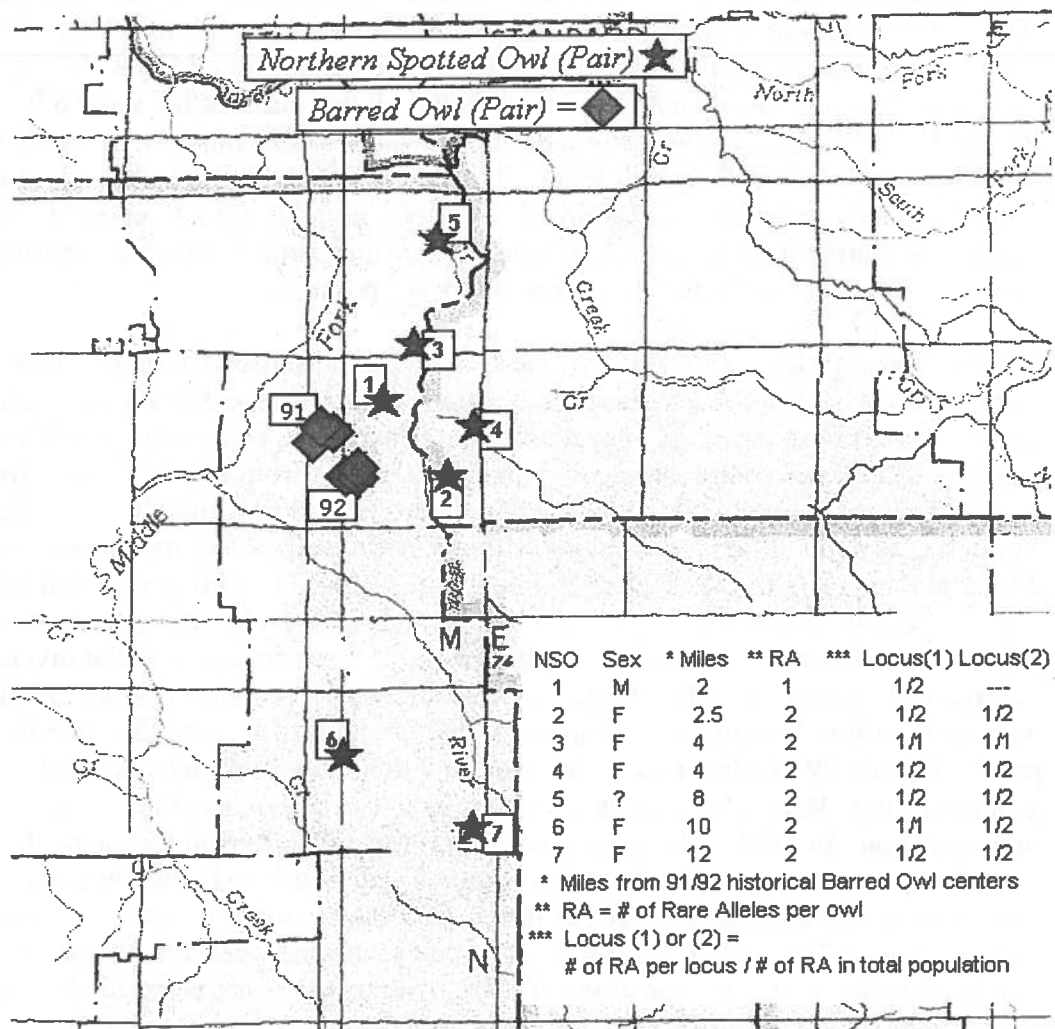


Fig. 2. Rare allele distribution among Northern Spotted Owl territories located adjacent to historical Barred Owl pair detections. Each star represents a Northern Spotted Owl territory. Barred Owl pair detections in 1991 and 1992 are represented by squares.

The close clustering of the 7 owls displaying rare alleles around the historical Barred Owl pair/s raises the suggestion of Barred Owl alleles entering the Northern Spotted Owl population through hybridization. Nesting between a Northern Spotted Owl and a Barred Owl was observed within the Mendocino National Forest in the early 1990's (personal communication from C. Furrer, U.S.F.S.). Successful matings between Barred Owls and Spotted Owls (that produced offspring that survived to adulthood) may immediately be detectable in allelic frequency data: 1) if that individual were sampled and 2) if Barred Owl allelic frequencies were known and no overlap occurred between Barred and Spotted Owl alleles. Confirmation of hybridization at the DNA level requires Barred Owl genetic samples, which are presently not available.

Incomplete sampling can also result in the identification of rare alleles that are actually more common in the total *N* population. Sampling error may be contributing to the geographic effect in Fig. 2. However, if this were the case, we would expect to find a more even distribution of rare alleles across the entire sampled Mendocino owl

population (assuming adequate gene flow). Rare allele frequencies can also be compared between the Mendocino and Marin populations. Marin County did not have any Barred Owl detections in the years sampled. Six of the 10 rare alleles identified in the MNF population are *not* present in the MC sampled population. These 6 rare alleles (distributed across 4 loci) are only present in Northern Spotted Owls #2 - #7 in Fig. 2. Each of those 6 owls carries rare alleles at 2 loci, and these alleles are present in different combinations together with common alleles across 4 loci. In addition, owls #2 - #7 are all *heterozygous* for all rare alleles.

Either these six owls together are representing under-sampled allelic diversity currently or historically present in the larger MNF population, or these alleles represent Barred Owl genetic variation within the Northern Spotted Owl population. Both conclusions ultimately pose a risk for the Northern Spotted Owl. If sampled rare alleles represent a residual fraction of the greater historical allelic diversity, theoretically some of the adaptive genetic character of the MNF Spotted Owl population has already been lost. Similarly, interspecific hybridization threatens the genetic integrity of the Spotted Owl and could contribute to a loss of Spotted Owl fitness or to a more general decline (Lande 1999). Recent studies of Barred Owl range expansions into the Western United States support this. In Oregon, Northern Spotted Owl annual occupancy in historic territories declined after the detection of Barred Owls near established territory centers (Kelley et al. 2003). Spotted Owls may be influenced by Barred Owls indirectly through competition for resources. In areas with reduced habitat suitability, these effects may be magnified if multiple species compete for the same niche. Given the low reproductive rate of Spotted Owls and an assumed small number of effective breeders (N_e), even low levels of hybridization have the potential to accelerate declines. Northern Spotted Owl fitness may be reduced if viable fertile hybrids, through introgression, alter local genetic adaptations.

Heterozygosity

Average gene diversity estimates were 24% larger for the MNF owl population ($H=0.687$) compared to MC ($H=0.556$) (Table 1). This estimated difference in gene diversity between the two populations was robust, and did not vary even with the exclusion of Northern Spotted Owls #2 - #7 in Fig 2. Because the MC owls display greater geographic isolation, a lower gene flow exchange rate with other owl populations is expected compared to owls located farther north in MNF (Fig. 1). Assuming a constant mutation rate across populations and no selection, if fewer immigrants are received into the MC population compared to MNF, eventually lower heterozygosity may result due to the tendency of alleles to drift towards fixation. Average gene diversity values for both Northern Spotted Owl populations were lower than the estimate of 79% (at 6 loci) reported for the threatened Mexican Spotted Owl (Thode et al. 2002). The differences in these estimates among subspecies cannot be evaluated without knowing more about how populations of Mexican Spotted Owls were sampled.

Hardy-Weinberg & Inbreeding Effects

Only one significant deviation from Hardy-Weinberg expected allelic frequencies was observed at a single locus for the MNF owl population (Table 1). Common explanations for non-equilibrium states include selection, the presence of null alleles, and inbreeding

effects. Selection is unlikely to affect microsatellite markers, but it is possible if there is a close linkage to nearby genes that are under selection. An additional consideration with this preliminary data set is low sampling sizes. This can exaggerate allelic typing errors, as in the possible case of null or non-amplifying alleles. However, if null alleles were present, they would be expected to occur evenly across all populations. This is not the case with the 4E10 marker, as comparisons with MC show nearly exact correlation of observed and expected heterozygote frequencies. The population characteristics of the MNF population are most consistent with an inbreeding conclusion. The MNF breeding owl population observed in 2002 was in partially clumped distributions and each breeding deme was somewhat isolated from other breeding pairs by unsuitable habitats. This was especially pronounced in the most southern located Spotted Owl territories. Increased inbreeding within the MNF population is supported by 1) F_{IS} estimates in both 4E10 and the linked locus 4E10.2, and 2) the small total numbers of Spotted Owl active territories detected forest-wide in 2002. Wide variation in reproductive success and/or the geographic distribution of breeding pairs (a nearest-neighbor breeding scenario) could also explain the deviation from Hardy-Weinberg expectations. The possibility of variable reproductive outcomes between low numbers of established breeding pairs seems plausible. If recruitment was low and extremely site-specific, only a small percentage of the breeding pairs might be contributing to the population gene pool. This scenario is possibly supported by the detection of only two subadult Spotted Owls in the MNF in 2002 (Chi & Henke, 2003). Power to detect inbreeding effects will increase with a larger sample size. Inbreeding hypotheses can be tested more completely by comparing allelic frequencies among different age classes, or by parental/offspring pedigrees (Tarr et al. 1998).

Population Divergence

Population divergence is measured by genetic distances and F-statistics. F-statistics are based on two different models of microsatellite evolution, with $F_{ST}(\theta)$ based on the Infinite Allele Model (IAM) and $R_{ST}(\rho)$ based on the Stepwise Mutation Model (SMM). Both statistics estimate the average level of gene flow, but R_{ST} is subject to bias when sample sizes are low and few loci are scored (Gaggiotti et al. 1999; Balloux & Goudet 2002). F_{ST} is recommended under these conditions and is expected to perform better for recently separated populations (Slatkin 1995). Other considerations that are accounted for by the SMM are the effect of homoplasy and the high mutation rate of microsatellites. Accounting for mutation rates may only be important for groups that have been separated long enough for mutation to become a significant factor. For these reasons, the F_{ST} values in Table 2 are suspected to better characterize the two Northern Spotted Owl populations.

Genetic differentiation between MNF and MC Northern Spotted Owls as estimated by F_{ST} was 12%. There is no reason to assume that the MNF and MC populations were historically reproductively isolated. Current distributions of owls from both populations reveal a 120 km gap between each population's nearest sampled owl territory (Fig. 1). Semi-forested corridors, and suitable habitat along the coastal mountains should provide 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

large, non-forested valleys acted as barriers to dispersal, but that owls would cross fragmented areas consisting of clear-cuts, roads, and variable forest cover types. The median dispersal distances reported for both females and males ranged from 13.5 – 24.5 km, with only 8.7% of 1,475 owls dispersing farther than 50 km.

The genetic distance observed between MNF and MC is also large ($D_a = 0.17$). Both measures imply that gene flow between the two populations is low. These results are consistent with other recent Spotted Owl genetic studies using RAPD markers and mitochondrial DNA. Using RAPDs, Haig et al. found a pattern of high F_{ST} estimates among local breeding areas, and suggested that genetic structuring among groups was due to genetic drift effects resulting from small, fragmented, declining populations of owls (Haig et al. 2001). This fine-scale analysis of two populations is consistent with this suggestion.

Genetic structuring should also be considered in the context of the population characteristics of both MNF and MC spotted owls. The MC population is one of the densest Northern Spotted Owl populations recorded, and should act as a source population. In contrast, the MNF owl population is fragmented, spread over a larger area, and is less densely populated due to large areas of unsuitable habitat (Chi & Henke, 2003). Using a population cluster modeling approach, the hypothesis that the allelic frequencies observed represented two distinct populations was tested. The number of populations inferred from the genetic data is presented in Table 3. A representation of individual owls sampled from each population shows the MC owls as more homogenous (i.e. tighter clustering) compared with the owls sampled in the MNF (Fig. 3). Attempts to cluster the MNF population into subpopulations were unsuccessful. Three owls (in the center) did not cluster with their respective populations, and may be recent immigrants or of immigrant ancestry. The clustering model results are in agreement with the classical measures of population differentiation, and suggest a high degree of isolation between the two Northern Spotted Owl populations.

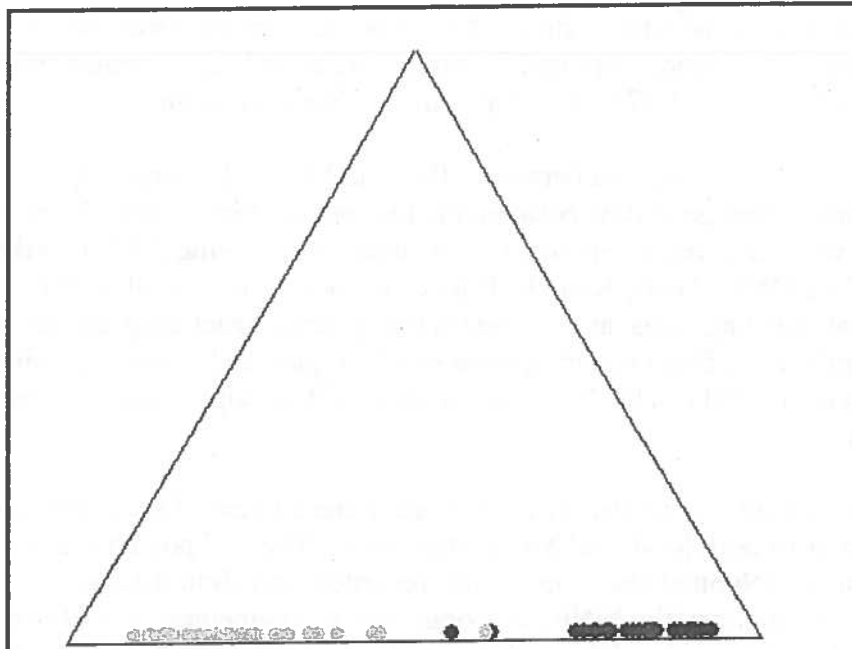


Fig. 3. Representation of population clustering analysis for both MNF (yellow) and MC (blue) assuming 2 populations. Each dot represents an individual. Three owls appear to be possible outliers.

CONCLUSION

Consideration of extinction risks and the interaction of multiple extinction factors are important in the management and recovery of this species. Small effective population size (N_e) is a concern because of the risk of magnifying genetic effects such as drift, hybridization, or inbreeding. Reliance on a few individuals for breeding 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. Genetic factors can combine with demographic factors, human-impacts, and environmental fluctuations to drive species to extinction (Lande 1999).

Both Northern Spotted Owl populations (MC and MNF) can be considered "small". The MNF owl population can be used to illustrate many of the threats and management concerns present throughout the range of the Northern Spotted Owl.

- As in many threatened species populations, the reproductive rates, survival rates, recruitment rates, and limiting factors of the MNF owl population are unknown.
- Spotted Owls within the MNF are subject to habitat alteration following timber harvesting as well as forest fragmentation.
- These owls are likely to be at risk in the future from the introduction of the West Nile virus.
- A closely-related competitor species, the Barred Owl, has been detected within the forest, and circumstantial genetic and field observations suggest hybridization.
- There is some support for inbreeding within demes (although this requires further study).

- Genetic variability is low across several genetic markers and compared to other avian species (Barrowclough & Gutierrez 1990; Barrowclough et al. 1999; Haig et al. 2001).
- Population structuring is high, suggesting low levels of gene flow, although genetic testing of additional Northern Spotted Owl populations is necessary to determine the extent of isolation with other local breeding areas.
- At any time, demographic and/or environmental stochastic factors may combine with any of the above to further threaten the population status.

Risk factors for the Marin County Spotted Owl population are similar, but are summarized specifically as follows:

- Compared to the MNF population, Spotted Owls in MC are closely monitored. Specific information on population demographic characteristics is available.
- This population is located on a mosaic of private and public lands, with most territories protected from habitat alteration.
- Disease vectors are a threat to population conservation.
- Ecological niche modeling has predicted Barred Owl invasion (Peterson & Robins 2003).
- Although significant inbreeding was not detected by microsatellite analysis, inbreeding depression is a concern for this population. Inbreeding, or the mating of close relatives, is more likely to occur in small populations with limited immigration. The geographic isolation of this population from other adjacent Spotted Owl populations increases the risk of inbreeding effects.
- Average gene diversity levels are low (56%). Heterozygosity levels have been positively correlated with fitness, and are a measure of evolutionary potential (Reed & Frankham 2003). In small populations, as is often the case with endangered species, genetic variation is lost as a result of genetic drift. Low heterozygosity is not only an indication of a loss of adaptive genetic variation, but can also suggest reduced reproductive fitness, which increases the risk of extinction (Reed & Frankham 2003).
- Gene flow (measured by F_{ST}) is low with Northern Spotted Owls located farther north in the MNF. This suggests that this population is relatively isolated from other Spotted Owl populations, and may benefit from improved wildlife corridors.

In summary, microsatellite markers suggest low levels of gene flow and low variability among two populations of Northern Spotted Owls. These results are in agreement with past allozyme, RAPD, and mtDNA genetic analyses. Careful consideration of genetic risk factors for Spotted Owls is required to address current management policies and decisions regarding the status of the Northern Spotted Owl.

We have shown that the microsatellites developed for the Mexican Spotted Owl (*Strix occidentalis lucida*) are useful in revealing fine-scale genetic structuring among Northern Spotted Owls. Combined with larger samples and additional populations, the availability of polymorphic microsatellites for assessing variability and gene flow will be useful to discern taxonomic relationships among Spotted Owl subspecies. We have also shown

that non-invasive genetic sampling (i.e. from feather samples) is a valid approach to genetic analysis.

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