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Author(s) :George F. Barrowclough, R. J. Gutiérrez, Jeff G. Groth, Jonas E. Lai and Dennis F. Rock

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THE HYBRID ZONE BETWEEN NORTHERN AND CALIFORNIA SPOTTED OWLS IN THE CASCADE–SIERRAN SUTURE ZONE

GEORGE F. BARROWCLOUGH^{1,4}, R. J. GUTIÉRREZ², JEFF G. GROTH¹, JONAS E. LAI¹, AND DENNIS F. ROCK³

¹Department of Ornithology, American Museum of Natural History, Central Park West at 79th St., New York, NY 10024

²Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, MN 55108

³Western Wildlife Program, National Council for Air and Stream Improvement, Inc., Amboy, WA 98601

Abstract. Both the geographic center and the shape of the hybrid zone between the Northern Spotted Owl (*Strix occidentalis caurina*) and California Spotted Owl (*S. o. occidentalis*) have been unknown because of a lack of samples from the transition zone between Mt. Shasta and Lassen Peak in northern California. However, the position of the boundary between these taxa, and their associated taxonomic rank, have important biogeographical, management, and political implications. We used sequences of the mtDNA control region from owls captured in that region to infer the quantitative structure of this contact zone. The proportion of Northern Spotted Owl haplotypes to total haplotypes fell below 50% approximately 24 km south of the Pit River; the 95% confidence interval for this transition extended from just south of the Pit River to just north of Lassen Peak. We estimated the standard width (20%–80%) of the hybrid zone, which appeared symmetrical, to be 94 km. We rejected the prior hypothesis that the boundary between Northern and California Spotted Owls was the Pit River; rather, the center of the zone did not appear to be closely associated with any obvious physical barrier to gene flow. This is the first described instance of a secondary contact zone between avian taxa that is clearly associated with Remington's Cascade–Sierran suture zone. This hybrid zone was too narrow to be consistent with neutral introgression; it is likely that there is a selective, behavioral, or demographic barrier to gene flow across the Pit River region.

Key words: control region, mtDNA, northern California, Spotted Owl, *Strix occidentalis*, suture zone.

Zona de Hibridación entre *Strix occidentalis caurina* y *Strix o. occidentalis* en la Zona de Sutura Cascades–Sierra Nevada

Resumen. La ubicación y forma de la zona de hibridación entre *Strix occidentalis caurina* y *Strix o. occidentalis* era desconocida por la falta de muestras de la zona de transición entre Mt. Shasta y Lassen Peak en el norte de California. Sin embargo, la posición del límite entre estos taxones y su categoría taxonómica tienen implicaciones importantes desde el punto de vista biogeográfico, de manejo y político. Se usaron secuencias de la región de control del ADN mitocondrial de lechuzas capturadas en esta región para caracterizar cuantitativamente la estructura de la zona de contacto. La proporción de haplotipos de *Strix o. caurina* pasó a ser menor del 50% a aproximadamente 24 km al sur del Río Pit; el intervalo de confianza (95%) de esta transición se extiende desde el sur del Río Pit hasta al norte de Lassen Peak. La zona de hibridación es simétrica y con un ancho estándar (20%-80%) de 94 km. La hipótesis de que el límite entre *Strix o. caurina* y *Strix o. occidentalis* es el Río Pit fue rechazada; el centro de la zona de hibridación no parece estar asociado a ninguna barrera física al flujo génico. Esta es la primera instancia descrita de una zona de contacto secundario en aves claramente asociada a la zona de sutura Cascades–Sierra Nevada de Remington. La zona de hibridación es muy estrecha como para ser consistente con una introgresión neutra; es probable que haya una barrera selectiva, comportamental o demográfica al flujo génico a través de la región del Río Pit.

INTRODUCTION

The careful description of hybrid zones has become an important element of evolutionary studies (Harrison 1993a). Analysis of the spatial distribution of genetic differences between hybridizing taxa can be used to make inferences about the genetic architecture of their divergence and the nature and magnitude of selection, if any, acting to maintain those differences (Barton and Hewitt 1985). For example,

Gay et al. (2008) and Brelsford and Irwin (2009) have used genetic markers to investigate the relative magnitudes of selection and gene flow in hybrid zones involving gulls and wood warblers, respectively, in western North America. In cases of secondary contact, the shape of a hybrid zone has often been important to taxonomic decisions: depending on the magnitude and nature of the differences between the populations, taxa interacting in hybrid zones may be ranked as species or subspecies.

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⁴E-mail: gfb@amnh.org

The classification of populations into subspecies has important implications for political and management decisions about endangered species (Zink 2004, Haig and D'Elia 2010). For example, if two subspecies have contiguous ranges and one is threatened while the other is not, then the precise boundary between the two subspecies may be important in where they are to be managed. Unfortunately, determining the exact boundary between most subspecies is difficult because, historically, subspecies were often based on quantitative attributes such as size or color that vary gradually over distance and are associated with large confidence intervals. As a consequence, many subspecies can be ascertained only at the level of the population (e.g., 90% of one sample distinguishable from 90% of the other: Mayr 1969, Patten and Unitt 2002); in such cases it is the populations—not the individual—that is diagnosable. The resulting inability to make individual assignments greatly complicates a fine-scale description of the transition.

In the case of the Northern (*Strix occidentalis caurina*) and California (*S. o. occidentalis*) Spotted Owls, the traditional subspecies were based on subtle differences in plumage. However, genetic markers, especially mtDNA, are superior to plumage characters for assigning individual owls to distinct genetic clades and hence to subspecies (Barrowclough et al. 2005, Gutiérrez and Barrowclough 2005). In fact, the concordance (>95%) of deep genetic divergence with geography indicates that these taxa are now in secondary, rather than primary, contact; hence, we treat their interaction as a hybrid zone (Harrison 1993b). Consequently, mtDNA sequences can be used to assign a boundary to the ranges of Northern and California Spotted Owls with a precision previously not possible, given an adequate sample from the relevant portion of the range, which lies between Mt. Shasta and Lassen Peak, California. We used dense sampling within the geographical transition zone between the Northern and California Spotted Owls to provide an improved description of the shape and position of the hybrid zone between those taxa. Because the U.S. Fish and Wildlife Service (USFWS) has listed the Northern Spotted Owl but not the California Spotted Owl as a threatened species, the position of the boundary between these two taxa may have important implications for management.

METHODS

FIELD WORK

Using historic location records combined with field surveys, we located Spotted Owls between Mt. Shasta and Lassen Peak, California. We used standard methods for locating and capturing owls (Franklin et al. 1996) on both public and private lands in the springs and summers of 2007–2010. We banded owls, upon their capture, with both color bands and USFWS locking, aluminum leg bands. In addition, we searched for contour feathers that were growing. If we found one or two such growing feathers, we plucked them for DNA analysis. If we found no growing feather, we plucked several small feathers for the same

purpose. In a few cases, we picked up fresh feathers that we observed being shed from an owl. We placed feather samples in DNA buffer and stored them at -30°C . We used the Global Positioning System (GPS) to record sample locations.

LABORATORY WORK AND ANALYSIS

We extracted, amplified, and sequenced DNA from the feather samples by methods previously described (Barrowclough et al. 1999). We used *Strix*-specific mtDNA control region PCR primers and standard *taq*-DNA polymerase amplification to obtain target DNA from each individual sample. Using fluorescent dye labeling and electrophoresis on an ABI 3730xl DNA analyzer, we obtained sequences from both strands of the amplified product. Individual sequences were edited with Sequencher software and aligned by eye. The new sequences were added to our database of previously sequenced samples from northern and central California.

We used PAUP* (Swofford 1998) to estimate the most parsimonious trees for our new samples plus the haplotypes described in Barrowclough et al. (2005). We searched for most parsimonious trees with a heuristic search with 10 replicates of random additions of haplotypes followed by tree-bisection reconnection (TBR) branch-swapping. Using the results of this analysis, we assigned each haplotype to either a Northern or California Spotted Owl mtDNA clade.

Using our GPS data, we placed assigned haplotypes on a spatially explicit map. We drew a straight line through the cloud of owl locations, passing through the centroid of the distribution and oriented along its major axis; it fell east of Mt. Shasta in the northwest and passed through Lassen Volcanic National Park in the southeast. The length of this transect was approximately 200 km. We projected the haplotypes perpendicularly onto this line and assigned each to a 5-km interval. This resulted in a histogram of categorized haplotypes in the region of transition between the Northern and California Spotted Owls. We did not use specimens from the coast ranges west of Mt. Shasta for this analysis.

Haplotypes observed along the transect belonged to either a Northern or a California Spotted Owl clade; consequently, the observations were categorically (binomially) distributed and standard regression approaches were inapplicable for describing the hybrid zone. Therefore, we used the distance from the northern end of the transect to each 5-km interval as the independent variable in a logit analysis (SAS 1985); the number of haplotypes observed in each interval was used as the observation size and the number of California Spotted Owl haplotypes observed in the interval was used as an effect. The probability distribution, $f(z)$, from the logit analysis using a maximum-likelihood estimator, was plotted along the transect; the center (with 95% confidence interval), the standard width (defined as the geographic distance between the 20% and 80% points of the probability distribution), and the maximum slope of the hybrid zone all were computed from the

logit equation, $f(z) = e^{w(x-c)} / [1 + e^{w(x-c)}]$, fit to the data. Here c is the center of the hybrid zone, w is four times its maximum slope, and the distance between $f(z) = 0.2$ and $f(z) = 0.8$ is its standard width (which is equal to $2.7 w^{-1}$ for this model).

We compared the proportion of Northern Spotted Owl haplotypes observed in the 50-km interval immediately north of the inferred center of the hybrid zone to the observed proportion of California Spotted Owl haplotypes in the 50-km interval immediately south of it. For a symmetrical hybrid zone, those proportions should be equal. We compared the 95% confidence intervals for the two proportions, by using a binomial distribution, to determine whether they differed statistically.

RESULTS

We obtained samples from 33 owls between Mt. Shasta and Lassen Peak from 2007 to 2010 (Appendix, Fig. 1) from which we obtained mtDNA control region sequences. The 33 sequences were edited and trimmed of flanking primer and threonine t-RNA sequence; the edited sequences varied in length from 1103 to 1104 contiguous base pairs. At a small number of bases (4 out of 36 432), we observed two peaks of approximately equal intensity on the DNA chromatograms; we assigned these bases standard ambiguity codes. The ambiguities were treated as uninformative in the parsimony analyses and did not affect the assignment of sequences

to haplotype clades. A single-base deletion was required for the sequences to be aligned; this was in a position known from our prior studies to involve an indel; we treated indels as a fifth state in phylogenetic analyses. One individual owl had an mtDNA haplotype identical to a Barred Owl (*S. varia*) haplotype known from Washington and Oregon. We identified nine previously unobserved haplotypes from the 32 new Spotted Owl sequences; one of these novel haplotypes occurred in two individuals. The other 22 individuals possessed mtDNA haplotypes found in prior DNA surveys. The GenBank accession numbers for the novel haplotypes found in this survey are HQ322396–HQ322405.

We used PAUP* to infer a phylogenetic tree for the new Spotted Owl sequences plus all haplotypes from Northern and California Spotted Owls that we had previously obtained. In addition, we added three Mexican Spotted Owl (*S. o. lucida*) and two Barred Owl sequences to the data as outgroups; the GenBank accession numbers of all haplotypes used in our phylogenetic analysis are provided in Figure 2. In total, we identified 266 504 trees of length 238; the topological differences among the trees concerned the relative placement of closely related haplotypes. The consistency index for informative sites was 0.81. The 50% majority rule consensus of those trees (Fig. 2) resembled that reported by Barrowclough et al. (2005); there was one clade of California Spotted Owls and two clades of Northern Spotted Owls.

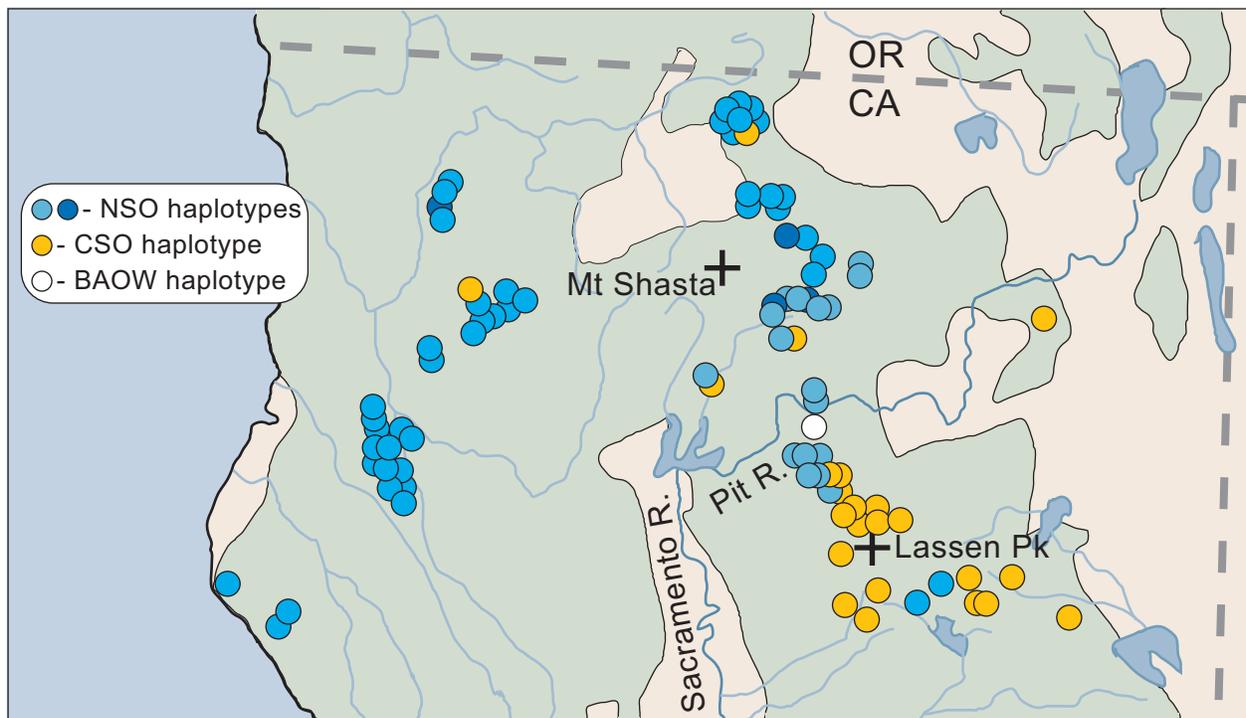


FIGURE 1. Distribution of known Spotted Owl mtDNA haplotypes in northern California. Approximate distribution of suitable Spotted Owl habitat is shown in green. On this map, the position of individual Spotted Owl haplotypes is only approximate: several haplotypes whose positions would appear identical at this scale have been displaced slightly for recognition. The position of a phenotypic Spotted Owl carrying a Barred Owl (BAOW) mtDNA haplotype is also shown. NSO, Northern Spotted Owl; CSO, California Spotted Owl.

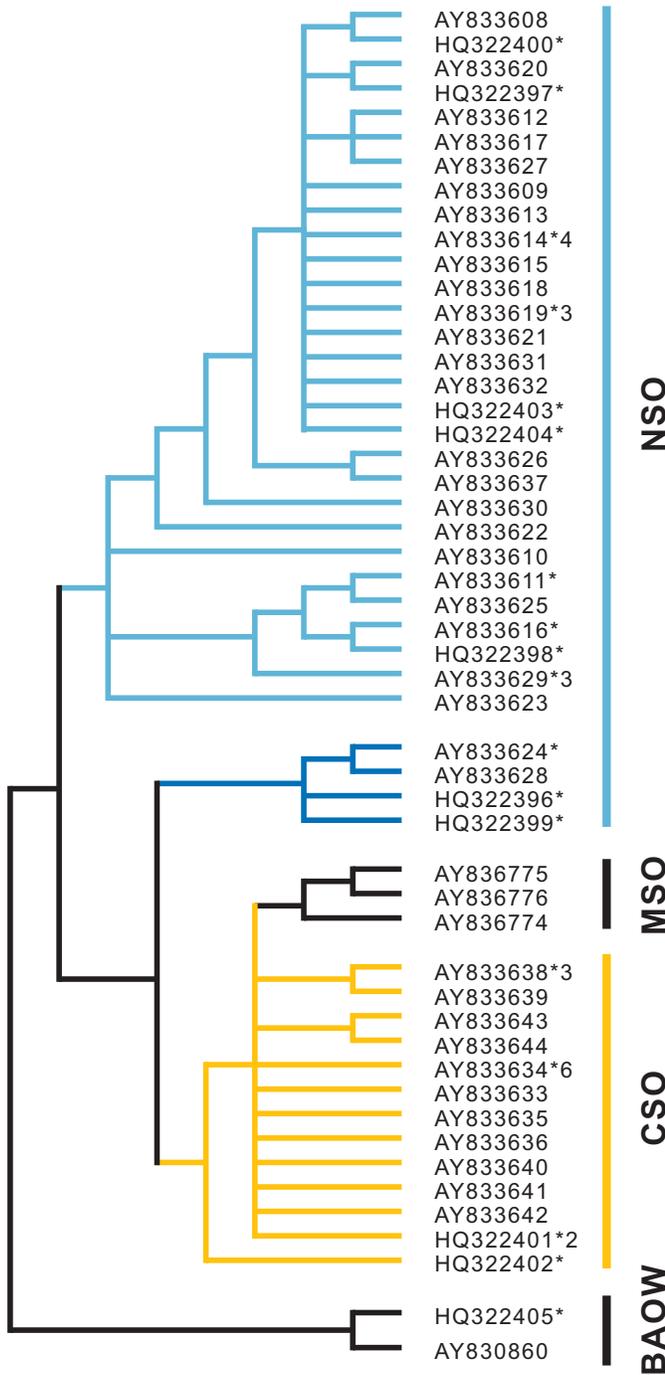


FIGURE 2. Phylogenetic relationships among mtDNA control region haplotypes of Spotted Owls. The 50% majority rule consensus of equally parsimonious trees is shown. Two clades of Northern Spotted Owls (incomplete lineage sorting) are indicated by two shades of blue. The relationships of 33 newly sampled birds, all taken from between Mt. Shasta and Lassen Peak, are indicated with asterisks; 10 of these new samples had 9 novel haplotypes, as indicated. The positions of three exemplar Mexican Spotted Owl (MSO) haplotypes and two of the Barred Owl (BAOW) as an outgroup are also illustrated. NSO, Northern Spotted Owl; CSO, California Spotted Owl.

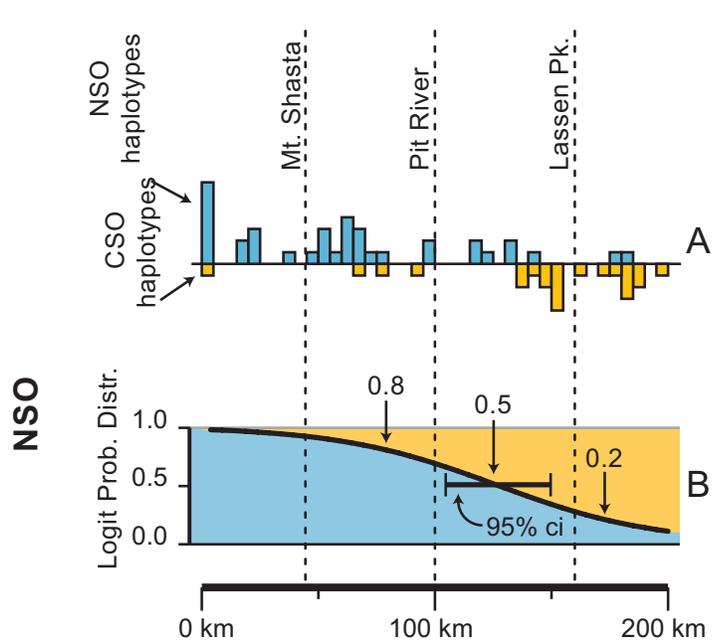


FIGURE 3. Geographic position and shape of the contact zone between Northern (NSO) and California (CSO) Spotted Owls. (A) Histogram of distributions of haplotypes along a transect from north of Mt. Shasta to southeast of Lassen Peak, California (see Fig. 1). (B) Inferred probability distribution of Northern Spotted Owl haplotypes along the transect, showing geographical positions of the 20%, 50%, and 80% points; 95% confidence interval for the 50% position is also indicated.

Following the logic of Barrowclough et al. (2005), we interpreted the latter two clades to be the result of incomplete lineage sorting. This reasoning, described in greater detail in that paper, was based on the occurrence of the second, rare clade only where the other, common clade of Northern Spotted Owl haplotypes was predominant and never south of the Pit River where the California Spotted Owl haplotypes predominated. Thus the occurrence of this clade in the north can not easily be interpreted as gene flow from the south because there is no apparent source population in the south. Second, this rare clade was sister to both the California Spotted Owl clade and the clade of Mexican Spotted Owls, suggesting it represents an ancestral clade. Third, an analysis of coalescence specifically designed to discriminate between gene flow and lack of lineage sorting favored an interpretation of incomplete lineage sorting. Consequently, incomplete lineage sorting seems to represent the most parsimonious interpretation of the distribution of haplotypes on the mtDNA trees. With this interpretation, we categorized each of the 32 new Spotted Owl samples as possessing a Northern or a California Spotted Owl haplotype, on the basis of its position in the consensus tree.

The approximate geographic distributions of the Northern and California Spotted Owl haplotypes are shown in Figure 1. The projection of haplotypes of the two subspecies, from north of Mt. Shasta to east of Lassen Peak, onto a linear

transect through that region is shown in Figure 3A along with the approximate position of the Pit River. The GenBank accession numbers of all individuals used in the transect are provided in the Appendix. The observed transition from predominantly Northern Spotted Owl to predominantly California Spotted Owl haplotypes was strikingly abrupt.

The logit distribution of proportions of Northern Spotted Owl haplotypes is shown in Figure 3B. The point estimate for the center of the distribution was 23.7 km south of the Pit River; however, this was approximate because the Pit River meanders and hence was not precisely perpendicular to our transect. The 95% confidence interval for the center reached from just south of the river to just north of Lassen Peak. Our estimate of the shape parameter of the logit equation, w , was 0.0295. The 20%–80% width of the hybrid zone (e.g., Endler 1977) was estimated to be 94 km, on the basis of the logistic regression. For purposes of population genetics modeling, a more useful parameter is the maximum slope, that is, the slope of the tangent to the transition curve at the 50% point (Barton and Gale 1993); for this zone, our estimate of the maximum slope, $w/4$, was 0.007 km^{-1} . From the relative proportions of haplotypes immediately north and south of the center of the zone, the shape of the hybrid zone appeared to be approximately symmetrical. In the 50-km interval immediately north of the inferred center of the zone, we found 6 Northern Spotted Owl haplotypes out of 8 total; in the same distance south of the inferred center, we encountered 12 California Spotted Owl haplotypes out of 16 total. Those fractions were not significantly different ($P > 0.05$).

DISCUSSION

CONTACT BETWEEN THE NORTHERN AND CALIFORNIA SPOTTED OWLS

The original descriptions of the Northern and California Spotted Owls were based on a small number of specimens, taken from Puget Sound and southern California, respectively (Merriam 1898); specimens from intervening localities were not available at the time of the original descriptions. A subsequent morphometric analysis of traits cited in the original description of the Northern Spotted Owl revealed only subtle clinal variation in size, plumage pattern, and color among population samples from throughout the range of the species (Barrowclough and Flesness 1996); thus the original recognition of the races was apparently due to sampling of individuals from distant ends of clines, which differed in color and spot size. However, subsequent mitochondrial DNA analyses demonstrated an abrupt discontinuity of genetic variation in northern California (e.g., Barrowclough et al. 2005); for example, north and west of Mt. Shasta, 79 of 81 (97.5%) individuals sampled carried Northern Spotted Owl haplotypes, whereas south of Lassen Peak, 87 of 89 (97.8%) individuals possessed California Spotted Owl haplotypes. Nuclear micro-

satellite markers also revealed a sharp genetic division concordant with the mtDNA results (Funk et al. 2008). Therefore, the subspecies are readily diagnosed on the basis of many DNA characters although not on the basis of the plumage traits cited in the original descriptions.

These DNA clades of haplotypes clearly meet between Mt. Shasta and Lassen Peak (Fig. 1), but is this a hybrid zone? An alternative interpretation is that these are two parapatric taxa that are in contact, without hybridization, with occasional limited dispersal resulting in a few apparently misplaced haplotypes. The plumage differences between the Northern and California Spotted Owls are too subtle to reveal whether hybrid offspring or even mixed pairs occur in northern California. However, in the region between Mt. Shasta and Lassen Peak where both haplotypes occurred in frequency sufficient for considerable hybridization, we caught both members of territorial pairs on five occasions during our field work; on three of these both individuals were Northern Spotted Owls and two were mixed pairs. Thus it appears that at least some hybridization is occurring in northern California and that the zone of contact can be characterized as a hybrid zone, *sensu* Harrison (1993b). Whether this hybrid zone is characterized by extensive continuing introgression, or instead represents a narrow tension zone stabilized by selection or a demographic filter, can be determined only by analysis of its shape and dynamics.

POSITION AND SHAPE OF THE HYBRID ZONE

Hybrid zones and other clines are attracted to physical barriers or regions of reduced population density, whether or not selection is involved in their maintenance (Barton 1979, Barton and Hewitt 1985). However, the Northern/California Spotted Owl hybrid zone apparently is not centered on the salient physical barrier, the Pit River, which is found between Mt. Shasta and Lassen Peak. Instead, the center is located 24 km south of the river. Whether that represents a region of particularly low population density is unknown; the entire Pit River region appears to have very few Spotted Owls (see below).

The general location of this hybrid zone, between Mt. Shasta and Lassen Peak, where the Cascades jog closer to the coast from the Sierra Nevada, was cited as a minor suture zone by Remington (1968), but the only avian contact zones he listed for the region were those between two subspecies of sapsuckers, *Sphyrapicus r. ruber* and *S. r. daggetti*, and two subspecies of juncos, *Junco hyemalis shufeldti* and *J. h. thurberi*. Howell (1952), however, thought the sapsucker zone was actually situated in southern Oregon, and Miller (1941) placed the junco zone within the Cascades and coastal ranges, again in southern and central Oregon. Thus neither of those avian transitions was actually situated where the Cascades abut the Sierra Nevada. In addition, although those two subspecific boundaries have not been examined in detail by modern molecular techniques, neither appears to be a zone

TABLE 1. Widths of mtDNA hybrid zones in North American birds.

Taxa	Width ^a	Region	Rank	Citation
<i>Larus glaucescens</i> / <i>L. occidentalis</i>	1140 km	Pacific coast	species	Gay et al. 2008
<i>Strix o. occidentalis</i> / <i>S. o. caurina</i>	94 km	Cascade-Sierran suture zone	subspecies	this study
<i>Colaptes a. auratus</i> / <i>C. a. cafer</i>	no fixed differences	Great Plains suture zone	subspecies	Moore et al. 1991
<i>Catharus u. ustulatus</i> / <i>C. u. swainsoni</i>	50 km	British Columbia coastal ranges	subspecies	Ruegg 2007
<i>Amphispiza belli nevadensis</i> / <i>A. b. canescens</i>	53 km	Great Basin/Mojave Desert	subspecies	Cicero and Johnson 2007
<i>Pheucticus ludovicianus</i> / <i>P. melanocephalus</i>	30–50 km	Great Plains suture zone	species	Mettler and Spellman 2009; Spellman, pers. comm.
<i>Passerina amoena</i> / <i>P. cyanea</i>	155 km	Great Plains suture zone	species	Carling and Brumfield 2008

^a20%-80%.

of secondary contact. Liston et al. (2007) identified a hybrid zone in this area between two cpDNA haplotype clades of sugar pine (*Pinus lambertiana*); it was centered close to Lassen Peak (Liston et al. 2007). In any case, the Spotted Owl hybrid zone is the first well-characterized avian zone of secondary contact associated with this suture zone.

At approximately 94 km in width, the Spotted Owl zone is quite narrow, somewhat surprisingly given that these owls are large, vagile predators capable of long-distance dispersal (Forsman et al. 2002). At present, there are only a few mtDNA studies of avian hybrid zones that have been sampled at geographical resolution sufficient for this zone to be placed in appropriate context. Avian hybrid zones vary in width by at least two orders of magnitude: a hybrid zone between two species of *Manacus* in Panama is only 7.7 km wide (Brumfield et al. 2001), whereas one between the Western and Glaucous-winged Gulls (*Larus occidentalis/glaucescens*) along the Pacific coast of North America extends for about 1140 km (Gay et al. 2008). Within the temperate zone of North America, the hybrid zone between these Spotted Owls is of the same order of width as that found between the few pairs of species-level or near-species-level taxa of hybridizing birds now in secondary contact that have been studied with mtDNA (Table 1).

We fit the pattern of categorized mitochondrial haplotypes to a logistic curve, as is standard for hybrid zones involving binary molecular characters (e.g., Barton and Gale 1993, Brumfield et al. 2001, Gay et al. 2008, Mettler and Spellman 2009). This procedure, however, would result in an inaccurate description of shape if the actual zone were substantially

asymmetrical, as is known to be the case in a few instances (e.g., Rohwer and Wood 1998). However, in this case, the proportional changes around the inflection point of the regression were not asymmetric.

EVOLUTIONARY DYNAMICS

In a prior analysis (Barrowclough et al. 2005), we estimated the gene-flow (σ) distance for Northern Spotted Owls in prime habitat to be on the order of 25–30 km per generation, on the basis of results from a study by Forsman et al. (2002). For a hybrid zone without selection, the width of the zone is expected to increase according to the relationship $W = 2.5\sigma\sqrt{T}$, where T is the number of generations since initial contact (Barton and Gale 1993). For the case of these Spotted Owls, with a hybrid zone less than 100 km wide and σ of approximately 25 km, T would have to be fewer than 2 generations, or less than 10 to 20 years, to be consistent with no selection. This value is unrealistically short, given the likely existence of forest in the Sierra Nevada and the Cascade Mountains for at least 6000 years (Thompson and Anderson 2000). Consequently, it is more likely that there is (1) selection against hybrids between the two taxa, (2) a local reduction of population density between the two volcanoes, or (3) a behavioral reluctance to disperse through this region.

In fact, local reductions in density or dispersal are not unreasonable. Much of the area southeast of Mt. Shasta, even prior to logging, was probably less suitable for Spotted Owls than the Sierra Nevada and the Coast Ranges because this region was dominated by either open mid-elevation ponderosa

pine (*Pinus ponderosa*) forest or high-elevation fir forest; neither of which is primary owl habitat (Gutiérrez et al. 1995). More recently, the area has been affected by both logging and large scale, stand-replacing fires with subsequent conversion to tree plantations. At present, the density of Spotted Owls, especially in the immediate vicinity of the Pit River, appears to be low. Whether there was a natural vegetation barrier in the past, future environmental conditions will likely contribute to the continuation of a habitat barrier. Any of these phenomena, continuing reduction of density or dispersal or selection against hybrids, would result in few Spotted Owls residing in or crossing the forested area between Lassen Peak and Mt. Shasta. This would effectively fix the position of the hybrid zone in that area and promote the long-term stability of a taxonomic boundary in the Pit River region (Barrowclough et al. 2005).

TAXONOMY

The northern subspecies of the Spotted Owl was originally described on the basis of plumage color from a few birds, all from near Puget Sound, in Washington state (Merriam 1898); subsequently, it has been determined that color changes from the Northern to the California Spotted Owl in a gradual, clinal fashion. Thus plumage is useful for the analysis of large population samples but not for individual specimens (Barrowclough 1991). Consequently, because mtDNA sequences allow a straightforward assignment of individual owls to the Northern or California clade, a logical geographical position for recognizing a boundary between the two subspecies is the point at which the proportions of haplotypes of the two taxa change from less than to greater than 50%. Our newly sequenced samples of Spotted Owls, along with those previously reported, indicated that the proportion of Northern Spotted Owl haplotypes falls below 50% at approximately 24 km south of the Pit River; the 95% confidence interval for that position lies between the Pit River and Lassen Peak.

Until recently, it was not realized that there was any contact between the Northern and California Spotted Owls. For instance, Grinnell and Miller (1944) mapped completely allopatric ranges for the two subspecies and, in its most recent treatment of subspecies of North American birds, the AOU (1957) also listed the ranges of the subspecies as allopatric. However, on the basis of much new data gathered since 1957, Gutiérrez and Barrowclough (2005) realized the taxa were probably parapatric and proposed a boundary between them based on a large-scale survey that suggested probable contact and occasional dispersal between the ranges (Barrowclough et al. 2005). The exact geographical characteristics of the contact were unknown at that time, so Gutiérrez and Barrowclough (2005) chose the Pit River as a boundary because it was a salient geographical feature located approximately halfway between populations known to consist largely of pure Northern Spotted Owl haplotypes at Mt. Shasta and largely of pure California Spotted Owl haplotypes at Lassen Peak. A subsequent study, using microsatellites, also indicated that the boundary between the taxa must

be in northern California or southern Oregon (Funk et al. 2008), but that study lacked dense sampling from the critical region in northern California. Our new results are incompatible with the Pit River hypothesis.

Given our new data, there are two reasonable options for delimiting the boundary between the Northern and California Spotted Owls. One might consider the boundary to be an arbitrary line, roughly oriented west-southwest to east-northeast, approximately 24 km south of the Pit River. An alternative would be to recognize birds found north of the Pit River as Northern Spotted Owls, those south and east of Lassen Peak as California Spotted Owls, and those owls in the narrow zone between the Pit River and Lassen Peak as intergrades. The former option is precise but perhaps more so than justified by evidence. The latter is more consistent with the data but would create some uncertainty for resource managers in the local area. We note that the second approach is consistent with AOU Check-list (1957, 1998) practice: geographically precise boundaries have rarely been provided for hybridizing parapatric taxa at either the subspecific (e.g., Northern Flicker, *Colaptes auratus*) or specific rank (e.g., Baltimore and Bullock's Orioles, *Icterus galbula/I. bull-ockii*). We favor this latter approach because it is biologically realistic, if politically problematic; a small number of owls in a 50-km-wide swath of low population density habitat will be treated somewhat arbitrarily. Haig et al. (2004) discussed aspects of the legal implications of treating some individuals as hybrids.

This report concerns the position and shape of a hybrid zone and does not seem to be an appropriate place to add to the debate about the use of the subspecific rank (e.g., Winker and Haig 2010), but the following point does seem to be in order. The Northern and California Spotted Owls are generally ranked as subspecies (e.g., AOU 1998); however, as pointed out by Barrowclough et al. (2005), comparison of their mtDNA sequences reveals multiple diagnostic characters that imply a long history of geographic isolation. Clearly the two taxa would be recognized as separate species by advocates of the phylogenetic species concept (Zink and McKittrick 1995). The results reported here do not negate that interpretation; rather, they indicate that an appropriate place to recognize a boundary between the two taxa, regardless of rank, is between the Pit River and Lassen Peak. In fact, our new results provide no evidence for unimpeded gene flow across the hybrid zone, as might be anticipated within a single biological species.

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APPENDIX. Spotted Owls sampled in this analysis.

Sample	Northing ^a	Easting ^a	Clade	Haplotype (GenBank)
Prior samples: north of Mt. Shasta				
1387-17835	4631607	569195	Northern	AY833619
1387-17839	4631607	569195	Northern	AY833611
1387-17841	4631607	569195	Northern	AY833611
1387-42107	4631607	569195	Northern	AY833619
1387-42110	4631607	569195	Northern	AY833632
1387-42164	4631607	569195	Northern	AY833619
1387-50967	4631607	569195	California	AY833633
1387-50975	4631607	569195	Northern	AY833619
1387-17843	4613251	583249	Northern	AY833619
1387-21339	4613251	583249	Northern	AY833626
1387-50919	4613251	583249	Northern	AY833611
1387-17844	4613103	569374	Northern	AY833616
1387-21347	4613103	569374	Northern	AY833619
1387-42100	4594921	597374	Northern	AY833629
1387-82028	4590015	603914	Northern	AY833619
1387-82026	4584508	600510	Northern	AY833614
New samples				
1857-09241	4598034	588753	Northern	AY833624
1387-17854	4591300	616009	Northern	AY833629
1177-20279	4591300	616009	Northern	AY833614
1177-20292	4575072	595468	Northern	HQ322396
1177-20295	4575072	595468	Northern	AY833616
1857-09238	4575030	595797	Northern	HQ322399
1857-09257	4574836	600875	Northern	AY833619
1857-09259	4574836	600875	Northern	AY833629
1177-20294	4574211	595248	Northern	HQ322397
1857-09237	4573467	593423	Northern	HQ322398
1177-20278	4571277	684801	California	HQ322401
1177-20290	4564889	589312	Northern	HQ322400
1857-09239	4564670	589276	California	HQ322401
1387-97872	4552882	562075	Northern	AY833619

(continued)

APPENDIX. Continued.

Sample	Northing ^a	Easting ^a	Clade	Haplotype (GenBank)
1387-97871	4552882	562075	California	HQ322402
1177-22184	4542117	603614	Northern	AY833619
1177-22185	4542117	603626	Northern	AY833629
1387-97873	4533534	601704	Barred	HQ322405
1177-20293	4517840	604370	Northern	HQ322403
1857-09260	4517812	601504	Northern	HQ322404
1857-09261	4517812	601504	Northern	AY833614
CC-1	4505011	609147	Northern	AY833611
CC-2	4505011	609147	Northern	AY833614
1387-78971	4504894	614141	California	AY833634
1857-09253	4504511	612811	California	AY833634
1387-84851	4498939	618475	Northern	AY833614
1387-78854	4498927	618463	California	AY833638
1807-49194	4496725	622443	California	AY833634
1387-84868	4496671	622852	California	AY833638
1807-49181	4492956	634596	California	AY833634
1387-84860	4492253	627826	California	AY833638
1387-97878	4492153	626878	California	AY833634
1387-78890	4491724	626272	California	AY833634
Prior samples: south of Lassen Peak				
1387-47443	4479311	615903	California	AY833634
1387-56366	4475381	674176	California	AY833634
1387-47436	4473218	660215	California	AY833634
1387-56362	4472339	652072	Northern	AY833614
1387-52699	4467757	624317	California	AY833638
1387-47439	4467298	663197	California	AY833635
1387-47442	4467298	663197	California	AY833634
1387-52693	4466222	693304	California	AY833636
1387-47438	4464288	642624	Northern	AY833637
1387-52695	4463814	614915	California	AY833634
1387-56371	4460697	620702	California	AY833634

^aNAD83: zone 10T.