The Auk 128(4):696–706, 2011

PHYLOGEOGRAPHY OF THE BARRED OWL (*STRIX VARIA*): SPECIES LIMITS, MULTIPLE REFUGIA, AND RANGE EXPANSION

George F. Barrowclough,[1] Jeff G. Groth, Karan J. Odom,[2] and Jonas E. Lai

Department of Ornithology, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024, USA

Abstract.—The Barred Owl (*Strix varia*) is a common nonmigratory owl distributed across southern Canada, south to California in the west, and to Texas and Florida in the east, with isolated populations in central Mexico. We examined the genetic structure of Barred Owl populations throughout their range using 500–600 base pairs each of one nuclear and three mitochondrial genes. In 75% of the shortest trees and 64% of the bootstrapped trees, Barred Owls were not monophyletic; rather, *S. v. sartorii* of Mexico was separated from the remaining taxa of *S. varia* by *S. fulvescens* of Central America. Consequently, the Barred Owls of Mexico are a species-level taxon. There was a large component (32%) of genetic variance distributed among population samples from the United States and Canada because of the occurrence of two clades of haplotypes (4.8% sequence divergence) with differing geographic distributions. One clade was predominant along the Atlantic Coast and the second in the south-central United States. The two clades co-occurred from the central Gulf Coast to the Upper Midwest, and across Canada to the Pacific. Nucleotide diversity was greatest where the clades overlapped in occurrence; mismatch distributions possessed the signatures of population expansion from the southern and eastern states to northern and western locations. These results suggest two Pleistocene refugia for northern populations of Barred Owls. Diversity within populations and divergence between haplotype clades varied by an order of magnitude among the three mitochondrial genes, but each recovered the overall phylogeographic pattern. The nuclear sequences showed much less variation and differentiation.
Received 9 March 2011, accepted 6 July 2011.

Key words: ACO1, Barred Owl, mtDNA, phylogeography, Pleistocene, *Strix varia*, suture zones.

Filogeografía de *Strix varia*: Límites Específicos, Refugios Múltiples y Expansión Geográfica

Resumen.—*Strix varia* es una lechuza no migratoria, común desde el sur de Canadá hasta el sur de California al oeste y hasta Texas y Florida al este, con poblaciones aisladas en el centro de México. Examinamos la estructura genética de poblaciones de *S. varia* en toda su distribución con 500–600 pares de bases de un gen nuclear y tres genes mitocondriales. En el 75% de los árboles más cortos y en el 64% de los árboles de "bootstrap" *S. varia* no fue monofilética debido a que *S. v. sartorii* de México estuvo más relacionada con *S. fulvescens* de Centroamérica que a otras subespecies de *S. varia*. Por lo tanto, las lechuzas *Strix* de México constituyen una especie distinta. La existencia de dos clados de haplotipos (4.8% de divergencia) con distintas distribuciones resultó en una alta variación genética entre poblaciones (32%). Uno de los clados fue predominante en la costa Atlántica y el otro lo fue en el centro sur de los EE.UU. Poblaciones con ambos clados se encontraron desde la costa central del Golfo de México hasta el centro norte de los EE.UU. y a través de Canadá hasta el Pacífico. La diversidad genotípica fue máxima donde ambos clados se superpusieron. Las distribuciones de substituciones sugieren eventos de expansión poblacional desde el sur y el este de los EE.UU. hacia el norte y el oeste. Estos resultados sugieren la existencia de dos refugios pleistocénicos para las poblaciones norteamericanas. La diversidad genética intrapoblacional y la divergencia entre los haplotipos variaron en un orden de magnitud entre los tres genes mitocondriales, pero los tres mostraron el mismo patrón filogeográfico.

Initial continent-wide surveys of avian phylogeographic patterns failed to reveal substantial genetic structure within eastern North America (e.g., Zink et al. 1991, Ball and Avise 1992, Zink 1996). This apparent homogeneity was consistent with a Mengelian worldview that a single, large, southern refuge for many elements of the eastern avifauna gave rise to postglacial expansions and occupation of the eastern deciduous and boreal coniferous forests (Mengel 1964, 1970). To some extent, this idea disagreed with palynological data that suggest multiple refugia in the east for plants (Pielou 1991), as well as Remington's (1968) identification of several suture zones between diverse (non-avian) floral and faunal elements of the eastern United States. In addition, phylogeographic surveys of non-avian taxa were uncovering deep structure in a wide array of plants and animals in the Southeast (Soltis et al. 2006). Nevertheless, following the early lack of dramatic results in the east, ornithologists turned their attention

[1]E-mail: gfb@amnh.org
[2]Present address: Department of Biological Sciences, University of Maryland, Baltimore County, Baltimore, Maryland 21250, USA.

The Auk, Vol. 128, Number 4, pages 696–706. ISSN 0004-8038, electronic ISSN 1938-4254. DOI: 10.1525/auk.2011.11057

to western North America, where fragmented forests associated with isolated mountain ranges harbored a rich store of morphologically differentiated species, all waiting to be studied using DNA technologies. For example, Spellman and Klicka (2007) surveyed the widespread White-breasted Nuthatch (*Sitta carolinensis*) and found a single mitochondrial DNA (mtDNA) clade in the eastern United States, but three distinct clades, each associated with a separate biogeographic region, in the west.

There are three caveats associated with the early studies of avian phylogeography in eastern North America: many of the species examined were migratory, the sampling in the southern portion of the ranges was often very sparse, and the surveys were frequently performed using restriction enzyme digests (RFLPs). First, the vagility of birds would seem to suggest that, if migratory behavior were associated with increased dispersal, then one might prefer to examine nonmigratory species in the search for historical biogeographic patterns. For example, six of the first seven species examined in the continent-wide surveys by Ball and Avise (1992) were migratory. Second, surveys were often characterized by six or fewer sampled locations scattered over the entire continent. Finally, restriction fragment analysis, the most feasible method for assaying DNA variation in the pre-PCR (polymerase chain reaction) era, accounts for only a limited portion of the mtDNA molecule and results in poor tree resolution vis-à-vis nucleotide sequences. In addition, RFLPs were often associated with unrooted networks due to problems of incorporating outgroups. Of course, the reason for several of these shortcomings was unavoidable; RFLP analysis was very expensive. Nevertheless, in light of the consistent findings in non-avian taxa (Soltis et al. 2006), it seemed desirable to investigate further the phylogeographic patterns of widespread eastern North American birds using dense sampling of nonmigratory species and rapidly evolving mtDNA gene sequences.

Prior phylogeographic surveys of owls in the genus *Strix* suggest that these birds might be useful tools for deciphering biogeographic history. A survey of Spotted Owls (*S. occidentalis*) in the western United States uncovered unexpectedly deep genetic divergences among morphologically poorly differentiated subspecies (Barrowclough et al. 1999), and a survey of Tawny Owls (*S. aluco*) across Europe revealed strong evidence for three distinct Pleistocene refugia in peninsular regions extending into the Mediterranean (Brito 2005).

We investigated the genetic population structure of the Barred Owl using mtDNA sequences. The Barred Owl is a large, nonmigratory, common and widespread owl continuously distributed from British Columbia to Nova Scotia and south to Florida and Texas with isolated populations in the southern highlands of Mexico (Mazur and James 2000). It has recently expanded its range into the Pacific Northwest, where it is the subject of controversy in the conservation community because of its potential threat to Northern Spotted Owls (*S. occidentalis caurina*; Gutiérrez et al. 2007). Barred Owls are divided into four subspecies based on size, plumage coloration, and amount of feathering on toes (American Ornithologists' Union [AOU] 1957). However, the subspecific variation is subtle and clinal at subspecific boundaries (Pyle 1997). Some accounts have recognized the Fulvous Owl (*S. fulvescens*) of southeastern Mexico, Guatemala, and western Honduras as a fifth subspecies (Peters 1940).

Methods

Samples.—We obtained samples of Barred Owls from throughout their range, including all four generally recognized (AOU 1957) subspecific taxa. Samples comprised birds not known to be matriarchally related from 19 localities, each of which was composed of one or more geographically proximal counties or parishes (Table 1 and Fig. 1). In three cases, samples were pooled between states: the New Jersey sample included two owls from southeastern New York, the Louisiana sample included one bird from southern Mississippi, and the Tennessee sample included three owls from northern Mississippi. The pooling of specimens over distances of up to several hundred kilometers allowed us to perform within and among

Table 1. Estimates of within-population genetic variation in Barred Owls based on control-region sequences from mtDNA (*$P < 0.01$).

Population	Number of individuals sampled	Number of haplotypes observed	Percent "Atlantic Coast"	Percent private haplotypes	Percent widespread haplotypes	Percent variable sites	π (95% confidence interval)	Tajima's *D*
Nova Scotia (NS)	5	4	100%	25%	50%	0.77	0.0031 (0.0008–0.0039)	–1.05
Maine (ME)	10	7	100%	43%	43%	2.50	0.0067 (0.0034–0.0086)	–1.09
New Jersey (NJ)	12	9	100%	78%	22%	2.30	0.0056 (0.0030–0.0069)	–1.13
North Carolina (NC)	16	14	100%	93%	7%	5.18	0.0112 (0.0077–0.0129)	–1.17
Georgia (GA)	10	9	60%	67%	11%	5.95	0.0266 (0.0135–0.0280)	1.28
Florida peninsula (FL-pen)	10	9	80%	89%	11%	6.14	0.0209 (0.0070–0.0273)	–0.18
Florida panhandle (FL-pan)	9	8	56%	50%	13%	6.53	0.0304 (0.0165–0.0313)	1.34
Ohio (OH)	11	10	64%	100%	0%	6.53	0.0280 (0.0136–0.0302)	1.20
Michigan (MI)	10	10	40%	90%	10%	7.49	0.0311 (0.0176–0.0322)	0.86
Minnesota (MN)	11	10	82%	80%	20%	6.91	0.0208 (0.0054–0.0290)	–0.55
Alberta–Athabasca (AB-a)	10	5	80%	40%	60%	5.37	0.0190 (0.0019–0.0270)	0.01
Alberta–Two Lakes (AB-t)	10	4	80%	0%	75%	4.80	0.0179 (0.0011–0.0261)	0.25
Washington (WA)	10	5	50%	40%	40%	4.99	0.0271 (0.0116–0.0276)	2.55*
Oregon (OR)	10	4	70%	50%	50%	5.95	0.0247 (0.0070–0.0290)	0.83
Kansas (KS)	12	10	0%	100%	0%	6.14	0.0145 (0.0083–0.0172)	–1.30
Louisiana (LA)	10	10	10%	100%	0%	6.53	0.0196 (0.0111–0.0254)	–0.73
Tennessee (TN)	11	9	0%	78%	0%	4.22	0.0132 (0.0083–0.0151)	–0.38
Texas (TX)	10	7	0%	100%	0%	3.07	0.0081 (0.0032–0.0110)	–1.19

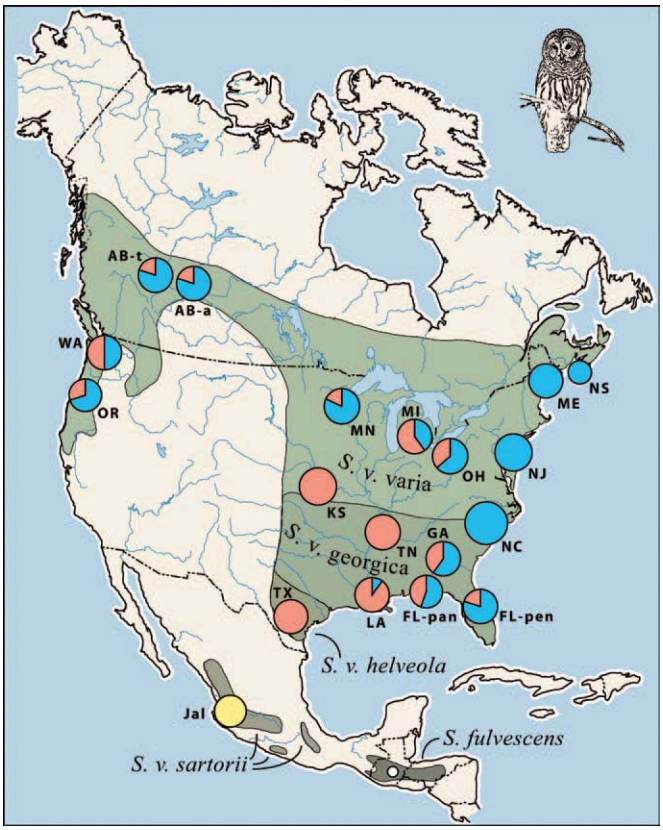

FIG. 1. Distribution, taxonomy, and phylogeography of Barred Owls across their range. Approximate distributions of four generally recognized (AOU 1957) subspecies are indicated by different shades of green; distribution of Fulvous Owl is also indicated. Approximate geographic locations of 19 population samples are indicated by pie-diagrams and locality abbreviations (Table 1). Observed proportions of sampled individuals in each of two major haplotype clades (Fig. 3) are indicated by different colors; area of each pie-diagram is proportional to sample size.

population analyses that seemed appropriate given the large overall geographic scale of this survey. We used fresh tissue, blood, and feather samples, obtained from natural-history museums, raptor rehabilitation centers, and raptor researchers, for samples from localities in the United States and Canada. However, we were unable to locate any recent samples from the endemic Mexican taxon, *S. v. sartorii*. Consequently, we used toe-pad samples from skin specimens in the American Museum of Natural History (AMNH) collection for that population. That sample was composed of nine specimens, all from Jalisco ("Jal" in Fig. 1), collected between 1892 and 1905; those skins were old and, hence, subject to DNA degradation. Some authors have treated the Fulvous Owl (*S. fulvescens*) of northern Central America as a subspecies of *S. varia* (e.g., Peters 1940). Consequently, we used a toe-pad of a skin specimen from Guatemala in the AMNH collection as a representative of that taxon; it was also an old specimen (date of collection unknown), presumably with lower-quality DNA. Spotted Owls were used as outgroups in several analyses.

Laboratory methods.—Laboratory methods were generally similar to those used in our laboratory in prior studies of Spotted Owls (Barrowclough et al. 1999) and Tawny Owls (Brito 2005). Total genomic DNA was extracted from tissues using DNeasy Tissue Kits (Qiagen, Valencia, California). We targeted three discrete regions of the mitochondrial genome for PCR amplification with *taq*-DNA polymerase; these included fragments of the CO3 and ND6 protein-coding genes, as well as a portion of the control region (CR). Novel primers were designed for CO3 and ND6 for Barred Owls. The CO3 primer sequences were L9880: 5′-GCAGTAGCTATAATCCAAGCCTACGT-3′ and H10526: 5′-ATGATTACGTGTAGTCCGTGGAATCC-3′. The ND6 primer sequences were PRO3: 5′-CCTCCGCCGCCAACTCCCAAAGCT-3′ and GLUB: 5′-GTCGCAGACCTTGGGTACAGTCCAA-3′. We used previously described primers N1 and D12 to amplify the CR sequences (Barrowclough et al. 1999: figure 2). Prior research on other *Strix* species (e.g., Brito 2005) had revealed a duplication of the control region in members of this genus, but with the two copies flanked by different transfer RNAs. This was also true of the Barred Owl. Because N1 is located outside the CR, in the flanking threonine t-RNA (at the 5′ end of the light-strand sequence), homologous CR sequences were always obtained. For fresh samples, these three sets of primers yielded PCR products 600–700 base pairs (bp) in length; these were isolated on agarose gels, sequenced using fluorescent dye labeling, and run out on either an ABI 377, ABI 3100, or ABI 3730xl DNA analyzer.

We were unable to amplify large pieces of mtDNA from the old toe-pad samples. Instead, we amplified a series of smaller, 100–200 bp, pieces of DNA for those specimens using a set of *S. varia*-specific primers designed for this study for the ND6 and CO3 genes. We were unable to use a series of closely spaced primers to amplify small fragments of the control region, however, because such internal primers will not yield consistently homologous sequence given the CR duplication in these owls. Consequently, we only obtained ND6 and CO3 sequence from the Mexican Barred Owls as well as from the Fulvous Owl. Sequences were aligned by eye.

We amplified and sequenced the intron between the 14th and 15th exons of the Aconitase gene (ACO1-I15) for a subset of the individuals used in the CR analysis. Aconitase is a sex-linked locus residing on the nuclear Z chromosome. Because nuclear loci are present in much lower copy number than mitochondrial loci, we only attempted to amplify the gene from higher-quality tissue samples. We designed a novel primer pair for Barred Owls (AI15fBi: 5′-GCCACTGGTAATTGCCTACGCAAT-3′ and AI-15rAi: 5′-GACGCTCAACAGCCTGAATCTCATT-3′) and used them for the PCR amplification and sequencing; otherwise, the laboratory methods for this locus were similar to those used for the mtDNA fragments. In order to determine the number of copies of alternate ACO1 alleles within populations, we sexed the owls using the standard CHD-Z/W intron system (Griffiths et al. 1998).

Analysis.—We edited sequences using SEQUENCHER (Gene Codes, Ann Arbor, Michigan). Some of our analyses were restricted to ND6 and CO3 sequences, some to CR sequences, and some to ACO1. We estimated most-parsimonious trees using heuristic TBR branch-swapping following random addition of sequences using PAUP*, version 4.0b10 (Sinauer Associates, Sunderland, Massachusetts); 10 random additions were used in each search; in some searches, individual replicates were limited to 10 min of computer time. In bootstrap analyses, we used 100 replicate searches. Haplotype networks for the ND6 plus CO3 data

were inferred using the methods described by Bandelt et al. (1999). For the CR analyses, a previously sequenced Spotted Owl haplotype (DQ230876) was used to root the trees.

We estimated the amount of genetic variation present within populations using the number of haplotypes observed in each population sample, the percentage of sites that were variable, and nucleotide diversity (π) with its bootstrap confidence interval (Nei 1987). We used Tajima's (1989a, b) *D* statistic to test for neutrality of the observed variation. We used mismatch distributions (Slatkin and Hudson 1991) to examine consistency with population growth for some individual population samples as well as for some pooled population samples. The significance of each mismatch distribution was estimated by comparison of the observed distribution to a Poisson distribution, with an identical mode, using a Kolmogorov-Smirnov one-sample test; mismatch distributions were compared to each other using Kolmogorov-Smirnov two-sample tests.

We estimated the amount of variation distributed among populations using the percentage of haplotypes in each population restricted to that population (private haplotypes), the percentage of haplotypes observed in each population that were also observed in an additional population ≥1,000 km distant (widespread haplotypes), and the G_{st} statistic of Holsinger and Mason-Gamer (1996). We estimated the extent of regional divergence across U.S. and Canadian population samples by computing the frequency of the major clades of DNA haplotypes observed in each of those population samples. We quantified the pattern of genetic divergence with geography using isolation-by-distance plots of the regressions of $G_{st}/(1 - G_{st})$ versus the common logarithm of geographic distance (Slatkin 1993, Rousset 1997). We tested the significance of the latter relationship using a randomized Mantel procedure on the genetic and geographic distance matrices (Dietz 1983); statistical significance was estimated by comparison of Spearman's rho to the observed distribution from 10,000 randomized matrices. We estimated the genetic distance matrix for our 18 samples using the pairwise $G_{st}/(1 - G_{st})$ estimates; geographic distances between all pairs of samples were estimated as the simple, straight-line distances separating them, independent of potential barriers such as major rivers, mountain ranges, or extralimital range. We estimated the relative efficacy of different genes for assaying variation within and among population samples by comparing the overall observed ratio of haplotypes to individuals (for mtDNA loci), observed alleles to number of gene copies (for ACO1), nucleotide diversity, overall G_{st}, and sequence divergence between haplotype clades, to the alternate genes and their sequence lengths. The extent of mitochondrial versus nuclear differentiation was then compared among populations to the expected divergence using the approach developed by Zink and Barrowclough (2008).

Results

Sequences.—We obtained 522 bp of ND6 sequence and 593 bp of CO3 sequence from 49 individual Barred Owls distributed among 8 populations from across their U.S. range (FL peninsula, KS, LA, MI, MN, NC, NJ, and WA; see Fig. 1 and Table 1), from 9 additional Barred Owls from Jalisco, Mexico, and from single Fulvous and Spotted owls from Guatemala and California, respectively. The ND6 and CO3 sequences were aligned without indels among Barred Owls and the Fulvous Owl; however, the Spotted Owl ND6 sequence was 3 bp shorter and required a one-amino-acid codon deletion for alignment. The ND6 sequences encompassed the entire gene, including the start and stop codons. The CO3 sequences included ~75% of the gene, including the start codon. The ratio of haplotypes to individuals (Table 2), along with the absence of internal stop codons, suggests that these were mitochondrial, rather than numt, sequences.

We obtained CR sequence from 187 Barred Owls distributed over 18 population samples from the United States and Canada (Table 1). These include the 8 population samples sequenced for the CO3 and ND6 genes plus 10 additional localities. The 5′ end of the CR was highly variable in length; a few individuals had large deletions, up to 30 bp in length, at or near the very beginning of the gene. In addition, most individuals possessed a variable-length homopolymer of up to 15 cytosines close to the 5′ end of the CR. Beyond the first 50 bp of the CR, there were a few 1-bp indels that were easily aligned. Because we were unable to accurately determine the number of cytosines in the homopolymer in some individuals, we excluded ~20 bp at the 5′ end of the CR in our analyses. Thus, the length of the aligned CR sequences used was 518 bp, when aligned with the Spotted Owl. Indels were not treated as informative variation in any of the analyses. The high ratio (0.65) of haplotypes to individuals suggests that these CR sequences are mitochondrial in origin.

We obtained 567 bp of ACO1-I15 sequence from 76 individuals distributed among 11 populations of Barred Owls from across the United States. No indels were required to align the sequences. No individual possessed more than a single heterozygous site in these sequences; consequently, phasing was not required to estimate allele frequencies.

The GenBank accessions for the individual CR (JN097839–JN098025), CO3 (JN112106–JN112165), and ND6 (JN112166–JN112225) sequences include geographic provenance and specimen voucher information. The ACON allele accessions (JF681361–JF681366) include similar data. The sequence used as

Table 2. Nucleotide variation within and among U.S. and Canadian populations of Barred Owls for one nuclear and three mitochondrial genes. Within-population variation was estimated as the ratio of distinct haplotypes observed to gene copies sequenced and as nucleotide diversity (π); variation among populations was estimated using variance partitioning (G_{st}) and as the percent sequence divergence between the most common haplotype observed in the "Atlantic Coast" (AC) and "south-central" (SC) haplotype clades.

Gene	Number of base pairs	Number of haplotypes copy^{-1}	π	G_{st}	Percent sequence divergence (AC vs. SC)
Among 18 populations					
Control region	518	0.65	0.018	0.32	4.8%
Among 8 populations					
Control region	518	0.92	0.020	0.32	6.0%
ND6	522	0.20	0.006	0.42	1.9%
CO3	593	0.24	0.002	0.31	0.3%
CR+ND6+CO3	1633	0.94	0.009	0.34	2.7%
ACO1-I15	567	0.06	0.001	0.05	<0.1%

an outgroup for the CR phylogenetic analysis had been deposited in GenBank as part of a prior Spotted Owl study (DQ230876).

Barred Owl paraphyly.—We found 28 unique haplotypes among 58 sequences of U.S. and Mexican Barred Owls and the single Fulvous Owl for the 1,115 bp of combined ND6 plus CO3 data. A minimum-length network for those sequences is shown in Figure 2. The Barred Owl sequences from the United States formed two clades of 6 and 13 haplotypes each that were ~1.3% divergent and heterogeneously distributed over geography. Eight haplotypes of Mexican specimens formed a third clade ~5% distant from those first two clades. The single Fulvous Owl haplotype was ~4.5% divergent from all of the Barred Owl haplotypes. Phylogenetic analysis indicated that this network could be resolved into 72 alternative trees of length 180, given a Spotted Owl outgroup. In a majority-rule consensus of those trees, the Fulvous Owl was sister to the U.S. haplotypes, to the exclusion of the Mexican Barred Owl haplotypes, 75% of the time. In a bootstrap analysis, the U.S. Barred Owl haplotypes plus the Fulvous Owl haplotype formed a clade in 54% of the replicates. In 10% of the replicates the Mexican Barred Owls plus the Fulvous Owl formed a clade, and in the remaining 36% of the replicates the U.S. and Mexican Barred Owls formed a monophyletic group. Thus, our data suggest that *Strix varia*, as currently classified, is not monophyletic and that the Mexican population is more divergent from the U.S. populations than is *S. fulvescens*.

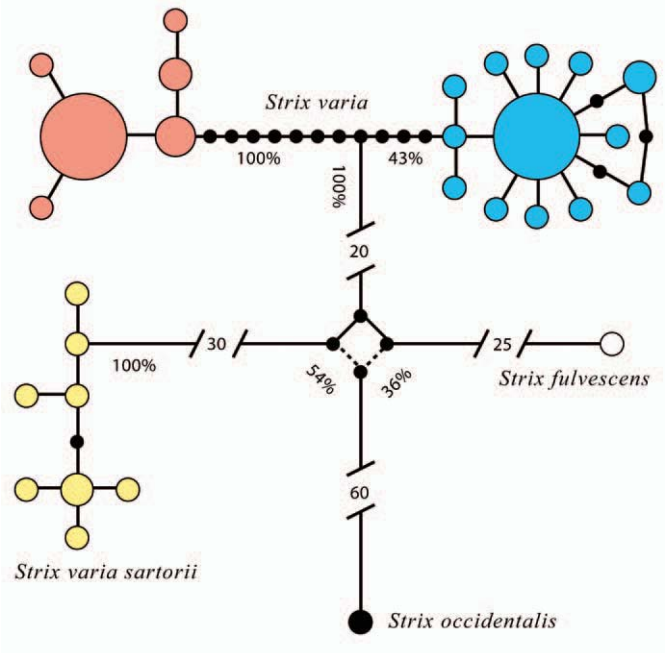

Fig. 2. Minimum-spanning network for 28 haplotypes (open circles) observed among U.S. and Mexican populations of Barred Owls, plus a Fulvous Owl, for 1,115 bp of ND6 plus CO3 mitochondrial sequence. Areas of circles are proportional to numbers of individuals that have that haplotype. Branch lengths within taxa are indicated by single steps; unobserved haplotypes within taxa are indicated by dots. Branch lengths between taxa, not drawn to scale, are indicated by numbers along branches. Bootstrap support values for some major nodes are shown below branches.

Genetic variation across the United States and Canada.—We used 518 bp of CR sequence to more fully resolve the distribution of the two clades in the United States and Canada. We observed 121 unique haplotypes among 187 individuals distributed among 18 populations north of Mexico (Fig. 1); individual sample sizes varied from 5 to 16, with a median of 10 (Table 1).

The ratio of private to total haplotypes was ≥50% for all samples south and east of Minnesota; for more northerly and western samples, the ratio was ≤50% (Table 1). Conversely, the relative frequency of haplotypes with a range >1,000 km was high in northern and western locations and low in the southern samples (Table 1).

There were large numbers of equally parsimonious trees for these haplotypes, each with length 299 and a consistency index (excluding uninformative sites) of 0.31. We terminated the PAUP* search at 250,000 trees; one of the trees is shown in Figure 3. The salient feature of 100% of those trees was the presence of the same two clades of haplotypes that had been found in the ND6 plus CO3 analysis. However, the CR had a substantially greater substitution rate than did ND6 plus CO3; hence, the mean divergence between the two clades was ~4.8% for CR. The geographic distribution of the two clades is shown in Figure 1. One clade was present at frequencies of nearly 100% in population samples from along the Atlantic Coast of the United States and Canada; the other clade occurred at similar frequencies in the samples from the south-central region of the United States (Table 1). From the central Gulf Coast of Florida north to the Upper Midwest and across Canada to the Pacific Northwest, the two clades of haplotypes co-occurred at intermediate frequencies. The heterogeneous distribution of the two clades resulted in a substantial portion of genetic variation being distributed among populations (G_{st} = 0.32; Table 2).

We observed six alleles among the ACO1 intron sequences (Fig. 4). One of these was present everywhere at high frequency; two others were present at overall frequencies of 8% and 9%, but nowhere at high frequency. One of the intermediate-frequency alleles was found only in populations that also possessed high frequencies of the Atlantic Coast mtDNA haplotype clade. The second intermediate-frequency allele was found both in some populations with a majority of Atlantic Coast mtDNA haplotypes and in some populations with a majority of south-central mtDNA haplotypes. Three alleles were observed in single individuals only. The six alleles formed a network, five steps in length, without homoplasy (Fig. 4). Owing to the quality of some samples, we were unable to compute G_{st} for ACO1 for precisely the same eight populations used for comparisons among the three mitochondrial genes. Instead, we replaced the FL, KS, LA, and MN samples with those from GA, TN, TX, and OH, respectively; these resulted in similar geographic coverage of the owl's range. The estimate of G_{st} for ACO1 among these populations was 0.05.

Variation within populations and demography.—The CR sequences had a relatively high substitution rate. The ratio of haplotypes to individuals was high (>0.7) everywhere except in the northern and western population samples from Alberta, Oregon, and Washington, where it was ≤0.5 (Table 1).

The interpretation of patterns of genetic variation within populations was complicated by the unequal distribution of the two clades of haplotypes among the population samples. Both nucleotide diversity and the percentage of sites that were variable were inflated in localities in which both clades were present, as compared

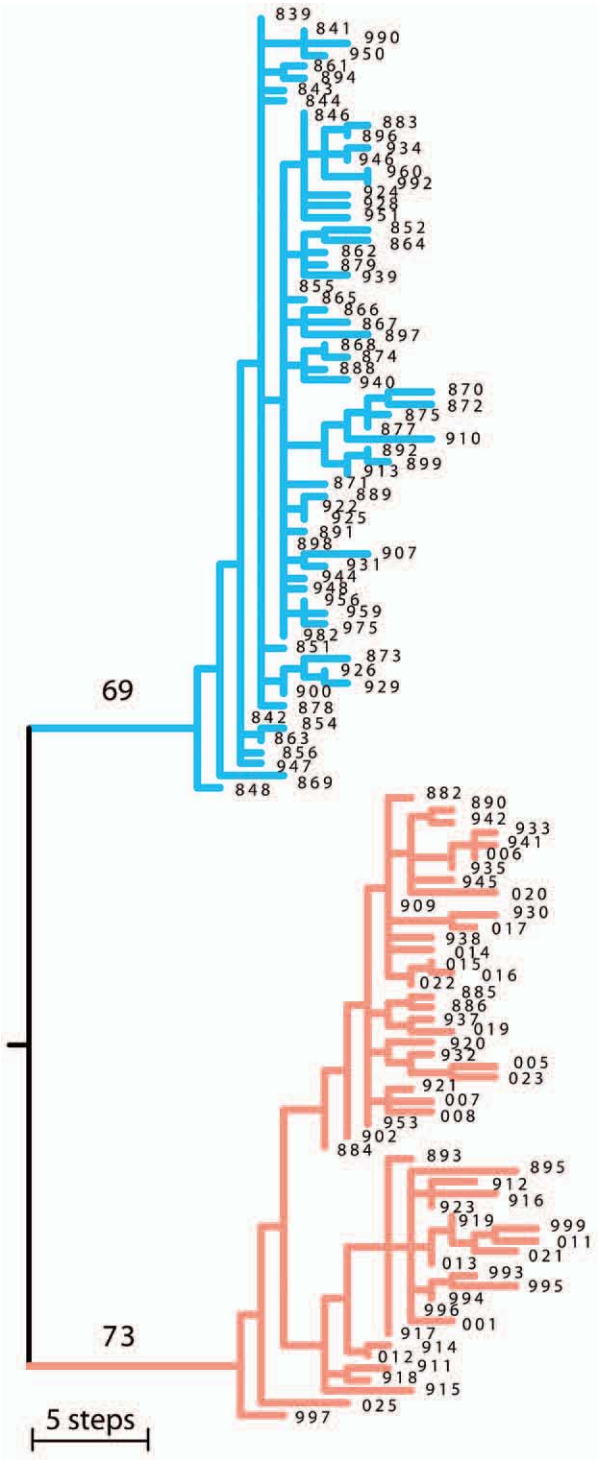

Fig. 3. Phylogram for 121 mitochondrial control-region (518 bp) haplotypes of Barred Owls observed among 18 populations distributed across the United States and Canada (Fig. 1). This is one of many equally parsimonious trees. Two major clades of haplotypes, present in 100% of the individual trees and ~4.8% divergent, are indicated by different colors; bootstrap proportions for the clades shown above branches. Individual haplotypes are designated by the last three digits of their GenBank accession numbers (JN097839–JN098025).

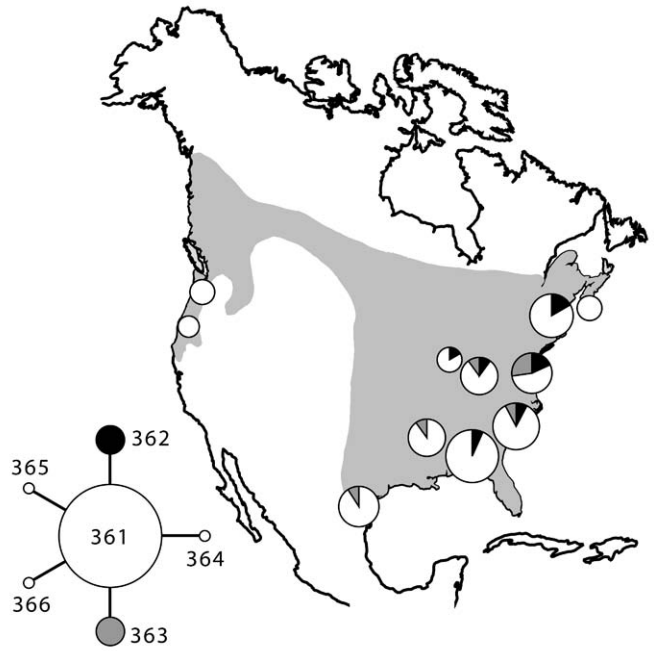

Fig. 4. Minimum-length allele network and geographic distribution of three widespread ACO1-I15 alleles in the U.S. range of the Barred Owl. Relative frequency of each allele is indicated by size of circle in network diagram. On the distribution map, the area of each pie-diagram is proportional to the sample size from that population; the observed proportions of each of three widespread alleles are indicated by different shading; the geographic distribution of three singleton alleles is not shown. Alleles are identified by the last three digits of their GenBank accession numbers (JF681361–JF681366).

with localities in which only one clade was observed (Table 1). Tajima's *D* also tended to be larger and positive in such samples; the sole instance of a statistically significant value of *D* occurred in the Washington state sample where the occurrence of the two clades was 50% each. Thus, potential patterns of populational variation differing over geography were compromised by the presence of two clades of haplotypes in some samples and not in others.

In the case of mismatch distributions, for example, and their use in the inference of population growth, the presence of two divergent clades in a sample will result in a second peak in the distribution situated on the abscissa at a value equal to the sequence divergence between the clades. This second peak, or any other measure of population growth dependent on haplotype divergence, will result in a pattern consistent with demographic stationarity, whether the population had been growing or not. We attempted to alleviate this difficulty by examining mismatch distributions for localities in which only one of the two clades were present and by computing mismatch distributions for the predominant clade at localities in which both were present. For these filtered mismatch distributions, none of the distributions was significantly divergent from a Poisson distribution; however, the modes of the distributions varied geographically: the modes were closer to the origin for samples farther from the Atlantic Coast (Fig. 5). This is consistent with a pattern of growing populations and expanding range in which, for the Atlantic Coast clade of

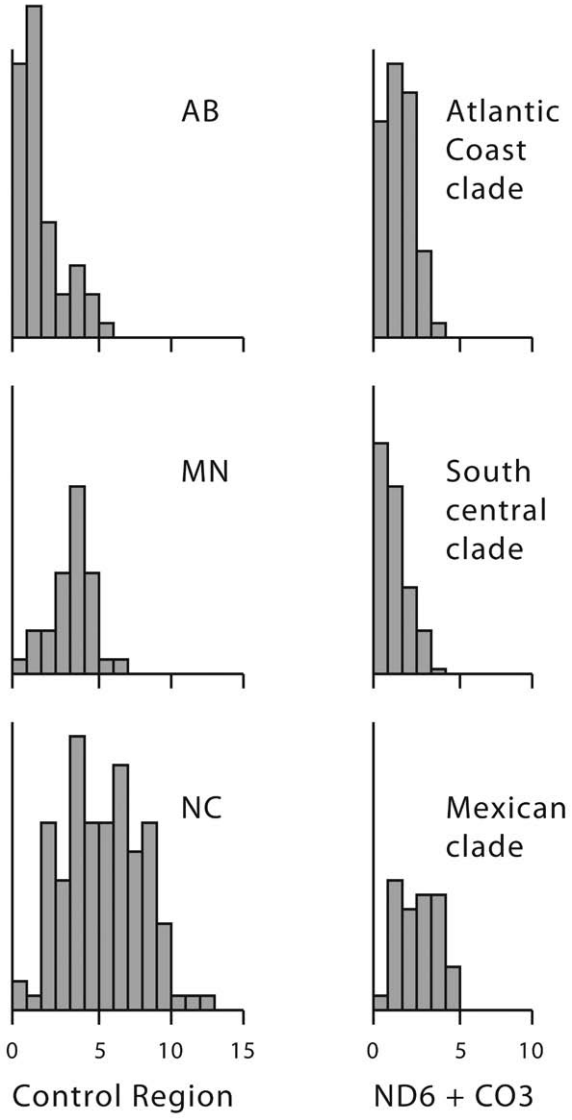

Fig. 5. Sample mismatch distributions for some populations of Barred Owls. Distributions for 518 bp of control region for "Atlantic Coast" clade of haplotypes for three localities (southeast to northwest: bottom to top) are shown at left. Distributions for 1,115 bp of ND6 plus CO3 sequence are shown at right for a Jalisco, Mexico, population, as well as for pooled samples for the "Atlantic Coast" and "south-central" clades, irrespective of location. Horizontal scale is constant for all plots; vertical scale is proportional to sample sizes.

haplotypes, the growth started in the east and moved north and west.

A second approach enabled us to compare the Mexican population with the U.S. and Canadian samples. For the 1,115 bp of ND6 plus CO3 sequence, there was less resolution and, hence, less variation within populations than for the CR sequences (Table 2), but by sorting these sequences into the three constituent clades of haplotypes, we obtained overall mismatch distributions for the Mexican, Atlantic Coast, and south-central clades (Fig. 5). None of those distributions differed from a Poisson distribution, but each was significantly divergent from the other two ($P < 0.01$). In particular, the distribution of the Mexican Barred Owl sample was farther from the origin than the Atlantic Coast clade sample, which, in turn, was farther from the origin than the south-central clade sample. This suggests that demographic histories have differed among the three populations represented by the haplotype clades, with the Mexican population being older. Within the United States and Canada, it appears that the Atlantic Coast population initiated its growth and expansion earlier than the south-central population.

Isolation-by-distance across the United States and Canada.—The heterogeneous distribution of the two clades of haplotypes also hampered our ability to examine patterns of isolation-by-distance. When all sequences were used, the genetic distance matrix was dominated by comparisons between population samples with and without both haplotype clades. Thus, subtle patterns of gene flow were obscured by large-scale patterns of clade introgression. For the overall comparison, the correlation between the genetic and geographic distance matrices (0.18) was not significant ($P = 0.245$). We separately estimated isolation-by-distance relationships for the Atlantic Coast and south-central clades of haplotypes, as we had for the mismatch distributions, where population samples were included only if five or more sequences from the clade of interest were present in the sample. Those regressions were positive for both clades of haplotypes (Fig. 6), and the correlations were statistically significant in both cases (Atlantic Coast clade, $P = 0.003$; south-central clade, $P = 0.035$). The steeper slope of the regression for the south-central clade (Fig. 6) suggests a more viscous population structure among those birds.

Comparative efficacy of alternate genes.—We used 500–600 bp sequences each of one nuclear and three mitochondrial genes to survey genetic variation and structure of Barred Owl populations

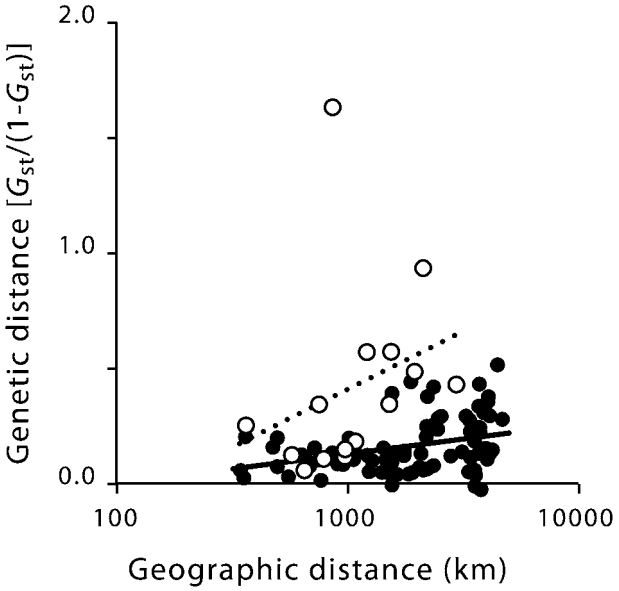

Fig. 6. Isolation-by-distance among U.S. and Canadian populations of Barred Owls, based on 518 bp of mitochondrial control-region sequence. Separate relationships are shown for populations containing five or more individuals from the "Atlantic Coast" clade: solid circles, solid regression line; and from populations containing five or more individuals from the "south-central" clade: open circles, dotted regression line.

(Table 2). These genes had rather different substitution rates, as reflected in both the ratio of observed number of haplotypes per gene copy and in our estimates of nucleotide diversity. Nucleotide diversity, averaged over 8 populations, varied by more than an order of magnitude, from 0.001 for ACO1 to 0.021 for CR. Likewise, the haplotype-to-individual (copy) ratio varied by a factor of 15. Clearly, it would be inappropriate to compare those statistics across genes, within or across taxa. The difference in substitution rates is also reflected in the divergence between the Atlantic Coast and south-central clades of haplotypes; those two haplotype clades were identified by all three mitochondrial genes, but they differed in percent sequence divergence by a factor of 20. Thus, the resolution of CR was greatest in identifying variation, followed by ND6, with CO3 trailing. However, when it came to identifying geographic structure—that is, partitioning rather than finding variation—there were no differences among the genes in identifying the two major clades with different geographic distributions, and there also was little difference in quantifying the magnitude of this structure, using G_{st}. Consequently, ~500 bp of any of the mitochondrial genes would have been adequate for detecting overall geographic structure. However, for analyses requiring more resolution of haplotypes, such as finding latitudinal trends in mismatch distributions or in π, or for quantifying patterns of isolation-by-distance, only our CR sequences were adequate.

The distribution of the ACO1 alleles revealed little geographic structure, consistent with the larger effective population size and slower coalescent time of nuclear loci vis-à-vis mtDNA (Zink and Barrowclough 2008). In addition to the high-frequency allele that was common everywhere, one allele was found only in populations with a majority of Atlantic Coast mtDNA haplotypes. This is consistent with incomplete lineage-sorting of an allele that was initially present at low frequency in the eastern Pleistocene refuge for Barred Owls. A second low-frequency allele, found in several populations extending from Texas to New Jersey, may have been present in multiple refugia. The heterogeneous frequency distribution of these two uncommon alleles may be due to small sample sizes or allelic surfing (Edmonds et al. 2004). Overall, the ACO1 data, while not confirming the mtDNA results, were nevertheless consistent with them, given the trailing nature of nuclear markers of population structure. For example, if the geographic distribution pattern of haplotypes across the United States and Canada was the result of prior glacial isolation, then the equation for the relationship between nuclear and mitochondrial fixation indices given by Zink and Barrowclough (2008) can be modified for a comparison between a sex-linked locus (Z chromosome) and a mitochondrial one. That relationship becomes

$$G_{st}(Z,t) \approx 1 - e^{\frac{\ln[1-G_{st}(mt,t)]}{3}}$$

For the Barred Owls, the predicted value of G_{st}(Z), given G_{st}(mt) of 0.34 (Table 2), is 0.13, much closer to the observation (0.05).

Overall, the ACO1 sequence data were consistent with the mitochondrial results. However, taken alone, they failed to reveal the salient geographic signal detected by even the least differentiated of the mitochondrial genes.

Discussion

Genetic structure, refugia, and introgression.—There are three divergent clades of Barred Owls based on our mtDNA data. Mismatch distributions suggest that these have had separate demographic histories. They probably are the result of isolation of populations in three geographically isolated refugia during or prior to several of the Pleistocene glacial maxima. The current distribution of clades of haplotypes suggests that these refugia were located in central Mexico, near the southern Atlantic Coast of the United States, and in the south-central states, perhaps along the Gulf Coast of Texas or Louisiana. Postglacial expansion of vegetation (Pielou 1991) enabled the two U.S. populations of Barred Owls to expand their ranges northward, resulting in extensive introgression through much of the Midwest. This introgression is reflected in elevated nucleotide diversity west of the Appalachian Mountains. More recently, the populations have expanded across the prairie provinces of Canadian to British Columbia and south into northern portions of the western United States. This expansion is reflected in the decreasing south-to-north modal values of the mismatch distributions, and in the different geographic distributions of private and widespread mtDNA haplotypes.

Timing of divergences.—Control-region sequences diverge between avian taxa at widely varying rates along the gene. For example, the 5′ end of the CR differs between congeneric Spotted and Barred owls to the point that alignment is almost impossible for the first 60 bp, exceeds 13% for next 500 bp, and is ~8% for the following 500 bp (Barrowclough et al. 1999). Consequently, divergence rates and time are difficult to correlate. The other two genes sampled, CO3 and ND6, differ in their divergence rates by a factor of 6 to 7 (Table 2). Of the two, CO3 is probably most similar to cytochrome-*b* in divergence rate because it is also a light-strand, protein-coding gene; ND6 is a heavy-strand, protein-coding gene with markedly differing nucleotide composition. At a 2% Ma^{-1} rate of divergence (Lovette 2004), the 2.9% divergence for CO3 between the Mexican and U.S. Barred Owls is approximately equivalent to 1.5 Ma. The 0.3% divergence between the Atlantic Coast and south-central clades suggests 100,000 to 200,000 years of isolation, that is, toward the end of the Pleistocene. Thus, the former event is of the order suggested by Klicka and Zink (1997) as typical of many songbird species-pairs that apparently predate the Late Pleistocene-origins model; the latter event is consistent with the traditional Pleistocene glaciation paradigm (Mengel 1964, Avise and Walker 1998).

Comparative biogeography.—Genetic structure similar to that found in the Barred Owl has not been a major feature of avian phylogeographic surveys in eastern North America (e.g., Zink 1996, 1997). This has been generally true of both passerines and nonpasserines (e.g., Ball and Avise 1992), including some recent studies that involved dense geographic sampling (e.g., McKay 2009). However, deep phylogeographic divisions across the southeastern United States are a common feature in many other organisms, including plants, fishes, amphibians, snakes, turtles, and mammals (Soltis et al. 2006). Birds are more vagile than most of these other organisms, and so it is plausible that separate refugia for some sedentary organisms are not effective for birds. A second possibility is that even if separate refugia existed for birds, longer-distance gene flow in birds than in other organisms may have subsequently obliterated the geographic signature of refugia in many cases.

In terms of the general patterns observed in non-avian taxa, the center of the division between the two Barred Owl clades must be close to the Apalachicola division identified for several

organisms along the U.S. Gulf Coast (Soltis et al. 2006). Our Florida panhandle sample was taken from just west of the Apalachicola River and our Georgia sample straddled the Chattahoochee River, a major tributary of the Apalachicola (Fig. 1).

We are aware of only a single avian species that possesses a biogeographic pattern reminiscent of that found in the Barred Owl: the Carolina Chickadee (*Poecile carolinensis*). The pattern of differentiation of two clades of Carolina Chickadees is quite similar to the Barred Owl pattern, although the chickadee's range does not extend as far north and west as the owl's. Previous RFLP analysis of mtDNA indicated a narrow east–west split of the chickadees centered on the Tombigbee River–Mobile Basin (Gill et al. 1993, 1999), slightly west of the apparent center of the Barred Owl division. The Carolina Chickadee is nonmigratory (Mostrom et al. 2002) and, thus, more sedentary than many of the avian species studied in past phylogeographic surveys.

Comparative phylogeography of Strix *owls*.—The partitioning of genetic variance indicated that ~32% of the mtDNA genetic variation was distributed among U.S. and Canadian populations of owls. Similarly, in a study of the Tawny Owl, Brito (2005) reported 35% of the variance distributed among populations that had expanded across Europe from three southern refugia; that variance component was associated with extensive introgression across the Alps from Italy into France, and across the Pyrenees from a widespread Balkan population into Iberia. By contrast, ~73% of the variation was distributed among populations of Spotted Owls in a study with range-wide sampling (Barrowclough et al. 1999). The salient difference between the results of these three studies is that, in the case of the Spotted Owls, there was little or no introgression between regions, whereas in the Barred and Tawny owls the introgression was extensive. Thus, fixed differences among regions of Spotted Owl populations, with a large variance component, contrast with intergradation and a much smaller variance component in the other two owls.

Taxonomy.—The Barred Owls of Mexico are genetically divergent from other Barred Owls. In fact, the Fulvous Owl is apparently more closely related to the Barred Owls of the United States and Canada than are the Mexican birds. Consequently, recognizing Barred Owls from Mexico plus the United States and Canada as conspecific, to the exclusion of the Fulvous Owl, would result in a paraphyletic taxon. Instead, one could lump the Fulvous Owl with the Barred Owl to create a monophyletic species, as has been done in the past by some authors (e.g., Peters 1940), but this seems unwarranted to us because the vocalizations of the Fulvous Owl, the Mexican Barred Owl, and the Barred Owls of the United States and Canada all differ substantially from each other (S. Howell pers. comm.). The Barred Owl of Mexico, the Barred Owl of the United States and Canada, and the Fulvous Owl of Mexico and Central America are each monophyletic and diagnosable on the basis of many mtDNA characters and vocalizations. They are weakly diagnosable on the basis of plumage (these are owls, after all). Thus, they certainly comprise three phylogenetic species and probably represent three biological species, given their allopatry, known vocal differences, and the understandable reluctance to recognize paraphyletic taxa (Johnson et al. 1999). The genetic divergences of 4–5% among the three suggest isolation over perhaps a couple of million years. We recognize three species: the Northern Barred Owl (*S. varia*), the Mexican Barred Owl (*S. sartorii*), and the Fulvous Owl (*S. fulvescens*).

Genetic variation within the Northern Barred Owls does not closely correspond to current subspecific ranges, which tend to be latitudinal, whereas the genetic variation tends to be longitudinal (e.g., Fig. 1). The three generally recognized subspecies do not delimit historical units and so will not be predictive of additional characteristics beyond those on which they were based. Although it is likely that during glacial maxima there would have been a close correspondence between the mtDNA clades and geography, during the present interglacial approximately one-third of the total geographic range of Barred Owls north of Mexico is a region of extensive introgression. Moreover, Barred Owls do not show vocal differentiation in the southern portion of their range in the United States coincident with the mtDNA variation we found (Odom and Mennill 2010).

One could recognize two taxa (the two clades) or three taxa (the two clades plus the intergrades) of Northern Barred Owls, probably with subspecific rank. In such a case, the name *S. v. varia* is available for the Atlantic Coast birds (type locality: Philadelphia, Pennsylvania); the name *S. v. helveola* is available for the south-central birds (type locality: Corpus Christi, Texas); and, if desirable, *S. v. brunnescens* is available for the intergrades (type locality: Lake of the Woods County, Minnesota). The latter is the earliest available name (Bishop 1931) clearly associated with a locality in the zone of introgression. However, this intraspecific taxonomy again has the problem of recognizing polyphyletic units and not being predictive for additional characters. Consequently, we believe that the extent of introgression in the United States and Canada is too great to treat birds that belong to these two clades as separate taxonomic units, at any rank.

Conservation.—The precise geographic distribution, abundance, and other aspects of the natural history of the Mexican Barred Owl are poorly known. It is quite possible that the range of the species, in higher-elevation pine–oak forest across the central volcanic belt, may be fragmented and the genetic structure of the populations may not be homogeneous. Additional study of that species is indicated.

Acknowledgments

Many field biologists, raptor rehabilitation centers, and museums provided us with critical samples used in this research; these include D. Rock (Western Wildlife Program, National Council for Air and Stream Improvement), G. Hill (Auburn University), M. Russell (Alberta Fish and Wildlife Division), the 2007 and 2008 Choctawhatchee River field crews (particularly B. Rolek), Arkansas Game and Fish Commission (T. Teel), Avian Haven Wild Bird Rehabilitation Center of Maine (M. Payne, D. Winn), Carolina Raptor Center (M. Engelmann), Chattahoochee Nature Center (K. Dudeck), Florida Audubon Society Center for Birds of Prey (C. Gilliland, D. Flynt, H. Kale), Georgia Southern University Lamar Q. Ball Raptor Center (S. Courdin), Hope for Wildlife of Nova Scotia (H. Swinimer), Last Chance Forever of Texas (J. Karger), Medina Raptor Center of Ohio (L. Jordan), Michigan State University Veterinary Clinic (J. Sikarskie, J. Lemson, E. Tullet), Mid-south Raptor Center of Tennessee (K. Martin), Mississippi Wildlife Rehabilitation Center (V. Smith), Ohio Wildlife Center (L. Fosco, S. Gibson), Raptor Trust of New Jersey (L. Soucy), Sardis Wildlife Center of Ferndale, Washington, Southeastern Raptor Center of Alabama (E. Crandall),

Texas State Aquarium (D. Prevratil), Cleveland Museum of Natural History (A. Jones), Louisiana State University Museum of Zoology (F. Sheldon, D. Dittmann, S. Cardiff), North Carolina Museum of Natural Sciences (D. Lee, J. Gerwin), Royal Alberta Museum (J. Hudon), University of Kansas Museum of Natural History (T. Peterson, M. Robbins), University of Michigan Museum of Zoology (B. Payne, J. Henshaw), University of Minnesota Bell Museum of Natural History (B. Zink, J. Klicka), and University of Washington Burke Museum (S. Rohwer, S. Birks). We are grateful to P. Capainolo, S. Haber, S. Kenney, P. Sweet, and J. Weicker for preparing many specimens for this research at the American Museum of Natural History. B. Jablonski, D. Kizirian, N. Klein, and G. Nunn assisted with some of the laboratory work associated with this project. We thank D. Mennill of the University of Windsor for logistical assistance and financial support for permits. R. Gutiérrez and B. Zink, two anonymous reviewers, and J. Klicka provided useful comments on earlier versions of the manuscript. We thank S. Claramunt for providing the Spanish abstract. This research was supported in part by the F.M. Chapman Memorial Fund, the L.C. Sanford Fund, the Leonard J. Sanford Trust, the Sackler Institute for Comparative Genomics, the Ambrose-Monell Molecular Laboratory, and the Lewis B. and Dorothy Cullman Program for Molecular Systematics.

Literature Cited

American Ornithologists' Union. 1957. Check-list of North American Birds, 5th ed. American Ornithologists' Union, Washington, D.C.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London, Series B 265:457–463.

Ball, R. M., Jr., and J. C. Avise. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. Auk 109:626–636.

Bandelt, H.-J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48.

Barrowclough, G. F., R. J. Gutiérrez, and J. G. Groth. 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.

Bishop, L. B. 1931. Three apparently undescribed owls. Proceedings of the Biological Society of Washington 44:93–95.

Brito, P. H. 2005. The influence of Pleistocene glacial refugia on Tawny Owl genetic diversity and phylogeography in western Europe. Molecular Ecology 14:3077–3094.

Dietz, E. J. 1983. Permutation tests for association between two distance matrices. Systematic Zoology 32:21–26.

Edmonds, C. A., A. S. Lillie, and L. L. Cavalli-Sforza. 2004. Mutations arising in the wave front of an expanding population. Proceedings of the National Academy of Sciences USA 101:975–979.

Gill, F. B., A. M. Mostrom, and A. L. Mack. 1993. Speciation in North American chickadees: I. Patterns of mtDNA genetic divergence. Evolution 47:195–212.

Gill, F. B., B. Slikas, and D. Agro. 1999. Speciation in North American Chickadees: II. Geography of mtDNA haplotypes in *Poecile carolinensis*. Auk 116:274–277.

Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071–1075.

Gutiérrez, R. J., M. Cody, S. Courtney, and A. B. Franklin. 2007. The invasion of Barred Owls and its potential effect on the Spotted Owl: A conservation conundrum. Biological Invasions 9:181–196.

Holsinger, K. E., and R. J. Mason-Gamer. 1996. Hierarchical analysis of nucleotide diversity in geographically structured populations. Genetics 142:629–639.

Johnson, N. K., J. V. Remsen, Jr., and C. Cicero. 1999. Resolution of the debate over species concepts in ornithology: A new comprehensive biological species concept. Pages 1470–1482 *in* Proceedings 22nd International Ornithological Congress (N. J. Adams and R. H. Slotow, Eds.). BirdLife South Africa, Johannesburg.

Klicka, J., and R. M. Zink. 1997. The importance of recent ice ages in speciation: A failed paradigm. Science 227:1666–1669.

Lovette, I. J. 2004. Mitochondrial dating and mixed support for the "2% rule" in birds. Auk 121:1–6.

Mazur, K. M., and P. C. James. 2000. Barred Owl (*Strix varia*). *In* The Birds of North America, no. 508 (A. Poole and F. Gill, Eds.). Birds of North America, Philadelphia.

McKay, B. D. 2009. Evolutionary history suggests rapid differentiation in the Yellow-throated Warbler *Dendroica dominica*. Journal of Avian Biology 40:181–190.

Mengel, R. M. 1964. The probable history of species formation in some northern wood warblers (Parulidae). Living Bird 3:9–43.

Mengel, R. M. 1970. The North American central plains as an isolating agent in bird speciation. Pages 279–340 *in* Pleistocene and Recent Environments of the Central Great Plains (W. Dart, Jr., and J. K. Jones, Jr., Eds.). University of Kansas Press, Lawrence.

Mostrom, A. M., R. L. Curry, and B. Lohr. 2002. Carolina Chickadee (*Poecile carolinensis*). *In* The Birds of North America, no. 636 (A. Poole and F. Gill, Eds.). Birds of North America, Philadelphia.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Odom, K. J., and D. J. Mennill. 2010. A quantitative description of the vocalizations and vocal activity of the Barred Owl. Condor 112:549–560.

Peters, J. L. 1940. Check-list of Birds of the World, vol. 4. Harvard University Press, Cambridge, Massachusetts.

Pielou, E. C. 1991. After the Ice Age: The Return of Life to Glaciated North America. University of Chicago Press, Chicago, Illinois.

Pyle, P. 1997. Identification Guide to North American Birds, part 1: Columbidae to Ploceidae. Slate Creek Press, Bolinas, California.

Remington, C. L. 1968. Suture-zones of hybrid interaction between recently joined biotas. Evolutionary Biology 2:321–428.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145:1219–1228.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279.

Slatkin, M., and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos, and P. S. Soltis. 2006. Comparative phylogeography of unglaciated eastern North America. Molecular Ecology 15:4261–4293.

Spellman, G. M., and J. Klicka. 2007. Phylogeography of the White-breasted Nuthatch (*Sitta carolinensis*): Diversification in North American pine and oak woodlands. Molecular Ecology 16: 1729–1740.

Tajima, F. 1989a. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Tajima, F. 1989b. The effect of change in population size on DNA polymorphism. Genetics 123:597–601.

Zink, R. M. 1996. Comparative phylogeography in North American birds. Evolution 50:308–317.

Zink, R. M. 1997. Phylogeographic studies of North American birds. Pages 301–324 *in* Avian Molecular Evolution and Systematics (D. P. Mindell, Ed.). Academic Press, San Diego, California.

Zink, R. M., and G. F. Barrowclough. 2008. Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology 17:2107–2121.

Zink, R. M., W. L. Rootes, and D. L. Dittmann. 1991. Mitochondrial DNA variation, population structure, and evolution of the Common Grackle (*Quiscalus quiscula*). Condor 93:318–329.

Associate Editor: J. Klicka